CLINICAL DIAGNOSIS

By

LABORATORY EXAMINATIONS

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BY

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Requiescat in Pace

No clinical or laboratory
examination can be better
than the thoroughness and skill
with which it is conducted.

PREFACE

I am very grateful for the generous reception afforded the first edition of this book and hope that this one will prove even more helpful to medical students, practicing physicians and surgeons, teachers, clinical pathologists and medical technologists. The kindness of those who have sent me helpful suggestions is also deeply appreciated.

The present edition has been heavily revised and inevitably enlarged by the inclusion of newer material and eighteen additional illustrations, some of which are in color. Space does not permit a detailed listing of this new material but mention may be made of the diagnostic value of acid phosphatase determinations; blood tests in relation to alcoholism; the posterior pituitary injection kidney function test; the secretin test for dysfunction of the pancreas; the cephalin-cholesterol flocculation, thymol turbidity, tyrosine tolerance and prothrombin liver function tests; the classification and differential laboratory diagnosis of jaundice; the barium strip and methylene blue tests for bilirubinuria; Watson's test for urobilinogenuria; infectious or viral hepatitis; homologous serum jaundice; Papanicolaou's cytology test for cancer; laboratory examinations in relation to sulfonamide and antibiotic therapy; Rh and Hr blood factors; anti-Rh agglutinins and "blocking antibodies" in relation to pregnancy and blood transfusion; conglutination; rickettsial agglutination tests; rickettsialpox; complement fixation and skin tests in mumps and histoplasmosis; complement fixation tests in yellow fever, psittacosis, and ornithosis; skin tests in the drug allergies; the agglutinin-inhibition test in influenza; primary atypical pneumonia and the cold agglutination test; the specificity and sensitivity of serologic tests and cardiolipin antigen in the diagnosis of syphilis, including the masking or suppression of the disease by penicillin in the treatment of gonorrhea; piedra, rhinosporidiosis, geotrichosis, etc.

As stated in the Preface of the first edition, the primary purpose of this book is to present the clinical interpretations of laboratory examinations and their practical applications in the diagnosis and differential diagnosis of various diseases. However, I have been mindful of the fact that not only may teachers of clinical pathology prefer that methods be included for the convenience of their students, but also that practicing physicians and surgeons who conduct the simpler examinations in their own laboratories may, likewise, prefer that the methods be included. Available space demanded that these be very carefully selected but I am not without hope that those chosen and presented in Part Three may prove adequate and satisfactory. After all, many clinical, pathologic, and especially biochemical, toxicologic, bacteriologic, mycologic, parasitologic and serologic examinations, to be properly conducted, require the services of laboratory specialists. So much skill and experience are required in conducting such examinations that they can be neither adequately taught students in the time allowed by curricula, nor conducted by the great majority of practitioners. The clinical or practical applications of laboratory examinations in the diagnosis and differential diagnosis of various diseases, however, concern medical students not only in the teaching of clinical pathology in the sophomore year but likewise during the junior and senior years, and especially since textbooks of medicine, surgery and the various specialties are not usually permitted to present laboratory diagnosis as thoroughly as indicated or required.

In conclusion I wish, once again, to express deep appreciation to the publishers for their excellent editorial work and for permission to revise the book heavily in order to bring its contents strictly up to date. I also remain deeply grateful to Mrs. Edna M. Kershaw for her secretarial assistance.

JOHN A. KOLMER

PREFACE TO THE FIRST EDITION

The past two or three decades have been extraordinarily fruitful in the development of clinical pathologic, biochemical, toxicologic, bacteriologic, mycologic, parasitologic, serologic and immunologic methods of great practical value in the diagnosis and treatment of disease. Under the circumstances, clinical diagnosis by laboratory examinations has become a large and important subject in medicine, surgery and the various specialties.

The preparation of this book was undertaken in response to suggestions by many teachers in medical schools for a work that would present fully the clinical interpretations of laboratory examinations and their practical applications in the diagnosis of disease, along with a brief section on laboratory technical methods sufficient for meeting the needs of teaching clinical pathology to medical and dental students. It was also motivated by the conviction that not only medical students but physicians and surgeons as well are far more interested in the clinical applications or interpretations of laboratory examinations in general than in their technic, since the majority have neither the time nor the inclination to bother with the latter except in the case of the simpler and frequently employed procedures. However, practitioners cannot escape a heavy responsibility in the proper collection of materials for laboratory examinations. For this reason methods for such purposes are described in detail. The same is true of glucose tolerance, kidney and liver function tests, etc., which, to be properly conducted, likewise require the close cooperation of clinicians. I have also included the technic of such tests as the various skin tests, which are conducted so frequently by physicians themselves and, rightly or wrongly, included in the category of laboratory examinations.

Quite commonly, various laboratory examinations are employed routinely. This is always advisable and commendable in view of the frequency with which clinically unsuspected abnormal states are discovered. Laboratory examinations are also widely employed as specific or helpful aids in the confirmation or exclusion of clinically suspected diseases, especially those presenting difficult diagnostic problems. Frequently, however, abnormal changes revealed by laboratory examinations are due to more than one cause. For this reason the clinical interpretation of laboratory examinations, which I have endeavored to cover in Part One, takes first place in importance. Furthermore, since abnormal changes revealed by laboratory examinations can never be fully appreciated or properly evaluated without a good working knowledge of functions and normal values, I have attempted, whenever possible, to give the latter and their physiologic sources or bases. Needless to state, however, normal values are subject to variation according to the methods employed, but, as far as possible, those given and the clinical interpretation of abnormal changes based upon them, have been selected on the basis of what are considered to be the usual methods properly conducted. It is hoped that the summaries included in this part of the book will be helpful not only to medical and dental students, but to busy practitioners as well.

In Part Two I have endeavored to summarize the important laboratory findings of specific or helpful value in the diagnosis and treatment of diseases of the blood and hemopoietic system, the kidneys and urinary tract, the venereal diseases and those of the stomach, intestines, pancreas, liver and biliary tract, cardiovascular and respiratory systems; also the diseases of metabolism and those due to the vitamin deficiencies, the diseases of the endocrine glands, and miscellaneous infectious diseases. In this manner it is hoped that practitioners and students may see at a glance the usual laboratory findings and the test or tests indicated when a certain disease is suspected; also those tests to employ for the purpose of confirming or excluding clinical diagnoses when laboratory examinations are required or advisable for such purposes.

As previously stated, I have been mindful of the fact, however, that teachers of clinical pathology naturally prefer that methods be included for the convenience of their students. Likewise, physicians and surgeons who conduct the simpler examinations in their own laboratories may prefer having the methods included. For these reasons, therefore, I have included the technic of such examinations of the blood, urine, gastric contents, feces, spinal fluid, etc., also some of the simpler and commonly employed blood chemistry determinations and functional, bacteriologic and serologic examinations, which I hope will meet both purposes and requirements. But since this book is so largely devoted to the practical aspects of laboratory examinations in relation to clinical diagnosis, I have thought it advisable to gather together, in Part Three, the technics of such methods instead of scattering them about in the text. Otherwise no attempt has been made to include descriptions of the more intricate laboratory procedures since they may be found, along with all technical details (which are so important in relation to their proper conduct), in manuals devoted to laboratory technic. After all, many clinical pathologic and especially biochemical, toxicologic, bacteriologic, mycologic, parasitologic and serologic examinations, to be properly conducted, require the services of laboratory specialists; also so much skill and experience is required that they can be neither adequately taught medical students nor conducted by the great majority of practitioners.

Needless to state, no examination can be better than the laboratory or individual conducting it. This fact deserves far more emphasis than commonly surmised. In this connection it is hoped that this book will be of interest and value also to medical laboratory technologists. If it does no more than emphasize the need for accurate and conscientious work on their part, it will have served at least one of its purposes.

No attempt has been made to render the book bibliographic, as a mere compilation of the enormous literature would have enlarged it too greatly and led me too far afield. I have endeavored, however, to give many of the principal references, especially those carrying good reviews of the literature, for those who are interested in consulting original sources of information.

Many illustrations have been included. It is hoped that they will serve to elucidate the text and to teach, rather than merely to embellish.

In conclusion, I am deeply grateful to my secretary, Mrs. Edna M. Kershaw, for valuable assistance in editing the manuscript. I also wish to express deep appreciation of the unvarying efficiency and courtesy of the publishers.

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INTRODUCTION

THE RELATION OF THE LABORATORY TO CLINICAL DIAGNOSIS

The diagnosis of disease requires that the physician and surgeon elicit as many facts as necessary or of possible bearing upon the complaints and health of the patient. When these are assembled in an orderly fashion logical deductions are made whenever possible. But when the data are insufficient for establishing a sure diagnosis, we may hope that experience and wise judgment, wherein lies art, may enable us to bridge the gap and make a good guess.

In relation to diagnosis the word "clinical" is worthy of a clearer definition than is commonly given it. It is generally used in the sense of meaning "at the bedside," with diagnosis based upon the history and physical examination of the patient, while a bacteriologic examination or a Wassermann test conducted in the laboratory is regarded as something quite separate and apart. But this is not true. A blood count, a test of the urine for sugar or albumin, a blood coagulation or some other test may be conducted if necessary at the bedside, but it happens that they can be done more conveniently and perhaps with greater accuracy in a laboratory. However, the information they give is of precisely the same order as that obtained by inspection, percussion and auscultation. Consequently, the word "clinical" in relation to diagnosis is not merely an adjective of place since diagnosis made in the laboratory is just as much clinical as that made at the bedside. In other words, "clinical" describes not the place where the examination is made but the purpose in view in making it.

The past thirty or forty years have been extraordinarily fruitful in the advancement of our knowledge of biochemistry, bacteriology, mycology, parasitology, immunology, serology and allied subjects. Hand in hand with pure science have been evolved a large number of new or improved laboratory methods of great practical value in the diagnosis of disease. It is true that but few are pathognomonic, as is likewise true of physical signs and symptoms, but not infrequently they are the only means for the diagnosis of disease as, for example, the Wassermann test in the detection of chronic asymptomatic or so-called "latent" syphilis, blood glucose tests in the detection of early or presymptomatic diabetes mellitus, etc. Furthermore, laboratory examinations are not meant to be short-cut or royal roads to diagnosis in the sense of supplanting careful histories and physical examinations. Indeed, they need not be resorted to at all except when they are the only means available for accurate diagnosis or for the purpose of eliciting additional clinical data upon which it may be based.

Unfortunately, however, not a few physicians lean too heavily upon histories, physical examinations and "hunches" for diagnostic purposes commonly referred to as the "art" of medicine. To me the latter has come to mean *exact* knowledge gained by experience, wise judgment and common sense along with a proper understanding of the psychology of the sick. I can see no reason for attempting to separate the art from the science of medicine and cannot conceive of any physician neglecting present-day scientific procedures in the diagnosis and treatment of disease. Indeed, I believe that the best healer of the sick is he or she who judiciously combines both the art and science of medicine in daily practice.

No one can have a higher respect and reverence than I for the great physicians of the past who, perforce, relied alone on the art of medicine. But if they could walk our wards today they would be among the first to confess their sins of commission in diagnosis while voicing a prayer that all of our present and future knowledge in both the art and science of medicine will continue to reduce progressively the errors of diagnosis revealed in the postmortem room and due, not so much to errors of commission, as to those of omission so frequently ascribable to lack of knowledge, carelessness or indifference.

Undoubtedly, pathology is and must remain the foundation on which the house of medicine is built. Under the influence and since the time of Virchow and Cohnheim, it has gradually become a highly specialized subject which may be pursued as a science apart from contact with the living sick. But one result has been the breeding of a race of pathologists who regarded contact with human beings as a contaminating influence while not a few physicians gained the impression that pathology was merely the technical process of cutting up organs in the postmortem room or laboratory for the purpose of demonstrating the final chapter of clinical medicine. Under such circumstances, comparatively few medical students have been and are attracted to this kind of pathology; indeed, many are repelled by such an approach which seems to them to have but little relation to their work as future physicians.

Fortunately, however, much of this has been and is being gradually changed. By insisting, if necessary, upon the presence of the attending physician or surgeon at a necropsy to summarize the history, signs, symptoms, x-ray and laboratory findings and declare the clinical diagnosis, if one has been made, before the necropsy has been conducted and to participate in a discussion or conference after it has been completed, unfailingly renders the necropsy of the utmost clinical interest and value be it conducted in a medical school or the humblest county hospital. The same applies to the teaching of pathology in the laboratory, since a synopsis of the chief clinical aspects of the organs and tissues being examined macroscopically or microscopically adds greatly to the interest and value of the subject in relation to the practice of medicine and surgery.

Undoubtedly, the eagerness and willingness with which Osler checked the signs and symptoms of disease by pathologic examinations after death contributed in large degree to his diagnostic ability and greatness as a physician. As is well known, many human beings recover from disease in spite of erroneous diagnoses and treatment, provided the latter has not been too meddlesome or too hazardous. But not infrequently the chances of recovery are greatly increased by accurate diagnosis as the basis for proper and intelligent therapy. Only those clinicians who rarely, if ever, check their diagnoses by postmortem examinations are likely to go serenely along with supreme confidence in their diagnostic ability. Consequently they are also likely to be among those who neglect laboratory methods as diagnostic aids under the erroneous impression that the art of medicine is necessarily separate and distinct from its science.

Since pathology in its broad sense is nowadays just as much concerned with the living as with the dead and since a large number of laboratory methods have been evolved during the past thirty or forty years of clinical value in the detection and diagnosis of disease as a result of extraordinarily fruitful investigations in hematology, biochemistry, toxicology, bacteriology, virology, mycology, parasitology, serology, endocrinology and vitaminology, a new kind of pathologist has been bred who, for the want of a better name, has been designated as the clinical pathologist. His true function, however, is not served by isolation in a laboratory conducting tests. Indeed, these can be done equally well by well-trained and experienced technologists under his supervision and direction. Rather he is expected to possess a special knowledge of those abnormal or pathologic changes occurring during life which may be detected by laboratory examinations and to interpret and appraise them expertly in relation to the diagnosis of disease. As a matter of fact, every practicing physician and surgeon is or should be a clinical pathologist in a broad sense insofar as the interpretation of laboratory examinations is concerned. The clinical pathologist, however, is expected to possess a special knowledge of such matters and in this capacity to serve not only as a consultant in clinical medicine but not infrequently to suggest therapeutic measures as well.

PART ONE

THE CLINICAL INTERPRETATION OF LABORATORY EXAMINATIONS

1

THE CLINICAL INTERPRETATION OF BLOOD EXAMINATIONS

Within recent years great advances of clinical value have been made in the science of hematology, and examinations of the erythrocytes, hemoglobin, leukocytes, platelets and other constituents of the blood are now among the most frequently employed laboratory procedures, being usually designated as "blood counts." They require, however, considerable time, experience and skill for their proper conduct in order to yield information of maximum value in the diagnosis and treatment of disease with special reference to diseases of the blood and blood-forming organs. The clinical interpretation of these is considered in this chapter with the normal ranges summarized in Table 1. Furthermore, chemical, serologic, immunologic, bacteriologic and parasitologic examinations of the blood are likewise of great clinical value and these are separately considered in succeeding chapters.

COMPOSITION OF THE BLOOD

The blood is a highly complex fluid in which are suspended the cellular elements composed of erythrocytes, leukocytes and platelets. The fluid part is called *plasma*, if the cellular elements are removed before clotting, or *serum* after this has occurred. The plasma carries the proteins as well as many organic and inorganic substances in solution—nutritive and excretory materials, antibodies, hormones and enzymes, as well as other substances of an unknown or imperfectly known chemical constitution. Under these conditions the composition of the blood may be expressed as follows (see also Table 1):

(A) *Cells* comprising the erythrocytes, leukocytes and platelets or thrombocytes.

(B) *Plasma* composed of 91 to 92 per cent water with 8 to 9 per cent solids. The latter are made up of 7 per cent proteins (serum albumin, serum globulin and fibrinogen), 0.9 per cent inorganic substances (sodium, calcium, potassium, magnesium, phosphorus, etc.) and organic constituents other than proteins (non-protein nitrogenous substances, neutral fats, phospholipids, cholesterol, glucose, etc.) in addition to respiratory gases (oxygen and carbon dioxide) and such constituents as complement, antibodies, hormones and enzymes.

The *total quantity* of blood has been estimated by various methods but none supplies a means for obtaining absolutely correct values. In general terms it

TABLE 1. SUMMARY OF NORMAL BLOOD

Constituents	Normal
Composition	(1) *Cells:* Erythrocytes, leukocytes and platelets. (2) *Plasma:* 91 to 92 per cent water; 8 to 9 per cent solids (albumin, globulin, fibrinogen); inorganic substances (sodium, calcium, potassium, magnesium, phosphorus, etc.); organic substances (nonprotein nitrogenous constituents, neutral fats, phospholipids, cholesterol, glucose, etc.); respiratory gases (O and CO_2); complement, antibodies, hormones and enzymes.
Total quantity	About 6 to 8 per cent of body weight in healthy adults (23 to 25 per cent increase in normal pregnancy).
Specific gravity	(1) Whole blood: 1.052 to 1.063 (slightly higher in men than in women and children); (2) serum: 1.026 to 1.031; (3) erythrocytes: 1.092 to 1.095.
Viscosity	3.5 to 5.4 (average 4.5). Slightly higher in men than in women and children.
Formation	By the mesenchyme or embryonic connective tissue. Theories: monophyletic, neo-unitarian and polyphyletic. Bone marrow most important source; extramedullary sources: spleen, lymph nodes, liver, suprarenal glands, cartilage, adipose and other tissues and heterotrophic bone marrow. Probably influenced by certain hormones.
Functions	(1) Respiratory; (2) nutritive; (3) excretory; (4) maintenance of water content of the body; (5) regulation of body temperature; (6) protective (antibodies); (7) vehicle for conveyance of hormones and enzymes to tissue cells.
Erythrocytes	Non-nucleated, round inert bodies. Many may be elliptical or oval in some persons (whites or negroes) due to heredity transmissible by either sex (sometimes mistaken for sickle cell anemia). Composed of water (64 per cent) and hemoglobin within a stroma (40 to 60 per cent protein, 10 to 12 per cent lipids and balance inorganic salts). "Longevity" apparently averages about 120 days with approximately 50 cc. packed cells destroyed daily in the average normal adult by fragmentation with phagocytosis by the cells of the reticulo-endothelial system. Hemoglobin is converted into bilirubin; urobilinogen and urobilin in urine and feces. Fate of globin unknown but probably converted into amino acids. Hemolysis may play a part in the normal destruction of erythrocytes in the body but there is no direct proof of its occurrence. Crenated and various degenerative forms (Maragliano bodies, "bacillary" degeneration, chromatin particles, Howell-Jolly bodies and Cabot's bodies) may occur.

TABLE 1. SUMMARY OF NORMAL BLOOD (continued)

Constituents	Normal
Total erythrocytes (per c.mm. of blood)	Under best of conditions at least 5 per cent error in counting. Increased by physiologic causes: (1) muscular activity; (2) psychic factors (excitement or fear); (3) high altitudes and (4) sex *after puberty* (higher in men than in women). Meals have no influence except reduction through high fluid intake. Climate, temperature and season have no influence except possible increase from dehydration (profuse sweating with low fluid intake). *Age has an important influence.* Infants under two weeks of age: about 5,100,000; children 1 to 15 years: about 4,600,000 to 4,700,000; adult men: 5,400,000 to 5,800,000; adult women: 4,600,000 to 4,800,000.
Reticulocytes	0.5 to 1.5 per cent of erythrocytes.
Volume packed erythrocytes (cc. per 100 cc. blood)	Infants 49.0 to 54.0 (± 10.0); children 35.0 to 39.0; adult males 47.0 (± 7.0); adult females 42.0 (± 5.0).
Volume index	Children 0.63 to 0.82; adults 0.80 to 1.00.
Mean corpuscular volume	In terms of cubic microns: (1) infants about 84; (2) children 72; (3) adult males 82 and (4) adult females 86.
Sedimentation rate	Men: up to 15 mm.; women: up to 20 mm. (Westergren); men: up to 6.5 mm.; women: up to 15 mm. (Wintrobe); men: up to 8 mm.; women: up to 10 mm. (Cutler).
Fragility of erythrocytes	Min. resistance (slight hemolysis): 0.40 to 0.46 per cent NaCl sol. Max. resistance (complete hemolysis): 0.30 to 0.36 per cent NaCl sol.
Hemoglobin	In terms of grams per 100 cc. of blood: (1) infants 11.8–19.5 (± 2.3–5.0) (2) children 11.2–13.4; (3) adult males 16 (± 2.0) and (4) adult females 14 (± 2.0).
Color index	1.0 in absolute terms.
Mean corpuscular hemoglobin	In terms of micromicrograms: (1) children 26 to 28; (2) adults 29 (± 2).
Mean corpuscular hemoglobin concentration	In terms of grams per 100 cc.: (1) children 32 to 34; (2) adults 34 (± 2).
Saturation index	0.9 to 1.2 in absolute terms.
Total leukocytes (per c.mm. of blood)	(1) Infants 8000–16,500 or higher; (2) children 6000–15,000 (av. 10,700); (3) adults of both sexes 5000–10,000 (av. 7000).

TABLE 1. SUMMARY OF NORMAL BLOOD (continued)

Constituents	Normal
Kinds of leukocytes	(1) Immature (nonfilamented or nonsegmented) neutrophils; (2) mature (filamented or segmented) neutrophils; (3) eosinophils; (4) basophils; (5) lymphocytes and (6) monocytes. Absolute numbers and percentages vary with age (Table 6).
Platelets	Varies according to different methods; in general terms 250,000 to 500,000 per c.mm. of blood.
Coagulation time	Lee and White method: 5 to 8 minutes; capillary tube method: 1 to 7 minutes; Howell method: 10 to 30 minutes.
Bleeding time	1 to 3 minutes (method of Duke).
Prothrombin time	10 to 20 seconds (method of Quick); 300 units per cc. of plasma (Warner *et al*).
Clot retraction time	Begins 1 hour; marked in 18 hours.
Capillary fragility	Suction cup method: — 20 to — 35 cm. of mercury.

appears that the amount in healthy adults probably makes up about 6 to 8 per cent of body weight, with quick adjustments in spite of the administration of fluids intravenously, as well as by mouth. Even blood transfusions cause only a temporary increase although in pregnancy both blood and plasma volumes increase 23 to 25 per cent, which probably explains why blood loss is so well borne by parturient women, with a return to normal within several weeks after delivery. Of course the immediate effect of severe hemorrhage is a reduction in total blood volume but the plasma, in particular, is restored very quickly by the passage of fluid from the tissues. Changes may also occur in polycythemia vera and other diseases which will be discussed later on.

The normal *specific gravity* of blood as determined by the copper sulfate method varies from 1.055 to 1.063 in normal men and from 1.052 to 1.060 in normal women; plasma varies from about 1.024 to 1.028 in both sexes. Since it depends particularly on the quantity of hemoglobin and the protein content of plasma, an increase is usually due either to dehydration or to increased globulins. A decrease usually follows a loss of proteins or decreased albumin formation.

The *viscosity* in healthy adults ranges from 3.5 to 5.4 (average 4.5), being slightly higher in men than in women and children. It is influenced by the numbers of erythrocytes, to a lesser extent by the leukocytes, and doubtfully by the platelets. While viscosimetry has received considerable attention in Europe, it has been used but little for clinical purposes in the United States. However, the suspension stability of the erythrocytes in plasma is in relation to viscosity and the sedimentation test is widely employed for clinical purposes.

All blood cells are derived from the mesenchyme or embryonic connective tissue. Primitive or nucleated erythrocytes (erythroblasts) first appear in the fetus but are rapidly replaced by non-nucleated cells so that by the third month of intra-uterine life the nucleated cells are already reduced to 8 per cent or less of the total. White corpuscles or leukocytes first appear as myeloblasts during the second month, lymphocytes about the fourth and monocytes about the fifth months. According to the *monophyletic theory,* either the lymphocytes of lymphatic tissue are the common source of all blood cells, whether erythrocytes or leukocytes (granulocytes and neonocyts), or myeloblasts are the common parent cells (neo-unitarian theory). According to the *polyphyletic theory,* however, erythrocytes and the different kinds of leukocytes are derived from two, three or more parent "blast cells." The "dualists" recognize myeloblasts and lymphoblasts as the parent cells, the "trialists" the lymphoblasts, myeloblasts and the pronormoblasts, while the "complete" polyphyletists maintain that there is a stem cell for erythrocytes and one for each of the leukocytes. All of these theories are discussed fully by a number of writers in Downey's *Handbook of Hematology* [1] but dogmatic statements on the interrelationship of the formation of blood cells cannot be made at the present time.

Undoubtedly, however, the bone marrow is the chief tissue concerned in the production of blood cells, both before and after birth, with the result that its examination during life is frequently of diagnostic clinical value. Its total weight varies from 1600 to 3700 gm. or roughly the weight of the liver, although during adult life only about half is in an active state. It is not yet clear just how the blood cells enter the circulation but the fact that the marrow is confined within a limited space may contribute to the expulsion of cells into the circulation. At any rate, its functional capacity is very great. Blood cells may also be produced by extramedullary tissues (particularly in the anemias of infants and children) with special reference to the spleen and lymph nodes, supplemented to a lesser extent by the liver, suprarenal glands, cartilage, the broad ligament, organizing thrombi and adipose tissue in various locations, as well as by nodules or large tumors composed of heterotopic bone marrow occurring in the kidneys, spleen, liver, adipose tissue or elsewhere in certain of the anemias.

Apparently some of the hormones have an influence on the formation of blood, but there are few facts commanding serious consideration. The spleen, hypophysis, suprarenal glands and the thyroid gland, however, appear to exert some influence and especially the spleen. At least the spleen plays an important rôle during fetal life and after birth, following which hematopoiesis may be resumed, while the spleen is at all times the site of formation of lymphocytes and is actively concerned in blood destruction and pigment metabolism. The possible influence of hormonal control by this organ is also suggested by the changes and associated cellular hyperplasia of the bone marrow which may occur after splenectomy, indicating that the spleen apparently inhibits the liberation of blood cells from the bone marrow.

FUNCTIONS OF THE BLOOD

Needless to state, the blood serves a large number of various important functions with the result that disturbances of any one or a combination of them may result in the production of signs and symptoms of disease. Among these may be mentioned the following:

(1) The transportation of oxygen from the air in the lungs to the tissues, and of carbon dioxide from the tissues to the lungs, largely through the hemoglobin of erythrocytes.

(2) The conveyance of glucose, amino acids and fats from the alimentary tract to the tissues.

(3) The removal of urea, uric acid, creatinine, creatine and other waste products largely by excretion through the kidneys, liver, etc.

(4) The maintenance of a normal water content of the body.

(5) The regulation of body temperature through its specific heat, high conductivity and high latent heat of evaporation.

(6) Furnishing a vehicle along with the lymph for the circulation of complex chemical substances called the antibodies (antitoxins, opsonins and lysins) as well as of complement and nonspecific bactericidal substances (leukins and plakins) of such vital importance in natural resistance to infection with the various living agents of disease and acquired immunity to them.

(7) Furnishing a vehicle by which the hormones of the different ductless glands, as well as certain enzymes, are conveyed to the cells of the tissues.

No wonder, therefore, that diseases of the blood and of the blood-forming organs, as well as of those concerned in blood destruction, are of such frequent occurrence and great clinical importance.

CHANGES IN THE ERYTHROCYTES

This refers not only to the total number of erythrocytes per c.mm. of blood but likewise to a detailed examination of them, which is of considerable clinical importance in relation to the diagnosis of the various kinds of anemia. In the bone marrow the progenitors of normal erythrocytes (normocytes) are the nucleated pronormoblasts, basophilic normoblasts, polychromatic normoblasts and the orthochromatic (acidophilic) normoblasts. In other words, the nuclei are lost in the erythrocytes of the circulating blood under normal conditions but even after they are lost some of the cells usually show a cytoplasmic basophilic substance (*diffuse basophilia*). When this substance occurs as blue or bluish-black granules stained by one of the Romanowsky methods, or seen in wet preparations by darkfield examination, the cells are called *reticulocytes*. These are usually increased when erythrocytes are being actively produced in the bone marrow during the anemias and when the basophilia is punctate the condition is called *basophilic stippling,* as seen in lead poisoning and certain other conditions.

Normal Erythrocytes. In other words, the normal erythrocytes or normocytes are non-nucleated cells and in the unstained state appear as greenish-yellow circular or slightly oval cells, with a thinner central area. They are composed of about 64 per cent water and hemoglobin within a stroma, the latter in a dried state being composed of 40 to 60 per cent proteins, 10 to 12 per cent lipids and the balance of inorganic salts. The chief function of the erythrocytes is to convey oxygen (of which they consume little or none themselves) from the lungs to the tissues and carbon dioxide from the tissues to the lungs. They are not living structures with a measurable life span but actually inert bodies. There is considerable difference of opinion regarding their "longevity" which has been estimated as varying from 30 to 100 days by Ashby [2] and from about 50 to 75 days by Dekkers,[3] while transfused erythrocytes are calculated by Vischer [4] to function only 12 to 13 days. On the other hand, Schoidt [5] believes that the functional activity of erythrocytes may be as short as 14 days, but it is more likely that the average "life span" is about 120 days with about 50 cc. packed cells replaced daily in the average adult. The manner of their destruction under normal conditions is also uncertain but it appears that this is largely through fragmentation with removal of the effete cells by phagocytosis by the cells of the reticulo-endothelial system, and especially those of the liver, as well as by those in the spleen, bone marrow and the subcutaneous tissues. The hemoglobin is probably first converted into biliverdin iron globin followed by removal of the iron and reduction to bilirubin by the reticulo-endothelial and mesenchymal cells of the body. The bilirubin remains attached to the original globin which is probably removed by liver cells. This constitutes the normal bilirubin of the blood, giving an icterus index of 4 to 6 units and an *indirect* van den Bergh reaction of 0.1 to 0.25 mg. per 100 cc. of serum with urobilinogen occurring in the urine and stercobilin in the feces. The ultimate fate of globin is unknown, but since it is a protein, it is probably converted into amino acids and used again in the production of hemoglobin. Whether there is any relationship of the porphyrins to the process of erythrocyte destruction is unknown. While hemolysis may play some part in the normal destruction of erythrocytes in the body, there is no direct proof of this although it may occur in certain diseases, as discussed later.

Counting the *normal number of erythrocytes* per c.mm. of blood by ordinary methods is about 95 per cent correct when skillfully made with accurate pipets and counting chambers, but even under the best of conditions about 5 per cent error will occur aside from variations due to physiologic causes. During complete inactivity there is probably little or no diurnal variations, but under activity the total number of erythrocytes tends to increase with as much as 11 per cent increase in the hemoglobin. In other words, muscular activity tends to increase both the total number of erythrocytes and the percentage of hemoglobin. No consistent variations can be ascribed to eating meats although these increase the leukocytes, as discussed later. Of course a high fluid intake tends to reduce the erythrocytes through dilution while the reverse occurs in states of dehydration. Psychic factors, such as excitement or fear, may cause a significant increase. Sex also has an influence *after puberty* with somewhat higher counts in men than in women. Low barometric pressure also causes a rapid increase of both erythrocytes and hemoglobin as seen in persons ascending to high altitudes. Climate, temperature and season probably have no influence except hot weather with low fluid intake when dehydration may result in an increase from hemoconcentration. However, striking variations occur according to age. Thus, within two weeks of birth, infants usually show about 5,100,000; children from 1 to 5 years about 4,600,000 and children from 6 to 15 years about 4,700,000 per c.mm., while adult males average 5,400,000 to 5,800,000 and adult females 4,600,000 to 4,800,000 per c.mm.,[6] as shown in Table 1.

As previously stated, erythrocytes are normally non-nucleated and round, but slightly elliptical-shaped cells may occur (*familial poikilocytosis* or ovalocytosis). Occasionally, however, as many as 50 to 90 per cent may be oval or elliptical in the absence of anemia or other evidences of blood destruction. Such oval cells may be found in the blood of white persons or negroes and in both sexes. Unlike the phenomenon of sickling, with which this anomaly has been confused, no change in the form or increase in number of these cells occurs in sealed fresh preparations of wet blood. It appears to be a hereditary trait, transmitted by either sex, possibly as a simple mendelian predominant and probably these cells are atavistic forms which represent a progressive structural adaptation to some unknown constitutional factor.

Abnormal Erythrocytes. It is always important to inspect the erythrocytes carefully as an essential part of differential leukocyte counts and to report the presence or absence of abnormal cells because these have an important bearing on the clinical differentiation of the anemias and help in establishing diagnosis in such diseases as sickle cell anemia, lead poisoning, etc.

Abnormal types of non-nucleated erythrocytes may not only show changes in staining designated (1) as *polychromatophilia* (in which the erythrocytes are normal in size), but likewise changes in size classified (2) as *microcytes* when smaller than normal and (3) *macrocytes* when larger than normal, either of these constituting *anisocytosis*, and along with polychromatophilia constituting the most common alterations from the normal. Alterations in shape are designated (4) as *poikilocytosis* with a great variety of bizarre forms and indicative of more severe anemia. Sickle-shaped cells are characteristic (5) of *sickle cell* anemia which is essentially peculiar to negroes although these cells may occur in the blood of healthy members of the white race. These cells should not be confused with the elliptical cells occurring normally in both whites and negroes, previously referred to, and sometimes responsible for an erroneous diagnosis of sickle cell anemia in whites. The significant factor in the latter is that sickling occurs when a drop of blood is sealed under a cover-slip on a slide or in test tubes. A few bizarre, pointed cells may be seen almost immediately, but marked sickling requires from two to six hours after blood has been drawn. It may be observed also in the counting chamber but more slowly because of dilution. If a rubber band is first placed around a finger for five minutes before puncture, a considerable number of sickle cells will be found in the dark blood even at the end of one hour in many cases (Table 2).

Nucleated erythrocytes are called "blasts" and do not occur normally in the blood. They represent immature erythrocytes swept out of the bone marrow into the blood because of a demand for erythrocytes in severe anemia and because of suddenly opened capillaries. When of normal size they are called (1) *normoblasts;* (2) *microblasts* when smaller than normal, and (3) *megaloblasts* when not only

TABLE 2. SUMMARY OF THE CLINICAL SIGNIFICANCE OF ABNORMAL CHANGES IN THE ERYTHROCYTES

Normal for infants: 5,100,000; children: 4,600,000 to 4,700,000; adult men: 5,400,000 to 5,800,000; adult women: 4,600,000 to 4,800,000 per c.mm.

Anemia (decreased)	Polycythemia (increased)
I. Normocytic: Post hemorrhagic Simple chronic Scurvy, hemophilia, purpura Effects of chemical, parasitic or bacterial poisons Incompatible transfusions Paroxysmal hemoglobinuria and hemoglobinemia Lederer's acute hemolytic anemia Congenital hemolytic jaundice Cirrhosis of the liver Sickle cell anemia Aplastic anemia (primary and secondary) Acute, subacute and chronic infections Acute and chronic nephritis Gaucher's disease Myelophthisic anemias Purpura haemorrhagica Anemia of pregnancy Anemias associated with splenomegaly Acute and chronic leukemia II. Macrocytic: Pernicious anemia Sprue, idiopathic steatorrhea, celiac disease, chronic diarrhea Pellagra Dietary deficiencies Pregnancy Diphyllobothrium latum infestation Hypothyroidism Chronic and extensive hepatic disease "Achrestic" anemia Some cases of aplastic anemia Sickle cell anemia Erythroblastosis fetalis Acute hemolytic anemia Chronic hemolytic anemia Multiple myeloma Leukemia III. Simple microcytic: Subacute and chronic inflammatory and chronic noninflammatory diseases Mediterranean anemia	Polycythemia vera Pseudopolycythemia due to dehydration and hemoconcentration (severe burns, shock, vomiting and diarrhea). Erythrocytosis due (1) to anoxemia (high altitudes, chronic pulmonary disease, congenital heart disease and chronic carbon monoxide poisoning); (2) rarely in myelogenous leukemia and multiple myeloma.

TABLE 2. SUMMARY OF THE CLINICAL SIGNIFICANCE OF ABNORMAL CHANGES IN THE ERYTHROCYTES (continued)

Anemia (decreased)	Polycythemia (increased)
IV. Hypochromic microcytic: Iron deficiency in diet (especially in infants) Achlorhydria Gastrectomy (total or partial) Sprue, celiac disease and chronic diarrhea Multiple hereditary telangiectasia Repeated pregnancies Chlorosis Chronic anemia of women Hypochromic anemia of infants Mediterranean anemia Chronic hemorrhage Hemolytic anemias	

larger than normal, but also presenting structural changes in the nucleus, characterized by fine chromatin without any tendency to clumping as the cell matures and seen only in pernicious anemia and related forms of liver-principle deficiency anemia. They are classified as promegaloblasts and as basophilic, polychromatic and orthochromatic megaloblasts. Nucleated erythrocytes are always likely to be present in severe anemias and especially in pernicious anemia, acute leukemia, Mediterranean anemia and erythroblastosis fetalis.

Because of their normal biconcave shape, erythrocytes appear more pale in the center than at the periphery. When this pallor is increased, due to a deficiency in hemoglobin, the condition is designated as *hypochromia* which in extreme instances may leave only a narrow rim of coloring matter in which case the cells are called *pessary forms.* A peculiar combination of poikilocytosis and hypochromia may be seen in hypochromic anemia, sickle cell anemia and erythroblastic anemia in which the corpuscles show a central rounded area of pigmented material, surrounded by a clear ring without pigment, outside of which is a pigmented border—these are called *target* or *Mexican hat* corpuscles. These cells are regarded by Dameshek [7] as being congenitally defective cells while Bohrod [8] regarded them as newly formed cells produced in the bone marrow in response to blood loss regardless of the cause. They may be found temporarily early in acute anemia as likewise in sickle cell anemia but are characteristic of Cooley's anemia occurring in infants and children as also of Mediterranean anemia which may be a benign form of the highly fatal Cooley's anemia.

Needless to state, *crenation* is not abnormal since it is due to shrinkage of corpuscles in a hypertonic medium. Degenerative forms, however, may be encountered under conditions of great bone marrow activity without being diagnostic of any particular type of anemia. These are especially apt to occur in anemias associated with severe changes in the spleen and after removal of this organ.

They are also important because they are sometimes mistaken for nuclei, inclusions or malarial plasmodia. These objects include the round or elliptical bodies (*Maragliano bodies*), rod-like, vibratory hyaline masses (*bacillary degenerations*) or dark bodies in the center of the corpuscles (*Ehrlich's hemoglobinemic degenerations*). Furthermore, and very occasionally, one may encounter nuclear remnants, especially in stained preparations, occurring as fine bluish dots (*chromatin particles*), spherical, eccentrically placed granules (*Howell-Jolly bodies*), or bluish, thread-like rings and convolutions (*Cabot's rings*). The nuclear derivation of these structures is indicated by the fact that they can be stained with stains specific for chromatin such as methyl green.

Anemia. A reduction in the total number of erythrocytes along with the presence of abnormal types and a reduction in hemoglobin, is designated clinically as *anemia.* At the present time this state is classified according to the size of non-nucleated erythrocytes and, on the basis of etiology, into the following types:

(1) *Normocytic anemia* (corpuscles normal in size) due (a) to sudden loss of blood; (b) acute and chronic destruction of blood; (c) lack of blood formation and (d) hydremia constituting, for example, the "physiologic" anemia of pregnancy.

(2) *Macrocytic anemia* (corpuscles larger than normal) due (a) to a deficiency of the antianemic principle in the liver and (b) to intense activity of the bone marrow.

(3) *Simple microcytic anemia* (corpuscles smaller than normal) due to imperfect formation of blood.

(4) *Hypochromic microcytic anemia* (corpuscles smaller than normal with marked reduction in hemoglobin) due to a deficiency of iron through deficient diet, deficient absorption, excessive demands for iron, deficient antenatal storage or postnatal supply of iron, continued loss of blood, and for unknown reasons as in Mediterranean anemia. A large number of diseases and states may be responsible for these various types of anemia, as summarized in Table 2.

Polycythemia. An increase in the total number of erythrocytes associated with an absolute or relative increase of hemoglobin is designated clinically as *polycythemia.* This typically occurs in polycythemia rubra vera (Vaquez's or Osler's disease) and to a lesser extent in the erythrocytoses due to anoxemia from high altitudes, chronic pulmonary diseases, congenital heart disease and chronic carbon monoxide poisoning as well as, rarely, in some cases of myelogenous leukemia and multiple myeloma. A pseudo or false type of polycythemia may be due to dehydration with hemoconcentration as from shock, severe burns, excessive vomiting, diarrhea, etc., in which the erythrocytes are temporarily increased above normal.

Reticulocytes. While basophilic or stippled erythrocytes occur very infrequently in the blood of normal persons, reticulated cells called *reticulocytes* constitute from 0.5 to 1.5 per cent of normal erythrocytes. They are counted by "wet" or "dry" methods until at least 1000 erythrocytes have been examined. Reticulocytes are somewhat larger than erythrocytes, the reticulum appearing as a narrow band traversing the cell, evenly distributed or so densely packed as to resemble a nucleus, the shape and density being influenced by such physical factors as the fixative, the concentration and *p*H of the stain, drying, heating, etc. They can be found in oxalated blood as long as twenty-four hours after collection.

An increase is designated as *reticulocytosis* and under physiologic conditions this may occur to a slight degree during the spring months. There may be a definite increase (2 to 6 per cent) in newborn infants in whom they reach normal numbers (0.5 to 1.5 per cent) in two to five days after birth. Reticulocytosis may also occur in pregnancy but is usually associated with anemia.

Otherwise, reticulocytosis is the surest index of accelerated hematopoiesis not only in remissions of pernicious anemia, but likewise in response to liver therapy of this disease as well as in response to the administration of iron in the anemias due to iron deficiency, and following acute hemorrhage. In these, the increase is usually temporary but in the anemias due to chronic blood destruction, as in congenital hemolytic jaundice or sickle cell anemia, they may show a continuous increase for many years. The degree of reticulocytosis depends on the size of the stimulus, the reactivity of the bone marrow and the quantity of blood-forming material available but does not necessarily indicate a physiologic and orderly response to treatment. Thus in the various types of myelophthisic anemia, leukemia, and after the administration of arsenic and other compounds, reticulocytosis is not usually of prognostic significance since it may be due to abnormal cells in the bone marrow acting as "irritants" or, through excessive multiplication, crowding out reticulocytes and other immature cells into the circulating blood. A slight increase may be observed also in lead and mercury poisoning and malaria but not usually in anemias due to chronic hemorrhage or chronic infection. A characteristic decrease to 0.4 per cent or less occurs in both idiopathic and symptomatic aplastic anemia (Table 3).

TABLE 3. SUMMARY OF THE CLINICAL SIGNIFICANCE OF CHANGES IN THE RETICULOCYTES

Normal for adults: 0.5 to 1.5 per cent of erythrocytes

Increased (reticulocytosis)	Decreased
In the newborn (return to normal in a few days)	Aplastic anemia (idiopathic or symptomatic)
Pregnancy (may be due to anemia)	
Lederer's and other acute hemolytic anemias	
Familial hemolytic jaundice	
Sickle cell anemia	
Pernicious anemia (during or preceding remissions) and with liver therapy	
After acute hemorrhages	
Lead and mercury poisoning	
Malaria	
Leukemias	
Myelophthisic anemia	
Iron therapy of iron-deficiency anemia	
Erythroblastosis fetalis	

Basophilic Stippling. In stained smears of normal blood about 1 per cent of the erythrocytes show a bluish pink color because of a slight affinity for the basic stain. This is due to traces of the primordial, cytoplasmic basophilic substance persisting after the nuclei have been lost. Under the circumstances such cells represent immature erythrocytes and the more immature they are, the more basic do they stain. When the number is increased it is called *polychromatophilia* or *polychromasia* which occurs when there is a demand for the rapid production of erythrocytes resulting in their liberation into the circulating blood before complete maturity has been reached. Polychromatophilia, therefore, is found not only in the newborn but likewise in many of the anemias, especially after acute hemorrhage, as well as after liver and iron therapy.

In severe anemia, however, this basophilia is more pronounced with the production of blue granules (*basophilic stippling*). The granules may be numerous and very fine or quite coarse and few in number. They usually occur in hypochromic or polychromophilic and occasionally in nucleated erythrocytes and especially in lead poisoning, in which disease they were formerly thought to be pathognomonic. It is now known, however, that stippling may occur in chronic mercurial poisoning, leukemia, pernicious anemia or any type of severe anemia, as well as in chronic malaria.

In this connection it should be remembered that some erythrocytes containing malarial parasites also show reddish granules (*Schüffner's granules*) due to a degenerative change in the erythrocytes; they should not be mistaken for basophilic stippling.

Size and Volume of Erythrocytes; The Volume Index and Mean Corpuscle Volume. As previously stated, erythrocytes are divisible into three kinds according to their size: *normocytes, microcytes* and *macrocytes*. If nucleated, they are designated as *normoblasts, microblasts* and *macroblasts*. Normocytes or normal erythrocytes have a mean diameter of 7.2 to 7.9 microns in dried films of blood, being 0.8 to 1 micron larger in wet preparations, although it is stated that the mean diameters of some corpuscles may be as small as 4.75 and as large as 9.5 microns in normal persons. Their average thickness is stated to vary from 1.84 to 2.14 microns. In general terms variations in size can be determined in stained smears but more accurate methods are available such as the method of Price-Jones,[9] which is quite laborious, or the method of Pijper which is simpler.[10]

But in the anemias, alterations in size of erythrocytes may occur in all dimensions. Consequently, changes in the size of cells which may be too slight to be perceived by measurements of their diameters, are better detected by a determination of their volume in a known amount of blood. For this purpose the hematocrit method of Wintrobe,[6] conducted with 1 cc. of blood, is especially recommended and, for smaller amounts, the microhematocrit method of Kato.[11] Normally the volume of packed cells in terms of cc. per 100 cc. of blood varies in infants from 49.0 to 54.0 (± 10.0), in children from 1 to 15 years from 35.0 to 39.0, in adult males 47.0 (± 7.0) and in adult females 42.0 (± 5.0). In macrocytosis it is increased and in microcytosis greatly reduced even though the total erythrocyte counts are not greatly increased or reduced respectively.

The volume of packed erythrocytes, therefore, affords the same kind of clinical information as total erythrocyte counts and hemoglobin estimations. According to Wintrobe,[6] it is the most useful single criterion of the degree of anemia or polycythemia now available. In routine hematologic examinations, if the volume is normal, nothing further need be done unless clinical examination makes additional examinations advisable or necessary.

However, if a careful erythrocyte count is made at the same time, the results are better expressed in terms of the *volume index*, determined as follows:

$$\frac{\text{vol. packed corpuscles, cc. per 100 cc.} \times 2.3}{\text{corpuscle count, millions per c.mm.} \times 20}$$

The volume index indicates, therefore, the relative mass of circulating erythrocytes as compared with the normal which, in children, varies from 0.63 to 0.82 and, in adults, from 0.80 to 1.00. In the macrocytic anemias the index may be 1.20 to 1.60 or higher. If increased, along with gastric anacidity, it is almost certain evidence of pernicious anemia even though the total erythrocyte count is not greatly below normal. In anemias due to acute hemorrhage, intoxications, infections and hemolysis, the index is normal or but slightly reduced. In microcytic anemias, however, and especially those due to chronic hemorrhage and chronic infections, the index is below normal.

Or, the results may be expressed in terms of the *mean corpuscular volume* which is determined as follows: $\frac{\text{vol. packed corpuscles, cc. per 1000 cc.}}{\text{corpuscle count, millions per c.mm.}}$ and expressed in terms of micra (cubic microns). Normally the value for infants is about 84, children 72, adult males 82 and adult females 86 micra.

Macroscopic Examination of the Blood. Furthermore, the hematocrit method for determining the volume of packed erythrocytes, the volume index and the mean corpuscle volume afford not only data of clinical value in the diagnosis of the anemias and polycythemia, but likewise useful information on the color and opacity of the plasma and quantitative changes in the leukocytes and platelets. A reddish-gray layer of packed leukocytes and platelets is found above the packed erythrocytes, the thickness of this layer depending upon the number and kinds of leukocytes and the number of platelets. In normal blood it varies from 0.5 to 1 mm. in thickness. If the platelets are normal in number the layer will be thicker if the leukocytes are increased with about 0.1 mm. increase corresponding to about every 2000 leukocytes per c.mm. above normal and especially if leukocytosis is due to cells of the myeloid series since they are larger than lymphocytes. If the platelets are greatly reduced (*thrombocytopenia*) the layer is greatly reduced. In cases of chronic myelocytic leukemia and in erythremia, in which both the leukocytes and platelets are increased, three well-defined layers present a very striking picture. Measurement of this reddish-gray layer, however, is not recommended as a substitute for leukocyte or platelet counts but it is evident that it can serve as a useful rough guide in calling attention to possible abnormal variations in the leukocytes and platelets.

Furthermore, the procedure yields information of clinical value in relation to the color and opacity of the plasma, as previously stated. Indeed, the icterus index can be readily determined by comparison with standards in tubes of the same size in the detection of latent jaundice. Lipemia is also easily detected, the plasma appearing quite opaque in such instances. If this increases when the hematocrit is placed in a refrigerator and disappears at room temperature, it is suggestive of the presence of multiple myeloma.[12]

The Sedimentation of Erythrocytes. The blood may be considered essentially as a suspension of cells in plasma. As discovered by Fahraeus,[13] its suspension stability is altered in pregnancy and many diseases. A test was thus discovered which, because of its simplicity and accuracy, when properly conducted, has become of considerable clinical value. It, however, is a nonspecific reaction so that the information given is of a general character as a measure of the presence and intensity of morbid processes. It may be accelerated when the temperature, pulse and even the leukocyte count are normal, particularly in chronic disorders and in localized infections.

Little is known, however, about the true nature of sedimentation; nor are the reasons why it is accelerated in the presence of pregnancy or disease well understood. Undoubtedly the speed of sedimentation is increased when the quantity of fibrinogen in the plasma is increased. An increase of serum globulin has the same effect but to a lesser degree. No relation, however, has been observed between sedimentation rate and total plasma proteins, albumin-globulin ratio, blood sugar, calcium or phosphorus. With a column of blood standing in the vertical position certain physical forces are involved and when the erythrocytes are reduced below normal, there is less retardation of sedimentation by these cells themselves. In the presence of pregnancy and certain other conditions, however, the *aggregation* of erythrocytes is greatly affected, probably because of an alteration in their surface changes, with rouleaux formation greatly increased. This is the chief cause of increased sedimentation in pregnancy and disease although the cause of increased aggregation is quite obscure. All that can be stated at present is that variations in sedimentation result from changes in the surface tension of erythrocytes which cause them to aggregate and that these are related to alterations in the physical state of the plasma colloids.

Various methods have been proposed for determining the sedimentation rate and index,[14, 15, 16] including micromethods for infants and children.[16, 17] The results depend on the type of tube employed so that normal values vary according to the technic employed. For this reason the graphic report should show the normal in terms of the technic. By all methods the rate is higher in normal women than in normal men, largely because the former have somewhat fewer erythrocytes. The tubes must be kept in an exact *vertical position* and since sedimentation is increased by heat, the test should be conducted at a *temperature* of not less than 22° C. or higher than 27° C. Methods have been proposed for "correcting" the effects of anemia and while these are far from satisfactory, it is advisable to record the "uncorrected" as well as the "corrected" sedimentation rate in severe anemias at least. The results are best expressed in terms of the *distance* the corpuscles have fallen in varying periods of time up to one hour, with a plotting of the curve in graphic form. Cutler [18] has demonstrated by such graphs that the process of sedimentation consists of three phases, namely, (1) a preliminary period during which aggregation takes place, (2) a period of rapid sedimentation and (3) the phase of packing when corpuscular masses, in accumulating at the bottom of the tube, slow up the rate of sedimentation.

As previously stated, normal values will vary according to the method employed which should always be stated by the laboratory. In general terms these may be expressed at the end of one hour in mm. as follows:

Methods	Children	Men	Women
Westergren	less than 10	up to 15	up to 20
Wintrobe	3–13 (av. 9)	up to 6.5	up to 15
Cutler	——	up to 8	up to 10

TABLE 4. SUMMARY OF THE CLINICAL INTERPRETATION OF CHANGES IN THE SEDIMENTATION RATE OF THE BLOOD

Normals: Men: up to 15 mm.; women: up to 20 mm. (Westergren method)
Men: up to 6.5 mm.; women: up to 15 mm. (Wintrobe method)
Men: up to 8 mm.; women: up to 10 mm. (Cutler method)

Normal	Increased	Decreased
Hypertrophic arthritis	Pregnancy after second month	Polycythemia vera
Cirrhosis of the liver	Ectopic pregnancy after 10 weeks	Malaria during paroxysms
Nonmalignant tumors unless disintegrating	During menstruation	Some cases of severe hepatic disease
Neuroses	Acute general infections	Sickle cell anemia
Essential hypertension	Acute localized infections	
Heart disease without infection	Rheumatic carditis	
Diabetes mellitus	Localized suppurations	
Allergic diseases	Chronic infections and especially when active (tuberculosis, syphilis, infectious arthritis, etc.)	
Uncomplicated peptic ulcers	Malignancy (especially late)	
Lithiasis without infection	Hyperthyroidism	
Hydronephrosis	Acute intoxications (especially arsenic and lead)	
	Severe alcoholism	
	Nephritis and especially nephrosis	
	Coronary thrombosis	
	Internal hemorrhages	
	Anemias	
	Leukemias	
	Some cases of severe hepatic disease	

As shown in Table 4, the sedimentation rate may be increased in a large number of acute and chronic diseases due to infection and in other conditions as well. It is not diagnostic, therefore, of any one disease. One of its most important clinical purposes is in the detection of more or less occult conditions. Not infrequently it may be found accelerated when clinical and other laboratory examinations are negative. On the other hand, a normal rate does not mean that all is well and occasionally, especially in cachexia, it may be normal in the presence of serious disease. In general terms an accelerated rate always suggests organic disease rather than a functional disorder, inflammation rather than a tumor and malignancy rather than a benign tumor. It is of particular value as a clinical guide to the progress and activity of an infection, with special reference to tuberculosis and rheumatic carditis as well as in the detection of complications. It also possesses prognostic value, as a progressive decrease to normal is indicative of favorable progress while a persistently increased rate indicates the reverse. For example, the rate is usually increased during the fifth to eleventh days of myocardial infarction and it is advisable to keep the patient at complete rest until it returns to normal. As with all laboratory tests, however, the sedimentation rate is not infallible; for example, the rate may be normal in as many as 20.8 to 36.9 per cent of cases of active pulmonary tuberculosis.[19] By all methods it is especially significant if sedimentation is abnormally rapid during the first fifteen minutes. In general terms a rate of 15 to 30 mm. at the end of an hour is indicative of a slightly positive, 30 to 50 mm. of moderately positive, and 50 to 100 mm. of a strongly positive reaction (Table 4).

Viscosity of the Blood; Formol-Gel Reaction. As previously stated, in this country blood viscosity is not usually determined for diagnostic purposes. According to Evans,[20] the relationships between whole-blood viscosity and the viscosity and quantity of plasma and corpuscles is expressed by the equation:

log blood viscosity = log plasma viscosity × proportion of plasma + log viscosity of packed corpuscles × proportion of corpuscles.

In late pregnancy the plasma and corpuscular viscosities are similar to those of nonpregnant women, but there is a parallel reduction of the blood viscosity and hematocrit readings. Plasma transfusion reduces blood viscosity while blood transfusion for hemorrhage retards the reduction of blood viscosity to a variable extent.[20]

The *formol-gel reaction* has been proposed as a means for determining the degree of hyperglobulinemia or hyperfibrinogenemia.[21] It depends upon a change in the viscosity of blood serum or plasma following the addition of formalin. Basically, the technic consists of adding two drops of formalin to 1 cc. of serum or one drop to 1 cc. of plasma and noting the degree of gelation at intervals from five minutes to twenty-four hours.[22] Normal sera and plasma undergo slight, if any, obvious physical change whereas serum and plasma containing large amounts of globulin or fibrinogen undergo a marked increase in viscosity which may be sufficient to result in complete coagulation. Scull and Pemberton [22] have found the reaction reasonably acceptable as an index of the extent of systemic activity in atrophic arthritis with the results closely paralleling the sedimentation time and rate of erythrocytes. In hypertrophic arthritis and moderate atrophic arthritis positive reactions were of lesser frequency and degree.

The Weltman Serum Coagulation Reaction. Weltman [23] has observed that when normal serum is heated in a boiling water bath, coagulation occurs after a few minutes and diffuse turbidity develops. If, however, the serum has been diluted 1:50 with distilled water, coagulation takes place only if certain electrolyte solutions are added. The test is conducted with ten test tubes carrying from 0.1 to 0.01 per cent concentrations of serum and the results expressed as a "prolonged coagulation band" (shift to right) or as a "shortened coagulation band" (shift to the left).

The mechanism of the reaction is unknown. It is nonspecific and therefore not diagnostic of any particular disease. Nevertheless it is regarded as possessing both diagnostic and prognostic values.[24, 25, 26] Thus, "shortened coagulation bands" are regarded as indicative of exudative inflammatory diseases such as septic febrile states, pneumonia, pulmonary tuberculosis, acute rheumatic fever, etc., while "prolonged coagulation bands" are indicative of diseases characterized by fibrotic changes such as cirrhosis of the liver, chronic nephritis, nonseptic febrile states, syphilis, scleroderma, etc. According to Siegel and Kraemer[27] the test appears to be of considerable value in indicating the presence of exudative or inflammatory disease of the gastrointestinal tract, including neoplastic lesions when the latter are accompanied by ulceration or exudation.

Hemolysis; Erythrocyte Fragility Test. While there is no direct proof of hemolysis playing a rôle in the physiologic or normal destruction of worn-out erythrocytes in the body, there can be no doubt that it is involved in blood destruction in some abnormal states. Thus, hemolysis *in vivo* is characteristic of paroxysmal hemoglobinuria upon exposure to cold and is particularly active in congenital or familial hemolytic jaundice. It apparently may also occur from the effects of the venoms of some of the poisonous snakes as well as from some of the bacterial toxins with special reference to those of hemolytic streptococci and staphylococci. The products of tissue destruction may be likewise feebly hemolytic *in vivo* as in the case of extensive burns and the autolysis of tumors. Furthermore, it may occur not only in incompatible blood transfusions from the effects of natural hemolysins, but even after repeated transfusions of compatible blood and especially of preserved citrated blood. In some instances this hemolysis evidently plays a part in the production of "hemolytic shock" with anuria, which is one of the gravest of the post-transfusion reactions. Certain drugs may also produce hemolysis *in vivo* with special reference to phenylhydrazine which is given for this purpose in the treatment of polycythemia vera. Apparently quinine may also cause hemolysis in malaria with the production of hemoglobinuria (blackwater fever), ascribed to a lowering of the resistance of erythrocytes to intravascular lytic agents, especially to the bile salts. But intravenous injections of only very large amounts of water and hypotonic saline solutions produce hemolysis since the erythrocytes are protected by the rapid passage of salts from the tissues into the blood for the maintenance of sufficient osmotic pressure to prevent its occurrence. In other words, substances which may be actively hemolytic in the test tube are usually counteracted in the body, although the reverse may occur, as in the case of phenylhydrazine, which is not hemolytic *in vitro* but actively so *in vivo*.

Little is known, however, of the mechanism of hemolysis in the test tube and in the body except that increased resistance to hypotonic saline solution may be related to an increased cholesterol content of the erythrocytes, probably due to absorption of cholesterol from the plasma.[28] Nevertheless, a determination of the resistance of erythrocytes to hypotonic solutions of sodium chloride, called the *erythrocyte fragility test,* is sometimes of clinical value and many methods have been evolved since the test was first proposed by Hamburger in 1883. For clinical purposes, a relatively simple test suffices in which whole blood is added to solutions of sodium chloride in test tubes varying from 0.28 to 0.6 per cent with gradations of 0.02 per cent. *A control test with normal blood should always be included.* The tubes, after *gentle* mixing of their contents, are placed in a refrigerator for two or three hours or left at room temperature for two hours, after which the readings are made. These may be facilitated by centrifuging but this step is not essential.

Hemolysis will vary according to whether whole blood or washed erythrocytes are used; also to some extent according to the amounts of whole blood employed in relation to the volume of hypotonic saline solution. When whole venous blood is employed, hemolysis with normal blood just begins at 0.4 to 0.46 per cent sodium chloride and the concentration, when this first slight hemolysis occurs, is called *minimum resistance.* Hemolysis is usually complete in solutions varying from 0.30 to 0.36 per cent sodium chloride which is called *maximum resistance.* The former is of more clinical value than the latter. Both values are essentially the same in children (Table 5).

TABLE 5. SUMMARY OF THE CLINICAL INTERPRETATION OF ERYTHROCYTE FRAGILITY TESTS

Normals: (1) *Minimum resistance* (slight hemolysis): 0.40 to 0.46; (2) *Maximum resistance* (complete hemolysis): 0.30 to 0.36 per cent NaCl sol.

Increased resistance	Decreased resistance
Pernicious anemia	Congenital hemolytic jaundice
Erythroblastic anemia (Cooley)	Aplastic anemia
Sickle cell anemia	
Myelophthisic anemia	
Tendency in hypochromic anemia	
Polycythemia vera	
Acute hepatitis; obstructive jaundice	
After splenectomy	
Lead poisoning	
Acquired hemolytic jaundice	

Decreased resistance (due to increased fragility) is especially marked in congenital or familial hemolytic jaundice and since resistance is usually normal or slightly increased in intrahepatic and extrahepatic obstructive jaundice, the test has been found of special clinical value in the differential diagnosis of these two kinds of jaundice. Resistance is also decreased to some extent in aplastic anemia while it is increased in pernicious and several other types of anemia, polycythemia vera, after splenectomy, etc., as summarized in Table 5.

CHANGES IN THE HEMOGLOBIN

Formation and Fate of Hemoglobin. Hemoglobin is a conjugated protein or complex consisting of iron in combination with protoporphyrin and globin (a protein of the histone class). It is formed in the bone marrow within erythrocytes and is responsible for the red color of the blood. The molecule is extremely large and contains one atom of iron. In erythrocytes the hemoglobin readily forms a loose combination with oxygen in the lungs to form *oxyhemoglobin* (responsible for the bright red color of arterial blood) and thereby enables these cells to convey oxygen to the tissues. In the tissues the hemoglobin gives up its oxygen to form *reduced hemoglobin* (responsible for the dark color of venous blood) and conveys carbon dioxide back to the lungs. Under normal conditions the total amount of hemoglobin in the body will carry approximately 1200 cc. of oxygen which is used by the tissues in about five minutes during rest and in less than a minute during muscular activity. Except for this carrying capacity of hemoglobin it would be necessary for the plasma to take on this important function and to do this it would have to be increased about 60 times, or from about 6 liters to over 350 liters, which is more than five times the bulk of the solid tissues.

As hemoglobin is released by the fragmentation of erythrocytes, it is apparently destroyed by the cells of the reticulo-endothelial system and especially those in the spleen, bone marrow and the Kupffer cells of the liver. The first step is the separation of globin and hematin. The former is converted into amino acids. The iron is separated from the hematin and stored in the liver and spleen to be used again for the synthesis of hemoglobin. The iron-free porphyrin is now converted to bilirubin and carried to the liver for excretion in the bile while a small amount is excreted by the kidneys in the urine.

Hemoglobinometry; Normal Hemoglobin; The Color Index and Other Indexes. Among laboratory examinations an estimation of the hemoglobin is one of the most widely employed in clinical medicine. However, few examinations are conducted less satisfactorily due not only to carelessness and the inaccuracy of instruments, but also to the fact that manufacturers have been unable to discover a standard on which hematologists would agree for the calibration of hemoglobinometers. As a result, many methods have been described with the hope of meeting the essential requirements of accuracy, speed and simplicity. Some are based on the direct matching of unchanged blood with color scales (Tallqvist and Dare methods) and others on the conversion of hemoglobin into acid hematin with the matching of colors (Sahli, Sahli-Hellige, Newcomer, Wintrobe, Haden-Hausser and Osgood-Haskins methods) including the carboxyhemoglobin method of Palmer, in which the hemoglobin in blood diluted with 0.4 per cent solution of ammonia is converted into carbon monoxide hemoglobin and the color compared with a standard. But the best are apparently the photo-electric cell methods of Exton and Sheard-Sanford although the instruments are expensive. Additional methods (Kennedy, Wong, Osterberg) are based on a determination of blood iron or on the oxygen-carrying capacity of the blood (Van Slyke), but while these are suitable for investigative work and the standardization of instruments, they are not commonly employed for routine clinical purposes.

Hemoglobinometers are standardized in relation to the oxygen capacity of the blood on the assumption that 1 gm. of hemoglobin combines with 1.338 cm. of oxygen at normal temperature and pressure (Hüfner). But it is now the consensus that the custom of expressing hemoglobin in percentages is grossly inaccurate because of normal variations according to age and sex. To be sufficiently accurate it would be necessary to set up an arbitrary normal for each sex and for children of varying ages. But for calculating the *color index* it is assumed that under normal conditions 5,000,000 erythrocytes per c.mm. of blood carry 100 per cent hemoglobin. By dividing the percentage of hemoglobin by the first two figures of the erythrocyte count, multiplied by 2, the index is determined and expressed in absolute terms. Thus, a hemoglobin estimation of 100 per cent, with an erythrocyte count of 5,000,000, would give a fraction $\frac{100}{100} = 1.0$ color index which is normal, whereas a hemoglobin of 80 per cent with an erythrocyte count of 4,500,000 would give a fraction $\frac{80}{90} = 0.8 +$ color index, indicative of hypochromic anemia.

At the present time hemoglobin is reported in terms of grams per 100 cc. of blood, which has been proposed by the American Society of Clinical Pathologists as a standard method. The calculation takes into account the age and sex of the individual. According to Wintrobe,[6] the average normals in grams per 100 cc. of blood from birth to adult age are as follows: First day, 19.5 ± 5.0; two to eight days, 18.3–19.0 ± 4.0; two to eight weeks, 14.0 ± 3.3; three to eleven months, 11.8–12.2 ± 2.3; one to two years, 11.2–11.8; three to ten years, 12.5–12.9; eleven to fifteen years, 13.4; adult males 16.0 ± 2.0 and adult females 14.0 ± 2.0.

If the hemoglobin is reported in grams per 100 cc. of blood, the color index may be calculated by multiplying by the factor 6.9 and dividing by the erythrocytes multiplied by 20. The *mean corpuscular hemoglobin* is determined by dividing the hemoglobin in grams per 1000 cc. by the erythrocytes in millions per c.mm. and expressing the results in micromicrograms, which normally for adults is 29 (± 2) and from 26 to 28 for children.[6] The *mean corpuscular hemoglobin concentration* is calculated by dividing the hemoglobin by the volume of packed corpuscles in cc. per 100 cc. of blood and multiplying by 100. The results are expressed in terms of grams per 100 cc. with a normal range of 32 to 34 for children and 34 (± 2) for adults.[6] The *saturation index* is determined by dividing the color index by the volume index which gives a normal range of 0.9 to 1.2 in absolute terms.

Hemoglobinemia and Hemoglobinuria. When rapid and severe destruction of erythrocytes occurs in the body, some free hemoglobin may be found in the plasma, constituting *hemoglobinemia;* likewise, other pigments of which the chief one has heretofore been regarded as methemoglobin, but which Fairley[29] has recently shown to be methemalbumin, a pigment formed from hematin by union with serum albumin. In addition, there is, of course, an increase of bilirubin (bilirubinemia) because hemoglobin is converted into this pigment very rapidly. All three pigments usually occur in the plasma when there is rapid and severe blood destruction, as in the acute and subacute hemolytic anemias due to malaria, bacterial toxins, chemical agents, vegetable poisons, venoms, extensive burns, incompatible blood transfusions, paroxysmal hemoglobinuria, Lederer's anemia, etc. When hemolysis is less severe and chronic, only bilirubinemia will be found, as in congenital hemolytic jaundice, sickle cell anemia, chronic "acquired" hemolytic jaundice, etc.

Experimentally it has been found that in normal subjects hemoglobin does not occur in the urine (*hemoglobinuria*) until the plasma hemoglobin is as high as 135 mg. per 100 cc. although, once hemoglobinuria has occurred, it persists until the plasma level is as low as 30 to 50 mg. per 100 cc.[30] This condition is especially observed in paroxysmal (cold) hemoglobinuria, chronic hemolytic anemia with paroxysmal hemoglobinuria and blackwater fever due to malaria or quinine.

Hyperchromemia. An increase in the total quantity of hemoglobin is designated *hyperchromemia;* it is uncommon and usually more apparent than real. Hyperchromemia accompanies an increase of erythrocytes as in polycythemia vera and in erythrocytosis due to high altitudes or other causes; likewise in diseases or states producing hemoconcentration by dehydration as in the severe diarrheas. In this connection it should be stated, however, that hyperchromemia is not synonymous with hyperchromia. For example, in the hyperchromic macrocytic anemias or pernicious anemia, pregnancy, pellagra and sprue, the total erythrocytes are reduced although each carries more than a normal amount of hemoglobin, with the total remaining less than normal.

Oligochromemia. A reduction in the total quantity of hemoglobin is designated *oligochromemia.* It is both common and important, being a distinct and striking feature of the anemias listed in Table 2 and especially of all hypochromic anemias. In other words, there is usually a relationship between oligochromemia and hypochromia because in the hypochromic anemias the erythrocytes are not only reduced in numbers, but each carries less than a normal amount of hemoglobin which reduces the total quantity of the latter.

CHANGES IN THE LEUKOCYTES

Total and differential leukocyte counts are likewise among the most frequent of laboratory examinations. When conducted with care and skill the information yielded is of great clinical value. A total leukocyte count, of course, requires much less time than a differential count but as a matter of fact the latter is frequently of more clinical value than the former, as when made according to the Schilling or "shift to the left" method. A differential count may reveal abnormal changes of clinical value when the total leukocytes show a normal count, especially in the diagnosis of acute and severe infections.

Formation and Fate of Leukocytes. The various theories concerning the origin and relationship of the cells of the blood have already been briefly discussed. Insofar as the leukocytes are concerned they are divisible into the myeloid, lymphocytic and monocytic series.

(1) The myeloid series begins with the *myeloblast* which normally occurs in the bone marrow but not in the circulating blood. In acute myeloid leukemia,

however, it occurs in the latter and is characteristic of the disease. It constitutes the parent cell of the polymorphonuclear neutrophil, eosinophil and basophil of the circulating blood. Myeloblasts vary in size and shape and not infrequently are difficult or impossible to differentiate from lymphoblasts and monoblasts. The nucleus may show several wide and deep indentations, in which case it is known as the *Rieder cell.* Large granules, globules or slender rods may be found in the cytoplasm—these are called *Auer bodies.* The *Türk irritation cell* is one resembling a plasma cell but retaining the nuclear pattern of the myeloblast.

On maturing the myeloblast gradually develops granules in the cytoplasm, without much indentation of the nucleus, to form the *myelocyte.* This cell does not occur normally in the blood but may be found in conditions and diseases causing great activity of the leukoblastic tissues of the bone marrow, such as acute infections, and especially in acute and chronic myeloid leukemia.

The next step is inaugurated by a deep indentation of the nucleus which becomes horseshoe- or sausage-shaped, after which it divides into two lobes to form the *metamyelocyte.* It occurs in normal circulating blood but is increased in the acute infections and even when the total leukocytes are within normal or but slightly increased. The detection of an increase in differential leukocyte counts constitutes the so-called "shift to the left."

The final step in maturity is the *polymorphonuclear neutrophil* in which there are two or more nuclei with neutrophilic granules in the cytoplasm. According to Arneth, the number of nuclei increases with age but this has not been conclusively proven. When unusually large, with 6 to 10 lobes in the nucleus, the cell is called a *macropolycyte,* which is rarely seen in health but is frequently found in pernicious anemia.[31]

Eosinophils exhibit the same processes of maturation but rarely have more than two lobes. They are characterized by having large acid-staining granules in the cytoplasm. The same is true of *basophils* which are characterized by large, coarse, basic-staining granules but with no lobulation of the nucleus.

(2) The lymphocytic series is thought to begin with the *lymphoblast* occurring in lymph glands, the spleen, thymus gland, tonsils, Peyer's patches and possibly the bone marrow. The difficulty of distinguishing them from myeloblasts has already been mentioned. Lymphoblasts do not occur normally in the blood but are characteristically present in acute lymphatic leukemia. It has not been shown conclusively that a true maturation cycle occurs as in the myeloid series, although Wiseman [32] has attempted a classification on this basis. At all events, the final cell is the *lymphocyte* which some have classified as large and small, usually on the assumption that the former are the more immature. Others, however, claim that a difference in size may be due to their origin, with the suggestion that large lymphocytes with kidney-shaped nuclei arise in the spleen while small round lymphocytes come from the lymph nodes. *Plasma cells* are thought to be derived from lymphocytes or primitive connective tissue cells. They are rarely seen in normal blood but are frequent in chronic inflammatory tissue and are normally present in the interstitial tissue of various organs and glands. When they contain granules and hyaline bodies in the cytoplasm they are known as *Russell body cells.* Plasma cell leukemia may occur, but is very rare.

(3) The monocytic series is thought to begin with the *monoblast* which, obviously, is difficult to distinguish from the myeloblast. The next cell in maturation is the young monocyte or *premonocyte* followed by the mature cell or *monocyte*. The latter is not found normally in the bone marrow but occurs in the circulating blood. Formerly it was called a "transitional cell" by Ehrlich in the belief that it represented a transitional stage between the lymphocyte and polymorphonuclear neutrophil, but was first clearly identified as a separate cell by Schilling in 1912. According to Sabin, the epithelioid cells of tuberculosis are derived from monocytes and in the opinion of some the tissue macrophage, clasmatocyte or histiocyte has the same origin, although it is admitted that monocytes may not be the sole source.

The *longevity* of leukocytes has not been definitely determined, but since more lymphocytes enter the blood through the thoracic duct than are normally present in the blood, it is thought that their span of life may be less than twenty-four hours while that of the granulocytes may be only a few days. Furthermore, their *fate* is not definitely known except that many are known to penetrate mucous membranes and are washed away. Effete cells may also be filtered out or phagocytosed by the cells of the reticulo-endothelial system and especially by those in the liver, spleen, lymph nodes and bone marrow.

Functions of the Leukocytes. Undoubtedly the various mature leukocytes possess various and important functions not only as they occur in the blood but also in the fixed tissues, an excellent review of recent advances in our knowledge being given by Rebuck.[33] One of the main functions of the polymorphonuclear neutrophils is that of *phagocytosis*, especially of bacteria and small particles. It is possible that they are also concerned in *antibody production*. The same is true of the monocytes with special reference to phagocytosis of protozoa, some of the bacteria and particulate matter including erythrocytes. Leukocytes also contain various *enzymes* and a bactericidal agent called *lysozyme* serving useful functions. Because they are more abundant in tissue fluids than in the blood and are found within the epithelial linings of the intestinal and respiratory tracts, as well as of the skin, the eosinophils are thought to play an important rôle in *detoxification*. They increase in number in foreign protein therapy and thereby may participate in its beneficial effects. Since they are also increased in parasitic diseases and allergy, it appears that they may be involved in these in some useful manner. Nothing is known, however, concerning the functions of the basophils and in man they may represent only functionless evolutionary cells. The function of the lymphocytes is also mysterious. However, since they are generally increased during convalescence from infections, they appear to be involved in some way in the *process of healing*. They may also play a rôle in the *fixations of toxins* as well as in resistance to antigens containing lipoids. At least they have been shown to participate in resistance to tuberculosis. They contain lipase and other enzymes but it is doubtful if the former is involved in fat metabolism. The possibility of their transferring fat through the intestinal mucosa to the lacteals has also been denied. Leukocytes consume oxygen and sugar, especially the neutrophils, but studies in their metabolism have yielded little information bearing on functions in relation to metabolism.

Normal Total Leukocytes. Age and other physiologic factors have a marked influence upon the total number of leukocytes per c.mm. of blood. In the *newborn infant* they average about 10,000 but may be much higher. *At birth* they average 10,000 to 20,000 or higher, dropping to 9000 to 11,000 three or four days thereafter; until six months of age they may be very irregular with variations from 5000 to 24,000 (averaging 8000–16,500) under normal conditions. In children four to seven years of age they vary from 6000 to 15,000 (average 10,700) and from eight to eighteen years 4500 to 13,500 (average 8300). In *adults* of both sexes the average is commonly stated to be about 7000 with a range of 5000 to 10,000.[6]

Fluctuations occur during the day and from day to day. They are increased by digestion and strenuous exercise. Conclusive demonstration of the effects of climate or season is lacking but some observers have thought a slight increase may be caused by natural or artificially induced heat (probably due to hemoconcentration from excessive perspiration) as well as by sunlight, ultraviolet radiation and high altitudes.[34]

Normal Kinds of Leukocytes. In 1904 Arneth divided the neutrophils into five classes with a number of subdivisions making a total of 20 in all. This is too complex for use and is rarely followed. However, he tabulated across a page with the first class of immature forms (single-lobed nuclei) to the left and the last class of mature forms (multiple-lobed nuclei) to the right. Thus the terms *shift to the left* and *shift to the right* were introduced and the former should always be used because of its diagnostic value in differential leukocyte counts (Table 6).

In 1911 Schilling divided the neutrophils into myelocytes, juveniles, stab-cells and segmented neutrophils, the first three representing immature and the last mature cells. Differences in interpretation, however, naturally introduced considerable variation in differential counts even by well-trained workers. To simplify matters Haden [35] introduced the method of dividing the neutrophils into nonfilament and filament cells, the latter referring to those in which two or more lobes of the nucleus are united by a filament of chromatin. All useful clinical purposes, however, appear to be met by simply dividing them into immature or nonsegmented cells (corresponding to the juveniles and stab-cells of Schilling and the nonfilament types of Haden) and mature or segmented cells (corresponding to the segmented cells of Schilling and the filament types of Haden).

The results are usually expressed in terms of percentages of the various leukocytes but are better expressed in terms of the absolute numbers of each per c.mm. of blood, since percentages do not always clearly differentiate between an absolute or a relative increase or decrease. All differential leukocyte counts, therefore, should not only give the percentages of each but, more importantly, the actual numbers of each calculated from the percentages and total leukocyte count. Furthermore, in the interests of accuracy, at least 200 leukocytes should be classified in order to render a change in percentages on the same individual at different times sufficiently reliable for clinical purposes.

Good staining is of course necessary, especially for the detection of *toxic granules* in the cytoplasm of both segmented and nonsegmented neutrophils, which are deeply staining basophilic granules of varying size, occurring in severe infections and thought to be due to the effects of toxins on the cells in the bone marrow during the stage of granule formation. For this purpose it is sometimes necessary to stain by the peroxidase method. Normally, neutrophils are full of peroxidase-staining granules whereas when toxic basophilic granules are present but few are found.

Under normal conditions the average percentages and absolute numbers of the various leukocytes vary according to age, since after the third week up to four years or even later the number of lymphocytes is higher than in adults (Table 6).

TABLE 6. SUMMARY OF NORMAL KINDS OF LEUKOCYTES

Kinds	Adults		Children (3 to 10 years)	
	Per cent	Absolute *	Per cent	Absolute *
Neutrophils Immature (nonfilamented or nonsegmented)	3–5	300 (150–400)	3–8	280 (150–300)
Neutrophils Mature (filamented or segmented)	54–62	4000 (3000–5800)	16–60 (av. 38)	3250 (3000–8000)
Eosinophils	1–3	200 (50–250)	1–3	300 (50–700)
Basophils	0–0.75	25 (15–50)	0–0.75	25 (0–50)
Lymphocytes	25–33	2100 (1500–3000)	42–48	4000 (3250–5000)
Monocytes	3–7	375 (285–500)	3–5	350 (250–700)

* Per 1 c.mm. of blood.

Abnormal Kinds of Leukocytes. Metamyelocytes may occur normally in the blood but this is not true of myeloblasts and myelocytes.

Myeloblasts are characteristic of acute myeloblastic leukemia but likewise occur to some extent in chronic myelocytic leukemia as well as in erythroblastosis fetalis.

Myelocytes are characteristic of chronic myelocytic leukemia but also occur in erythroblastosis fetalis and to some extent in pernicious anemia and other severe anemias.

Lymphoblasts do not ordinarily occur in normal blood but are characteristic of acute lymphoblastic leukemia while large and normally small lymphocytes are characteristic of subacute and chronic types of lymphocytic leukemia.

Large mononuclear leukocytes do not occur normally in the blood but are commonly present in infectious mononucleosis along with a great increase of monocytes and small lymphocytes.

Leukopenia. A reduction in the total number of leukocytes below the lower limits of normal is called *leukopenia.* It is usually due to a marked reduction in the neutrophils. When leukopenia is marked, however, all types are reduced.

The causes of leukopenia may be grouped as follows: (1) Certain bacterial, viral and protozoal diseases and all types of overwhelming infections; (2) cachectic and debilitated states and inanition; (3) diseases of the hemopoietic system, especially those involving the spleen; (4) the effects of certain drugs and poisons; (5) the effects of physical agents; (6) certain diseases of the liver and conditions of unknown cause and (7) anaphylactoid shock and the early stages of reactions to parenteral injections of nonspecific protein agents (Table 7).

TABLE 7. SUMMARY OF THE CLINICAL INTERPRETATION OF LEUKOPENIA AND SIMPLE LEUKOCYTOSIS

Leukopenia	Simple leukocytosis
Typhoid and paratyphoid fevers	Digestion
Undulant fever; tularemia (some cases)	Strenuous exercise
Influenza, measles, psittacosis, dengue and rubella	Convulsions and chorea
	Fear and pain
Malaria	Pregnancy, labor and the puerperium
Relapsing fever	Eclampsia
Kala-azar	Ether anesthesia
Miliary tuberculosis	Paroxysmal tachycardia
Overwhelming septicemias	Coronary occlusion
Cachectic and debilitated states	Acute and chronic infections
Banti's and Gaucher's diseases	Acute hemorrhage
Relapse of pernicious anemia	Acidosis and diabetic coma
Chronic hypochromic anemia	Uremia
Aplastic anemia	Congenital hemolytic jaundice
"Aleukemic" leukemia	Burns
Agranulocytosis	Lymphosarcoma
Amidopyrine, sulfonamides, barbiturates, benzol, etc.	Fractures
	Intestinal obstruction
Roentgen rays and radium	Osteomyelitis
Portal cirrhosis of the liver	Salpingitis
Infectious hepatitis	Sickle cell anemia
Felty's syndrome	Polycythemia vera
Anaphylactoid shock	Smallpox
Nonspecific protein therapy	Scarlet fever
	Syphilis
	Gonorrhea
	Coccidioidal granuloma
	Rheumatic fever
	Tetanus

Leukocytosis; Relation to Diagnosis and Prognosis. An increase in the total number of leukocytes above the upper limits of normal according to age is called *leukocytosis.* When there is neither an absolute nor a relative lymphocytosis, along with the fact that the eosinophils, basophils and monocytes have not completely disappeared, it is designated as *simple leukocytosis*. As shown in Table 7, this may be due not only to physiologic causes including those of the later stages of pregnancy, during labor and the puerperium, but likewise to a large number of diseases. Very marked leukocytosis is characteristic of the acute and chronic leukemias and is also usually observed in infectious mononucleosis and Lederer's acute hemolytic anemia.

TABLE 8. SUMMARY OF THE CLINICAL INTERPRETATION OF NEUTROPHILIA, EOSINOPHILIA AND BASOPHILIA

Neutrophilia	Eosinophilia	Basophilia
Acute systemic infections Acute localized infections Acute complications Metabolic intoxications Intoxications by drugs and poisons Insect venoms Foreign protein reactions Acute hemorrhage Coronary thrombosis Rapidly growing tumors Incompatible transfusions Myelocytic leukemia Erythroblastosis fetalis Neutrophilic leukemia	Allergic diseases; asthma Skin diseases (especially pemphigus, Duhring's disease and erythema multiforme) Scarlet fever; chorea Helminthiasis Chronic myelocytic leukemia Hodgkin's disease After splenectomy Pernicious anemia; liver therapy Following irradiation Periarteritis nodosa Certain poisons Loeffler's syndrome Tumors of the ovary and those of serous surfaces and bones; malignant tumors As a family anomaly Coccidioidomycosis (Valley fever)	Basophilic leukemia Chronic myelocytic leukemia Pernicious anemia Polycythemia vera Tendency in chronic hemolytic anemia, chlorosis, Hodgkin's disease, chronic sinusitis, smallpox, chickenpox, after splenectomy and after the administration of nonspecific proteins

Leukocytosis due to disease may also be characterized chiefly by an absolute increase of the neutrophils (*neutrophilia*), the eosinophils (*eosinophilia*) or the basophils (*basophilia*), summarized in Table 8. Leukocytosis may also be due chiefly to an absolute increase of the lymphocytes (*lymphocytosis*) or the monocytes (*monocytosis*), as summarized in Table 9.

Differential leukocyte counts, therefore, may not only be helpful in discovering hidden subacute and chronic infections and acute infections during the early stages (by a shift to the left) when the total leukocytes are within normal according to age, but they are also useful in relation to *prognosis*.

Unfavorable signs include: an extremely high leukocytosis and a high percentage of neutrophils with the immature outnumbering the mature cells; also the finding of numerous toxic and degenerated neutrophils along with a complete absence of eosinophils and a marked absolute reduction of lymphocytes. Probably still worse is when the patient fails to develop leukocytosis at all.

TABLE 9. SUMMARY OF THE CLINICAL INTERPRETATION OF LYMPHOCYTOSIS AND MONOCYTOSIS

Lymphocytosis	Monocytosis
Lymphocytic leukemia	Monocytic leukemia
Infectious mononucleosis	Infectious mononucleosis
Acute infectious lymphocytosis	Hodgkin's disease
Mumps; pertussis	Gaucher's disease
German measles	Certain bacterial infections (tuberculosis, subacute bacterial endocarditis, undulant fever, typhus fever)
Chronic infections (tuberculosis, syphilis and undulant fever)	During convalescence from acute infections and agranulocytosis
During convalescence from an acute infection	Malaria
Exophthalmic goiter (usually relative)	Kala-azar
Rickets	Rocky Mountain spotted fever
Malnutrition of children	Tetrachlorethane poisoning
Pernicious anemia (relative)	
Gaucher's disease	

Favorable changes indicative of recovery are: falling total leukocyte counts with diminishing numbers of neutrophils (especially of immature forms) and toxic cells; also an increase of lymphocytes and monocytes along with a reappearance or an increase of eosinophils.

Leukemoid Reactions. Cases have been reported in which the total and differential leukocyte counts, along with severe anemia, fever, hemorrhages and splenomegaly or adenopathy, have suggested lymphocytic and myelocytic leukemia and even myeloblastic leukemia. These conditions have occurred in pneumonia, meningococcus meningitis, diphtheria, tuberculosis, pertussis, chickenpox and, of course, in infectious mononucleosis; also in such intoxications as eclampsia and mercury poisoning, as well as after severe burns, hemorrhage and hemolysis, and in carcinoma with bone metastases, multiple myeloma, myelosclerosis and Hodgkin's disease. Human beings never recover from true leukemia or Hodgkin's disease; in alleged recoveries from any of these diseases the blood pictures were doubtless leukemoid rather than leukemic.

CHANGES IN THE PLATELETS

The blood platelets or thrombocytes play an important rôle in the coagulation of the blood. Their estimation, therefore, in terms of numbers per c.mm. of blood is always required in the study and diagnosis of bleeding states and the hemorrhagic diseases, although failure of coagulation may be due to defective function rather than to a numerical decrease of them.

Origin of the Platelets. The exact origin of platelets is still unknown. From time to time they have been thought to be derived from the plasma itself, from the endothelium of blood vessels, from erythrocytes or their nuclei, from the nuclei of leukocytes or the granules of eosinophils, from the reticulo-endothelial cells of the lymph nodes and spleen, or from megakaryocytes in the bone marrow or lungs.

At the present time it is thought that their source is the megakaryocytes of the bone marrow, as stated by Wright in 1906, although the mechanism of their production by the cytoplasm of these cells is by no means clear. Howell and Donaghue [36] have maintained that they are normally produced in the lungs from megakaryocytes acting as they do in the bone marrow. Others, however, regard these cells in the lungs as effete cells and have been unable to obtain satisfactory evidence of platelet production in these organs.[37]

Megakaryocytes or fragments of their nuclei or cytoplasm are sometimes found in the circulating blood. They may also occur in myelocytic leukemia, pernicious anemia, polycythemia vera, purpura haemorrhagica during the phases of platelet increase, in acute infections (*e.g.*, lobar pneumonia), plumbism and Hodgkin's disease. A rare form of megakaryocytic leukemia has been described as "aleukemic megakaryocytic myelosis." Italian hematologists have designated a flooding of the blood with megakaryocytes by the term "plastrinemia."

The rate of production of platelets varies considerably but it is thought that about 100,000 are formed per c.mm. daily. They appear to have a short life span, as the entire number in circulation can be replaced in three to five days. Rapidity of production is indicated by the fact that following splenectomy for thrombocytopenic purpura, as many as 1,000,000 or more per c.mm. have been produced within twenty-four hours. Nothing is known, however, of their fate.

Functions of the Platelets. As previously stated, the platelets are concerned in the *coagulation* of the blood as well as in clot contraction or *syneresis,* and through these functions in *hemostasis* and doubtfully in thrombosis. Their exact rôle in coagulation, however, is still a matter of conjecture. Most investigators believe that they carry a lipid (possibly a phosphatide) which influences the rate of transformation of prothrombin to thrombin by neutralizing antiprothrombin (Howell), by uniting with prothrombin in the presence of calcium (Fuchs, Bordet, Fischer), by acting as an activator of prothrombin (Morawitz) or by uniting with calcium and fibrinogen to form fibrin (Mills). When the platelets are numerically reduced, blood clots show less adhesiveness, firmness, rigidity and contractility. Normal syneresis is important in hemostasis because it causes the walls of capillaries to collapse. The number of platelets, however, is relatively unimportant in thrombosis which is no more frequent after splenectomy with an increase of them (thrombocytosis), which often develops, than after other surgical operations.

Because of the readiness with which platelets adhere to foreign particles, and because this has been observed shortly after the intravenous injection of bacteria, some investigators have thought that they play a rôle in *resistance to infection* and are concerned in the removal of foreign material,[38] but there is no evidence that they or their products take part in the destruction of bacteria about which they adhere.

Normal Platelets. Many methods have been proposed for counting the platelets but none is satisfactory in every respect. This is due not only to their small size and ready attachment to instruments but, likewise, because of their tendency to agglutination and disintegration. Indirect and direct methods are employed as well as a determination of their volume by means of a special thrombocytocrit which normally shows about 0.3 cc. per 100 cc. of blood.

The number per c.mm. of blood under normal conditions varies according to the method employed. Thus, with the direct method of Rees and Ecker, the normal varies from 200,000 to 500,000 per c.mm. while, according to the brilliant cresyl blue moist preparation method (indirect), the normal is stated to average around 800,000. In general terms, however, the normal may be taken as 250,000 to 500,000 per c.mm. Since errors in counting are possible, only fluctuations of 50,000 to 100,000 per c.mm. possess clinical significance.

Newborn infants are said to show fewer platelets (150,000 to 250,000). A daily fluctuation of 6 to 10 per cent may occur. No differences have been ascribed to sex although during the first day of menstruation they are sharply decreased. Severe exercise and high altitudes are said to increase them.

TABLE 10. SUMMARY OF THE CLINICAL INTERPRETATION OF CHANGES IN THE PLATELETS

Normal varies with the method of counting: usually 250,000 to 500,000 per c.mm. of blood

Thrombocytopenia (Decrease)	Thrombocytosis (Increase)	Essentially Normal
Primary or idiopathic purpura haemorrhagica (Werlhoff's disease)	Polycythemia vera	In the nonthrombocytopenic purpuras:
Secondary or symptomatic purpura haemorrhagica due to:	Hemolytic anemias	Purpura simplex
Chemical agents	Chr. myelocytic leukemia	Henoch's purpura
Physical agents	Posthemorrhagic anemia	Schönlein's purpura
Acute leukemias	Chlorosis	Some acute infections due to chemical agents
Chronic leukemias	Cachexia and malnutrition	Hereditary hemorrhagic diathesis and telangiectasia
Aplastic anemia	Acute rheumatic fever	Agranulocytosis
Chr. hypochrom. anemia	Suppurative infections	Hemorrhagic disease of the newborn
Myelophthisic anemia	Fractures of bones (especially of the neck of the femur)	Scurvy
Pernicious anemia	Severe exercise	Certain skin diseases
Hemolytic jaundice	High altitudes	Usually in infectious mononucleosis
Banti's disease	Asphyxia	Usually in multiple myeloma
Gaucher's disease		Usually in chronic leukemia
Septicemia		
Bacterial endocarditis		
Onset of pneumonia		
Typhoid fever		
Diphtheria		
After splenectomy		
First day of menstruation		

Thrombocytosis and Thrombocytopenia. A definite increase of the platelets due to an increase of the number or activity of megakaryocytes in the bone marrow, is called *thrombocytosis*. A definite decrease, due to injury or destruction of megakaryocytes or destruction of platelets in circulation, is called *thrombocytopenia* and is of more clinical importance. Owing to inevitable errors in counting, slight alterations from the normal possess no clinical value, as previously stated. Platelet counts, however, are of great value in differentiating the hemorrhagic diseases. In hemophilia they are normal in number but probably deficient in function (*thrombasthenia*), while in purpura haemorrhagica they are functionally normal but greatly reduced in number. Numerical changes of diagnostic value are observed in other diseases, including the various nonthrombocytopenic purpuras, after splenectomy and in blood diseases in which platelet counts are usually made (Table 10).

Changes may also occur in the size and morphology of the platelets, especially when they are reduced and then increased, as seen after splenectomy. Unusually large platelets, sometimes with very coarse granules, are frequently observed in purpura haemorrhagica. Basophilic platelets may be observed in thrombocytopenia, particularly when their numbers are increasing.

CHANGES IN THE COAGULATION, BLEEDING, PROTHROMBIN AND CLOT RETRACTION TIMES; CAPILLARY FRAGILITY

Whatever may be the exact mechanism of coagulation of the blood, it appears to be at least well established that thrombin changes fibrinogen into fibrin. The formation of thrombin, on the other hand, depends on the interaction of at least three substances: (1) One called *thromboplastin* derived from the platelets, which is also present in the tissues and may be present in traces in platelet-free plasma; (2) *calcium;* and (3) *prothrombin* present in plasma. The nature of thromboplastin is not clear. It is not only a constituent of platelets and of cells in the brain, lungs, thymus gland and other organs, but is released by these into the tissue fluids with traces in the plasma. Prothrombin is believed to be formed in the liver with the antihemorrhagic vitamin, K, intimately concerned in some way in its production, as discussed in more detail in Chapter 3. Why the blood does not coagulate *in vivo* is likewise uncertain, although a matter of great importance in relation to the etiology and possible prevention of thrombosis, but it is generally believed that the stability of the platelets is the determining factor. At least it appears that disintegration of them, or some change which does not necessarily involve their complete dissolution, furnishes something to the plasma (apparently thromboplastin) that breaks down normal equilibrium, resulting in the coagulation of the blood. In this connection it is significant that substances preventing coagulation, such as hirudin, heparin, oxalates, citrates, etc., apparently owe this property to the preservation of platelets.

Be that as it may, a prolongation of the coagulation time of the shed blood is not only characteristic of hemophilia and hemorrhagic disease of the newborn, or erythroblastosis fetalis (especially in icterus gravis), but it may also occur in some cases of multiple myeloma and Hodgkin's disease (when there is a thrombocytopenia). In these, as well as in other diseases where coagulation time is normal, clot retraction (*syneresis*) is poor. On the other hand, bleeding time may be prolonged with a normal coagulation time, as is commonly observed in hemophilia, idiopathic and symptomatic purpura haemorrhagica, the leukemias, pernicious anemia, etc. (Table 11). Either or both may be primarily responsible for the hemorrhagic diseases, along with or without an increase of capillary fragility as an additional factor in etiology.

Normal Coagulation Time; Causes of Prolonged Coagulation Time. Coagulation time must be distinguished from bleeding time since it measures the clotting time of blood alone in the absence of tissue factors. For this reason it is necessary to avoid traumatization or squeezing of the tissues as much as possible when blood is being taken for the test. Various methods have been proposed, such as the capillary tube method and those of Boggs, Gibbs, Howell, Lee and White, Cannon and Mendenhall. The normal varies with the method employed which should always be specified. Thus, by the method of Lee and White, which is recommended for routine use, it varies from 5 to 8 minutes; by the capillary tube method 1 to 7 minutes, and by the method of Howell from 10 to 30 minutes. But a determination of the coagulation time, no matter how accurate, is of limited clinical value, not only because it fails to distinguish between the various factors which may be responsible for delayed coagulation, but because these are present in such excess that any one of them must be greatly reduced before coagulation is affected.

One of the most important of these factors is a deficiency of thromboplastin due to thrombocytopenia. But even when the platelets are numerically normal, thromboplastin production may be decreased if they are functionally defective with the possibility that this may be due to an increased resistance to disintegration, as is believed by some to be responsible for hemophilia. Or, delayed coagulation may be due to a deficiency in prothrombin either because the liver is unable to utilize vitamin K in its production, as occurs in intrahepatic and extrahepatic obstructive jaundice and hepatic necrosis, or because of a dietary deficiency or defective absorption of the vitamin, as is discussed more fully in Chapter 3. Delayed coagulation may also be due to a decrease of fibrinogen in severe disease of the liver which is concerned in its production (Table 11); also to an excess of antithrombin in the blood, which largely accounts for delayed coagulation in anaphylactic shock due to the liberation of heparin, rather than to thrombocytopenia which may also occur. On the other hand, delayed coagulation may hardly be ascribed to a deficiency of calcium, since it does not occur until the total calcium is reduced to 2.5 mg. or less per 100 cc. of blood,[39] or to variations in the plasma proteins which might alter the proportions of diffusible and nondiffusible calcium. For this reason a determination of the calcium time is of little clinical value.

Normal Bleeding Time; Causes of Prolonged Bleeding Time. The method of Duke is that usually employed. A moderately deep cut is made in the finger or ear lobe with a sharp blood lancet. Tocantins[40] has devised a special device for making an incision of approximately uniform size. The cut should be sufficiently deep to cause the blood to flow without any pressure. At intervals of one-half minute the drop of blood exuding is removed by filter paper, but the skin should not be touched. The time required for bleeding to cease spontaneously is noted and normally this varies from 1 to 3 minutes. A time of 5 minutes or longer indicates prolongation.

Prolongation of bleeding time may be due to decreased fibrinogen in hepatic disease but recent studies indicate that the chief cause may be a defective capillary contractility [41] in explanation of the paradox of a prolonged bleeding time with a normal coagulation time. It is also possible that a deficiency of thromboplastin in the tissue fluids may be a factor.

TABLE 11. SUMMARY OF THE CLINICAL INTERPRETATION OF THE COAGULATION, BLEEDING AND CLOT RETRACTION TIMES OF THE BLOOD; CAPILLARY FRAGILITY

Diseases	Coagulation Time	Clot Retraction Time	Bleeding Time	Capillary Fragility
Pernicious anemia	Normal	Poor	Prolonged	Variable
Aplastic anemia	Normal	Poor	Prolonged	Negative
Sickle cell anemia	Normal	Normal	Normal	Negative
Hypochromic microcytic anemia	Normal	Normal	Normal	Negative
Agranulocytosis	Normal	Normal	Normal	Variable
Infectious mononucleosis	Normal	Normal	Prolonged	Negative
Purpura haemorrhagica	Normal	Poor	Prolonged	Negative
Henoch's and Schönlein's purpura	Normal	Normal	Normal	Negative
Hemophilia	Prolonged	Normal once formed	Normal	Negative
Hemorrhagic dis. newborn	Prolonged	Poor	Prolonged	Variable
Hereditary hemorrhagic telangiectasia	Normal	Normal	Normal	Negative
Acute leukemias	Normal	Poor	Prolonged	Negative
Chr. lymph. leukemia	Normal	Normal	Prolonged	Negative
Hodgkin's disease with thrombocytopenia	Prolonged	Poor	Prolonged	Negative
Multiple myeloma	Prolonged	Poor	Prolonged	Positive

Prothrombin Time; Plasma Coagulation Time. Various methods have been proposed for the quantitative determination of prothrombin. By the two-stage method of Warner, Brinkhous and Smith [42] human plasma normally contains 300 units per cc. For clinical purposes, however, this method is too time-consuming and the simpler plasma prothrombin time method of Quick and his colleagues [43] has been found of practical value in spite of the fact that it does not control such variables as prothrombin conversion rate, differences in time required for the reaction of thrombin with fibrinogen, delayed coagulation due to deficiency of fibrinogen or excess of antithrombin. It is essentially an improvement on Howell's prothrombin or plasma coagulability test and the normal varies from 10 to 20 seconds, depending on the activity of the thromboplastin employed (prepared of the fresh brain of the rabbit). Bleeding does not usually occur until the time is as low as forty seconds or longer (hypoprothrombinemia) which may occur in obstructive jaundice or hepatic necrosis, as well as in states due to deficient intake or absorption of vitamin K. Recent studies indicate that Russel viper venom ("stypven") may be used instead of thromboplastin in an optimal strength of 1:20,000.[44]

Recently the coagulation time of recalcified oxalated blood plasma has been reintroduced by Cheney [45] as a simple test for vitamin K deficiency. As variations in temperature affect the results, the tests should be conducted at room temperature varying from 23° to 26° C. Under these conditions clotting occurs with the plasma of normal individuals and those with nonhemorrhagic conditions in 4 to 7 minutes, with an average of 5.25 minutes.

Clot Retraction Time. A convenient test is conducted by collecting 6 cc. of blood from a vein and placing 1 cc. in each of three small test tubes with 3 cc. in a fourth. The tubes are placed in an incubator and inspected at the end of one hour and again after eighteen to twenty-four hours. Normally, retraction of the clot with separation of serum is appreciable after one hour and marked after eighteen hours. Occasionally, the clot of normal blood fails to separate from the walls of the test tubes, but if it is loosened with a platinum wire, retraction promptly occurs. Delay of clot retraction (syneresis) occurs in different diseases (Table 11) and almost invariably in diseases due to thrombocytopenia (Table 10).

Capillary Fragility Test. The capillary fragility or tourniquet test for the Rumpel-Leede phenomenon is conducted by applying a *blood pressure cuff* about the upper arm in the usual manner and keeping it inflated for five minutes slightly above the diastolic pressure. The arm is first inspected for purpuric spots or pigmentation which may be mistaken for such spots. Normally a few tiny spots may appear but if the test is positive, a crop of purpuric spots develop in the skin below the cuff within a few minutes. These spots are roughly proportional to the hemorrhagic tendency but not necessarily to the number of platelets.

The *suction cup* also offers a simple and quick method for measuring capillary resistance. A specific negative pressure is maintained for one minute and for a reading to be positive at least two petechiae must be clearly discernible. The lowest negative pressure giving a positive reaction is the capillary resistance. Normally this is usually — 20 to — 35 cm. of mercury.[46] Below — 20 indicates increased fragility which usually occurs in purpura haemorrhagica with variable results in several other diseases (Table 11). Its importance in relation to thrombocytopenic purpura is indicated by the observation that, following splenectomy, resistance rises abruptly during the first twenty-four hours, as bleeding ceases, although the platelet count often increases more slowly.[36] It is also lowered in scurvy, scarlet fever, measles, influenza, gastric achylia, some cases of chronic nephritis and in vitamin K deficiency. Likewise capillary resistance is lowered during the few days prior to and on the first day of menstruation, as well as one or two days after menstruation in some cases. This increased capillary permeability is due to rhythmic changes in the capillaries associated with the cyclic menstrual rhythm.[47] In functional uterine bleeding similar changes occur in the capillaries which, however, are completely dissociated from the menstrual rhythm. These facts indicate that menstruation, which is evidenced as a local vascular phenomenon, is part of a general vascular change throughout the body. Capillary fragility may also result in excessive bleeding following the extraction of teeth and even following scaling of the teeth and needle punctures in the induction of anesthesia. The administration of vitamin P (citrin) is stated to maintain normal capillary permeability [48] although this has not been definitely proved; its administration, however, along with vitamin C and rutin, is commonly employed in treatment.

A *venom skin test* is also employed on the basis that hemorrhage will result from its action on the capillaries if they are fragile.[49] This consists in the intradermal injection of 0.1 cc. of a sterile 1:3000 standardized solution of moccasin snake venom. A control injection of the same amount of normal sodium chloride solution is made in the other arm. The reaction is read at the end of one hour when, if positive, a hemorrhage 1 cm. or more in diameter develops.

CHANGES IN BONE MARROW

Examinations of the bone marrow during life are not infrequently of considerable clinical value in the diagnosis of diseases of the blood and of the blood-forming organs. This is especially true when the blood findings are atypical or inconclusive as in differentiating "aleukemic" leukemia from agranulocytosis, purpura haemorrhagica and aplastic anemia.

It should be stated, however, that bone marrow examinations require unusual skill and experience. This is because of the difficulty so frequently experienced in the recognition of the many different kinds of cells encountered belonging to the granulocytic, lymphocytic and erythrocytic series, since the marrow consists of a variety of blood cells and their precursors, as well as fat cells, blood vessels and a framework of reticulum. During the first few years of infancy and childhood practically all of the marrow is of the red variety and highly cellular. Fat cells begin to appear between the ages of five and seven years. At about maturity the actively hematopoietic marrow is found only in the sternum, ribs, vertebrae, bones of the skull, the innominate bone and to some extent in the proximal epiphyses of the femur and humerus.[50] The total bone marrow in adults has been calculated to vary from 1600 to 3700 gm. Of this total amount, however, only about half is in an active state.

Because of rapid autolysis, marrow examinations conducted postmortem are frequently unsatisfactory unless removed within two hours after death. Specimens should be taken from several bones. If only one is to be taken, Custer [50] recommends the middle of the femur because the marrow is more labile than that of the tibia or even that of the ribs.

Technic of Sternal Biopsy. From the standpoint of clinical diagnosis, examinations of the marrow are of great interest and value, especially since 1923 when the method of *sternal trephining* was introduced by Seyfath. This method is generally favored because it yields material which may be used for preparing smears, touch preparations, wet preparations for supravital staining and blocks for the preparation of sections. As compared with *sternal puncture,* however, it has the disadvantage of not being as readily repeated at short intervals.

The technic of the latter, introduced by Arinkin [51] in 1927, is much simpler. The upper portion of the bone, between the second and third ribs, is the site of choice because it is less likely to bend or give at this point. Furthermore, marrow is present in this area even in infants of one to two years of age. The manubrium is more likely to contain fat and the lower third of the sternum is unsatisfactory because congenital abnormalities are common in this region.

The skin is prepared for sternal trephining and the whole operation is conducted with scrupulous aseptic precautions. A cushion may be placed beneath the shoulders. The skin, deeper tissues and periosteum are infiltrated with a sterile 1 per cent solution of procaine. A short-beveled, 18-gauge needle with a lumen of 1 to 2 mm. is employed. It should be short to avoid bending and preferably provided with an adjustable guard, the Sharp needle (A. S. Aloe Company, St. Louis, Mo.) being recommended. The guard is set at 1 cm. if the patient is an adult, or at 0.6 to 0.2 cm. if a child; the outer lamina of the sternum varies considerably in thickness, ranging from 0.2 to about 5 mm.

The needle is pushed vertically with a slight rotating or boring motion into the sternum in the midline or slightly to the side. A "give" is felt as the marrow cavity is entered. The needle is then passed about 1 or 2 mm. farther in as the cavity is normally 5 to 15 mm. in depth.

The stylet is then removed and a sterile, 2 or 5 cc., tight-fitting syringe is attached for the aspiration of 1 or 2 cc. of liquid or semiliquid marrow without too much suction to avoid pain. If no marrow is obtained, the stylet may be replaced and the marrow penetrated more deeply. If this fails, 0.5 cc. of sterile saline solution or the patient's own plasma (prepared beforehand and in readiness) may be injected to aid the dislodgment of cells and then removed. Upon removal of the needle the puncture wound is sealed with collodion and cotton.

The material is then sent to the laboratory for examination of smears, dry imprints, supravital preparations, wet preparations and possibly sections. Smears stained by the Wright or May-Grünwald-Giemsa stains are of particular value in a study of the cells. Smears may also be stained by the peroxidase method.

When properly conducted, the method is safe, causes but little discomfort and may be classed with such routine examinations as spinal puncture and pleural tap. Hemophilia is not usually a contraindication.[52] The most important criticism is that only a small and not necessarily representative sample of marrow is obtained with the chances of missing lesions which are patching in character, as in Gaucher's disease, myeloma of bone, etc.

Normal Bone Marrow. Erythrocyte counts and hemoglobin estimations may be made. The results are about the same as those of blood or slightly lower. The leukocytes range from 10,000 to 190,000 per c.mm. Differential counts are of most value. At least 500 to 1000 cells should be examined. Even under these circumstances, considerable variations may be found not only between several preparations of the same material, but especially between specimens removed at different times. The relative percentages of nucleated cells for adults are approximately as follows:[6]

Cells	Range	Average
Myeloblasts	0.3 — 5.0	2.0
Promyelocytes	1.0 — 8.0	5.0
Myelocytes: Neutrophilic	5.0 —19.0	12.0
Eosinophilic	0.5 — 3.0	1.5
Basophilic	0.0 — 0.5	0.3
Metamyelocytes	13.0 —32.0	22.0
Polymorphonuclear neutrophils	7.0 —32.0	20.0
Polymorphonuclear eosinophils	0.5 — 4.0	2.0
Polymorphonuclear basophils	0.0 — 0.7	0.2

Cells	Range	Average
Lymphocytes	3.0 —17.0	10.0
Plasma cells	0.0 — 2.0	0.4
Monocytes	0.5 — 5.0	2.0
Reticulum cells	0.2 — 2.0	0.2
Megakaryocytes	0.03— 3.0	0.4
Macroblasts	1.0 — 8.0	4.0
Normoblasts	7.0 —32.0	18.0

In children the leukocyte: nucleated erythrocyte cell ratio is about 8:1, or even 2:1, while in adults it is normally 3 or 4:1. In young children there are relatively more immature neutrophilic cells and more lymphocytes than in adults.[53]

Bone Marrow in Disease. In many diseases, gross as well as microscopic changes may occur in the bone marrow. For example, the marrow shows an unusual red color in pernicious and other severe anemias and there is a displacement of fat by gelatinous albuminoid material in starvation states and wasting diseases.

TABLE 12. SUMMARY OF THE CLINICAL INTERPRETATION OF BONE MARROW EXAMINATIONS

Disease	Important Changes
Pernicious and related anemias	Untreated or during relapse: (1) Increase of nucleated erythrocytes; (2) preponderance of megaloblasts; (3) increase of reticulum (Ferrata) cells; (4) abnormal leukopoiesis especially lymphocytes; (5) reduction in megakaryocytes.
Aplastic anemia	Chiefly red blood corpuscles. Relative lymphocytosis constituting 60 to 100 per cent of the nucleated cells. Striking immaturity of the red and white corpuscles, but may be normally cellular or hyperplastic.
Acute hemolytic anemias	Markedly hyperplastic; 60 per cent or more of the nucleated cells belong to the erythrocytic series; leukocytes relatively reduced.
Chronic hemolytic anemias	Normoblastic hyperplasia characteristic (normoblasts or macroblasts); no megaloblasts.
Sickle cell anemia	Largely nucleated red cells (chiefly normoblasts). May be moderate "shift to the left" of the myeloid leukocytes; eosinophils relatively increased; megakaryocytes may be increased.
Hypochromic microcytic anemia	Hyperplastic; increase of normoblasts; no megaloblasts; granulopoiesis usually normal.
Congenital hemolytic jaundice	Erythropoietic hyperplasia of the normoblastic type; no megaloblasts or giant, abnormal leukocytes.
Purpura haemorrhagica	Many megakaryocytes; usually an increase of erythyroid elements due to severe hemorrhage and anemia.

TABLE 12. SUMMARY OF THE CLINICAL INTERPRETATION OF BONE MARROW EXAMINATIONS (continued)

Disease	Important Changes
Polycythemia vera	Dark and red and very cellular; hyperplasia of all elements; moderate increase of nucleated erythrocytes; sometimes an increase of megakaryocytes, myelocytes and myeloblasts.
Myeloblastic and myelocytic leukemia	Marked hyperplasia; crowded with myeloblasts and more primitive cells. In eosinophilic leukemia preponderance of eosinophils. In monocytic leukemia myelocytes and myeloblasts; also "monoblasts" and "monocytes" in some cases. In chronic myelocytic leukemia the differential count is similar to that of the blood.
Lymphocytic leukemia	May be only slightly changed; usually, however, a well-marked lymphocytosis (30 to 90 per cent of the cells).
"Aleukemic" leukemia	Of great value in diagnosis. Frequently the changes are identical with those with typical blood findings. Sometimes misleading when only a few cells are obtained. But pronounced immaturity of the leukocytes may be observed when cellularity is reduced. In leukopenic cases of lymphocytic leukemia, marrow lymphocytosis may be slight or absent.
Infectious mononucleosis	Increase of lymphocytes or moderate shift to left of myeloid leukocytes. Chiefly of value from a negative standpoint in the sense that findings characteristic of leukemia are absent.
Agranulocytosis	Normal erythropoietic tissue and normal numbers of megakaryocytes. Striking lack of granulocytes. Plasma cells, lymphocytes and reticulum cells may be increased.
Hodgkin's disease	Findings variable and nonspecific. May be slight shift to left in the myeloid cells; also slight monocytosis or moderate eosinophilia. No lymphocytosis. Relative reduction in nucleated red cells.
Multiple myeloma	Various types described as myeloblastic, lymphoblastic, erythroblastic, etc., with "plasma cells" the usual designation. *Myeloma cells* most characteristic, constituting 3 to 65 per cent of all cells present.

Of special clinical importance is the information sometimes obtainable from a skilful microscopic examination in differential diagnosis. This is particularly true in those diseases of the hemopoietic system in which no definite changes in the blood are found. Sternal biopsies have frequently demonstrated the presence of "aleukemic" leukemia with the ruling out of aplastic anemia or Banti's disease.

Furthermore, they are frequently helpful in distinguishing leukemoid conditions from true leukemia, pseudo pernicious anemia from true pernicious anemia, granulocytopenia due to marrow aplasia from that due to drug allergy. Biopsies also frequently reveal primary or secondary bone tumors.[54, 55] The important changes found in many of these diseases are summarized in Table 12.

The clinical interpretation of *bacteriologic examinations* of the blood is discussed in Chapter 15 and of *parasitologic examinations* in Chapter 12.

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2

THE CLINICAL INTERPRETATION OF URINE EXAMINATIONS

Of all laboratory examinations, that of the urine is undoubtedly the most frequently employed, being commonly a routine procedure. This is right and proper because of the information of clinical value to be gained not only in relation to diseases of the kidneys and their functional capacity for eliminating the waste products of normal metabolism and digestion brought to them by the blood for elimination, but likewise in relation to diseases of the lower urinary tract, as well as to some involving the cardiovascular, hemopoietic, metabolic, digestive, endocrine and nervous systems.

It is too bad, however, that the results of so many routine urine examinations are of limited clinical value because of technical errors and indifference on the part of technicians. Not infrequently physicians are partly to blame because of the scant attention given reports which, in hospital practice at least, may amount to no attention at all. Another important source of error may occur in the collection of the specimen submitted for analysis, since the urine may vary greatly at different hours of the same day. It is evident, therefore, that *no quantitative test can be of real clinical value unless a sample of the mixed twenty-four hour urine is used and percentages mean little or nothing except as they furnish a means of calculating the twenty-four hour elimination.* Otherwise, separate samples of urine voided at night and in the morning are to be preferred, as a specimen collected at random during the day is of limited value.

FORMATION OF THE URINE

The mechanism of the formation of urine is not yet definitely known but is believed to be due to the simultaneous operation of the following two processes: (1) the filtration of blood plasma by the glomeruli or renal corpuscles followed (2) by selective tubular reabsorption of the glomerular filtrate resulting in the complete absorption of glucose and some water with the concentration of other substances, under normal conditions. Some investigators [1] have thought that a third process may be involved, namely, the secretion by the cells of the proximal convoluted tubules of soluble substances brought to them in the blood plasma by the peritubular capillaries. But while under certain conditions dyes and urea may be excreted by the tubular epithelium, such a process appears to be of relatively little importance and probably only a functional relic from primitive forms without relation to the activity of the kidneys of man. In other words, when various plasma proteins pass into the urine they do so in amounts which are inversely related to their molecular weights, indicating that secretion plays no part, but that they escape through "pores" in the glomerular membrane. Hemoglobin, for example, passes freely, serum albumin more than serum globulin, while fibrinogen rarely escapes at all.

Glomerular filtration through the capillary endothelium and investing squamous epithelium is purely a physical process resulting from an excess of systolic blood pressure within capillary loops over that due to colloid osmotic pressure (25 to 30 mm. Hg) and the pressure within the capsule of Bowman (5 to 10 mm. Hg). Tubular reabsorption, however, of water and glucose, with the concentration of threshold and other substances for the corrective conservation of solids, water and glucose wastefully passed by the glomeruli, is a "vital" process during which the reabsorbed fluid is not always of constant composition but essentially resembling that of Locke's solution plus small amounts of urea, uric acid and phosphates. This is best illustrated by Table 13 after Cushing.[2]

TABLE 13. SUMMARY OF THE CONSTITUTION OF THE BLOOD PLASMA AND URINE

	Blood Plasma per cent	Urine per cent
Water *	90–93	95
Proteins, fats, and other colloids	7–9	0
Glucose *	0.1	0
Sodium *	0.30	0.35
Chlorine *	0.37	0.6
Urea	0.03	2.0
Uric Acid	0.004	0.05
Potassium *	0.020	0.15
NH_4	0.001?	0.04
Calcium	0.008	0.015
Magnesium	0.0025	0.006
PO_4 *	0.009	0.15
SO_4	0.002	0.18
Creatinine	0.001?	0.075

* Threshold substance.

In other words, the formation of urine under normal conditions is one of physical filtration of the blood plasma of the glomeruli, followed by selective or "vital" reabsorption by the tubules with the remainder constituting urine.

Normal Urine. Under the conditions it is not possible accurately to define a normal urine, even if the type and amount of diet and the final fluid intake are known, because variations may occur according to external and internal temperatures, physical activity, physiologic variations in metabolism and the composition of the plasma reaching the kidneys. The *average* physical, chemical and microscopic composition of the urine of normal adults is summarized, along with possible abnormal changes of clinical diagnostic value, in Tables 14, 16 and 18.

CHANGES IN AMOUNT

The excess of water absorbed from the gastro-intestinal tract over that excreted by the skin, lungs and bowels, tends to dilute the plasma but this is prevented by glomerular filtration; tubular reabsorption helps to maintain a normal water balance. Thus it has been calculated that the quantity of glomerular filtrate produced by normal adult kidneys may be as high as 75 to 100 liters in twenty-four hours; since 97 to 98 per cent is reabsorbed by the tubules, the average output of urine is from 1000 to 1600 cc., or about 40 to 50 ounces per day. Usually from 40 to 60 per cent of the total fluid intake is excreted by the kidneys but this must necessarily vary inversely with the quantity eliminated by the skin, lungs and bowels. After abstention from fluids for 24 hours the average normal excretion per 12 hours (6 P.M. to 6 A.M.) is about 381 cc. with $\pm$ 113 cc.[3] The output is greater on a high than on a low protein diet because the end products of protein metabolism and especially urea exert a diuretic effect. High temperature and humidity, as well as strenuous exercise accompanied by profuse sweating, reduce the output. The volume is less in the standing position than in recumbency chiefly due to the greater concentration of plasma protein in the former position. Young children excrete for their weight from 3 to 4 times more urine than adults. The urine of the day is normally from 2 to 4 times greater in amount than that secreted during the night even though the total fluid intake is the same for both periods. An increase of night urine above 500 cc. with a specific gravity of less than 1.018 is usually defined as *nocturia* or *nycturia* but the name is frequently and erroneously used for increased frequency of urination during the night without an actual abnormal increase of the total urinary output above the normal. True nocturia, however, may occur in renal disease. The terms *polyuria* and *oliguria* are applied respectively to the excretion of supernormal and subnormal quantities of urine; total cessation of urinary excretion is termed *anuria* but the term is likewise sometimes used for failure to pass urine because of complete ureteral or urethral obstruction (Table 14). Obviously this is an error when urine is secured by catheterization.

Polyuria. Polyuria or the excessive production of urine must be distinguished from frequency of micturition. In case of doubt, the total output in twenty-four hours should be carefully collected in a clean vessel, kept in a cool place, and measured. To avoid error specific directions should be given to empty the bladder

TABLE 14. SUMMARY OF THE CLINICAL INTERPRETATION OF PHYSICAL CHANGES IN THE URINE

	Normal	Abnormal Changes
Formation	(1) Glomerular filtration followed by (2) selective tubular reabsorption. Tubular excretion and secretion doubtful.	
Amount	Adults: 1000 to 1600 cc. per 24 hours; children 3 to 4 times as much as adults per kilogram of body weight. During the day two to four times as much voided as during the night.	*Nocturia:* 500 cc. more voided at night than during the day with a specific gravity below 1.018. *Polyuria:* supernormal excretion; may be physiologic or pathologic. *Oliguria:* subnormal excretion; may be physiologic or pathologic. *Anuria:* total cessation of excretion; always pathologic but may be temporary.
Turbidity	Normally clear when freshly voided but may be cloudy due to phosphates. All urine becomes cloudy or turbid upon standing. Alimentary lipuria may occur (opalescence); also from accidental contamination with oils.	Cloudy or turbid when freshly voided: usually due to pus, bacteria or blood from the urogenital tract or menstrual blood. *Lipuria* may occur in lipemia due to various causes. *Chyluria* (milky) from lymphatic obstruction.
Color	Yellow-amber due to normal pigments (see Table 15).	Lighter in polyuric and darker in oliguric states; various changes due to excess of normal pigments or the presence of abnormal pigments from drugs as well as those from chemical agents employed in renal function tests.
Odor	Normally aromatic due to volatile oils; especially marked in concentrated urine. Ammoniacal and "urinous" upon decomposition. Peculiar odors may be due to various articles of diet and drugs.	Ammoniacal upon decomposition in cystitis and retention. Sometimes "fruity" in diabetes and other states. May have putrid odor in suppurative states of the urinary tract. Fecal odor in bladder-intestinal fistula.
Reaction	Acid due to acid phosphates and traces of organic acids. Occasionally alkaline due to alkaline salts. All urine without preservatives becomes alkaline upon standing due to micro-organisms.	Increased acidity in diabetic acidosis; also in contracted kidneys of chronic nephritis. May be alkaline in cystitis and pyelonephritis. Can be rendered more acid or more alkaline by diets and drugs.

TABLE 14. SUMMARY OF THE CLINICAL INTFRPRETATION OF PHYSICAL CHANGES IN THE URINE (continued)

	Normal	Abnormal Changes
Specific Gravity	Varies according to solids in solution from 1.010 to 1.030 averaging 1.015 to 1.025. Of little value with random specimens; best determined with a sample of total 24-hour output.	May vary from 1.001 to 1.060 or higher. Relationship to volume may be lost. *Reduced* in chronic nephritis with tendency to fixation at 1.010; also in diabetes insipidus. *Increased* in diabetes mellitus, fevers and acute nephritis. Of clinical value in renal function tests.
Total Solids	Related to specific gravity under normal conditions. Accurate chemical methods preferred for estimation. Vary from 60 to 70 gm. in 24 hours. Decreases after 45 years of age.	Vary greatly according to kinds of solids in solution. Determinations are of value in relation to the functional condition of the kidneys.

at 8 A.M. and to discard the urine, followed by the collection of all passed to 8 A.M. the following day, at which time the bladder should be emptied and the urine added to the collection.

In general terms the causes of polyuria may be referable (1) to an increased rate of glomerular filtration due to increased pressure of the blood in the capillary loops with increased renal blood flow; (2) to a reduction of osmotic pressure of the plasma in the glomerular blood due to loss of protein or other colloids; (3) to a reduction in the tubular reabsorption of water, urea or other diuretic substances due to injury of the epithelium since the process is not subject to direct influence of the nervous system except insofar as this affects circulation; (4) to changes in the activity of certain of the endocrine glands with special reference to the pituitary and suprarenals or (5) to a combination of two or more of these factors. The pituitary gland hormone probably exerts its antidiuretic effects by increasing tubular reabsorption.

Polyuria, however, is not always a sign of disease since it may occur under normal or physiologic conditions. Thus excessive fluid intake and chilling of the body, reducing perspiration, may produce it temporarily, as may also mental excitement and apprehension (witness the frequency of micturition from increased urine excretion of medical students before examinations), as well as the effects of caffeine in coffee, tea and coca-cola due to renal vasodilatation. Alcohol is also a powerful diuretic resembling water as well as diuretics like salyrgan and the xanthines which operate mainly by depressing tubular reabsorption. Polyuria may also follow the administration of large amounts of isotonic saline or glucose solutions largely due to dilution of plasma proteins or it may follow the administration of adrenalin because of a rise in glomerular blood pressure. It may also occur temporarily or intermittently in neurosis ("hysteria"), essential phosphaturia, during the absorption of large exudates or transudates, after an attack of asthma, angina pectoris, migraine or epilepsy as well as during malarial chills, convalescence from typhoid fever, during pregnancy and an intermittent hydronephrosis from ureteral obstruction or floating kidney following the oliguria of a Dietl's crisis.

Polyuria, however, may be more or less permanent—a fact of much value in diagnosis—because of abnormal thirst and high fluid intake in diabetes mellitus and especially diabetes insipidus, resulting in the passage of three to ten liters of urine per day due to tubular rejection. An increased output is also encountered in contracted kidneys or any disease of these organs which destroys a large number of renal nephrons such as congenital polycystic kidneys, renal tuberculosis, amyloid nephrosis and nephrosclerosis (in spite of decreased glomerular filtration), because of decreased tubular reabsorption and possibly diminished contact of fluid with epithelium in dilated tubules. Polyuria may also occur in acromegaly and myxedema, and less commonly in anemia, hypopituitarism, tabes dorsalis, paralysis agitans, Christian's disease and tumors of the brain or spinal cord. Nocturia in such states is presumably due to the effort of inefficient kidneys to compensate for deficient excretion of urea and other low threshold substances during the day, made possible by improved circulation in the kidneys during the more restful hours of night.

Oliguria and Anuria. True oliguria, or the diminished excretion of urine, may be due to deficient fluid intake or to excessive fluid loss through the skin, lungs or gastro-intestinal tract (vomiting or diarrhea). Otherwise, it is generally to be ascribed to (1) a decreased rate of glomerular filtration due to low blood pressure following hemorrhage, shock, nervous states (including melancholia) or other causes; (2) impairment of tubular reabsorption which, paradoxical as it may seem, finally leads to a reduction in urine volume through its unfavorable effect upon filtration because of a rise in pressure within Bowman's capsules or (3) a combination of these factors. A deficiency in the output of urine may also be due to pressure on one or both of the ureters by abdominal tumors, the blockage of both by calculi (including the uroliths composed of crystals of the sulfonamide compounds deposited in the tubules) or by pus, which likewise reduces glomerular filtration by raising pressure within the capsules of Bowman. One side may be blocked with reflex inhibition of glomerular filtration on the other.

True oliguria may be prolonged in degenerative types of nephritis (especially in acute glomerular nephritis), in chronic glomerular nephritis and lipoid nephrosis with edema and in acute nephritis due to poisoning by turpentine, phenol, the mercurial compounds, phosphorus, etc. Microscopic examination of the kidneys and the results of renal function tests in degenerative types of nephritis may, however, give but little evidence of glomerular damage, the chief changes being found in the tubules. Regeneration of the exfoliated tubular cells, however, may occur with a constant preponderance of young irritated cells along with direct evidence of fluid resorption by them, resulting in scanty urine possibly because of their state of irritation. In acute nephritis there is usually diminished glomerular filtration due to glomerular obstruction. The oliguria of congestive heart failure is apparently largely due to diminished filtration because of impaired renal circulation and low blood pressure.

Other causes for oliguria may be dilatation of the stomach from pyloric stenosis, attacks of lead colic, acute intestinal obstruction, acute general peritonitis, portal cirrhosis and acute yellow atrophy of the liver, thrombosis of the inferior vena cava or renal veins, and sunstroke.

True *anuria* or the complete suppression of the excretion of urine may result from any of the causes of true oliguria, especially in acute nephritis with uremia. Symptomatic anuria, of course, may follow complete obstruction of the ureters or urethra with resulting hydronephrosis or the surgical removal of a congenital single kidney, which fortunately is a rare accident these days. Temporary anuria may also occur during hypertensive states as the result of vasospasm due to exposure to cold.

PHYSICAL CHANGES

Turbidity. Normally, freshly voided urine is usually clear but it may be cloudy due to phosphates, especially in individuals of nervous temperament or after the ingestion of large amounts of fresh fruits (Table 14). All specimens of urine will normally develop cloudiness upon cooling ("nubecula") due to mucus, leukocytes and epithelial cells particularly in the case of women; likewise upon standing, because of bacterial contamination and the precipitation of amorphous urates (if acid) or of phosphates (if alkaline).

Abnormally, a fresh cloudy or turbid urine may indicate the presence of pus and bacteria from somewhere along the urogenital tract, including the prostate gland (especially after massage), the vagina and the urethra. The presence of a small amount of blood may also render it cloudy without being sufficient to change the color. A great excess of epithelial cells and casts may contribute to turbidity. It is stated that spontaneous precipitation of proteins may occur as a cause for cloudiness of the urine but, if so, its occurrence must be very rare.

Opalescence may be due also to the presence of highly refractile droplets of fat constituting *lipuria*. This may occur in normal individuals following the ingestion of a high fat diet (alimentary lipuria) or the administration of excessive amounts of cod liver or other oils. Lipuria may also occur in association with lipemia in severe diabetes mellitus, lipoid nephrosis, following fractures of long bones with injury to the marrow, after crushing injuries of the subcutaneous fat and possibly after poisoning with alcohol and phosphorus. *Chyluria* due to filariasis or some other obstruction to the flow of lymph in the thoracic duct and sometimes to the rupture of the lymphatics in the kidneys or bladder, produces turbidity or a milky appearance. Of course it must be remembered that fat appearing in the urine following catheterization may be due to the use of petrolatum or other lubricant employed. Hysterical individuals and malingerers have been known to add milk to their urine and this has occurred accidentally from the use of unwashed milk bottles for collection of urine containing traces of cream.

Color. Under normal conditions the color of urine ranges from yellow to amber due to the presence of *urochrome* (the chief normal pigment of unknown source but highly constant from day to day and independent of diet), supplemented by traces of the following additional pigments: *urobilin,* formed by the oxidation of urobilinogen; *uro-erythrin* which is chiefly responsible for the deep reddish color of the urine in acute febrile states and the *coproporphyrins* of which only types I and III occur in plant and animal foods. Normally, the 24-hour urine contains less than 100 gammas of total coproporphyrins (60–90 per cent Type I and 10–40 per cent Type III).

In various diseases and intoxications and following the administration of various drugs and the use of chemical agents in the conduct of renal and hepatic function tests, the color of the urine may undergo many changes of clinical significance owing to the presence of pigments or the compounds administered (Table 15). Among the more common of the pigments occurring in abnormal states are *bilirubin* which appears in excessive amounts in the blood from intrahepatic or extrahepatic obstructive lesions of the liver or the excessive hemolysis of erythrocytes; *biliverdin,* due to the oxidation of bilirubin usually after the urine has been voided and allowed to stand; *hemoglobin,* resulting from paroxysmal hemoglobinuria, severe primary anemias, chronic malaria (blackwater fever), severe burns, hemoglobinemia from incompatible blood transfusions, etc.; the *alkapton bodies* or aromatic oxyacids and especially homogentisic acid, resulting from the abnormal metabolism of tyrosine and phenylalanine producing alkaptonuria (a rare disorder and frequently hereditary); *melanin,* a sulfur-containing pigment probably derived from aromatic protein derivatives and producing melanuria in melanotic sarcoma and possibly (rarely) from excessive protein destruction, and the *porphyrins* in congenital or chronic and acute porphyrias (of toxic or idiopathic origin). As previously stated, porphyrins occur normally in the urine and especially Type I uroporphyrin with smaller amounts of Type III coproporphyrin. In congenital or chronic porphyria, however, excessive amounts of Type I uro- and coproporphyrins or Type III coproporphyrin are present while in acute porphyria discoloration is usually due to Type III uro- and coproporphyrins, particularly the former which may be excreted as a zinc metal complex, as discussed more fully in Chapter 19. The porphyrins are identified by spectroscopic examinations. The total porphyrins are uniformly increased in hepatic disease (obstructive or parenchymatous) and especially in parenchymatous hepatitis, as discussed more fully in Chapter 26. Porphyrinuria is also stated to occur in pellagra but not constantly enough to be of diagnostic value or a guide in its treatment.[3] Indeed it appears that reports of its occurrence in this disease may have been based upon faulty chemical methods, since the only reliable test for porphyrin is by spectroscopic examination of the urine, preferably compared at the same time with a known solution of porphyrin.

Increases of urinary urobilinogen are consistently associated with the increase of bile pigment in the urine, indicating that pathologic urobilinogenuria does not occur from excessive hemolysis of erythrocytes unless there is concomitant liver damage. Determinations of urobilinogen in the urine are, therefore, of clinical value in the differential diagnosis between jaundice of intrahepatic and extrahepatic origin and for following the progress of hepatic damage.[4] Since small amounts of bilirubin in the urine are readily missed, it is advisable to use the spot method of Godfried or some method of concentration.

TABLE 15. SUMMARY OF THE CLINICAL INTERPRETATION OF THE COLOR OF URINE

Color	Due to	Causes
Amber	Urochrome Urobilin Uro-erythrin Porphyrins	Normal
Dark amber	Same highly concentrated; especially urochrome	Conditions producing oliguria Hyperthyroidism Wasting states Starvation
Pale yellow or greenish-yellow	Same highly diluted	Conditions producing polyuria especially diabetes mellitus Hypochromic anemias
Golden-yellow	Santonin Chrysarobin Senna	Administration
Yellowish-green	Bilirubin Biliverdin *	Obstructive and hemolytic jaundice Acute yellow atrophy of liver
Yellowish-green	Acriflavine	Administration
Orange-red	Pyridium	Administration
Red to reddish-brown or black	Hemoglobin	Paroxysmal hemoglobinuria Severe primary anemias Blackwater fever (malaria) Incompatible transfusion of blood Severe burns Severe prolonged exposure to cold Hemolytic jaundice (some cases) Severe intra-abdominal hemorrhage
Pink after alkalinization	Phenolsulfonephthalein	Renal function test
Red to brownish-red; smoky	Erythrocytes	Bleeding in urogenital tract or contamination with menstrual blood
Rose red	Pyramidon	Administration
Dark red	Neoprontosil Rose bengal	Administration Liver function test
Port wine	Porphyrins	Congenital or chronic porphyrinuria

* Due to oxidation of bilirubin after voiding.

TABLE 15. SUMMARY OF THE CLINICAL INTERPRETATION OF THE COLOR OF URINE (continued)

Color	Due to	Causes
		Acute porphyrinuria (toxic and idiopathic) Hemochromatosis Regurgitant jaundice Toxic hepatitis Infectious hepatitis Portal cirrhosis of the liver Pellagra (?)
Dark brown	Urobilinogen and urobilin	Hepatic insufficiency Pernicious anemia Malaria Acute infections
Brown turning black *	Homogentisic acid and other alkapton bodies	Alkaptonuria Imperfect protein metabolism (?)
Brown turning black *	Melanin	Melanotic sarcoma Protein destruction (?)
Dark brown to black; smoky	Hydroquinone Pyrocatechin	Poisoning: phenol, guaiacol, creosote, salol, resorcin
Greenish-blue	Methylene blue Thymol	Administration; renal function tests

* On standing and alkalinization.

Odor. The characteristic aromatic odor of fresh urine is generally attributed to volatile acids although a substance called "urinod" has also been held responsible. It is more marked in urines of high concentration. If the urine undergoes decomposition, either within the bladder or on standing, an ammoniacal or so-called "urinous odor" develops which is due to the decomposition of proteins. Should such an odor appear in freshly voided urine, it is evidence of marked cystitis or retention.

Various articles of diet and drugs may impart peculiar odors. Notable among these are asparagus, which gives a characteristic offensive odor; oil of turpentine which produces a distinct odor of violets; menthol which causes an odor of peppermint while cubebs, copaiba, sandal-wood oil, tolu, and saffron produce peculiar spicy odors.

A fruity odor is sometimes noted in diabetes mellitus, due probably to acetone, as well as sometimes in febrile states and in some stomach and intestinal disorders.

Freshly voided urine may have a putrid odor attributable to the decomposition of pus forming hydrogen sulfide along with ammonia, especially if the urine contains cystine. This change is particularly likely to occur in extensive infections of the urinary tract like pyelitis and pyelonephritis as well as in cystitis with retention and in carcinoma of the bladder. In *cystinuria*, a metabolic disorder of but little clinical significance and occurring mostly in children, the urine upon standing gives off the odor of hydrogen sulfide since the amount of sulfur present in the neutral form is greatly increased. A distinctly fecal odor may be present in cases of perforation of the intestines into the bladder.

Reaction. Normally, the mixed twenty-four hour urine is slightly acid in reaction largely due to the monobasic (chiefly sodium) salts of phosphoric acid plus small amounts of free organic acids (uric, lactic and hippuric). As a result the usual total titratable acidity of twenty-four hour urine is between 200 and 400 cc. of N/10 standard acid but in health may vary from 100 to as much as 600 cc. In terms of *p*H the average normal is about 6.0 but the range in health may vary from as low as 4.6 to as high as 8.0.[5] No single test can be accepted as giving the range for any individual. Indeed normality is characterized by variability. Intelligent therapy for altering the reaction requires a knowledge of the acid-base curve of the patient. Water excretion, emotional status, exercise, fatigue, meals and rate of pulmonary ventilation are all factors affecting urinary reaction. As shown by Leathes,[6] the urine passed a short time after rising in the morning may be less acid than that found during sleep (*morning alkaline tide*) which he ascribed to depression of the respiratory center during sleep with the retention of carbon dioxide. An alkaline tide also occurs within an hour after a meal (*postprandial alkaline tide*) and sometimes freshly voided urine is both cloudy and highly alkaline due to phosphates (*phosphaturia*).

The acidity of the urine is normally influenced by diet, fluid-intake and also by various drugs. Cereals, meat and fish tend to increase it while most fruits (except plums, prunes and cranberries) tend to reduce it because of the rejection of basic radicals (alkaline ash) by the tubules. A high protein diet, however, increases acidity because of a production of an excess of sulfuric and phosphoric acids which are eliminated by tubular rejection. Acidity is also increased by a diet sufficiently high in fats to produce ketosis; also by an acid-ash diet which is more effective in increasing acidity in the long run. Fasting and starvation, in which the body proteins are metabolized, also tend to increase the titratable acidity.

The administration of large amounts of nitro-hydrochloric, phosphoric and mandelic acids, as well as of ammonium chloride, ammonium nitrate, ammonium mandelate and calcium chloride, tends to reduce the alkali reserve of the plasma and to increase the acidity of the urine because the ammonium radical becomes detached and converted to neutral urea by the liver with the liberation of the chlorine radical while in turn is promptly rejected by the tubules. The maximum acidity attainable, however, is about *p*H 5. Such acidity would be exceeded and the urine rendered extremely irritating to the urinary tract but for a local protective mechanism of the tubular epithelium which has a capacity for the production of ammonia equivalent to rejection of acid. There is no evidence, however, that the prolonged administration of an acid salt is injurious to the kidneys.

In severe diabetes the titratable acidity may be two or three times higher than average normal, because of the excretion of aceto-acetic and beta-hydroxybutyric acids (acetone bodies), unless ammonia excretion is high. But ketones may be present in abnormal amounts in urine of any reaction. There is usually fixation of urinary *p*H at 5.0 to 6.0 in cardiorenal disease with 4.7 to 5.3 in cardiac decompensation.[7] In nephritis, with contracted kidneys, the excess acid is not completely neutralized by ammonia as by normal kidneys; as a result, the urine tends to be a little more acid than normal. In terminal nephritis neutrality usually occurs.

Many physicians make a routine practice of prescribing alkalies and an alkaline-ash diet in the treatment of nephritis. And it is a notorious and regrettable fact that the public has been "sold" on the idea that all human ills result from a "too acid" condition for which alkalies are a panacea. As a matter of fact, alkalinization should be attempted only with definite justification as when extreme acidity is accompanied by frequency of micturition and a burning sensation. There is no merit in striving for continuously alkaline urine; indeed, it may be harmful to the kidneys by producing irritation of the tubular epithelium. The administration of alkalies, however, is sometimes required as in the treatment of urinary tract infections with the sulfonamide compounds, in the treatment of post-transfusion hemoglobinuria, peptic ulcer and other hyperchlorhydrias, for increase of weight, etc. Under these conditions the administration of large amounts of alkalies tends to augment the alkali reserve of the plasma with a reduction in the excretion of total phosphates resulting in a lowering of total acidity. But the reaction of the urine is not always a safe guide in the avoidance of alkalosis in sodium bicarbonate therapy. In other words, any attempt to change the urinary reaction should be undertaken only by the competent physician and achieved by means which do not ignore the body's other needs, as to do so may defeat the very purpose of urine excretion.

Alkaline urines are frequently observed in cystitis and pyelonephritis because of the formation of ammonia from urea by bacteria. Unless properly preserved, urine, on standing, ultimately becomes alkaline because of the formation of ammonia by contaminating bacteria.

Specific Gravity and Total Solids. The *normal* specific gravity of the urine varies directly according to the amounts of solids in solution (chiefly chlorides and urea) and inversely according to volume. Since the solids fluctuate according to diet, and the volume according to fluid intake versus fluid loss through the skin, lungs and bowels, the range of normal may be anywhere between 1.010 to 1.030, with a general average between 1.015 and 1.025. Because samples are so commonly taken at random and hence may go far above or below these figures, it is apparent that the specific gravities ordinarily reported by laboratories are of but limited clinical value. For example, urine first voided in the morning is generally more concentrated and of higher specific gravity than that passed during the day; a high fluid intake may readily reduce the urine below 1.010 while a low fluid intake or much fluid loss through the skin and lungs, by exercise or from the bowels after laxatives, may raise it to 1.030 or higher. Under the conditions, the specific gravity of a single random specimen of urine in health may readily enough fall within the upper and lower limits of the urine in disease and readily result in error in interpretation.

In other words, variability in function according to the needs of the moment is one of the pronounced characteristics of the normal kidney. On the other hand, the diseased kidney may be unable to vary the specific gravity over a wide range because its ability to alter volume of excretion may be greatly impaired. For example, normal kidneys should be able to dilute urine to a specific gravity of 1.003 or less following the ingestion of 1500 cc. of water on an empty stomach in the morning, and should be able to concentrate the urine to about 1.030 on a diet of solid food without liquids for a day. Inability to dilute or concentrate is evidence of defective renal function (Volhard and Fahr), as are variations in specific gravity of specimens, taken at stated intervals during the day, when the patient is on a standard diet with no intake between meals (Mosenthal).

In *disease* the specific gravity of a mixed twenty-four hour specimen of urine may vary from as low as 1.001 to as high as 1.060. The relationship between specific gravity and volume may be lost. Thus, while the fluid intake may be normal or rigidly restricted, a kidney whose function is seriously impaired may be unable to concentrate the urine sufficiently to raise the specific gravity to 1.010; on the other hand, when the fluid intake is high the excretion of a urine of low specific gravity, a feat readily accomplished by the normal kidney, is beyond the capacity of a seriously diseased one. Consequently, in *chronic nephritis* the specific gravity is low, followed by a tendency to fixation at about 1.010, apparently because of a sustained compensatory overactivity of surviving nephrons. A sudden drop without a corresponding increase in volume may indicate approaching uremia. It is also low in *diabetes insipidus* and many functional nervous disorders.

On the other hand, in *acute glomerular nephritis* it is apt to be high, due to concentration, as likewise in *febrile states* for the same reason because of fluid loss. But it is well established that the capacity of the kidney to concentrate urine with an increase of specific gravity may be impaired in acute nephritis.[8] Indeed in most, if not all, cases with high blood nitrogen retention there is a failure to concentrate urine because of impaired tubular function due to interference with tubular blood supply, or the effects of a toxic agent upon tubular epithelium or the peritubular capillaries. Certainly the necessity for making corrections in the specific gravity of urines which contain large amounts of protein is to be emphasized.

Specific gravity is apt to be highest in *diabetes mellitus* in spite of large urine output owing to the presence of sugar in solution, although a normal or low specific gravity may occur in this disease.

There is a relationship between the specific gravity and *total solids* of normal urine. A rough method for their estimation consists in multiplying the last two figures of the specific gravity by coefficients like 2.66 (Long) or 2.33 (Haeser) to obtain total solids in grams per liter, or by 1.1 (Haines) to obtain them in grains per ounce. But these simple methods may not be reliable for some pathologic states, since different substances vary in the extent to which they contribute to the specific gravity of a solution; thus, less than 1.5 gm. of sodium chloride will produce as great a rise in specific gravity of urine as nearly 4 gm. of albumin.

Therefore, accurate chemical methods for the estimation of total solids are to be preferred and of course with the total twenty-four hour output of the urine. Accurate methods, along with uniform conditions of diet, exercise and fluid intake, furnish important data on the functional capacity of the kidneys. Under normal conditions the total solids present in about 1500 cc. of urine varies from 60 to 70 gm. for an adult of 150 pounds. After about the forty-fifth year of age they become gradually less, amounting to about half of the normal after seventy years of age.

CHEMICAL CHANGES

While urine may contain many different organic and inorganic substances, only those showing abnormal changes in relation to health and disease will be considered (Table 16).

Albumin and Globulins. Of the organic constituents the proteins are among the most important, especially albumin and the globulins (pseudoglobulin and euglobulin). The quantity of the albumin usually exceeds that of the globulins and while the term "proteinuria" is more exact and desirable than "albuminuria," the latter term has become so firmly rooted in medical literature that it will be used to designate that group of protein substances responding to the commonly employed qualitative and quantitative tests for albumin.

Undoubtedly, albumin and the globulins are derived from the blood plasma and while the normal glomerular membranes are regarded as impermeable to the passage of these colloids, yet as a matter of fact it appears that both, especially albumin, may occur in *normal urine* in the extremely minute total amount of about 0.075 gm. per twenty-four hour output, which is too small for detection by the routine tests commonly employed. In a series of 74 normal medical students Addis observed 0.010 to 0.30 gm. in 12-hour samples of night urine.

In the various types of renal disease the urinary albumin usually exceeds the globulins because the size and viscosity of the molecule of the former is much less than that of the latter, permitting greater glomerular filtration, while the molecule of fibrinogen is so much larger that it rarely escapes into the urine. The number of milligrams of albumin per liter divided by the globulin content in milligrams gives the *urinary albumin-globulin ratio* (A-G) but this determination does not possess as much clinical value as the A-G ratio of the blood plasma where it is normally 1.5 to 2.5:1. In chronic lipemic nephrosis, which is only a term that attempts to differentiate as a distinct disease part of the usual picture of subacute or subchronic glomerulonephritis, unusually high urinary A-G ratios may occur, ranging from 10 to 20, while in amyloid nephrosis it is usually quite low—between 1 and 2. It is also usually low in acute nephritis (4 to 6), usually as the acute process subsides. In chronic nephritis it may be between 3 and 10, while a ratio below 5 appears to be a grave prognostic sign. As the urinary A-G rises, that of the blood plasma falls, with the result that plasma albumin may not be regenerated sufficiently rapidly to maintain its normal level; consequently the plasma A-G may be inverted with globulin exceeding albumin giving a ratio as low as 0.2.

The *causes of albuminuria* (*proteinuria*) are many and diverse. Certainly the physician can make no greater mistake than to regard all cases as indicative of renal disease. Proteins at least may gain access to the urine in the lower genito-urinary tract (ureters, bladder, urethra, vagina, prostate gland, etc.) from inflammatory states or hemorrhage and constitute *extrarenal albuminuria,* although it is

TABLE 16. SUMMARY OF CLINICAL INTERPPETATION OF CHEMICAL CHANGES IN THE URINE

	Normal Urine	Abnormal Changes
Albumin and Globulins	About 0.075 gm. in 24-hour output and too small in amount for detection by ordinary qualitative and quantitative tests. Physiologic albuminuria may occur from excessive proteins in diet.	Albuminuria (proteinuria): (1) transitory, functional and orthostatic; (2) renal (nephritic or nephrotic); (3) extrarenal (pus, blood or both from pelves of kidneys, ureter, bladder, urethra, vagina, etc.).
Bence-Jones protein	Contained in normal bone marrow. Normally absent in the urine.	Occurs in 60 to 80 per cent of cases of myeloma of bone; sometimes in other bone tumors and osteomalacia; occasionally in leukemia.
Proteoses and Peptones	Absent.	May be present in states accompanied by the autolysis and absorption of exudates and tissues.
Mucin	Traces may be present.	Increased by irritation or inflammation of the urinary tract.
Nucleoprotein	Absent.	May be present in pyelitis, pyelonephritis, cystitis and other suppurative conditions.
Urea	Principal waste product of metabolism; 20 to 30 gm. in total 24-hour urine on average diet. Estimations based on random samples of urine are of no value.	*Decreased formation* in hepatic disease and acidosis; *increased formation* in wasting diseases, fevers and from absorption of exudates. *Decreased elimination* in acute nephritis and late stages of chronic nephritis.
Ammonia	In terms of NH_3 about 0.6 gm. per 1000 cc. Free ammonia produced from decomposition of urea after voiding or in cystitis with retention.	*Decreased* in mild acidosis of diabetes mellitus (not in nephritic acidosis); also by the administration of alkalies and in alkalosis. *Increased* in severe acidosis of diabetes and the acidosis of infants; by the administration of mineral acids; in hepatic insufficiency reducing the synthesis of urea (cirrhosis, acute yellow atrophy, poisoning by cinchophen, phosphorus, arsenic); also in pernicious vomiting of pregnancy and conditions associated with deficient oxygenation.

TABLE 16. SUMMARY OF CLINICAL INTERPRETATION OF CHEMICAL CHANGES IN THE URINE (continued)

	Normal Urine	Abnormal Changes
Uric Acid	0.4 to 1 gm. per 24-hour urine; increased by exercise and foods rich in the purines. Excreted as urates and in the free state.	*Increased* in the leukemias, polycythemia vera, diseases of the liver, acute fevers, absorption of exudates, nephritis, toxemia of pregnancy, x-ray treatment and after acute attacks of gout. *Decreased* before attacks of gout.
Creatine	In adult males 0 to 196 mg. per 24 hours; more commonly found in the urine of women; regularly in children (10 to 15 mg. daily).	Probably *increased* by muscular activity; independent of protein intake; otherwise of no clinical significance.
Creatinine	About 1.25 gm. per 24 hours. Constant and independent of diet.	*Decreased* in renal insufficiency and the primary myopathies; *increased* in wasting diseases due to increased tissue catabolism; also by unusual muscular activity.
Amino acids	Total of free and combined 0.5 to 1.0 gm. in 24 hours.	*Increased* in severe hepatic disease resulting in autolysis of its proteins and reduced deamination; also in wasting from tissue autolysis (protracted fevers) and diabetic acidosis.
Glucose	Reducing substances normally present (glycuresis) including trace of glucose; total 0.61 to 1.38 gm. daily; reduction of Fehling's and Benedict's reagents may also occur by uric acid, nucleoprotein, conjugate glycuronates and chloroform.	*Glycosuria* due to (1) renal glycosuria or (2) hyperglycemic glycosuria occurring in diabetes mellitus, hyperthyroidism, acromegaly, hyperadrenalism (fear, anger, anxiety), intracranial pressure, hypertension, hepatic and renal disease and acidosis from anesthesia, asphyxia or other factors. *Renal glycosuria* of two types: (1) normoglycemic glycosuria (rare) including the glycosuria of pregnancy (common) and (2) cyclic glycosuria (alimentary glycosuria) (not uncommon).
Pentose	Traces usually as the optically inactive form of arabinose.	Chief clinical significance is possibility of mistaking pentosuria for renal glycosuria or diabetes mellitus. Increased excretion may occur in the latter. "Alimentary pentosuria" may occur after the ingestion of fruits. "Essential pentosuria" uncommon but not rare.

TABLE 16. SUMMARY OF CLINICAL INTERPRETATION OF CHEMICAL CHANGES IN THE URINE (continued)

	Normal Urine	Abnormal Changes
Levulose	Usually absent.	Chief clinical significance is the possibility of mistaking levulosuria (fructosuria) for renal glycosuria or diabetes mellitus. "Alimentary levulosuria" may occur. "Essential levulosuria" rare. Increased excretion may occur in severe diabetes.
Lactose	Minute traces may occur.	Lactosuria occurs in a considerable proportion of women during lactation; does not occur during pregnancy. Of no clinical significance except for the possibility of being mistaken for glycosuria.
Galactose	Usually absent.	Galactosuria may occur in nursing infants. The galactose tolerance test has been proposed for the estimation of liver function.
Acetone	Minute traces may be present, especially in urine of young children. Should always be tested for routinely in preoperative surgical cases.	*Acetonuria* may occur in fevers, gastro-intestinal disturbances, nervous disorders, cachectic states, toxemias of pregnancy, after anesthesia and especially in diabetes mellitus. Progressive increase gives warning of acidosis and impending diabetic coma.
Diacetic and Beta-oxy-butyric acids	Absent.	*Ketonuria* due to same causes producing acetonuria alone but always of more serious import. Especially apt to occur in severe diabetes, dehydrated states due to excessive vomiting and diarrhea, starvation states, after ether and chloroform anesthesia and in alkalosis from hyperventilation, intestinal obstruction and excessive administration of alkalies.

TABLE 16. SUMMARY OF CLINICAL INTERPRETATION OF CHEMICAL CHANGES IN THE URINE (continued)

	Normal Urine	Abnormal Changes
Erythrocytes and their pigments	Vary from occasional to 425,000 (average 65,750) in 12-hour night urine (Addis). When containing 70,000 to 100,000 the urinary sediment may show 4 to 10 per high-power field which may be normal. An occasional erythrocyte therefore is within normal. Increased through contamination by uterine hemorrhage (menstruation, etc.); also by trauma from catheterization.	*Hematuria* from (1) trauma or lesions in the kidneys, ureters, bladder, prostate gland or urethra; (2) from the blood dyscrasias (hemophilia, the purpuras, etc.); (3) from nephritis and especially glomerular nephritis; (4) following the administration of sulfapyridine or sulfathiazole. *Hemoglobinuria* from excessive hemolysis due to poisons, malaria, blood transfusion and types of paroxysmal hemoglobinuria. *Urinary siderosis* from pernicious anemia, hemochromatosis, etc. *Hematoporphyrinuria* (see color of urine).
Bile and its pigments	No bilirubin. Trace of urobilin. Urobilinogen: (0.02 to 0.29 mg. or 0.03 to 0.97 Ehrlich units per 100 cc.) reacting in dilutions up to 1:20 of urine. No salts of biliary acids.	*Bilirubinuria:* (1) obstructive (intrahepatic and extrahepatic) jaundice; (2) hemolytic jaundice. *Urobilinuria:* jaundice due to obstruction associated with infection of biliary passages. *Urobilinogenuria:* (1) excessive hemolysis, pernicious anemia; hemolytic anemia; (2) hepatitis, toxemias of pregnancy; (3) hepatic disease without jaundice (portal cirrhosis, pneumonia, septicemia, congestive heart failure); (4) rarely in obstructive jaundice unless infection is present.
Diazo substance	Absent.	Positive diazo reactions occur especially in (1) typhoid fever (of diagnostic and prognostic value); (2) pulmonary tuberculosis (of prognostic value); (3) measles but not in rubella and (4) in some nonfebrile diseases and after the administration of certain drugs.

TABLE 16. SUMMARY OF CLINICAL INTERPRETATION OF CHEMICAL CHANGES IN THE URINE (continued)

	Normal Urine	Abnormal Changes
Indican	4 to 20 mg. per 24-hour urine. Increased by meat diet.	*Indicanuria* occurs especially in (1) intestinal putrefaction from obstruction, paralytic ileus, peritonitis, typhoid fever, cholera, achlorhydria, obstructive jaundice, pernicious anemia, sprue and pellagra; also in (2) bacterial decomposition of tissue proteins and exudates (gangrene, empyema, tuberculous cavities, bronchiectasis, etc.).
Chlorides	Largely sodium chloride: 10 to 16 gm. in 24 hours. Influenced by diet. Normal "renal threshold" 560–570 mg. per 100 cc. of blood plasma.	*Reduced* in (1) starvation states; (2) by excessive elimination through the skin or gastro-intestinal tract and (3) in conditions producing hypochloremia (with the exception of Addison's disease) as pyloric stenosis: pneumonia before the crisis: other acute infections, especially those producing large exudates; glomerulonephritis and nephrosis with edema; congestive heart failure with edema or anasarca; extensive burns and to some extent in the anemias and carcinoma of the stomach. *Increased* during the absorption of exudates and transudates.
Phosphates	2.5 to 3.5 gm. in 24 hours: two-thirds as alkaline phosphates and one-third as earthy phosphates.	*Increased elimination* in osteitis fibrosa diffusa, alkalosis, non-nephritic acidosis and after the administration of parathyroid hormone. *Decreased elimination* in nephritic acidosis, parathyroid tetany and anesthetics (ether, chloroform, ethylene).
Drugs and Poisons	Lead: 0.010 to 0.100 mg. per liter; arsenic: 0 to 0.15 mg. per 24-hour urine; mercury: none unless exposed to it.	Qualitative or quantitative tests for various drugs and chemical agents sometimes required for therapeutic guides in dosage or for the detection of poisoning.

to be admitted that in the final analysis albuminuria arising from the kidneys is indicative of some change in the glomeruli or tubules, or both, even though these may be so minor in degree or of such short duration as to be designated *transitory albuminuria.* Possibly the only exceptions are so-called "alimentary albuminuria" following the ingestion of large amounts of albumin, especially that of raw eggs, and those occasional instances of hemoglobinuria and methemoglobinuria, which, because they may pass through apparently normal glomeruli, may be designated as *functional albuminuria.* Otherwise, the temporary albuminuria following severe exercise or mental strain and after prolonged exposure to severe cold, as well as premenstrual albuminuria, is to be regarded as due to minor but nevertheless definite changes in the kidneys like hyperemia with increased permeability of the glomerular membranes.

Apparently the transitory albuminurias occurring in congestive heart failure, ascites, pregnancy and intra-abdominal tumors are to be included in the same category as due to renal stasis, including those resulting from convulsive states and coma (brain tumors, epilepsy, cerebral and meningeal hemorrhage) which produce vasomotor disturbances with hyperemia and increased glomerular permeability due to local or generalized asphyxia.

Orthostatic albuminuria (postural, lordotic, or cyclic albuminuria) occurs most frequently in young neurasthenic individuals and is characterized by the presence of albumin in the urine during periods of ordinary activity in the erect posture (especially late in the afternoon), which disappears during recumbency. Orthostatic albuminuria is now believed to be due to renal lesions and is not always to be regarded as "functional" since it is now known that nephritis may begin in this manner. Undoubtedly lordosis, movable kidneys and other conditions which interfere with the renal veins and thus cause passive congestion of the kidneys with increased glomerular permeability may be responsible for the albuminuria. As shown by Rytand[9] in roentgenographic studies of the kidneys following injections of diodast, as well as by the Addis urea ratio, the Rehberg glomerular filtration rate and the Addis count of the urinary sediment in five cases, there may be transitory renal degenerative lesions as well. However, the albuminuria may be due to only some minor disturbance in renal circulation leading to venous stasis, since most individuals are in good health without progressing to nephritis, and it has been found in 4 per cent of soldiers[10] and 5.25 per cent of healthy students.[11]

Transitory albuminuria may also occur in hepatic disease with jaundice as well as in intestinal obstruction and eclampsia, presumably because of the presence in the blood of toxic protein products or as the result of alkalosis resulting in the irritation of the nephrons. It is to be stated, however, that the term "transitory" is not always well chosen, as the albuminuria may be constant, although cyclic, as in orthostasia, or prolonged, although intermittent, as in congestive heart failure.

Of course, *glomerulonephritis* (acute, subacute or chronic) with secondary tubular changes, *nephrosis* (tubular nephritis) with secondary glomerular changes, and *nephrosclerosis* (vascular nephritis) primarily affecting the glomeruli and peritubular capillaries, are the most serious causes of albuminuria. Together these albuminurias may be classified as renal in origin; the designation "organic albuminuria" is not well chosen because, as previously mentioned, those albuminurias classified as transitory or orthostatic are likewise usually due to organic changes in or affecting the kidneys.

Undoubtedly, urinary albumin in glomerulonephritis is largely due to the escape of albumin, globulins and whole blood through the damaged glomeruli, with the probability that small additional amounts escape from exfoliated tubular cells and the peritubular capillaries. The albuminuria of nephrosis, however, is more difficult to explain since the glomeruli may appear to be normal or show but minor changes. For example, nephrosis due to larval causes, like that occurring in the fevers, or to diabetes, jaundice, hyperthyroidism and the blood dyscrasias, or resulting from severe necrosis of tubular epithelium by necrotizing chemical agents and drugs like sublimate nephrosis, is hardly to be explained on the basis of tubular injuries alone. Apparently some change in the glomerular membranes occurs, responsible for increased permeability sufficient for the escape of the comparatively small molecules of albumin, especially if there is a disturbance of protein metabolism, as Epstein believes to be the case in the so-called genuine "lipoid nephrosis." But, as a matter of fact, this disease entity is extremely rare, most cases being chronic lipemic nephrosis (Fahr) and part and parcel of subacute or subchronic glomerulonephritis.

The albuminuria of nephrosclerosis is likewise largely due to glomerular injuries. Certainly this is true of those due to arteriolarsclerosis, as in primary or essential hypertension (hyperpiesia), while in those due to arteriosclerosis involving the larger branches of the renal arteries with secondary hypertension, the responsible lesion appears to be one of increased glomerular permeability resulting from nutritional changes and asphyxia. At least there can be no doubt of the important relationship between blood pressure and albuminuria, since albuminuria has been observed in 25 per cent of hypertensive groups as compared with about 8 per cent among those with normal or low pressures on the basis of but single examinations.[12] Even secondary hypertension with albuminuria, therefore, is not a benign disease as albuminuria is likely to be an early sign of renal impairment and, as shown by life insurance statistics, deaths from Bright's disease are about two and one-half times the normal death rate.

In summary, albuminuria may be due to many causes producing different kinds of lesions which are seldom pure types but frequently with one or the other predominating, as summarized in Table 17. "A few gray hairs in the kidney" may be all right for explaining its presence to the patient but the physician must realize that the renal types at least cannot be dismissed as irrelevant to the patient's present and future health.

Bence-Jones Protein. The protein known by this name is regularly present in the normal bone marrow and apparently the leukocytes are concerned in its production.[13] Sometimes such enormous amounts are excreted in the urine in individuals with myeloma of bone (of which the plasma cell is only one type) that some have thought it may be derived from food, but is now known to be endogenous in origin and a protein of such low molecular weight (around 35,000) that it readily escapes through the healthy glomerular membrane. It occurs in

TABLE 17. A SUMMARY OF THE CLINICAL INTERPRETATION OF ALBUMINURIA (PROTEINURIA)

Classification	Lesions	Etiology and Occurrence
(A) Physiologic	None	Excessive ingestion of proteins Hemoglobinemia
(B) Transitory and Orthostatic	Acute congestion	Severe exercise Severe mental strain Prolonged exposure to cold Trauma to the kidney Vasomotor instability Premenstrual Convulsive states and coma
	Passive congestion	Congestive heart failure
	Venous stasis (pressure or kinking of renal veins)	Ascites Pregnancy Intra-abdominal tumors Movable kidney Postural or lordotic (orthostatic)
	Irritation of nephrons by toxic protein substances (?) Alkalosis (?)	Hepatic disease and jaundice Intestinal obstruction Eclampsia
(C) Renal	Primary glomerular and secondary tubular lesions (*nephritis*)	Glomerulonephritis Pyelonephritis Tuberculous nephritis Polycystic disease Renal infection and hemorrhage
	Primary tubular with secondary glomerular lesions (*nephrosis*)	Larval: fevers; diabetes, jaundice, hyperthyroidism, blood dyscrasias Necrotizing: chemical agents and drugs Lipoid or chronic lipemic nephrosis Renal amyloidosis
	Primary arteriolar or arterial sclerosis with secondary interstitial fibrosis (*nephrosclerosis*)	Primary or essential hypertension (hyperpiesia) Secondary hypertension Arteriosclerotic nephritis
(D) Extrarenal	Exudates, blood or both gaining access to the urine from the renal pelvis, ureters, bladder, urethra, vagina, prostate gland, etc.	Pyelitis Ureteritis Cystitis Urethritis Vaginitis Prostatitis Ureteral and vesical calculi

the urine in fully 60 to 80 per cent of cases of myelomas (especially late in the disease) as well as in the blood and serous exudates of this disease. Under the circumstances it was thought to be pathognomonic for this particular bone tumor but it is now known to occur sometimes in the urine of individuals with osteogenic sarcoma, osteomalacia and carcinomatous metastases of the bone marrow. Since the leukocytes apparently have something to do with its production, it is not surprising that it has also been found in the urine of individuals with both lymphoid and myeloid leukemia and even empyema, although but rarely.

This urinary protein precipitates by heating at 50° to 60° C., and wholly or partially disappears as the temperature approaches the boiling point, to reappear as the urine cools, and is further markedly influenced by acidity and salt concentration. It is therefore readily understood that its presence may escape detection, especially in routine urine examinations. Bannick and Greene [14] have observed that multiple myelomata may be accompanied by kidney disease in the form of tubular destruction with subsequent fibrosis of pyelonephritis, which adds greatly to the proteinuria. They advise that the urine be examined for Bence-Jones protein in all individuals showing marked proteinuria with marked secondary anemia, blood nitrogen retention and delayed phenolsulfonphthalein excretion with little or no edema, hematuria, hypertension or retinitis.

Other Urinary Proteins. Primary and secondary *proteoses* may be excreted in the urine in the presence of considerable autolysis and absorption of exudates and tissues, as in chronic suppurations, pulmonary tuberculosis, resolving pneumonia, leukemia, carcinomatosis, osteomalacia, peptic ulcer, severe liver disease, during pregnancy from the absorption of amniotic fluid and after labor during the involution of the uterus. At one time proteosuria was thought to be of significance in allergic states but Tuft and Brodsky [15] have found that positive skin reactions may occur with urinary proteose in nonallergic conditions. The urinary proteoses, therefore, possess but little clinical interest and tests for them are of no value when albumin is also present. *Peptonuria* is extremely rare and most reported cases have represented, perhaps, proteosuria.

The exact chemical nature of *mucin* has not been determined but it appears to be a glycoprotein, which sometimes occurs in normal urine and which may be increased as the result of irritation and inflammation of the urinary tract (especially in cystitis) and the vagina. It must be differentiated from the Bence-Jones protein.

Nucleoprotein may also be present in the urine in abnormal amounts not only in pyelitis but especially in pyelonephritis and cystitis. The term is sometimes incorrectly applied to include other protein substances such as mucin and phosphoprotein but, as in the case of mucin and the proteoses, examinations for it are not ordinarily required for clinical purposes.

Urea. Urea is formed in the liver from ammonia derived from amino acids during the process of deamination. As the principal waste product of metabolism, it is the most important constituent of the urine and under normal conditions is promptly eliminated since the storage capacity of the body for nitrogen is limited. On a low protein diet it constitutes about 60 per cent of the total urinary nitrogen, about 90 per cent on a high protein diet and averages generally about 80 per cent on a mixed diet. It constitutes about one-half of the solids excreted, amounting to about 20 to 30 gm. in twenty-four hours, or 25 gm. per 1000 cc. of urine.

It is evident, therefore, that under normal conditions the urea nitrogen of the urine largely depends upon the nitrogen intake and while its quantitative estimation is simple, though not a very accurate method of ascertaining the state of nitrogen excretion, the estimation of urea is of no value whatever when, as is so frequently the case, a small quantity of urine is taken at random for its examination because excretion fluctuates. When, however, estimations are made at intervals under the same conditions of diet and exercise, employing samples of the *mixed twenty-four hour urine,* the results may be of clinical value, since a steady decline in elimination is of bad prognostic significance in nephritis and usually a forerunner of uremia. But, as a matter of fact, estimations of the blood urea nitrogen are much more helpful clinically and for this reason estimations of urinary urea are made much less frequently than formerly.

The excretion of urea as well as of other nitrogenous substances (uric acid, creatinine, etc.) is (1) directly proportional to their concentration in the blood when the urine volume is above a certain limit (augmentation limit), as well as (2) to the square root of the urine volume when this is below the augmentation limit and (3) to the amount of active renal tissue when this is less than 50 per cent of the normal. Therefore, various renal function tests have been devised for determining the capacity of the kidneys to excrete urea (these are described in Chapter 5). But while the quantity eliminated varies considerably according to protein intake, the percentage of urea in twenty-four hour urine is quite constant under *normal* conditions although increased by exercise, copious fluid intake and the administration of ammonium salts of organic acids.

In general, a pathologic decrease of urea in the urine is due to (1) either a lessened formation of it in the liver or (2) to diminished excretion by the kidneys. Since the quantity formed by the liver appears to be determined by the efficiency of deamination of amino acids, a *decreased formation of urea* may occur in such diseases of the liver as marked cirrhosis, toxic hepatitis, carcinoma and acute yellow atrophy, the latter being characterized by the presence of leucine and tyrosine in the urine as the result of a failure of deamination of amino acids. Severe acidosis also decreases urea formation because of a great increase in the formation of urinary ammonia by the kidneys, probably from amino acids, in an attempt to combat acidosis by a conservation of the plasma alkali reserve. An *increased formation of urea* above nitrogen intake, however, may occur in wasting diseases, diabetes without acidosis, fevers, and especially during the resolution of pneumonia and the absorption of large exudates because of excessive tissue catabolism. In deciding whether or not an increase of urea is due to increased metabolism, the relation between the amounts of urea and of sodium chloride in the urine may be clinically helpful since, on a mixed diet, the amount of urea is normally about twice that of the chlorides. If the proportion is much increased above this, increased tissue destruction may be inferred since other conditions which increase urea also increase the chlorides. Increased formation of urea may also cause an increased output of urine because it possesses a diuretic effect by rendering the glomeruli more active, along with an increase of osmotic pressure of the urine which reduces tubular reabsorption.

Retention of urea occurs in most cases of nephritis. In acute nephritis the amount of urinary urea is usually markedly decreased, and a return to normal indicates improvement. In the early stages of chronic nephritis the total eliminated in twenty-four hours may be normal because the ability to concentrate urea is impaired. In the late stages, however, it is usually decreased and the retained nitrogen frequently cannot be entirely accounted for by the concentration of nonprotein nitrogen in the blood, where it may or may not be increased, suggesting that the excess may be retained in the liver and muscles.

Ammonia. Related to the clinical significance of urinary urea is that of ammonia since there is convincing evidence that the latter is formed almost entirely by the kidneys from the urea and probably the amino acids [16] conveyed to them in the blood.[17] It appears, therefore, to have two sources and the two mechanisms are not necessarily related since individuals with renal disease may lose one mechanism of its production without losing the other, which gives a certain margin of safety. This ammonia aids in the preservation of the acid-base equilibrium of the blood and tissues by the neutralization of acids in the kidneys.

Urinary ammonia refers to the ammonium salts and not to the accumulation of free ammonia, which is derived from the decomposition of urea after the urine is excreted or in cystitis with retention of urine. Calculated in terms of NH_3 the total output under normal conditions is about 0.6 gm. per 1000 cc. of urine.

Urinary ammonia, therefore, bears no relation to that in the blood. It is, however, a very important index of the degree of acidosis in diabetes mellitus since it is reduced by diacetic and oxybutyric acids through neutralization of them in the kidneys. In mild diabetic acidosis the output may be as low as 1 to 1.5 gm. per twenty-four hour urine, but in severe acidosis it may rise to 4 or 5 gm. and even 8 or 10 gm. because of combination with these acids and when excreted is accompanied of course by a reduction in urinary urea. The urinary ammonium salts are also increased in the acidosis of infants but not in the acidosis of nephritis.

Urinary ammonia is likewise increased by the ingestion of mineral acids, in pernicious vomiting of pregnancy and in states of hepatic insufficiency which reduce the synthesis of urea, like cirrhosis of the liver and the hepatitis of phosphorus, arsenic and cinchophen poisoning, acute yellow atrophy, etc.; also in conditions associated with deficient oxygenation. It may be decreased by the administration of fixed alkalies and the salts of organic acids and of course by any other factors producing alkalosis.

Uric Acid, Creatine, Creatinine, Amino Acids and Nitrogen Partition. These additional nonprotein nitrogenous substances may be discussed briefly because determinations of them in the blood possess far more clinical value than determinations of their elimination in the urine.

The site of formation of *uric acid* is unknown but it appears to occur in the liver. At all events it is formed from the purines of foods and especially the meats, liver, kidney, thymus and pancreas (exogenous) and from the purines of the body from the breakdown of nuclear material and free mononucleotides (endogenous). Anywhere from 30 to 70 per cent of the total uric acid so formed is destroyed (probably in the liver), some is retained in the blood (2 to 4 mg. per 100 cc.) and the balance eliminated in the urine as urates of sodium, potassium or ammonium. In the free state, the balance eliminated in the urine averages about 0.4 to 1 gm. per twenty-four hours of which 0.3 to 0.4 gm. is thought to be endogenous in origin under normal conditions. Excretion is increased by muscular exercise and especially by foods rich in the purines like kidney, liver and sweetbreads. Normal urine, therefore, always contains urates which are readily deposited after voiding and standing as a sediment of "amorphous urates" or crystals. It is a common error to consider these deposits as evidence of excessive excretion unless they are unusually heavy.

An increase of urinary urates and uric acid is commonly observed in the leukemias, probably due to the disintegration of the tissues generally, rather than to leukocytes, in polycythemia vera, in diseases or dysfunction of the liver, during the absorption of exudates (especially during the resolution of pneumonia), during the acute fevers and x-ray therapy as likewise in nephritis and the toxemias of pregnancy. It is decreased before an attack of gout and increased for several days thereafter but its etiologic relationship to the disease is uncertain.

Creatine is not usually regarded as present in the urine of normal adult males but may occur in them in amounts varying from 0 to 196 mg. estimated as creatine nitrogen per twenty-four hours.[18] It is much more likely to occur in the urine of women and is regularly found in the urine of children (10 to 15 mg. daily). Under the conditions, it does not appear to be one of the waste products of metabolism and is without relation to protein intake, but a substance which probably serves a useful function in the muscles. It may be increased by unusual or sudden change in muscular activity but is of no further clinical interest.

Creatinine, however, is a product solely of endogenous protein metabolism and, as such, the quantity excreted in the urine is an index of its level, amounting to approximately 1.25 gm. per twenty-four hour urine for adults under normal conditions. It is quite constant and quite independent of diet, provided no preformed creatinine is ingested, so that its excretion may be taken, probably, as an index of muscular activity. Preformed creatinine is almost completely eliminated in the urine in which it has been designated as the "creatinine coefficient," expressed in terms of milligrams excreted per kilogram of weight in twenty-four hours and averaging 8 to 11 for normal adult males and 5 to 8 for females, which has been employed as a test of renal function (Rehberg). Its excretion is reduced in renal insufficiency and the primary myopathies, while increased in the wasting diseases due to increased tissue catabolism.

Those *amino acids* absorbed from the small intestine following the hydrolysis of protein and escaping deamination in the liver or resynthesis to protein in the tissues, are eliminated in the urine in both the combined and the free state, amounting to a total of 0.5 to 1.0 gm. per twenty-four hours, of which 0.1 to 0.15 gm. is free amino acid nitrogen. Increased urinary output is especially likely to occur in diseases of the liver which result in autolysis of its proteins and in reduced deamination like eclampsia, acute yellow atrophy, poisoning by cinchophen, chloroform, phosphorus and arsenic, as well as in states of wasting from tissue autolysis (protracted fevers) and in diabetic acidosis.

It is apparent, therefore, that the proportion of nitrogen distributed among the nonprotein nitrogen substances of the urine is subject to great variation and the study of its distribution (*nitrogen partition*) and that of relation of excretion to intake (*nitrogen equilibrium*) is an important one but of more concern to the biochemist than to the clinician. Fortunately, nephritis patients losing large amounts of protein excrete only traces of lipid nitrogen and lipid phosphorus.[19] On a mixed diet, under normal conditions, the partition of nitrogen is somewhat as follows: urea nitrogen, 86.9 per cent; ammonia nitrogen, 4.4 per cent; uric acid nitrogen, 0.75 per cent; creatinine nitrogen, 3.6 per cent and "undetermined nitrogen," chiefly in amino acids, 4.3 per cent.

Melituria and Glycuresis. The term "melituria" is properly employed to designate the presence of an *abnormal* amount of sugar in the urine. Glucose (dextrose) is by far the most common and is termed glycosuria. Other sugars occasionally found in the urine and of lesser clinical importance, are levulose (levulosuria), lactose (lactosuria), galactose (galactosuria), pentose (pentosuria), etc. Since all meliturias are not glycosurias, the identification of the sugar present in urine frequently becomes a matter of clinical importance.

In the normal individual, glucose is excreted by the renal glomeruli but, being one of the "threshold substances," it is largely reabsorbed into the blood through the tubular epithelium for conservation. However, traces of a copper-reducing substance or substances are to be found—these are designated as *normal urine sugar*. The amount is variously stated but appears to vary from 0.03 to 0.10 per cent in terms of glucose [20] of which at least 25 per cent is glucose (Benedict). For this physiologic phenomenon Benedict, Osterberg and Neuwirth have proposed the term *glycuresis* to distinguish it from glycosuria which is usually a pathologic condition. According to Folin and Berglund, glycuresis follows every ordinary carbohydrate meal, the increase in reducing substances being independent of the amount of glucose in the blood and largely due to the excretion of foreign unassimilable carbohydrates and carbohydrate decomposition products produced during the preparation of food a portion of which is glucose.

Glycuresis, however, is not synonymous with so-called "alimentary glycosuria," a term employed to designate the urinary excretion of glucose by certain apparently normal individuals after the ingestion of excessive amounts of glucose, saccharose or, at times, starch. Indeed, as much as 200 gm. of glucose may be given normal individuals without the production of glycosuria. Under the circumstances, "alimentary glycosuria" must be due either to a lowering of the "renal threshold" for glucose or to the absorption of it from the intestine at a rate too rapid to allow for its adequate removal from the blood by the liver. Others maintain that it may be due to some disturbance of intermediary carbohydrate metabolism originating in the liver, endocrine glands, or muscles, but no such disturbances are to be demonstrated satisfactorily in most cases and it appears to be a type of so-called renal glycosuria to which further reference will be made shortly because of its clinical importance.

In this connection it is also to be emphasized that both the Benedict and Fehling solutions may be reduced not only by the substances occurring in glycuresis but likewise by substances other than sugars, if present in sufficient amounts, among which are included uric acid, nucleoprotein and conjugate glycuronates formed after the ingestion of antipyrine, menthol, phenol, camphor, chloral, etc., as well as by chloroform used as a preservative. The Benedict reagent, being less susceptible to reduction by uric acid and chloroform, is to be preferred and, since the glycuronates do not give osazones and are not permeated by yeast, and since they cease to be excreted after stopping the drug, differentiation from glucose is not difficult. But at all events, reduction of metallic oxides of copper in alkaline solution does not always mean the presence of sugars and when it occurs the cause should be ascertained whenever possible.

Glucose. As previously stated, the term "glycosuria" signifies the excretion, in the urine, of abnormal amounts of glucose. The normal fasting blood sugar is 80 to 120 mg. per 100 cc. (Folin-Wu) and glucose does not normally "spill over" into the urine until it reaches about 160 mg. per 100 cc., which is designated as the *renal threshold.* But glucose may occur in the urine at normal blood sugar levels or, temporarily, when it is increased up to but not above the normal threshold by a meal or the administration of glucose. This is commonly called "renal glycosuria," "benign glycosuria," "renal diabetes" or "orthoglycosuria." When this occurs constantly with a normal blood sugar it is sometimes termed "normoglycemic glycosuria" or "glycosuria innocens"; when occurring only after a meal or the administration of glucose, but at blood levels below the threshold, it is sometimes called "cyclic glycosuria" or "cyclic renal glycosuria" and is a type of renal glycosuria best detected by the so-called sugar tolerance test.

On the other hand, glycosuria may occur only when the blood sugar is materially above the threshold—up to 180 and even 200 mg. per 100 cc. as in diabetes mellitus. Under the conditions, glycosuria may be stated to be of two types, namely, (1) renal glycosuria and (2) hyperglycemic glycosuria.

Renal glycosuria of the normoglycemic type in which the normal fasting blood sugar levels are constant at 85 to 120 mg. per 100 cc., is a rare condition, having been observed in only 22 cases of 9000 individuals with glycosuria in Joslin's Clinic,[21] except in the *glycosuria of pregnancy* where it may occur in as high as 10 to 15 per cent of normal pregnant women, particularly in the late months, and more frequently in primagravidae than in multigravidae. The cause of the glycosuria of pregnancy is unknown but is apparently due to a lowering of the renal threshold for glucose which some have ascribed to a physiologic hypertrophy of the pituitary gland. However, renal glycosuria of the cyclic type, or that occurring when the blood sugar is increased by a meal or the administration of glucose (alimentary glycosuria), is not uncommon and requires careful differentiation from incipient diabetes.

The cause of renal glycosuria occurring in males and nonpregnant females is unknown. In the final analysis, it may be due to some factor or factors lowering the renal threshold for glucose. As previously stated, no morphologic tissue changes attributable to nephritis, nephrosis or other injury have been detected. Renal glycosuria usually occurs in individuals under 30 years of age and may be due to the influence of heredity since a history of glycosuria in the relatives of the patient was obtained in 11 of 15 cases.[21] Autonomic instability may be a factor and some have thought it may be due to a disturbance of calcium metabolism, but evidence is lacking. The cause or causes, whatever they may be, may act by reducing tubular reabsorption of glucose. True or normoglycemic renal glycosuria, including the glycosuria of pregnancy, apparently do not progress to diabetes mellitus but the cyclic or alimentary type undoubtedly may, requiring a careful supervision of diet to ward off this end result as long as possible.

Nonhyperglycemic glycosuria may occur in a considerable proportion of individuals with *glomerulonephritis, nephrosclerosis* and *nephrosis,*[22] the frequency and degree increasing with increasing severity of the renal lesions. This, however, is not renal glycosuria, even though occurring with normal fasting blood sugars or those below the renal threshold, since the cause is reasonably ascribed to lesions causing increased glomerular excretion of glucose, tubular lesions resulting in decreased reabsorption, or to a combination of the two.

Hyperglycemic glycosuria, or the occurrence of abnormal amounts of glucose in the urine in association with hyperglycemia, is readily understood. Diabetes mellitus is, of course, a frequent cause but it is erroneous to conclude that this disease is always responsible since the glycosuria may be due to hyperthyroidism, hyperpituitarism (acromegaly), hyperadrenalinism (due to anger, fear or anxiety leading to the sudden mobilization of glucose stored as glycogen), severe exercise not greatly prolonged, increased intracranial pressure (brain tumors, cerebral hemorrhage, fractured skull, etc.), hypertension, chronic hepatic disease, and acidosis due to anesthesia, asphyxia or other factors. Indeed, the differential diagnosis between diabetes and these other diseases or states causing hyperglycemic glycosuria, sometimes entails a study of sugar tolerance and the respiratory quotient variations following the administration of glucose, basal metabolic rate determinations, the serum phosphate curve, etc. As shown by Robinson and his colleagues,[23] glycosuria after the intravenous injection of glucose may be explained on the basis of the participation of phosphorylation in the reabsorption of the glomerular filtrate.

Pentose. Pentoses, usually the optically inactive form of arabinose, are more or less constantly present in the urine, the quantity excreted bearing no relation to the amount ingested. Increased amounts of pentose may, however, occur in the urine in some cases of diabetes mellitus and sometimes temporarily in normal individuals after the ingestion of large quantities of fruits which have a high pentose content (prunes, cherries, grapes, plums), constituting *alimentary pentosuria,* although normal individuals can utilize or destroy xyloketose when it is administered by mouth.

Essential pentosuria is uncommon although not rare [24] and may be mistaken for diabetes mellitus with resultant unnecessary dietary restrictions and the possibility of producing hypoglycemic reactions by the needless administration of insulin. Indeed, every case of glycosuria showing the regular presence of 1 per cent or less of reducing substances in the urine should be investigated for possible pentosuria. The ingestion of amidopyrine increases the excretion of pentose which is not influenced by diet, rest, exercise or thyroid extract. Otherwise, it is of no particular clinical importance aside from the possibility of being mistaken for renal glycosuria or diabetes mellitus. The metabolism of other carbohydrates is unimpaired. It appears to be familial and hereditary in nature.

Levulose. Traces of this fruit sugar may occur in normal urine with increased excretion in severe diabetes, always in association with glucose. *Alimentary levulosuria* or fructosuria may occur, following the ingestion of large amounts of levulose in fruits and honey or its administration, particularly in individuals with hepatic insufficiency. Approximately 10 per cent of normal individuals excrete levulose in the urine following the ingestion of 100 gm. so that the levulose tolerance test has not proved satisfactory as a test of hepatic function. It is stated that the rectal administration of the sugar results in a higher excretion than oral administration.

Essential levulosuria occurs but is rare with only 33 cases recorded in the literature.[25] Its chief clinical significance lies in the possibility of mistaking it for renal glycosuria or diabetes mellitus; the metabolism of other carbohydrates is undisturbed. It is regarded by some as due to an inborn error of metabolism, localized primarily in the liver where a specific enzyme deficiency exists, resulting in impaired ability to convert fructose into glycogen. Others regard it as being due, not to hepatic insufficiency, but to increased permeability of the glomeruli for the levulose molecule.[25] Its clinical importance lies in its early recognition, before the patient is subjected to the rigors of a diabetic regimen and before the needless administration of insulin which may handicap the growing child.

Lactose. Minute traces of lactose may occur in normal urine and especially after the ingestion of large amounts. *Lactosuria* occurs in a considerable proportion of women during the period of lactation but is apparently physiologic and has no apparent clinical significance except the possibility of being mistaken for glycosuria. Lactosuria does not occur normally during pregnancy but it frequently occurs during lactation.

Galactose. Galactosuria has been found in nursing infants in association with derangements of gastro-intestinal function (from lactose). It is converted into glycogen largely in the liver and a galactose tolerance test has been proposed for the determination of liver function. Tolerance is believed to be diminished during pregnancy and menstruation, after the menopause and in dysfunctions of certain of the endocrine glands.

Acetone. Minute traces of acetone, too small for detection by ordinary tests, may be present in the urine of adults under normal conditions. This is especially true of young children in whom larger amounts are apt to occur in the urine.

Large amounts (acetonuria) are not uncommon when the intake of carbohydrates is limited in fevers, gastro-intestinal disturbances, certain nervous disorders, and especially in cachectic states, pernicious vomiting of pregnancy, eclampsia, and in the serious and often fatal toxic condition which is a not infrequent late effect of anesthesia (especially chloroform anesthesia). This post-anesthetic acetonuria seems more likely to appear and to be more severe when the urine contains any notable amount of acetone before operation, which *in surgical cases* suggests *the importance of routinely testing for acetone as well as for sugar.* It is likely that the acetone, as well as the diacetic and beta-oxybutyric acids which may also be present in these conditions, are found secondarily, as a result of primary conditions, so that the symptoms are not necessarily due to acidosis;

indeed, in many cases there may be little or no acidosis as determined by the carbon dioxide combining power of the plasma which is the best method for its detection. According to Folin, in these states acetone is usually present in only small amounts, the substance shown by the usual tests, particularly after distillation of the urine, being diacetic acid. Under the conditions, the Frommer test is to be preferred, since it does not require distillation and does not react to diacetic acid unless too much heat is applied.

The chief clinical significance of acetonuria, however, is in connection with diabetes mellitus. It occurs intermittently in some mild cases, fairly regularly in most advanced cases, although much depends upon the diet, and is always present in severe cases. On the whole, tests for acetone are fully as important as tests for glucose in diabetes. A progressive increase is always a warning of impending acidosis since the acetone bodies are probably the chief cause of the dreaded diabetic coma. But, owing to the marked and variable loss of acetone through the lungs, a quantitative estimation is of little value; when acidosis is present it is better to rely upon an estimation of the ammonia insofar as the urine is concerned, as a measure of its severity.

Diacetic and Beta-oxybutyric Acids. These two acids, along with acetone, are commonly designated as the "acetone bodies" or ketones and together are responsible for the clinical state designated as acidosis or ketosis. Diacetic and beta-oxybutyric acids do not occur normally in the urine under average conditions but, together, may occur and produce *ketonuria* under the same conditions as those producing acetonuria. Their presence is always of more serious significance, especially when beta-oxybutyric acid is present.

Not only may ketonuria develop in diabetes mellitus through an excessive fluid loss with excessive loss of base, resulting in changes of electrolytic and acid-base balance of the blood plasma due to alkali deficit, but likewise after the excessive loss of fluid base and electrolytes caused by severe vomiting, especially the pernicious vomiting of pregnancy, severe diarrhea and other states of dehydration. In normal pregnancy, after the second month there is a progressive tendency to ketosis and especially on carbohydrate-poor diets. Starvation states, especially those including restriction of carbohydrates, may also produce ketosis due to incomplete combustion of the fatty acids in exactly the same manner as it is produced in diabetes. However, instead of ketosis being due to the incomplete combustion of fats, it now appears that diacetic acid, from which acetone and beta-oxybutyric acid are derived, is a normal intermediate product of fat metabolism which is formed by the liver and excreted like the excretion of glucose to meet the energy requirements of the body.[26, 27] According to this point of view, the production of the acetone bodies is simply an expression of accelerated fat metabolism. It will be observed that either view is consistent with the fact that a ketosis develops in any of the clinical states of deficient carbohydrate metabolism. It may also occur after ether and chloroform anesthesia, especially the latter, but much less frequently after ethylene and nitrous oxide anesthesia, which some investigators have ascribed to the excessive production of lactic acid as a result of anoxemia and imperfect glucose combustion. Ketosis with ketonuria may also occur in alkalosis due to hyperventilation, intestinal obstruction and the excessive administration of alkalies due, presumably, to a disturbance of the oxidation of the fatty acids but with little effect on the acid-base equilibrium of the plasma.

Erythrocytes and Their Pigments. The sediment from a twelve-hour specimen of urine will usually show an occasional erythrocyte per low-power field under *normal* conditions, amounting to about 70,000 cells, as determined by the count method of Addis. In my experience when urine contains 70,000 to 100,000 erythrocytes per cubic centimeter by actual count, anywhere from 4 to 10 per high-power field may be found upon microscopic examination of sediment obtained by centrifuging 15 cc. at high speed for at least five minutes. This is important to remember because not infrequently the presence of a few erythrocytes in urinary sediment is erroneously interpreted as indicative of glomerular injury.

In women the number may be increased during menstruation or other types of uterine hemorrhage with contamination of the urine. Examinations for blood should be deferred during these states unless a specimen is secured with special precautions or by catheterization. Not infrequently urine collected by ureteral catheterization will show an increase of erythrocytes due to trauma which may lead to diagnostic errors in interpretation. Of course, the presence of small numbers of erythrocytes does not alter the color of the urine; they are detected by microscopic examination supplemented by the chemical tests for occult blood. The former is preferred and is the more accurate, as weakly positive benzidine and other chemical reactions may be due to pus or other substances.

Hematuria may occur in many *diseases of the kidneys* and especially if the glomeruli are involved, as in acute and chronic glomerulonephritis (including the focal type of subacute bacterial endocarditis), to a lesser extent in nephrosis (tubular nephritis) unless acute, tuberculosis, malignant hypertension, nephrolithiasis, pyelitis, pyelonephritis and pyonephrosis, hydronephrosis, tumors (including congenital polycystic disease and especially intrapelvic papillomas), trauma, etc., also from passive congestion of cardiac failure as well as in some of the blood dyscrasias such as hemophilia, the purpuras, polycythemia vera and the leukemias. It may also result from lesions of the *ureters* (calculi, strictures, trauma) as well as of the *bladder* (cystitis, tumors, calculi, foreign bodies) and of the *prostate gland* (prostatitis, tumors) and *urethra* (urethritis, foreign bodies).

Hemoglobinuria, unaccompanied by hematuria, may occur from excessive hemolysis in poisoning (mushrooms, favism and various chemical agents and drugs), estivo-autumnal malaria (blackwater fever), blood transfusions due to incompatibility, and the four types of paroxysmal hemoglobinuria.

Hemosiderosis with the production of hemosiderin crystals in the urine (*urinary siderosis*) may occur in pernicious anemia, hemochromatosis and other diseases causing siderosis of the kidneys.[28]

Porphyrinuria has already been considered in a discussion of the clinical interpretation of the color of urine.

Bile and Its Pigments. The constituents or derivatives of bile (excepting the normal urinary pigments) which may be excreted into the urine are bilirubin, bile salts, urobilin and urobilinogen.

An excess of *bilirubin* (bilirubinuria) occurs in both obstructive (intrahepatic and extrahepatic) and hemolytic jaundice. But since the bilirubin giving a direct van den Bergh reaction is more diffusible than the relatively poorly diffusible pigment-complex giving the indirect reaction, it appears earlier in the urine in obstructive than in hemolytic jaundice when the "threshold" of bilirubin in the blood (about 1.6 mg. per 100 cc.) has been exceeded. Consequently, clinical jaundice usually appears later in hemolytic than in obstructive jaundice, and bilirubinuria may be absent in spite of relatively high grades of hyperbilirubinemia. In conditions of moderate hepatic damage, incomplete biliary obstruction, cholangitis and hemolytic jaundice, the liver is unable to metabolize all of the bilirubin absorbed from the intestine and some consequently passes into the blood with excretion into the urine when the threshold has been passed.

The *bile salts* are those of the series of cholic and deoxycholic acids and their derivatives. The acids are decreased in the bile in clinical states of extensive hepatic damage, and especially in total bile stasis, from complete biliary obstruction present for a week or longer. When the salts are absent from the urine in the presence of bilirubinuria, which may occur in hemolytic or acholuric jaundice, the state is termed "dissociated jaundice."

Urobilin occurs normally in the urine only in traces. Bacterial activity or putrefaction is necessary for the oxidation of urobilinogen into this pigment. This occurs normally in the intestine but may also occur in the biliary passages as the result of infection. Under the circumstances, it is particularly likely to occur in the urine in jaundice due to obstruction associated with infection of the bile ducts.

Urobilinogen occurs normally in the urine to the extent of about 0.2 to 0.29 mg. per 100 cc. and a positive reaction may occur normally in dilution up to 1:20; a positive reaction with higher dilutions is considered indicative of the presence of excessive amounts as is, likewise, more than 1 Ehrlich unit per 100 cc. by Watson's method. The morning urine is likely to contain more than that passed during the day but the rate of elimination varies so much from hour to hour and day to day that all estimations should be made on twenty-four hour specimens, repeated for at least five consecutive days, before a negative reaction is accepted.

Increased amounts of urobilinogen and urobilin may be found in states of excessive hemolysis, hepatocellular jaundice (hepatitis, acute and subacute hepatic necrosis, toxemia of pregnancy), hepatic disease without jaundice (portal cirrhosis, congestive heart failure, pneumonia, the septicemias and malaria) but an excess of urobilinogen in the urine rarely, if ever, occurs in complete obstructive jaundice unless infection is present.

Urobilinuria and urobilinogenuria, therefore, are not pathognomonic of any single disease since their chief diagnostic significance, apart from their occurrence in hemolytic anemia, pernicious anemia and chronic hemolytic jaundice, is that some bile pigment is entering the intestine and that the functional activity of the liver is either considerably impaired or that there is active infection of the bile ducts. When properly interpreted, a determination of urobilinogen excretion is of great value not only in relation to hepatic function but also in differentiating between complete and incomplete obstruction to the flow of bile. This may be helpful in distinguishing between jaundice due to a stone in the common duct and that due to neoplasm of the duct or head of the pancreas. In the latter, a simultaneous determination of the urobilin content of the feces is of great value.

Diazo Substance. In typhoid fever, measles, tuberculosis and less frequently in other conditions, the urine contains a substance which, when treated with the Ehrlich diazo reagent, produces a characteristic red color constituting a positive diazo reaction. The substance responsible for this reaction is probably in the nature of a phenol, or phenol derivative, but its exact identity is not known. Urine which responds to the diazo test will be found always to give the permanganate test for urochromogen.

Practically all cases of typhoid fever give a positive reaction which first occurs about the fourth or fifth day of the disease. It begins to decrease about the end of the second week and to disappear during the third week, its early disappearance being considered of favorable prognostic import and its reappearance indicative of a relapse but not a complication. If negative upon successive days during the first to the third week of illness, typhoid fever is almost certainly absent so that the test may be said to be "negatively pathognomonic."

A positive diazo reaction is also frequently observed in measles prior to and during the eruptive stage; since it does not occur in rubella (German measles) it may be an aid in differential diagnosis.

Positive reactions may also occur in pulmonary tuberculosis where the continued presence of the diazo substance is of grave prognostic import, even when the physical signs are slight. After it once appears it generally persists more or less intermittently until death, the average duration of life after its appearance being about six months.

The diazo substance has been occasionally observed, although much less frequently, in scarlet fever, erysipelas and typhus fever, and rarely in diphtheria, rheumatic fever and pneumonia. Positive reactions may also occur in nonfebrile disorders such as carcinoma of the stomach, leukemia, cirrhosis of the liver, congestive heart failure and after the administration of certain drugs such as alcohol, creosote, cinchophen, the salicylates, menthol, opium, etc.

Indican. Indol is produced in the intestines by the action of bacteria on tryptophane. It is oxidized into indoxyl and absorbed with transformation, probably in the liver, into an ethereal sulfate by combining with sulfuric acid, known as indican, or indoxyl potassium sulfate, which is eliminated in the urine. Because of its origin, indican may be assumed to be an index of the putrefaction of protein and especially in the small intestine.

Under *normal* conditions, from 4 to 20 mg. are eliminated in the twenty-four hour output of urine but this varies with the amount of protein ingested, being particularly high in an excessive diet of meats.

Indicanuria is especially observed in conditions producing increased intestinal putrefaction such as intestinal obstruction, paralytic ileus, generalized peritonitis, typhoid fever, cholera, achlorhydria and hypochlorhydria (although curiously also in gastric ulcer with hyperchlorhydria), obstructive jaundice, pernicious anemia, sprue and pellagra with loose, putrid stools and, rarely, in simple constipation. It is also observed in association with bacterial decomposition of tissue proteins and purulent exudates, as in gangrene, empyema, pulmonary suppuration, large tuberculous cavities, bronchiectasis, etc. But because of improved and more direct diagnostic methods, the determination of urinary indican is not now resorted to as frequently as formerly.

Chlorides. These are mainly in the form of sodium chloride which, after passage through the glomeruli, is largely reabsorbed through the tubules to maintain a normal concentration in the whole blood of about 450 to 500 mg. per 100 cc., or 550 to 650 mg. per 100 cc. of plasma, the normal threshold being 560 to 570 mg. per 100 cc. of plasma, below which excretion decreases and finally ceases. Sodium chloride is derived from foods and is much affected by diet, the total urinary excretion ranging from 10 to 16 gm. in twenty-four hours. As expected, the urinary excretion of sodium chloride is reduced in starvation states as likewise by excessive elimination through the skin (excessive perspiration) or gastro-intestinal tract (vomiting or diarrhea).

A decrease of urinary chloride also occurs in all conditions associated with a decrease of blood chlorides (hypochloremia), with the notable exception of Addison's disease which is the basis of a test proposed by Cutler for its diagnosis. Thus a decrease is commonly observed in pyloric stenosis due to vomiting; in pneumonia until the crisis when excretion rapidly returns to normal; in other acute infections in which the decrease is due to reduced food intake and impaired glomerular permeability and especially those resulting in the formation of large exudates which "lock up" the chlorides (pleurisy, etc.) which is followed by increased elimination during their absorption; in congestive heart failure with edema or anasarca; in glomerulonephritis and nephrosis especially with edema in which the glomeruli are less permeable or tubular reabsorption reduced; in extensive burns and to some extent in anemia and carcinoma of the stomach. According to some investigators, urinary excretion is apt to be extremely low in severe diabetes insipidus but Blotner [29] has recently reported a total excretion within normal limits in two cases with no appreciable influence by pituitrin therapy or restricted fluid intake providing a normal amount of salt was ingested.

Phosphates. Phosphates are largely derived from foods and are absorbed from the intestines under the influence of the same factors which influence the absorption of calcium and its utilization in the body. A marked diminution in the alkalinity of the small intestine favors the absorption of phosphates but since a large amount is secreted in the large intestine, reabsorption is largely from this area, being inversely proportional to the excretion of calcium. Vitamin D appears to increase the degree of both absorption and utilization. Under normal conditions about 30 per cent of ingested phosphate is eliminated in the feces and 70 per cent in the urine, chiefly in the form of the acid salt (BH_2PO_4) amounting to about 2.5 to 3.5 gm. per twenty-four hour urine, about two-thirds of which occurs as *alkaline phosphates* of sodium and potassium and one-third as *earthy phosphates* of calcium and magnesium ("amorphous phosphates").

"Phosphaturia" is merely evidence of a diminished acidity of the urine, or of an increase in the proportion of phosphoric acid eliminated as earthy phosphates and may follow the excessive ingestion of fruits or occur in neurasthenia or hysteria.

Increased elimination of urinary phosphates commonly occurs in hyperparathyroidism (osteitis fibrosa diffusa), in alkalosis (increase of alkaline phosphates), in acidosis, as one of the compensatory mechanisms for aiding in the maintenance of acid-base balance, and after the administration of parathyroid hormone.

Deficient urinary elimination of acid phosphate, however, with its consequent retention in the blood and tissues, is an important factor in the production of acidosis in nephritis. In other words, while acidosis may be present in the absence of inorganic phosphate retention, it is almost invariably present when such retention exists.

A diminution in elimination of phosphates occurs in parathyroid tetany, in rachitis from inadequate absorption and sometimes after anesthesia by ether, chloroform or ethylene secondary to their hyperglycemic effects.

Drugs and Poisons. Many chemical agents which may be administered therapeutically or taken as poisons either accidentally or with suicidal intent, are excreted in the urine. Qualitative or quantitative tests for them may be required for the diagnosis of poisoning or for therapeutic guides in dosage. For this purpose relatively simple tests are available for the detection of barbital, acetanilid, phenacetin, antipyrine, chloral hydrate, morphine, hexamethylamine, bromides, iodine, phenol, quinine, salicylates, aspirin and the sulfonamides, although a determination of the blood concentrations of the latter is to be preferred.

Of special importance is the detection of poisoning due to lead, arsenic and mercury. Excretion of these, however, is usually irregular and especially in chronic intoxications. Estimations based upon the examination of random samples of urine are not always satisfactory; samples of twenty-four hour urine are preferred and tests on different days may be required. The *normal urinary lead* varies according to the method employed but by chemical procedures is stated to vary from 0.010 to 0.100 mg. per 1000 cc.; that of *arsenic* varies from 0 to 0.15 mg. per twenty-four hour urine, while chemical methods may show no *mercury* at all unless the individual has been exposed to contact with it.

The determination of urinary bismuth, however, is advisable as a therapeutic guide in its administration in the treatment of syphilis as the dosage should be such as to yield at least 2 to 4 mg. metallic bismuth in the daily total urine over long periods of time.[30] The same is true in relation to the treatment of syphilis with the organic arsenicals although not to the same extent since the compounds are given intravenously without involving the question of absorption from the muscles or gastro-intestinal tract.[31]

MICROSCOPIC CHANGES

The results of careful and properly conducted microscopic examinations of urine for casts, erythrocytes, leukocytes, epithelial cells and other organized substances possess so much clinical value that they should be part of every routine examination. With a few exceptions, however, the unorganized constituents, such as various crystals and amorphous deposits, possess much less clinical significance and frequently none at all (Table 18) except in the case of crystals of the sulfonamide compounds.

TABLE 18. SUMMARY OF THE CLINICAL INTERPRETATION OF MICROSCOPIC CHANGES IN THE URINE

	Normal	Abnormal Changes
Casts	Hyaline casts vary from none to 5000 (averaging about 1040) in 12-hour concentrated night urine (Addis). By ordinary methods of examination usually absent except occasional hyaline casts in the urine of elderly individuals.	*Cylindruria* due to the presence of hyaline, granular, fatty, waxy, blood, epithelial, leukocytic and renal failure casts. Their presence indicative of the condition of the tubules but not necessarily of the kidney as a whole. May be absent in extensive destruction of the kidneys. Occur in all types of acute and chronic nephritis. Blood casts especially characteristic of glomerular nephritis. Epithelial casts common in degenerative or tubular nephritis. Leukocytic or pus casts especially in suppurative nephritis.
Leukocytes and Pus	Few present; increased by contamination with vaginal discharges.	Excess of leukocytes common in acute nephritis and conditions producing hematuria. *Pyuria* indicative of suppuration in the genito-urinary tract, in pyelitis, pyelonephritis, tuberculosis, cystitis, urethritis, seminal vesiculitis, etc.
Epithelium	Few cells always present. Three main varieties: (1) small round; (2) transitional and (3) squamous.	Of but little clinical significance. Small round tubular cells especially in degenerative or tubular nephritis; transitional cells especially in cystitis; squamous cells in cystitis and from vaginal discharges.
Mucus	Traces.	Increased in cystitis, oxaluria and other irritations of the genito-urinary tract. Should not be confused with "gonorrheal shreds."
Spermatozoa	Usually absent but may occur after nocturnal emissions, prolonged continence and in both sexes after coitus.	After convulsive seizures; after massage of the seminal vesicles; spermatorrhea.
Tissue fragments	Absent.	Broken-down tumors in the urinary tract.

TABLE 18. SUMMARY OF THE CLINICAL INTERPRETATION OF MICROSCOPIC CHANGES IN THE URINE (continued)

	Normal	Abnormal Changes
Crystals	Commonly: uric acid and urates: calcium oxalate; phosphates; calcium carbonate; ammonium biurate; sulfonamide compounds during treatment. Rarely: calcium sulfate; cholesterol; hippuric acid.	Usually of but little diagnostic or prognostic significance. Excess of uric acid and urates may occur in disturbances of uric acid metabolism and in the concentrated urine of febrile states. *Oxaluria* may be due to diet; common in nervous dyspepsia and neurasthenia; great excess with clumping suggestive of calculus and especially in association with hematuria. *Phosphaturia* usually due to diet and nervous states. *Tyrosinuria* due to autolysis of liver and inefficient deamination of amino acids (acute yellow atrophy of the liver; eclampsia; obstructive jaundice of long standing due to stone; occasionally extrahepatic autolytic states). *Sulfapyridine*, *sulfadiazine* and *sulfathiazole* crystals producing uroliths and hematuria.

The Importance of Technic; Method of Addis. Since hyaline casts and erythrocytes are especially apt to dissolve and disappear in alkaline urine, it is important for the specimens to be kept at a low temperature or preserved by the addition of 4 drops of formalin or 5 grains of boric acid for each 4 ounces. Undoubtedly samples of twelve to twenty-four hour collections are preferred, as specimens of any single urination are usually unsatisfactory. A high protein diet tends to increase the acidity of urine which in turn preserves casts before voiding.

The sediment is best obtained by centrifuging well-mixed urine. Otherwise urine may be allowed to stand in a conical glass for six to twelve hours for the collection of sediment but best results are undoubtedly obtained by centrifuging before the formation of heavy deposits of crystals which, unless removed, may obscure casts, erythrocytes and leukocytes.

Since hyaline casts tend to disappear in urine of low specific gravity and to dissolve in that of low sodium chloride and reduced *p*H concentration, Addis [32, 33] has advised that when the diagnosis of nephritis is involved, all of the urine voided over a period of 12 hours, between 6 P.M. and 6 A.M., be carefully collected and accurately measured following abstention from fluids during the day. With this urine a count is made of the casts, erythrocytes and other cells (leukocytes and epithelial cells together) by a method which Addis has described expressing the approximate total numbers of these elements in terms of the twelve-hour night urine. This method results in the microscopic examination of concentrated urine with greatly improved chances for detecting hyaline casts although the latter may not be found if the blood urea concentration is high, due to its diuretic effects.

However, the method of Addis, because of the time and work involved, cannot be employed as a routine procedure but it is undoubtedly of value in the diagnosis of incipient and chronic nephritis where quantitative changes in the excretion of casts, erythrocytes, leukocytes and epithelium are of clinical importance. While routine qualitative examinations may suffice for general clinical purposes, the results are subject to so much variation that a quantitative microscopic method is always to be preferred. In this connection the method of Exton [34] for quantitative microscopic urinalysis is highly recommended as a routine procedure since it is not only rapid and easy but much more accurate than current ordinary methods. A special counting chamber is required and the results may be expressed in terms of the number of casts, erythrocytes, leukocytes, etc., per cubic centimeter of urine.

In other words, if urinary sediments are subjected to microscopic study at all, the examinations should be conducted with sufficient skill and care to render the results worth while. After the first routine examination it is well for physicians to manifest their interest by specifying the element or elements in which they are particularly interested so that laboratory technicians will know that the results of their examinations will be awaited with interest and appreciation.

Normal Results. By the method of Addis the normal urine of young adults may show 0 to 5000 (average 1040) hyaline casts in the twelve-hour concentrated night urine; epithelial casts are only very rarely found. Erythrocytes vary from 0 to 425,000 (average 65,760) and leukocytes and epithelial cells, counted together, from 32,400 to about 1,000,000 (average 322,550). According to Lyttle,[35] the urine of normal children may show slightly more casts but slightly fewer erythrocytes and leukocytes than the average excreted in twelve hours by adults.

It is apparent, therefore, that the difference between the microscopy of urinary sediments of normal individuals and those with Bright's disease is quantitative, which adds greatly to the value and clinical significance of quantitative methods of examination.

When examined by current qualitative methods employing drops of sediment picked up from the bottoms of bottles or conical glasses, casts are not ordinarily found under normal conditions except an occasional hyaline cast in the urine of individuals past middle life. A few leukocytes are invariably present, especially in the urine of women; likewise an occasional erythrocyte and many epithelial cells.

The unorganized elements usually found in acid urines are uric acid, amorphous urates and calcium oxalate, while in alkaline urines, phosphates, calcium carbonate and ammonium biurate are the usual findings. However, the characteristic crystals of acid urine may remain after the urine has become alkaline, while the alkaline crystals may be precipitated in a urine which is still acid.

Casts. The presence of casts in the urine is designated as *cylindruria*. They are molds of the interior of tubules and indicate the condition of those in which they are formed although not necessarily of the kidney as a whole. Nor is there any constant relation between the renal lesions of Bright's disease and the production of casts or the excretion of erythrocytes. Indeed, they are not apt to be formed at all when there is extensive destruction of renal tissue as in suppurative nephritis or new growths; furthermore, the urinary sediments of patients with the most severe renal lesions of nephritis may show no casts at all. As observed by Addis, hyaline casts may be found in the concentrated night urine of normal young adults but only rarely by ordinary methods of examination and then usually in the urine of elderly persons in whom their increased production and excretion is indicative of arteriosclerotic nephritis. Otherwise, however, the discovery of casts in the urine along with albumin usually has the same clinical significance as albuminuria of renal origin and their presence should never be ignored.

Hyaline casts are apparently composed of an altered protein compound of chondroitin-sulfuric acid. The source of this protein is not definitely known but is thought to be a product of perverted activity of tubular epithelium, a product of degeneration of these cells, or an exudate from the blood in the glomeruli undergoing inspissation in the tubules. If there is no exfoliation of the tubular epithelium, the casts are of the narrow type, but if the epithelium is lost they are broad and therefore indicate more extensive tubular damage.

As previously stated, a few casts may be found in concentrated night urine of normal individuals by the Addis quantitative method of examination and in the urine of elderly persons with arteriosclerotic nephritis by ordinary methods of examination. They may occur, however, in increased numbers after ether anesthesia, severe exercise and other factors producing renal congestion. Under these conditions *cylindroids* may also occur along with hyaline casts and have practically the same significance.

Hyaline casts may also occur during the course of prolonged febrile diseases due to cloudy swelling of the kidneys and of course in acute, subacute and chronic glomerular nephritis in which, however, blood casts are of more significance and especially in the initial or acute stages. They may also occur in nephrosis or tubular nephritis but are less significant than granular and epithelial casts. They are particularly important in arteriosclerotic or chronic interstitial nephritis.

Finely and coarsely *granular casts* are apparently of the same composition as hyaline casts, with granules derived from degenerative changes in the tubular epithelium or altered blood pigment. They probably are never produced in normal kidneys with the result that their presence is usually indicative of glomerular nephritis or nephrosis.

Fatty casts are those containing droplets of fat, of either isotropic or anisotropic properties, due to fatty degeneration of epithelial cells, or composed of fat extruded as such from glomerular tufts where fatty degeneration may occur, with imbedding of droplets in hyaline or granular casts or their adherence to them. They are not found under normal conditions and are especially apt to occur in chronic glomerular nephritis and so-called lipoid nephrosis.

Waxy casts are especially likely to occur in amyloid disease of the kidneys and likewise in chronic glomerular nephritis but are not as distinctive as sometimes stated.

Blood casts are so characteristic of glomerular nephritis in which hemorrhage is so likely to be the first sign to appear, and the last to disappear, that Addis prefers to designate it as "hemorrhagic Bright's disease." The blood casts are due to glomerular bleeding, with the passage of a coagulable protein, probably fibrinogen, which cements the erythrocytes into coagulated molded masses in the tubules. They also include yellow or orange colored casts full of granules or agglutinated masses and shadows due to hemolysis of the cells.

Epithelial casts are those composed of swollen and degenerated tubular cells and are especially indicative of tubular nephritis or nephrosis (degenerative nephritis) occurring in the late toxemias of pregnancy, general infections, jaundice, severe malaria, amyloid disease and poisoning with mercury, uranium, chromium, etc. When of the broad type, indicative of severe tubular injury, uremia is almost sure to develop (Addis).

Leukocytic or pus casts, composed almost entirely of pus cells, are uncommon and indicate the presence of suppurative nephritis, usually a pyelonephritis. True *bacterial casts* are rare and likewise indicate the presence of suppurative nephritis.

Renal failure casts are formed in the terminal dilatations of the ducts of Bellini, just at their joint of entry into the pelvis of the kidney, and occur as loose packings of heterogenous debris originating in the upper tubules. They are characterized by fragility and occur most frequently in oliguria or upon a sudden output of urine following a period of reduced excretion.

There are, therefore, many different kinds of casts, as determined by their morphology. But, as stated by Addis and Oliver,[33] they may be divided into two groups insofar as the different forms of Bright's disease are concerned. In the first group, with specific characteristics other than their appearance, are to be placed hyaline casts, distinguished by their chemical nature; the blood casts, characterized and separated from all others by the nature and site of the inflammatory processes resulting in their formation; and the renal failure casts, the formation of which is determined by the site of their origin and a condition of decreased function of the renal tissue. In the second group, embracing less well-defined types of various origin and significance, are to be included the granular, epithelial, leukocytic and fatty casts.

Erythrocytes. When blood in the urine (*hematuria*) is not due to contamination from menstrual discharge it is always pathologic although, as previously stated, an occasional erythrocyte may be found under normal conditions amounting to an average of about 65,750 per twelve-hour concentrated night urine but reaching as high as 425,000.[36] The urine of healthy infants sometimes contains an increased number for weeks at a time which, however, may be due to slight renal injury. Erythrocytes are not ordinarily difficult of detection in urinary sediments by microscopic examination but, on the other hand, inexperienced workers may mistake them for yeast and other cells. If in doubt a chemical test for hemoglobin should be conducted, but even with a chemical test a few erythrocytes may be present when negative reactions are observed.

Since erythrocytes, like hyaline casts, may dissolve and disappear in alkaline urines as well as in those with low sodium chloride concentration, it is always advisable to examine freshly voided urine when hematuria is suspected.

Blood in the urine is always a very important finding. The source may be renal or extrarenal with many different causes previously discussed. In glomerular nephritis, hematuria is always likely to be the first sign to appear and the last to disappear. On the other hand, it is unusual as a result of passive congestion of the kidneys from cardiac failure. Subacute bacterial endocarditis with focal glomerular nephritis is the only form of heart disease in which striking hematuria may occur.[36]

Since crystals of sulfapyridine and sulfathiazole in the terminal uriniferous tubules may cause bleeding, it is always advisable carefully to examine urinary sediments not only for such crystals, but likewise for erythrocytes in the case of individuals to whom these compounds are administered in large doses.

Leukocytes and Pus. As previously stated, a very few leukocytes occur in normal urine, particularly when mucus is present, amounting to about 3 to 5 per field (6-mm. objective with 5× eyepiece) upon ordinary examination of sediment. By the Addis method the total leukocytes and epithelial cells together, in concentrated twelve-hour night urine of normal individuals, vary from 32,400 to about 1,000,000, averaging from 300,000 to 400,000. Their numbers are increased by contamination with vaginal discharge. When numerous and especially when occurring in clumps, they constitute pus and their presence is designated as *pyuria.*

Pyuria usually produces proteinuria and it has been estimated that 80,000 to 100,000 leukocytes per cubic centimeter of urine add about 0.1 per cent of albumin. If the latter is present in higher proportions, the excess is probably derived from the kidney. When pus is abundant the freshly voided urine is turbid and a white sediment forms, resembling that of amorphous phosphates. On the other hand, turbidity of fresh urine may be due to phosphates which immediately dissolve with clearing of the urine following the addition of glacial acetic or nitric acids.

Pyuria indicates suppuration in some part of the genito-urinary tract due to pyelitis or pyelonephritis, tuberculosis of the kidneys, cystitis, urethritis, prostatitis, seminal vesiculitis, etc. Chronic cystitis usually produces by far the largest amount of pus; a considerable amount appearing suddenly, usually originating from a ruptured abscess somewhere in the genito-urinary tract. Trauma, including that of instrumentation, may produce slight temporary pyuria. Of course an excess of leukocytes may occur in acute nephritis or any other cause for hematuria. In general, the source of increased numbers of leukocytes and pus may be determined only by other urinary findings and clinical signs.

Epithelial Cells. A few epithelial cells from various parts of the urinary tract occur normally in the urine. A marked increase indicates exfoliation due to some pathologic condition at the site of their origin as, for example, exfoliation of tubular epithelium in degenerative or tubular nephritis. It is sometimes, but by no means always, possible to designate their probable source according to size and morphology, notably in the case of the vaginal epithelium. Epithelial cells are, however, subject to considerable alteration from degenerative changes, especially cloudy swelling and fatty degeneration.

A large excess of *small round or polyhedral cells,* which are uncommon in normal urine, are apparently derived from the tubules and, especially when occurring in epithelial casts, may indicate degenerative nephritis (tubular nephritis or nephrosis). They are nearly always fatty and may show the presence of yellow granules or altered hemoglobin in renal infarction, chronic passive congestion of the kidneys from cardiac failure, and in hemochromatosis.

Transitional cells (pear or spindle shaped) are usually derived from the pelves of the kidneys, ureters or bladder. Similar cells may be derived from the prostate gland and seminal vesicles.

Squamous or pavement cells, when small, thin and scale-like, may originate in the bladder and be particularly numerous in cystitis. When especially large, thin, and angular, their source is usually the vagina and, when present in large numbers associated with pus, indicative of contamination of the urine with leukorrheal discharge.

With these possible exceptions, a study of the epithelium in urinary sediments has little if any clinical value but technicians should at least report on the type of cell or cells predominating in them.

Mucus. Long ribbon-like strands of mucus with tapering ends and faint longitudinal striations form part of the nubecula of normal urine but may be increased in chronic cystitis or any irritation of the urinary tract and especially in oxaluria. They are sometimes mistaken for hyaline casts or cylindroids. However, being microscopic in size they should not be mistaken for "gonorrheal shreds" which are macroscopic and composed of a matrix of mucus in which many epithelial and pus cells are imbedded.

Spermatozoa. These are generally present in the urine of men after nocturnal emissions, massage of the seminal vesicles, epileptic or other convulsive seizures, prolonged continence, prostatic hypertrophy and in spermatorrhea. They may be found in the urine of both sexes following coitus.

Tissue Fragments. Small fragments of tissue may occur in the urine and especially in broken-down tumors of the bladder and renal pelves. For this reason it is always advisable to tease out blood clots for possible fragments. When found, sections should be prepared and examined microscopically unless diagnosis has been previously established.

Crystals. As a general rule the unorganized portions of urinary sediments have but little diagnostic or prognostic significance. Most crystals and amorphous substances occur normally in solution which has become precipitated either because these crystals occur in excessive amounts or, more frequently, because of some alteration in the urine upon standing.

Uric acid crystals possess no clinical significance unless they occur in large amounts in freshly voided urine, when they are suggestive of disturbances of uric acid metabolism or of a calculus in the urinary tract, and especially if associated with hematuria. *Urates* are commonly increased in the concentrated urine of febrile states.

An excess of *calcium oxalate* (oxaluria) is suggestive of possible calculus, especially if the crystals occur in clusters or clumps. This may also result from a diet of vegetables rich in oxalic acid, particularly tomatoes, spinach, rhubarb and asparagus. Oxaluria, however, may also occur in digestive disturbances with carbohydrate fermentation and especially in nervous dyspepsia and neurasthenia. It is sometimes associated with an excess of mucus in the urine from irritation and in men with spermatorrhea.

Phosphates occurring in excess constitute phosphaturia due to the excessive excretion of phosphoric acid.

Ammonium biurate is found only when free ammonia is present from decomposition of the urine due to its retention in cystitis or after standing.

Leucine and *tyrosine,* which are cleavage products of the protein molecule, may occur in states associated with marked autolysis of the tissues and especially in severe fatty degeneration of the liver as in acute yellow atrophy, in phosphorus, chloroform, carbon tetrachloride and arsphenamine poisoning and in some cases of eclampsia. Their production is ascribed to inefficient deamination of amino acids brought to the liver through the portal circulation and in part to autolysis of its tissues. Others believe that tyrosinuria originates chiefly from autolyzed liver tissue and may occur in the absence of apparent disturbance in the metabolism of amino acids with significance in the diagnosis and prognosis of diseases of the liver and biliary passages, such as subacute atrophy of the liver, degenerating neoplasms of this organ, toxic hepatitis and, rarely, in patients with obstructive jaundice of long standing due to stone. Tyrosinuria has not been observed in uncomplicated inflammatory lesions of the bile ducts but has been noted occasionally in the presence of extrahepatic autolytic conditions like degenerating tumors of the lung and extensive sloughing of the skin. During the phase of recovery, tyrosine may disappear from the urine. Increasing tyrosinuria is assumed to indicate a rapidly progressive degeneration of the hepatic parenchyma.

Cystine, a sulfur-containing amino acid and a normal product of intermediary protein metabolism, does not occur in the urine of normal individuals except possibly in minute traces. Cystinuria is one of the "inborn errors of metabolism" and while uncommon, occurs more frequently than alkaptonuria. Apparently it is of but little clinical significance except in children in whom it may be associated with evidences of severe malnutrition. Because of the poor solubility of cystine, individuals with cystinuria may develop stones in the kidneys or bladder, a phenomenon which may be prevented or inhibited by a low-protein diet with the administration of alkalies. Urine containing cystine, on standing, gives off the characteristic odor of hydrogen sulfide, the proportion of sulfur present in the neutral form being increased.

It was not long after the introduction of *sulfapyridine* into clinical use that the first report of hematuria appeared. Experimental studies revealed that the drug, when given in large doses, might precipitate in the urine in the form of crystals. These, by forming concretions in the kidney and lower urinary tract, produce mechanical damage and bleeding, and also obstruction to the flow of urine. Studies on the fate of sulfapyridine in the body have demonstrated that a varying proportion of the drug is converted to acetylsulfapyridine, and many authorities believe that the concretions observed in the urinary tract are composed largely of the latter substance.[37,38] Some time later, when *sulfathiazole* and *sulfadiazine* came into general use, similar disturbances in the urinary tract were noted following the administration of large doses. Here again, there was evidence that the drugs were excreted partially unchanged and partially as the acetyl derivatives. It is important, therefore, for technicians to be able to recognize the crystals of these compounds occurring in the urine and for physicians to request examinations for them when the compounds are administered in large doses and especially over long periods of time. Sulfanilamide and neoprontosil, however, do not ordinarily produce crystals of acetylated compounds in the kidneys and consequently they are less likely to occur in the urine.

The clinical interpretation of *bacteriologic examinations* of the urine is discussed in Chapter 15 and of *parasitologic examinations* in Chapter 17.

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3

THE CLINICAL INTERPRETATION OF BLOOD CHEMISTRY EXAMINATIONS

In no other field has clinical chemistry contributed as much aid in the diagnosis of diseases and as a guide in their treatment as in the clinical examination of the blood for its various constituents concerned in the metabolism of carbohydrates, proteins, lipids and various inorganic substances with special reference to chlorides, calcium and phosphorus. This is amply indicated by a glance at Table 19 summarizing the normal range of a large number of substances to be found in the blood, each of which may be altered under normal or abnormal conditions to be considered separately in this chapter.

PRINCIPLES

Analytical methods are available of sufficient accuracy for clinical purposes when *properly conducted* but all require the cooperation of the physician with special reference to the preparation of the patient before blood is taken as well as for the proper collection of the latter. As a general rule digestion has a profound influence upon most of the chemical constituents of the blood, requiring that the latter should usually be taken after a period of fasting and preferably in the morning before breakfast. Furthermore, the physician should always deliver the specimen as promptly as possible to the laboratory (especially in the case of blood sugar determinations), since spontaneous changes may occur and interfere with the accuracy of results and their clinical interpretation. It scarcely requires emphasis that the physician must know the normal range for each constituent. Also, the normal range varies in the case of many substances according to the method of analysis, which requires that the laboratory should state the normal range in relation to the method employed.

Some determinations require whole blood while others are conducted with plasma or serum. In Table 19 the required specimen is specified with each constituent for the guidance of the physician. *Whole blood* should be collected with an anticoagulant and for this purpose sodium oxalate is recommended in about 0.030 gm. for 5 to 15 cc. of blood. This oxalate is less soluble than potassium oxalate so that an excess does not interfere with protein precipitation in the preparation of protein-free filtrates in the laboratory. As a general rule, 3 to 5 cc. of blood is sufficient for any single determination except in the case of guanidine which requires 8 cc. Micromethods are available for some of the determinations (like blood sugar), require only a few drops of blood obtained from a finger and are especially useful in the case of infants and young children. *Plasma* is also collected in the same manner, employing sodium oxalate for the prevention of

TABLE 19. SUMMARY OF NORMAL BLOOD CHEMISTRY VALUES

Constituent	Required for Determination	Average Range (mg. per 100 cc. for adults unless otherwise stated)
Glucose (fasting)	Whole blood	80–120 (Folin-Wu); newborn 55–75
CO_2 capacity of the plasma	Plasma	55–80 volumes per cent
Total fixed base	Serum	15–15.5
*p*H	"	7.3–7.5 (average 7.35)
Total protein	Plasma	6.0–8.0 (gm. per 100 cc.)
Fibrinogen	"	0.2–0.4 (gm. per 100 cc.)
Albumin	"	3.6–5.6 (gm. per 100 cc.)
Globulin	"	1.3–3.2 (gm. per 100 cc.)
Albumin-Globulin ratio	"	1.5–2.5:1
Total nonprotein nitrogen	Whole blood	25–35
Urea	" "	20–30
Urea nitrogen	" "	9–17
Creatinine	" "	1–2
Uric acid	" "	2–4
Amino acid nitrogen	" "	5–8 (plasma 3.75–5.56)
Ammonia nitrogen	" "	0.1–0.2
Undetermined nitrogen (residual)	" "	5–18
Total lipids (fasting)	Plasma	400–600
Neutral fat	"	Adults: 0–370 (average 170) Children: lower (average 100–182 mg.)
Fatty acids	"	190–450 (average 353)
Phospholipids	"	60–350 (average 196)
Free cholesterol	"	40–50 (average 47)
Cholesterol ester	"	190–200 (average 192)
Bilirubin	Serum	Van den Bergh {direct: negative; indirect: 0.1–0.25} Icterus index: 4–6 units
Carotene	Plasma or serum	0.1
Prothrombin (time)	Plasma	10–20 seconds (method of Quick)
Lactic acid	Whole blood	6–20
Chlorides (as Cl)	Serum	340–370
Chlorides (as NaCl)	Plasma	570–620
Sulfates (inorganic)	Serum	2.5–5.0
Sodium	"	315–340 (average 330)
Potassium	"	16-22 (average 19)
Magnesium	"	1.8–3.6
Calcium (total)	"	8.5–11.5
Phosphorus (as inorganic phosphate)	"	Adults: 3–4.5 (average 3.5) Children: 4.0–6.0 (average 5.0)
Iron	Whole blood or serum	Organic (whole blood): average 52 men; lower in women (average 45) Inorganic (serum): 1–0.17
Copper	Whole blood	0.14–0.15

TABLE 19. SUMMARY OF NORMAL BLOOD CHEMISTRY VALUES (continued)

Constituent	Required for Determination	Average Range (mg. per 100 cc. for adults unless otherwise stated)
Iodine	Plasma or serum	4 to 10 (av. 6–7) micrograms per cent
Alkaline phosphatase	Serum	Bodansky units { Adults: 1.5–4.0; Children: 5–14
Acid phosphatase (units)	"	Less than 3
Lipase	"	1–1.5 cc. of N/20 NaOH
Amylase	"	70–200 mg. glucose from starch by 100 cc.
Guanidine	"	0.032–0.48 (average 0.40)
Phenols and other organic compounds	"	Xanthoproteic reaction (0 to 50)
Bromine	"	0.33–1.73

coagulation, while *serum* requires the collection of blood in a test tube without the prevention of coagulation by oxalate, heparin or other anticoagulants.

GLUCOSE

Normal Blood Glucose. Under normal conditions the chief source of the glucose of the blood is the sugars and starches ingested with foods. The disaccharides and polysaccharides are converted into monosaccharides, with glucose as the most important end product. The glucose is not absorbed to any appreciable extent from the stomach and only to a slight degree from the colon but chiefly from the small intestine, the rate depending upon phosphorylation under the influence of the adrenal cortical hormone. Small amounts of glucose are also derived from certain amino acids resulting from the digestion of proteins as well as from glycerol and perhaps fat and fatty acids under the influence of the anterior pituitary hormone. An additional source is lactic acid released by the muscles and carried to the liver where it is converted into glycogen and subsequently into glucose as required.

The glucose carried to the liver is mostly converted into glycogen (*glycogenesis*) by a process of polymerization and stored. Part of this stored glycogen is utilized by the liver (*glycolysis*) but the larger part is converted into glucose as required for the maintenance of normal blood sugar. However, some glucose carried to the liver passes through this organ unchanged, to be converted into glycogen and stored in the muscles (*tissue glycogenesis*). During this phase of tissue glycogenesis inorganic phosphate is withdrawn from the blood, as it appears to be intimately related to the intermediary metabolism of glucose. Furthermore, vitamin B_1 also appears to play an important rôle, probably as a catalyst in some oxidizing capacity essential for the normal metabolism of pyruvic acid. In other words, the chief function of glycogen stored in the liver is the maintenance of normal glucose of the blood. Balance is aided by its withdrawal from the blood and oxidation with deposition as glycogen and conversion to fat in the tissues;

glycogen stored in the muscles is chiefly for their own use with no direct part in the maintenance of normal blood glucose.

In the blood the larger part of glucose is contained in the plasma and the balance in the erythrocytes. The plasma glucose may increase without a corresponding increase of corpuscular sugar; in fact, the corpuscles appear to have a diminished capacity for absorbing glucose in diabetes mellitus. Some investigators have raised the question of the advisability of determining plasma and corpuscular sugar separately but this does not appear to have any clinical value. Under the circumstances whole blood is used for the determination of glucose but the method employed is likely to be complicated by the presence in the plasma, and especially in the corpuscles, of certain nonsugar copper-reducing substances, one of which is probably glutathione.

Insulin is important for the deposition of glycogen in the liver and muscles at physiologic blood sugar levels. Among its well-established effects are (1) increased oxidation of sugar and (2) increased deposition of glycogen in muscle. Both processes can take place in the absence of insulin. Other important effects are (3) inhibition of glycogenolysis in the liver and (4) suppression of protein and fat catabolism by an indirect sparing action; this leads to a decrease both of gluconeogenesis from amino acids and of ketone body formation from fatty acids in the liver.

Blood Glycolysis. As previously stated, it is clinically important to make a blood sugar determination quite promptly after withdrawal of blood and to prevent its coagulation with sodium oxalate. This is because glycolysis or destruction of glucose may otherwise occur with erroneous results. For example, when blood is kept at 37° C., from 13 to 16 mg. of glucose are lost per 100 cc. per hour with an increase of inorganic phosphorus. At the end of six hours the glucose is practically exhausted, with nothing remaining except about 20 mg. per 100 cc. of the nonsugar copper-reducing substances. If the examination cannot be promptly made, about 0.060 gm. of sodium fluoride per 10 cc. of blood should also be added and thoroughly mixed as it tends to prevent glycolysis although the specimen is not suitable for the determination of urea under these conditions. Glycolysis does not occur after laking.

Hyperglycemia. An increase of glucose in the blood is called *hyperglycemia.* This occurs normally after a meal and especially after the ingestion of carbohydrates. For this reason most blood sugar determinations for clinical purposes should be made after a period of at least five hours of fasting and preferably in the morning before breakfast. As summarized in Table 20, hyperglycemia may be due to (1) *an increased rate of hepatic glycogenolysis* resulting in the passage of glucose into the blood at a rate too rapid for the removal of the excess by the tissues, as encountered in conditions producing an increased secretion of epinephrine, increased hydrogen-ion concentration of the blood as in ketosis due to diabetes mellitus, anesthesia, asphyxia or acidosis of other causes (fever, nephritis, dehydration), hyperthyroidism and after convulsions or severe muscular exercise, and (2) to *decreased tissue utilization of glucose* (tissue glycogenesis) as is thought to occur in the hypo-insulinism of diabetes mellitus and possibly in hypopituitarism.

TABLE 20. SUMMARY OF CLINICAL INTERPRETATION OF CHANGES IN BLOOD SUGAR

Normal: 80 to 120 mg. per 100 cc. for adults

Hyperglycemia (increased): Slight: 130–140; Moderate: 150–180; Severe: 190 or higher	Hypoglycemia (decreased): Slight: 60–70; Moderate: 40–50; Severe: 30 or less
After meals.	Overdosage of insulin.
Diabetes mellitus.	Renal glycosuria.
Acidosis due to diabetes, severe vomiting of pregnancy, anesthesia, asphyxia, acute infections like pneumonia, nephritis and dehydration.	Hyperinsulinism (pancreatic tumors or disease): imbalance of autonomic nervous system (neurocirculatory asthenia).
Convulsions (epilepsy, eclampsia).	Progressive hepatic disease (acute hepatitis, cirrhosis, carcinoma, acute yellow atrophy, etc.).
Muscular exercise.	Starvation; anorexia nervosa.
Hyperpituitarism (early acromegaly).	Pregnancy and lactation (tendency).
Hyperadrenalism (tendency).	Pernicious vomiting.
Hyperthyroidism (tendency).	Pituitary cachexia (Simmonds' disease, tumors, etc.).
Ether and chloroform anesthesia.	Cortical hypoadrenalism (Addison's disease) and adrenal apoplexy.
Administration of morphine.	Late acromegaly.
Acute myocardial infarction.	Epilepsy (some cases) and lesions of central nervous system.
Acute hepatitis (slight).	Severe muscular exercise.
Hypertension (some cases).	Hypothyroidism (tendency) and after thyroidectomy.
Pancreatitis (some cases).	Status thymicolymphaticus (tendency).
Excessive smoking (possible).	Progressive muscular atrophy (tendency).
Methyl salicylate poisoning.	"Smoke" drinkers (denatured alcohol).
Caffeine, quinine, benzedrine sulfate.	Excessive smoking (tendency).

Hypoglycemia. A decrease of fasting blood sugar is called *hypoglycemia.* It is less frequent than hyperglycemia although, as shown in Table 20, there are many causes for this state with the result that it commands much more clinical interest and attention than was formerly the case. In general terms, the causes may be (1) *decreased hepatic glycogenolysis,* as in hypothyroidism, hypo-adrenalism and pituitary cachexia; (2) *depletion of hepatic glycogen,* as in acute rapidly progressive diseases of the liver seen in yellow atrophy, infections, phosphorus and arsenical poisoning, etc., including the terminal stages of cirrhosis and (3) *increased tissue utilization of glucose,* as in hyperinsulinism from overdosage of insulin or other causes, hypopituitarism, hypothyroidism (especially after thyroidectomy), status thymicolymphaticus, progressive muscular atrophy, etc.

ACID-BASE BALANCE

As carbohydrate utilization diminishes and hyperglycemia develops, especially in diabetes mellitus, the combustion of fats increases until a point is eventually reached at which the oxidation of fatty acid becomes impaired, with the production of acetone, aceto-acetic acid (diacetic acid) and betahydroxybutyric acid (the ketone bodies), constituting the clinical state designated as *ketosis*. As the saying goes, "fat burns in the fire of carbohydrates." In the absence of carbohydrates the fat "smokes," the "smoke" being represented by the ketone bodies which are better detected by an examination of the urine than of the blood. At present, however, objections have been raised to the view that the formation of the ketone bodies is dependent directly upon this mechanism with the suggestion that they are formed only in the liver and that the antiketogenic effect of insulin and glucose cannot be related to the utilization of carbohydrate in the tissues generally but must be due to effects produced in this organ. Be that as it may, this disturbance in the intermediary metabolism of fat may result in an increase of the lipids of the blood (cholesterol, fatty acids, neutral fat and phospholipid) which constitute lipemia, shortly to be discussed in more detail.

In diabetes mellitus, however, an accumulation of ketone bodies in the blood, along with the excessive loss of water in the urine and an associated depletion of the alkali reserve or base balance of the blood, may finally result in the production of the clinical state designated as *acidosis* which is best detected by a determination of the CO_2 combining power of the plasma, drowsiness or coma almost invariably occurring when the CO_2 combining power is reduced to 28 to 10 volumes per cent.

In other words, a profound disturbance occurs in the acid-base balance or equilibrium of the blood and tissues. Normally the hydrogen-ion concentration of the blood is kept remarkably constant (7.3 to 7.5 with an average of 7.35), even in disease the range being rarely beyond 7 to 7.8, largely because of the presence of "buffers" in the blood with special reference to bicarbonates. For example, the acids formed during metabolic activity combine with bicarbonate to eliminate the CO_2 that is produced through the expired air of the lungs. Furthermore, since CO_2 is constantly formed through oxidative processes, any excess of alkali is normally transformed into bicarbonate. The blood bicarbonate, therefore, represents the main supply of base available for the neutralization of acids and constitutes the main "alkali reserve" not only of the blood but of the whole body as well, as far as clinical purposes are concerned.

But additional "buffers" are the monosodium and disodium phosphates of the body and especially in the fixed tissues with much less importance in relation to the blood. Furthermore, the plasma proteins (albumin and globulin) and hemoglobin also act in this capacity, the former constituting about one-tenth of the total buffering capacity of the blood, while the latter acts as a weak acid capable of combining with alkali or base.

Acidosis and Alkalosis. Normally *acidosis* is prevented by the elimination of volatile acids, of which carbon dioxide (CO_2) is the most important, through the lungs. As is well known, the respiratory center is remarkably sensitive to even very slight variations in the CO_2 plasma tension and changes in the *p*H of the blood and the elimination of CO_2 through the lungs is the most important and efficient mechanism for keeping the *p*H of the blood within normal limits. Additional amounts of CO_2, however, are eliminated through the gastro-intestinal tract by vomiting or diarrhea so that the excessive loss of fluids and hydrochloric acid through this channel, resulting in dehydration and hypochloremia, involves the loss of electrolytes with a disturbance of acid-base balance. Undoubtedly the kidneys rank next to the lungs in the maintenance of this normal balance not only by possessing an extremely delicate mechanism for the elimination of excessive quantities of fixed acids and bases but also because of their capacity for producing ammonia.

Acidosis, therefore, may be defined as that condition resulting from the formation or absorption of acids at a rate exceeding that of their neutralization or elimination or resulting from the excessive loss of base from the body which is of much less frequent occurrence. Acidosis may result from (1) *an increased accumulation of carbon dioxide in the blood* as from the breathing of air containing an excess of CO_2, or because of factors interfering with its elimination in the expired air of the lungs as in mechanical asphyxia, the narcoses of morphine or other opiates, severe attacks of asthma, pneumonia and especially bronchopneumonia, the terminal stages of pulmonary tuberculosis, extensive emphysema, congestive heart failure, etc.; or (2) *from states producing a primary deficiency of alkali* as in severe diabetes mellitus (especially in coma), starvation states and carbohydrate restriction, renal failure with the retention of acid radicals (phosphoric and sulfuric) in decompensated nephritis and nephrosis, pyelonephritis, nephrosis, pyelonephrosis, polycystic disease and renal tuberculosis, also in ether, chloroform and (to a lesser degree) ethylene and nitrous oxide anesthesia, dehydration and especially excessive vomiting in uremia and cyclic vomiting of children, pregnancy and its toxemias (with special reference to the excessive vomiting of eclampsia), intestinal and pancreatic fistulas, etc., as summarized in Table 21. As the carbon dioxide content of the blood increases, however, certain compensatory mechanisms come into play by means of which the body attempts to maintain the hydrogen-ion concentration of the blood within normal limits; these embrace (1) increased ventilation due to increased stimulation of the respiratory center, (2) increase in alkali reserve, (3) increased ammonia formation and (4) increased urinary acidity.

Alkalosis is a state in which either excessive amounts of acid are lost without a comparable loss of base (alkali), or alkali is formed or supplied at a rate exceeding that of its neutralization or elimination. Consequently alkalosis may result from either (1) *a primary decrease in the carbon dioxide of the blood* due to hyperventilation as in hysteria, fever, high external temperatures, ascending high altitudes (anoxic anoxemia) and encephalitis, or (2) *a primary increase in blood bicarbonate (alkali reserve)* due to excessive loss of hydrochloric acid from the stomach in severe vomiting (pyloric and high intestinal obstruction, pylorospasm, generalized peritonitis, the alimentary toxicoses of infants and children, etc.), to the administration of excessive amounts of alkalis (particularly sodium bicarbonate), and to roentgen ray and ultraviolet irradiation and radium therapy, as summarized in Table 21. Compensatory mechanisms consist of (1) increased alkali excretion, (2) decreased acid excretion, (3) decreased ammonia formation, (4) retention of acid metabolic products (ketosis) and (5) decreased pulmonary ventilation.

TABLE 21. SUMMARY OF CLINICAL INTERPRETATION OF CHANGES IN ACID-BASE BALANCE OF THE BLOOD

(A) CO_2 Capacity of the Plasma: (Normal) 55 to 75 volumes per cent
(B) Alveolar CO_2 Tension: (Normal) 5.2 to 5.7 volumes per cent

Acidosis	CO_2 plasma	Slight: 55–40 Moderate: 40–30 Severe: 25–10	Alkalosis	CO_2 plasma	Slight: 78–80 Moderate: 85–90 Severe: 100 or higher
	CO_2 alveolar air	Slight: 5–3.5 Moderate: 3–2.5 Severe: 2 or less		CO_2 alveolar air	Slight: 6–6.5 Moderate: 7–8 Severe 9 or higher
Severe diabetes mellitus and especially coma. Starvation states and carbohydrate restriction. Renal failure (from nephritis and nephrosis, pyelonephritis, pyelonephrosis, polycystic disease, renal tuberculosis, etc.). Anesthesia (ether and chloroform). Excessive vomiting (dehydration). Excessive diarrhea (dehydration). Pancreatic and intestinal fistulas (dehydration). Pregnancy and its toxemias (eclampsia). Ingestion of excessive amounts of acids. Breathing excess of CO_2. Mechanical asphyxia. Narcosis of morphine and other opiates. Severe attacks of asthma. Pneumonia (especially bronchopneumonia). Terminal stages of pulmonary tuberculosis. Extensive emphysemia. Congestive heart failure.			Fevers (especially pneumonia). Exposure to high external temperatures. High altitudes (anoxic anoxia). Hysteria. Encephalitis. Pyloric obstruction and other high intestinal obstruction with severe vomiting. Pylorospasm and alimentary toxicoses of infants and children. General peritonitis (vomiting). Excessive administration of alkalies. X-ray, ultraviolet light or radium therapy.		

Perhaps the most satisfactory method for detecting disturbances of acid-base balance, from a clinical standpoint, is to consider all disturbances in terms of the bicarbonate system, and to consider the hydrogen-ion concentration of the blood as dependent upon the ratio between the concentration of carbon dioxide and bicarbonate in the blood. Consequently three variable factors must be considered: (1) the hydrogen-ion concentration of the blood, (2) the blood carbon dioxide concentration (carbon dioxide tension) and (3) the blood bicarbonate concentration (alkali reserve). Obviously, a distinct disturbance may exist in blood carbon dioxide or blood bicarbonate concentrations which, by virtue of a compensatory change in either, is associated with no significant alteration in the hydrogen-ion concentration of the blood. In other words, the primary cause of the disturbance is compensated or corrected. Under such conditions the true state of the acid-base balance can be determined accurately and most satisfactorily only by the determination of at least two of the three factors involved, namely, hydrogen-ion concentration of the blood, the blood carbon dioxide concentration, or the blood bicarbonate.

Various laboratory methods have been employed for the detection of disturbances of acid-base balance but from the clinical standpoint the most satisfactory approach is to consider all disturbances in terms of the bicarbonate system and hydrogen-ion concentration of the blood as dependent upon the ratio between carbon dioxide and bicarbonate. For clinical purposes the alkali reserve of the blood is best determined by the CO_2 capacity of the plasma. It is possible to determine directly by titration the bicarbonate concentration of the plasma but this offers no advantage over the simpler and more available CO_2 capacity determination. The determination of the carbon dioxide of the blood may be made directly but its indirect determination, based on the CO_2 content (tension) of the alveolar air, is much simpler and equally satisfactory for clinical purposes. Other methods include: the determination of the *p*H of plasma or serum which, when done in connection with one of the tests previously mentioned, indicates the degree of compensation or decompensation of an existing disturbance; the alkali tolerance test of Sellards which possesses more negative than positive value but is not without danger in renal failure, since it may convert an acidosis into one of severe alkalosis; the determination of urinary ammonia which in the acidosis of uremia may show no comparable increase with variations due to dietary factors and advanced hepatic disease; the determination of the titratable acids of the urine which may be of some value for indicating the presence or absence of acidosis in diabetes but not as a measure of the degree in advanced cases; finally, the detection of the ketone bodies in the urine which is one of the first manifestations of beginning acidosis and serves as a valuable therapeutic guide.

NITROGENOUS CONSTITUENTS

The blood normally contains a number of nitrogenous substances embracing (1) the plasma proteins like fibrinogen, albumin, pseudoglobulin and euglobulin totaling 6.0 to 8.0 gm. per 100 cc. and (2) various nonprotein nitrogenous substances like urea, creatinine, uric acid, amino acids, ammonia and undetermined or "rest nitrogen" in varying amounts, as shown in Table 19, which total 25 to 35 mg. per 100 cc. of plasma.

Fibrinogen is apparently formed only in the liver. The site of formation of serum albumin and the globulins is conjecturable except that it seems likely they are formed in those organs where capillary permeability is of sufficient degree to permit ready diffusibility like the liver, the blood-forming organs, the spleen and perhaps the intestinal mucosa. Certainly their formation is influenced by diet and the globulins are regenerated more rapidly than albumin. At least, a state of dynamic equilibrium exists between the blood and tissue proteins, since it appears that the reserve stores in the latter are adequate for the restoration of the former in tiding the body over any emergency demands; but when this reserve is exhausted the tissues contribute only small amounts to the blood and with considerable difficulty. The blood, however, appears to be able to contribute its proteins much more readily to the tissues, although when the plasma proteins are reduced (hypoproteinemia) the healing of wounds may be delayed.

Of the latter or nonprotein nitrogenous substances, urea is one of the chief products of protein digestion as well as of protein metabolism of the tissues; creatinine is solely a product of protein metabolism and is practically unaffected by diet except insofar as the latter influences the breakdown of tissue proteins; uric acid is the chief end-product of exogenous and endogenous metabolism of nucleoproteins; the amino acids are the chief end products of protein metabolism which undergo deamination and synthesis into urea in the liver; ammonia is a product of those amino acids escaping deamination in this organ as likewise conversion into urea while the undetermined or "rest nitrogen," which constitutes as much as 45 per cent of the total and residing chiefly in the corpuscles, is of unknown nature or origin with a possible important relationship to the toxic manifestations of uremia and eclampsia.

In other words, ingested proteins (exclusive of nucleoproteins) are broken down in the stomach and smaller intestine by hydrochloric acid and various proteolytic enzymes into proteoses, peptones, peptides and amino acids, the latter constituting the chief end products which are absorbed as such from the small intestine and carried to the liver and other tissues. From the liver a portion of the amino acids passes into the general circulation unchanged, a portion is utilized for the formation of proteins by this organ, while the balance is formed into a non-nitrogenous residue or "carbohydrate moiety" which undergoes a variety of transformations. Thus amino acids (alanine, glycine, serine, cystine, aspartic and glutamic acids, proline and arginine) are converted into glucose and finally into glycogen which is stored in the liver and muscles, others (glycine and cystine) are partly utilized for the formation of the bile acids, while others (leucine, tyrosine and phenylalanine) yield fatty acid molecules and acetone. Indeed, it has been calculated that glucose formed from protein constitutes 58 per cent, by weight, of the amount ingested, whereas fatty acids so formed constitute 42 per cent.

While the details of the digestion and intermediary metabolism of the nucleoproteins are not as yet clearly understood, it appears that they are decomposed into protein and nucleic acid in the stomach and small intestine while nucleic acid is further broken down into purine and pyrimidine nucleotides and nucleosides. These are likewise absorbed from the small intestine, and are converted in the liver and the tissues throughout the body into the purine and pyrimidine bases, pentose and phosphoric acid, the purine basis (adenine and guanine) being eventually transformed into uric acid by enzymes present in the tissues.

All excess of such nonprotein nitrogen constituents like urea, uric acid, creatinine, amino acids and ammonia are normally excreted in the urine but some are retained in the blood (Table 19) where all may show abnormal concentrations in disease, to be discussed in more detail.

FIBRINOGEN

As previously stated, fibrinogen appears to be formed in the liver with an average normal range of 0.2 to 0.4 gm. per 100 cc. of plasma. Its most important function is in the coagulation of the blood, being transformed into fibrin by the action of thrombin in this process.

Fibrinogen is not significantly altered by disturbances of protein metabolism per se. As would be expected, however, mild hepatitis and cholecystitis sometimes produce a slight increase while severe haptic injury frequently results in a sharp decrease and especially in acute yellow atrophy and toxic hepatitis (arsphenamine, chloroform, phosphorus, carbon tetrachloride) as well as in some cases of extensive and progressive cirrhosis of the liver (Table 22).

TABLE 22. SUMMARY OF CLINICAL INTERPRETATION OF CHANGES IN FIBRINOGEN IN THE BLOOD

Normal: 0.2 to 0.4 gm. per 100 cc. of plasma

Increased	Decreased
Tendency in mild hepatitis and cholecystitis.	Acute severe hepatitis (arsphenamine, phosphorus, chloroform, carbon tetrachloride) and acute yellow atrophy.
Acute infections including the septicemias (except typhoid fever) and notably in pneumonia.	Progressive cirrhosis of the liver.
Active pulmonary tuberculosis.	Typhoid fever.
Tendency in chronic focal infections.	Cachectic states, notably malignancy.
Tendency during pregnancy and menstruation.	Temporarily after extensive hemorrhage.
Tendency after x-ray irradiation.	Severe anemias.
Marked in nephrosis.	Constitutional fibrinogenopenia.
	Disorders of the bone marrow.

An increase of plasma fibrinogen may also result from tissue destruction and increased capillary permeability in the course of most of the acute infections (except typhoid fever), notably in pneumonia, as well as in the septicemias, active tuberculosis and to some extent in chronic infections of the nasal accessory sinuses, tonsils, etc. In these infections it is likely that the increased fibrinogen is responsible for the increase of the sedimentation rate and time of the erythrocytes. In typhoid fever, however, a decrease is usually observed as likewise in cachectic states with special reference to carcinoma. As expected, a temporary decrease also occurs following extensive hemorrhage and in severe anemia.

A slight increase may be observed during pregnancy and menstruation and following x-ray irradiation but a marked increase is not unusual in nephrosis, apparently in an attempt to compensate for the diminution in plasma albumin in this state. This increase is perhaps made possible by the existence of a general increase in capillary permeability which facilitates the more ready entrance of the comparatively large fibrinogen molecule into the blood.

ALBUMIN AND GLOBULIN

Normally the blood plasma contains from 3.6 to 5.6 gm. of albumin and 1.3 to 3.2 gm. of globulin (average euglobulin 0.7 gm.; pseudoglobulin 1.7 gm.)

per 100 cc. giving a ratio of 1.5 to 2.5:1. One of the chief functions of albumin and globulin is the maintenance of a normal colloid osmotic pressure in the capillaries (25 to 30 mm. Hg) for a normal distribution of fluids between the blood and the tissues, the crystalloid osmotic pressure of the plasma being balanced by that of the tissue fluids. But since the plasma has a higher protein content than the tissues there is a tendency for fluids to flow from the tissues into the blood which normally is counterbalanced by capillary blood pressure (16 to 35 mm. Hg). Because of the lower pressure in the venules, fluid tends to pass from the plasma into the tissues through the arterioles but from the tissues into the blood through the venules, which is also favored by the increased osmotic pressure of the plasma resulting from an increased concentration of the proteins incident to a loss of fluid through the capillaries. Furthermore, under conditions of increased tissue metabolism the osmotic pressure of the tissue fluids is raised by the decomposition of carbohydrates, proteins and fats into relatively much

TABLE 23. SUMMARY OF CLINICAL INTERPRETATION OF CHANGES IN THE ALBUMIN AND GLOBULIN IN THE BLOOD

Normal in gm. per 100 cc. Total protein: 6.0 to 8.0; albumin: 3.6 to 5.6; globulin: 1.3 to 3.2; albumin-globulin ratio: 1.5 to 2.5:1.

Hyperproteinemia (Increased)	Hypoproteinemia (Decreased)
Severe rapid dehydration (cholera, diarrhea and alimentary toxicoses especially in children, intestinal fistulas, vomiting from high intestinal obstruction, diabetic acidosis, shock, severe burns, severe infections, heat exhaustion, marked restriction of fluid intake, etc.).	Excessive and prolonged albuminuria in nephritis and nephrosis.
Addison's disease	Cirrhosis of the liver, especially with ascites.
Due principally to increase of globulins in:	Some cases of hepatocellular hepatitis, especially acute yellow atrophy and toxic hepatitis (less frequently in extrahepatic obstructive jaundice).
Multiple myeloma	Malnutrition (diabetes mellitus, anemia, malignancy, tuberculosis, etc.).
Pneumonia	Excess loss of protein in severe prolonged vomiting or diarrhea.
Active tuberculosis	Protein-deficit diet.
Subacute bacterial endocarditis	Tendency in pregnancy and lactation.
Tendency in rheumatic fever	Toxemias of pregnancy and especially in eclampsia
Rheumatoid arthritis	Tendency in congestive heart failure and especially with edema
Osteomyelitis	Severe thyrotoxicosis
Pulmonary abscess	Severe hemorrhage
Lymphogranuloma venereum	Extensive burns
Kala-azar	Polyserositis (Pick's disease)
Malaria	
Filariasis	
Schistosomiasis; trypanosomiasis	
Lupus erythematosis	
Leprosy; syphilis	
Periarteritis nodosa	
Sarcoid of Boeck	
The leukemias	

smaller molecules which facilitates their passage from the tissues into the plasma. In hypoproteinemia the colloid osmotic pressure in the capillaries and venules is reduced which plays an important rôle in the production of edema of nephritis as well as in nutritional edema. It is apparent, therefore, that the plasma albumin and globulin play an important rôle in maintaining a normal balance between the fluids of the blood and the tissues as well as greatly aiding in the regulation and maintenance of a normal acid-base or hydrogen-ion equilibrium of the blood, as previously discussed.

Primary alterations in abnormal states occur more commonly in the albumin than in the globulin fractions of the plasma but since variations in the one are usually associated with compensatory changes in the other, they may be considered conjointly (Table 23).

Hypoproteinemia. A reduction in the total amount of these proteins is designated as *hypoproteinemia* and possesses great clinical significance. Hypoproteinemia may result in the production of edema because of a reduction in the colloid osmotic pressure of the plasma and particularly since there is but little evidence of an effective compensatory increase of fibrinogen. There is considerable difference of opinion, however, in regard to the degree of hypoproteinemia in relation to edema because of the complicating influences of tissue and plasma sodium chloride balance and acid-base equilibrium, but it appears that a reduction of total protein below 5.5 gm. per 100 cc. favors its occurrence. Hypoproteinemia, with special reference to reduction of serum albumin, may occur in many conditions among which may be mentioned (1) the loss of protein by the excessive and prolonged albuminuria of acute nephritis as well as in chronic active glomerulonephritis and intercapillary nephritis, the reduction possessing prognostic significance in acute glomerulonephritis, and possibly due in part to interference with the regeneration of serum albumin; (2) in advanced cirrhosis of the liver and especially with ascites in which the serum albumin is especially decreased (although sometimes associated with an actual increase of the serum globulin) which some ascribe to coexisting malnutrition and others to impairment of the liver in the synthesis of the plasma proteins; (3) in some cases of hepatocellular hepatitis (less frequently in extrahepatic obstructive jaundice) and especially in acute yellow atrophy of the liver and toxic hepatitis; (4) in states of malnutrition as diabetes mellitus, anemia, malignancy, tuberculosis partly due to low protein intake; (5) in states accompanied by excessive loss of protein in prolonged diarrhea or vomiting; (6) in lipoid nephrosis which is primarily a disturbance of protein metabolism characterized by the excretion in the urine of large amounts of albumin with extreme reduction in plasma albumin but usually with an increase of globulin resulting in a reversal of the albumin-globulin ratio; (7) sometimes during pregnancy and lactation due either to low protein intake, impairment of the regeneration of the serum proteins or a drain on the woman by the synthesis of body proteins by the fetus; (8) the toxemias of pregnancy, particularly eclampsia, with special reference to reduction in serum albumin, and (9) sometimes in congestive heart failure with edema and especially anasarca.

Hyperproteinemia. An increase in the plasma proteins is designated as *hyperproteinemia.* It is much less commonly observed than hypoproteinemia, since in numerous diseases an increase of serum globulin is offset by a reduction in serum albumin. As a matter of fact, hyperproteinemia due to an increase of serum albumin is seldom encountered. But hyperproteinemia due to an increase of both fractions may occur in (1) states of severe rapid dehydration as in cholera, other types of diarrhea and alimentary toxicoses (especially in children), intestinal fistulas, severe vomiting as in pyloric or other forms of high intestinal forms of obstruction as well as after marked restriction of fluid intake and in diabetic acidosis; (2) extensive burns; (3) Addison's disease and (4) in some fulminating infections.

Hyperproteinemia due to an increase of plasma globulins occurs much more frequently, although it may escape detection unless the albumin-globulin ratio is determined as in (1) multiple myeloma; (2) acute and chronic infections like pneumonia, active tuberculosis, subacute bacterial endocarditis, occasionally rheumatic fever, rheumatoid arthritis, osteomyelitis and pulmonary abscess, with some evidence indicating that amyloidosis may be due to a prolonged increase of serum globulin in chronic suppurative infections; (3) lymphogranuloma venereum, kala-azar, malaria, filariasis, schistosomiasis, trypanosomiasis, leprosy and syphilis; (4) sarcoid of Boeck and (5) the leukemias (myelogenous, monocytic and lymphogenous).

In this connection it may be stated that relatively simple tests are available for the detection of an increase of serum globulin, such as (1) the Takata-Ara reaction, especially in cirrhosis of the liver although it is now known to occur in the presence of any disease with increased globulin and a simultaneous decrease of albumin; (2) the formol-gel reaction; (3) Naumann's CO_2 saturation test; (4) Napier's aldehyde test; (5) Henry's melanin reaction; (6) Hanger's cephalin-cholesterol flocculation test and (7) the Weltman reaction.

UREA, UREA NITROGEN AND CREATININE

The *urea* content of the whole blood ranges from 20 to 30 mg. per 100 cc. As previously stated, it is the chief end product of protein metabolism and is normally produced practically solely by the liver through successive stages of deamination of the amino acids, formation of ammonium carbonate and dehydration of this substance to ammonium carbamate and, finally, to urea. It is an extremely diffusible substance and, as such, exists in all body fluids (spinal fluid, saliva, transudates) in practically the same amount as in the blood. It is eliminated chiefly in the urine, but some is lost through the skin and particularly if perspiration is active.

At the present time, however, urea of the blood is seldom determined, since preference has been shown for its determination in terms of its constituent nitrogen (*urea nitrogen*), which ranges, approximately, from 9 to 17 mg. per 100 cc. This nitrogen usually rises markedly following a meal high in protein and tends to maintain a higher level in individuals on a high protein diet than in those on a low protein intake. It is essential, therefore, that blood be taken in a fasting state (usually before breakfast) and preferably after the patient has been on a standard diet.

Creatinine, however, is solely a product of endogenous protein metabolism and is practically unaffected by the character of the diet except insofar as this influences the breakdown of tissue protein. It is the anhydride of creatine which is normally present in the muscles. It is likewise highly diffusible and is excreted largely by the kidneys. However, the methods employed for its determination are nonspecific and indeed it is questionable whether creatinine exists as such in the blood. But it is remarkably constant in health, with a normal range of 1 to 2 mg. per 100 cc. of whole blood, and variations are so definite in disease that the question as to whether or not it is in reality creatinine is of more academic than practical clinical interest.

An increase of creatinine may occur in any condition in which the urea nitrogen is increased but in chronic nephritis its retention is of far more serious diagnostic import. Indeed, retention of 5 or more mg. per 100 cc. usually indicates a grave prognosis. But in acute nephritis extremely high concentrations may occur, which, with subsidence of the nephritis, may return to normal. The same is true in urinary tract obstruction without permanent renal damage in which, since the process is largely mechanical, extremely large amounts of blood creatinine may return to normal following relief from the obstruction. And while the degree of increased creatinine is roughly proportional to that of the urea nitrogen it is in striking contrast to the findings in chronic glomerulonephritis, in which the high urea nitrogen may be observed in association with a normal creatinine. Under such circumstances and with these exceptions in mind, those renal or extrarenal conditions producing an increase or decrease of urea nitrogen and creatinine may be listed together as summarized in Table 24.

An *increase of urea nitrogen and creatinine* may occur in many diseases of renal or extrarenal (azotemic) origin, as (1) in subacute and chronic nephritis and especially glomerulonephritis, particularly in uremia due to reduced glomerular filtration; (2) in conditions associated with marked oliguria or anuria, like acute glomerulonephritis, toxic nephrosis (bichloride of mercury poisoning), obstructing ureteral calculi or unilateral calculus with reflex suppression of function of the opposite kidney and postoperative urinary suppression of very severe congestive heart failure; (3) from actual destruction of kidney parenchyma as in tuberculosis, pyonephrosis, hydronephrosis, renal cortical necrosis or congenital polycystic disease of the kidneys; (4) in prostatic obstruction; (5) from excessive endogenous protein metabolism due to the fever of acute infections; (6) in dehydration from severe hemorrhage (especially gastro-intestinal) and in states of renal azotemia with hemoconcentration, increased tissue protein destruction with an attempt to preserve the plasma osmotic pressure which is reduced by the loss of plasma chloride from the loss of gastro-intestinal secretions (pylorospasm, acute intestinal obstruction, gastric tetany, vomiting and diarrhea), the loss of extracellular fluids (profuse sweating and alkalosis, postoperative or from limitations of fluid intake) or from the loss of fluid and chloride in extensive burns and histamine shock and adrenal insufficiency (Addison's disease); (7) in congenital hypoplasia of the kidneys; (8) in renal amyloidosis and hemoglobinuria due to quinine or transfusion reactions with blocking of the uriniferous tubules by precipitates as well as with the crystals of the sulfonamide compounds; (9) as a terminal event in

TABLE 24. SUMMARY OF CLINICAL INTERPRETATION OF UREA NITROGEN AND CREATININE IN THE BLOOD

Normals: urea nitrogen 9 to 17 mg. and creatinine 1 to 2 mg. per 100 cc.

Increased	Decreased
Subacute and chronic nephritis and especially glomerulonephritis.	Urea nitrogen in habitual low protein intake.
Uremia.	Tendency in hepatocellular hepatitis.
In oliguria or anuria (acute nephritis, toxic nephrosis, urinary tract obstruction and postoperative suppression, very severe congestive heart failure).	Acute yellow atrophy of the liver.
Renal tuberculosis.	Toxic hepatitis.
Pyonephrosis and hydronephrosis.	Operations on the biliary tract.
Renal cortical necrosis.	Rarely in cirrhosis of the liver, passive congestion or malignancy.
Polycystic disease of the kidneys.	Tendency in normal pregnancy, especially after six months.
Prostatic obstruction.	Sometimes in lipoid nephrosis.
Acute high fevers of infections.	Sometimes in celiac disease.
Pylorospasm and intestinal obstruction.	Tendency in acromegaly.
Gastric tetany.	
Vomiting and diarrhea.	
Profuse sweating and alkalosis.	
Postoperative.	
Limitation of fluid intake.	
Extensive burns and histamine shock.	
Adrenal insufficiency (Addison's disease).	
Congenital hypoplasia of the kidneys.	
Renal amyloidosis.	
Hemoglobinuria, especially post-transfusional.	
Hyperthyroidism (terminal).	
Sometimes in diabetic coma.	
After operations on the liver and especially for decompression of the ducts.	
Shock and hemoconcentration.	
Multiple myeloma.	
Renal rickets (dwarfism).	

the renal failure of hyperthyroidism due in part to extensive calcification of the renal tubules or the development of renal calculi; (10) sometimes in diabetic coma probably due in part to dehydration, excessive catabolism of proteins or perhaps actual renal impairment; (11) not infrequently after operations on the liver and particularly after decompression of obstructed bile ducts, for which the term "hepato-renal syndrome" is sometimes used; (12) from extreme reduction of the blood pressure as in Addison's disease or shock with marked oliguria or even anuria; (13) in multiple myeloma and (14) in renal rickets (renal dwarfism).

A *decrease of urea nitrogen and creatinine* is encountered much less frequently but may occur (1) from a habitual and very low protein intake in the case of urea nitrogen; (2) sometimes slightly in hepatocellular jaundice but much more markedly in hepatic insufficiency from acute yellow atrophy of the liver, toxic hepatitis (phosphorus, chloroform, carbon tetrachloride, cinchophen, arsphenamine) and following operations on the biliary tract although but rarely in chronic hepatic disease (cirrhosis, passive congestion or malignancy) because of the enormous reserve capacity and regenerative powers of the liver; (3) sometimes in normal pregnancy, especially after six months although it is here likely to be accompanied by an increase of the undetermined or "rest nitrogen"; (4) sometimes in lipoid nephrosis attributed to some disturbance of protein metabolism which is believed by some to be a fundamental factor in its etiology; (5) sometimes in celiac disease and (6) sometimes in acromegaly as a result of increased elimination in the urine.

URIC ACID

In man, uric acid is the chief end product of exogenous and endogenous nucleoprotein metabolism, the normal amount in whole blood varying from 2 to 4 mg. per 100 cc. It is probable that certain organs, particularly the kidneys, may store relatively large amounts which may be subsequently liberated and destroyed in the tissues and the liver, but if such destruction occurs nothing is known of the end products. Under normal conditions the blood uric acid is affected but slightly, if at all, by the ingestion of proteins and purine-rich foods, and bears no direct relation to the total nonprotein or urea nitrogen of the blood. It is normally excreted almost entirely by the kidneys. As is the case with glucose determinations, it appears advisable to use whole blood filtrates for uric acid determinations.

Increased blood uric acid (Table 25) may occur (1) because of reduced elimination in the urine as in both acute and chronic nephritis, which may precede an increase of urea nitrogen and creatinine, and increased in such persons by the administration of purine-rich foods which does not occur in normal individuals; (2) because of a primary alteration in the metabolism of nucleoproteins or purines as in gout. The exact nature of this metabolic disease is still unknown. It is characterized by a rise in the resting level of blood (and, perhaps, tissue) uric acid immediately before and during acute attacks which may be precipitated by purine-rich foods, and in chronic gout nephritis is a common complication with eventual increase of blood urea nitrogen and creatinine. Clinically, gout may be apparently hereditary or acquired through excessive eating, excessive use of fermented malt beverages, lack of exercise and lead poisoning. The relation of these factors to the disease is, however, not understood. Some investigators have thought that the fault lies in the intermediary metabolism of purines due to their defective deamination in the liver, with the result that they cannot be dissociated and eliminated by the kidneys, which leads to a retention of uric acid in the blood followed by the deposition of urates in the cartilages, which appear to have a specific affinity for them and particularly those of the great toes. Other investigators have thought that in gout the metabolism of purines is normal but that the kidneys are primarily at fault, not only in the way of failure to store uric acid in them for gradual return to the blood and its destruction in the blood and tissues, but likewise because of failure of excretion in the urine. But the question as to whether the fundamental defect resides in the kidneys or in some abnormality of intermediary purine metabolism is as yet unsettled.

TABLE 25. SUMMARY OF THE CLINICAL INTERPRETATION OF URIC ACID IN THE BLOOD

Normal: 2 to 4 mg. per 100 cc. of whole blood

Increased	Decreased
Acute and chronic nephritis. Gout. Chronic leukemia (especially myelogenous). Multiple myeloma. Remissions of pernicious anemia. Urinary tract obstruction and reflex anuria. Pyelonephrosis and hydronephrosis. Renal tuberculosis. Polycystic kidneys. Some cases of osteoarthritis. Chronic lead poisoning. Eclampsia and severe vomiting of pregnancy. Tendency in normal pregnancy. Intestinal obstruction. Severe acute hepatitis. Hypertension. Congestive heart failure. Tendency in pneumonia. Some chronic dermatoses.	Tendency in pernicious anemia during relapses. Tendency in celiac disease.

Blood uric acid may be also increased in (3) chronic leukemia (especially myelogenous) and (4) multiple myeloma. In both the increase is, in all probability, due to an active and greatly increased metabolism of nucleoprotein (cell nuclei). Also (5) during spontaneous remissions of pernicious anemia or those induced by the administration of liver or liver extracts. This is usually ascribed to the increased activity of the hematopoietic tissues with an increased production of uric acid by the nuclei of erythrocytes lost during maturation in the bone marrow; (6) as the result of urinary tract obstruction, urinary suppression (reflex anuria) or destruction of the renal parenchyma (tuberculosis, pyonephrosis, hydronephrosis, polycystic kidneys, etc.), resulting in the retention of uric acid in the blood along with other constituents; (7) in some cases of osteoarthritis which may be due in reality to early chronic nephritis; (8) in chronic lead poisoning with defective elimination due to chronic nephritis as the cause of the uric acid retention; (9) in eclampsia (especially during convulsions and before delivery), and in severe vomiting of pregnancy in which the increase of uric acid in the blood is sometimes attributable to increased endogenous nucleoprotein metabolism with mild grades of renal insufficiency; (10) sometimes in normal pregnancy; (11) in intestinal obstruction; (12) in severe acute hepatitis and especially toxic hepatocellular jaundice; (13) frequently in hypertension and congestive heart failure; (14) sometimes in pneumonia and (15) in some of the chronic dermatoses characterized by pruritus in which the cause of the uric acid retention may not be discoverable.

A slight *decrease of uric acid* may occur in celiac disease and in some cases of pernicious anemia during relapses.

AMINO ACID, AMMONIA AND UNDETERMINED NITROGENS

Since amino acids normally undergo deamination in the liver with the consequent formation of urea, the *amino acid nitrogen* of the blood (which is normally 5 to 8 mg. per 100 cc.) increases (hyperamino-acidemia) and that of urea diminishes when the deaminizing function of this organ is seriously impaired, as in acute yellow atrophy, phosphorus, arsenic, chloroform and carbon tetrachloride poisoning and, occasionally, in eclampsia and even in severe catarrhal jaundice. Indeed, this increase in amino acid nitrogen is partly due to extensive destruction (autolysis) of liver tissue. But high values are not ordinarily found in chronic hepatic disease such as cirrhosis, obstructive jaundice, syphilis or malignancy because of the large functional reserve and marked regenerative powers of the liver. But the blood amino acids may also show a slight increase not only in myelogenous leukemia but likewise when there is a deficient excretion of them by the kidneys, as in some cases of advanced chronic nephritis with marked retention of urea nitrogen, as well as in urinary tract obstruction or suppression of urine. High values are, however, rare under these circumstances, unless there is an associated hepatic insufficiency; indeed, in most cases of nephritis the amino acid content of the blood is within normal limits or but slightly increased.

The normal average plasma amino acid nitrogen is about 4.47 mg. ($\pm$ 0.46) per 100 cc. A decrease (hypoamino-acidemia) has been reported as occurring in young children with the nephrotic syndrome [1] as, likewise, in the early stage of a small percentage of cases of pneumococcus pneumonia.[2]

The *ammonia nitrogen* which is also produced by the deamination of amino acids by the liver and represents largely that portion escaping conversion into urea, is of little clinical interest and is normally present in only 0.1 to 0.2 mg. per 100 cc. of blood. It is not increased in nephritis or other states of renal insufficiency although, as previously stated, since ammonia itself is formed by the kidneys, diseases of the kidneys may reduce its production and result in disturbances of the acid-base balance with the production of acidosis.

The balance of the nonprotein nitrogen of the blood amounts to 4 to 18 mg. per 100 cc. and is designated as the *undetermined nitrogen* or residual nitrogen. It is chiefly contained in the corpuscles and is believed by some to occur in the form of hippuric acid, nucleotides and histones. In some cases of eclampsia and advanced chronic nephritis with uremia and high nitrogen retention it may be increased much more, proportionately, than urea nitrogen, creatinine or uric acid. Its significance, however, has not been determined although some investigators believe that it may be responsible for the toxic manifestations of eclampsia and uremia.

TOTAL LIPIDS

The blood normally contains true fats, including neutral fat and the fatty acids; also additional substances resembling fats in their general properties and particularly in their solubilities, embracing the lipids (phospholipids or phosphatides) and the sterols (the most important of which is cholesterol), which are not saponifiable and are without any close chemical relation to fats. All of these together constitute the "lipids" of the blood, which total in the *plasma* about 400 to 600 mg. per 100 cc. under fasting or postdigestive conditions. An increase is designated as *lipoidemia*. Lipoidemia largely due to an increase of neutral fat and fatty acids may be designated as *lipemia* or hyperlipemia; as *phospholipidemia* when mainly due to an increase of lipids as *hypercholesterolemia* when due to an increase of cholesterol which is the sterol of chief interest from the standpoint of clinical interest.

Under normal conditions neutral fat and fatty acids are derived from ingested fats following their breakdown into fatty acids and glycerol in the gastro-intestinal tract through the agency of lipases aided by the bile salts. But small amounts of fat usually escape this process with absorption after emulsification into the lacteals and finally into the blood, where the fat may render the plasma or serum milky or chylous in appearance, especially immediately after meals, due to the presence of minute globules (chylomicrons) detected by microscopic examination.

The fatty acids, however, are absorbed as such through the intestinal mucosa, with the assistance of phospholipids in the epithelial cells, into the lymphatics and lacteals with ultimate arrival in the blood. Some investigators believe that they may be partly absorbed directly into the portal circulation but the evidence is not conclusive. During absorption the fatty acids are resynthesized into neutral fat closely resembling body fat, although not as perfectly resynthesized in this particular as the synthesis of protein and carbohydrate. This neutral fat is then stored in the fatty depots of the body until called upon to furnish fuel through a process of beta-oxidation or combustion, with fatty acids, carbon dioxide and water as the end products. Part of these fatty acids is deposited in the tissues apparently with the aid of the phospholipids, since the latter are increased in the blood during digestion, part combines with cholesterol to form its esters, and a part remains in the plasma where it reaches a normal concentration of 190 to 450 mg. per 100 cc. (Table 19). The fatty acids are excreted in relatively large amounts by the intestinal mucosa, to a less extent by the bile, and in minute amounts by the sebaceous secretions of the skin with none normally in the urine.

The phospholipids or phosphatides ingested in foods are probably not absorbed as such from the intestinal tract but under the influence of lipases are apparently broken down into their component parts which, after absorption, undergo resynthesis to phospholipids in the body. Man also appears able to synthesize them from fat in the diet. They are eliminated chiefly in the bile and intestinal secretions, being normally in the feces, but in the urine only occasionally and then only during albuminuria due to adsorption by urinary protein under such circumstances.

As previously stated, cholesterol is the chief sterol concerned in lipid metabolism and blood chemistry, with the liver playing an active part in removing it from the blood and storing it within its substance. It is widely distributed and present in all living matter. In the plasma it exists in the free state (40 to 50 mg. per 100 cc.) and as cholesterol esters through combination with fatty acids (190 to 200 mg. per 100 cc.). It appears to be of both endogenous and exogenous origin, the former being fairly competent to maintain a normal amount if the exogenous supply fails. In other words, synthesis of cholesterol has been proved, as has the fact that it can be destroyed and removed from the body under normal conditions. Indeed, it is now believed to be continually formed and destroyed in the tissues with a positive or negative balance depending upon whether synthesis is in excess of destruction, or the reverse. But there are still differences of opinion regarding where the synthesis and destruction of cholesterol occurs, being attributed to the liver, spleen, adrenals, thyroid, pancreas and hypophysis by different investigators. Apparently endogenous cholesterol may originate in the cells in which it occurs but it is now widely thought that cholesterol metabolism is chiefly regulated by the cells of the reticulo-endothelial system rather than by any one organ, either by controlling the normal disposition of lipids or by the synthesis of cholesterol itself. However, there can be no doubt about the importance of the liver in the metabolism of cholesterol, not only because it is an important storehouse of this substance, but likewise because of its excretion of free cholesterol esters in the plasma.

When cholesterol of animal origin is ingested it is not readily absorbed in the absence of fat and bile acids. Apparently little or none of the plant sterols are absorbed at all. After ingestion and before absorption cholesterol appears to be hydrolyzed by the esterases into esters which are resynthesized in the intestinal mucosa before reaching the lymph stream, which is the chief channel of absorption. But a portion passes through the gastro-intestinal tract unchanged while a portion is reduced to coprosterol by the anaerobic bacteria and likewise excreted in the feces. It is also probable that some of the cholesterol of the bile is reabsorbed in this manner from the small intestine. While free cholesterol is eliminated in the bile it is now known that cholesterol is largely eliminated in the intestinal secretions in the form of cholesterol and betacholestanol, the former being converted into coprosterol, with small amounts lost in desquamated skin, by excretion in milk and in the urine after conjugation, with the balance completely destroyed in the body.

NEUTRAL FAT AND FATTY ACIDS

As previously stated, the term *lipemia* or *hyperlipemia* may be employed for designating an increase of the neutral fat and fatty acids of the blood and *hypolipemia* for their decrease. The former may result from either diminished removal of these substances from the blood or because of their increased absorption as a result of increased ingestion of fat or of increased mobilization of fat from the reserve depots in the body. Lipemia occurs most frequently in conditions in which the body is forced to mobilize its fat reserves for fuel because other material, chiefly carbohydrate, is lacking or cannot be adequately utilized as in diabetes mellitus, malnutrition and fasting, although in diabetes at least it may be partly due to a state of hemoconcentration due to polyuria.

Under normal conditions and in the fasting state the neutral fat of the plasma varies from 0 to 370 mg. per 100 cc. with an average of about 170 mg. in adults. Considerably smaller amounts are observed in children under ten years of age (100 to 182 mg. per 100 cc.). The amounts of fatty acids in the plasma vary from 190 to 450 mg. per 100 cc., with an average of about 353 mg. in adults. Both show a physiologic increase one to two hours after the ingestion of fat, generally reaching a maximum within four to six hours, designated as *alimentary lipemia.* For this reason oxalated blood for the determination of neutral fat and fatty acids should be drawn in the morning before breakfast.

Although neutral fat and fatty acids vary in certain conditions, they are not usually determined for clinical purposes largely because of technical difficulties and the fact that alterations in plasma cholesterol, which usually occur under the same circumstances, can be detected much more satisfactorily.

TABLE 26. SUMMARY OF CLINICAL INTERPRETATION OF NEUTRAL FAT AND FATTY ACIDS OF THE BLOOD

Normals in plasma for adults: (1) Neutral fat: 0 to 370 averaging 170 and (2) Fatty acids: 190 to 450 averaging 353 mg. per 100 cc.

Hyperlipemia (increase)	Hypolipemia (decrease)
Alimentary after meals.	Tendency in schizophrenia.
After prolonged fasting or an exclusive meat diet.	Hyperthyroidism.
Malnutrition.	
Normal pregnancy and especially during lactation.	
Diabetes mellitus with acidosis and ketonuria.	
Ether and chloroform anesthesia.	
Alcoholism.	
Chronic glomerulonephritis and especially nephrotic type.	
Nephrosis.	
Pernicious anemia.	
Acute and chronic hemolytic anemias.	
Idiopathic hypochromic anemia.	
Leukemia.	
Tendency in obstructive jaundice.	
Hypothyroidism.	
Hyperthyroidism (alimentary).	
Tendency in essential hypertension.	
Tendency in manic depressive psychosis.	
Von Gierke's or glycogen storage disease.	

Hyperlipemia. An increase of neutral fat and fatty acids occurs not only post-digestively, as previously stated, but also to some extent during normal pregnancy and particularly during lactation. Importantly enough it also commonly occurs after complete fasting or on an exclusive diet of meats because of a continuous demand for fat as fuel resulting from the absence of adequate amounts of carbohydrate for purposes of combustion and heat production.

In abnormal states it is particularly increased in (1) severe diabetes mellitus with acidosis and ketonuria and occurs not infrequently after (2) ether narcosis, in which it may be reduced or abolished by the administration of insulin, and (3) in alcoholism. Hyperlipemia also occurs in many individuals with (4) chronic glomerulonephritis and especially in nephrotic types as well as in (5) nephrosis; (6) pernicious anemia; (7) acute and chronic hemolytic anemias; (8) idiopathic hypochromic anemia; (9) the leukemias; (10) sometimes in obstructive jaundice; (11) hypothyroidism as well as in (12) hyperthyroidism due to increased alimentary lipemia; (13) to some extent in essential hypertension; (14) manic depressive psychosis and (15) in von Gierke's or glycogen storage disease, as summarized in Table 26.

Hypolipemia. A decrease in the neutral fat below normal limits is of much less frequency but may occur in schizophrenia and hyperthyroidism.

TABLE 27. SUMMARY OF CLINICAL INTERPRETATION OF THE PHOSPHOLIPIDS OF THE BLOOD

Normal: 60 to 350 mg., averaging 196 mg., per 100 cc. of plasma.

Hyperphospholipidemia (increase)	Hypophospholipidemia (decrease)
Advanced diabetes mellitus with malnutrition.	Pernicious anemia during relapses.
Glomerulonephritis with edema.	Hemolytic anemia with jaundice.
Nephrosis.	Idiopathic hypochromic anemia.
Anemia due to chronic hemorrhage.	During acute febrile infections.
B-avitaminosis.	Sometimes in hyperthyroidism.
Niemann-Pick disease (some cases).	
Sometimes in epilepsy.	
Sometimes in essential hypertension.	
Sometimes in hypothyroidism.	
Sometimes in syphilis.	
Sometimes in necrosis of the liver.	

PHOSPHOLIPIDS

Normally phospholipids occur in the plasma in amounts varying from 60 to 350 mg. with an average of 196 mg. per 100 cc. An increase is designated as *hyperphospholipidemia* and a decrease as *hypophospholipidemia*. The phospholipids are chiefly composed of lecithin, cephalin and sphingomyelin, the latter occurring particularly in the brain and nerve tissue and of least chemical interest. As previously stated, they are found in all living matter and may be regarded as essential components of protoplasm. There is little exact knowledge regarding their functions but it is believed that they are intermediary products of fat metabolism and intimately concerned with the absorption of fatty acids across the intestinal mucosa, or that they may act as agents for the transportation of oxygen to the tissues, or find their chief function in contributing to the constitution of protoplasm and the construction of cell membranes, the first of these hypotheses being now most widely accepted. They may also play some part in immunologic reactions and certainly cephalin is actively concerned in the coagulation of the blood, since it is the thromboplastic agent supplied by the platelets.

Hyperphospholipidemia. A significant increase of phospholipids may occur (1) in advanced diabetes mellitus and particularly poorly nourished individuals as the result of malnutrition and hemoconcentration; (2) in glomerulonephritis with edema and (3) in nephrosis due either to deficient removal of phospholipids from the blood or to their increased liberation from fat depots; (4) in the anemia due to chronic hemorrhage; (5) in B-avitaminosis; (6) in Niemann-Pick disease (some cases) and occasionally but with little clinical interest in (7) epilepsy, (8) essential hypertension, (9) syphilis, (10) hypothyroidism and (11) necrosis of the liver (Table 27).

Hypophospholipidemia. A decrease of phospholipids is of frequent occurrence in (1) pernicious anemia during relapses, (2) hemolytic anemia with jaundice and less consistently in (3) idiopathic hypochromic anemia, (4) during acute febrile infections and (5) in hyperthyroidism.

CHOLESTEROL

As previously stated, cholesterol occurs in the blood in two forms, free cholesterol in amounts varying from 40 to 50 mg., and esterified cholesterol in amounts varying from 190 to 200 mg. per 100 cc. of plasma. The cholesterol content of the erythrocytes is slightly lower but the normal total for plasma varies from about 130 to 250 mg. per 100 cc. In man it is so slightly influenced by food that for clinical purposes this factor may be disregarded. Age, however, has an important influence as cholesterol appears to be extremely low at birth but rapidly increases until reaching about 20 to 25 mg. below that of normal adults within a few days. Some investigators have also found it increased just before and decreased during the menstrual period, which is of some clinical interest when blood cholesterol determinations are made in women. Cholesterol is also increased during normal pregnancy, reaching the maximum about the thirtieth week, followed by a decrease of the free form but an increase of the esters up to delivery, the total cholesterol, however, remaining above normal until about eight weeks thereafter. No satisfactory explanation can be offered for these changes.

Hypercholesterolemia. An increase of cholesterol in the plasma is designated *hypercholesterolemia* or *cholesteremia.* This especially occurs (1) in severe and progressive diabetes of adults and particularly during coma but not usually in uncomplicated cases of the disease in children. Occasionally subnormal values occur in advanced diabetes mellitus which is of serious prognostic importance. Both the free and ester fractions are usually increased in this disease and are probably indicative of a greater demand upon fat metabolism because of the unavailability of other fuel and therefore similar to the lipemia of starvation. Diabetic acidosis is also accompanied by an increase of serum proteins due to dehydration so that hemoconcentration is likewise an important factor in the production of cholesteremia. As is well known, there is an intimate relationship between the carbohydrate metabolism and plasma cholesterol and although the absorption and oxidation of fats are unimpaired in diabetes mellitus, the lipemia and cholesteremia appear to indicate an increased demand for the metabolism of fat because of the unavailability of carbohydrates, with neutral fat, fatty acids and cholesterol merely representative of the quantities in process of transportation. At least, there is no definite parallelism between cholesteremia and glycemia, glycosuria, ketonuria, or acidosis in diabetes. The determination of blood cholesterol, however, may serve as a valuable measure of the necessity for insulin therapy after the blood sugar has reached the normal.

TABLE 28. SUMMARY OF CLINICAL INTERPRETATION OF CHOLESTEROL OF THE BLOOD

Normal: Free cholesterol 40 to 50 mg. and cholesterol esters 190 to 200 mg. per 100 cc. plasma with total 140 to 250 mg.

Hypercholesterolemia (increase)	Hypocholesterolemia (decrease)
Before menstruation.	At birth.
Normal pregnancy.	During menstruation.
Severe diabetes mellitus.	Pernicious anemia.
Ether narcosis.	Severe hypochromic anemia.
Chronic glomerulonephritis (nephrotic type).	Hemolytic anemia with jaundice.
Lipoid and amyloid nephrosis.	Hepatocellular jaundice.
Obstruction of common bile duct.	Portal cirrhosis (terminal stage).
Biliary fistula.	Acute infectious diseases.
Hepatocellular jaundice (some cases).	Advanced pulmonary tuberculosis.
Myxedema (especially of children).	Hyperthyroidism.
Primary multiple xanthomatosis.	Malnutrition with cachexia.
After acute severe hemorrhage.	Terminal stages of chronic glomerulonephritis (nephrotic type).
Possibly in vitamin A deficiency.	Urinary tract obstruction.
Possibly in atherosclerosis.	Arteriosclerosis.
Hypertrophic osteoarthritis.	Congestive heart failure.
Senile cataract.	Coronary artery thrombosis.
Psoriasis.	Acute high intestinal obstruction.
Celiac disease of children.	Acute pancreatitis.
Gaucher's and Niemann-Pick disease.	Celiac disease of children.
Hand-Schüller-Christian disease.	Diabetes mellitus (terminal stage).
Von Gierke's disease.	Polyneuritis with undernutrition.
	Schizophrenia.

An increase of plasma cholesterol also commonly occurs (2) after ether narcosis, (3) in chronic glomerulonephritis with edema (nephrotic type) and (4) in lipoid and amyloid nephrosis for which the responsible factors are not clearly understood; also (5) in uncomplicated obstruction of the common bile duct, (6) in biliary fistula and (7) in some cases of hepatocellular jaundice which is due in some way to the absence of bile or certain of its constituents in the intestine; likewise in (8) hypothyroidism (myxedema) especially of children; (9) primary or essential multiple xanthomatosis; (10) after acute severe hemorrhage; possibly (11) in vitamin A deficiency; doubtfully and irregularly in (12) atherosclerosis and occasionally in (13) hypertrophic osteoarthritis; (14) senile cataract; (15) psoriasis; (16) celiac disease of infants and sometimes in (17) Gaucher's, Niemann-Pick, Hand-Schüller-Christian and von Gierke's diseases (Table 28).

Hypocholesterolemia. A decrease of cholesterol in the blood is designated as *hypocholesterolemia* and commonly occurs in (1) pernicious anemia; (2) severe hypochromic anemia; (3) hemolytic anemia with jaundice; (4) hepatocellular jaundice (due to drugs, pneumonia, yellow fever and other infections, including catarrhal jaundice) and (5) in the terminal stages of portal cirrhosis apparently due to faulty absorption from the intestines, impaired esterification in the liver and storage of esters in this organ. A pronounced discrepancy is usually observed between the degree of bilirubinemia and cholesterolemia in hepatocellular damage of the liver, in that the more severe the damage the greater the hypocholesterolemia, due particularly to a decrease in cholesterol esters, which is of differential diagnostic value, since hypercholesterolemia commonly occurs in extrahepatic or obstructive jaundice.

Hypocholesterolemia may also occur (6) in the course of acute infectious diseases and advanced pulmonary tuberculosis; (7) hyperthyroidism; (8) malnutrition with cachexia and wasting; (9) chronic glomerulonephritis in the terminal states; (10) urinary tract obstruction; (11) the nephrotic syndrome; (12) arteriosclerosis; (13) congestive heart failure; (14) coronary artery thrombosis; (15) acute high intestinal obstruction; (16) acute pancreatitis; (17) celiac disease of children; (18) terminal stage of diabetes mellitus; (19) polyneuritis with undernutrition and (20) schizophrenia.

BILIRUBIN

Bilirubin, the chief pigment of human bile, is derived from hemoglobin which is first converted into biliverdin iron globin followed by removal of iron and reduction to bilirubin by the reticulo-endothelial and mesenchymal cells of the body without the production of hematin as an intermediate body. When first formed it remains attached to the original globin which is probably removed by the liver. In other words, the parenchymal cells of the liver are not concerned in its production as formerly believed, but nevertheless have as one of their chief functions its withdrawal from the blood and excretion in the bile.

In the gastro-intestinal tract hemoglobin is converted into hematin and protoporphyrins but not into bilirubin; consequently jaundice is not produced in gastrointestinal bleeding even though hemolysis and hematin production occur. In connective tissues and serous cavities (pleural, peritoneal, subarachnoid), however, hemoglobin is converted directly into bilirubin so that hemorrhages may produce hyperbilirubinemia with or without jaundice. Consequently, bloody fluids obtained from the serous cavities should be centrifuged and tested for bilirubin because if a positive van den Bergh reaction is observed bleeding occurred before paracentesis was conducted.

The van den Bergh Reactions. Since bilirubin is being constantly produced by the reticulo-endothelial and mesenchymal cells from the hemoglobin of broken-down erythrocytes, it is always to be found normally in the blood on its way to the liver for excretion, being apparently adsorbed by the serum globulins which prevents its oxidation and elimination by the kidneys. The amount, however, is too small for the production of jaundice and varies from 0.1 to 0.25 mg. per 100 cc. of serum as determined by the *indirect* van den Bergh reaction, which is the most accurate and widely employed, clinically available method for its quantitative estimation. By the more sensitive method of Thannhauser and Anderson, however, larger amounts are found, varying from 0.1 to 0.8 mg. or more per 100 cc. of serum.

Passage of bilirubin through the parenchymal cells of the liver for excretion into the biliary ducts, however, renders it more easily oxidizable, more dialyzable, more readily adsorbed by precipitated serum proteins, but insoluble in chloroform and so best detected by the *direct* or immediate van den Bergh reaction. Since under normal conditions none of this modified bilirubin is reabsorbed into the blood, the direct van den Bergh test, therefore, gives a negative reaction. But if the normal flow of bile on its way to the duodenum is obstructed intrahepatically, and especially extrahepatically, it is readily reabsorbed, in which case the direct van den Bergh test gives a positive reaction, with the possible production of jaundice. Van den Bergh clearly recognized that this direct or immediate reaction takes place within one minute and normally gives a negative reaction by his technic. But Watson [3] has recently developed a quantitative test in which the normal value is less than 0.2 mg. per 100 cc. of serum and the total bilirubin 1.0 mg. or less per 100 cc. All or nearly all of the bilirubin of human fistula bile reacts within one minute but in congenital hemolytic jaundice it reacts slowly, after one minute.[3]

For this reason the indirect and direct van den Bergh reactions have been proposed for the differential diagnosis between hemolytic and obstructive jaundice. This is based upon the hypothesis that hemolytic jaundice is due to the production of bilirubin from the hemoglobin by excessive destruction of erythrocytes by the reticulo-endothelial and mesenchymal cells at a rate and in amounts beyond the capacity of the parenchymal cells of the liver to excrete it fast enough, with consequent accumulation in the blood beyond 0.25 mg. per 100 cc. of serum as measured by the indirect reaction but with a negative direct reaction if there is no reabsorption of the bilirubin excreted by the liver. In intrahepatic and extrahepatic obstructive jaundice, however, the excreted bile is reabsorbed with the production of positive direct and indirect reactions.

Clinically, however, the differentiation is not always so easy and care is required in the use and interpretation of the term "obstructive." For example, the excretion of normal bilirubin may be, and usually is, sufficiently "obstructed" because of impairment in the excretory capacity of the parenchymal cells in hepatocellular hepatitis due to infection or toxic agents to produce an increase of it in the serum, as determined quantitatively by the indirect test, as well as a positive direct reaction due to the absorption of excreted bile because of coincident obstruction of the bile ducts from cholangitis, increased permeability of the cholangioles (toxic) or actual rupture of them due to increased biliary pressure and especially of the ampullae of the bile capillaries. Likewise in extrahepatic obstruction the direct reaction is not only positive, due to the absorption of excreted bilirubin, but the indirect reaction also usually shows an increase because back pressure on the parenchymal cells is always apt to reduce their capacity for removing the pigment from the blood. In hemolytic jaundice resulting from the excessive destruction of erythrocytes, however, the indirect reaction only may be positive because bilirubin is produced in too large amounts for the parenchymal cells to excrete it fast enough, plus the fact that the same cause for excessive hemolysis may also injure them sufficiently to reduce their excretory capacity; but if the bile ducts are also sufficiently swollen at the same time from cholangitis to produce obstruction some of the excreted bile is absorbed, with the result that a positive direct reaction may be likewise observed.

The Icterus Index. An increase of bilirubin in the serum is also detected by the *icterus index* which is a simpler procedure than the van den Bergh tests, since it merely compares the color of the serum with a standard 1:10,000 solution of potassium bichromate in a colorimeter with the results expressed in terms of units. The normal, due to bilirubin in the blood before excretion, varies from 4 to 6 units. Values of 7 to 15 indicate an increase, but usually without jaundice of the tissues, and constitute the zone of "latent jaundice." Values above 16 are usually associated with frank jaundice, particularly in bilirubinemia due to obstruction, with a rough correlation between the index and the indirect van den Bergh reaction, since an index of 10 usually corresponds to about 1 mg. and an index of 25 to about 2.5 mg. per 100 cc. of serum in the latter.

The icterus index, however, is unable to differentiate between bilirubin before and after excretion by the liver; it is, therefore, a measure of the total bilirubin which is no aid in differential diagnosis between hemolytic and obstructive jaundice. But since the capacity of the van den Bergh test in this connection is of limited clinical value, as previously discussed, the icterus index is a very useful procedure for the detection of bilirubinemia. However, the presence of free hemoglobin resulting from hemolysis interferes with the accuracy of the test and for this reason it is advisable not only to draw blood with a dry needle into a dry tube which may be centrifuged and to separate promptly the serum from the corpuscles with the minimum of trauma to the erythrocytes, but to conduct the test before the serum has become cloudy. Furthermore, the blood should not be drawn soon after a meal, as the presence of lipemia may also render the serum too cloudy for use. Another important source of error is the fact that substances other than bilirubin may impart a yellow color to the serum, the most important of which are carotene and xanthophyll to which further reference will be made shortly.

It is apparent, therefore, that the concentration of bilirubin in the blood as determined by the van den Bergh or icterus index tests is dependent upon the number and rate of destruction of erythrocytes, the functional capacity of the cells of the reticulo-endothelial cells, the functional capacity of the parenchymal cells of the liver and the patency of the biliary ducts. It should be emphasized that its determination is not only the most certain means of detecting latent jaundice in both calculous and noncalculous cholycystitis, hepatic disease, congestive heart failure and other extrahepatic diseases, but that it gives a much more definite idea of the severity and fluctuations of frank jaundice than examinations of the skin, urine or feces. This is particularly true before operations on the biliary tract in patients with jaundice in whom serial determinations are of far greater value in determining the most favorable time for surgical intervention (especially for the removal of stones in the common bile duct) than observations on the color of the skin and conjunctivae. It is true, however, that the amount of bilirubin in the blood required for the production of clinical jaundice varies in different types because of variations in the size of its molecule. For example, it has long been recognized clinically that jaundice and the presence of bile pigment in the urine occur more commonly in the obstructive than in the hemolytic types, probably because in the former bilirubin is free in the serum and therefore more diffusible, while in the latter it is adsorbed by the globulins and consequently less diffusible.

Hyperbilirubinemia. An increase of bilirubin in the blood either before hepatic excretion, after excretion, or a combination of both is designated as *bilirubinemia.* This may occur not only (1) as the result of prolonged fasting and (2) in untrained individuals going suddenly into high altitudes with resulting over-activity of the blood-making organs and increased blood destruction by the cells of the reticulo-endothelial system, but also and more especially in (3) extrahepatic obstruction of the biliary ducts by calculi, adhesions, parasites, kinks from hepatoptosis and compression or stenosis by carcinoma of the head of the pancreas or pancreatic calculi, aortic aneurysms, etc., as well as from (4) intrahepatic biliary obstruction from cholangitis, malignant tumors, atresia, parasites, cirrhosis (portal and biliary), etc.; also as a result of (5) acute and chronic hepatitis from infections (infectious hepatitis, yellow fever, infectious or leptospiral jaundice, syphilis, etc.); (6) acute hepatitis due to drugs and toxic agents (cinchophen, arsphenamine, chloroform, phosphorus, carbon tetrachloride, x-rays, etc.); (7) in eclampsia; (8) acute yellow atrophy of the liver; (9) in congestive heart failure because of functional incapacity due to anoxemia and (10) in celiac disease of children.

Hyperbilirubinemia may also result from excessive blood destruction but is seldom as severe as in the conditions just mentioned, since values above 10 mg. per 100 cc. of serum are extremely rare and above 6 mg. quite unusual. Under such conditions, however, hyperbilirubinemia may occur (1) in hemolytic jaundice of the congenital, familial or acquired types; (2) paroxysmal hemoglobinuria; (3) acute hemolytic anemia of Lederer (acute febrile anemia); (4) pernicious anemia; (5) sickle cell anemia; (6) splenic anemia; (7) polycythemia and especially if phenylhydrazine hydrochloride is being administered; (8) in the newborn (icterus neonatorum); (9) occasionally from the absorption of blood in concealed hemorrhages; (10) following hemolysis in post-transfusion reactions and (11) from acute or chronic infections producing excessive hemolysis, as in malaria, oroya fever, hemolytic streptococcus or staphylococcus septicemias, etc., as summarized in Table 29.

TABLE 29. SUMMARY OF THE CLINICAL INTERPRETATION OF THE BILIRUBIN OF THE BLOOD

Normal in the serum: Direct van den Bergh none; indirect 0.1 to 0.25 mg. per 100 cc.; icterus index: 4 to 6 units.

Hyperbilirubinemia (increase)	Hypobilirubinemia (decrease)
Prolonged fasting.	Postdigestional.
High altitudes.	Aplastic anemia.
Extrahepatic biliary obstruction (calculi, adhesions, parasites, kinks, compression, etc.).	"Secondary" anemias (especially those due to malignancy and nephritis).
Intrahepatic biliary obstruction (cholangitis, malignant tumors, atresia, parasites, portal and biliary cirrhosis, etc.).	
Acute and chronic hepatitis due to infections (infectious jaundice, yellow fever, leptospiral jaundice, syphilis, etc.).	
Toxic hepatitis due to cinchophen, arsphenamine, phosphorus, x-rays, nicotinic acid, etc.	
Diabetes mellitus (some cases).	
Acute yellow atrophy of the liver; eclampsia.	
Congestive heart failure.	
Celiac disease of children.	
Hemolytic jaundice (congenital, acquired).	
Paroxysmal hemoglobinuria.	
Acute hemolytic anemia.	
Pernicious anemia.	
Sickle cell anemia.	
Splenic anemia.	
Polycythemia (phenylhydrazine).	
Icterus neonatorum.	
Concealed hemorrhage.	
Post-transfusion hemolysis.	
Malaria; Oroya fever (Carrión's disease).	
Severe burns.	
Streptococcus septicemia.	
Staphylococcus septicemia.	

Hypobilirubinemia. A decrease of bilirubin in the blood is designated as *hypobilirubinemia* and is of much less frequency and clinical importance. It may occur (1) in three to six hours after a meal and is not unusual in (2) aplastic anemia and (3) all "secondary" anemias, with special reference to those due to malignancy and nephritis. Curiously enough, a reduction in the hyperbilirubinemia of patients with marked jaundice due to carcinoma of the head of the pancreas may occur even though there is complete obstruction of the common bile duct, probably due to a decrease in the rate of destruction of erythrocytes with consequent reduction in the rate of formation of bilirubin.

Carotene. As previously stated, the presence of carotin or carotene in the serum may introduce error into the conduct of the icterus index because of its yellow color. This lipochrome, which is regarded as being pro-vitamin A, is present in many common vegetables, principally carrots, pumpkins, yellow squash, yellow turnips, parsnips, spinach, lettuce, green and yellow beans, kale and oranges as well as in eggyolk, butter and milk. Bile acids are necessary for its absorption from the intestine. Normally it may be found in an amount of about 60 to 368 micrograms per 100 cc. of plasma or serum. An increase is designated as *carotinemia* which occurs principally in children following the ingestion of large amounts of carrots and producing a canary-yellow discoloration of the skin and mucous membranes. It may also occur in diabetic individuals chiefly because of a large intake of carotene-containing vegetables in the diet along with a possible diminished capacity for converting this substance into vitamin A.

PROTHROMBIN

As is well known, prothrombin is essential for the coagulation of shed blood since, according to the theory of Howell, it is converted into thrombin by the action of calcium ions (after neutralization of antiprothrombin by the thromboplastin of platelets) which in turn converts fibrinogen into fibrin. It has some of the characteristics of an enzyme and for this reason has been called "prothrombase" by Mellanby, who does not believe that calcium ions are necessary for its conversion into thrombin.

TABLE 30. SUMMARY OF THE CLINICAL INTERPRETATION OF PROTHROMBIN IN THE BLOOD

Normal prothrombin time of plasma: 10 to 25 seconds.

Hyperprothrombinemia: due to functional incapacity of the liver to utilize vitamin K.	Hypoprothrombinemia: due to lack of vitamin K or failure of its absorption.
Extrahepatic obstructive jaundice due to calculus, carcinoma of the head of the pancreas, etc. Intrahepatic obstructive jaundice due to cirrhosis, malignancy, etc. Hepatocellular jaundice due to acute hepatitis from infection or toxic agents. Acute yellow atrophy of the liver. Pernicious anemia in relapse.	Dietary deficiency. Biliary fistula with loss of bile and bile salts in the small intestine required for absorption. Intestinal diseases interfering with absorption. Defective digestion and absorption of fats due to deficiency in lipases. Icterus neonatorum.

Prothrombin is now believed to be formed exclusively in the liver without the storage of any material excess, probably from circulating platelets, by the action of vitamin K. Bleeding from the failure of coagulation may be due to a deficiency of the vitamin, a functional incapacity of the liver to utilize it in the production of prothrombin, or to a combination of these factors. Insofar as the liver is concerned, it is at least well established that the coagulation of the blood is generally reduced in individuals with obstructive jaundice as well as, occasionally, in those with jaundice due to hepatic disease (hepatocellular jaundice) which accounts for the hemorrhagic tendency manifested under these conditions (Table 30). This hemorrhagic tendency is also noted in cases of biliary fistula because of the absence in the small intestine of bile salts which are essential for the absorption of vitamin K. It is doubtful that an increase of prothrombin ever occurs or can be produced.

Unfortunately, there are no direct methods for the accurate quantitative estimation of prothrombin. The indirect method of Quick is commonly employed with plasma which normally produces coagulation in 10 to 25 seconds. There is, however, a wide margin of safety. When it is reduced in the plasma to between 30 and 40 per cent of the normal, the "prothrombin time" is likely to be at the upper limit of normal or about 20 seconds; when reduced to about 10 per cent of normal it is likely to be around 40 seconds and when reduced to about 5 per cent around 70 seconds.

Vitamin K. As previously stated, vitamin K is intimately involved in the production of prothrombin. For this reason it is commonly referred to as the "coagulation vitamin" and is discussed more fully in Chapter 20. It occurs as a fat-soluble substance in leafy green vegetables, as well as in certain tissues, and has been produced synthetically in the form of 2-methyl-1,4 naphthoquinone which may be administered orally or by intramuscular or intravenous injection. When given orally, the presence of whole bile or bile salts in the small intestine is required for the absorption of either the natural or synthetic vitamin. A deficiency of vitamin K and consequently hypothrombinemia may occur as a result of (1) insufficient intake of the vitamin; (2) a lack of bile salts in the intestine sufficient for its absorption; (3) an impaired absorption of the vitamin from the intestine because of intestinal disease, or (4) because of defective digestion and absorption of fats due to a lack of lipases.

The administration of vitamin K, therefore, has proved of value in the treatment of hypothrombinemia, provided the liver is capable of utilizing it in the production of prothrombin; also in the prevention of icterus neonatorum by its administration to the mother before delivery or to the infant at the time of birth. It may fail, however, in the treatment of some cases of severe disease of the parenchyma of the liver as well as in patients with severe chronic septic infections. In these, transfusions of whole fresh, not "bank" blood, are likely to be more efficacious.

LACTIC ACID

Lactic acid is a product of the chemical processes involved in muscular activity and the amount present in the blood results from the breakdown of glycogen in the muscles. In the tissues part of the lactic acid undergoes immediate oxidation while the remaining portion is carried to the liver where the amount escaping oxidation is reconverted into glycogen. Normally the venous blood contains from 6 to 20 mg. per 100 cc. but this may be increased by muscular exercise.

The lactic acid of the blood may be increased (1) in states of anoxemia resulting in decreased oxidation and (2) in diseases of the liver resulting in an impairment of its glycogenic function. Consequently several investigators have found an increase of lactic acid in the blood in individuals with congestive heart failure after muscular activity and in severe cases even at rest, although the results reported have not been uniform and indicate the need of further study of lactic acid metabolism in heart disease. But there can be no doubt of the fact that when the glycogenic function of the liver is seriously impaired the blood lactic acid undergoes a distinct increase, as reported in many cases of extensive acute hepatitis from infections or toxic agents as well as in the terminal stages of cirrhosis of this organ. Indeed, a "lactic acid tolerance test" conducted by the intravenous injection of lactic acid or sodium lactate has been proposed for the detection of impairment of the glycogenic function of the liver. The lactic acid of the blood also tends to become increased in lobar pneumonia, apparently as a result of anoxemia, and likewise during attacks of familial periodic paralysis, evidently because of a disturbance in its metabolism in the muscles in this disease.

CHLORIDE

The chlorides of the blood, other body fluids and the tissues play an important and essential part in the maintenance of normal acid-base equilibrium, the osmotic equilibrium and the water balance of the body. They exist in the plasma chiefly in the form of sodium chloride, the amount and distribution of which may be affected by factors which exert their influence primarily upon the concentration and distribution of sodium in the body fluids. In other words, the amounts of chlorides and sodium in the blood are usually altered simultaneously and in the same direction, but exceptions may occur and notably in pyloric and upper intestinal obstruction, to which further reference will be made.

The erythrocytes contain only about one-half as much chloride as the plasma because of their relatively high content of protein (hemoglobin) which limits the quantity of base available for combination with electrolytes and ions, and their relatively low water content which limits the concentration of electrolytes compatible with the maintenance of isotonicity. As a general rule, the chloride of the blood is expressed in terms of the chloride ion, which varies normally from 340 to 370 mg. per 100 cc. of serum, or in terms of sodium chloride which varies normally from 570 to 620 mg. per 100 cc. of plasma (Table 19). For this purpose oxalated blood is used but the specimen should be sent to the laboratory as soon as possible for the removal of erythrocytes to avoid a shift of chloride from plasma to these cells incident to the formation of acid which occurs upon standing. Furthermore, because of the shift of chloride to the erythrocytes, and in the opposite direction, with changes in the HCO_3 concentration of the plasma, blood is preferably collected under oil, for, if exposed to air, carbon dioxide escapes with an increase of plasma chloride.

Sodium chloride is eliminated from the body chiefly by the kidneys in the urine and, to a lesser extent, in the sweat and feces. Under normal conditions it undergoes but slight variations in the blood, due to the regulatory mechanism of the suprarenal cortex in maintaining normal potassium, sodium and water content and distribution in the body fluids. It may fall slightly during periods of active gastric secretion and rise in the postdigestive period, owing probably to its reabsorption from the intestines. An excessive sodium chloride intake with foods causes only a slight increase in the plasma and complete withdrawal rarely results in a reduction below the low limit of normal (570 mg. per 100 cc.). In starvation states, however, the plasma chloride is likely to be definitely reduced by sodium chloride deprivation and particularly by acidosis resulting from its passage from plasma to erythrocytes.

Hyperchloremia. An increase of sodium chloride in the plasma is designated as *hyperchloremia*. Since sodium chloride is normally excreted largely by the kidneys, it would naturally be expected that nephritis and other diseases of these organs producing deficient elimination would result in its accumulation in the blood but, as a matter of fact, this does not always occur because of the intervention of extrarenal factors. Certainly it is becoming increasingly apparent that nephritis cannot be classified into forms characterized by sodium chloride and water retention and others by nitrogen retention. However, hyperchloremia may occur (1) in chronic glomerulonephritis, especially in the nephrotic type, as well as (2) in nephrosis, but does not bear any constant relation to the occurrence or degree of edema. Rather the edema appears to be due to a decrease of plasma protein although in patients with nephrotic edema, with previously normal plasma sodium chloride, hyperchloremia may occur in association with diuresis and the elimination of large quantities of edema fluid. One apparent reason for the less frequent occurrence of hyperchloremia in nephrotic patients, in spite of large amounts of salt in the diet, is the fact that the retained sodium chloride is rapidly distributed throughout the edema fluid. Under the conditions hyperchloremia is seldom observed in the advanced stages of chronic glomerulonephritis unless large amounts of salt are being taken without a large amount of fluids at the same time. The same is true (3) in acute glomerulonephritis, in which hyperchloremia may occur, however, if there is hypoproteinemia without excessive fluid loss by vomiting or diarrhea.

Hyperchloremia may also occur (4) in complete obstruction of the urinary tract, but because of the frequent coincidence of factors tending to produce hypochloremia it is not commonly observed.

Contrary to widespread impressions, the level of plasma sodium chloride in chronic nephritis bears no constant relation to the occurrence or degree of hypertension, but hyperchloremia may be observed (5) in some cases of essential hypertension complicated by nephritis; also occasionally (6) in cardiac decompensation with edema; (7) in states of hyperventilation, as in hysteria and the postencephalitic syndromes, because of the excessive loss of CO_2 through the expired air; (8) following the parenteral administration of sodium chloride for the purpose of inducing diuresis in patients with urinary suppression, in which the hyperchloremia is frequently accompanied by acidosis and may terminate fatally; (9) after the crisis in pneumonia and (10) in some cases of hypopituitarism (Table 31).

Hypochloremia. A decrease of sodium chloride in the plasma is designated as *hypochloremia*. Since a large amount of chloride is normally secreted into the stomach in addition to that ingested in foods, hypochloremia is especially likely to occur (1) because of a loss of the gastro-intestinal secretions through excessive vomiting resulting from pylorospasm, upper intestinal obstruction, gastro-enteritis, and such toxic states as uremia and the toxemias of pregnancy. The reduction in plasma sodium chloride is apparently due to its loss in the vomitus and not, as formerly believed, to its retention in the tissues. The same may occur (2) because of diarrhea or the constant loss of gastric juice, pancreatic secretions or bile through external fistulas. In all of these conditions, however, the actual degree of hypochloremia may be masked by the associated state of dehydration, so that the plasma sodium chloride content may not be a true index of the chloride requirements of the body.

TABLE 31. SUMMARY OF THE CLINICAL INTERPRETATION OF CHLORIDE OF THE BLOOD

Normal: Plasma (as NaCl): 570–620; serum (as Cl): 340–370 mg. per 100 cc.

Hyperchloremia (increased)	Hypochloremia (reduced)
Slight in postdigestive period.	Slight during active digestion.
Slight with excessive intake of salt.	Starvation states.
May occur in chronic glomerulonephritis with hypoproteinemia and high salt intake; also in nephrosis under the same conditions.	Excessive vomiting resulting from pylorospasm, intestinal obstruction, uremia, toxemias of pregnancy, alimentary toxicoses of infants, etc.
May occur in acute glomerulonephritis with hypoproteinemia without excessive fluid loss.	Excessive diarrhea or loss of gastric and pancreatic secretions and bile through external fistulas.
May occur in complete obstruction of the urinary tract	May occur in advanced chronic nephritis and especially in uremia.
May occur in essential hypertension with associated nephritis.	Common in nephrosis, especially that due to bichloride of mercury poisoning.
Sometimes in cardiac decompensation with edema.	Hepatic cirrhosis with low salt intake following withdrawal of ascitic fluid and hepatocellular jaundice.
In states of hyperventilation like hysteria, postencephalitic syndromes, etc.	Severe diabetes with acidosis. Some of the infectious diseases (rheumatic fever, meningitis, lobar pneumonia, active pulmonary tuberculosis).
After the parenteral administration of sodium chloride in the presence of suppression of the urine.	Emphysema (some cases).
After the crisis in lobar pneumonia.	Addison's disease.
Simmonds' disease (pituitary cachexia).	Excessive sweating, with the drinking of large amounts of salt-free water.
Cushing's syndrome.	Tendency in hyperparathyroidism.
	Tendency after operations, especially those upon the gastro-intestinal tract.

Hypochloremia may also occur (3) in advanced nephritis and especially in uremia because of a failure to conserve sodium chloride through tubular reabsorption, as likewise (4) in nephrosis and especially that due to bichloride of mercury poisoning, in which case the low plasma chloride is also due to a retention of other acid ions producing a decrease in plasma bicarbonate and the passage of chloride from the plasma into the erythrocytes. The hypochloremia, however, may be masked to some extent by dehydration and hemoconcentration. It is less frequently observed than hyperchloremia in nephritic edema or that due to congestive heart failure, although it sometimes occurs because of a "locking" of sodium chloride in the edema fluid, especially if the patient is on a low salt diet.

Hypochloremia may also occur (5) in hepatic cirrhosis of patients on a low salt intake following the withdrawal of large amounts of ascitic fluid and likewise in acute hepatitis resulting in hepatocellular jaundice; (6) in some cases of severe diabetes, owing to polyuria and acidosis; (7) in several of the infections, including rheumatic fever, meningitis, lobar pneumonia and active pulmonary tuberculosis, for reasons as yet unknown but apparently not because of retention of sodium chloride in the tissues ("historetention") as commonly believed, or the retention of sodium rather than chloride; (8) in some cases of emphysema, because of increased CO_2 tension of the alveolar air with passage of chloride from the plasma to the erythrocytes; (9) in Addison's disease, because of a deficiency of the adrenal cortical hormone producing an increased elimination of sodium; (10) in individuals exposed to high external temperatures with excessive sweating and the drinking of large amounts of salt-free water; (11) in some cases of hyperparathyroidism and (12) occasionally after operation, especially those on the gastro-intestinal tract.

SULFATES

The importance of sulfates in the blood in relation to renal insufficiency has commanded considerable attention in recent years with special reference to the inorganic and conjugated sulfates representing the oxidized portion of the non-protein sulfur. The normal range of inorganic sulfates is from 2.5 to 5 mg. per 100 cc. of serum, corresponding to 0.8 to 1.7 mg. in terms of sulfur. In advanced renal damage there is a consistent relationship between sulfate and phosphate retention and together they appear to account for the total increase in undetermined acid in nephritic acidosis. An increase of the inorganic sulfates of the blood, therefore, may occur in advanced glomerular nephritis, nephrosclerosis, the nephritis of pregnancy, pyelonephritis, the uropathies due to obstruction and polycystic kidney disease. As a general rule, there is a coincident retention of urea nitrogen, creatinine and inorganic phosphate, but the retention of inorganic sulfates with an increase in the blood may occur before other tests reveal any change in kidney function. As renal insufficiency advances, the sulfates continue to increase and may reach as high as 50 to 100 mg. per 100 cc. of serum.

SODIUM AND POTASSIUM

Since the greatest portion of the sodium of the body exists as sodium chloride, changes of sodium balance and concentration in the serum are usually accompanied by simultaneous and similar changes in chloride balance and concentration. For this reason a determination of the sodium chloride of the plasma is usually sufficient for clinical purposes. However, exceptions may occur, notably in patients with excessive loss of gastric juice from vomiting, as in pyloric stenosis or other types of upper intestinal obstruction, in which the loss of chloride in the form of hydrochloric acid is greatly in excess of that of sodium.

Sodium and potassium constitute about 95 per cent or more of the total base of the blood plasma and both (especially sodium) are extremely important in the maintenance of normal osmotic pressure of the tissue fluids and in the maintenance of normal acid-base equilibrium, apart from serving other physiologic functions. Consequently, disturbances in their metabolism are intimately associated with disturbances of water balance and acid-base equilibrium.

Under normal conditions both sodium and potassium salts are completely absorbed from the gastro-intestinal tract; at least 90 to 95 per cent of each is excreted in the urine. Sodium in normal blood serum ranges from 315 to 340 mg. and potassium from 16 to 22 mg. per 100 cc. There is little or no sodium in erythrocytes but these cells carry about 420 mg. of potassium per 100 cc.

TABLE 32. SUMMARY OF THE CLINICAL INTERPRETATION OF SODIUM AND POTASSIUM IN THE BLOOD

Normal sodium: 315 to 340 mg. and potassium: 16 to 22 mg. per 100 cc. of serum.

Sodium	Potassium
(1) Increased: Cushing's disease (basophilic adenoma of anterior lobe of the pituitary gland). (2) Decreased: Addison's disease. Pyloric stenosis and high intestinal obstruction. Severe and prolonged diarrhea. Pancreatic, biliary and jejunal fistulas. Excessive sweating with large intake of salt-free water. Chronic glomerulonephritis with uremia. Diabetes mellitus with acidosis. Lobar pneumonia before the crisis. During ether anesthesia. Some cases of congestive heart failure. Some cases of hepatic necrosis. Some cases of hypoparathyroidism.	(1) Increased: Addison's disease. Uremia. Portal cirrhosis with ascites. Epilepsy after convulsions. Some cases of lobar pneumonia. Hyperparathyroidism. Acute high intestinal obstruction. (2) Decreased: Hyperpituitarism (Cushing's disease). Diabetic acidosis.

A *decrease of the sodium of serum* is especially encountered (1) in Addison's disease, because of a deficiency of the cortical hormone resulting in the increased excretion of sodium, chloride and water by the kidneys, with retention of potassium, to which further reference will be made shortly. A decrease is seen also in states characterized by the excessive loss of the gastro-intestinal secretions, as from severe vomiting in (2) pyloric stenosis or other types of high intestinal obstruction, (3) severe and prolonged diarrhea and (4) pancreatic, biliary or jejunal fistulas. A decrease may also occur (5) from excessive perspiration, with a large intake of salt-free water; (6) in advanced chronic glomerulonephritis with uremia; (7) in advanced diabetes mellitus, especially in the presence of marked acidosis; (8) in lobar pneumonia before the crisis; (9) during ether anesthesia; (10) in some cases of congestive heart failure; (11) occasionally in hepatic necrosis and (12) in some cases of hypoparathyroidism (Table 32).

An *increase of the sodium of the serum* is not frequently encountered, except in hyperpituitarism or Cushing's disease (basophilic adenoma of the anterior lobe of the pituitary gland) when the increase is accompanied by an increase of plasma chloride, a decrease in serum potassium and a tendency to alkalosis. These changes have been ascribed to hyperfunction of the adrenal cortex resulting from overstimulation by the adrenotropic hormone. It has also been suggested that certain sex hormones may cause a decrease of excretion of sodium in the urine, with a consequent increase in the blood serum, which may have a bearing upon the occasional occurrence of edema in connection with menstruation.

An *increase of serum potassium* is characteristic (1) of Addison's disease and may occur in (2) uremia, portal cirrhosis, especially in patients with ascites and on a high potassium intake with frequent drainages; (3) epilepsy after convulsions; (4) possibly during or after lobar pneumonia; (5) hyperparathyroidism (generalized osteitis fibrosa) and (6) acute high intestinal obstruction. A *decrease* may occur in Cushing's disease and diabetic acidosis.[20]

Magnesium. The absorption of magnesium from the small intestine resembles that of calcium in many respects. The influence of vitamin D is uncertain but it may play some rôle in its absorption and retention. It is eliminated in the feces and urine, with some evidence that urinary excretion is reduced in the presence of marked renal impairment.

In the blood, magnesium occurs in both plasma and erythrocytes. The amounts observed have varied according to different methods of analysis employed but the normal range of serum apparently varies from 1.8 to 3.6 mg. per 100 cc. From 60 to 90 per cent (average 80 per cent) is in a diffusible state, with the balance non-diffusible and combined with serum protein.

Significant deviations from the normal do not occur consistently enough to be of clinical importance. An increase may occur more frequently in chronic glomerulonephritis than in any other disease, especially during uremia. Slightly increased values have been reported in hypertrophic arthritis, essential hypertension, atherosclerosis and following the administration of parathyroid hormone.

CALCIUM

Calcium is among the important mineral constituents of the blood in relation to disease. It is chiefly absorbed from the upper part of the small intestine, the degree of absorption being dependent upon factors influencing the solubility of calcium salts, among which hydrogen ion-concentration and the phosphate present are the most important. Thus, absorption is diminished by increased alkalinity, by a high phosphate content with the formation of insoluble tertiary calcium phosphate, and by large amounts of fatty acids with the formation of insoluble calcium soaps as observed clinically in obstructive jaundice, sprue, celiac disease and similar disorders. It is believed by some that vitamin D and ultraviolet light promote the absorption of calcium but it appears that their main influence is upon the intermediary metabolism of calcium and phosphorus.

After absorption, calcium is stored in the bones (with very little present in the soft tissues) where it is readily called upon under physiologic and pathologic conditions. In the maintenance of a normal equilibrium, from 10 to 30 per cent is eliminated in the urine and from 70 to 90 per cent in the feces, the calcium being actively excreted into the large intestine as calcium phosphate and calcium carbonate.

The calcium of the blood is contained entirely in the plasma. Determinations are best made by using serum, which normally contains from 8.5 to 11.5 mg. per 100 cc., although some investigators place 12 mg. as the upper limit of normal. This total serum calcium exists as diffusible and nondiffusible fractions, the latter occurring in combination with serum proteins. The former is or contains the physiologically active calcium in an ionizable state and constitutes about half the total serum calcium, the condition of acid-base balance being apparently important in determining the degree of ionization.

The intermediary metabolism of calcium and the factors involved in the maintenance of normal serum calcium and its partition are extremely important in relation to changes in disease. The parathyroid glands are intimately involved, since the administration of the hormone produces an increase of both the diffusible and nondiffusible calcium of the serum, due either to a direct effect upon calcium metabolism or primarily upon phosphorus metabolism with a secondary effect upon the calcium. Vitamin D and ultraviolet irradiation also increase serum calcium through promotion of calcium absorption and an influence upon the intermediary metabolism of calcium and phosphorus. Under normal conditions the effect of vitamin D appears to be dependent upon a normal supply of parathyroid hormone, although in the absence of the latter a deficiency may be largely corrected by the administration of excessively large doses of calcium with proper dietary regulation. In other words, vitamin D appears to have the same effect upon serum calcium as does parathyroid hormone. Some observers believe that it stimulates the production of the hormone but apparently the action of the two substances is not fundamentally the same.

Since about half the calcium of the blood is bound to the serum proteins, especially the albumin (nondiffusible calcium), although these proteins are not completely "saturated" with calcium, alterations in the amount of serum calcium may vary with alterations in the serum proteins. Furthermore, there is a roughly reciprocal relationship between the serum calcium and the serum inorganic phosphate; as the latter ion increases the calcium ion decreases, and vice versa.

Hypercalcemia. An increase of total serum calcium is designated *hypercalcemia.* It occurs characteristically (1) in hyperparathyroidism due to the over-production of parathormone and (2) in multiple myeloma, in which the cause is still uncertain, since it cannot be ascribed to hyperparathyroidism, although in some cases it is apparently due to an increase of serum proteins. Not infrequently it is also increased (3) in neoplastic disease of bone and especially metastatic carcinoma and (4) to a mild degree in diseases in which the CO_2 tension of the blood is increased, thereby enhancing its capacity for maintaining calcium in solution, as in extensive emphysema, pneumoconiosis, congestive heart failure or any cause of asphyxia. It is also sometimes observed (5) in polycythemia vera, for unknown reasons, (6) in Addison's disease, (7) in Cushing's disease (pituitary basophilism), (8) in chronic nephritis with uremia, (9) sometimes in pregnancy and (10) after the administration of excessive amounts of vitamin D or viosterol (Table 33).

TABLE 33. SUMMARY OF THE CLINICAL INTERPRETATION OF BLOOD CALCIUM

Normal total serum calcium: 8.5 to 11.5 mg. per 100 cc.; of this about one-half is diffusible calcium.

Hypercalcemia (increased)	Hypocalcemia (decreased)
Hyperparathyroidism. Multiple myeloma. Neoplastic disease of bones. Extensive emphysema. Pneumoconiosis. Congestive heart failure. Polycythemia vera. Sometimes in Addison's disease. Sometimes in Cushing's disease. Sometimes in nephritis with uremia. Sometimes after excessive amounts of vitamin D or viosterol.	Hypoparathyroidism (infantile tetany; idiopathic juvenile or adult tetany; after parathyroidectomy; sometimes after thyroidectomy). Deficiency of vitamin D. Osteomalacia. Hunger osteopathy. Celiac disease. Sprue. Hypoproteinemia. Occasionally in nephrosis. Occasionally in non-nephrotic chronic glomerulonephritis. Kala-azar. Prolonged obstructive jaundice. Malignancy and cachectic states. Sometimes in advanced pregnancy. Sometimes in manic depressive psychosis. Sometimes in renal rickets (renal dwarfism) but usually in ordinary rickets.

Hypocalcemia. A decrease in the total serum calcium is designated *hypocalcemia.* It is characteristically found (1) in hypoparathyroidism with tetany (infantile, or spasmophilia) as well as in idiopathic juvenile and adult tetany (occupational) and in the tetany following parathyroidectomy or thyroidectomy (disturbed blood supply); also (2) in osteomalacia; (3) in hunger osteopathy due to a deficiency in calcium, phosphorus and vitamin D; (4) in celiac disease and (5) sprue, in both of which the decrease is due to inadequate absorption of calcium and vitamin D; (6) occasionally in nephrosis as well as (7) in non-nephrotic cases of chronic glomerulonephritis with hypertension and nitrogen retention; (8) in kala-azar as a result of a decrease in serum albumin; (9) in prolonged obstructive jaundice; (10) in malignancy and other cachectic conditions because primarily of a decrease of serum albumin and sometimes (11) in advanced pregnancy due to the demands on maternal calcium by the fetus; (12) in manic depressive psychosis and (13) in renal rickets (renal dwarfism) but not usually in ordinary rickets which is due to vitamin D deficiency with a characteristic decrease of serum phosphate, although hypocalcemia occurs in some cases along with the manifestations of tetany.

PHOSPHORUS

Phosphorus is present in the blood in the form of inorganic phosphates, nucleotides, phosphate esters, and as lipids such as lecithin, cephalin and sphingomyelin. Determinations of inorganic phosphate in the serum, however, serve all practical clinical purposes in relation to inorganic phosphorus metabolism. In adults it varies normally from 3 to 4.5 mg. and in infants and young children from 4 to 6 mg. per 100 cc. of serum. The amounts, however, vary directly with the concentration of solar ultraviolet rays, being highest in summer and lowest in winter. Variations also occur in relation to carbohydrate utilization, since combinations of carbohydrate and phosphoric acid (hexone-phosphate) play an important part in the intermediary metabolism of carbohydrate. Thus, following the ingestion of carbohydrates there is a gradual and progressive decrease of serum phosphate which persists during the period of increased glucose utilization, returning to normal in four to five hours. This fall occurs independently of the blood sugar, since it depends entirely upon the utilization of glucose in the tissues.

An enzyme, phosphatase, normally present in the bones, kidney, striated muscle and other tissues, including the blood, also liberates inorganic phosphate by the hydrolysis of phosphoric esters for the deposition of calcium phosphate in the bones as well as serving other physiologic functions, shortly to be discussed in more detail.

The absorption and utilization of inorganic phosphate is greatly influenced by the amount of calcium in the intestinal tract as well as by vitamin D. Some phosphate is undoubtedly absorbed from the upper portion of the small intestine, especially when there is a diminution in the alkalinity of the intestinal contents. A large amount, however, is also excreted into this part of the bowel with reabsorption from the lower intestine. But the degree of reabsorption is inversely proportional to the excretion of calcium because of the formation of tertiary calcium phosphate which is insoluble. Therefore, if there is a decreased utilization of calcium in the bones it is excreted in relatively large amounts into the intestine, with a correspondingly reduced absorption of phosphates. Vitamin D appears to increase the degree of both absorption and utilization of ingested phosphate. Under normal conditions about 30 per cent of the latter is eliminated in the feces and 70 per cent in the urine (chiefly in the form of the acid salt).

Hyperphosphatemia. An increase of serum phosphate is designated *hyperphosphatemia.* This may occur to a slight degree (1) during the healing of fractures and (2) as the result of excessive administration of vitamin D in fish liver oils or viosterol, as well as after ultraviolet irradiation. A slight increase may also occur (3) in hypoparathyroidism which is more or less proportional to the reduction of serum calcium. Phosphate retention may likewise occur (4) in chronic glomerulonephritis and nephrosclerosis with uremia as the result of renal insufficiency in which retention contributes to the production of acidosis because of a decrease in alkali reserve. However, acidosis may exist without hyperphosphatemia but the latter, when it occurs, has approximately the same clinical significance as retention of creatinine and is of serious prognostic import. A similar increase of serum phosphate may occur in renal insufficiency due to (5) pyelonephritis, (6) tuberculosis, (7) polycystic disease and (8) multiple myeloma as well as (9) in acute high intestinal obstruction. An increase may also occur (10) in acute yellow atrophy of the liver, (11) in myelogenous leukemia, (12) Addison's disease and (13) in renal rickets or renal dwarfism (Table 34).

TABLE 34. SUMMARY OF THE CLINICAL INTERPRETATION OF PHOSPHATE IN THE BLOOD

Normal for adults: 3 to 4.5 mg. and for infants and children: 4 to 6 mg. per 100 cc. of serum.

Hyperphosphatemia (increased)	Hypophosphatemia (decreased)
During healing of fractures.	Temporarily after the oral or intravenous administration of glucose.
Excessive administration of vitamin D.	Temporarily in hyperinsulinism following the administration of insulin or epinephrine; and after administration of parathormone.
Hypoparathyroidism.	Rickets.
Renal insufficiency (nephritis with edema, pyelonephritis, tuberculous nephritis, polycystic disease, etc.).	Osteomalacia.
Multiple myeloma.	Osteitis fibrosa diffusa and osteoporosis.
Acute high intestinal obstruction.	Celiac disease.
Acute yellow atrophy of the liver.	Tropical and nontropical sprue.
Myelogenous leukemia.	Severe diabetes mellitus.
Addison's disease.	Tendency in normal pregnancy.
Renal rickets (renal dwarfism).	

Hypophosphatemia. A decrease of serum phosphate is designated *hypophosphatemia.* It is commonly observed (1) in rickets due fundamentally to a deficiency of vitamin D which operates either by reducing the absorption of phosphorus and calcium or, more especially, by interfering with their proper utilization in the process of ossification. In some cases, however, the serum phosphate is normal but the serum calcium diminished. The same is true (2) of osteomalacia in which hypophosphatemia is a characteristic change. A primary decrease is also commonly observed (3) in osteitis fibrosa diffusa due to hyperparathyroidism and resulting from an increased elimination of phosphate in the urine, although this may be followed by a secondary increase. Hypophosphatemia is also commonly observed in idiopathic steatorrhea or conditions associated with fatty diarrhea, such as (4) celiac disease, (5) tropical sprue and (6) nontropical sprue. In these conditions demineralization of bones, dwarfism, rickets and tetany may occur because of abnormalities in calcium and phosphorus metabolism secondary to excessively large amounts of fat in the intestine and defective absorption of calcium, phosphorus and vitamin D. There is also a tendency toward a decrease (7) in normal pregnancy. As previously stated, serum phosphate diminishes during periods of increased carbohydrate utilization. Consequently it is decreased temporarily (8) after the oral or intravenous administration of glucose and a decrease is frequently observed (9) in diabetes in which it is proportional to the severity of the disease. It is also occasionally observed (10) in hyperinsulinism following the administration of insulin or epinephrine and (11) after the administration of parathormone.

IRON AND COPPER

Because iron is an essential constituent of hemoglobin and of chromatin substances, it is an element of great importance in the fundamental processes of nutrition. The mechanism of its absorption from the gastro-intestinal tract (chiefly from the stomach and duodenum) is still subject to considerable controversy but the old idea that inorganic iron is not absorbed is no longer tenable. Both inorganic and organic iron are absorbable but absorption of either or both may be deficient when there is a relatively high *p*H of the jejunum with the production of insoluble basic iron compounds, by the absence of free hydrochloric acid in the stomach, the absence of sufficient bile in the duodenum, the presence of large amounts of phosphates with the production of insoluble compounds of iron phosphate and by the administration of alkalis. Theoretically, ferrous salts may be absorbed more readily than ferric salts because of fewer possibilities of the formation of unabsorbable compounds.

Following absorption, iron is carried to the liver by the blood, where most of it is removed and the balance stored in the other tissues of the body in two functionally different forms, designated (1) *parenchymal iron* and (2) *available storage iron*. Hemoglobin iron constitutes about 57 per cent of the total. Hematin iron is not available for utilization or the production of hemoglobin by the body.

Normally the *organic iron* content of whole blood averages about 52 mg. per 100 cc. in men, corresponding to about 15.6 gm. of hemoglobin per 100 cc.; it is somewhat less in women (average 45 mg.). The hemoglobin contains 0.335 per cent iron and a determination of this blood iron furnishes a method for determining the hemoglobin content, although this method is not commonly employed clinically since, from a practical standpoint, it parallels the hemoglobin of the blood when the latter is accurately determined. The *inorganic* iron of the serum or plasma varies from 1.0 to 0.17 mg. per 100 cc., which is too small for accurate determination, as far as clinical purposes are concerned, and its exact significance is not clearly understood except that it is increased in aplastic anemia and during relapses of pernicious anemia and reduced in the anemias due to iron deficiency. The factors responsible for iron deficiency, therefore, may be a low intake, failure in absorption, or failure in utilization. These, acting singly or in combination, are responsible for the reduction in organic (hemoglobin) iron in hypochromic anemia of infancy and childhood, chlorosis, "idiopathic" hypochromic anemia and hypochromic anemia associated with pathologic conditions of the gastro-intestinal tract as well as in acute and chronic hemolytic and posthemorrhagic anemias in which the reduction is sometimes quite pronounced.

Copper. Copper in minute amounts is also essential for the production of erythrocytes, probably by promoting the utilization of iron and thereby the formation of hemoglobin. It is normally present in 0.14 to 0.15 mg. per 100 cc. of whole blood. A deficiency may be a factor in the hypochromic anemia of infancy and an increase from chronic poisoning was formerly thought to be responsible for hemochromatosis.

IODINE

Within recent years the determination of iodine in the blood by chemical methods has commanded increasing interest because of its importance in relation to the functional activity of the thyroid gland. Both food iodine and inorganic iodine are readily absorbed from the gastro-intestinal tract, with the liver playing an important part in their excretion, especially as a regulator of blood iodine. Most of the iodine excreted in the bile is reabsorbed into the portal blood, since only relatively small amounts are excreted in the feces. A large part is also excreted in the urine with small amounts in the sweat and other secretions. In hyperthyroidism, during menstruation and pregnancy, as well as after partial thyroidectomy, there is usually an increased urinary excretion.

In the blood, iodine appears in two forms: (1) one insoluble in alcohol or the "organic" fraction, presumably thyroid hormone, and (2) one soluble in alcohol, designated the "inorganic" fraction, and largely the iodine of nutrition. The total is regarded as varying from 4 to 10 micrograms per 100 cc. of plasma or serum averaging about 6 to 7 micrograms. It tends to be slightly increased (1) during summer months as well as (2) during menstruation and (3) pregnancy (Table 35).

TABLE 35. SUMMARY OF CLINICAL INTERPRETATION OF IODINE IN THE BLOOD

Normal: 4 to 10 μg. per 100 cc. of plasma or serum.

Increased	Decreased
Slightly during summer months and after exercise. Slightly during menstruation. Slightly during latter half of pregnancy and labor. Markedly in hyperthyroidism. Extrahepatic obstructive and hepatocellular jaundice. Malignancy and remissions of pernicious anemia. Slightly in leukemia. Some cases of hypertension. Some cases of severe infection. After administration of iodine or thyroid substance.	Hypothyroidism.

In pathologic states the blood iodine is commonly increased (1) in hyperthyroidism with a decrease in the thyroid gland but the increase has no constant relationship to the basal metabolic rate; (2) in extrahepatic obstructive and hepatocellular jaundice and sometimes to a slight extent (3) in leukemia, (4) hypertension, (5) during severe infections and (6) after the administration of iodine or iodides (Table 35). A decrease may occur in hypothyroidism but general experience has shown that the determination of blood iodine is of little practical value, since significant variations occur only in clinically obvious conditions. At any rate, the clinical value of blood iodine determinations appears to be limited to (1) an aid in establishing the diagnosis of borderline cases of hyperthyroidism and (2) the apparent fact that normal values in hyperthyroidism are indicative of severe intoxication. An iodine tolerance test has been proposed for diagnostic purposes which is described in Chapter 6.

PHOSPHATASE

Alkaline phosphatase, or phosphate esterase, discovered by Robison in 1923, is an enzyme capable of hydrolyzing the phosphoric esters of erythrocytes and plasma derived from hexosephosphate, glycerophosphate and nucleoprotein with the liberation of inorganic phosphate. Originally found in rice and wheat bran, phosphatase is now known to occur in practically all of the tissues of the body, being in largest amounts in the bones and teeth during infancy and childhood. It is well established, however, that phosphatase occurring in the erythrocytes is apparently different from that present in the tissues, plasma and leukocytes so that two phosphatases probably exist. That present in the serum, as well as in most of the tissues, is activated by magnesium, iron, ascorbic acid, glycine and other substances while apparently inhibited by cholic acid, copper and zinc.

The liberation of inorganic phosphate by alkaline phosphatase not only (1) increases the deposition of calcium phosphate in the bones, but (2) promotes the excretion of phosphates by the kidneys and thereby (3) aids in the maintenance of a normal phosphate buffer system in the tissues as well as (4) possibly playing a rôle in the activity of the muscles.

The normal serum *alkaline* phosphatase range for adults is 1.5 to 4 and for infants and children 5 to 14 Bodansky units per 100 cc., a unit being the amount of phosphatase equivalent to the actual or calculated liberation of 1 mg. of phosphorus as the phosphate ion during the first hour of incubation at 37° C. and a *p*H of 8.6, with the substrate containing sodium beta-glycerophosphate and hydrolysis not exceeding 10 per cent of the substrate. The phosphatase activity of oxalated plasma may be about 10 per cent lower than that of serum if diluted with a solution of oxalate.

Normal serum also contains small amounts of *acid phosphatase.* This may have its origin in the liver, spleen, bones, kidney and prostate gland, but it is not entirely or even largely of prostatic origin, since it is present in women and children in essentially the same amounts as in adult men. Because of the presence of large amounts in erythrocytes, care must be taken to avoid hemolysis in plasma or serum in which this determination is to be made. The normal is stated to be less than 3 units per 100 cc.[4, 5]

Hyperphosphatasemia. An increase of serum phosphatase is designated as *hyperphosphatasemia* and it is better to use this term for designating an increase of phosphatase activity than of phosphatase enzyme, since increased values may be sometimes more dependent upon increased activation of the enzyme than upon an actual increase of it.

Alkaline Phosphatase. Under physiologic conditions alkaline phosphatase is not only increased (1) during infancy and childhood in relation to the normal development of the teeth and bones, but also to slight degree (2) during the healing of fractures (although here it is not an index of the degree or ability of healing); (3) during the progress of pregnancy; (4) during alimentary hyperglycemia; (5) following exposure to ultraviolet light, and (6) after the administration of small doses of irradiated ergosterol.

In pathologic states phosphatase is increased by conditions producing abnormal cellular activity associated with increased utilization of inorganic phosphates, or by an increased activation of normal phosphatase by a coenzyme present as part of the disease. Under the conditions the serum phosphatase is consistently increased (7) in rickets because of a deficiency in plasma inorganic phosphate or, more importantly, to a deficiency of plasma and particularly of erythrocytic phosphoric ester substrate. Indeed, determinations are ordinarily an excellent means for showing the rate and progress of healing. An increase is also commonly observed (8) in generalized osteitis fibrosa cystica of hypoparathyroidism, osteitis deformans (Paget's disease) and in such additional diseases of bones as (9) severe osteogenesis imperfecta (not in mild types); (10) osteomalacia; (11) metastatic carcinoma (osteoblastic type); (12) osteogenic (osteoblastic) sarcoma and (13) in Gaucher's disease with absorption of bones and fragility. Since the serum phosphatase is generallv normal in bone tuberculosis, osteomyelitis, atrophic and hypertrophic arthritis, Ewing's tumor and benign giant cell tumors, an increase of it is of particular value in the differential diagnosis between Paget's disease, osteitis fibrosa cystica and osteoplastic types of osteogenic sarcoma (Table 36).

An increase is also uniformly observed (14) in the jaundice of acute and chronic hepatitis (cirrhosis) and sometimes (15) in cancer of the liver as well as (16) in some cases of extrahepatic obstructive jaundice, but not in hemolytic jaundice. The cause is unknown. However, attempts to utilize this change in serum phosphatase in the differential diagnosis between hepatocellular (toxic) and obstructive jaundice have been unsatisfactory owing to irregular results in the former; indeed, if there is marked necrosis of the liver cells, there is usually no increase of serum phosphatase at all. On the other hand, some observers believe that the absence of an increase rules out common duct obstruction. An increase is also commonly observed (17) in cases of biliary fistula and sometimes (18) in chronic myelogenous leukemia; possibly (19) in some cases of renal rickets (renal dwarfism); (20) active tuberculosis and (21) during periods of calcification of hemorrhages in scurvy (Table 36).

Acid Phosphatase. The discovery by Kutscher and Wolbergs[6] that normal prostatic tissue is rich in a phosphatase with an optimum activity at a *p*H of 4.9 has been elaborated by Gutman and his coworkers.[7] They showed that this *acid phosphatase* is also formed in carcinomatous prostate tissues and at the site of skeletal metastases, with an increase in the serum in a large percentage of cases. Use has been made of this finding in distinguishing between osteitis fibrosa cystica and skeletal metastases due to prostatic carcinoma. The differential diagnosis of these diseases is often difficult in the early stages, yielding only to biopsy or long observation. In the bony metastases of prostatic cancer the alkaline phosphatase of the serum may be increased just as in osteitis deformans, but in the latter the acid phosphatase is but slightly increased or lies within the normal range. Estimation of the acid phosphatase should therefore be a useful supplement to the clinical and radiologic examination of patients with carcinoma of the prostate, especially if operation is in question. An increase may be the first indication of metastases in the bones.

TABLE 36. SUMMARY OF THE CLINICAL INTERPRETATION OF BLOOD ALKALINE PHOSPHATASE

Normal for adults: 1.5 to 4 and for infants and children: 5 to 14 Bodansky units per 100 cc. of serum.

Hyperphosphatasemia (increase)	Hypophosphatasemia (decrease)
During healing of fractures.	Chronic nephritis with destruction of renal tissue.
During pregnancy.	Celiac disease.
During alimentary hyperglycemia.	Idiopathic and renal dwarfism in the absence of rickets.
Following ultraviolet irradiation.	Hypothyroidism of children.
Administration of irradiated ergosterol.	
Rickets.	
Generalized osteitis fibrosa cystica.	
Osteitis deformans.	
Severe osteogenesis imperfecta.	
Osteomalacia.	
Metastatic carcinoma of bones (osteoblastic type).	
Boeck's sarcoid (some cases).	
Marked hyperthyroidism and active tuberculosis.	
Osteogenic sarcoma (osteoblastic).	
Gaucher's disease.	
Obstructive and hepatocellular jaundice.	
Cancer of liver with jaundice.	
Portal cirrhosis (some cases).	
Biliary fistula.	
Myelogenous leukemia.	
Renal rickets (renal dwarfism).	
Calcification of hemorrhages.	

For clinical purposes, serum acid phosphatase below 3 units per 100 cc. may be regarded as negative and over 10 units as positive in the diagnosis of metastasizing carcinoma of the prostate gland. A sharp drop follows successful treatment, particularly castration and estrogen administration. Consequently, changes in acid phosphatase are of value not only in estimating the efficacy of treatment but in prognosis as well. Normal values of serum acid phosphatase, however, may be observed in metastasizing prostatic carcinoma if the prostatic cells elaborate very little of the enzyme because of anaplasia or very low androgen levels and, likewise, if there is not sufficient invasion of the lymph or blood channels to permit entrance into the circulation of significant amounts of the enzyme.[8] Abnormally high values have been observed, however, in about 85 per cent of cases of metastasizing carcinoma of the prostate and normal values in about 95 per cent of non-prostatic disease, including skeletal and other diseases accompanied by an increase of alkaline phosphatase. A slight increase of acid phosphatase (rarely over 6 and not over 10 units) may occur in advanced Paget's disease, osteoporosis, hyperparathyroidism and carcinoma of the mammary gland with extensive skeletal metastases.[8]

Hypophosphatasemia. A decrease in serum alkaline phosphatase is designated as *hypophosphatasemia.* It occurs much less frequently, being encountered (1) in chronic nephritis, regarded as being due to the destruction of renal tissue which is a rich source of the enzyme, and (2) in some cases of celiac disease.

Recently Talbot[9] has advocated alkaline-phosphatase estimation as an index of thyroid deficiency during infancy and childhood. He has shown conclusively that the value is low in cretinism—an indication that osteoblastic activity is very low at an age when the reverse normally holds good. He also points out that in idiopathic dwarfism a low level of phosphatase may be expected, and the same would apply to renal dwarfism and celiac disease in the absence of rickets. But to diagnose hypothyroidism simply on a low value for plasma phosphatase may readily lead to the unnecessary, and harmful, administration of thyroid. There is, however, undoubtedly a place for this estimation in assessing the progress of a patient under treatment (Table 36).

TABLE 37. SUMMARY OF THE CLINICAL INTERPRETATION OF LIPASE AND AMYLASE OF THE BLOOD

Normal lipase: 1 cc. or less in terms of N/20 NaOH; amylase: 70 to 200 mg. glucose from starch by 100 cc. of serum.

Lipase	Amylase
Increased: Acute pancreatitis. Carcinoma of pancreas. Some cases of duodenal ulcer. Cholelithiasis with jaundice. Cirrhosis of the liver. Carcinoma of the liver. Intestinal obstruction. Mumps. Chronic alcoholism (some cases).	Increased: Pancreatitis; carcinoma of pancreas. Renal failure; perforated peptic ulcer. Mumps; alcoholism. Decreased: Severe hepatitis; cirrhosis of liver. Acute and chronic cholecystitis. Thyrotoxicosis; severe burns. Diabetes mellitus; toxemia of pregnancy. Pneumonia (some cases). Congestive heart failure (some cases).

LIPASE AND AMYLASE

Lipase. An increase of lipase of the serum (*hyperlipasemia*) is a valuable procedure in the diagnosis of acute pancreatitis and of carcinoma of the pancreas, as it is regarded as evidence of disturbed pancreatic function. It is expressed in terms of N/20 sodium hydroxide per 1 cc. of serum which normally has been found to be about 1 cc. or less, although some investigators have placed it as high as 1.5 cc. Increased values may also occur in some patients with duodenal ulcer. in cholelithiasis with jaundice, in cirrhosis and carcinoma of the liver, in intestinal obstruction, in mumps and in some cases of chronic alcoholism (Table 37).

Amylase. This starch-splitting enzyme of the blood (diastase) is also characteristically increased in the serum or plasma in pancreatic disease and especially in acute pancreatitis as well as in carcinoma of this organ. It is commonly stated that a normal value is important in the differential diagnosis between acute pancreatitis and other acute abdominal states but it has been reported as increased in perforating peptic ulcers. An increase may also occur in severe renal impairment while a decrease has been reported in hepatic disease with dysfunction (especially hepatic necrosis), severe burns and to some extent in pneumonia and congestive heart failure. The results are expressed in milligrams of glucose formed by the action of 100 cc. of serum on starch which is normally 70 to 200 mg. (Table 37).

GUANIDINE

The exact chemical nature of guanidine (imido-urea) is unknown but it is apparently a product of some abnormality of protein metabolism due to hepatic insufficiency. Thus, Minot and his colleagues [10] have noted a similarity between the toxic manifestations produced in dogs with acute toxic hepatitis by carbon tetrachloride and experimental guanidine poisoning. An increase of guanidine in the blood has been observed in human beings with acute hepatitis due to arsphenamine, as well as in acute catarrhal jaundice and eclampsia, but not in extrahepatic obstructive jaundice or chronic hepatic disease due to syphilis, cirrhosis or carcinoma. In other words, the guanidine of the blood is likely to be increased only in hepatocellular jaundice due to toxic agents or infection of sufficient degree to produce marked insufficiency of the liver in relation to protein metabolism (Table 38).

TABLE 38. SUMMARY OF THE CLINICAL INTERPRETATION OF GUANIDINE IN THE BLOOD

Normal: 0.032 to 0.48 mg. in 100 cc. serum averaging 0.040 mg.

Increased	Within Normal
Acute toxic hepatitis (arsphenamine, etc.).	Obstructive jaundice.
Acute infectious jaundice.	Portal cirrhosis of the liver.
Eclampsia.	Syphilitic hepatitis.
Uremia.	Carcinoma of the liver.
Hypertension.	Coma due to alcoholism, diabetes mellitus or narcosis.

Furthermore, the symptoms of uremia in human beings are quite similar to those observed in experimental guanidine poisoning and several investigators have reported an increase of guanidine in the blood of patients with uremia and marked retention of urea nitrogen. This suggests that although urea itself apparently plays little or no part in the production of the toxic manifestations of uremia, it may be indirectly toxic by favoring the retention of guanidine and other poisonous products of intermediary metabolism. At least, an increase of guanidine in the blood is to be expected in decompensated chronic glomerulonephritis with uremia, so that a guanidine determination may be of value in the detection of coma due to this cause, since guanidine does not appear to be increased in the coma of acute alcoholism, diabetes mellitus or narcosis. Apparently calcium inhibits the action of guanidine in the production of muscular twitchings and convulsions in uremia, and when there is a deficit of calcium they are likely to be more severe, presumably because there is insufficient calcium ion acting on the central nervous system to oppose adequately the effects of guanidine. Several observers have also noted that blood guanidine is increased in hypertension.[11,12]

Unfortunately, methods available for the quantitative determination of guanidine in the blood are not highly specific and the procedure is not commonly employed, but the normal is stated to vary from 0.032 to 0.48 mg. per 100 cc. of serum with an average of about 0.040 mg.

PHENOLIC AND OTHER ORGANIC COMPOUNDS

Of further interest is the possibility that in renal insufficiency and especially in uremia, there may occur in the blood an accumulation of aromatic intestinal putrefactive products comprising an ether-soluble group (phenols, cresols, aromatic oxyacids and indoxyl) and an ether-insoluble group (amino acids, tyrosine, phenylalanine and tryptophane) which appear to be formed chiefly, if not entirely, as a result of bacterial action on protein derivatives in the intestines involving deamination, decarboxylation and oxidation followed by absorption and detoxification by conjugation with sulfuric or glycuronic acids in the liver and possibly other organs.

Xanthoproteic Reaction. In 1924 Becker[13] described the yellow color obtained when nitric acid reacts with aromatic amino acids, phenols and cresols in the blood serum as the *xanthoproteic reaction.* The normal reaction using serum without preliminary hydrolysis and ether extraction was given as 25, which represents a measure of the percentage of color observed by comparison with an 0.3874 per cent potassium bichromate solution. Since then other investigators have placed the normal as 15 to 35 but Ragins and his colleagues[14] have recently placed the normal range as 0 to 50 with 60 or higher as definitely abnormal.

In 1925 Becker and Koch[15] reported markedly increased xanthoproteic reactions of 80 to 250 in patients with uremia and maintained that uremia was less dependent on retention of nitrogenous products than on the retention of these aromatic substances. They stated that many of the signs and symptoms of uremia resembled those of chronic phenol poisoning. Since then various investigators[16,17] have reported xanthoproteic reactions above the normal in uremia and claim that, except in acute nephritis, they give information of clinical value in relation to the diagnosis and prognosis of renal insufficiency with special reference to uremia. The xanthoproteic reaction has also been found of value in differentiating pseudo-uremia (especially in hypertensive encephalopathy) from true uremia with low or normal values significant of extrarenal azotemia occurring in states of dehydration and hemorrhage into the gastro-intestinal tract.[14] This reaction is believed, therefore, to possess clinical value in the differential diagnosis of uremia, suspected uremia, pseudo-uremia, coma and nephritis. Furthermore, it seems probable that the prognostic value of variations in the xanthoproteic index in nephritis results from several causes. Increases in the index measure the degree of general retention, just as increases of urea and creatinine. In addition the variations measure, at least to some extent, variations in the phenol compounds present in the blood, and among the phenols are included definitely toxic compounds. The index also measures other nonphenolic compounds of unknown nature, probably substances containing the benzene ring, which are markedly increased in renal failure, and which parallel the retention of urea quite closely.[16]

DRUGS

Sulfonamide Compounds. It is now well established that chemical examination of the blood for free sulfanilamide, sulfapyridine, sulfathiazole and sulfadiazine is always desirable and of great value in gauging dosage and frequency of administration, particularly in the treatment of severe infections. On absorption from the gastro-intestinal tract or after parenteral administration, portions of these compounds are conjugated or acetylated in the liver. Therapeutic efficacy largely depends upon the amounts escaping acetylation (free) in the blood while toxic effects are mainly due to the acetylated compounds. The amounts of both present in the blood are subject to some variation in individual cases, depending upon dosage in relation to body weight, the rate of absorption, the degree of acetylation and the rate of excretion by the kidneys. Furthermore, the rate and degree of acetylation varies with different compounds, being highest with sulfapyridine and sulfadiazine, next highest with sulfathiazole and lowest with sulfanilamide. Hematuria is due to crystals of acetylated compounds in the uriniferous tubules and therefore occurs most frequently after the prolonged administration of sulfapyridine and sulfadiazine, much less frequently after sulfathiazole and least frequently after sulfanilamide.

Ordinarily, effective concentrations of free compounds are reached in six to eight hours after large initial doses by mouth, but two or three days may be required for establishing equilibrium between intake and urinary output and after administration has been stopped a similar length of time is required for complete elimination. Therefore, the most effective method for gauging dosage and to guard against toxic concentrations is by determining the actual amounts of free and acetylated compounds in the blood. Oxalated blood is used and micromethods are available. In the treatment of severe infections, dosage and intervals of administration should be such as to maintain a concentration of free sulfanilamide between 10 to 15 mg. per 100 cc. of plasma and, in the case of sulfadiazine, between 5 to 15 mg. Since sulfathiazole is absorbed and eliminated more rapidly than sulfadiazine, it is more difficult to maintain effective blood levels of free sulfathiazole but the advisable concentration is likewise 5 to 15 mg., while with sulfapyridine levels of 4 to 8 mg. appear to be satisfactory. These should be maintained until there is definite improvement before dosage is materially reduced.

Bromide. The estimation of bromide in the blood is of value in gauging bromide therapy in the treatment of epilepsy and certain psychiatric conditions. Excretion lags behind absorption and after a period of time there is retention of the salt at the expense of chloride which it replaces. Such replacement, if more than 40 per cent of the blood chloride, is usually fatal and toxic manifestations may occur at 25 to 30 per cent. Not infrequently it is difficult to determine whether certain symptoms are caused by bromide medication or by the condition under treatment and in such circumstances estimations of blood bromide are of clinical value. A blood bromide concentration of 125 mg. per 100 cc. is considered the upper limit of safety and amounts above 150 mg. are usually accompanied by signs of intoxication. Omission of the drug and the administration of increased amounts of sodium chloride usually promote satisfactory elimination. According to Wikoff and his colleagues,[18] the normal bromine of the blood serum varies from 0.33 to 1.73 mg. per 100 cc.; according to Flynn,[19] the ingestion of 30 to 45 grains of sodium bromide daily over a period of four months does not result in a high blood bromide.

Thiocyanate. The blood concentration of potassium or other thiocyanates is also important in relation to the treatment of hypertension with these compounds. They are slowly excreted unchanged in the urine. A method for their determination in the blood has been described by Barker.[20] For effectiveness, a concentration of 8 to 12 mg. per 100 cc. of serum is required over long periods of time. Toxic symptoms may occur with blood levels of 15 to 20 mg. and these may be dangerous at 35 to 50 mg. Blood determinations at frequent intervals are, therefore, the only safe guide in dosage.

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4

THE CLINICAL INTERPRETATION OF GLUCOSE TOLERANCE TESTS

The purpose of glucose tolerance tests is the detection of disturbances of carbohydrate metabolism. By means of blood sugar tests one seeks to determine the rapidity and degree of utilization of glucose over an arbitrary period of one to three hours following a carbohydrate meal or the administration of glucose. When properly conducted these tests may not only reveal hidden disturbances in carbohydrate metabolism, but yield information of clinical value in relation to the severity of manifest abnormalities. But while the technic of the tests is simple, there is no unanimity of opinion regarding the best technic to employ. Furthermore, difficulties are often encountered in the interpretation of the results obtained due not only to the complexity of the physiologic processes involved but also to modifications in technic, for which there may be inadequate standards of the normal blood sugar curves for purposes of comparison.

FACTORS INFLUENCING GLUCOSE TOLERANCE TESTS

In the first place, various physiologic and pathologic factors are capable of influencing or modifying the results of glucose tolerance tests as follows (see also Table 39):

The Kind of Blood Employed. The normal fasting blood sugar is not only slightly higher in capillary blood obtained from a finger than in venous blood, but after the administration of glucose the blood levels at varying intervals over a period of one to three hours are considerably higher in the former in both normal and diabetic individuals (Fig. 1). This is because arterial blood contains more glucose than venous blood, and capillary blood is practically arterial.[1]

The Effect of Preceding Diet, Starvation and the Fasting Period. The nature of the diet preceding the test has a distinct influence on the results in both adults and infants. As shown over fifty years ago by Hofmeister, the administration of a carbohydrate meal to previously starved dogs results in glycosuria called "starvation diabetes." Since then it has been amply proved that restriction of carbohydrates in man results in a marked decrease in ability to utilize carbohydrate.[2,3,4] Under these conditions the ingestion of a test dose of glucose produces a marked hyperglycemia, glycosuria and a marked delay in clearing the blood of absorbed glucose; on the contrary, however, if a high carbohydrate diet precedes the test, the degree and prolongation of hyperglycemia are much less (Fig. 1). Furthermore, a previous diet high in fat also produces a much greater and more prolonged degree of hyperglycemia when glucose is given than does one rich in carbohydrate. Consequently, in cases of mild or questionable diabetes, as well as in the differential diagnosis between nondiabetic and diabetic glycosurias, in both of which the glucose tolerance test becomes of major diagnostic importance, the physician should first eliminate any influence which the previous diet may have on the results. For this purpose it is generally sufficient for the patient to be placed on a diet of 80 gm. protein and 300 gm. carbohydrate (furnishing 2800 calories daily) for several days before the test is conducted.[5]

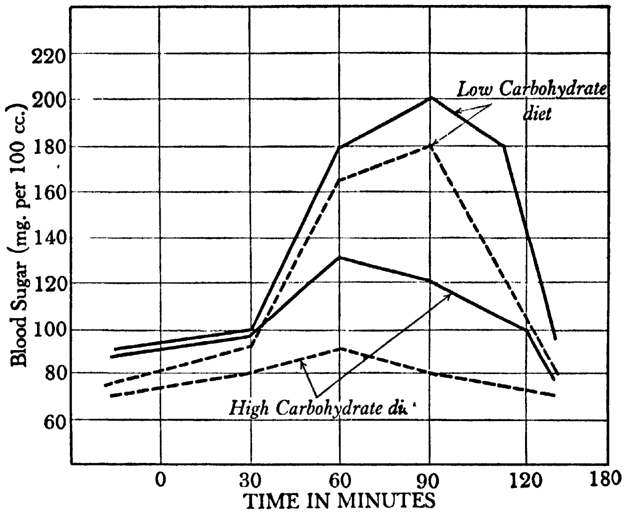

Fig. 1. The Influence of High and Low Carbohydrate Diets Preceding the Oral Administration of 50 Gm. of Glucose (Solid line: capillary blood sugar; broken line: venous blood sugar.)

Prolonged abstinence from food before the test is conducted and especially of protein and fat, may also result in the "diabetic" type of blood sugar curve. A few days of carbohydrate feeding, however, is usually sufficient for restoring to normal the decreased utilization of glucose[6] although a single preliminary dose of glucose may fail to do so.[7] Starvation also tends to give a high and prolonged type of curve, similar to that produced by a preceding low carbohydrate diet, through the stimulation of utilization of large amounts of fat from depots. Indeed, an additional four to eight hours of fasting beyond the usual overnight period may cause a similar curve, while a period of fasting of twenty-four hours usually yields a preliminary blood sugar level that is either low or definitely within hypoglycemic levels.[4]

Malnutrition. Malnutrition due to an actual lack of food or to an associated vitamin deficiency, especially B complex, may also alter the results of glucose tolerance tests.

Exercise. Increased muscular activity before or during the test may likewise modify the results, since it not only increases glucose production from liver glycogen but also increases the utilization of glucose to some extent.

TABLE 39. SUMMARY OF FUNDAMENTAL FACTORS INFLUENCING GLUCOSE TOLERANCE AND RELATED TESTS

The tests are simple to conduct but there is no unanimity of opinion on the best technic to employ. Those commonly used are (1) the carbohydrate meal test; (2) the standard one-dose three-hour test; (3) the Exton-Rose two-dose one-hour test and (4) the intravenous test. They are also applicable to infants and children.

Blood sugar is higher in capillary blood obtained from a finger than in venous blood.

The diet preceding the conduct of a glucose tolerance test has a marked influence on the results. A standard diet should be used for several days before it is conducted.

Starvation and prolonged abstinence from food have an important influence and should be corrected before the test is conducted. The duration of the fasting period before the test is conducted also has some influence.

Malnutrition, exercise, age and disturbances of the pituitary, thyroid and adrenal glands also have an important influence.

Failure to absorb glucose normally from the intestinal tract, as well as abnormally rapid absorption, has a marked influence entirely independent of disturbances in carbohydrate metabolism.

Glucose tolerance tests only express the sum total of disturbances in carbohydrate metabolism without showing whether they are due to a failure in hepatic or tissue glycogenesis, inability to maintain a normal blood sugar by glycogen or protein break-down, or inability on the part of the tissues to utilize glucose.

Glucose tolerance tests should not be used promiscuously but only when there is doubt about diabetes or under other special circumstances.

The *insulin sensitivity test* is sometimes advisable for determining the activity of insulin in promoting the withdrawal of glucose from the blood.

The *epinephrine test* is sometimes advisable for determining the ability of the liver to convert glycogen into glucose and to discharge it into the blood.

The *fat tolerance test* may be a useful procedure in elucidating disturbances in fat metabolism and its endocrinal regulation.

Age. Age also has an influence. In older adults both the degree and duration of the hyperglycemia may be more marked than in young adults, while infants and young children are stated to require the administration of relatively more glucose per body weight than adults to induce the same degree of hyperglycemia.

Endocrine Dysfunctions. The glucose tolerance test is used chiefly for the detection of impairment of carbohydrate metabolism in diabetes mellitus due to a deficiency in the production of insulin by the pancreas. But other endocrine dysfunctions may likewise affect carbohydrate metabolism and the results of glucose tolerance tests. For example, an exaggerated response, due to diminished glucose tolerance, may occur in hyperpituitarism similar to that observed in mild diabetes mellitus, while hypothyroidism and hypoadrenalism may show just the reverse or a decreased response because of increased glucose tolerance.

Absorption of Glucose. Furthermore, low blood sugar curves following the oral administration of glucose in the conduct of glucose tolerance tests may not be due to increased glucose tolerance at all but merely to the fact that it is not absorbed in a normal manner. For example, this may occur in cases of celiac disease, intestinal tuberculosis, etc. On the other hand, glucose may be absorbed from the intestinal tract at a greater rate and degree than normal and thereby yield high blood sugar curves suggestive of diminished glucose tolerance, as in cases of intestinal hypermotility, after gastro-enterostomy, hyperthyroidism and emotional disturbances, all of which are capable of speeding up the absorption of glucose within half an hour after its ingestion.

Limitations of Glucose Tolerance Tests. It is apparent, therefore, that the physician must keep in mind several important factors capable of altering the results of glucose tolerance tests. But even when these are eliminated, the tests only express the total of disturbances in carbohydrate metabolism without revealing just where the breakdown occurs.

As previously stated, a low or flat blood sugar curve may not be due to a disturbance of carbohydrate metabolism at all but merely to a failure of adequate absorption of glucose from the intestinal tract, while a high curve may indicate an abnormal or enhanced absorption. Consequently, when the factor of absorption is in doubt it is better to conduct the test by giving the glucose intravenously.

Even when absorption is normal, glucose tolerance tests do not reveal information bearing upon the reasons for failure to maintain normal blood sugar levels by glycogenesis in the liver and tissues or by protein breakdown when glycogen reserves are exhausted. It is possible that the fasting respiratory quotient may be of value in this connection as an index of the degree of combustion of glucose, but in its absence one must interpret glucose storage and utilization together from the height and the rate of fall of the blood sugar curves if abnormalities in absorption are to be definitely eliminated.

Nor do glucose tolerance tests give information bearing on the ability to build up glycogen reserves or the ability of the tissues to utilize glucose. Consequently, too little is known at the present time concerning the fundamental physiology of these integral processes of metabolism to make it possible to apply tests for the solution of clinical problems with entirely satisfactory results. As stated by Livingston and Bridge,[4] the hope of progress lies in the ability to interpret abnormal blood sugar curves in terms of disturbances in the physiologic processes involved.

Furthermore, levulose and galactose tolerance tests have not proved as useful as glucose tolerance tests except when the rôle of the liver in carbohydrate metabolism is of primary clinical importance. Under these conditions the tests are useful as an index of the rapidity with which these sugars are converted into dextrose and stored as glycogen, as one measure of the functional capacity of the liver which is discussed in more detail in Chapter 3.

THE RENAL THRESHOLD FOR GLUCOSE

By the renal threshold for glucose is generally understood that concentration of glucose in the blood above which glucose appears in the urine (Table 40). From the clinical standpoint it is of particular interest when glucose is detected in the urine, since this may indicate either hyperglycemia or a so-called "renal glycosuria" ("renal diabetes"). Under fasting conditions the threshold in normal individuals varies from 150 to 180 mg. of glucose per 100 cc. of blood, with a general average of about 160 mg.

But there is no uniformity of opinion on what constitutes the normal renal threshold. Certainly any effort to fix it at definite levels of the blood sugar appears to be untenable, since many factors may cause it to vary in different normal individuals although it may be fairly uniform in the same individual at different times when determined under identical conditions.[8] Under ordinary circumstances the figure found simply represents the situation as it exists at any particular time. As a matter of fact, it may be impossible to produce an alimentary glycosuria in some normal persons by the ingestion of large amounts of glucose. Campbell and his associates[9] state that the threshold for true sugar varies from 99 to as much as 228 mg. per 100 cc. of blood, with 80 per cent of normal individuals ranging from 140 to 190 mg. In this connection it must be remembered that not all of the copper-reducing substances in either urine or blood are glucose. In determining the renal threshold for glucose only the fermentable reducing substances should be determined.

TABLE 40. SUMMARY OF THE RENAL THRESHOLD FOR GLUCOSE
The renal threshold for glucose may vary greatly in normal individuals but is usually from 150 to 180 mg. per 100 cc. of blood (general average about 160 mg.).
There is no fixed threshold applicable to all normal individuals.
It may be impossible to produce an alimentary glycosuria in some normal individuals because of a normally high threshold.
In chronic diabetes the threshold may be increased until the blood sugar is over 200 mg. and even 250 mg. per 100 cc.
The threshold is characteristically low in "renal glycosuria" in which glycosuria may occur with a normal blood sugar.
The threshold may be temporarily low in pregnancy, returning to normal after the period of lactation has ended.
The threshold may be likewise low in hyperthyroidism and nephrosis.

In chronic diabetes the threshold may be so high that glucose does not appear in the urine until the blood sugar is over 200 and even as high as 250 mg. per 100 cc. At all events, individuals may have diabetes mellitus without showing any glucose in the urine, a fact which renders fasting blood sugar tests of great clinical value in the detection of the disease, especially in early or incipient cases.

The threshold is particularly and characteristically low in "renal glycosuria" in which case sugar occurs in the urine even when the blood sugar is within normal. In case of doubt, (1) the patient should empty the bladder and discard the urine; (2) then a sample of venous blood should be taken for sugar test; (3) one-half hour later urine is obtained and (4) at the end of another thirty minutes a second specimen of blood. If both blood sugars are normal and the urine contains sugar, a diagnosis of renal glycosuria may be made, particularly if a second specimen of urine and a third blood sugar test one-half hour later show the same results.

In 10 to 15 per cent of normal pregnant women the threshold is temporarily low, with a return to normal after the end of the period of lactation. Low thresholds are likewise not unusual in cases of hyperthyroidism and nephrosis.

METHODS FOR CONDUCTING GLUCOSE TOLERANCE AND RELATED TESTS

As glucose tolerance tests in infants and children under two years of age require some modifications in technic, they will be considered separately. In older children and adults various methods may be employed, although it is apparent that no one ideal test has yet been devised.

Indications. Glucose tolerance tests should not be used promiscuously but only when there is doubt about the diagnosis of diabetes or under other special circumstances as follows:

(1) To differentiate between diabetes mellitus and "renal glycosuria."

(2) To detect latent or incipient diabetes mellitus at a time when a modification in diet may prevent the progress of the disease.

(3) When necessary to determine the state of carbohydrate metabolism in an individual suspected of being diabetic even though the blood sugar is normal and there is no glycosuria, since it is known that mild diabetes may be present at a time when the blood sugar is normal with no sugar in the urine.

(4) In individuals from diabetic families contemplating marriage or who for other reasons may want assurance that they do not have diabetes in a latent or potential form.

(5) To remove the suspicion that diabetes is present when it does not exist.

Carbohydrate Meal Test. This test is conducted by determining the fasting blood sugar followed by a meal containing about 100 gm. of carbohydrate consisting of a banana, one shredded wheat biscuit, one cup of milk, two slices of bread and two teaspoonfuls of butter. Two hours later another blood sugar determination is made. Should this exceed 160 mg. of glucose per 100 cc., it is good evidence of an impaired carbohydrate metabolism. If there is doubt or if the blood sugar does not exceed 140 mg., a glucose tolerance test should be conducted.

Standard Test. This is the one-dose three-hour test originally described by Janney and Isaacson [10] and widely used in this country. It has the disadvantage of requiring five venipunctures and three hours of time and is conducted as follows:

1. For three days before the test there are no restrictions on carbohydrate in the diet as restriction may give a diabetic type of curve and lead to error. On the contrary, it is advisable for the patient to have a daily intake of at least 300 gm. of carbohydrate.
2. The patient should report in the morning in a fasting state, having eaten nothing since the previous evening.
3. The bladder is emptied and a sample of blood taken for sugar determination.
4. A solution prepared by dissolving 100 gm. of glucose in 300 cc. of water and flavored with the juice of a lemon is administered orally.
5. Specimens of blood and urine for sugar determinations are secured one-half hour later, and at one, two and three hours.

Examples of blood sugar curves commonly occurring in normal and diabetic individuals are shown in Figure 2. Diabetes can be excluded with reasonable accuracy if (1) the fasting blood sugar is normal or below 120 mg. per 100 cc. and (2) if the blood sugar after the ingestion of the glucose returns to normal within two hours. Under these conditions the actual height reached by the blood sugar is of little importance. The important point is that the patient provides an insulin response sufficient to bring about a rapid fall in the curve to normal and usually no sugar in the urine.

In diabetes, however, (1) the fasting blood sugar may be normal or slightly increased; (2) the blood sugar following the administration of glucose rises to or above 180 mg. per 100 cc. during the test and (3) does not return to normal within three hours. Sugar usually occurs in at least two or three of the specimens of urine voided when the blood sugar is above 160 mg. but glycosuria may not occur if the renal threshold is high.

In renal glycosuria the blood sugar curve is quite similar to that observed in normal individuals but sugar is present in all specimens of urine except the preliminary one.

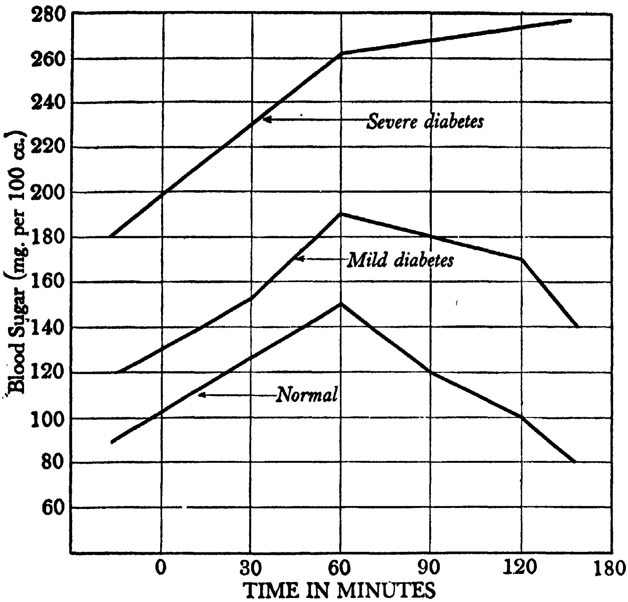

FIG. 2. BLOOD SUGAR CURVES IN THE STANDARD ONE-DOSE THREE-HOUR GLUCOSE TOLERANCE TEST

The Exton-Rose Test. The one-hour two-dose test of Exton and Rose,[11] however, requires fewer venipunctures, fewer blood sugar and urine tests and only one hour of time. For these technical reasons and because it is generally considered more specific in differentiating between diabetes and nondiabetic states,[12] it has to some extent replaced the standard test, although Langner and Dewees [13] and Langner, Romansky and Robin [14] have recently reported the latter as more reliable.

The Exton-Rose test is based on the hypothesis that in normal individuals the first dose of glucose stimulates the pancreas to produce more insulin, in consequence of which the blood sugar falls instead of rising after the second dose, which is known as the Staub-Traugott effect. In diabetes, however, the pancreas is unable to respond in this manner, with the result that the blood sugar does not fall after the second dose of glucose. The test is conducted as follows:

1. Over a period of three days before the test the patient takes at least 100 gm. of carbohydrate daily.
2. The patient should report in the morning in a fasting state by abstaining from all food since the previous evening.
3. The bladder is emptied and a sample of blood taken for a sugar determination.
4. Immediately thereafter one-half of a solution, prepared by dissolving 100 gm. of glucose in 650 cc. of water and flavored with lemon juice, is administered orally.
5. After an interval of thirty minutes a second sample of blood is taken and urine collected, followed immediately by the oral administration of the second portion of the solution of glucose.

6. After another thirty-minute interval specimens of blood and urine are obtained.

Examples of blood sugar curves commonly observed in normal and diabetic individuals are shown in Figure 3. The normal tolerance curve is indicated by (1) a normal preliminary fasting blood sugar level; (2) by a one-hour level not exceeding 160 mg. per 100 cc. and (3) by a lower blood sugar at the end of one hour than at the end of the first half hour.

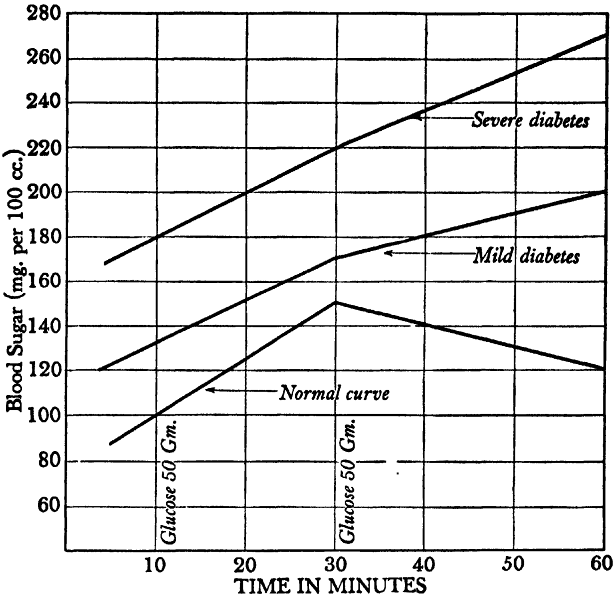

FIG. 3. BLOOD SUGAR CURVES IN THE EXTON-ROSE ONE-HOUR TWO-DOSE GLUCOSE TOLERANCE TEST

In diabetes, however, (1) the preliminary blood fasting sugar is normal or in excess of 120 mg. per 100 cc.; (2) it continues to increase by more than 25 mg. during the second half hour and (3) by a blood sugar in excess of 160 mg. per 100 cc. at the end of the hour.

A six-hour two-dose method has been described by Traugott [15] but it is of no more clinical value and has the disadvantages of requiring a great deal more laboratory work and causing a good deal of discomfort to the patient.[12]

The Intravenous Test. When it is thought that the absorption of glucose from the gastro-intestinal tract is abnormal, an intravenous glucose tolerance test may be conducted. The technic is the same as employed in the oral tests except that 0.5 gm. of chemically pure glucose per kilogram of body weight, in 20 per cent solution, is injected intravenously over a period of twenty to thirty minutes. Blood is collected for glucose determinations at intervals of thirty minutes over a period of two hours, although the maximum blood sugar concentration should occur within one minute after the completion of the injection. As shown in Figure 4, the blood levels are higher in both normal and diabetic individuals than after the oral administration of glucose. In normal individuals the blood sugar remains high during the first half hour, reaching normal within one and one-half hours after the injection and never in less than one hour. In diabetes, however, a return to the preliminary blood sugar level does not occur in one and one-half or two hours. The test should be used only as an aid in the diagnosis of doubtful or mild cases of diabetes and never in severe cases of the disease.

Glucose Tolerance Tests in Infants. Livingston and Bridge [4] have recommended the following method for infants and children under two years of age:

1. A preceding diet for several days of normal caloric value with normal proportions of fat, carbohydrate and protein. The tests should not be repeated at intervals of less than five days.

2. A preliminary fasting period of twelve hours for infants receiving three, four or five feedings a day, and nine hours for premature infants and those receiving six or more feedings.

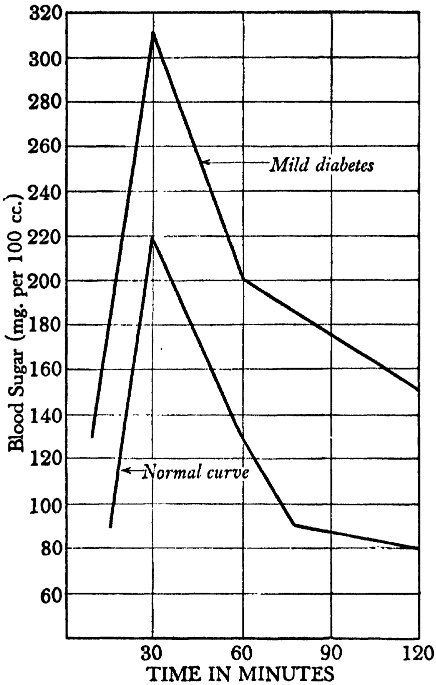

FIG. 4. BLOOD SUGAR CURVES IN THE INTRAVENOUS GLUCOSE TOLERANCE TEST

3. A test dose of 3 gm. of glucose per kilogram of body weight administered in 30 to 60 cc. of orange juice.

4. In intravenous tests a dose of 1 gm. of glucose per kilogram in 50 per cent solution given over a period of two to four minutes.

Otherwise, the tests are conducted in the same manner as in adults. Capillary blood is employed. On a regular diet the normal blood sugar after fasting six to twelve hours is about 90 mg. per 100 cc., and about 70 mg. on a preceding high fat diet. A maximum rise of blood sugar during the test to 160 to 180 mg. per 100 cc., with a return to normal at the end of three hours, is regarded as a normal response.

The Insulin Sensitivity Test. This test is sometimes advisable for determining the activity of insulin in promoting the withdrawal of glucose from the blood in a study of disturbances of metabolism with special reference to diabetes mellitus.

As a general rule, the standard test should be repeated five or more days later under exactly the same conditions except that one-quarter unit of insulin per kilogram of weight is injected subcutaneously about ten minutes after the administration of the glucose. This dose of insulin does not produce hypoglycemia which may confuse the interpretation of the results. In normal individuals the insulin test shows about 45 mg. less glucose per 100 cc. of blood about one hour after the ingestion of the glucose.

The Epinephrine Test. It is also sometimes advisable to determine the ability of the liver to convert glycogen into glucose and to discharge it into the blood by conducting the epinephrine test.

For this purpose, for three days the patient is placed on the same preparatory carbohydrate diet as used in the standard test. In the same manner, the test is conducted after fasting for twelve hours except that the test dose of glucose is omitted and 0.03 cc. of a 1:1000 solution of adrenalin chloride per kilogram of weight is injected subcutaneously. In normal individuals the blood sugar is increased one hour later.

The Fat Tolerance Test. In this connection brief mention may be made of the fat tolerance test based upon determinations of the total blood ketones expressed as acetone in milligrams per 100 cc. as determined by the gravimetric method of Van Slyke, in which readings of zero mean less than 1 mg. which is the limit of sensitivity and accuracy of this method. As conducted by Kauvar,[16] the technic is as follows:

1. The patient should report in the morning in a fasting state, having eaten no food since the previous evening.
2. A preliminary specimen of blood is taken.
3. A meal consisting of 75 gm. of butter spread on 15 gm. of gluten bread is then administered.
4. Samples of blood are taken after two, three and five hours with no additional food during this period of time.

Normal individuals do not show ketonemia before or during the test. Pregnant women may show 1.2 to 8.6 mg. in the fasting state, with an increase due to the fat meal during the period of five hours.

Diabetic individuals and those with glycogen disease may show a fasting ketonemia of 5.5 mg. or higher, depending upon the severity of the disease, which increases during the test.

Cases of myxedema do not show ketonemia in the fasting state, but may show 3.3 to 6.8 mg. within five hours after the ingestion of the fat meal.

In acromegaly, Cushing's disease and Simmonds' disease there is likewise no ketonemia in the fasting state, but cases of Cushing's disease have shown 2.7 to 7.5 mg. per 100 cc. of blood two hours after the fat meal. Under the circumstances, Kauvar believes that a fat tolerance test may be a useful procedure in elucidating disturbances in fat metabolism and its endocrinal regulation.

TABLE 41. SUMMARY OF THE CLINICAL INTERPRETATION OF GLUCOSE TOLERANCE TESTS

Decreased Tolerance (Exaggerated curve)	Increased Tolerance (Low or "Flat" curve)
Due to any one or a combination of the following factors: (1) a decrease of tissue utilization of glucose; (2) an increase of hepatic glycogenolysis or (3) inadequate hepatic glycogenesis: Diabetes mellitus and acidosis. Essential hypertension; arteriosclerosis. Hyperpituitarism; hyperthyroidism. Hyperadrenalinism; Paget's disease. Malnutrition; severe dehydration. Chronic obesity (some cases). Hepatic disease and especially acute diffuse hepatitis; not uniform in jaundice. Acute and chronic infections. Chronic arthritis (especially during acute stages). Pregnancy (late stages). Severe anemias; hemochromatosis; malignancy. Chronic nephritis with renal failure.	Due to any one or a combination of the following factors: (1) decreased hepatic glycogenolysis; (2) accelerated hepatic glycogenesis; (3) increased tissue utilization of glucose: Hyperinsulinism; von Gierke's disease. Increased sensitivity to insulin. High carbohydrate diet. Adrenal cortical insufficiency. Hypopituitarism and hypothyroidism. Celiac disease and sprue. Low or "flat" curves may be also due to: (1) poor or slow absorption of glucose from the digestive tract, or (2) the excessive excretion of glucose in the urine in individuals with a low renal threshold (renal glycosuria).

GLUCOSE TOLERANCE CURVES

The results of glucose tolerance tests, expressed in terms of curves prepared by plotting the results of blood sugar determinations, are not pathognomonic of any particular disease or diseases although very useful diagnostic aids. They are not only subject to considerable variation in relation to the technic employed but to various physiologic and pathologic factors as well (Table 41). In general terms, however, three principal types of curves occur: (1) the normal curve indicative of normal tolerance; (2) the exaggerated curve indicative of decreased tolerance, and (3) the low or "flat" curve indicative of increased tolerance. Typical examples

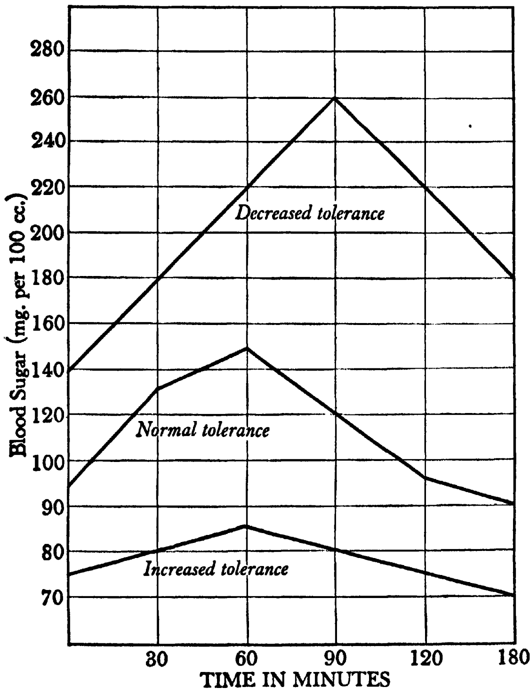

FIG. 5. TYPES OF GLUCOSE TOLERANCE CURVES IN STANDARD TESTS

of these three curves are shown in Figure 5 based upon standard or one-dose three-hour tests following the oral administration of 100 gm. of glucose. When this test is conducted with 50 gm. of glucose the height of the blood sugar curves is, of course, lower than those shown.

Decreased Tolerance. A decreased or diminished tolerance for glucose means that an individual is unable to handle glucose administered orally or intravenously as efficiently as a normal person. It may be due to any one or a combination of causes as follows:

(1) *A Decrease of Tissue Utilization of Glucose.* This most commonly occurs in *diabetes mellitus* due to hypo-insulinism resulting in a decrease in the rate of removal of glucose from blood entering the tissues. It is the most characteristic

feature of the disease and occurs regardless of the postabsorptive blood sugar concentration.

In all but mild cases the preliminary or fasting blood sugar is above normal. Following the administration of glucose the maximum concentration in the blood usually occurs about one and one-half hours later, but in severe cases this may not be reached for two or three hours. The most characteristic feature of the curve, however, is a failure to return to the preliminary or fasting blood sugar level at the end of two or three hours. The higher the increase the slower the fall, both paralleling the severity of the disease. The respiratory quotient may remain at the postabsorptive level instead of showing the normal increase which attends active carbohydrate storage and combustion. The serum phosphate concentration usually remains unchanged instead of decreasing during the period of hyperglycemia. In *glycogen storage* or *von Gierke's disease* a similar tolerance curve may occur and likewise be characterized by a delay or failure to return to normal in two or three hours.

Since *obesity* precedes diabetes in about 40 per cent of cases, glucose tolerance is of unusual interest. Ogilvie [17] has found that in about a third of cases there is a preliminary phase of increased tolerance, with low or "flat" curves due to the overproduction of insulin resulting from hypertrophy of the islands of Langerhans. In the majority of early cases, however, glucose tolerance is normal, although with advancing age decreased tolerance develops which may ultimately result in diabetes.

Curves similar to those observed in mild diabetes may also occur in *hyperpituitarism* (acromegaly and pituitary dwarfism) because an excessive production of pituitrin excessively inhibits insulin. The respiratory quotient and serum phosphate are likewise similar to those observed in diabetes mellitus. Decreased tolerance also occurs in Paget's disease.[18]

(2) *An Increase of Hepatic Glycogenolysis.* This most commonly occurs in *hyperthyroidism* and *infections* and is due to an increased passage of glucose into the blood at a rate too rapid for the removal of the excess by the tissues. The fasting blood sugar is likewise usually above normal, but not always. After the administration of glucose the peak of the blood sugar curve usually occurs within one to one and one-half hours. But since tissue utilization is unimpaired, the return to normal is relatively rapid, occurring usually within three hours unless the degree of hyperglycemia has been extremely high. For the same reason the respiratory quotient rises and the serum phosphate falls during the test.

(3) *Inadequate Hepatic Glycogenesis.* This most commonly occurs in cases of *diseases of the liver* severe enough to interfere with conversion of glucose into glycogen. But because of the great functional reserve and remarkable regenerative powers of this organ, normal or approximately normal curves may be observed in chronic diseases of the liver as in the various types of cirrhosis, passive congestion, carcinoma and syphilis. Decreased tolerance is much more likely to occur in acute diffuse types of liver disease.

The preliminary or fasting blood sugar is usually low, normal or subnormal. After the oral administration of glucose, however, it may reach 160 mg. or higher within one to one and one-half hours. But it falls rapidly and usually returns to normal within two to three hours. The respiratory quotient usually rises and the serum phosphate usually falls during the period of hyperglycemia. In jaundice, however, the results are not uniform.[19]

A decreased tolerance for glucose is also commonly observed during the latter stages of *pregnancy,* although seldom to a marked degree. It is believed to be due to temporary increased activity of the thyroid and pituitary glands. Decreased tolerance may also occur in *chronic arthritis,* especially during acute exacerbations, it is believed as a result of the effects which focal infections have on the endocrine system in either increasing hepatic glycogenolysis or suppressing the activity or production of insulin. Abnormal curves may also occur in gout but possess no diagnostic significance. Exaggerated curves may occur also in cases of *severe anemia,* especially pernicious anemia, and particularly when hypochlorhydria or anacidity are present. Fasting hyperglycemia and decreased glucose tolerance may likewise occur in patients with *glomerulonephritis* and *nephrosclerosis,* especially if renal failure is present, presumably because of a retarded transfer of glucose from the blood to the tissues rather than a retarded combustion of glucose in the latter.

Decreased tolerance also commonly occurs in patients with *essential hypertension;* indeed, in severe cases it may approach the curves observed in diabetes.

Increased Tolerance. Increased tolerance means that an individual has an increased ability to handle glucose. Consequently the tolerance curve is lower than normal or of the "flat" type. As a general rule, the preliminary fasting blood sugar is at low normal or below normal and after the administration of glucose the peak of the blood sugar curve is usually betwen 80 and 100 mg. per 100 cc. and therefore below normal. This increased tolerance may be due to any one or a combination of the following causes:

(1) *Decreased Hepatic Glycogenolysis* and (2) *Accelerated Hepatic Glycogenesis.* Decreased hepatic glycogenolysis refers to a deficiency on the part of the liver in converting stored glycogen into glucose. Accelerated glycogenesis refers to an excessive rate of conversion of glucose into glycogen by the liver. Since both result from endocrine disturbances, they are being discussed together. It is possible that an accelerated combustion of glucose is also involved.

Increased tolerance or "flat" tolerance curves due to these factors are observed not only in individuals on a *high carbohydrate diet* but also in cases of *hyperinsulinism* in which there is either excessive production of insulin by the pancreas or increased sensitivity to insulin. Low or "flat" curves in hyperinsulinism may also be attributable to an increased tissue utilization of glucose characterized by a slow and progressive fall to very low levels over a period of five or six hours. Indeed, the sugar tolerance curves in hyperinsulinism are so varied as to reduce the clinical value of the test. Consequently it should be conducted under rigid standard conditions.[20]

Increased glucose tolerance may also result from a lack of antagonists to insulin. For example, this may occur in *hypo-adrenalism* (Addison's disease), *hypopituitarism* (Simmonds' disease, tumors or congenital defects) and in *hypothyroidism* (myxedema and cretinism). An increased tolerance is not specific for adrenocortical insufficiency but possesses diagnostic value. When suspected, the intravenous test is probably to be preferred.[21]

Increased Tissue Utilization of Glucose. This is observed typically in *hyperinsulinism* which either results from a functional disturbance of the pancreas or occurs in association with hyperplasia, adenoma or carcinoma of the gland involving the islands of Langerhans in which there is a constant and increased production of insulin instead of its periodic secretion in response to requirements. On the other hand, however, tumors of the pancreatic islet cells may not produce increased tolerance with "flat" curves, especially when the liver is saturated with glycogen. While the fasting blood sugar may be below normal, the increase following the administration of glucose may reach normal or above and subsequently fall to a relatively normal level within three hours. However, it may continue to fall and reach an extremely low level within five to six hours. Consequently, the glucose tolerance test should be extended to five or six hours in all cases of suspected hyperinsulinism.

As previously stated, low or "flat" glucose tolerance curves may also indicate a poor or slow adsorption of glucose from the digestive tract due to edema, atrophy of the mucosa or circulatory disturbances (sprue, celiac disease, malnutrition, anorexia nervosa, vitamin B complex deficiency, etc.), inflammatory changes (tuberculous enteritis, ileitis, etc.) or neoplasms. Low curves also indicate the excessive excretion of glucose by the kidneys owing to a low renal threshold as in so-called renal glycosuria.

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5

THE CLINICAL INTERPRETATION OF KIDNEY FUNCTION TESTS

As modern physiologic investigations have progressively contributed to our knowledge of the normal functions of organs, it has been but natural that praiseworthy efforts should be made to devise clinically applicable tests for detecting disturbances of them. In relation to the kidneys these are not only valuable to the physiologist and chemist in a study of the mechanisms involved in the performance of their functions, as well as the influence of various extrarenal factors upon them, but also as important aids in the diagnosis of kidney diseases, especially when the clinical manifestations of dysfunction are indefinite or not apparent. Furthermore, they have proved particularly valuable for determining the degree and progression of nephritis in relation to treatment and prognosis, as likewise for estimating the functional capacity of the kidneys in relation to surgical risks. Owing to the special adaptability of the kidneys to such experimental and clinical studies, our knowledge of their functions is probably more complete than that of other organs, although the mechanisms involved in their performance are less well understood and still controversial in some particulars.

FUNCTIONS OF THE KIDNEYS

From a practical or clinical standpoint, it may be stated that the chief functions of the kidneys are: (1) to aid in the elimination of the waste products and toxic substances of the body; (2) to regulate water balance and maintain a normal crystalloid and colloid osmotic equilibrium between the blood plasma and tissues and (3) to regulate and maintain a normal acid-base equilibrium of the body. These functions involve the following mechanisms (Table 42):

1. The excretion of the nonvolatile end products of metabolism, chiefly those of protein metabolism like urea, creatinine, etc.

2. The excretion of useless foreign substances such as foreign proteins, toxins, drugs, dyes, etc.

3. The excretion of water in accordance with the requirements of the body.

4. The excretion of certain salts (sodium chloride, phosphate, etc.) to maintain a normal crystalloid osmotic equilibrium between the blood plasma and tissues.

5. The retention of normal protein constituents of the blood to maintain a normal colloid osmotic equilibrium between the plasma and the tissues.

6. The retention through reabsorption through the tubules of necessary substances like glucose, sodium chloride, etc., which pass in excessive amounts into the glomerular filtrate.

7. The synthesis of ammonia in the maintenance of acid-base equilibrium and doubtfully of hippuric acid.

TABLE 42. SUMMARY OF THE FUNCTIONS OF THE KIDNEYS

	Functions
Elimination and retention	(1) The excretion of the nonvolatile end products of metabolism. (2) The excretion of useless foreign and toxic substances. (3) The retention through reabsorption of necessary substances.
Regulation of water balance Maintenance of normal osmotic and acid-base equilibria	(1) The excretion of water in accordance with the requirements of the body. (2) The excretion of certain salts to maintain a normal crystalloid osmotic equilibrium. (3) The retention of normal protein constituents of the blood to maintain a normal colloid osmotic equilibrium. (4) The synthesis of ammonia in the maintenance of normal acid-base equilibrium.

In general terms, all of these together render the elimination of solid substances in solution in water the chief function of the kidneys. As stated in Chapter 2, it is the consensus that normal urine is essentially a simple colloid-free ultrafiltrate of the blood plasma by the glomeruli from which the glucose and part of the water, urea, sodium chloride and other solutes are removed by reabsorption by the tubules which exercise a fine selective discrimination. It is apparent, therefore, that the functions of the kidneys are not alone those of excretion but of conservation as well, requiring an examination of both in a study of renal function. In other words, while glomerular filtration of blood plasma is apparently largely one obeying physical laws, that of selective reabsorption of solutes by the tubules involves vital activities largely of an unknown and mysterious nature, including the synthesis by the tubules of ammonia from a certain amount of urea which is substituted for other bases in the urine. Furthermore, while glomerular function is influenced by nervous and hormonal control (pituitrin, epinephrine, etc.) exercised through vasomotor reactions, it is probable that both may likewise influence tubular functions.

PRINCIPLES AND PRACTICAL VALUE OF KIDNEY FUNCTION TESTS

For clinical purposes, the primary object of renal function tests is the detection of the presence and degree of renal insufficiency or the reduced capacity of the kidneys to carry out their functions (Table 43). An ideal test would be one determining not only the degree of insufficiency but the exact mechanism involved. But the tests commonly employed are a measure of total functional capacity rather than of glomerular filtration and tubular reabsorption separately. Indeed,

TABLE 43. SUMMARY OF THE PRINCIPLES AND PRACTICAL VALUE OF KIDNEY FUNCTION TESTS

Subject	Summary
Principles	(1) Available tests divisible into two main kinds: (a) those that determine capacity for eliminating waste products and (b) those that determine power to dilute or concentrate the urine. (2) "One-sided" tests based upon examinations of the blood or urine alone may be unsatisfactory. (3) Urea clearance test advisable for determining the total functional capacity. Inulin clearance test best for measuring glomerular function alone. (4) Dilution and concentration tests especially advisable for determining the functional capacity of the tubules. (5) Advisable to use at least two tests routinely. (6) Kidney insufficiency may depend not only on renal but upon extrarenal factors as well; important in relation to the interpretation of results.
Practical Value	(1) May not be a measure of the anatomic extensiveness of kidney lesions because of compensatory hyperfunction. (2) They estimate functional capacity at the particular time conducted. (3) Dysfunction may be only temporary. (4) Of special value in chronic kidney disease in relation to diagnosis and prognosis; also as a measure of therapeutic effectiveness and as a guide in treatment. (5) Of particular value in relation to urologic surgery, especially in the obstructive uropathies.

it appears that only the inulin clearance test is a measure of glomerular function alone, as it does not appear to be either excreted or reabsorbed by the tubules and none of the other tests employed measures the functional capacity of the tubules alone. But, after all, this is of more interest to the physiologist than to the clinician because sooner or later a glomerular nephritis is followed by tubular changes and a tubular nephritis or nephrosis by glomerular changes, while in arterial and arteriolar nephrosclerosis both glomeruli and tubules are ultimately involved with special reference to the former. In other words, while it was formerly thought essential to study renal function from the standpoint of the individual functions of the kidneys on the basis of selective injury in the various types of nephritis, this is no longer tenable since modern studies have clearly demonstrated that variations in the clinical manifestations of renal insufficiency are dependent largely not only upon renal but upon extrarenal factors as well, such as variations in the acid-base balance and in the crystalloid and colloid osmotic balance of the blood and tissues.

In general terms, however, renal function tests may be divided into two general groups: (1) those that determine the capacity of the kidneys for eliminating their excretory products and (2) those that determine the power of the kidneys to dilute or concentrate the urine. Apparently the urea clearance test is one of the best for measuring the former and a combined test for the volume of urine and its specific gravity one of the best for the latter. Insofar as a single test is concerned, however, it appears that urea clearance is the most satisfactory method for estimating the total active renal tissue. But in view of the multiple functions of the kidneys, it is apparent that two or more tests should be routinely employed whenever possible. Furthermore, there is a growing conviction that all one-sided tests, *i.e.*, all methods in which the blood or the urine alone is examined depend too much on extrarenal influences [1,2] such as fluid intake, diurnal diuresis, the administration of caffeine and other stimulants, severe acidosis and alkalosis, edema and large exudates "locking" urea, congestive heart failure, severe anemia, urinary tract obstruction, acute infections, hepatic disease with jaundice, etc.

Indeed, extrarenal factors may influence function not only when the kidneys are normal but likewise when they are diseased, while some functions may be temporarily exalted in the early stages of nephritis. Furthermore, the results of function tests never give one the right to judge of the anatomic extensiveness of lesions, because other portions of a partly diseased kidney may show compensatory hyperfunction. In other words, when one kidney is diseased or has been removed, the remaining healthy kidney may show compensatory hyperfunction so effectively that all function tests are normal.

In fact, it has been estimated that only about 10 per cent of the glomeruli may be active at any one time and that about 75 per cent of the nephrons must be involved before evidences of dysfunction are to be expected. Thus, Hayman [3] has calculated that urea and creatinine clearance are closely related to the number of inactive glomeruli in chronic glomerular nephritis and nephrosclerosis, with a drop in the specific gravity of the urine when 700,000 to 800,000 per kidney are defective after which specific gravity becomes fixed with an increase of systolic blood pressure above 150 mm. Addis and Oliver [4] have calculated that 27 mg. of urea (not urea nitrogen) per 100 cc. of blood indicates the pressure of 99 per cent normal kidney while 50 mg. indicates 35 per cent and 100 mg. about 6 per cent. Incidentally Peters [2] states that it is a mistake to assume that body weight parallels the weight of the kidney parenchyma and that diuresis of normal kidneys is reduced in proportion to the decrease in weight of the parenchyma, as seen in children and small adults.

Furthermore, it is evident that renal function tests estimate the functional capacity of the kidneys only at the particular time they are conducted and that evidences of dysfunction may be only temporary, as in acute nephritis, acute infections, urinary tract obstruction, cardiac failure, etc. As a general rule, glomerular function is the first to be involved. In chronic disease of the kidneys, however, renal function tests possess far more prognostic value and are a measure of the effectiveness of therapeutic measures, since progressive evidences of dysfunction usually indicate the relentless progress of the disease.

METHODS FOR CONDUCTING KIDNEY FUNCTION TESTS

No less than fifty renal function tests have been proposed but only a few have been widely accepted. In general terms, these may be classified as follows:

(1) Tests based on the retention of urea nitrogen, total nonprotein nitrogen, creatinine, uric acid and sodium chloride in the blood, summarized in Table 44.

TABLE 44. SUMMARY OF RENAL FUNCTION TESTS BASED ON THE RETENTION OF UREA NITROGEN, TOTAL NONPROTEIN NITROGEN, CREATININE, URIC ACID AND SODIUM CHLORIDE IN THE BLOOD

<table>
<tr><th>Substances</th><th>Principles</th><th>Normal (mg. per 100 cc.)</th><th>Impairment (mg. per 100 cc.)</th></tr>
<tr><td>Urea Nitrogen</td><td rowspan="3">Essentially tests of glomerular filtration and tubular reabsorption (concentrating power).
May be normal in kidney disease if there is an increased elimination of water to compensate for diminution of concentration.
Retention may be increased in the absence of renal disease by (1) prerenal deviation of water; (2) dehydration states and (3) excessive protein catabolism.</td><td>8–18</td><td>Slight (20–27)
Moderate (28–44)
Marked (45–64)
Maximal (65 or more)</td></tr>
<tr><td>Nonprotein Nitrogen</td><td>20–35</td><td>Slight (40–45)
Moderate (46–65)
Marked (66–90)
Maximal (91 or more)</td></tr>
<tr><td>Creatinine</td><td>1–2</td><td>Slight (3–4)
Moderate (5–7)
Marked (8–16)
Maximal (17–35 or more)</td></tr>
<tr><td>Uric Acid</td><td>Essentially a test of glomerular filtration and tubular reabsorption (concentrating power).
Retention may be increased in gout, hypertension, arthritis, leukemia, plumbism, pregnancy, intestinal obstruction, cardiac decompensation and hepatic dysfunction.</td><td>2–4</td><td>Usually increased in renal impairment but less satisfactory as an index of the degree or presence of renal disease than urea nitrogen retention.</td></tr>
<tr><td>Sodium Chloride</td><td>Essentially a test of glomerular filtration and tubular reabsorption (concentrating power).
A "threshold" constituent jealously preserved to maintain normal osmotic processes.
Blood concentration may be reduced by extrarenal factors as (1) edema; (2) vomiting and diarrhea and (3) acidosis.</td><td>Whole blood: 450–500

Plasma (always preferred): 570–620</td><td>Plasma concentration may be reduced by the lower renal threshold of nephritis. May be reduced to less than 350 mg. in elderly persons with arteriosclerosis through loss by vomiting producing pseudo-uremia.
Retention in some cases of acute and chronic nephritis usually without marked edema but severe oliguria; also in urinary tract obstruction. Rarely above 750 mg. but may be higher.</td></tr>
</table>

(2) Tests based on the elimination or clearance of urea nitrogen, creatinine and inulin, summarized in Table 45.

(3) Tests based upon the concentration (specific gravity) and dilution of urine, summarized in Table 46.

(4) Tests based on the elimination of such foreign substances as phenolsulfonephthalein and sodium ferrocyanide, summarized in Table 47.

Probably the most widely employed at present are the urea clearance test of Van Slyke,[5] the concentration tests of Mosenthal[6] and of Soderman and Engelhardt,[7,8] the concentration tests of Lashmet and Newburgh[9] and of Addis and Shevky,[10] the phenolsulfonephthalein test of Rowntree and Geraghty[11] including the fractional method of Chapman and Halstead,[12] and the blood chemistry tests for retention of urea nitrogen, total nonprotein nitrogen and creatinine. Only the phenolsulfonephthalein test has been commonly used for testing each kidney separately by ureteral catheterization. As previously stated, the urea clearance and concentration tests appear to meet average clinical requirements. Indeed, it appears to be the consensus that when a concentration test yields a urine of more than 1.026 specific gravity, it may be assumed that renal function is normal and that the urea clearance test may be omitted.[13] Freyberg,[14] after comparing the Lashmet-Newburgh test with urea clearance and phenolsulfonephthalein excretion (15-minute and 2-hour tests), concluded that the concentration test was the most sensitive in demonstrating impairment of renal function. In this connection, it should be emphasized that in conducting concentration tests, specific gravities should be corrected by subtracting 0.003 for each gram of albumin per 100 cc. Furthermore, if several specimens have been collected, the temperature of each should be approximately the same, because if some have been kept at room temperature and others in a refrigerator, error in the readings may interfere with their proper interpretation.

Since a measure of the retention of urea in the blood alone cannot be unreservedly relied upon for the detection of slight insufficiency in view of the influence of extrarenal factors, Ambard and others were among the first to measure the ability of the kidneys to eliminate this waste product by a comparison of its concentration in the blood with that in the urine, the relationship being expressed numerically by the so-called "Ambard's coefficient" simplified by McLean's index. But it is now known that one of Ambard's laws is inaccurate,[15] since the proportion between urea excretion and the volume of urine is altered when diuresis reaches the "augmentation limit." Similar tests for determining the urea ratio have been advocated by Mosenthal and Bruger, Addis and Watanabe and others, but the urea clearance test of Van Slyke is most widely employed at present. Peters,[2] has recently described a modification of the urea clearance test (ureosecretory index) but its value cannot be expressed at the present time. On the supposition that xylose is not metabolized and has a constant rate of excretion in normal individuals, Fishberg and Friedfield[16] have proposed a "xylose clearance" test. After the ingestion of 50 gm., normal kidneys are stated to concentrate xylose to 2.5 per cent within two hours and to excrete 25 per cent or more within twenty-four hours. In severe cases of renal insufficiency the total excreted in

TABLE 45. SUMMARY OF RENAL FUNCTION TESTS BASED ON THE ELIMINATION OR CLEARANCE OF UREA NITROGEN, CREATININE AND INULIN

Tests	Mechanism of Elimination	Normal Elimination	Impairment
Urea Clearance	Glomerular filtration with some tubular reabsorption. Slight diurnal variations. Increased by high protein diet and caffeine. Decreased by low protein diet, epinephrine and pituitrin.	Average normal adult excretes the amount of urea contained in 60 to 95 cc. of blood per minute (average 75 cc.).	Slight (30–60) Moderate (20–40) Marked (10–30) Maximal (below 10) *Decreased* in: acute nephritis; chronic glomerular nephritis in active stage; usually in progressive essential hypertension and nephrosclerosis; passive congestion due to cardiac decompensation and shock; in renal angiospasm. Normal in eclampsia but later may show a decrease due to permanent renal damage. Inaccurate in edema with a relative oliguria to urea. May be *increased* during acute infections under 40 years of age.
Creatinine Clearance	Exogenous by both glomeruli and tubules. Endogenous probably by glomeruli alone (partly by tubules in advanced renal disease).	Varies widely; 70 to 238 cc. of plasma cleared per minute (mean 148 $\pm$ 34).	Below 60 indicates decreased clearance. Decreased in most cases of acute and chronic glomerular nephritis, nephrosis and nephrosclerosis.
Inulin Clearance	Glomerular filtration; apparently not excreted by the tubules or reabsorbed by them. A measure of glomerular filtration rate.	Body surface area 1.73 sq. m. = 120 to 140 cc. per minute (rate of glomerular filtration).	Clearance reduced in glomerulonephritis. Useful for the determination of excretion of water under the influence of diuretics in diabetes insipidus, etc.; also for the estimation of the excretion of dextrose and uric acid.

twenty-four hours may be no more than 1 gm. It is possible that a significant amount of xylose is reabsorbed by the tubules of normal kidneys.

As far as tests based on the retention of urea nitrogen, total nonprotein nitrogen and creatinine are concerned (Table 44), it is to be remembered that these tests are of limited value in the detection of slight degrees of renal insufficiency, particularly because of the possible wide ranges due to extrarenal factors as well as the practical difficulties of controlling diet, metabolic activities and the remarkable reserve powers of the kidneys. These tests are, however, far more valuable and reliable in the latter stages of nephritis as well as for differentiation between the effects of congestive heart failure on kidney function and those of nephritis. In this connection it appears that a retention of the inorganic sulfates, with a consequent increase of them in the blood, may occur before other tests reveal any change in kidney function. The normal range of these inorganic sulfates is from 2.5 to 5.0 mg. per 100 cc. of serum, corresponding to 0.8 to 1.7 mg. in terms of sulfur.

It is hoped that the principles, mechanism of elimination, normal values and signs of impairment in relation to the four groups of renal function tests summarized in Tables 44 to 47 will suffice for clinical purposes. The purely laboratory phases of the various tests are being omitted since these, along with all of the important technical details, are to be found in laboratory manuals; but all of the tests require the cooperation of the clinician to the following extent:

Urea Clearance Test. 1. This test is usually performed in the morning, as excretion is less liable to fluctuate during this period, but, if necessary, it may be done at some other time, providing at least five and a half hours have elapsed since the preceding meal. It is advisable for the patient to rest in a reclining position.

2. The patient is requested to drink a glass of water and immediately thereafter empty the bladder. This specimen is not saved but the time of voiding is carefully recorded.

3. At the end of approximately one hour, the patient again voids; the time is accurately recorded and the specimen saved. At about this time (± 15 minutes) blood is withdrawn from a vein (2 to 3 cc. is sufficient).

4. A second glass of water is then taken.

5. One hour later the patient again completely empties the bladder. The time is again accurately recorded and the specimen saved.

The two specimens of urine and the blood are then sent to the laboratory for analysis.

Creatinine Clearance Test. 1. At 7 A.M. give orally 3 to 5 gm. of creatinine dissolved in 400 cc. of water.

2. At 8 A.M. a sample of blood is withdrawn from a vein and the bladder emptied (specimen discarded).

3. At 9 A.M. a second sample of blood is taken. The bladder is again emptied and the specimen saved.

4. At 10 A.M. a third sample of blood is taken. The bladder is again emptied and the specimen saved.

The three samples of blood and the two of urine are sent to the laboratory for analysis.

Inulin Clearance Test. 1. This test may be started any time during the day but preferably in the morning. It is advisable to keep the patient reclining. The height and weight are recorded for calculation of the surface area.

2. At 6:30 A.M. give the patient a glass of water and thereafter every half hour until the test is completed.

3. Breakfast at 7:30 A.M.: One-half glass of milk and one slice of toast and butter.

4. At 8 A.M. withdraw a sample of blood from a vein as a control. A control specimen of urine is not required unless the kidneys are severely damaged. Give 10 gm. of inulin

TABLE 46. SUMMARY OF RENAL FUNCTION TESTS BASED ON THE CONCENTRATION (SPECIFIC GRAVITY) AND DILUTION OF URINE

Tests	Principles	Normal Values	Impairment
Concentration (Volume and specific gravity test of Mosenthal)	Based on the volume and specific gravity of the urine during the day and night with ordinary food and fluid intake. If albumin is present correct the specific gravity of each specimen by subtracting 0.003 for each gram per 100 cc.	*Volume:* 8 A.M. to 8 P.M.: about 1100 cc.; 8 P.M. to 8 A.M.: less than 725 cc. (usually 360–500 cc.). Total for 24 hours about 1500 cc. *Specific gravity:* 8 A.M. to 8 P.M.: 1.010 to 1.020; 8 P.M. to 8 A.M.: 1.026 or over.	Valuable for detection of slight degrees of renal dysfunction; may fail to show differences between moderate and severe renal injury. Concentrating power may be normal in *acute glomerular nephritis* but not if filtration rate is seriously impaired. Failure of concentration usual in *chronic glomerular nephritis.* Polyuria or nocturia interferes with the accuracy of the test.
Concentration (Specific gravity test of Fishberg)	Based on the specific gravity of three specimens of urine voided at hourly intervals in the morning, after supper at 6 P.M. (considerable protein) with minimal amounts of fluid.	The specific gravity of at least one specimen should be 1.025 or higher.	Renal insufficiency indicated by low specific gravities not exceeding 1.020; in severe cases may be 1.010 or less.
Dilution (Water function test of Fishberg)	A test of water excretory function; primarily a measure of the blood supply to the kidneys. Makes use of normal diurnal diuresis which begins in the early morning.	First hour specimen: about 400 cc. with specific gravity of 1.001 to 1.003. Thereafter less volume and higher specific gravity with about 100 cc. at 1.012 to 1.016 at fourth hour. Total volume should be 80 to 120 per cent of the intake (1200 cc.).	Test of no value in dehydration states (diarrhea, vomiting, inanition, etc.) or in the presence of prerenal deviation of water by edema (congestive heart failure, salt and phosphorus retention of chronic nephritis). Compensatory polyuria of chronic nephritis may mask impaired ability to eliminate water. In renal insufficiencies, output reduced to 200 cc. or less in first hour or two with specific gravity 1.010 or higher.

TABLE 46. SUMMARY OF RENAL FUNCTION TESTS BASED ON THE CONCENTRATION (SPECIFIC GRAVITY) AND DILUTION OF URINE (continued)

Tests	Principles	Normal Values	Impairment
Posterior Pituitary Injection Test (Soderman and Engelhardt)	Based on the principle that the antidiuretic effects of posterior pituitary injection normally increase the reabsorption of water by the renal tubules, with reduction in urinary volume and increased specific gravity of the urine. Useful for the detection of disturbances of renal tubular function. If the urine has a specific gravity of 1.022 or higher, the test need not be done. Contraindicated in oliguria, pregnancy, coronary sclerosis, myocardial infarction, angina pectoris, epilepsy and hypersensitivity to pituitary extract (rare).	A definite increase in the specific gravity of the urine collected one or two hours or both after the injection. No significant electrocardiographic changes and only a transient rise in blood pressure with slight acceleration of pulse rate.	In uncompensated renal impairment there is but slight or no increase in the specific gravity of urine collected one or two hours or both after the injection.
Concentration Test (Lashmet and Newburgh)	Based upon the specific gravity of urine voided during the day on a special diet with no fluids.	1.029 to 1.032.	Lower specific gravity in renal impairment.
Concentration Test (Addis and Shevky)	Based upon specific gravity of urine passed from 8 P.M. to 8 A.M. after patient abstains from fluid for approximately 24 hours.	1.026 to 1.028 or higher; average 1.032.	Lower specific gravity in renal impairment.

TABLE 47. SUMMARY OF RENAL FUNCTION TESTS BASED ON THE ELIMINATION OF FOREIGN SUBSTANCES

Tests	Mechanism of Elimination	Normal Elimination	Impairment
Phenolsulfonephthalein (1-2 hour method)	Normally independent of urine volume. May be employed for testing each kidney separately. Ureteral catheters may cause some inhibition of excretion. 40 per cent retained in the liver; balance eliminated by glomerular filtration and tubular excretion.	*Both kidneys:* First hour: 40–60 per cent; second hour: 20–25 per cent (total 60–85 per cent). *Kidneys separately:* First appears in 5 to 10 minutes after intramuscular injection; 3 to 5 minutes after intravenous injection.	Total both kidneys: Slight (59–40) Moderate (39–25) Marked (24–11) Maximal (10–0) Most useful in chronic nephritis in which output runs fairly parallel with course of the disease. In early acute nephritis may be normal or increased. In severe renal disease, elimination varies directly with urine volume and can be increased by the administration of water. May be normal in advanced kidney disease during stage of compensation. *Reduced in:* Chronic nephritis. Congestive heart failure. Hepatic disease (cirrhosis) with normal kidneys. By edema and other prerenal deviation of water. Often low in urinary tract obstruction. In most cases of kidney disease, retention parallels that of nitrogen in the blood. May be ***increased*** in: Hypertension Hyperthyroidism
Phenolsulfonephthalein (Fractional method)	Should not be used if patient is taking saline cathartics as they tend to delay elimination.	15 min. specimen: 28–51 per cent. 30 min. specimen: 13–24 per cent. 60 min. specimen: 9–17 per cent. 120 min. specimen: 3–10 per cent. (total 63–84 per cent).	
Phenolsulfonepthalein (Two kidneys separately)		First appearance of dye 3 to 5 minutes. May be delayed by reflex inhibition. Total for two kidneys together: 15 min.: 35–45 per cent. 30 min.: 50–60 per cent. 60 min.: 65–80 per cent.	

TABLE 47. SUMMARY OF RENAL FUNCTION TESTS BASED ON THE ELIMINATION OF FOREIGN SUBSTANCES

Tests	Mechanism of Elimination	Normal Elimination	Impairment
			Severe nephritis with increased urine volume. Hepatic disease with hepatic dysfunction.
Sodium Ferro-cyanide	Glomerular filtration; apparently partly reabsorbed by the tubules. Part retained in the body (only 84.6 per cent of injected dose theoretically available for elimination in second interval).	Percentage of injected dose "uncorrected": 30 min.: 15.4 per cent. 60 min.: 12.3 per cent. 120 min.: 9:8 per cent. 180 min.: 6.5 per cent. "Corrected": 30 min.: 15.4 per cent. 60 min.: 14.54 per cent. 120 min.: 13.55 per cent. 180 min.: 10.41 per cent.	Decreased elimination in hypertensive arterial disease, especially during the first hour. An excretion test and may give erroneous results because it does not take into account the concentration of the compound in the blood.

dissolved in 100 cc. of sterile saline solution at body temperature intravenously at the rate of 10 cc. per minute.

5. Have the patient empty the bladder one hour after completion of the injection of inulin and discard the urine; also two and three hours after the injection. These two specimens should be accurately timed, measured and saved.

6. Collect 15 cc. of blood in oxalated tubes one and one-half and two and one-half hours after completing the injection of inulin.

The two specimens of urine and the two of blood are then sent to the laboratory for analysis for inulin, using the colorimetric method of Alving, Rubin and Miller.[17]

The Concentration (Volume and Specific Gravity) Test of Mosenthal. 1. The patient is permitted the usual food and fluid intake.

2. Carefully collect in one container all urine voided from 8 A.M. to 8 P.M. This should be carefully measured and the specific gravity ascertained.

3. Carefully collect in one container all urine voided after 8 P.M. to 8 A.M. of the following day. Carefully measure and take the specific gravity.

The Concentration Test of Volhard and Fahr. 1. Allow no foods from the evening before the test until it is finished with no food between meals.

2. Breakfast at 8 A.M.: Dry cereal with sugar, syrup, or honey; no milk; one egg; toast or plain bread with butter.

3. Dinner at noon: Roast beef, steak, or chops; potatoes boiled or baked; bread and butter; jam.

4. Supper at 5 P.M.: Two eggs; bread and butter; jam.

5. At 8 A.M. of the same day have the patient void and save the specimen. Thereafter have the patient void at 11 A.M., 2 P.M., 5 P.M., and 8 P.M. Save each specimen in a separate container. After 8 P.M. to 8 A.M. of the following day collect all urine in one container.

6. Note the quantity and specific gravity of each of the six specimens of urine. Normally, the specific gravity of at least one specimen should be 1.030, or at least 1.025.

The Concentration (Specific Gravity) Test of Fishberg. 1. Have the patient eat a regular evening meal at 6 P.M. with a minimal amount of fluid and a considerable amount of protein.

2. Discard all urine voided during the night.

3. Save the first sample of urine voided in the morning.

4. Have the patient rest in bed for an hour, when a second sample of urine should be voided and saved.

5. If possible, the patient should arise and after one hour's activity a third sample of urine should be collected and saved.

Send the three samples of urine to the laboratory for accurate determination of the specific gravity of each. If much albumin is present the specific gravity should be corrected by subtracting 0.003 for each per cent of protein present.

The Dilution or Water Function Test of Fishberg. 1. Omit breakfast. For dinner and supper give the usual routine nephritic diet or the diet to which the patient has been accustomed. Permit one glass of water after supper.

2. At 8 A.M. have the patient empty the bladder and give 1500 cc. of water. Discard the urine.

3. Collect urine in separate containers at hourly intervals, beginning at 9 A.M. and ending at 12 noon, making four specimens in all.

4. Carefully measure and take the specific gravity of each specimen.

The Posterior Pituitary Injection Test of Soderman and Engelhardt. This test is based upon the principle that the antidiuretic effects of posterior pituitary injection normally increase the reabsorption of water by the renal tubules, with consequent reduction in urinary volume and an increased specific gravity of the urine. It is, therefore, useful for the detection of disturbances of renal tubular function and particularly valuable in edematous patients, in surgical patients in whom restriction of fluids may be inadvisable and in patients who cannot be relied upon for co-operation in restricting fluid intake. The Soderman-Engelhardt test also has the advantage of being technically simple and applicable in unprepared patients at any time of the day. Posterior pituitary injection, in the dose employed, has been found to produce no significant electrocardiographic changes and only a transient rise in blood pressure, even in the presence of severe hypertension and uremia, with slight acceleration of the pulse rate. It is contraindicated, however, in oliguria, pregnancy, coronary artery disease (arteriosclerotic heart disease with angina, acute coronary insufficiency or myocardial infarction), angina pectoris, epilepsy and individuals who are hypersensitive to posterior pituitary injection which, fortunately, is but rarely observed. The test may be conducted as follows:

1. The unprepared subject empties the bladder completely. The specific gravity is taken and if 1.022 or higher, the test need not be done.

2. If 1.021 or less, 10 units (1 cc. of obstetric or 0.5 cc. of surgical) of posterior pituitary injection is given subcutaneously.

3. The subject receives nothing by mouth until the test has been completed.

4. Specimens of urine are collected at the end of the first and second hours.

5. The specific gravities of the three specimens are then taken, and the usual corrections for albumin and temperature are made, if necessary.

Normally there is a definite increase in the specific gravity of the urine collected one or two hours, or both, after the injection. In uncompensated renal impairment there is but very slight or no increase of specific gravity in either or both specimens.

Concentration Test of Lashmet and Newburgh. 1. After 10 P.M. of the day preceding the test all fluid and food, except a special diet, are withheld from the patient. This special diet contains protein 40 gm., fat 104 gm., carbohydrate 240 gm., and sodium chloride 1 gm. It is equivalent to 1900 calories.

2. Under the conditions of this test normal kidneys are able to concentrate the urine to a specific gravity between 1.029 and 1.032, while diseased kidneys cannot. The lower the concentrating ability, the greater the renal damage.

Concentration Test of Addis and Shevky. 1. The patient abstains from fluid approximately 24 hours (after breakfast of one day until arising the next day).

2. Urine voided during the first 12 hours is discarded.

3. Urine voided during the last 12 hours (8 P.M. to 8 A.M.) is collected and the specific gravity determined.

4. Normal kidneys show a specific gravity above 1.026 (100 per cent) and 1.028 or above in 95 per cent. The average is about 1.032.

Phenolsulfonephthalein Test. 1. Give the patient 300 to 400 cc. (about 2 glasses) of water to promote diuresis. Smoking and the taking of coffee or tea should be forbidden at least two hours prior to, and during, the test.

2. Twenty minutes later have the patient empty the bladder and discard the urine. In case of necessity catheterize.

3. Then inject *intramuscularly* exactly 1 cc. of the sterile phenolsulfonephthalein solution. If there is general edema the solution should be injected intravenously.

4. Exactly one hour and ten minutes later have the patient empty the bladder (or catheterize) and save the urine.

5. Exactly one hour later (two hours and ten minutes after the injection) have the patient empty the bladder (or catheterize) and save the urine.

Send both specimens to the laboratory for an estimation of the phenolsulfonephthalein in each.

Shaw [18] has advocated the intravenous injection of the dye and the collection of urine 15, 30, 60 and 120 minutes thereafter (*fractional method*), with the estimation of the phenolsulfonephthalein in each. The total elimination may be normal in cases of acute nephritis, but on elimination of less than 25 per cent of the compound in the first fifteen minutes after its injection may be the earliest evidence of renal disease.

The Phenolsulfonephthalein Test Applied to the Two Kidneys Separately. 1. Give the patient two glasses of water about one-half hour beforehand.

2. Introduce catheters into the two ureters.

3. Immediately inject the phenolsulfonephthalein solution intravenously.

4. Collect urine directly into two test tubes which contain a few drops of 10 per cent sodium hydroxide solution.

5. The time of first appearance of the dye in the urine, indicated by the appearance of a red color in the tubes, is noted.

6. Collect urine for one-half to one hour thereafter at intervals of fifteen or thirty minutes. When the catheters are removed, care should be taken to ascertain whether any urine has leaked past them into the bladder, as this accident would confuse results.

7. Send specimens to the laboratory for estimation of dye in each.

Under normal conditions the dye first appears in the urine in three to five minutes, although it may sometimes be delayed for one or both kidneys as a result of reflex inhibition due to catheterization. The total output for each kidney is more important than the time of appearance. Output, for the two kidneys together, is about 35 to 45 per cent in fifteen minutes, 50 to 60 per cent in the first half hour, and 65 to 80 per cent in the first hour. When one kidney is defective the time of appearance of the dye is delayed and the total elimination is reduced. Under such circumstances, the other kidney may compensate to a greater or less degree by increased elimination.

The Sodium Ferrocyanide Test. This test proposed by Stieglitz and Knight [15] for glomerular function consists in the slow *intravenous* injection of a clear solution of 0.5 gm. of pure sodium ferrocyanide dissolved in 10 cc. of sterile distilled water. It is immaterial just how much water is consumed before or during the test, since the rate of excretion appears to be independent of the volume of urine. But the intake of ample amounts is desirable to make possible the prompt voiding of urine 30, 60, 120 and 180 minutes after the injection. The four specimens are sent to the laboratory for titration of ferrocyanide employing a 0.4 per cent solution of cupric sulfate.

THE CONGO RED TEST FOR AMYLOIDOSIS AND NEPHROSIS

Since Bennhold [19] discovered that Congo red injected intravenously is rapidly absorbed by amyloid deposits in advanced amyloidosis, during the past several years the test has been used very extensively for diagnostic purposes and especially for the detection of amyloidosis in patients with chronic pulmonary tuberculosis.[20,21] Furthermore, in lipoid nephrosis it is more rapidly excreted into the urine than in normal individuals and for that reason is being included in this chapter. The test is conducted as follows: [21]

1. Inject intravenously 1 cc. of a 1 per cent aqueous solution of Congo red per 10 pounds of body weight.

2. Exactly four minutes later, and again at the expiration of one hour, remove 10 cc. amounts of blood and place them in clean, dry test tubes for coagulation and centrifuging for the separation of serum; or, each specimen of blood may be collected in test tubes carrying 20 mg. of powdered potassium oxalate and thoroughly mixed for the separation of plasma.

3. Urine should be collected at the end of one hour.

4. All specimens are sent to the laboratory for colorimetric determinations of the dye.

In normal individuals less than 40 per cent of the dye disappears from the blood in one hour. In amyloidosis the disappearance of 60 to 100 per cent of the dye in four minutes is a consistent finding. In some cases of amyloidosis, however, it is necessary to examine blood two minutes after the injection of the dye and compare the results with the four-minute specimen. Since small amounts of amyloid may not absorb more than 40 per cent of the dye in an hour, the absorption of 40 or less per cent does not exclude amyloidosis.

In lipoid nephrosis from 40 to 60 per cent of the dye disappears from the blood in an hour. But if the urine shows the presence of the dye with the disappearance of more than 40 per cent in the blood, nephrosis is the most probable diagnosis.

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6

THE CLINICAL INTERPRETATION OF THE BASAL METABOLIC RATE AND THE IODINE TOLERANCE TEST

Metabolism is the total of various chemical processes within the tissues upon which growth, repair and heat production depend and from which energy is derived for muscular activity and the maintenance of vital functions. Metabolism is concerned with the physicochemical changes associated with and regulated by the availability, utilization and disposal of protein, fat, carbohydrate, minerals, water and vitamins, along with the influence of the endocrine secretions upon these changes. *Anabolism* refers to the building up or assimilative processes in growth, maintenance and repair while *catabolism* refers to the breaking down of substances into simple constituents which are readily excreted under normal conditions. Alterations in these normal metabolic processes constitute the diseases of metabolism.

Basal metabolism, also known as "standard metabolism" or "postabsorptive metabolism," refers to the basal or minimum of energy (heat) output of the body at complete mental and physical rest. Under these conditions approximately 25 per cent of heat production is due to the functional activity of the kidneys, heart, liver and the respiratory movements, while the remaining 75 per cent represents heat production by the resting tissues, especially the muscles. Since metabolic processes primarily involve the utilization of oxygen, the liberation of carbon dioxide and the production of energy in the form of heat, basal metabolism may be expressed in terms of any one of these three factors, namely, (1) as oxygen utilized, (2) as carbon dioxide liberated or (3) as calories of heat produced, the basal metabolic rate being expressed in terms of calories per square meter of body surface per hour (Table 48).

OXYGEN EXCHANGE

In view of the importance of oxidative processes in metabolism, brief reference may be made to the disturbances in oxygen exchange before basal metabolism is discussed further.

External respiration refers to the interchange of oxygen and carbon dioxide between the blood and the lungs, while *internal respiration* refers to the transportation of these gases by the blood and their exchange in the tissues.

By far the greater part of oxygen in the blood exists in a loose combination with hemoglobin, since only about 0.25 to 0.3 volume per cent exists free in physical solution in arterial blood and about 0.1 volume per cent in venous blood. The actual amount in combination with hemoglobin, however, varies not only accord-

TABLE 48. SUMMARY OF GENERAL CONSIDERATIONS IN RELATION TO METABOLISM AND OXYGEN EXCHANGE

Subject	Clinical Interpretation
Metabolism	*Metabolism* is the total of various chemical processes within the tissues upon which growth and heat production depend and from which energy is derived. *Anabolism* refers to building-up or assimilative processes and *catabolism* to the breakdown of substances into their simpler constituents. *Basal metabolism* refers to the minimum of energy (heat) produced by the body at complete mental and physical rest. It involves essentially the utilization of oxygen, the liberation of carbon dioxide and the production of energy in the form of heat.
Respiration	*External respiration* refers to the interchange of oxygen and carbon dioxide between the blood and the lungs. *Internal respiration* refers to the transportation of these gases by the blood and their exchange in the tissues.
Oxygen Content and Capacity of the Blood	The oxygen content of the arterial blood varies from 16 to 24 and of venous blood from 10 to 18 volumes per cent. The oxygen capacity and content of the blood varies with the hemoglobin, being about 20.9 volumes per cent in normal adult males and about 1.5 volumes per cent lower in adult females. The normal hemoglobin in adult males is about 16 ($\pm$ 2.0) and in adult females about 14 ($\pm$ 2.0) gm. per 100 cc. of blood. *Anoxemia* refers to a deficiency of oxygen in the blood.
Anoxemia and Anoxia	*Anoxia* refers to an inefficiency in the utilization of oxygen by the tissues. *Anoxic anoxia* may occur in high altitudes, from the mechanical interference of oxygen absorption or in congenital heart disease. *Anemic anoxia* may occur in the anemias, carbon monoxide poisoning, methemoglobinemia and sulfhemoglobinemia. *Stagnant anoxia* may occur in cardiac decompensation, shock, Raynaud's disease, etc. *Histotoxic anoxia* may occur in tissue injuries by various poisons.
Respiratory Quotient	Refers to the ratio of the volume of carbon dioxide expired to the volume of oxygen inspired during the same interval of time. Varies according to the relative amounts of oxygen and carbon in foodstuffs. Its determination is of value in estimating the severity of diabetes mellitus. It is usually subnormal in hyperthyroidism.

ing to the tension of oxygen and carbon dioxide in the lungs and tissues but to the temperature as well. Consequently, in the lungs where oxygen tension is high and carbon dioxide tension is low, the venous blood readily takes up oxygen, whereas in the tissues where oxygen tension is low and carbon dioxide tension and temperature are high, the arterial blood readily gives up oxygen and takes up carbon dioxide. Under the conditions, the average oxygen content of arterial blood has been variously estimated at from 16 to 24 and of venous blood from 10 to 18 volumes per cent, the exchange of oxygen in the tissues concerned in internal respiration being dependent upon the amount in the blood along with the efficiency of peripheral (capillary) circulation.

According to Hüffner, 1.34 cc. of oxygen combines with each gram of hemoglobin so that the oxygen content capacity of the blood varies with the hemoglobin. Under normal conditions, it has been estimated to average for males from sixteen to sixty years about 20.9 volumes per cent, being about 1.5 volumes per cent lower in females in this age range. Consequently, utilizing Hüffner's factor (1.34), the hemoglobin concentration of the blood of normal males would be $\frac{20.9}{1.34}$ or about 15.6 gm. of hemoglobin per 100 cc. of blood. While Hüffner's factor is now considered slightly inaccurate it is, however, generally used for estimating hemoglobin which, in normal adult males, is commonly stated to be about 16 ($\pm$ 2.0) and in normal adult females about 14 ($\pm$ 2.0) gm. per 100 cc. of blood.

Anoxemia and Anoxia. *Anoxemia* refers to a deficiency of oxygen in the blood and *anoxia* to an inefficiency in the utilization of oxygen by the tissues. Four clinical types are recognized, namely, (1) *anoxic anoxia* due to high altitudes as in mountain sickness, rapid and shallow respiration as in pneumonia, mechanical interference with oxygen absorption as in pneumonia, pulmonary congestion and edema, emphysema, asthma, etc., or to congenital heart disease resulting in a mixture of arterial and venous blood; (2) *anemic anoxia* accompanying severe anemia which reduces hemoglobin, carbon monoxide poisoning, methemoglobinemia (due to drugs and other toxic agents, certain hemolytic poisons, enterogenous cyanosis, etc.) and sulfhemoglobinemia (due to nitrites, coal-tar preparations, sulfur, etc.); (3) *stagnant anoxia* resulting from circulatory inefficiency as in cardiac decompensation, shock, Raynaud's disease, etc., and (4) *histotoxic anoxia* (Peters and Van Slyke) in which the oxygen supply is normal but the degree of oxygen utilization by the tissues is diminished because the tissue cells are injured as in poisoning by alcohol, the cyanides and probably by formaldehyde and acetone.

THE RESPIRATORY QUOTIENT

The *respiratory quotient* or R.Q. refers to the ratio of the volume of carbon dioxide expired to the volume of oxygen inspired during the same interval of time. It varies according to the relative amounts of oxygen and carbon contained in foodstuffs, being 1.0 on a pure carbohydrate diet, 0.80 on a pure protein diet, 0.71 on a pure fat diet and about 0.85 on a mixed diet. Consequently, the R.Q. is taken as an index of the *type* of food undergoing combustion, although it is not a quantitative measure of metabolism since several other different metabolic processes are involved.[1]

In general terms, however, when the R.Q. is around 1.0 it indicates that the food being used is chiefly carbohydrate and when around 0.70 that it is mainly fat. When carbohydrates, which are rich in oxygen, are converted into fats, which are poor in oxygen, the volume of oxygen inspired may be relatively much less than the volume of carbon dioxide expired, in which case the respiratory quotient may be increased to 1.3 or higher. On the other hand, the quotient may be reduced to 0.6 or less during periods of conversion of fats to carbohydrates. Consequently, a determination of the respiratory quotient is of particular value in determining the severity of diabetes mellitus, since it affords an accurate index of the degree of impairment of carbohydrate utilization. In hyperthyroidism it is usually subnormal because of a depleted state of the glycogen reserve in the tissues resulting from an increased rate of carbohydrate utilization in this condition.

THE BASAL METABOLIC RATE

This refers to the production of heat by an individual who, though awake, is as nearly as possible at complete *muscular* and *mental* rest and in the *postabsorptive state* (*i.e.*, from twelve to twenty-four hours after a light meal when, it is assumed, the digestive processes are quiescent). These and additional precautions in its determination are required because heat production is increased by (1) muscular movements, (2) emotional disturbances, (3) food, (4) a fall in room temperature, or (5) a rise in body temperature (fever).

Methods. The basal metabolic rate, or B.M.R., may be determined directly by placing the patient in a calorimeter and actually measuring the amount of heat produced in a given time but the extreme complexity and expense of the apparatus renders this method unavailable and impractical for clinical purposes. Fortunately, however, indirect methods in their present state of perfection are not only much simpler but possess a high degree of accuracy. Two are available, namely, the so-called "open or gasometric" method commonly employed abroad, and the "closed or spirometric" method commonly employed in this country. By the latter, oxygen consumption is determined directly in terms of cubic centimeters per minute and the B.M.R. calculated in one or both of two ways, *i.e.*, on the basis of either oxygen consumption or heat production. Corrections must be made according to age, sex, height and weight (in terms of surface area in square meters). The usual method is to compare oxygen consumption per square meter of body surface with normal standards. Of course, the apparatus should be checked at frequent intervals for leaks and compared with normal controls. The report should also include a record of the temperature by mouth, pulse rate, blood pressure and the length of the rest period before the test is conducted; also whether or not the results are reliable from the standpoint of muscular movements, restlessness, fatigue or apprehension. In borderline cases, from − 15 to − 20 or + 15 to + 20 per cent of normal, it is always advisable to repeat the test, as two or more determinations are of much greater clinical value than a single test.

It is apparent, therefore, that an accurate and clinically acceptable determination of the basal metabolic rate requires not only perfect apparatus along with skill and experience on the part of the technologist, but proper attention to many important details as follows:

1. The patient should have stopped taking thyroid or dinitrophenol for at least one to three weeks, depending upon the dosage; likewise caffeine or epinephrine (adrenalin) for at least twenty-four hours, since all of these raise the basal metabolic rate, while sedatives (morphine, chloral hydrate, barbital, nirvanol, etc.) tend to reduce slightly oxygen consumption and thereby reduce the B.M.R.

2. A light meal with the minimum amount of protein should be taken not later than 7 P.M. and the test conducted the following morning before breakfast.

3. The patient should retire early and secure a good night's rest. For this reason I believe it is advisable for the individual to enter a hospital late in the afternoon previous to the test. In the case of nervous and apprehensive individuals, and especially children, the apparatus should be shown and a mock test conducted in order to allay all apprehension before the test is conducted the following morning.

4. The bladder should be emptied, as discomfort from distention tends to increase the B.M.R.

5. If the test is not conducted at the bedside the patient should not be allowed to walk but should be transported to the basal metabolism room on a stretcher or wheel chair and allowed to lie motionless and in comfort for twenty to thirty minutes before the test is conducted. The error introduced by muscular movements or emotional disturbances is greater in hyper- than in hypothyroidism.

6. The room temperature should be 20° C.

7. The technologist should not only be skilful but kindly, unhurried and very careful in conversation in order that no undue importance be placed on the test by the patient, especially in reference to operations.

The Normal Basal Metabolic Rate; Physiologic Influences. Under these technical conditions a large volume of accumulated data indicates that about 75 per cent of individuals without thyroid disease have a B.M.R. ranging from + 10 to − 10 per cent whereas about 95 per cent fall within the limits of + 15 to − 15 (DuBois standards). It is probable, therefore, that + 10 to − 10 more accurately represents the strictly normal range, although this is commonly taken as + 15 to − 15.

Age has an influence, in that heat production per square meter of body surface diminishes progressively from infancy to old age. *Sex* also has an influence and females usually have a B.M.R. a little lower than males in the same age group. *Race* may also have an effect, since Orientals and Negroes frequently show a somewhat lower rate than whites living under the same climatic conditions. Likewise *occupation* affects the B.M.R.; athletes and laborers have, in general, a somewhat higher rate than persons leading a sedentary life. The B.M.R. during *pregnancy* shows but little or no change until the sixth or seventh month when the fetus causes an appreciable increase, at a rate proportional to the combined surface area of mother and fetus. During *lactation* high rates are particularly frequent which may be ascribed to the increased activity of the mammary glands. *Diet* seems to have little or no influence insofar as vegetarians and meat eaters are concerned. The rate is reduced when the *barometric pressure* falls to half an atmosphere. Extremely high and extremely low *external temperatures* may also cause a rise in the basal metabolic rate.

Even light *muscular exertion, e.g.*, standing or undressing, may raise the B.M.R. from 25 to 60 per cent above the normal. *Mental effort* has little or no influence but *emotional disturbances* like fright, anger, etc., may raise it from 5 to 20 per cent above normal. During *sleep* it usually falls below the basal level. The *ingestion of food* increases heat production (its specific dynamic action) and the B.M.R. An *environmental temperature* sufficiently high to raise the temperature of the tissues will, by increasing the velocity of chemical reactions, also increase heat production and consequently the B.M.R.

Pathologic Increase of the Basal Metabolic Rate. An increase in the B.M.R. is perhaps the most characteristic manifestation of *hyperthyroidism* in toxic adenoma, exophthalmic goiter, carcinoma or thyroiditis (Riedel's struma) due to the effects of an increased production of thyroxin (Table 49). Indeed, it affords the best index of the severity of the diseases producing hyperthyroidism and is invaluable for determining the operative risk in any case. Values of + 20 to + 50 show slight to moderate severity, + 50 to + 75 severe, and above + 75 very severe. The latter are only exceptionally observed.

TABLE 49. SUMMARY OF THE CLINICAL INTERPRETATION OF THE BASAL METABOLIC RATE

Normal: — 10 to + 10; — 15 to + 15 borderline or upper limits of normal.

Increased (above + 15)	Decreased (below — 15)
(1) *Physiologic factors:* Pregnancy after the sixth or seventh months; lactation; muscular movements and exercise; emotional disturbances; ingestion of food; high environmental temperature.	(1) *Physiologic factors:* Sex (females); drop in barometric pressure; sleep.
(2) *Drugs:* Thyroid; dinitrophenol; caffeine; epinephrine (adrenalin).	(2) *Drugs:* Morphine, chloral hydrate, barbital, nirvanol and other sedatives and hypnotics.
(3) *Disease:*	(3) *Disease:*
Hyperthyroidism	Hypothyroidism (cretinism and myxedema)
Acromegaly (early stage)	Hypo-adrenalism
Pituitary basophilism	Hypopituitarism (Simmonds' disease and Fröhlich's syndrome)
Diabetes insipidus (some cases)	Lipoid nephrosis
Diabetic pseudodwarfism	Shock
Hyperadrenalism (some cases)	Starvation and malnutrition
In some cases immediately after smoking.	Sometimes in diabetes mellitus, nephritis and various lowgrade chronic diseases.
Essential hypertension (some cases)	Severe anemias
Cardiorenal disease with decompensation.	Anorexia nervosa
Fevers and especially with toxemia.	Chronic arthritis
Leukemia	Peptic ulcer
Polycythemia vera (some cases)	Autonomic imbalance
Pernicious anemia (some cases)	Schizophrenia
Steatorrhea or sprue (some cases)	

In the majority of cases of *acromegaly* the B.M.R. is within normal but in the early stage of the disease it may range from + 20 to + 35, probably due to hypersecretion of the pituitary gland. After a short time, however, the rate is reduced and may fall below normal due to hypopituitarism. Increased rates may also occur in patients with ***pituitary basophilism*** (Cushing's syndrome or basophilic adenoma of the pituitary gland). ***Diabetes insipidus*** may also be associated with an increased metabolic rate, amounting in some cases to + 25 to + 60, probably because of a great increase in the work of the kidneys incident to the elimination of enormous amounts of water. Diminution of the urine output due to the administration of pituitrin is usually followed by a return of the rate to normal limits. Increased rates are also observed in *diabetic pseudodwarfism.*

The B.M.R. is also increased in many cases of *hyperadrenalism* resulting from tumors of the adrenal glands like hypernephroma or adenoma. Injections of epinephrine are rapidly followed by an increase of the rate. It is believed that these effects are not due to a specific calorigenic influence of epinephrine as in the case of thyroxin, but rather to the generalized increase in muscle tonus which results from stimulation of the sympathetic nervous system. Since smoking, or *nicotine,* apparently stimulates the adrenal glands with an increased secretion of adrenalin, there appears to be a widespread impression that smoking is followed by a temporary rise in basal metabolism.[2,3] Since an increase in blood sugar has also been observed[4] a higher rate may be due to an increase of oxidation of carbohydrate. Goddard and Voss[5] have recently reported that the apparent basal metabolic rate after smoking generally shows an appreciable deviation from a control rate determined prior to smoking but that an increase in the B.M.R. is by no means of universal occurrence.

In the great majority of cases of *essential hypertension* without cardiac insufficiency the rate is within normal but increased rates have been reported in about 25 per cent.[6] In the absence of myocardial insufficiency and dyspnea the cause is unknown but some investigators have attributed the increased rate to overactivity of the sympathetic nervous system with increased secretion of epinephrine or thyroxin and increased cardiac work. Heart disease itself has apparently no effect although rates as high as + 25 to + 40 have been reported in cases of *cardiorenal disease* with cardiac decompensation which have been ascribed to an increased activity of the respiratory muscles or an increase of oxygen consumption resulting from reduced circulation in the tissues.

Practically all *fevers* also cause an increase of the rate, amounting to as much as 7.2 per cent for each degree Fahrenheit or about 13 per cent for each degree Centigrade. This is particularly true when fever is associated with toxemia and the toxic destruction of body protein. In tuberculosis, however, the increase is usually slight, since the toxemia is comparatively mild and malnutrition tends to give a low metabolic rate.

Next to hyperthyroidism, increases in the B.M.R. are particularly high in the *leukemias* (+ 21 to + 80), both lymphogenous and myelogenous, even in the absence of fever. The exact cause is unknown. The increased rate does not appear to parallel the total leukocyte count but may be due to an increased activity of the bone marrow or the lymphoid tissues as well as to an increased rate of protein metabolism.

High rates ranging from + 20 to + 40 may occur in some cases of *polycythemia vera* or erythremia, probably as a result of excessive destruction of nuclear material, although in some cases the rate may be within normal. For unknown reasons, increased rates may also occur in *pernicious anemia* and the *severe secondary anemias.*

The rate is also increased in about 50 per cent of cases of *steatorrhea* in tropical and nontropical sprue although, occasionally, subnormal rates occur. The cause of the high rate is unknown and seems especially hard to explain, since abnormally low rates usually appear as the result of undernutrition and emaciation.

Pathologic Decrease of the Basal Metabolic Rate. A decrease in the B.M.R. is practically just as characteristic and diagnostic of ***hypothyroidism*** in myxedema and cretinism as an increase is characteristic of hyperthyroidism. Rates below — 35 per cent are uncommon, although rates as low as — 42 per cent have been observed in severe cases of myxedema. A determination of the rate, therefore, is almost essential for the detection of mild grades of hypothyroidism as well as being a valuable guide in thyroid therapy.

Hypo-adrenalism, either functional or due to Addison's disease, may also be associated with low rates but are not usually as pronounced as in hypothyroidism, since rates below — 25 per cent are very unusual.

Similar results may be observed in ***hypopituitarism,*** reaching as low as — 40 per cent in pituitary cachexia (Simmonds' disease) or — 25 per cent in adiposal dystrophy (Fröhlich's disease).

The B.M.R. is also usually subnormal in patients with ***lipoid nephrosis,*** being frequently about — 20 per cent and occasionally as low as — 35 per cent, especially during periods of marked edema, although the low rate cannot be explained satisfactorily in the latter state in many cases.

Sometimes it is also greatly reduced in ***shock,*** the degree of diminution being of some value from the standpoint of prognosis.

Low rates are also commonly observed during starvation, falling progressively through the period of fasting. In this, as well as in ***malnutrition*** (inanition), in which the rate may be as low as 70 per cent or less of the normal in severe cases, the decrease is apparently due to a deprivation of protein. Indeed, normal individuals on a low caloric diet deficient in protein may show rates as low as —25 to —30 per cent. Similarly, individuals with *diabetes mellitus* and ***nephritis,*** who are frequently malnourished, may show similar findings. Likewise in ***anorexia nervosa,*** which may be difficult to distinguish from pituitary cachexia, the B.M.R. may be as low as — 40 per cent due to inanition. Slightly reduced rates may also occur in various low-grade chronic illnesses like chronic arthritis, paralysis agitans, toxemia of pregnancy, menstrual disorders, peptic ulcer and other gastro-intestinal diseases.[7] In chorea the rate is usually within normal.[8]

THE SPECIFIC DYNAMIC ACTION OF PROTEIN

When proteins are ingested there is a greater rise in the B.M.R. than can be accounted for by the mere food value they represent. The phenomenon has been called the *specific dynamic action of protein.* Neither fats nor carbohydrates produce as marked an increase of the B.M.R. The effect of proteins is thought to depend primarily upon the anterior lobe of the pituitary gland.

The test is conducted as follows: (1) Determine the basal metabolic rate under standard conditions, as previously described; (2) give the patient a meal of three boiled eggs with a slice of toast and a little water; (3) have the patient rest in a reclining position for two hours; (4) at the end of this period make another basal metabolic test and compare the results.

In *normal* individuals the rate is usually increased by 14 to 18 per cent, with occasional higher rates in constitutionally thin rather than obese individuals.

A greater increase commonly occurs in *hyperpituitarism* and *hyperthyroidism*, either independently or as a result of gonadal failure (castration, menopause, etc.). In hypo-ovarian cases the rate is increased about 20 per cent, while in secreting pituitary tumors it may be 35 per cent or more. However, these changes in pituitary gland disorders may be due to disturbances of the vegetative nervous system independent of the gland.

In *hypopituitarism, hypothyroidism* and various *gastro-intestinal disorders* the rate may not be increased at all or, at most about 10 per cent, because of a marked decrease in metabolism.

IODINE TOLERANCE TEST

The total iodine in blood varies normally from about 8 to 16 micrograms per 100 cc., with a general average of 6 to 7 micrograms. A microgram is $\frac{1}{1000}$ of a milligram so that it is apparent that the method employed must be very sensitive and accurate.

In hyperthyroidism the total blood iodine has been found to vary from 2 to 155 micrograms per cent with an average of 21 micrograms. However, about 25 per cent of cases may have normal values in spite of severe symptoms. Consequently, a determination of the total blood iodine is not ordinarily employed for diagnostic purposes, as discussed more fully in Chapter 3.

Perkin, Lahey and Cattell,[9] however, have suggested that an iodine tolerance test may be of clinical value in differentiation between borderline cases of toxic and nontoxic cases of thyroid disease.

In conducting this test the patient should not have been taking iodine or iodides for at least a week. Blood is taken for a preliminary total blood iodine determination in the morning under fasting conditions. From 0.3 to 0.5 cc. of Lugol's solution is then given in milk, administering about 37.5 mg. of iodine. Blood is taken at intervals of one-half hour over a period of two and one-half hours for total blood iodine determinations.

As shown in Figure 6, the blood level in *normal* individuals reaches about 160 micrograms per 100 cc. one-half hour later, decreasing to about 150 micrograms per cent at the end of two and one-half hours because the normal thyroid has but little avidity for iodine, so that most of it is rejected and appears in the blood.

In *colloid goiter* and *hyperthyroid states* the level at the end of half an hour is likely to be lower, averaging about 100 micrograms per cent and decreasing to a level of about 50 to 60 micrograms at the end of two and one-half hours because the thyroid gland utilizes some of the iodine administered. The total blood iodine may not return to normal levels for three to four hours.

In *hyperthyroidism,* however, the curve is likely to be of the "flat" type, the total blood iodine reaching only 30 to 40 micrograms per cent at the end of half an hour, with only a very slight decrease to between 20 and 30 micrograms at the end of two and one-half hours. It may not reach normal levels for five to six hours or longer. These results would seem to indicate, therefore, that in hyperthyroidism the ingested iodine is taken up by the thyroid gland or tissues or that it is rapidly absorbed and eliminated. Elmer [10] has observed that after the injection of about 1300 micrograms of iodine as potassium iodide, about the same amount is excreted in individuals with hyperthyroidism as in normal individuals.

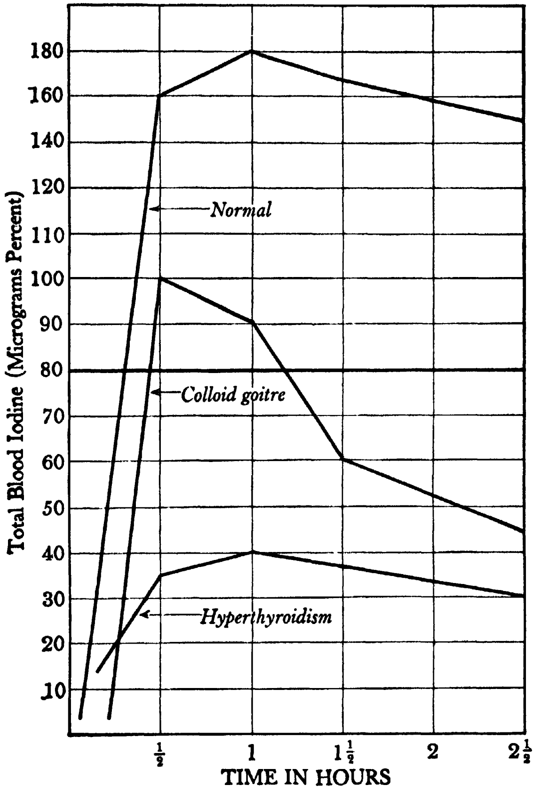

FIG. 6. IODINE TOLERANCE CURVES

The "flat" curve in hyperthyroidism, however, indicates undersaturation of the individual with respect to iodine. The test has not been used to a sufficient extent to state its exact value, but it appears to possess some clinical value in the diagnosis of doubtful or borderline cases of hyperthyroidism in exophthalmic goiter or toxic adenomas.

POTASSIUM TOLERANCE TEST

As previously discussed in Chapter 3, the blood serum normally contains from 16 to 22 mg. of potassium per 100 cc. with about 420 mg. per 100 cc. of cells.

In hypo-adrenalism due to Addison's disease, the basal metabolic rate is not only decreased but the serum potassium is likely to be increased and act as a toxic agent. With this there is a simultaneous decrease of serum sodium. Consequently, a replacement of the excess potassium by sodium, effected by the administration of sodium chloride and a diet low in potassium, appears to remove or reduce the toxic factor with marked therapeutic benefit.

In doubtful cases of the disease the potassium tolerance test of Zwermer may be of clinical value. This test is based upon the observation that a patient with cortical deficiency shows an increased rise and duration of the serum potassium level after the ingestion of a potassium salt. Needless to state caution is necessary in selecting cases for the test, since a severe crisis may be precipitated. The test is conducted as follows: (1) Blood is taken in a fasting state for a determination of the serum potassium; (2) a potassium salt is given orally in such dose as to administer 10 mg. of potassium per kilogram of body weight; (3) blood is taken for potassium estimations at intervals of one-half hour over a period of two hours.[11]

Normally the serum potassium rises from about 20 mg. to 40 mg. per cent one-half hour later, returning to the preliminary level in one and one-half to two hours. In Addison's disease or other states of hypo-adrenalism, however, the rise is much higher and longer sustained.

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7

THE CLINICAL INTERPRETATION OF TOXICOLOGIC EXAMINATIONS

Unfortunately, poisoning is not always easy to recognize during life. Such symptoms and signs as weakness and prostration, nausea, vomiting, diarrhea, gastro-intestinal colic, pupillary changes, cyanosis, respiratory difficulties, delirium, coma, paralysis, etc., are encountered in many diseases. Consequently, unless circumstances arouse suspicion, poisoning may be readily overlooked, especially chronic poisoning by carbon monoxide, arsenic, lead or mercury. Therefore, since only a few of the clinical manifestations are sufficiently pathognomonic to be employed as diagnostic criteria, laboratory examinations are frequently required in cases of suspected poisoning in relation to industrial hazards as well as in cases where individuals are suspected of having taken overdoses of drugs or poisons either accidentally or with suicidal intent.

Poisons are divisible into (1) those which occur as gases; (2) inorganic poisons, including the corrosives, metallic substances and their salts; (3) organic poisons, including those which are volatile as well as alkaloidal and nonalkaloidal compounds and (4) such miscellaneous poisons as those associated with food intoxications and infections, venins, etc. During life, chemical analyses of the blood, urine, feces, gastric contents or vomitus are generally resorted to as diagnostic aids. Chemical methods are also available for the detection of an extraordinarily large number of drugs and poisons by analyses of the tissues after death; these are adequately described in books on legal medicine and toxicology.

Only the more common and important poisons are considered here. Some, like carbon monoxide, alcohol, lead, arsenic, copper and mercury, may occur in the blood, urine, feces, etc., as the result of exposure to small amounts, without the production of manifest ill effects. Consequently, their presence in small amounts may not constitute poisoning and such "normal" values must be carefully considered in the interpretation of laboratory examinations. Furthermore, individuals not only vary greatly in susceptibility to drugs and poisons but amounts detected by chemical analyses vary to some extent according to the methods employed.

CARBON MONOXIDE

The affinity of carbon monoxide for hemoglobin is 200 times greater than that of oxygen, and a compound, carboxyhemoglobin, is formed which is more stable than oxyhemoglobin. Its presence in the blood under "normal" conditions is subject to wide fluctuations, dependent upon unavoidable exposure to illuminating, coal, and automobile-exhaust gases, tobacco smoke, etc. The poisonous

TABLE 50. SUMMARY OF THE CLINICAL INTERPRETATION OF TOXICOLOGIC EXAMINATIONS

Substance	Materials for Examination	"Normal"	Abnormal
Carbon Monoxide	Blood for the estimation of carboxyhemoglobin.	0.00–0.84 (av. 0.27) volume per cent. May be as high as 6 per cent in habituated individuals.	Symptoms usually produced by 15 to 20 volumes per cent. 30 to 75 per cent or over volumes per cent is usually fatal.
Ethyl Alcohol	Blood, urine and breath.	*Blood:* less than 0.05 per cent does not usually produce intoxication; 0.05 to 0.15 per cent is significant of being "under the influence" of alcohol. *Urine:* less than 0.1 per cent does not usually produce intoxication.	*Blood:* 0.15 per cent may produce slight, 0.21 to 0.30 per cent moderate, and 0.41 to 0.50 per cent severe intoxication. *Urine:* 0.1 to 0.2 per cent mild, 0.2 to 0.3 per cent moderate, and 0.4 per cent or higher marked intoxication.
Methyl Alcohol	Urine for formic acid.	0.251 gm. formic acid in total 24-hour urine.	Highly poisonous but susceptibility varies greatly.
Phenol	Stomach contents, urine, feces, blood and tissues for total phenol.	Total phenol in the *blood:* 0.0 to 0.8 mg. per cent; *urine:* 13 to 42 mg. per cent; *feces:* 1.9 to 4.2 mg. per cent.	May produce acute or chronic poisoning. Oxidized internally to hydroquinone and pyrocatechin. Excreted largely in the urine as phenol or various combinations of glycouronic and sulfuric acids with phenol, hydroquinone or pyrocatechin.
Arsenic	Stomach contents, urine and feces in acute poisoning; urine (24-hour output) in subacute and chronic poisoning. Tissues after death.	*Urine:* varies according to diet; usually 0 to 0.15 mg. per 24-hour output. *Blood:* usually none or not more than 0.02 mg. per 100 cc. *Feces:* usually none or not in excess of 0.1 mg. per 100 gm.	Inorganic compounds highly toxic. Pentavalent organic compounds less toxic than trivalent. Largely stored in the liver, bones and hair.

			Unless suspected, clinical detection may be impossible. Chemical examinations are of great value in diagnosis and especially of the urine.
Lead	Urine, feces and blood, particularly urine. Tissues after death.	Varies greatly according to occupation. With no exposure *urinary* lead varies from 0.010 to 0.100 mg. per liter. With industrial exposure from 0.05 to 0.336 mg. per liter. About 0.050 mg. is safe; 0.097 mg. is near the toxic threshold; 0.175 mg. is dangerous and 0.352 mg. is very hazardous. *Feces* with no exposure 0.110 to 0.380 mg. per diem; with exposure 0.470 to 7.60 mg. per diem. *Blood:* about 0.060 mg. per 100 cc.	Chemical analyses of the urine, feces or blood of diagnostic value although limited. General examination of the urine and blood (especially for basophilic stippling) of supplementary value. There are many possible sources of error in collection of materials and in the methods of analysis. Plumbism is most frequently due to absorption of lead through the respiratory tract in industrial exposures. Domestic poisoning is usually due to absorption through the gastro-intestinal tract. Absorption through the skin is doubtful.
Mercury	Gastric contents, vomitus. urine and feces. Kidneys, liver and other tissues after death.	None in the urine and feces if there has been no exposure.	Traces in the urine and feces after even trivial exposure. Examinations of gastric contents and vomitus of value in the detection of acute poisoning by ingestion. Examinations of the urine and feces of diagnostic value in chronic poisoning.
Copper	Urine and feces.	*Urine:* 0.04 to 0.52 (average 0.3) mg. per liter.	Acute poisoning may occur from the accidental swallowing of copper sulfate or for suicidal purposes. Chronic poisoning is usually due to industrial exposure to copper.

concentration of carbon monoxide in the air is estimated at 0.02 to 0.05 per cent by volume. Individuals with a minimum exposure may show from 0.00 to 0.84 (av. 0.27) volume per cent or 0.0 to 4.1 percentage saturation in the blood.[1] Human beings, however, may become habituated to carbon monoxide through occupational exposure and show as high as 6 per cent carboxyhemoglobin in the blood. Susceptibility to the effects of the gas varies considerably, but 15 to 20 per cent of carboxyhemoglobin usually produces symptoms of intoxication, while concentrations of 30 to 75 per cent or over are usually fatal unless prompt remedial measures are applied. The blood should be examined within twelve hours after exposure and kept in a tightly stoppered bottle under oil until the analysis is made. Spectroscopic tests are preferred, as chemical tests are inadequate unless the concentration is 5 to 10 per cent or higher (Table 50).

ETHYL ALCOHOL

The frequency with which clinical examinations for alcoholic intoxication result in inconclusive results has rendered laboratory methods advisable for its detection with special reference to medicolegal examinations. For such purposes the methods of Gettler and Freireich [2] and Turner [3] are commonly employed with blood serum or plasma as they appear superior to those which depend on the colorimetric estimation of reduced dichromate solutions, since bumping over of blood or contact with inorganic material from imperfectly cleaned glassware does not influence the results. The methods may also be used with urine, spinal fluid or minced tissue, but if the material is obtained after death the body must not be embalmed. Examinations of serum or plasma are satisfactory if kept in a refrigerator as long as five days, or for several weeks, if 10 mg. of sodium fluoride is added per cc. of blood. Examinations of fresh serum or plasma, however, are always to be preferred whenever possible. The micromethod of Harger [4] and particularly the method of Cavett [5] are also widely employed and are stated to give reliable results.

It is now well established that there is a close relationship between blood alcohol and intoxication. Consequently, determinations of blood alcohol are always to be preferred since they furnish adequate criteria for medicolegal purposes.[6–9] Determinations of urine alcohol are practically identical with those of cerebrospinal fluid and likewise of practical value.[10–12] The percentage of alcohol in the urine, however, is lower than in the blood and therefore of advantage to the accused in medicolegal cases; a second specimen taken about half an hour later gives a truer result.[12] The results of tests conducted with expired breath closely reflect the blood alcohol and their value has also been established.[13–15]

It is difficult to define the stages of intoxication on the basis of percentages of ethyl alcohol in the blood, urine or breath because of differences of behavior in individuals under its influence. The results of blood alcohol determinations have shown variations in different investigations but in general are in agreement. Turner [3] has reported the following as averages, subject to variation in individual cases:

Clinical	Blood Alcohol
No evidences of intoxication	0.10 to 0.20 per cent
Slight intoxication (stands and walks but talks incoherently)	0.21 to 0.30 per cent
Marked intoxication (falls frequently)	0.31 to 0.40 per cent
Alcoholic stupor (attempted answers and movements)	0.41 to 0.50 per cent
Alcoholic coma	0.51 per cent or above

Jetter,[16] in a study of 1150 cases of suspected intoxication, found 18 per cent intoxicated with a blood concentration of 0.10 per cent alcohol and 47 per cent with a blood concentration of 0.15 per cent. But, in general terms, intoxication is not noticeable until the blood alcohol concentration has reached 0.15 to 0.20 per cent or higher; above 0.5 per cent coma or death may result. Several states have legislated that a blood concentration above 0.150 gm. per 100 cc. (adopted by the National Safety Council) constitutes legal evidence of an individual's being "under the influence" of alcohol, although Newman and Fletcher[17] maintain that this means punishment for drinking, rather than drunkenness, in relation to automobile driving. Concentrations below 0.05 per cent are of doubtful significance; concentrations of 0.05 to 0.15 per cent are significant but the physical signs and symptoms must be carefully considered in rendering the opinion that an individual is "under the influence" of alcohol.

In the urine a concentration of less than 0.1 per cent indicates no intoxication; mild intoxication may occur in about 50 per cent of individuals with a concentration of 0.1 to 0.2 per cent; moderate intoxication with 0.2 to 0.3 per cent and marked intoxication with 0.4 per cent or higher.[10,18]

METHYL ALCOHOL

Methyl alcohol (methanol) is far more toxic than ethyl alcohol. Its action is more prolonged and the products of its decomposition are more toxic. It is more difficult to oxidize than ethyl alcohol and may be eliminated in the breath for a week or longer. A considerable quantity is slowly oxidized into formic acid and slowly eliminated as such in the urine over a period of several days. Qualitative chemical tests for formic acid, however, are of no value in the detection of poisoning, as normal individuals are stated to eliminate about 0.251 gm. of formic acid in 24 hours;[19] therefore, quantitative tests are required.

PHENOL

Phenol is readily absorbed from the gastro-intestinal tract, skin or lungs. It is commonly used for suicidal purposes. Large amounts depress the central nervous system and cause severe irritation of the kidneys. It is oxidized internally to hydroquinone and pyrocatechin. It is excreted in the urine mostly as phenol, or as various combinations of glycouronic and sulfuric acids with phenol, hydroquinone and pyrocatechin. The urine containing these substances becomes dark and smoky, especially on standing, and usually shows the presence of albumin and blood because of renal irritation.

Phenol may produce chronic poisoning when absorbed over long periods of time, producing such symptoms as nausea, anorexia, headache, diarrhea, skin eruptions and renal irritation. Ochronosis, resembling the alkaptonuric variety, may occur with the deposit of bluish-black or brown pigment in the skin, tracheal cartilages, aorta and kidneys.

When phenol has been swallowed, examinations of the gastric contents or vomitus possess diagnostic value; also examinations of the urine, blood and feces during life, and of the tissues after death. By means of chemical and colorimetric methods it is possible to estimate total phenol. Deichmann and Schafer [20] have recently described a spectrophotometric method for estimating free and conjugated phenol separately in the tissues, blood, urine and feces. They state that the blood may normally contain from 0.0 to 0.08 mg. of total phenol per 100 cc., the urine from 13 to 42 mg. per 100 cc. (0.0 to 3.8 mg. as free and from 10 to 39 mg. as conjugated phenol) and the feces from 1.9 to 4.2 mg. of total phenol per 100 gm.

ARSENIC

Arsenic is also frequently taken by ingestion for suicidal purposes, especially "Paris green" (copper acetoarsenite) or "white arsenic" (arsenic trioxide). These inorganic compounds are far more poisonous than the trivalent organic compounds (arsphenamine, neoarsphenamine, mapharsen) and the pentavalent compounds (sodium cacodylate, tryparsamide, stovarsol), the latter being less toxic than the former. Arsenic is largely stored in the liver, bones and hair, being mainly excreted in the bile and, possibly, by the duodenal mucosa. In poisoning by ingestion it may be found in the gastric contents or vomitus. It may be found in the urine in all cases. The blood is not usually examined for it; the same is true of the feces except in acute poisoning from ingestion, since it is not possible to differentiate between arsenic excreted in the feces and that never absorbed from the gastro-intestinal tract.

Large doses of the inorganic compounds taken for suicidal purposes, or administered surreptitiously in foods or beverages for purposes of murder, may produce the paralytic type of poisoning. Smaller but lethal amounts produce the gastro-intestinal type characterized by nausea, vomiting and colic which is more common, while still smaller amounts absorbed frequently over varying periods of time produce the subacute or chronic types of intoxication. Unless the suspicions of the physician are aroused by clinical manifestations, poisoning may not be detected, especially in the subacute and chronic types.

Chemical analyses of the gastric contents or vomitus and of the urine may be and frequently are the only means available for the definite diagnosis of arsenic poisoning, since there may be no pathognomonic clinical signs or symptoms. After death chemical analyses of the organs, particularly of the liver, kidneys, hair and bones, are especially valuable provided the body has not been embalmed with fluids containing arsenic.

"Normal" Arsenic. Since arsenic is widely distributed in nature, occurring in water, soil, some unwashed vegetables and fruits and likewise in some meats (especially sea foods), and because of the possibility of its absorption from hair dyes and skin lotions and the administration of arsenical compounds for medicinal purposes, it is quite usual to find traces of it normally in the *urine*. However, the amounts of "normal" urinary arsenic reported by different investigators have varied according to the methods of analyses employed and blank determinations are always required for the detection of possible traces due to the reagents used in analyses. In individuals on a regular diet it has been reported [21] as occurring in the total twenty-four hour urine in amounts varying from 0 to as high as 0.85 mg. (average 0.26 mg.) and on low arsenic diets from 0 to 0.20 mg. (average 0.07 mg.). In general terms, therefore, the "normal" urinary arsenic appears to vary from 0.0 to 0.15 mg. per twenty-four hour output or 0.40 mg. per 100 gm. of dried urine.[22]

The *blood* is much less likely to contain arsenic and the upper limit of "normal" is probably not higher than 0.02 mg. per 100 cc. or about 0.5 mg. per 100 gm. of dried blood.[22] It is difficult to express the "normal" arsenic occurring in the *feces* because that ingested in foods is so likely to be absorbed and excreted in the urine. Consequently, the feces do not usually show any at all, or not in excess of about 0.1 mg. per 100 gm. of dried material.

LEAD

Chemical analyses of the urine, blood and feces for lead are of but limited value in the diagnosis of plumbism. At least, this is frequently true of chronic cases due to the irregular elimination of lead, since known cases of chronic poisoning may at any particular time show no more in the urine or blood than do normal individuals. Therefore, repeated examinations are frequently required under well-controlled conditions. An abnormal elimination may only indicate a recent exposure and does not necessarily constitute proof of lead poisoning. Consequently, the diagnosis of plumbism not infrequently rests more upon the clinical skill and judgment of the physician than upon chemical examinations of the urine, blood or feces for lead. If, however, due precautions are taken in the collection of urine, feces and blood, and if the "normal" lead contained in them in relation to exposure is carefully considered, the results of laboratory analyses are not without distinct value in diagnosis. Further aid is given also by the presence of albumin, erythrocytes and casts in the urine due to renal irritation; also by the detection of toxic effects on the bone marrow resulting in an early transient polycythemia,[23] followed by polychromatophilia, reticulocytosis, increased or decreased platelets and basophilic stippling of the erythrocytes. Stippling does not occur in all cases of plumbism and may be due to other conditions, but the presence of over 5 per cent of stippled erythrocytes in stained smears of blood is more common in lead poisoning than in any other disease.

Lead is a slow-acting and insidious but powerful poison. The early symptoms may be mild and, unless detected and further poisoning is stopped, it may progress to severe incapacitating illness. If exposure is slight years may elapse before signs and symptoms of intoxication appear. From the clinical standpoint plumbism may be divided into incipient, latent, acute and chronic stages or types. Needless to state, detection in the incipient stage is especially desirable in order to stop further exposure and to institute early treatment, the situation being similar in many respects to that in syphilis, since many of the same organs are vulnerable to attack with varied signs and symptoms. The susceptibility of human beings to lead varies greatly.

Lead is slowly absorbed. Industrial poisonings occur most frequently because of lead absorption in dusts or fumes through the respiratory tract. Household poisonings are not uncommon and are due usually to absorption through the gastro-intestinal tract from the use of stagnant water in lead pipes, the making of wines, cider or beer in glazed pottery containers, the eating of paint from cribs and woodwork by children, etc. Absorption through the skin is now regarded as very doubtful although it may occur through wounds. Under general conditions the intake varies from 0.05 to 2.0 mg. per day (average 0.33 mg.).

When swallowed, lead is absorbed into the portal blood and largely excreted by the bile before reaching the systemic circulation. Frequent exposures by this route may result in its reabsorption from the feces. Some of the lead found in feces may be that escaping absorption. During exposure, however, the amount of lead in the feces is generally proportional to the degree of exposure. Inhalation of lead in fumes and dusts is far more dangerous, since the lead absorbed reaches the systemic circulation directly and in large amounts. After absorption by either route, storage occurs primarily in the liver and kidneys with early migration to the bones where the lead is deposited as an insoluble tertiary phosphate persisting over years of time, usually with no deleterious effects as far as the bones are concerned.

Excretion is mainly by way of the intestinal tract and kidneys. Toxicity or poisoning is mainly determined by the balance existing between absorption, storage and elimination. When excretion keeps pace with absorption, storage does not occur. The metabolism of lead is closely linked not only with that of calcium [24] but with phosphates as well.[25] Acidosis produced by the administration of acids and acid-forming salts like ammonium chloride, as well as potassium iodide and parathyroid hormone, has been proposed for the treatment of chronic lead poisoning based upon promoting excretion.[24] Vitamin D, in association with adequate amounts of calcium and phosphates in the diet, is regarded as promoting the deposition of lead in the bones, although Shelling [25] believes that a high-calcium, low-phosphate diet with vitamin D is less favorable in this regard than the diet alone.

"Normal" Lead. The amount of lead excreted in the *urine* affords some indication of its amount in the blood although both lag behind the amount absorbed from the gastro-intestinal tract. The amounts of "normal" lead in the urine vary considerably not only in relation to occupation but according to the method of analysis employed. For example, electrometric and spectrographic methods are more sensitive and show larger amounts in the urine, blood and feces than chemical methods of examination, although the diphenylthiocarbazone (dithizone) procedure is widely employed. Smith and his colleagues [26] have recently described this method in detail, along with methods for deleading glassware, syringes and needles and the purification of reagents. In other words, there are many possible sources of laboratory error due to traces of lead not only in reagents, but even in filter paper, distilled water and laboratory dusts. Consequently, the analyst must be constantly on guard and always include "blanks" with every examination. But these sources of error are nothing compared to the chances of contamination in the collection of specimens, with special reference to bed pans, urinals, rubber catheters, and needles and syringes used for the collection of blood. Even the excessive drinking of water may result in error in the estimation of urinary lead while a dose of Epsom salts will spoil a sample of feces for analysis because of the lead content of the compound.

With no unusual exposure, Kehoe and his associates [27] found from 0.021 to 0.038 mg. per liter of *urine* (average 0.030 mg.) by chemical methods. Other investigators have reported the "normal" urinary lead as varying from 0.010 to 0.080 mg. per 100 cc. In general terms, therefore, the "normal" urinary lead may be regarded as varying from 0.010 to 0.100 mg. per liter. It rarely reaches a concentration of 0.2 mg. According to Kehoe and his associates, however, among those exposed occupationally to lead, the "normal" urinary amounts may vary from about 0.05 mg. per liter, in the case of garage mechanics, to 0.07 and as high as 0.336 mg. in the case of workmen engaged in the manufacture of tetra-ethyl and white leads, storage battery workers and brass foundrymen. Under the conditions a urinary excretion not exceeding 0.052 mg. per liter is regarded as safe in the case of those exposed occupationally, 0.097 mg. as near the toxic threshold, 0.175 mg. as dangerous, and 0.352 mg. as indicative of very hazardous exposure.[27] As shown by Webster [28] fractional-day samples give no indication of the total daily output and no measure of lead absorption; the total twenty-four hour urine should be examined but if this is not possible morning urine is preferred.

The "normal" lead in the *feces* is stated to vary from 0.110 to 0.380 mg. (average 0.240 mg.) per diem in those without occupational exposure, from 0.470 to 0.540 mg. in garage mechanics and from 0.480 to as high as 7.60 mg. in workmen engaged in the manufacture of tetra-ethyl and white leads, storage battery workers and brass foundrymen.[16] In terms of ash of stool, the normal is stated to vary from 0.05 to 0.25 mg. per gm.; from 0.15 to 2.0 mg. is hazardous and almost always indicative of plumbism.

By spectrographic methods, the upper limit of "normal" lead in the *blood* (occurring as a colloidal phosphate and possibly as diphosphoglycerate) appears to vary from 0.005 to 0.100 mg. per 100 cc., with a general average of about 0.060 mg. per cent.[29] Smith and his colleagues [30] have found none per 10 gm. of blood serum, 0.002 to 0.011 mg. per 10 gm. of cells and fibrin fraction and from 0.001 to 0.005 mg. per 10 gm. of whole blood. These ranges were found independent of sex, age, climate, fatigue, exercise, meals and menstruation. From 0.07 to 0.4 mg. per 100 gm. of whole blood is regarded as hazardous and usually indicative of plumbism. In this connection it may be stated that 0–12 "stippled" *erythrocytes* per 50 oil immersion fields (12,500 cells), averaging 4 per 50 fields, is regarded as being within normal lead levels; 16 to 500 per 50 fields (averaging 75) is regarded as hazardous and indicative of plumbism.

The "normal" lead of *spinal fluid* ranges from 0.0005 to 0.03 mg. (average 0.001 mg.) per 100 cc.; more than 0.004 mg. per 100 cc. is regarded as hazardous and indicative of plumbism.

MERCURY

All compounds of mercury are potential sources of the mercuric ion which is highly toxic and a "general protoplasmic poison" because of the precipitation of proteins. Mercury can be absorbed from all channels of the body including the gastro-intestinal and respiratory tracts, vagina and skin. Once gaining access to the blood, it is rapidly taken up by the tissues and especially the kidneys, liver, spleen, intestinal wall, muscles and lungs with, possibly, some storage in the bones. Excretion begins soon after absorption, being found in the urine within fifteen hours after ingestion and in the feces within thirty to forty hours. Most of the mercury is excreted within six days after its administration, but in poisoning excretion may continue for much longer periods and be readily detected in the liver, kidneys, intestinal wall, muscles, etc., after death.

Acute poisoning usually results from the accidental or suicidal ingestion of highly ionized inorganic compounds, especially mercuric chloride. Acute poisoning has also followed the use of this compound in excessively strong solutions for vaginal douching. Frequently, individuals taking mercuric chloride for suicidal purposes deny doing so. Under these circumstances, a chemical examination of the gastric contents or vomitus for mercury is of great diagnostic aid. Acute poisoning has also followed the administration of mercurial diuretics (when diuresis fails to occur) although the incidence is very low.[31] As a rule, chronic poisoning follows industrial exposure or the medicinal use of mercurial compounds, as in the treatment of syphilis.

In the absence of all exposure to mercury none is to be found in the urine or feces. Very delicate chemical and electrolytic methods, however, may detect minute traces in the urine after what may be regarded as even trivial exposures to the metal or its salts in the industries and in medicinal or cosmetic preparations.

OTHER SUBSTANCES

Copper compounds are not often swallowed for suicidal purposes but accidental poisoning may occur, as well as chronic intoxication due to industrial exposure. Since copper is widely distributed in nature, especially in foods, the normal intake is approximately 2.0 to 2.5 mg. per day in the case of adults and from about 0.07 to 0.12 mg. in children. From three to nine times as much is eliminated in the feces as in the urine. From 0.026 to 0.62 mg. (average 0.16 mg.) is eliminated daily in the urine, corresponding to 0.04 to 0.52 mg. (average 0.3 mg.) per liter.

In addition to tests for these poisons, qualitative and quantitative chemical methods are available for the detection of many other poisons and drugs taken accidentally or with suicidal intent. As a general rule poisons are swallowed. Consequently, chemical examinations of the stomach contents or vomitus are indicated when specimens are obtainable and especially in suspected poisoning due to phosphorus, dinitrophenol or morphine, supplemented by examinations of the urine and blood.

Otherwise, chemical examinations of the urine are required as in suspected poisoning with the barbituric acid derivatives, acetanilid, acetphenetidin, aspirin, morphine, heroin, codeine, chloral hydrate, strychnine, digitalin, iodoform, fusel oil, isopropyl alcohol, formaldehyde, pyrogallol, etc. With some of these compounds examinations of the blood and likewise of the feces are also indicated or required.

As discussed in Chapter 3, chemical examinations of the blood are also frequently indicated for determining the concentrations of the sulfonamide compounds, thiocyanates and bromides in relation to treatment. Under the circumstances, these are not toxicologic examinations unless required in suspected cases of overdosage with toxic manifestations. In this connection it is to be stated, however, that chemical examinations of the urine for quinine are sometimes indicated for determining whether or not it is being adequately absorbed from the gastro-intestinal tract.

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8

THE CLINICAL INTERPRETATION OF LIVER FUNCTION TESTS AND EXAMINATIONS OF THE BILE

No other organ has as many functions to perform as the liver. Indeed, its rôle in maintaining a constant supply of utilizable food materials requires its participation in every phase of metabolism, so that it has been aptly described by Mann as the "commissariat of the body." Furthermore, it is the site of many syntheses as well as being an organ concerned in detoxication, secretion and excretion. Owing, however, to its large size, enormous reserve capacity, and remarkable regenerative powers, it has been estimated that about 80 per cent must be destroyed functionally or anatomically before gross impairment of its functions occurs. For this reason and because of its multiple functions, no one test for functional capacity has been found satisfactory, as will be discussed later in more detail.

FUNCTIONS OF THE LIVER

One of the most important functions of the liver is (1) *its relation* to *carbohydrate metabolism* (Table 51). As previously discussed in Chapter 3, the liver transforms into glycogen not only the glucose resulting from the gluconeogenesis of protein and perhaps of fat, but also the glucose over and above the amount required for immediate needs brought to it from the intestinal tract by the portal blood. This glycogen, in addition to that which the liver forms from lactic acid, is then largely stored within the liver where it constitutes the main reserve for the maintenance of normal blood sugar. Because of these functions, the determination of the fasting blood sugar level and the blood sugar curve following the administration of glucose is frequently employed for an estimation of the functional capacity of the liver, as are also the levulose, galactose and lactic acid tolerance tests.

The liver also plays an important part (2) *in protein metabolism* not only because it is the sole site of deamination of amino acids absorbed from the intestine, but likewise because it forms urea from ammonia derived from these amino acids as well as being involved in the destruction of uric acid. Furthermore, it appears that the liver is the sole site of fibrinogen production and also plays a major rôle in the formation of plasma albumin and globulin and prothrombin as well. Consequently, estimations of the amino acids, urea nitrogen, uric acid, albumin, globulin and prothrombin (time) of the blood are in relation to the functional capacity of the liver in protein metabolism but, otherwise, the only functional test employed for this purpose is that of Takata-Ara (mercuric chloride reaction) for the detection of an increase of plasma globulins.

TABLE 51. SUMMARY OF FUNCTIONS OF THE LIVER

Functions	Mechanism
Carbohydrate Metabolism	(1) Conversion of glucose into glycogen. (2) Conversion of lactic acid into glycogen. (3) Storage of glycogen.
Protein Metabolism	(1) Deamination of amino-acids. (2) Production of urea. (3) Production of fibrinogen. (4) Formation of plasma albumin and globulin. (5) Production of prothrombin.
Lipid Metabolism	(1) Through bile promotes the digestion and absorption of fats and storage of fats. (2) Possibly involved in the synthesis, storage and destruction of cholesterol. (3) Desaturation and oxidation of fatty acids; formation of ketone bodies.
Bile Metabolism	(1) Production of bilirubin from hemoglobin by the Kupffer cells. (2) Excretion of bilirubin. (3) Formation and destruction of bile salts.
Additional Functions	(1) Excretion and metabolism of the porphyrins. (2) Involved in phosphatase activity. (3) Detoxification and conjugation of various toxic substances (indole and other substances absorbed from the intestinal tract, drugs, etc.). (4) Important storehouse of iron. (5) Stores the antianemic principle involved in the production of erythrocytes. (6) Stores the fat-soluble vitamins A, D and K. (7) Serves as a reservoir for venous blood. (8) Intimately concerned in the elimination of certain dyes.

The liver is also concerned (3) *in lipid metabolism.* This is not only owing to the importance of the bile on the absorption of fats from the intestines through activation of pancreatic lipase, emulsification of fats by the bile salts, and the formation of soluble compounds of fats with them, but also because the liver may be involved to some extent in the synthesis and destruction of cholesterol. At least, it appears that esterification and storage of cholesterol esters may be impaired in hepatic disease, while the absence of bile from the intestines results in its defective elimination and the development of hypercholesteremia. Its rôle in lipid metabolism includes, therefore, not only the desaturation and oxidation of fatty acids and the storage of fat, but the formation of ketone bodies as well. But, aside from an estimation of fat in the feces (total fat, soap fat and free fatty acids) and of cholesterol and cholesterol esters in the blood, there are no direct tests for hepatic function in relation to lipid metabolism.

Furthermore, the liver is (4) *intimately concerned in the excretion of bile as well as to some extent in the formation of bilirubin* from hemoglobin by the Kupffer cells. Consequently, an estimation of the blood bilirubin by the van den Bergh and icterus index tests, as well as examinations of the feces for bile pigments and of the urine for bilirubin and urobilin, are of value in the detection of hepatic insufficiency. Furthermore, a bilirubin excretion test is available for an estimation of this phase of the functional capacity of the liver. In this connection, it also appears that the liver is not only (5) *the site of formation of the bile salts, but that it also plays a dominant rôle in their destruction.*

Moreover, it may be stated that the liver (6) *is apparently involved in the excretion and metabolism of the porphyrins.* For example, deficient elimination of coproporphyrin not only results in its accumulation in the blood in amounts sufficient for detection, but an increase of urinary porphyrin is apparently an early indication of hepatic insufficiency.

That the liver is also (7) *involved in phosphatase activity* is indicated by the fact that serum alkaline phosphatase is almost invariably increased in the presence of jaundice due to extrahepatic obstruction of the bile ducts as well as in intrahepatic obstruction with or without hyperbilirubinemia.

Furthermore, an important additional function of the liver is (8) *in the detoxification and conjugation of various toxic substances* which are subsequently eliminated in the bile and urine. This not only refers to indole and other products absorbed from the intestinal tract, but other substances as well, including salicylic acid, menthol, camphor, phenol, etc., which are transformed in conjugate glycuronates and eliminated in the urine. For this reason an estimation of the inorganic and ethereal sulfates in the blood and urine, and of conjugate glycuronates in the blood following the administration of such substances, has been proposed as a method of estimating liver function but has proved of little clinical value. However, tests based on the oxidation of cinchophen and the synthesis of hippuric acid are not without clinical value, especially the latter, to which further reference will be made.

The liver is also (9) *an important storehouse of iron* as likewise (10) *of the antianemic principle* involved in the production of erythrocytes and (11) *of the fat soluble vitamins A, D and K.*

It also acts (12) *as a reservoir for venous blood* governing the rate of delivery of blood to the right auricle of the heart. This, to a certain degree, regulates cardiac output and controls dilution of blood after intravenous infusions.

Finally the liver (13) *is intimately concerned in the elimination of dyes* just as phenolsulfonephthalein is excreted almost entirely by the kidneys. Among these dyes are azorubin S, rose bengal, phenoltetrachlorphthalein, phenoltetraiodophthalein and phenoltetrabromphthalein (bromsulfalein). Several liver function tests have been proposed on the basis of elimination of these substances, of which the bromsulfalein test has proven the most satisfactory because it is much less toxic and irritating than rose bengal and other dyes.

TABLE 52. SUMMARY OF THE PRINCIPLES AND CLINICAL VALUE OF LIVER FUNCTION TESTS

Principles	Clinical Value
Many difficulties due to (1) multiplicity of functions and (2) the remarkable reserve and regenerative powers of the liver as well as (3) because the functions of the liver are intimately associated with other organs. Nevertheless, liver function tests are of value in estimating damage of this organ, especially in relation to surgery, although of but little assistance in disease diagnosis. Hepatic dysfunction may be detected by (1) blood chemistry tests (fasting blood sugar, amino-acids, urea nitrogen, fibrinogen, albumin-globulin ratio, prothrombin, fibrinogen, bilirubin, cholesterol, phosphatase); (2) urine examinations (bile pigments, porphyrins) and (3) feces examinations (fats, bile pigments). Liver function tests have been devised based upon (1) tests for carbohydrate metabolism (glucose, galactose, levulose and lactic acid tolerance tests); (2) for pigment metabolism (bilirubin tolerance test); (3) for detoxifying and conjugation functions (hippuric acid and cinchophen oxidation tests); (4) for the capacity to eliminate dyes (bromsulfalein, rose-bengal), and (5) cephalin flocculation and thymol turbidity tests.	(1) Frequently impossible to detect evidences of dysfunction in the presence of advanced anatomic lesions, especially in chronic liver disease. (2) Of more value in estimating the degree of damage in acute infections. (3) Of special value in the preoperative study of patients with biliary tract disease and thyrotoxicosis, in order to avoid or reduce postoperative complications by preoperative preparations and postoperative treatment. (4) Also of value in deciding upon the choice of anesthetic in patients in whom liver damage is discovered. (5) Of value for the detection of residual liver damage following recovery from acute hepatitis due to infections or toxic agents. (6) Of value for the detection of secondary liver damage by extrahepatic disease. (7) Of value in deciding treatment of syphilis with the organic arsenicals when toxic reactions have occurred (dermatitis, jaundice, etc.).

THE CLINICAL VALUE OF LIVER FUNCTION TESTS

As previously stated, a determination of the efficiency of the liver offers many difficulties, not only because of the many functions involved but likewise because of the remarkable reserve and regenerative powers of this organ (Table 52). It is frequently impossible to detect evidences of dysfunction even in the presence of advanced anatomic damage either resulting from primary disease of the liver, or secondary to lesions of the gallbladder and extrahepatic ducts. This is especially true of chronic diseases such as cirrhosis, syphilis, carcinoma, etc., which, because of their slow progress, are associated with compensatory regeneration with little or no impairment of functions. In acute hepatic disease, however, and especially in hepatocellular necrosis, the functional tests yield more satisfactory results. Then, too, some of the functions of the liver are so intimately associated with other organs that it is difficult or impossible, as stated by Mann, to determine definitely the hepatic factor.

Nevertheless, valuable information is frequently obtained in acute and chronic diseases of the liver and the biliary tract by the utilization of function tests. This is particularly true in relation to the preoperative study of patients with biliary tract and thyroid disease, since some patients who seem to be in satisfactory condition for operation, nevertheless develop severe and even fatal complications. It is true that in most cases of chronic cholecystitis with no jaundice the lesions of the liver are confined to the gallbladder fossa, with little or no effect upon hepatic functions, but in recent acute cholecystitis or biliary colic it is apt to be otherwise. In other words, the presence of gallstones, with or without clinical or x-ray evidences of obstruction, is frequently associated with hepatic damage and when gallstones are present surgical operations are attended by increased mortality unless adequate preoperative measures are employed. The cause of complications in relation to the liver, including the so-called "liver deaths," is not understood but it has been suggested that damaged liver cells, failing in their functions, may produce toxic substances which overtax the kidneys in their elimination. In relation to thyrotoxicosis, it is also to be stated that concomitant liver damage is not infrequent with postoperative crisis or the "thyroid storm" attributed to hepatic insufficiency, against which the preoperative detection of liver dysfunction would lead to more adequate preparation.

Under the circumstances, therefore, it is well for surgeons to remember that liver function tests may not only prove of value in estimating surgical risk, but may indicate the need for a careful choice of anesthetic along with special preoperative and postoperative measures. For example, it is recognized that the use of certain anesthetics, such as chloroform, ether, and spinal analgesia, is associated with depression of hepatic function. Thus, if the liver is known to be damaged, the selection of another less toxic anesthetic like ethylene is permissible.

In other words, it is possible that the liver, in spite of extensive chronic disease, may still be capable of carrying on its functions in an essentially normal manner but, conversely, the latter may be impaired in the absence of anatomic changes only to give serious evidence of dysfunction as the result of further injury or extra strain. With these facts in mind, it is seen that the liver function tests possess clinical value but are of little help in relation to disease diagnosis although they are of aid in differentiating between obstructive and hepatocellular jaundice.

Finally, the liver function tests may furnish supplementary information in patients known or suspected as having liver disease. For example, these tests may aid in the detection of residual liver damage after apparent recovery from acute hepatitis due to infections or toxic agents; likewise, in the detection of secondary liver damage resulting from extrahepatic disease, particularly congestive heart failure; also before the administration of the organic arsenicals in the treatment of syphilis, to determine whether treatment should be continued when dermatitis, jaundice or other severe toxic reactions have occurred.

CHOICE OF LIVER FUNCTION TESTS

In view of the multiplicity of the functions of the liver it is apparent, therefore, that many laboratory examinations other than function tests may reveal the presence of damage to this organ. Among these may be mentioned various blood chemistry determinations, *i.e.*, those for fasting blood sugar, amino acids, urea nitrogen, uric acid, fibrinogen, total proteins (with the albumin-globulin ratio), prothrombin, bilirubin, total cholesterol and cholesterol esters and alkaline phosphatase, discussed in Chapter 3; of bile pigments and the porphyrins in the urine (Chapter 2); and of fats and bile pigments in the feces (Chapter 11).

Since liver damage may impair the ability of this organ to synthesize albumin and globulin, the production of albumin being reduced before that of globulin, serum albumin below 2.5 gm. per 100 cc. is indicative of maximum liver damage with an unfavorable prognosis. Dysfunction may also result in a reduction in the synthesis of prothrombin in the presence of adequate amounts of vitamin K, and this reduction is the basis of a prothrombin function test based upon a failure in the response of the liver to an intramuscular injection of menadione, values 10 to 15 per cent of normal or lower being likewise indicative of serious liver damage. Determinations of plasma alkaline phosphatase are also of value, since in obstructive jaundice of adults 10 to 20 or more Bodansky units may be observed with approximately 10 units in nonobstructive jaundice; according to Wade,[1] the test is particularly valuable in conjunction with the cephalin-cholesterol flocculation test, since a positive reaction with a negative cephalin is indicative of obstructive jaundice while a negative or slightly positive phosphatase with a positive cephalin reaction is indicative of hepatocellular or hepatogenous jaundice. The same is true of cholesterol determinations, since more than 220 mg. of total cholesterol per 100 cc. of plasma, with cholesterol esters less than 60 per cent of the total, are indicative of impairment of liver function. Quantitative urinary urobilinogen tests by Watson's method[2] are also of value, since more than 1 Ehrlich unit per 100 cc. of urine collected between 2 and 4 P.M. is likewise indicative of liver dysfunction with particular reference to hepatocellular jaundice and portal cirrhosis. It may also be stated that hepatic disease may reduce the capacity of the liver for storing the antianemic substance, with an increase of the mean corpuscular volume of erythrocytes.

When any of the laboratory examinations indicate liver damage under circumstances where nephritis and other extrahepatic disease may be excluded, function tests may not be required. But, otherwise and especially when it is important to estimate the degree of liver damage, in relation to surgical operative risks, the function tests are advisable.

A large number of function tests have been proposed (Table 53). Some, like the glucose, galactose, levulose and lactic acid tolerance tests, are based upon the assumption that disturbances of the liver in relation to carbohydrate metabolism are sufficient for indicating dysfunction of this organ as a whole, while the Takata-Ara test has been proposed for the detection of disturbances in the formation of plasma proteins (especially of globulins) in relation to protein metabolism. The bilirubin tolerance test was designed for the detection of disturbances of pigment metabolism while the hippuric acid, tyrosine tolerance and cinchophen oxidation tests were designed to detect disturbances in the detoxifying and conjugation functions. Others, like the bromsulfalein, rose bengal and phenoltetraidophthalein (iso-iodeikon) tests, are based upon the assumption that the ability

TABLE 53. SUMMARY OF THE CLINICAL INTERPRETATION OF LIVER FUNCTION TESTS

Tests	Principles	Normals	Impairment
Glucose Tolerance Test	Failure of glycogen storage with increased or prolonged hepatic glycogenolysis.	Venous blood sugar 140–160 mg. per 100 cc. within an hour. Return to normal in 2½ hours. Arterial (capillary) blood sugar increases more rapidly and from 10–15 mg. higher. The return to normal is not as rapid.	Abnormal increase in venous blood sugar within ¾ to 1½ hours. Falls rapidly within 2 to 3 hours. Arterial-venous difference normal or increased. Impairment common in hepatic disease but the test is of little practical value because of extrahepatic factors. Fasting blood sugar abnormally low in von Gierke's or glycogen disease.
Galactose Tolerance Test	A test for the efficiency of glycogen storage. Galactose is not a renal threshold substance. Retention in the blood indicates failure of the liver to metabolize carbohydrates.	Excretion of galactose in the urine (melituria) not higher than a total of 3 gm. Blood (2 hrs.) 30–120 mg. per 100 cc.	Of no value in the absence of jaundice or in chronic liver disease. Especially likely to be positive in extrahepatic obstructive jaundice. Usually positive in hepatocellular jaundice but may be negative in mild or chronic cases. Does not uniformly distinguish between obstructive and hepatocellular jaundice. 6 gm. or more in the urine indicative of serious hepatic injury. Greatly impaired in hyperthyroidism. Negative in portal cirrhosis and cancer of the liver; also in congestive heart failure.
	Transformed into glycogen only by the liver. Increase in blood above normal indicates	Less than 20 mg. per 100 cc. within first hour; falls below 8 mg. by end of two hours.	Of no value in the presence of diabetes. An increase of 35 mg. or more any time during the test with a delay of

Levulose Tolerance Test	impairment of the liver in converting the sugar into glycogen.		return to normal beyond two hours is positive. Especially positive in severe hepatocellular jaundice; less pronounced in obstructive jaundice, cirrhosis and carcinoma. Chiefly of value in the absence of other disturbances of carbohydrate metabolism.
Lactic Acid Tolerance Test	Lactic acid resulting from a breakdown of glycogen in the muscles and escaping immediate combustion is converted by the liver into glycogen.	Increase of 15 to 24 mg. per 100 cc. above fasting level. Peak reached in 5 minutes returning to normal within 30 minutes.	Increase of 5 mg. or more above fasting level at end of 30 minutes is positive. Essentially normal in extrahepatic obstructive jaundice. Positive in acute diffuse hepatocellular jaundice. May prove of value in differentiating between obstructive and hepatocellular jaundice.
Takata-Ara Test	Based on the assumption that mercuric chloride and sodium carbonate form mercuric oxide in the presence of proteins. In pathologic states precipitation of protein (especially globulins) results. May depend upon a change in the constitution of the proteins without necessarily a change in the albumin-globulin ratio.	Usually negative.	Not specific for any single disease of the liver. Value in prognosis uncertain. Especially apt to be positive in portal cirrhosis; also in severe hepatocellular jaundice and liver abscess. May be positive in carcinoma and passive congestion of the liver; also in pulmonary tuberculosis, hyperthyroidism, chronic alcoholism, multiple myeloma and nephritis with edema. Generally negative in infectious hepatitis, hepatic syphilis and cholelithiasis without obstruction.

TABLE 53. SUMMARY OF THE CLINICAL INTERPRETATION OF LIVER FUNCTION TESTS—(Continued)

Tests	Principles	Normals	Impairment
Bilirubin Tolerance Test	Based upon the excretion of bilirubin by the parenchymal cells of the liver.	All or nearly all excreted in four hours; does not show a retention above 6 per cent at the highest.	Above 6 per cent retention usual in cirrhosis, carcinoma, diffuse hepatitis (after jaundice has subsided) and other diseases of the liver. Also positive in a large percentage of cases of pregnancy after the fourth month.
Hippuric Acid Test (Oral and Intravenous)	Based upon the capacity of the liver to conjugate glycine and benzoic acid into hippuric acid with elimination in the urine. Values below 50 per cent of normal are indicative of severe liver damage and unfavorable prognosis.	In the *oral* test the excretion of approximately 1 gm. or more of hippuric acid during the second and third hours with a total of 3 to 3.5 gm. in the total 4-hour urine. In the *intravenous* test a total excretion of 0.7 gm. in the total one hour specimen of urine.	Of proved value in cases of known liver disease in determining operative risks. Of lesser value in determining operative risks in hyperthyroidism. Diminished elimination usual in intrahepatic obstructive jaundice, hepatic syphilis, infectious hepatitis, acute and subacute hepatic necrosis and carcinoma of the liver; also after anesthesia and operations on the biliary tract. May be likewise diminished in nephritis, cachectic states and anemia. Usually normal in cholecystitis and in uncomplicated obstruction of the common bile duct (except in prolonged cases with hepatic damage).
Cinchophen Oxidation Test	Based upon the conversion of a part of a dose of 0.45 gm. into an intermediary product (oxy-cinchophen) excreted in the urine.	7 to 21 per cent (0.030 to 0.100 gm.) eliminated in 24-hour urine as oxycinchophen.	Increased excretion in the urine in acute and chronic hepatic disease. Biliary stasis alone without influence unless of extremely long duration.
	Based upon excretion by the parenchymal cells of the liver aided by	Not more than 10 per cent of the dye 30 minutes after injection: usually	10 to 40 per cent retention in the blood indicates slight impairment; 50

Bromsulfalein Test	destruction of the dye by the Kupffer cells. Some may also undergo destruction in the tissues. Test less reliable if the serum is discolored by jaundice. Very high retention (60 min. reading) indicative of severe liver damage and unfavorable prognosis.	only a faint trace or none at all. By the *serial* method all dye should be eliminated in 20 minutes.	to 80 per cent moderately severe; 90 per cent or higher very severe. Obstructive jaundice; 10 per cent in early and 40 per cent in late (average). Acute and chronic cholangitis. Acute and chronic hepatocellular hepatitis. Portal and biliary cirrhosis. Syphilitic hepatitis. Chronic passive congestion. Hepatic damage in thyrotoxicosis, amyloidosis, alcoholism, severe anemia, pneumonia, malaria, etc.
Rose Bengal Test	Based upon the excretion of the dye in the bile by the liver. May produce photosensitization. Feces discolored red.	Concentration of second specimen of blood (six minutes) at least 50 per cent less than first specimen (two minutes).	Retention of more than 50 per cent indicates impairment.
Phenoltetraiodophthalein (Iso-iodeikon) Test	Based upon the excretion of the dye in the bile by the liver. In the gallbladder it casts a shadow upon x-ray examination (cholecystography).	10 to 15 per cent retained in the blood 30 minutes after intravenous injection.	Retention of 20 per cent or more abnormal. Of particular value in the preoperative study of patients requiring operations on the galltract. Retention of 50 per cent or more indicates need for special preoperative preparations.
Cephalin-Cholesterol Flocculation Test	Capacity of serum to flocculate a colloidal suspension of cephalin and cholesterol.	Negative or + reactions.	Positive reactions are ++, +++ or ++++. Affords a quantitative degree of liver impairment. Of most value for estimating prognosis in cirrhosis of the liver.
Colloidal Gold Test	Precipitation of colloidal gold by serum.	Reactions less than 5 in the first tube like 432, 321, 221, etc.	Positive reactions show complete precipitation in the first tube (5) like 553, 543, 522, etc. Very sensitive but may yield falsely positive reactions.

TABLE 53. SUMMARY OF THE CLINICAL INTERPRETATION OF LIVER FUNCTION TESTS—(Continued)

Tests	Principles	Normals	Impairment
Thymol Turbidity Test	In hepatitis the blood serum produces turbidity when added to a barbital buffer saturated with thymol. Reacts chiefly if not exclusively with the beta globulin fraction of serum.	0–5 units (av. 2.66). Thymol reagent with pH of 7.55 increases sensitivity; range 6½–9½ units (av. 7.5).	Of greatest value in following the progress of infectious hepatitis. Frequently positive if obstruction is complicated by cholangitis. Sometimes positive in lymphogranuloma venereum and rheumatoid arthritis.
Tyrosine Tolerance Test	In hepatic disease (especially in cirrhosis) the liver fails to metabolize tyrosine (an important amino acid). Based upon determinations of blood tyrosyl (tyrosine and closely related metabolic products).	Fasting: 1.0–1.8 mg. per 100 cc. (av. 1.4). 1 hr.: 4.0–6.4 (av. 5.4) 2 hrs.: 3.6–5.0 (av. 4.6) 3 hrs.: 2.9–4.0 (av. 3.4)	Marked increase in cirrhosis of the liver. In hepatic disease without cirrhosis normal fasting levels but slight increase in 1–2 hours.
Prothrombin Test	Plasma prothrombin is intimately related to the functional state of the liver. Determination an excellent differential diagnostic aid in jaundice. Based upon response of liver to the intramuscular injection of menadione (synthetic vitamin K).	Prothrombin time (method of Quick): 10–25 seconds (av. 20).	Average in hepatocellular jaundice 40 seconds; in early obstructive jaundice 20 seconds; in late obstructive jaundice 50 seconds; in cirrhosis of liver 40 seconds. Values 10–15 per cent of normal or lower are indicative of severe liver damage and unfavorable prognosis.

of the liver to excrete these dyes is a sufficient measure of its functional capacity for clinical purposes.

In 1938 Hanger [3] proposed a simple test by which disturbances of the liver parenchyma may be detected by noting the capacity of blood serum to flocculate a colloidal suspension of a cephalin-cholesterol emulsion. The test has the advantage of simplicity and is applicable to jaundiced and nonjaundiced individuals; apparently it has proved of most value in relation to prognosis in cirrhosis of the liver. The reactions expressed in terms of +, ++, +++ and ++++ afford a quantitative estimation of the degree of liver impairment. It combines marked sensitivity with reliability, provided unripened cephalin is used and false weakly positive (+) reactions are ignored and regarded as negative. In this connection it may be stated that flocculation by normal sera occurs when the diluted reagent is exposed to light and permitted to age. For this reason the modified method of Frisch and Quilligan [4] is to be preferred.

Recently Gray [5] has also described a test based upon a colloidal gold reaction conducted with blood serum. The Klaas modification of Patterson's method for preparing the reagent is employed. Gray advised checking the standardization and acid requirements of the reagent every two weeks, as otherwise difficulties may arise because of change in *p*H with an increase of acid requirements. The test is conducted with three test tubes carrying varying amounts of serum diluted with saline solution with 5 cc. of reagent in each. The readings are made in twelve to twenty-four hours and recorded as *positive* (555, 543, 522, etc.) while readings like 432, 321, 221, etc., are regarded as *negative*. The test possesses marked sensitivity but as a routine laboratory procedure it is too complicated and time-consuming. Furthermore, it is susceptible of yielding falsely positive reactions due to considerable variation in the reactions observed with the sera of normal individuals. These, however, may be controlled and the reliability of the test increased by conducting tests with the sera of 10 to 20 normal individuals every two weeks when restandardizing the reagent for its altered acid requirements.

An additional liver function test is the azorubin S test of Tada and Nakashima [6] which depends on the speed with which the dye appears in the bile. Under normal conditions its appearance time (deep cherry red color of the bile collected by duodenal drainage) is 25–30 minutes. In cirrhosis and acute and chronic diffuse hepatitis the appearance time is delayed or the bile fails to become more than reddish brown in color.[7,8]

The thymol turbidity functional test of Maclagan [9] is of particular value in following the progress of infectious hepatitis. According to Mateer and his colleagues,[10] reducing the *p*H of the thymol buffer from 7.8 to 7.55 increases the sensitivity of the reaction which, according to Cohen and Thompson,[11] is one chiefly, if not exclusively, with the beta globulin fraction of serum. Frequently positive reactions have been observed by Stillerman [12] in obstructive jaundice complicated by cholangitis. Stillerman likewise, observed falsely positive reactions in some cases of lymphogranuloma venereum and rheumatoid arthritis. Apparently the test is not as sensitive as the cephalin-cholesterol flocculation test in the detection of subclinical cases of liver impairment, although both used conjointly were found particularly valuable for "screening" purposes.[10]

As is well known, tyrosine rarely occurs in the urine under normal conditions. But in severe hepatic damage tyrosine may occur because of increased amounts of amino acids in the blood. This is always of serious import. Bernhardt and Schneider [13] have described a tyrosine tolerance test based upon the principle that impairment of the liver to metabolize tyrosine indicates the presence of hepatic disease. A modification of the Millon reaction is employed, using a photoelectric colorimeter. The test is actually for blood tyrosyl which is composed of tyrosine and closely related metabolic products.

With so many tests proposed for estimating the functional capacity of the liver it is difficult to appraise them from the standpoint of choice.

Apparently the bromsulfalein test is the one of first choice and especially when conducted by the serial method.[14] It determines the detoxifying function of the liver and possesses both sensitivity and reliability and especially when 5 mg. of the dye per kilogram of weight is employed.[15] However, it is not applicable or accurate in the presence of jaundice. Recently Wirts and Cantarow [16] have described a procedure whereby the curve of elimination of the dye in the bile is determined simultaneously with the estimation of the degree of its retention in the blood. By this method instances of apparent dissociation of two phases in the mechanism of excretion of the dye were observed, namely, the first phase of its removal from the blood, largely ascribed to a function of the Kupffer cells, and a second phase comprising its gradual excretion in the bile as a function of the polygonal cells of the liver.

The hippuric acid test is probably second choice as a means for estimating the synthetic and detoxifying functions of the liver. Furthermore, it is applicable to jaundiced and nonjaundiced individuals. The intravenous method combines marked sensitivity with reliability and measures maximum hepatic work over a short period of time.[17] The oral test is likewise simple, reliable and inexpensive as well as of proved value in cases with known liver disease in determining operative risk, since the excretion of 1.5 gm. in four hours (less than 50 per cent of normal) indicates a poor operative risk.[14] Some observers have considered the test of particular value in estimating operative risks in hyperthyroidism. Undoubtedly the excretion of hippuric acid in the urine is reduced in a significant number of cases of hyperthyroidism because of functional disturbance of the liver, but according to Haines and his colleagues,[18] excretion is not uniformly reduced in patients who, by other criteria, may be assumed to have a high surgical risk. Nor do all patients with marked reduction in the excretion of hippuric acid present other criteria suggesting high surgical risk. Consequently, the test cannot replace any factor of clinical importance or judgment in relation to hyperthyroidism. Also, dehydration, nephritis or urinary tract obstruction may retard the elimination of hippuric acid and these conditions must be carefully considered or excluded when either the oral or intravenous test is conducted.

For these reasons the galactose tolerance test is considered superior as a test for hepatic function in relation to hyperthyroidism and is undoubtedly the test of choice for estimating the functional capacity of the liver in relation to carbohydrate metabolism, although it appears that galactose tolerance depends upon a multiplicity of factors,[19] including its increased rate of absorption from the intestinal tract. Thus, Meranze and his colleagues[20] have observed positive reactions in 33 of 36 cases of hyperthyroidism and consider the test a valuable adjunct to the diagnosis of this disease, although all attempts to correlate galactose tolerance with other liver function tests have failed. In this connection it should also be stated that individuals with chronic nephritis, upper respiratory tract infections, malignant disease and those who have recently received sulfonamide therapy frequently show impairment of galactose tolerance.[21] Indeed, insofar as cancer of the gastro-intestinal tract in concerned, Abels and his associates[22] have shown that a very high percentage of patients show hepatic dysfunction by various tests, which fact is important because it increases both operative and postoperative risks through the production of hypoproteinemia, hypoprothrombinemia, delayed wound healing, refractory anemia, hemolytic transfusion reactions, etc.

According to Mateer and his colleagues,[10] the cephalin-cholesterol flocculation, bromsulfalein, intravenous hippuric acid tolerance and thymol turbidity tests have been found most sensitive in the detection of hepatic dysfunction; the oral hippuric acid tolerance and urinary urobilinogen tests ranked next in sensitivity while the less sensitive ones embraced the prothrombin (vitamin K response) serum albumin, oral galactose tolerance and plasma cholesterol (total and esters) tests in the order given. For "screening" purposes in the detection of early liver impairment, the cephalin-cholesterol flocculation, thymol turbidity, bromsulfalein, and serum bilirubin tests are to be recommendea. For the early differentiation of obstructive and hepatocellular (hepatic) jaundice, the quantitative urinary urobilinogen, oral galactose, oral hippuric acid tolerance tests and the prothrombin test for vitamin K response are especially indicated. For the detection of initial impairment and progress of acute hepatitis, the serum bilirubin, cephalin-cholesterol flocculation, thymol turbidity, urinary urobilinogen and hippuric acid (oral or intravenous) tolerance tests are recommended. For the detection of chronic hepatitis and cirrhosis, the bromsulfalein, cephalin-cholesterol flocculation, thymol turbidity, oral hippuric acid tolerance, serum albumin and prothrombin tests may be employed, while the bromsulfalein and oral hippuric acid tests are preferred in the detection of metastatic carcinoma of the liver.

The bilirubin tolerance and other tests for hepatic function are also of clinical value and are included in this chapter. Since clinicians must participate in their conduct, methods are described herewith without including the purely laboratory phases of the technic involved.

METHODS FOR CONDUCTING LIVER FUNCTION TESTS

Glucose Tolerance Test. 1. Collect a specimen of venous blood in oxalate twelve hours after last meal (preferably before breakfast).

2. Administer 50 to 100 gm. of glucose in solution flavored with lemon.

3. Remove specimens of blood ½, 1, 2 and 3 hours later.

4. Advisable also to collect arterial (capillary) blood before and at the same intervals after the administration of the glucose.

5. Send specimens of blood immediately to the laboratory for glucose determinations.

Galactose Tolerance Test. This test, first suggested by Bauer in 1906, may be conducted as follows:

1. Collect blood (oxalate) and urine before breakfast.

2. Administer 1 gm. of galactose per kilogram of weight (usually 40–50 gm.) dissolved in 500 cc. of cold water flavored with lemon. The same dose per kilogram is administered to infants and children.

3. Collect urine and blood hourly for five hours thereafter. Water may be allowed, but no food.

4. Send specimens immediately to the laboratory. Each specimen of urine should be tested by the Benedict qualitative method. All positive specimens are mixed, measured and a quantitative test is made by the Benedict quantitative method. Proof that the sugar is galactose is made by the yeast fermentation test. A quantitative galactose determination of each specimen of blood may be made according to the method of Roe and Schwartzman.[23]

Levulose Tolerance Test. This test, as suggested by Tallerman, is conducted as follows:

1. Collect blood in oxalate before breakfast.

2. Administer 40 to 50 gm. of glucose-free levulose dissolved in 250 cc. of water flavored with lemon. For infants and children the dose is 1 gm. per kilogram of weight.

3. Collect blood at one-half hour intervals for two hours.

4. Send specimens to the laboratory for quantitative determinations for levulose (fructose) by Patterson's modification of the Radt test.

Lactic Acid Tolerance Test. This test, suggested by Soffer and his colleagues, is conducted as follows:

1. Collect blood in *fluoride* before breakfast.

2. Inject *intravenously* a solution of sodium-d-lactate in dose of 75 mg. per kilogram of body weight in a 10 to 14 per cent solution in sterile distilled water.

3. Collect blood in fluoride thirty minutes later and send to laboratory for a determination of lactic acid.

The Takata-Ara Test. This test may give positive results in a wide variety of diseases and especially in parenchymatous diseases of the liver, pulmonary tuberculosis, and nephritis. It is correlated to some extent with changes in the ratio of serum albumin to globulin, but the correlation is not absolute, the mechanism uncertain and the test not very sensitive.[24] Many modifications in the technic have been proposed, along with different methods for interpreting the results.[25-28] All that is required is a specimen of blood collected in a dry test tube for the separation of serum.

Bilirubin Tolerance Test. This test, proposed by Eilbott, has its greatest field of usefulness in cases of liver disease in which the serum bilirubin does not exceed 1 mg. per 100 cc. Soffer [29] believes that it may be the most sensitive single function test of all but it has the drawbacks of high cost of bilirubin and the analytical precision essential for eliciting small differences, especially when the total amount in the blood is small. It is conducted as follows:

1. Prepare a solution of chemically pure bilirubin by dissolving 1 mg. per kilogram of body weight (should not exceed 70 mg.) in 15 cc. of a $\frac{1}{10}$ molar solution of sodium carbonate which has been previously brought to the boiling point and allowed to cool to 80° C.

2. Take a sample of venous blood in oxalate (preferably in a paraffined tube) with a dry syringe and then inject the solution of bilirubin through the same needle.

3. Obtain samples of blood in the same manner from the other arm five minutes and again four hours later. Allow no food or water.

4. Send samples of blood to the laboratory for estimations of bilirubin. In conducting these 2 cc. amounts of plasma of the control and four-hour samples from the patient are thoroughly shaken with 2 cc. of redistilled acetone, while 1 cc. of plasma of the five-minute sample is thoroughly shaken with 4 cc. of acetone. The plasma and acetone mixtures are then centrifuged and filtered directly into a dry microcolorimeter cup and compared with a standard 1:6000 solution of potassium bichromate. The difference in bilirubin concentration between the first two specimens is taken to represent 100 per cent of the injected pigment and is the basis upon which is computed the per cent retention at the end of four hours.

Hippuric Acid Test. The recent investigations of Quick[30] have shown that the liver is perhaps the principal site of the formation of hippuric acid by the conjugation of glycine and benzoic acid, with its elimination in the urine. In certain types of hepatic disease this function is impaired due primarily to a diminished capacity of the liver to synthesize glycine, which is essential for the formation of hippuric acid, and in part to damage of the enzymatic mechanism which unites benzoic acid with glycine. The urinary elimination of hippuric acid after the ingestion of benzoic acid is, therefore, regarded as a measure of the capacity of the liver to furnish glycine (amino-acetic acid) and also as an index of its detoxifying activity as well. In this connection, it is to be remembered, however, that deficient elimination may be due to impairment of kidney function. For this reason it is also advisable to conduct the urea clearance test when nephritis is suspected. Defective elimination of hippuric acid may also occur in cachectic states, anemia and chronic passive congestion. However, when these disturbing factors can be eliminated, the test appears to be of value, especially in relation to "surgical" types of jaundice. This is because not only operations but anesthesia as well may result in a sharp reduction in the formation and elimination of hippuric acid which may be partly averted by the administration of glucose, which seemingly hastens the recovery of these functions postoperatively. The technic of the *oral test* is as follows:

1. Give 5.9 gm. of sodium benzoate dissolved in 30 cc. of water (flavored with a teaspoonful of cherry syrup) one hour after breakfast (coffee and toast). In the same glass (to be certain that all of the solution is taken) give one-half a glass of water.
2. Immediately thereafter have the patient void urine and discard the specimen. Thereafter collect urine at hourly intervals for four hours. These specimens are preserved with toluene and sent to the laboratory for a hippuric acid determination of each.

The *intravenous test* should be used in the case of patients who are nauseated or vomiting or who are too ill to be kept off fluids for the five hour period necessary for the oral test. The technic is as follows:

1. Give a breakfast of toast and coffee. If the patient is vomiting, or cannot take solid food, the entire meal, or the toast, may be omitted.
2. One hour later have the patient void urine and inject intravenously 20 cc. of a sterile aqueous solution containing 1.77 gm. of sodium benzoate during a period of 5 to 8 minutes. A period of 5 to 8 minutes is preferred,[14] as the slower injection tends to minimize or prevent the occasional symptoms of transient faintness, sweating, slight epigastric distress, or flushing of the face. Any symptom which may occur tends to disappear if the rate of injection is further retarded, or momentarily stopped.
3. One hour after the completion of the injection, collect urine (by catheterization if necessary) and send it to the laboratory.

Cinchophen Oxidation Test. This test, introduced by Lichtman,[31] is based on the hypothesis that when 0.45 gm. of cinchophen is given orally to a normal individual, a relatively constant amount is converted into an intermediary product (oxy-cinchophen) by the liver and excreted in the urine, while the remainder is disposed of in some other manner. In the presence of liver damage, a much larger percentage of oxy-cinchophen appears in the urine. But the test has not proved sufficiently dependable for clinical purposes and, furthermore, several investigators have properly questioned the advisability of giving cinchophen to individuals with suspected or known damaged livers. The test is conducted by administering 0.45 gm. of cinchophen orally and collecting all of the urine over a period of twenty-four hours which is sent to the laboratory for an estimation of the total oxy-cinchophen excreted.

Bromsulfalein Test. This test, introduced by Rosenthal and White, is regarded by many investigators as being as accurate for the determination of liver function as any employed.[29,32] Normally, the dye is rapidly removed by the liver and excreted in the bile, with some evidence that it is also removed in part by the Kupffer cells of this organ. It has also been suggested that considerable amounts may undergo destruction in the tissues, but it appears that defective elimination of the dye, with a consequent retention of it in the blood, is largely due to defective excretion by the parenchymal cells of the liver and, possibly, in part to impaired function of the Kupffer cells. Originally,[33] the test dose was 2 mg. per kilogram of body weight, but 5 mg. is more generally employed at the present time, the technic of the test being as follows:

1. Weigh the patient and calculate the dose required, allowing 5 mg. for each kilogram of weight. The dye is available in a 5 per cent solution of which 0.1 cc. contains 5 mg. (Hynson, Westcott, and Dunning) so that the dose is 1 cc. for each 10 kilograms, equivalent to 22 pounds of body weight.

2. Withdraw 5 cc. of blood in a dry test tube as a control and then *slowly* inject the dye through the same needle with due care against extravascular injection.

3. At the end of forty-five minutes and again one hour after the injection secure 5 cc. of blood from the other arm, using a dry needle and syringe to avoid hemolysis.

4. The specimens then should be promptly sent to the laboratory for the separation of the sera and a determination of the dye in each by a colorimetric method (photoelectric preferred).

In the presence of complete obstruction of the common bile duct, the degree of retention of the dye in the blood increases progressively with that of bilirubin so that but little information is obtained by this test which cannot be obtained by the van den Bergh and icterus index tests. However, in prolonged incomplete obstruction with superimposed chronic biliary duct disease and hepatocellular damage, the dye may be retained out of all proportion to that of bilirubin; the test, therefore, is of particular value in estimating surgical risks in cases of cholelithiasis. It has also been observed that following relief from obstruction, dye retention, while diminishing, frequently persists for a variable time after the serum bilirubin has returned to normal. This is apparently due to residual hepatitis which so commonly occurs in nearly all patients with prolonged biliary obstruction.

Abnormal retention due to hepatocellular damage also occurs almost invariably in acute and chronic hepatitis and may likewise persist longer than bilirubinemia. Furthermore, retention of the dye may be greater than that of bilirubin, as in congestive heart failure with passive congestion of the liver, thyrotoxicosis, pneumonia and severe anemia. Indeed, most observers agree that in portal cirrhosis retention of the dye may occur without bilirubinemia at all, as likewise in cholecystitis, cholelithiasis without obstruction, toxemia of pregnancy, syphilis and various acute infections. Moreover, the test has proved to be of value for the early detection of hepatitis in patients receiving such hepatotoxic agents as arsphenamine, cinchophen, tetrachlorethylene, etc.

In conducting the test by the *serial* method the dose is 2 mg. per kilogram of weight injected intravenously. Blood (4 cc.) is withdrawn from the opposite arm every 5 minutes over a period of 30 minutes. The per cent of dye in each specimen is determined by the colorimetric method.[14,15] The normal clearance time is about 20 minutes. Any dye remaining at the expiration of 25 or 30 minutes constitutes a positive reaction.

Rose Bengal Test. This dye test, devised by Delprat,[34] also depends upon its excretion by the liver in the bile. It has about the same clinical applications as the bromsulfalein test but the preference is for the latter. The dye is photosensitizing, so patients should keep out of direct sunlight for a few hours at least. It also turns the feces red which patients may mistake for hemorrhage unless previously warned. The test is conducted as follows:

1. Inject intravenously 10 cc. of a specially prepared 1 per cent solution of the dye (Coleman and Bell).

2. Two minutes after completing the injection take 8 cc. of blood from the other arm and place in a tube containing oxalate.

3. *Exactly* six minutes after completing the injection take a second specimen of blood in a tube containing oxalate.

4. Send both specimens to the laboratory for an estimation of the dye in the plasma of each.

Phenoltetraiodophthalein (Iso-iodeikon) Test. This test, originally devised by Graham, to be combined with cholecystography in the preoperative study of patients requiring operations on the gallbladder and common duct, may be conducted as follows:

1. Dissolve 2.0 gm. of sodium-phenoltetraiodophthalein in 50 cc. of freshly distilled water with the aid of gentle heating. Filter through paper. Sterilize by boiling or in autoclave for 15 to 20 minutes. Cool. This 4 per cent solution therefore contains 40 mg. per cc. It may be administered immediately or stored in sealed ampules (keeps 2 to 3 weeks). If a precipitate forms, filter or draw off the supernatant fluid with a syringe.

2. Inject intravenously 1 cc. (40 mg.) per kilogram of weight but not exceeding a total of 62 cc. (2.5 gm.). The dose is diluted with 300 to 500 cc. of sterile saline solution and *slowly* administered intravenously by gravity (with particular care against extravasation in the tissues) followed by saline solution.

3. Collect 12 cc. of blood from the other arm in a plain test tube exactly 30 minutes after the completion of the injection.

4. Send the specimen to the laboratory for the separation of serum and an estimation of the dye.

Cephalin-Cholesterol Flocculation Test. In conducting this test it is only necessary to collect 5 to 10 cc. of blood in a clean, dry test tube after a period of fasting.

Colloidal Gold Test. The conduct of this test likewise requires only 5 to 10 cc. of blood collected in a clean, dry test tube after a period of fasting.

Azorubin S Test. This test may be conducted as follows: [8] 1. Pass a tube into the duodenum of the patient after an overnight fast and confirm its position fluoroscopically.

2. When bile begins to flow through the tube inject 4 cc. of a sterile 1 per cent aqueous solution of azorubin S (a dye of the monoazo group) intravenously.

3. Five minutes later give 40 cc. of a 25 per cent aqueous solution of magnesium sulfate through the duodenal tube.

4. Collect duodenal contents in separate test tubes at intervals of one to two minutes.

5. Note the color: "appearance time" occurs when the bile becomes a deep cherry red in color.

Thymol Turbidity Test. The conduct of this test also requires only 5 to 10 cc. of blood collected in a clean, dry test tube after an overnight fast.

Tyrosine Tolerance Test. This test may be conducted as follows: [13] 1. After an overnight fast, water being allowed until midnight, withdraw 5–10 cc. of blood and place in a dry, clean test tube.

2. Place 4 gm. of tyrosine (S.M.A. Corporation, Chagrin Falls, Ohio), 5 gm. of casein and 4 to 5 drops of 1 per cent phenolphthalein solution in 250 cc. of water in a 1 liter Erlenmeyer flask. While the flask is heating over a Bunsen burner add 5N sodium hydroxide solution, drop by drop, with shaking of the flask until the tyrosine and casein are dissolved with persistence of the phenolphthalein color.

3. Cool and add 2 drops of oil of peppermint to disguise the alkaline taste.

4. Administer orally. Take blood samples 1, 2 and 3 hours thereafter in clean, dry test tubes and send to laboratory along with the preliminary specimen, for the determination of blood tyrosine with a photoelectric colorimeter.

Prothrombin Test. This test may be conducted as follows: 1. Determine the prothrombin time of the plasma according to the method of Quick.

2. Give 10 mg. of menadione (synthetic vitamin K) in oil by intramuscular injection.

3. Determine the prothrombin time 24, 48 and 72 hours after the injection. A control test employing normal plasma should be included.

FUNCTIONS OF THE BILE

Although the functions of the liver and the formation of bile have been previously discussed, a brief consideration of the functions of the latter is advisable at present, especially since it has been shown experimentally that the presence of bile in the intestine appears to be essential to life. Apparently this is largely due to its important rôle in the processes of digestion and absorption of foods with special reference to the fats. Under the circumstances one of the chief functions of the bile is (1) *in the digestion of fats.* Bile salts activate steapsinogen and act as a coferment on the fat-splitting steapsin. Bile also acts as an emulsifying agent which not only facilitates the absorption of fat but exposes small droplets, with a greater surface area, to the activity of digestive enzymes. Normally the feces contain less than 3 per cent fat but in the complete absence of bile the content may be as high as 25 to 27 per cent. However, digestion of fat is not entirely lost as indicated by the presence of free fatty acids and soaps in the feces. The relation of neutral fat to these is not greatly different from the normal but the total amount of each is greatly increased (Table 54).

TABLE 54. SUMMARY OF THE FUNCTIONS OF THE BILE AND GALLBLADDER

	Normal Functions
Functions of the Bile	(1) In the digestion of fats. (2) As an aid in the digestion of other foods. (3) Laxative. (4) Promotes absorption of fats. (5) Promotes absorption of vitamin K and the fat-soluble vitamins. (6) Provides an excretory medium for metabolic products, drugs, etc.
Functions of the Gallbladder	(1) Provides for the storage of bile during interdigestive periods. (2) Concentrates the bile 8 to 10 times. (3) Provides for the equalization of pressure in the biliary duct system. (4) Secretory.

Bile also (2) *promotes the digestion of other foods,* not only because it prevents fats from protecting proteins and carbohydrates against enzymes, but also because the bile salts enhance the activity of proteolytic and amylolytic enzymes in the intestine. Under the circumstances, when bile is absent, or diminished, large amounts of partially digested food reach the colon followed by putrefactive changes. Furthermore, bile (3) *is naturally laxative* by stimulation of peristalsis, so that when absent or diminished, constipation adds to the stasis and putrefaction of fats, carbohydrates and proteins.

The fecal excretion of large amounts of fatty acids and soaps, in the absence of bile, also indicates that the bile (4) *promotes the absorption of fats.* Thus, the bile salts not only play a large part in the solution of substances insoluble in water, due to their hydrotropic effects, but they promote the absorption of emulsified fats by the lacteals as well as act as "carriers" of the fatty acids through the intestinal mucosa. The solubility of neutral fat, as well as of fatty acids, is also greatly increased by the presence of bile acids, and the presence of lecithin further enhances their solubility. Bile also (5) *promotes the absorption of vitamins* with special reference to K and those which are fat-soluble (D and E). Furthermore, the bile (6) *provides a medium for the excretion of various substances.* This refers not only to bile pigments and acids, but to cholesterol, lecithin and other metabolic substances, as well as to the salts of the heavy metals and many drugs. Indeed, this excretory function is of considerable importance, since certain substances excreted in the bile increase biliary secretion and act as cholagogues, with special reference to the bile salts and drugs of the salicylate and benzoate series.

FUNCTIONS OF THE GALLBLADDER

The presence or absence of the gallbladder does not greatly influence the flow of bile into the duodenum during digestion, except in the case of the early excretion which appears to be more concentrated when the gallbladder is present. But if the gallbladder is absent the bile continues to flow into the duodenum during interdigestive periods which does not occur if it is present. Consequently, one of the main functions of the gallbladder is (1) *the storage of bile* until required for the purposes of digestion. Normally the excretion of bile by the liver is a continuous process, with a daily output of 500 to 1200 cc. Excretion is increased by digestion, with the volume varying according to the amount and character of the food; thus excretion is greatest when the diet is rich in protein and least when composed entirely of carbohydrates. Obviously the gallbladder cannot be filled by gravity but during interdigestive periods the sphincter at the duodenal end of the common bile duct is normally closed, which forces the bile to pass through the cystic duct into the gallbladder, because of the secretory pressure of the liver, with only minimal amounts escaping into the duodenum. After the ingestion of food the tone of the sphincter is decreased or abolished by the passage of gastric contents into the duodenum. At the same time, cholecystokinin is secreted by the duodenal mucosa and, after absorption into the blood, causes the musculature of the gallbladder to contract. The net results are a flow of stored bile from the latter into the duodenum (Table 54).

But since the capacity of the normal adult gallbladder is only about 35 cc., storage of bile immediately involves the second main function of this organ, namely, (2) *the concentration of the bile* which, of course, may be greatly reduced or entirely abolished in cholecystitis or cholelithiasis. Normally gallbladder bile is concentrated eight to ten times when compared to bile derived directly from the liver. In other words, the capacity of the gallbladder is only sufficient for the storage of bile excreted during a very short period of time, as the amount excreted in twenty-four hours is about twenty times greater than could be accommodated by the gallbladder. In other words, through this concentrating function, the normal gallbladder appears to be capable of storing bile in amounts approximately that secreted by the liver in two days. Indeed, as shown experimentally by Rous and McMaster,[35] the mere passage of bile through the gallbladder may increase its concentration nearly five times.

Absorption of water and inorganic salts is largely responsible for this concentration, as the composition of the absorbed fluid is essentially that of physiologic saline solution. As a result the solids are increased and largely composed of bile salts and cholesterol, which are not absorbed to any appreciable degree, as is likewise true of the pigments. Readily diffusible substances like sodium chloride, however, are absorbed with the result that while the *p*H of hepatic bile ranges from 7.4 to 8.5, that of gallbladder bile is slightly more acid or neutral, with a *p*H range of 5.4 to 6.9. Calcium is probably absorbed to some extent but its larger amount in gallbladder bile is largely due to concentration from the absorption of water. Other diffusible substances, like drugs and chemical agents, are likewise absorbed. But others, such as iodized or brominated phenolphthaleins, are but slightly absorbed and thus become sufficiently concentrated to cast a shadow by cholecystography, the degree of concentration being closely parallel to that of bile pigments and bile salts.

This function of concentration results not only in furnishing bile salts for the digestion and metabolization of fats in the small intestine but also (3) *in an equalization of pressure within the biliary duct system.* The loss of this function results in a dilatation of the bile ducts. When due to removal of the gallbladder, the flow of bile into the duodenum is at first almost continuous, but later the adaptation of the ducts permits intermittent discharge.

Furthermore, the gallbladder (4) *possesses secretory functions* although their physiologic importance is in doubt. Thus it adds to the viscosity of the bile by the secretion during twenty-four hours of about 20 cc. of a thick mucinous material composed largely of nucleo-albumin and little or none of which is furnished by the bile ducts. Of course, this secretion is increased by acute irritation of the wall of the gallbladder and contributes to the production of so-called *white bile,* which contains no bile salts or pigments, and is not uncommonly seen during operations on obstructed bile ducts associated with chronic cholecystitis. When the gallbladder is completely functionless it is furnished solely by the mucosa of the ducts.

Under normal conditions, the secretions of the gallbladder do not contain significant amounts of cholesterol although it may be excreted by the mucosa.[36] A diffuse deposition of cholesterol ester in the mucosa and connective tissue of the gallbladder may occur, however, as a pathologic condition designated *cholesterolosis* or the "strawberry gallbladder." The cause is unknown but its existence cannot be used as evidence of the secretion of cholesterol by the mucosa under normal conditions. It is also probable that certain toxic agents are at least partly secreted by the mucosa of the gallbladder and are capable of producing acute cholecystitis, as shown experimentally in dogs by the intravenous injection of such substances as chlorinated soda and material obtained from obstructed loops of the small intestine.

COLLECTION OF BILE

From the clinical standpoint, it is obvious that examinations of the bile obtained without the need of surgical intervention possess, potentially at least, most interest and value from the standpoint of diagnosis of diseases of the galltract, although the examination of specimens obtained during operations or immediately after death are of particular value in relation to investigative work. Fortunately, it is usually possible to obtain bile preoperatively by the duodenal drainage method of Lyon.[37] Owing, however, to the chances of contamination with gastric and duodenal juices, the method has been severely criticized. It is true that gross contamination reduces the value of macroscopic examinations and of microscopic examinations as well, as far at least as cytology is concerned. Contamination may likewise interfere with the accuracy of chemical analyses but, nevertheless, when bile is properly collected by this method it would appear that macroscopic examinations for color, viscosity, flocculi, "sand," etc., are of distinct diagnostic value, as are also microscopic examinations for the kinds and numbers of cells and especially for crystals and parasites. Obviously, however, even minor contamination renders bacteriologic examinations worthless, but here again it is apparently possible in many instances to obtain bile acceptable for this purpose, providing all of the precautions against contamination are taken as so carefully described and emphasized by Lyon.

According to Lyon's method, the bile first obtained is from the common duct following relaxation of its sphincter and is designated "A" bile. This is thought to be followed by bile from the gallbladder designated "B" and finally by that freshly excreted by the liver designated "C." If the bile has escaped from the common duct because of relaxation of the sphincter, its segregation into these three categories cannot be made and the bile collected is then assumed to be a mixture of that from the gallbladder and biliary ducts, designated "BC." Needless to state, considerable skill and experience in the microscopy of the bile are essential in relation to the clinical value and interpretation of the results.

1. *Equipment.* The Lyon tube is 130 cm. in length, with a pear-shaped metallic tip having an elongated grooved shank securing it to the tube without tying by thread, thereby minimizing trauma. This tube has two marks equidistant from the ends, and 20 cm. apart. The single mark at 55 cm. from the tip represents the average distance from the lips to the greater curvature of the stomach, and the double mark at 75 cm. the distance to a position approximately at the level of the ampulla of Vater. To the outer end is attached a glass observation cannula or window, which, in turn, connects a larger size tubing, 30 to 50 cm. in length. Because the marks on this tube are equidistant from the ends, the tube may be reversed when wear and tear begin to appear on the swallowed portion. These tubes are also equipped with an adjustable rubber collar at the duodenal mark, which serves to record the variations in length effective for individual patients, and to enable the patient to feel its contact on the lips without difficulty.

Other useful tubes are those of Rehfuss, Twiss, Levin and Jutte (well adapted for use in infants).

2. The patient is also provided with a tray containing a one-ounce capacity Asepto bulb syringe, a kidney basin, a clamp, a percolator or funnel, two 250 cc. graduates, one 125 cc. graduate, and three or more 250 cc. bottles fitted with perforated rubber stoppers with glass tubing inserted for the collection of bile.

3. *Stimulants for Bile Flow.* (a) Saturated solution of magnesium sulfate diluted with two volumes of sterile water to make a 33 per cent volumetric solution. With this the patient is stimulated one or more times, depending upon the amount of magnesium sulfate solution that has been retained. The following fractional dose is advised: first, stimulation of 45 cc., recovering as much as possible; second, stimulation of 30 cc.; third, stimulation of 30 cc. Care should be taken that not more than 90 cc. of the 33 per cent solution (equivalent to one ounce of the saturated solution) is retained at any one treatment, because of the danger of a severe adynamic ileus of the upper bowel. (b) Fifty to 100 cc. of a 5 per cent sterile solution of peptone (boiled and filtered). (c) Fifteen to 30 cc. of olive oil warmed to body temperature. Adding 15 cc. of hot water facilitates its delivery through the tube. Since the oil rises to the top of the specimens and does not dilute the flow of bile, it affords the most satisfactory results when quantitative chemical analyses are to be carried out. Olive oil frequently produces a more prompt flow of B-bile than occurs with magnesium sulfate, and occasionally will cause such a flow after previous drainages with the magnesium

salt have failed to do so. The chief objection to the use of olive oil, however, is that it interferes with proper microscopy, especially at the hands of a beginner. (d) Five to 10 cc. of oleic acid, chemically pure, in 15 to 30 cc. of water.

4. *Preparation of the Patient.* The most satisfactory time for doing a diagnostic drainage is in the morning on a fasting stomach. In preparation, the patient may be instructed to eat a meat sandwich, 20 raisins, and a glass of milk or water at 9 P.M. the previous night, as an optional motor test meal. The reason for this test meal is to introduce easily recognized food material so that if stasis occurs, it may be detected in the aspirates the following morning.

FIG. 7. METHOD OF PASSING THE DUODENAL TUBE

(Courtesy of Dr. Wm. A. Swalm)

After a twelve-hour fast, drainage is then performed the following morning at nine o'clock. Brushing of the teeth should be omitted to prevent the swallowing of blood from bleeding gums. A few moments of simple explanation as to the procedure generally gains the confidence of the patient. For the beginning of the drainage, the patient, if not too ill, sits on a chair, having removed any dentures or tight clothing.

5. *Intubation.* (a) In passing the tube, stand a little to the right of the patient, facing her with the tip of the tube in the right hand (Fig. 7), and explain that when the tip is placed in the back of the mouth she should alternately swallow and breathe naturally through the nose, holding the head in a natural position, until the tip has passed the glottis, after which the tube may easily be slipped down to the stomach mark in the absence of esophageal obstruction or an excessive gag reflex.

(b) The fasting gastric residuum is now extracted by gravity. Only occasionally is the syringe needed to start the flow, but always with minimum suction to avoid trauma. This residue should be described and examined, especially for free and total hydrochloric acid, occult blood, bile, mucus, and detailed microscopy.

(c) The stomach is now washed with several 250 cc. units of sterile water at body temperature through a percolator or funnel placed about 18 inches above the patient's head. This is returned by syphonage into a graduate on the floor (Fig. 8).

(d) Following this, 100 cc. of sterile water may be introduced through the tube into the stomach to encourage gastric peristalsis in carrying the tip through the pyloric canal, and the tube is then clamped.

FIG. 8. METHOD OF GASTRIC LAVAGE IN DUODENAL DRAINAGE

(Courtesy of Dr. Wm. A. Swalm)

(e) The patient is instructed to lie on the bed on the right side (Fig. 9) and *slowly* swallow the tube, taking 20 minutes to get the duodenal mark on the lips. One minute for each centimeter on the Lyon tube will usually engage the tip synchronously with the frequent peristaltic waves which will carry it through the pyloric canal.

(f) The tube is then unclamped and connected to the first bottle. The first fluid to appear will be either pearly gray or yellow duodenal-pancreatic fluid. The fasting duodenum frequently already contains bile.

(g) In locating the position of the duodenal tip and assuring oneself that it is at the proper level, fluoroscopy is rarely necessary. If there exists any doubt as to the location of the tip, stethoscoping the abdomen for maximum air explosions over the stomach and duodenum with air introduced under syringe pressure through the tube will prove highly reliable. An experienced technician, however, can easily and quickly locate the position of the tip when using an Asepto bulb syringe, and, with an "educated thumb" constantly controlling the pressure on the bulb, by injecting a little water will find that if the tip is in the stomach. all fluid will return quickly; if in the pyloric canal, the water will go in very slowly and there will be no return of the fluid, but only a decided tug on the bulb; if in the duodenum, the water will enter slowly and only a portion, generally bile-stained, will return. If the tube is not in the duodenum, it is then withdrawn well up in the stomach and slowly reswallowed. A tube buckled in the stomach or at the pyloric orifice may be partially withdrawn without any initial resistance, but if the tip is in the pylorus or duodenum a slight initial resistance is felt. Rarely a worm tube knots itself in the stomach.

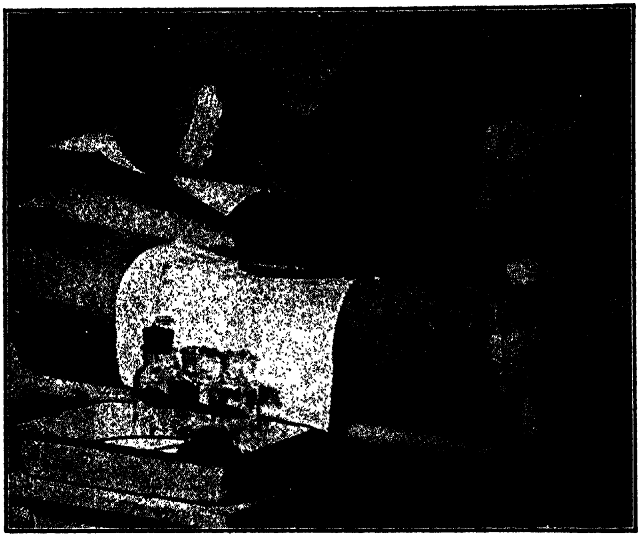

FIG. 9. DUODENAL LAVAGE IN THE SIMS' POSITION

(Courtesy of Dr. Wm. A. Swalm)

6. *Stimulation for Bile Flow.* (a) With the tip definitely in the duodenum, bile drainage is established by serial stimulations with one or more of the stimulants described above. These solutions should all be introduced at body temperature and the tube clamped for 2 to 5 minutes before syphonage is established. This will begin immediately, provided the tube remains filled with fluid before being clamped. A mixture of magnesium sulfate and peptone will sometimes give better results than either alone, and both are satisfactory for bile microscopy and culture.

(b) Over a three-hour drainage period the amount of bile mixture usually recovered by an *adequate drainage* will total 250 to 400 cc., including 20 cc. of pearly gray or yellow duodenal fluid (D-bile), 10 to 20 cc. of golden-yellow duct bile (A-bile), 30 to 75 cc. of dark yellow-brown, mahogany, dark green, or black gallbladder bile (B-bile), and at least 200 cc. of golden to lemon liver bile (C-bile).

Cultures are made as desired from any bile sample. For gallbladder culture the best material is afforded by the last of the B-bile (the dregs from the floor of the gallbladder). This is theoretically so but is difficult in practice to accomplish. Cultures are taken directly into glucose hormone bouillon in the special flasks devised by Richardson. Or the bile may be collected in a sterile vial and plated in the laboratory.

Withdrawal of Tube. Before withdrawing the tube from the duodenum to the stomach and from the stomach to the esophagus, a little air should be blown through the tube to balloon the walls away from the metal tip so as to avoid scratching the mucous membrane. As the tube reaches the glottis, a swallowing movement will facilitate its withdrawal. The duodenum and stomach may be lavaged during removal as desired. A stomach wash with water during withdrawal to remove any regurgitant bile, and a mouth wash, a cup of broth, and some crackers after withdrawal add to the comfort of the patient. Tubes should be flushed out and sterilized by boiling after use. Good rubber should be used in the manufacture of these tubes.

Sources of Difficulty. Occasionally difficulty may be experienced in getting the tip through the pylorus, or the tube may be sharply buckled in the stomach. In such a situation withdrawal of the tip well up into the fundus is necessary prior to reswallowing. The following conditions must be considered: faulty technic; pylorospasm; gastroptosis and gastric atony; organic disease of the stomach and duodenum and accidents.

According to Swalm, if bile is not obtained at the first attempt, the procedure should be repeated after sedatives and antispasmodics have been administered orally for several days. In case of thick gastric mucus, spirits of ammonia and sodium bicarbonate, one-half dram of each, may be added to 250 cc. units. If, after the tube reaches the duodenum, drainage does not proceed properly, it may be started by tilting upward the glass connection, thus reducing air pressure. In stubborn cases, 1/100 grain of atropine in 25 cc. of warm water, clamped off for five minutes, will often facilitate the procedure. If duodenal mucus is found to be blocking the tube, lavage several times with 25 cc. magnesium sulfate in 225 cc. of warm water will usually overcome the difficulty.

In obstinate cases, in which there is difficulty in obtaining "B" bile after ordinary stimulation, the use of a mixture of peptone (one dram in 75 cc. of water, boiled and filtered) with 25 cc. of magnesium sulfate solution and 1/100 grain of atropine will often succeed. Drainage in difficult cases can also be sometimes started by instructing the patient to rise on the elbow for two or three minutes while air is syringed in and out of the tube. In the case of nervous individuals it is advisable to give bromide and atropine orally on arising in the morning. Most patients relax better when given some light reading during the drainage.

EXAMINATIONS OF THE BILE

Color and Viscosity. Normally, "A" bile is of a light golden yellow color; "B" bile is slightly darker, golden yellow to yellow-brown and likewise more viscid; "C" bile is lighter, of a lemon to straw color and less viscid (Table 55). An admixture of any with gastric juice will frequently cause the specimen to turn greenish on standing. Pathologically, any of these may be "off color," ranging from yellow-brown into green-yellows, green-browns, green-blacks and blacks, with an increased viscosity from that of thick syrup to tar. These changes, especially when occurring in "B" bile, are stated to be characteristic of atonic catarrhal cholecystitis and, according to the investigations of Lyon, are thought to be a potential forerunner of cholelithiasis.

Mucus and Flocculi. The normal bile contains but very little mucus with only an occasional floccule. But in catarrh of the duodenum or of the bile ducts, the quantity of mucus is usually increased. Mucus strands enmeshing exfoliated epithelial and pus cells form flocculi appearing as fine and feathery, as thick clumps or granular shaggy masses, the amount being of diagnostic importance and usually expressed as + to ++++. Duodenal mucus usually occurs in ribbons or bands enmeshing oval or cuboidal epithelial cells, whereas mucus originating in the ducts is apt to be in twists and spirals, bile stained and enmeshing mostly

TABLE 55. SUMMARY OF THE CLINICAL INTERPRETATION OF EXAMINATIONS OF THE BILE COLLECTED BY DUODENAL DRAINAGE

Constituents	Normal	Abnormal
Color and Viscosity	"A" bile: light golden-yellow. "B" bile: darker and more viscid. "C" bile: lemon to straw color and less viscid.	"Off color": yellow-brown, brown-greenish, green-black and black; highly viscid. Characteristic of atonic cholecystitis (often a forerunner of cholelithiasis and especially in the case of "B" bile).
Mucus and Flocculi	Very little mucus; occasional floccule.	Increased in duodenitis (ribbons and bands enmeshing oval or cuboidal cells) and catarrh of the ducts (twists and spirals enmeshing columnar cells) and bile stained. May be oleaginous (also occurs occasionally in cholesterosis of the gallbladder).
Epithelium and Leukocytes	Few epithelial cells and occasional leukocyte.	Epithelial cells (oval or cuboidal) and leukocytes increased in duodenitis. Epithelial cells (columnar and polygonal) as well as leukocytes (pus cells) increased in choledochitis and cholecystitis. In empyema of the gallbladder almost pure pus.
Crystals	Normally absent or very few. Crystals of calcium bilirubinate may precipitate out of normal bile permitted to stand for any considerable length of time.	Crystals of cholesterol and calcium bilirubinate most important; diagnostic in about 90 per cent of cases of cholelithiasis. Their absence a strong argument against stone in the gallbladder. May also occur in noncalculous cholecystitis. Crystals of calcium, calcium carbonate and tyrosine may occur.
Chemistry	Insufficient data on bile obtained by duodenal drainage. Chemistry of normal bile otherwise obtained varies with source (hepatic or gallbladder); consult text.	Insufficient data on bile obtained by duodenal drainage in diseases of the liver and galltract; consult text.

columnar and polygonal cells. Sometimes a yellow oily fluid may melt out from the strands of twisted mucus not only in catarrh of the bile ducts but occasionally in cholesterosis of the gallbladder as well.

Epithelium and Leukocytes. Duodenal epithelial cells, therefore, are oval or cuboidal in shape and when present in large numbers indicate the presence of duodenitis, degenerated cells being predominant in chronic duodenitis. Layers of simple tall columnar cells with collections of polygonal cells arranged in rows or rosettes and bile-stained usually indicate a cholecystitis and many antedate all other changes. Bile-stained leukocytes (pus cells) also suggest choledochitis or cholecystitis but if the nuclei are prominent with digestion of the cytoplasm they are apt to be from the stomach or duodenum. In empyema of the gallbladder the cells may be practically pure pus.

Crystals. The presence of large amounts of crystals is especially valuable as indicative of "gallsand," of calculi which have formed or of calculi in the process of forming. The most important of these are crystals of cholesterol occurring in flat, thin, colorless plates with edges like splintered glass. Crystals of calcium bilirubinate are likewise important, with the same significance. These crystals occur in lustrous bright yellow-orange or coarsely granular and gritty clusters. Calcium occurs as dirty grayish-white, thick crystals of irregular size and shape, while crystals of bile pigment are reddish-black and those of tyrosine occur in long slender needles. Calcium carbonate crystals may also be found. Cholesterol crystals and those of calcium bilirubinate are the ones most frequently found preoperatively in calculous and noncalculous cholecystitis and their presence has been regarded as being not only of diagnostic significance in about 90 per cent of cases of cholelithiasis but even more helpful than cholecystography.[38–41] Conversely, the absence of crystals is a strong argument against the presence of stone in the gallbladder, if one obvious source of error has been avoided—namely, that calcium bilirubinate will precipitate out from even normal bile that is allowed to stand for any considerable length of time.

Parasites or their ova may also occur in the bile, as discussed in Chapter 12, while the *microscopy* of duodenal contents is considered in Chapter 10. The *bacteriology* of bile is discussed in Chapter 15.

Chemistry. Much added information, however, is needed on the chemistry of bile obtained by duodenal drainage in relation to clinical diagnosis. That significant changes occur in the gallbladder bile in disease has been shown by Ravdin[42] and others with bile chiefly obtained at operation, largely referable to changes in *p*H concentration, basic ions and chlorides. But few similar studies have been made with bile obtained preoperatively by duodenal drainage. The normal bilirubin of liver or "C" bile obtained by this method ranges from 2 to 10 mg. per 100 cc., whereas the dark bile of galltract disease ranges from 11 to 40 mg. per 100 cc. Chiray and Marcotte[43] have observed that in noncalculous cholecystitis the concentration of cholesterol and bilirubin is high, whereas when the gallbladder contains stones with a patulous cystic duct, the reverse obtains. However, it is necessary that studies on the chemistry of biles obtained preoperatively by duodenal drainage be compared with those obtained at operations on the same patients before any definite statements can be made concerning their diagnostic value. At the present time such studies by Shay and Riegel[41] have indicated that a close correlation has not been obtained, either in respect to chemical changes or in connection with the results of cholecystography.

As far as normal bile obtained at operation or postmortem is concerned, chemical analyses have shown 8 to 18 per cent total solids, 1 to 4 per cent mucin and from 0.5 to 1.1 per cent inorganic material. But values outside of these limits are not uncommon. As previously stated, hepatic duct bile is alkaline (*p*H 7.4–8.5) while gallbladder bile is slightly acid or neutral (*p*H 5.4–6.9). The chief ingredients are the bile acids, in combination as alkaline salts, bile pigments, lipids (including cholesterol), mucoprotein and electrolytes (approximately equivalent to that of plasma in the case of liver bile while higher in gallbladder bile). The concentration of cholesterol in liver bile is less than that of the blood, most values being below 100 mg. per 100 cc. Gallbladder bile, however, contains more cholesterol (150 to 200 mg. per 100 cc.) and particularly in diseases of the liver and galltract.[44]

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9

THE CLINICAL INTERPRETATION OF EXAMINATIONS OF THE SALIVA AND SPUTUM

From the clinical standpoint, examinations of specimens of saliva are of most interest in relation to alterations in the amounts secreted and their chemical composition. The latter is particularly important because of the relation of inorganic constituents to the etiology of dental caries. Bacteriologic and mycologic examinations also possess clinical value, as discussed in Chapters 15 and 16.

In the great majority of instances, examinations of sputums are confined to their bacteriologic aspects with special reference to tubercle bacilli. But their physical properties in relation to quantity, consistency, odor, color, blood and various other gross and microscopic constituents are not without clinical value. Strictly speaking, sputum does not include saliva or the secretions of the nose, nasopharynx or pharynx, and the time spent by the physician in explaining its proper collection is usually well repaid.

EXAMINATIONS OF THE SALIVA

Formation. Saliva is secreted mainly by the parotid, submaxillary and sublingual glands, with small amounts contributed by small glands scattered over the buccal mucous membrane. The cells of the parotid gland are of the serous variety and secrete a thin watery fluid, while those of the submaxillary and sublingual glands are a mixture of serous and mucous type and secrete a slightly viscid watery fluid. Secretion is mainly excited through the mediation of nerves, probably through the agency of a substance liberated at their endings in the glands. This serves for the immediate need of the secretions for the mastication and digestion of foods. Otherwise, secretion is controlled by hormones. The salivary cells, however, are by no means unsusceptible to chemical influences, for a number of substances, *e.g.*, drugs and abnormal metabolic products, reaching them through the blood are capable of influencing their activity (Table 56).

Functions. These are numerous and not without importance. They include (1) *digestion* due to the presence in saliva of ptyalin (salivary amylase) which digests starch into the disaccharide maltase with possible further digestion by maltase which is likewise present. The rapid passage of food through the mouth precludes the possibility of starch being acted upon to any appreciable extent but it is now definitely established that the enzymes may continue with its digestion in the stomach for some time. Saliva is also important (2) *for the alteration of the consistency of food in preparation for swallowing,* as likewise (3) *as a solvent* to stimulate the taste buds. Furthermore, it possesses (4) a *moistening, lubricating* and *cleansing action,* the latter being in particular relationship to the cleansing of

TABLE 56. SUMMARY OF THE CLINICAL INTERPRETATION OF CHANGES IN THE SALIVA

Constituent	Normal	Abnormal
Formation	Secreted by the parotid, submaxillary and sublingual glands; also by small glands in the buccal mucosa.	Reduction or suppression of excretion (*xerostomia* or *aptyalism*) may be permanent (idiopathic) but is commonly temporary due to emotional states, sialolithiasis, dehydration or drugs (belladonna, opium, etc.). Increased excretion (*salivation, sialorrhea* or *ptyalism*) may occur in pregnancy, nausea, hysteria, migraine, emotional shock and other disturbances of the nervous system as well as from reflex stimuli arising in the stomach, duodenum, or oral cavity; also by certain drugs and poisons.
Functions	(1) Digestive; (2) preparing solid foods for swallowing; (3) a solvent in relation to taste; (4) lubricating and cleansing; (5) antiseptic and feebly bactericidal; (6) secretory; (7) as an aid to the regulation of water balance.	
Amount	1200 to 1500 cc. per 24 hours.	
Specific Gravity	1.002 to 1.008.	
Reaction	Slightly acid (*p*H 5.75 to 7.05) when collected without loss of CO_2; otherwise 7.14 $\pm$ 0.04.	
Chemical Composition	About 99.5 per cent water with about 0.2 per cent inorganic substances (chlorides, sodium bicarbonate, carbonates, phosphates and potassium sulfocyanate) and about 0.3 per cent organic substances (enzymes, serum albumin and globulin, urea and mucin). Sodium bicarbonate and phosphates important as buffering substances. Urea about 80 per cent that of the blood. Best determined as combined urea and ammonia nitrogen. Normal "salivary index" varies from 30 to 50.	The calcium salts may be precipitated and, combining with mucin, produce "tartar" deposits on the teeth or salivary calculi. Potassium sulfocyanate is increased in habitual smokers and decreased in pellagra. Reduction of buffering activity, an increase of mucin and other chemical changes may be local factors in relation to the etiology of dental caries. "Salivary indexes" of 50 to 60 or higher indicative of nitrogen retention due to nephritis, etc.
Microscopy	Few epithelial cells, "salivary corpuscles" and bacteria.	Bacteriologic and mycologic examinations only of significance; see Chapters 15 and 16.

the mouth and teeth of food detritus, shed epithelial cells, foreign particles, bacteria, molds and yeasts. Indeed, the normal saliva is now known to possess (5) feeble *antiseptic and bactericidal properties* for staphylococci, streptococci, pneumococci, diphtheria bacilli and possibly some of the viruses through the presence of lysozyme [1,2] and inhibitins.[3] Consequently, the diminution of saliva in febrile and other states readily results in the formation of sordes about the teeth and lips.

In addition, the saliva possesses (6) *secretory functions,* and many substances, both organic and inorganic, are excreted in it. These include not only certain drugs (mercury, bismuth, iodides, lead, etc.) but urea in chronic nephritis, sugar in severe diabetes mellitus as well as such viruses as those of rabies and acute anterior poliomyelitis. Finally the saliva plays an important rôle in (7) *the regulation of water balance,* since dehydration with consequent dryness of the mouth stimulates the afferent nerves with the production of thirst which thereby warns the individual that the body supply of water requires replenishing.

Amount and Reaction. Normally from 1200 to 1500 cc. of mixed saliva is excreted in twenty-four hours (Table 56). When collected with precautions against the loss of CO_2, the reaction is slightly acid with a *p*H varying from 5.75 to 7.05 and in relation to the CO_2 content of the blood; otherwise the *p*H of mixed saliva is about 7.14 ± 0.04. Consequently an increase of CO_2 in the blood results in an increase of the gas in the saliva with an increase of acidity.

Reduction or suppression of the salivary secretion is known as *xerostomia* or *aptyalism.* On rare occasions it is permanent or idiopathic but little is known of its cause. Temporary xerostomia, however, is not uncommon and may be due not only to emotional states or salivary calculi blocking the ducts (*sialolithiasis*), but to fluid loss in fevers, diabetes mellitus, diabetes insipidus, chronic nephritis with edema, severe diarrhea, severe vomiting, excessive perspiration, etc., as likewise after the administration of belladonna or opium.

An increase of saliva, known as *salivation, sialorrhea* or *ptyalism,* may have many causes. It is not uncommon in pregnancy, due to reflex origin or to some metabolic product, as likewise in nausea, hysteria, migraine, facial paralysis, paralysis agitans, tic douloureux, emotional shock, etc., of nervous origin.

It may also result reflexly from stimuli arising in the esophagus, stomach or duodenum, as in peptic ulcer, carcinoma of the stomach, the passage of a stomach tube, etc., as well as in hepatic and pancreatic disease. Under these circumstances the excessive saliva may escape notice, pass down the esophagus and collect above the cardiac sphincter, especially after meals, to be later brought up in gushes without vomiting or nausea and constituting *water-brash.*

Since the salivary glands respond to stimuli, painful or otherwise, salivation may be associated with abnormal conditions of the mouth, including not only teething of infants but caries of the teeth and ill-fitting dentures, as well as with stomatitis, gingivitis, scurvy, purpura, pernicious anemia, etc. Furthermore, salivation may be produced by various drugs and poisons like mercury, bismuth, copper, lead, iodides, bromides, pilocarpine, potassium chlorate, tobacco, etc.

Chemical Composition; Relation to Dental Caries. About 99.5 per cent of the saliva is normally water and about 0.5 per cent total solids. Of the latter, about 0.2 per cent is composed of inorganic substances (chiefly chlorides, sodium bicarbonate, calcium carbonate, phosphates and potassium sulfocyanate) and about 0.3 per cent of organic substances (ptyalin, maltase, serum albumin and globulin, urea and mucin). It also contains gases (CO_2 and O) and varies in specific gravity from 1.002 to 1.008 (Table 56). Total nitrogen averages 45.9 to 49.5 mg. and ammonia nitrogen 7.6 to 8.3 mg. per 100 cc.[4]

Sodium bicarbonate and, to some extent, the phosphates act as "buffers." The chlorides (40 to 50 mg. per 100 cc.) are necessary for the activation of ptyalin (amylase). The calcium salts, being insoluble in alkaline media, tend to be precipitated when the *p*H rises. Under these conditions they may form salivary calculi or, in combination with mucin, be deposited on the teeth as "tartar." The potassium sulfocyanate is probably formed in the body from the metabolism of protein as a detoxicating process, but is stated to be present in excess in the saliva of habitual smokers while reduced in pellagra.[5]

The chemical constitution of the saliva may have, therefore, an important etiologic relationship to dental caries, especially when there is a reduction of its buffering activity in preventing excessive acidity. Undoubtedly calcium salts, which constitute a large proportion of tooth structure, are soluble in acid so that an increased acidity of the saliva is thought to favor decalcification of the teeth and in this way expose them to infection with streptococci or other microorganisms. This is the chemicoparasitic theory of dental caries.

Some of the bacteria commonly occurring in the mouth, especially *L. acidophilus* and other lactobacilli, grow best in an acid medium which they create through their own metabolic activities by the fermentation of food and exudates in the crevices of the teeth as well as at the margins between the teeth and gingivae. Saliva of high buffering value tends to inhibit this bacterial activity, with a reduced tendency to caries, while saliva with a low buffering activity tends to increase susceptibility. Sugars and starches are believed to reduce the buffers while bitter substances, like quinine, tend to increase them.

Another local factor predisposing to caries is thought to be deposits of mucin as tenacious plaques on the teeth protecting underlying bacteria from the buffering action of the saliva. Furthermore, saliva rich in mucin increases its viscosity and reduces its ability to penetrate into, and wash out, small crevices where bacteria lurk and flourish on food debris.

It would appear, therefore, that alterations in the buffering activity of the saliva and its content of mucin are important local factors in the etiology of dental caries, but probably other factors affecting tooth structure (like heredity), as well as the mineral and vitamin constituents of the diet, are of greater importance. Indeed, recent studies by Arnold and McClure[4] on the chemistry of saliva in caries-inactive and caries-active individuals, in relation to *L. acidophilus,* have shown that none of the chemical properties of saliva have any real significant relationship to numbers of the organism in saliva or the occurrence of caries. Furthermore, the *p*H at which maximum coagulation or precipitation of mucin occurs by acids (normally less than *p*H 2.0 to *p*H 3.2) was apparently found to be without relationship to the disease. Krasnow,[6] however, has found that in individuals with erosion the average range of the *p*H of the saliva, as well as its content of calcium, magnesium, inorganic phosphate, protein, lipid phosphorus, and cholesterol, varied to a slight extent from the normal, with significant changes in *p*H, magnesium, lipid phosphorus and cholesterol in caries-active individuals. Alkaline and acid salivas were observed in both erosion and caries cases, with the majority falling on the acid side; no explanation has been found, however, for the occurrence of cases with alkaline saliva. Karshan [7] has likewise observed that the calcium, inorganic phosphorus and CO_2 capacity of the saliva may have a relation to caries susceptibility.

Otherwise, however, the chemistry of saliva has but little clinical interest, except possibly in relation to its urea and ptyalin content. As shown by Hench and Aldrich,[8] the normal *urea* is about 80 per cent that of the blood; parotid saliva is stated to contain 8 to 15 mg. per 100 cc.[2] The combined urea and ammonia nitrogen, as determined by the mercury-combining power of the saliva, more closely approximates the concentration of urea nitrogen of the blood because of the fact that urea in saliva is readily broken down into ammonium carbonate through bacterial action. Normally, 100 cc. of saliva contains enough urea to combine with 30 to 50 cc. of a 5 per cent solution of mercuric chloride which constitutes the mercury-combining or *salivary index* from which the blood urea may be roughly calculated. When the index is below 50 there is not likely to be an increase of blood urea nitrogen above normal. The method is both rapid and simple as an office procedure but is by no means as accurate as the determination of blood urea nitrogen. However, it may be serviceable under special circumstances as a preliminary test for nitrogen retention as well as when venipuncture is impossible or impracticable.

Sometimes it is advisable to examine the saliva for the presence of *ptyalin* (amylase). A simple test may be employed by mixing a few cubic centimeters in a test tube with a few drops of a 1 per cent suspension of starch and placing the tube in a water bath at 37° C. for 10 to 15 minutes. At intervals a drop of the mixture is removed and mixed with a drop of Lugol's solution on a white porcelain plate. If ptyalin is present, the successive drops will turn blue, purple, red and finally yellow, due to erythrodextrin and achroodextrin with maltase, which is capable of reducing Benedict's copper solution, as the final product of digestion.

Nothing of clinical value is to be gained by a *microscopic examination* of the saliva except for bacteria, yeasts and molds, discussed in Chapters 15 and 16. Normally, it contains a few squamous epithelial cells and a few "salivary corpuscles" which are chiefly mononuclear leukocytes, generally much swollen and often filled with granules showing active brownian movement in fresh wet preparations.

EXAMINATIONS OF THE SPUTUM

Formation. Sputum consists of material usually brought up from the lungs, bronchi or trachea by coughing. The amount of normal mucus secreted by these organs is too small to excite expectoration. Strictly stated, sputum does not include saliva or the nasopharyngeal secretions but it is not always possible to exclude them in its collection.

As a general rule, sputum is largely composed of mucous or inflammatory exudates produced in the alveoli of the lungs, bronchi, or trachea, or from a combination of these sources. Extensive accumulations in the lung, however, can be expectorated only when there is a communication with a bronchus and when the material is sufficiently fluid for expectoration. The absence of sputum, therefore, does not exclude the possibility of accumulated materials in the lungs.

Sputum may be largely composed of a serous transudate in pulmonary edema. Or it may be derived primarily from adjacent parts such as the pleural or peritoneal cavities, cysts of the liver, the esophagus, tracheobronchial glands, etc., by rupture into the trachea, bronchi or lungs. Consequently, sputum does not always indicate that its primary source is the lower respiratory tract. Blood may also enter the bronchi and be expectorated, originating in passive congestion of the lungs, ulceration of the bronchi or trachea, hemorrhagic inflammation involving the alveoli, bronchi or trachea, or from blood vessels and various other sources communicating with the latter (Table 57).

Collection. Patients should be instructed to collect only material brought up by coughing without admixture with saliva or secretions aspirated into the throat from the nasal cavity or nasopharynx. It is also advisable for them to rinse the mouth thoroughly with water before collection, as contamination with food particles may prove misleading.

As a general rule, morning sputum, or the entire amount collected over twenty-four hours, is advisable for examination. In some cases of chronic pulmonary tuberculosis there may be no cough at all but only small masses of sputum rising to the larynx and swallowed without patients realizing their significance. The sputum of infants and young children is usually swallowed unless special measures are taken with the latter to encourage expectoration.

As a receptacle for sputum a clean, wide-mouthed bottle with a tightly fitting cork stopper or a sputum cup may be employed. For cultures the bottle and stopper should be sterilized. Patients should be particularly cautioned against smearing any of their sputum on the outside of the container, since it may be a source of danger from infection to the examiner. Disinfectants like phenol, formalin, etc., should not be added. In case of delay in the delivery of the specimens to the laboratory, sputums should be kept in a cold place. After examination the specimen should be destroyed by heat or disinfected with a chemical agent.

Quantity. It is frequently desirable to obtain a general idea of the quantity expectorated over daily periods, but accurate measurement is seldom necessary.

The amount per twenty-four hours usually varies greatly (Table 57). As previously stated, it may be so slight in early tuberculosis as to be entirely overlooked. It is usually scanty in acute bronchitis, bronchial asthma, and in the early stages of pneumonia. Large amounts varying from 25 to 100 cc. or more are the rule, however, in chronic bronchitis, bronchiectasis, gangrene and abscess of the lungs, advanced pulmonary tuberculosis with cavitation, pulmonary edema and pulmonary hemorrhage. Likewise, following the rupture of an empyema, a subphrenic abscess or a liver abscess into bronchi there may be sudden violent coughing and expectorating, with a gush of large amounts of pus.

Alterations in the daily output are of some prognostic value. Thus, increasing amounts in chronic bronchitis, bronchiectasis, tuberculosis and lung abscess indicate progression, while a gradual decrease indicates healing. A sudden cessation, however, is usually due to bronchial plugging followed by a flare-up of fever and constitutional symptoms unless drainage is reestablished.

TABLE 57. SUMMARY OF THE CLINICAL INTERPRETATION OF SPUTUM EXAMINATIONS

Constituent	Abnormal Changes
Formation	Consists of material usually brought up from the lungs, bronchi or trachea by coughing. Should not include saliva or nasopharyngeal secretions. May be composed of (1) mucous or inflammatory exudates from the lower respiratory tract; (2) of transudates in pulmonary edema; (3) blood; or (4) derived from the rupture of material from adjacent parts into a bronchus.
Collection	Should not be contaminated by saliva, nasopharyngeal secretions or food debris from the mouth. Morning specimens are advisable; twenty-four hour collections are preferred. Clean containers required; should be sterilized if cultures are to be made. Outside smearing of the container should be avoided. Preservatives are inadvisable. Specimens should be disinfected after examination before disposal.
Quantity	Accurate measurement seldom necessary. Usually scanty in acute bronchitis, bronchial asthma and the early stages of pneumonia. Large amounts usual in chronic bronchitis, bronchiectasis, gangrene and abscess of the lungs, advanced pulmonary tuberculosis, pulmonary edema and pulmonary hemorrhage; also following rupture of an empyema, subphrenic abscess, abscess of the liver, or broken down mediastinal glands into a bronchus. Alterations in quantity of some prognostic significance. Sudden cessation usually indicative of bronchial obstruction.
Consistency	Serous, frothy, mucoid or glairy, purulent, seropurulent, mucopurulent or hemorrhagic. Mucoid and "rusty" in the early stages of pneumonia. Mucoid in acute bronchitis and bronchial asthma. Serous and frothy in pulmonary edema. Thin and watery or seropurulent in bronchiectasis, tuberculous laryngitis, and perforating abscesses. "Mummular masses" may occur in sputum from bronchiectatic and tuberculous cavities.
Odor	Due to retention and decomposition: sweet, putrid, sour or cheese-like. Sweetish sickening odor frequent in the sputum of bronchiectatic and tuberculous cavities. Foul and putrid in gangrene of the lung and sometimes late in malignancy with necrosis. Sometimes a fecal odor in ruptured subphrenic and liver abscesses.

TABLE 57. SUMMARY OF THE CLINICAL INTERPRETATION OF SPUTUM EXAMINATIONS—(Continued)

Constituent	Abnormal Changes
Color	Mucus usually colorless and transparent. Mucopurulent whitish-yellow; may be streaked with blood. Greenish due to *Ps. aeruginosa* or bile pigment. Dark gray in anthracosis and excessive smoking. "Anchovy sauce" appearance in ruptured amebic abscess of the liver. "Prune juice" color in the late stages of pneumonia; may be of bad prognostic import when due to an associated pulmonary edema.
Blood	Bright red streaks strongly suggestive of tuberculosis; also occur in bronchiectasis. Dark red in capillary oozing in tuberculosis or when retained in cavities. "Rusty" in the early stages of pneumonia owing to the decomposition of hemoglobin; may also occur in pulmonary infarction. A similar color may be due to iron oxides. Brown color not infrequent in congestive heart failure. *Hemoptysis* may occur in pulmonary tuberculosis (60 to 80 per cent of all cases); pneumonias of all types; passive congestion; infarction; bronchiectasis; abscess and gangrene; malignant neoplasms; hypertension; ulcerations; foreign bodies and injuries; ruptured aneurysm of the thoracic aorta; hemorrhagic diseases; pulmonary spirochetosis; gassing in warfare; mycotic infections; infestments with animal parasites, etc.
Bronchial Casts	Small and without branching in the later stages of lobar pneumonia. Medium sized in fibrinous or chronic plastic bronchitis. Large branching casts are rare but may occur in diphtheria of the trachea and bronchi; likewise in severe fibrinous bronchitis.
Dittrich's Plugs	Sometimes expectorated by apparently healthy individuals. May be erroneously regarded as evidence of tuberculosis. May occur in chronic bronchitis, bronchiectasis and bronchial asthma. Caseous masses from the tonsillar crypts may be mistaken for them.
Pneumoliths	Usually calcareous nodules of tissue in chronic tuberculosis. May be small foreign bodies retained in the lungs over long periods of time, encrusted with calcium salts.
Elastic Tissue	Presence indicates destructive lesions of the alveoli and bronchioles. Of important diagnostic and prognostic significance. Usually due to active tuberculosis with cavitation. May occur in asbestosis, abscess and gangrene of the lungs and in ulcerating malignant tumors of the lungs. "Asbestos bodies" may occur in asbestosis with or without elastic tissue.
Curschmann's Spirals	Fairly characteristic of bronchial asthma. Occasionally in chronic bronchitis or catarrhal conditions but usually with an underlying asthmatic tendency.

TABLE 57. SUMMARY OF THE CLINICAL INTERPRETATION OF SPUTUM EXAMINATIONS—(Continued)

Constituent	Abnormal Changes
Eosinophils	Characteristic of bronchial (allergic) asthma. Absent in "cardiac asthma" or paroxysmal nocturnal dyspnea.
Pigmented Cells and Myelin Globules	Large mononuclear cells bearing hemosiderin in congestive heart failure ("heart-failure cells"); may also occur in pulmonary infarction and for some time after pulmonary hemorrhage. Carbon-laden cells in anthracosis and excessive smoking. Myelin globules of little significance. May occur in the scanty morning sputum of apparently healthy individuals; also in the mucoid sputum of bronchitis.
Charcot-Leyden Crystals	May occur characteristically in the sputum of bronchial asthma, especially after standing for some time.
Bacteria, Yeasts and Molds	See Chapters 15 and 16.
Animal Parasites	See Chapter 12.

Consistency. Sputums are usually classified as serous, frothy, mucoid, or glairy, purulent, seropurulent, mucopurulent, or hemorrhagic; these terms are self-explanatory.

The rusty sputum of lobar pneumonia is usually so tenacious that the patient may have difficulty in raising and expectorating it and especially because of pleuritic pain associated with coughing. It is also highly mucoid and sticky in acute bronchitis, as likewise following an asthmatic attack.

In pulmonary edema, however, it is characteristically serous and frothy with traces of blood. It is likewise usually thin and watery in bronchiectasis, tuberculous laryngitis and perforating abscesses (hepatic and diaphragmatic). In these conditions, as well as in lung abscess and gangrene, it is apt to separate into three layers when allowed to stand in a tall vessel. Sputums derived from tuberculous and bronchiectatic cavities frequently show the presence of mucopurulent masses which flatten out into coin-like or "mummular masses" when mixed with water.

Odor. Odor is due to the decomposition of sputum from prolonged retention and may be sweet, putrid, sour or cheese-like. In acute infections it is absent or very faint. But sputums derived from tuberculous and large bronchiectatic cavities sometimes possess a peculiar sweetish sickening odor, while in gangrene of the lung the sputum is usually so foul as to require the isolation of the patient. A foul odor may also result from necrosis of malignant tumors of the bronchi (especially carcinoma) but it is a late sign more often absent than present. Sputums from ruptured subphrenic and liver abscesses may have a fecal odor, usually due to the presence of *Esch. coli* or other intestinal bacteria.

Color. The color of sputum is frequently of clinical significance and it is always advisable for the physician to inspect it instead of relying on the reports of technicians. A great variety of colors may be seen, as determined by the nature of the substances present.

When composed largely of mucus the sputum may be colorless and translucent. Mucopurulent sputums are usually whitish-yellow and sometimes streaked with green, because of the presence of *Ps. aeruginosa,* other chromogenic bacteria or old pus. In jaundice, caseous tuberculosis and slowly resolving pneumonia, the sputum may assume a uniform bright green color due to biliverdin. A dark gray or blackish sputum is usually due to anthracosis or excessive smoking, especially of cigarettes. When there is a rupture of an amebic abscess of the liver into a bronchus, the sputum has a characteristic anchovy-sauce appearance. Of particular importance is the color in relation to the presence of blood (hemoptysis). Bright red blood, most commonly in streaks, is strongly suggestive of tuberculosis. Blood from tuberculous cavities, however, is usually very dark if due to oozing with temporary retention. Blood-stained sputum is likewise common in bronchiectasis. Needless to state, blood may be derived from the nasopharynx, and tuberculous individuals frequently mistake this for blood-streaked sputum.

An orange or rusty red tenacious and scanty sputum is the rule in the early stages of pneumonia as likewise in pulmonary infarction; the color indicates decomposed hemoglobin. A similar color may be caused by iron oxides. Later the sputum frequently becomes more fluid and "prune juice" in color. This type is stated to be characteristic of "drunkard's pneumonia" and of bad prognostic import because so likely to be due to an associated pulmonary edema. A brown color, indicating altered hemoglobin, frequently results from oozing in chronic passive congestion of the lungs and especially in congestive heart failure. Other causes for hemoptysis are listed in Table 57.

Bronchial Casts. These are usually, but not always, composed of fibrin and grayish in color unless stained with hemoglobin when they are of a reddish or brown color. They vary greatly in size and are usually rolled into balls or tangled masses best detected by floating out sputum in water over a black background. They are usually small and without branching in lobar pneumonia during the latter stages, while of medium size in fibrinous or chronic plastic bronchitis. Large casts of the bronchi are rare but may occur in fibrinous bronchitis and especially in diphtheria as a result of extension of the disease into the trachea and bronchi.

Dittrich's Plugs. These consist of yellowish or gray caseous masses, usually about the size of a pin-head but sometimes larger, emitting a foul odor when crushed. Microscopically they consist of granular debris, fat globules, fatty acid crystals and bacteria. Sometimes they are expectorated alone. They are formed in the bronchi and especially in chronic bronchitis, bronchiectasis and bronchial asthma, although they are sometimes expectorated by apparently healthy individuals. The laity not infrequently regard them as evidence of tuberculosis. Caseous masses from the crypts of the tonsils may be mistaken for them.

Pneumoliths. At times during the course of chronic tuberculosis, small calcified nodules of tuberculous tissue may be expectorated and constitute the great majority of the so-called "pneumoliths." However, they may be composed of small foreign bodies remaining in the lung tissue or bronchi over long periods of time and encrusted with calcium salts which, upon ulceration, are expectorated, usually with hemorrhage.

Elastic Tissue. Since elastic fibers are normally distributed in the walls of the alveoli and bronchioles, their presence in the sputum indicates destructive disease of these structures and is consequently of very important diagnostic and prognostic significance, providing they do not come from food about the teeth, which is not an infrequent source of error. They may occur in the sputum in asbestosis, abscess and gangrene of the lungs and in ulcerating malignant tumors, but in the great majority of instances elastic fibers indicate the presence of active tuberculosis with cavitation; on rare occasions, however, they may occur in early tuberculosis before tubercle bacilli are found. However, they are not usually found in uncomplicated bronchiectasis, uncomplicated nontuberculous pneumonias, nonprogressive tuberculous cavities, or in material gaining access to the bronchi from empyema, subphrenic or liver abscess, and caseous mediastinal lymph nodes.

In asbestosis the sputum may show the presence of "asbestos bodies" formed in the alveoli by the deposition around asbestos fibers of an iron-containing silica gel, derived partly from the fibers and partly from the surrounding fluids.[9,10] Their presence indicates exposure to asbestos dust. Elastic tissue may be present, indicative of destruction of lung tissue, with or without "asbestos bodies" or clumps.[11]

Curschmann's Spirals. These peculiar structures occur as small whitish or yellow wavy threads, frequently coiled into loosely or tightly wound little balls. Their exact nature has not been definitely determined but microscopically they are usually found to be composed of twisted strands of mucus enclosing leukocytes (especially eosinophils) and sometimes Charcot-Leyden crystals. Their presence in sputum is fairly characteristic of bronchial asthma although these spirals are not present in every attack. Sometimes they can be found only near the end of a paroxysm. However, they may occasionally be met with in chronic bronchitis or other catarrhal conditions, but usually only when there is an underlying asthmatic tendency.

Eosinophils. The presence of an excess of eosinophils in the sputum of bronchial (allergic) asthma is so constant that in their absence a diagnosis of this disease is seldom justified. In "cardiac asthma" or paroxysmal nocturnal dyspnea due to left ventricular failure from hypertension, aortic stenosis or aortic insufficiency, they are almost invariably present in normal numbers.

Pigmented Cells and Myelin Globules. Granules of pigment are sometimes seen in ordinary leukocytes or pus cells, but the more common and important pigment-containing cells are large mononuclear cells whose origin is still in some doubt.

The pigment is chiefly hemosiderin. Since such cells are most frequently found in chronic passive congestion of the lungs from congestive heart failure, they are usually designated as "*heart-failure cells.*" They may also occur in pulmonary infarction, as likewise for some time after pulmonary hemorrhage; they may be so numerous as to give the sputum a brownish color.

Carbon-laden cells are less important and characteristic of anthracosis and excessive smoking. *Myelin globules* are colorless, round, oval, or pear-shaped globules of various sizes showing peculiar concentric or irregularly spiral markings. They may occur in the scanty morning sputum of apparently healthy persons, but are especially likely to occur in the mucoid sputum of bronchitis. Otherwise, however, they have little or no clinical significance except for the chances of mistaking them for more important structures, notably blastomyces.

Charcot-Leyden and Other Crystals. The sputum may contain various crystals such as those of hematoidin, cholesterol and fatty acids, especially when retained with decomposition, as in bronchiectasis and abscess of the lungs. Fatty acid crystals are regularly found in Dittrich's plugs and may be mistaken not only for elastic fibers but for clumps of *Actinomyces bovis*.

The most important, however, are the colorless, pointed and often needle-like Charcot-Leyden crystals, whose exact nature is still unknown. They seem to be in some way connected with the presence of eosinophils and are rarely present except in the sputum of bronchial asthma in which they frequently adhere to Curschmann's spirals. They may be absent when the sputum is expectorated but appear in large numbers after it has stood for some time.

Bacteriology, Mycology and Animal Parasites. Undoubtedly the most frequent examinations of sputum are for tubercle bacilli. This subject, as well as bacteriologic examinations for pneumococci, other bacteria, yeasts and molds, is discussed in Chapters 15 and 16. Various animal parasites, or their ova, may also occur in the sputum, as discussed in Chapter 12.

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10

THE CLINICAL INTERPRETATION OF EXAMINATIONS OF THE STOMACH, DUODENAL CONTENTS AND PANCREAS FUNCTION TESTS

Whether examinations of the stomach contents are worth the time involved and the discomfort given patients in their collection has been sometimes questioned, especially by surgeons, but it is the consensus that they are frequently capable of yielding valuable information bearing on the diagnosis of functional, ulcerative, neoplastic or obstructive lesions of this organ. It is true that they are not indicated or required as routine procedures, but when properly conducted in selected cases with an understanding of their sources of errors and limitations the results, while seldom pathognomonic, are frequently of diagnostic value and especially in connection with roentgenologic or other special examinations.

FUNCTIONS OF THE STOMACH

The stomach serves many useful but not necessarily vital functions in relation to digestion and nutrition (Table 58). For example, one of its chief functions is (1) *as a reservoir for foods and fluids* to avoid the need for frequent ingestion of them. This involves (2) *the function of absorption* although it has long been known that the stomach is not an important absorbing organ and cannot be relied upon to help very much when the pylorus is closed. However, while but little water is absorbed, alcohol is not only readily absorbed but facilitates the absorption of other substances as well. Furthermore, glucose and the other sugars, peptones, sodium chloride and some drugs are also absorbed to some extent.

The stomach also possesses (3) *the function of digestion* through the agency of hydrochloric acid and its enzymes (pepsin, rennin and lipase). Hydrochloric acid is produced by the parietal cells although the exact mechanism of its production is still unknown. It serves the useful function of converting pepsinogen into pepsin as well as effecting a certain degree of splitting of disaccharides. Pepsinogen is produced by the chief cells of the fundic glands and when converted into pepsin initiates the digestion of proteins, with the production of acid metaproteins followed by primary and secondary proteoses and peptones. Rennin, or the milk-clotting enzyme, which is also produced by the chief cells of the fundic glands as renninogen, in amounts closely paralleling that of pepsinogen, and converted into rennin by the hydrochloric acid, acts on casein to form paracasein and whey albumose. Its exact function is not understood although its activity is doubtless helpful in the digestion of milk and particularly in infants and adults on a milk diet. Furthermore, the existence of rennin as a separate enzyme is in doubt, since it has never been isolated; indeed, the clotting of milk may, in reality, be due to pepsin. While gastric lipase is a weak fat-splitting enzyme, it is nevertheless highly active on fats which are thoroughly emulsified, with the production of glycerol and fatty acids. It differs from the lipases of the pancreas and intestinal mucosa in being active in an acid medium, the optimum *p*H varying from 4 to 5.

TABLE 58. SUMMARY OF THE FUNCTIONS OF THE STOMACH

Functions	Mechanism
Retention	For the temporary retention of foods and liquids.
Absorption	Relatively unimportant. Little water absorbed. Alcohol absorbed more readily. Glucose and other sugars as well as sodium chloride and some drugs absorbed to a slight degree.
Digestion	(1) By the production of hydrochloric acid for the conversion of pepsinogen into pepsin and a certain degree of splitting of disaccharides. (2) By the production of pepsin for the digestion of proteins. (3) By the production of rennin for the clotting of milk and the digestion of casein although the existence of rennin as a separate enzyme is in doubt, since it has never been isolated. (4) By the production of lipase for the digestion of fats, especially those highly emulsified.
Motility	For the thorough mixing of the gastric contents and the propulsion of chyme into the duodenum where it causes the production of secretin which in turn stimulates the production of pancreatic secretion and bile for purposes of further digestion.
Production of Mucin	For (1) checking acidity; (2) protecting the gastric mucosa and (3) for checking peptic activity.
Production of the "Intrinsic" Anti-anemia Factor	For the production of erythrocytes and the prevention of pernicious anemia.
Bactericidal and Antiseptic Action	Through the bactericidal and antiseptic action of normal hydrochloric acid which renders the chyme practically but not absolutely sterile with a large measure of protection against infection from the swallowing of bacteria and other living agents of disease.

The products of this gastric digestion constitute the chyme. This is a more or less homogenous and creamy material composed not only of the products of protein digestion, including casein, but of sugars in solution from foods or derived from the partial digestion of starches, as well as the balance of the latter in a fine state of mechanical division. This chyme is passed from time to time into the duodenum which involves (4) *the motor function of the stomach.* The chyme in turn causes the production of secretion by the intestinal mucosa which, after being absorbed into the blood, stimulates the production of pancreatic secretions and bile for purposes of further digestion.

The stomach also (5) *produces mucin.* It is a product of the pyloric and cardiac glands, with a *p*H of 7.0 to 7.5 which not only serves to check acidity because of its high acid-combining power but also protects the gastric mucosa against the action of its secretions as well as serving as a check on peptic activity through its mucoitin-sulfuric acid. Wilhelmj,[1] however, has advanced the view that the intensity of the stimulus for acid secretion is itself the most important intragastric factor in the regulation of gastric acidity.

From the investigations of Castle it also appears that the stomach (6) *produces the intrinsic antianemic factor* by some change on meat (muscle), although all attempts to correlate this function with known digestive agents have so far failed. It appears, however, to be concerned in the normal production of erythrocytes, and its absence is involved in the etiology of pernicious anemia.

Finally, the stomach (7) *exerts a bactericidal or antiseptic action* through the hydrochloric acid which, when present in normal amounts, renders the chyme practically but not absolutely sterile. This in turn affords a large measure of resistance to bacteria and other living agents of disease, particularly those swallowed in raw foods and fluids, with special reference to typhoid, paratyphoid and undulant fevers, dysentery and other enteric diseases.

THE COLLECTION AND EXAMINATION OF STOMACH CONTENTS

As previously stated, the clinical value of analyses of the stomach contents has been much disputed. This is largely because of the sources of error incident to the kind of test meal given and the collection of stomach contents, with special reference to determinations of the free hydrochloric acid and total acidity under physiologic conditions. According to Bloomfield and Polland,[2] a satisfactory test meal should not only be capable of being applied under standard conditions, with similar results upon repetition of real diagnostic value, but it should also impose a load on the function to be tested.

Sources of Error. Unfortunately, there are several possible sources of error involving the accuracy and clinical value of stomach analyses (Table 59). The passage of the tube for the removal of residuum and again after the test meal involves not only an important psychic stimulation to gastric secretion, but frequently entails the swallowing of considerable saliva which, because of its alkalinity, is capable of neutralizing unknown amounts of free hydrochloric acid, in addition to that neutralized by the alkaline mucus secreted by the gastric mucosa which cannot be estimated by any method of analysis. Furthermore, the regurgitation of alkaline bile-containing duodenal contents, which is bound to occur in a small percentage of all patients, but especially in neurotic individuals as well as those with gallbladder disease, is increased by psychic disturbances, with sufficient neutralization of acid in some instances to produce a false achlorhydria. Therefore, the presence of saliva and bile in the gastric contents must be reckoned with in the clinical interpretation of acid determinations, not to mention the advisability of keeping the patient as quiet and as free as possible of anxiety, and of having the stomach contents removed by someone possessing adequate skill and experience.

Moreover, the amount of water or tea given with a test meal involves error due to dilution [3] which in turn involves the emptying time of the stomach, especially when its contents are removed one or two hours after a test meal. Furthermore, the bread, soda or arrowroot crackers, or cereals given with the meal will combine with and neutralize surprisingly large amounts of hydrochloric acid up to a certain point, following which free acid will remain in the contents, which likewise involves the important factor of emptying time of the stomach. Gross amounts of blood will also reduce acidity so that no value can be attached to free hydrochloric acid determinations in cases of actively bleeding peptic ulcers or neoplasms. And even traces of blood from trauma of the gastric mucosa or the result of swallowing may interfere with the clinical interpretation of occult blood tests and microscopic examinations.

The *kind of stomach tube* employed is also a matter of importance, as is the gentleness and skill with which it is used. The Rehfuss tube is best known but the Jutte tube is stated to be more readily passed with less psychic disturbances. Apparently one of the best is that devised by Miss Sawyer of the Mayo Clinic (V. Mueller and Company, Chicago) because the walls are of such thickness and size that no wire stylet is necessary as in the Jutte tube, nor is it necessary to use a metal tip as in the Rehfuss tube.

TABLE 59. SUMMARY OF EXAMINATIONS OF CLINICAL VALUE AND SOURCES OF ERROR IN GASTRIC ANALYSIS

Examinations	Sources of Error
Should include the following examinations of the residuum removed before, and of the stomach contents removed after, a test meal: (1) *Physical examination* for amount and color and for mucus, food remnants and tissue fragments. (2) *Chemical examinations* for free hydrochloric acid and total acidity, acid deficit, lactic acid and occult blood. (3) *For enzymes* and particularly pepsin. (4) *Microscopic examinations* (of limited value) for remnants of food from previous meals, erythrocytes, pus cells, sarcinae, yeasts and Boas-Oppler bacilli.	(1) Psychic stimulation of gastric secretion by the passage of the stomach tube. (2) Swallowing of considerable alkaline saliva. (3) Regurgitation of alkaline bile-containing duodenal contents. (4) Dilution by the fluid of the test meal. (5) Combination and neutralization of hydrochloric acid by the test meal when foods are given. (6) Reduction of acidity by blood.

Examinations of Clinical Value. At the present time it is thought advisable to examine not only the stomach contents after a test meal but also the gastric residuum removed before it has been given (Table 59). The amount of the residuum, as well as the presence or absence of retained food from the previous regular meal, or after a special meal containing easily recognizable materials (rice pudding with raisins, jam with seeds, spinach, etc.) and given six or seven hours previously, are important in relation to the motility of the stomach and especially in atony, dilatation and pyloric obstruction.

Physical examinations, therefore, are of clinical value and should include a search for particles of food and bits of tissue from the gastric mucosa or neoplasms and also determinations of the amounts and character of the mucus as well as the color in relation to bile and blood.

Undoubtedly, however, *chemical examinations* are of most importance. This is true not only in regard to qualitative tests for free hydrochloric acid, but more especially in relation to its quantitative estimation, including that of total acidity due to free and combined hydrochloric acid, organic acids and acid salts. An estimation of combined hydrochloric acid alone may be helpful but when the free acid is absent, it is probably better to estimate the *acid deficit* which shows how far the acid secreted by the stomach falls short of saturating the protein and bases of the test meal. The acid deficit represents, therefore, the amount of hydrochloric acid which must be added to the fluid before a qualitative test for free acid can be obtained. It is determined by titrating with decinormal hydrochloric acid, using dimethyl-amino-azobenzol as indicator, until the fluid assumes a red color. The amount of deficit is expressed by the number of cubic centimeters of the decinormal solution required for 100 cc. of the stomach fluid.

A determination of hydrogen ion concentration is hardly worth while for clinical purposes but qualitative tests for lactic acid should always be included, especially when hydrochloric acid is absent, since it is the most important of the organic acids which may be present from stasis, dilatation or obstruction. Acetic and butyric acids are sometimes present under the same conditions but are not tested for. The presence of butyric acid may be detected by its odor, resembling that of rancid butter, especially after heating.

Chemical tests for occult blood should always be included, as its detection by these is more reliable than microscopic examinations for erythrocytes. When found, the possibility of its presence being due to swallowed blood or blood due to injury by the stomach tube, must be carefully considered or excluded. Tests for absorptive and motor powers are sometimes required.

Examinations for the gastric enzymes also possess value. Pepsin is rarely or never absent in the presence of free hydrochloric acid, consequently the absence or marked diminution of this enzyme indicates organic disease of the stomach, since it is not influenced by neuroses or circulatory disturbances. The absence or reduction of rennin has the same significance as that of pepsin. It is more easily recognized but if an examination has been made for pepsin it is hardly worth while to test for rennin, especially since the curdling of milk may, in reality, be due to pepsin anyway. Examinations for lipase, however, possess so little clinical value as to be hardly worth while.

As a general rule, *microscopic examinations* are of limited clinical value. They include examinations for partially digested starch, remnants of food from previous meals, erythrocytes, pus cells, sarcinae, yeast cells and bacteria with special reference to Boas-Oppler bacilli.

TEST MEALS AND METHODS

Since different foods stimulate the gastric mucosa in different degrees, certain standard "test meals" have come into general use. It is customary to give them in the morning after a period of fasting.

The usual or *one-hour test meal method* is to pass the tube for the removal of the stomach residuum. The tube is then removed and the meal given because patients cannot usually swallow it with the tube *in situ*. However, some meals or other stimulating substances, like alcohol, are readily administered by injection through the tube. If, however, the matter of possible residuum is not of clinical importance, its preliminary removal may be omitted, which is particularly advisable, whenever permissible, in nervous individuals in order to reduce to a minimum sources of error due to the swallowing of saliva and the psychic stimulation of increased gastric secretion and regurgitation of bile. After the meal has been given (and thoroughly masticated), the tube is re-introduced and the stomach contents removed one hour afterward, counting from the beginning, not the end of the meal. The entire collection is then sent to the laboratory for examination.

This method, however, has several shortcomings. For example, the amounts of hydrochloric acid and total acidity found are not necessarily certain indexes of secretory activity; furthermore, considerable variations may occur not only in different normal individuals but even in the same individual at different times. These, as well as such changeable factors as the diluting effect of the meal, the degree of duodenal regurgitation and the rate of evacuation of the stomach contents, interfere with the clinical value of the test for diagnostic purposes.

Therefore, since it is now well recognized that there is great variation in the time at which the maximum of secretion of hydrochloric acid and enzymes is reached, the *fractional method* of removal has come into wide use and is generally preferred. By this method, 5 to 10 cc. of stomach contents are removed by suction at intervals of 15 minutes over a period of two to three hours. Its main disadvantage is the possible discomfort of retaining the tube for this period of time with considerable gagging and the swallowing of saliva by nervous individuals. Each specimen is sent to the laboratory for examination for the amounts of free hydrochloric acid and the total acidity which should be separately reported and charted in curves.

According to Rehfuss, the curves of free hydrochloric acid may occur *normally* as the isosecretory (37 per cent), the hypersecretory (33 per cent) or the hyposecretory (30 per cent), as shown in Figure 10. In *hyperacidity* five different curves may occur, namely, (1) the *laval* in which there is a sharp rise within the first hour with remainder normal; (2) the *digestive* which resembles the normal except that it is greatly exaggerated and prolonged; (3) the *postdigestive* in which it increases steadily throughout the entire digestive period and is maintained in the postdigestive period; (4) the *interdigestive* which may occur independently and (5) the *plateau* curve.

Recent investigations, however, have thrown considerable doubt upon the value of the method as originally conducted with the Ewald test meal. For example, specimens removed from different parts of the stomach at the same intervals may vary considerably in acidity, particularly in individuals with gastric disease. For this reason it has been suggested that just before each fifteen-minute extraction the portions be sucked into the syringe and forced back several times. Probably the best method of fractional analysis is to give a series of test meals on successive days and to remove the entire contents of the stomach on the respective days at different periods of digestion. But this is not always a practical procedure in view of the discomfort to the patient and the time involved.

The kind of meal to be given depends to some extent on individual circumstances. Possibly the best known and most frequently used is that of *Ewald,* because of its blandness, consisting of either one roll or two slices of plain or toasted wheat bread (without butter) weighing about 35 gm., with 300 to 400 cc. of water, or weak tea without cream or sugar. However, because of its blandness it provides a weak stimulus to the secretion of hydrochloric acid, with the result that it yields too high a percentage of cases of false achlorhydria,[4, 5] especially if removed one hour later. Furthermore, the use of rolls or bread introduces the possibility of their containing lactic acid and sarcinae. For this reason shredded wheat biscuit, soda crackers or arrowroot cookies are preferred. Otherwise, the *Boas meal* may be used with a preliminary stomach washing the previous evening. This meal consists of oatmeal boiled in 800 cc. of water until the volume is reduced to 400 cc. However, if retention is suspected because of atony of the stomach, dilatation or pyloric obstruction, rendering the removal and examination of the residuum particularly of interest, a special meal of easily recognizable materials should be given six or seven hours before, composed of rice pudding with currants or raisins, jam with seeds, spinach, etc.

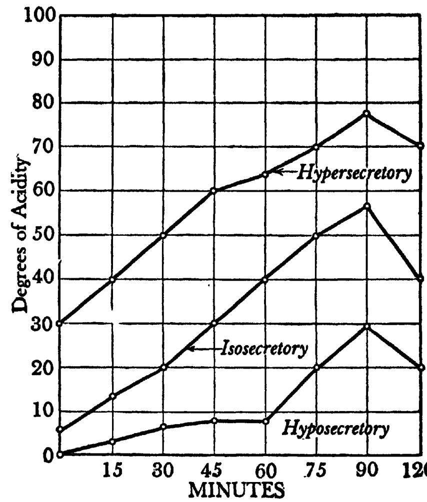

Fig. 10. Normal Free HCl Curves

But if a more stimulating meal is indicated, as when hypoacidity or achylia are suspected, the *Riegel meal* may be given. This consists of about 200 cc. of beef broth, 150 to 200 gm. of broiled beef steak (which should be thoroughly masticated) and about 100 gm. of mashed potatoes.

On the other hand, if it is desired to give a meal through the stomach tube in order to require its passage but once, the residuum may be removed by suction with the syringe and the *Heckman meal*[6] administered, which consists of 80 cc. of freshly prepared egg albumin and 130 cc. of distilled water, to which is added 2 drops of a 2 per cent solution of methylene blue, with filtration through gauze after being gradually and carefully heated to body temperature.

Although these meals may yield satisfactory results from a physiologic standpoint, there has been a great deal of discussion by gastro-enterologists as to the clinical value of the findings. The result has been an increasing tendency to study gastric function without a meal, using either a small amount of alcohol as a direct stimulant of secretion, or a small dose of histamine injected subcutaneously for the same purpose.

The *Ehrman alcohol meal*, as recommended by Bloomfield and Keefer,[7] consists of 50 cc. of 7 per cent solution of ethyl alcohol. It possesses the advantages of readiness of administration through the tube which thereby is introduced but once, ease of withdrawal, and the possibility of yielding more exact quantitative results, since there is no insoluble or a buffer-containing residue. However, it has the disadvantage of affording but little information on the motor activity of the stomach in addition to being a somewhat unphysiologic method of gastric stimulation. A modification of this alcohol meal has been proposed,[8, 9] consisting of the addition of 1 cc. of a 0.1 per cent solution of phenolphthalein in 95 per cent alcohol to 49 cc. of 7 per cent alcohol. This is believed to make possible a determination of the acidity of pure gastric juice and of the exact emptying time, the stomach contents being removed 40 minutes after the administration of the meal and sent to the laboratory for an estimation of acid by the Dunning colorimeter. But, while it is simple and rapid to perform, Reich [10] has recently reported that it yields a large percentage of error in normal individuals and those with gastrointestinal disease, while being valueless in cases with jaundice, cardiorenal disease and blood dyscrasias. Toxic reactions may occur and the test contraindicated in patients with hematemesis. On the other hand, the plain alcohol meal yields the two most essential items of information, namely, the acidity of the pure juice and the volume of gastric secretion with the advantage of being administered through the tube. The same is true of the *Lewin meal* [11] of alcohol and bouillon.[12]

The *histamine meal* is particularly of value for differentiating between true and false achylia. This meal provides an adequate and physiologic stimulus, since histamine may be not only a normal constituent of the gastric mucosa but also a gastric secretory hormone,[13] and it induces the maximum of secretion (especially of hydrochloric acid) with similar results on repeated tests. And while the histamine meal has been objected to on the basis that it does not stimulate the pepsinogen producing glands,[14, 15] yet the results observed have firmly established its many advantages,[16] particularly since it stimulates the production of hydrochloric acid more effectively than the Ewald and alcohol meals.

This test is conducted as follows: 1.—The patient is requested to fast for at least twelve hours (overnight) and to lie in bed on the back or left side.

2. The tube is passed and the gastric residuum removed. The *Ewald meal* may be then given or the test conducted without it.

3. A standard amount of histamine is injected subcutaneously in dose of 0.1 mg. per 10 kg. (22 pounds) of body weight. *A total dose of 0.25 mg. may be used satisfactorily.*[17] Each 0.1 mg. of histamine is equivalent to 0.19 mg. of histamine phosphate. As a reaction of flushing, increased pulse rate and sometimes physical discomfort develops rapidly, caution must be exercised in the selection of patients and the test should not be used routinely.

4. Gastric secretions are removed ten minutes later and subsequently at ten-minute intervals over as long a time as desired (usually one to two hours). All specimens are sent to the laboratory for measurement of their amounts and titrations for free hydrochloric acid, total acidity, etc.

However, since one negative histamine test is not conclusive evidence of a true or absolute achlorhydria, a *double histamine test* has been proposed by Rivers, Osterberg and Vansant,[18] for determining not only the maximum potentiality of acid and pepsin secretion of the gastric mucosa, but also the capacity for maintaining the increased secretory rates over relatively long periods of time.

The gastric residuum is removed as completely as possible by several aspirations at ten- or fifteen-minute intervals. Histamine (0.1 mg. per 10 kg. of body weight) is then injected subcutaneously, following which the gastric juice is aspirated for six periods of ten or fifteen minutes each. At the conclusion of the sixty or ninety minutes, a second dose of histamine similar to the first is injected and specimens again collected at ten- to fifteen-minute intervals. Normally there is an initial rise in acidity, followed by a reduction about sixty to ninety minutes after the first injection; a second injection produces a second rise. In patients with peptic ulcer the acidity is maintained at a comparatively high level.

THE NORMAL GASTRIC CONTENTS

Pure human gastric juice obtained through a fistula has been found to be a colorless fluid containing 0.40 to 0.60 per cent free hydrochloric acid, a total acidity of 0.45 to 0.60 per cent, organic solids (including mucin and the various enzymes) 0.42 to 0.46 per cent, and inorganic solids 0.13 to 0.14 per cent, with a specific gravity varying from 1.006 to 1.009. Total nitrogen varies from 0.051 to 0.075 per cent. In other words, gastric juice is composed essentially of hydrochloric acid, mucin, enzymes and inorganic salts.

The Gastric Residuum. The normal gastric residuum obtained by the stomach tube during an interdigestive period usually varies in *amount* from 20 to 100 cc., with an average of 30 to 50 cc. (Table 60). It may be slightly yellow or green in *color* due to the regurgitation of small amounts of bile. It is fluid in consistency and contains only a small amount of ropy *mucus,* derived from the stomach and nasopharynx, but normally contains no solid food particles. The *free hydrochloric acid,* expressed in terms of the number of cubic centimeters of decinormal sodium hydroxide solution required for the neutralization of 100 cc., usually varies from 0 to 30 degrees (average 18.5) or from 0 to 0.1095 per cent (average 0.0675 per cent). *Total acidity* due to hydrochloric acid, hydrochloric acid combined with protein and acid salts (phosphates and carbonates), and possible traces of organic acids, varies from 10 to 50 degrees or from 0.0365 to 0.1825 per cent (average 0.1095 per cent). *Organic acids* (lactic, butyric and other volatile fatty acids) are not normally present. The same is true of *blood* unless it has been swallowed or emanates from trauma by the stomach tube. *Enzymes* (pepsin, rennin and lipase) are present. Abnormal changes are summarized in Table 60.

TABLE 60. SUMMARY OF THE CLINICAL INTERPRETATION OF THE GASTRIC RESIDUUM

Constituents	Normal	Abnormal
Amount and Color	20 to 100 cc. (average 30 to 50 cc). May be slightly yellow or green due to a trace of bile.	Less than 20 cc. indicative of increased motor activity of the stomach or incomplete removal of contents. More than 100 cc. usually indicative of retention due to decreased motor activity, pyloric obstruction, or excessive secretions.
Mucus	A small amount.	Increased by gagging and swallowing of mucus. Increased in chronic gastritis.
Food	Absent.	Presence indicative of decreased motility of the stomach or pyloric obstruction.
Free HCl	0 to 30 degrees (average 18.5) or 0 to 0.1095 per cent (average 0.0675).	Increased in states producing hyperchlorhydria. Decreased in states producing hypochlorhydria.
Total Acidity	10 to 50 degrees or 0.0365 to 0.1825 per cent (average 0.1095).	
Lactic and Other Organic Acids	Absent.	Presence indicative of stagnation of the stomach contents with fermentation; usually associated with hypochlorhydria in carcinoma, atrophic gastritis, pyloric obstruction, etc.
Blood	Absent unless due to the swallowing of blood, trauma from the tube or the presence of blood in meat (Riegel test meal).	Acid in reaction, dark red ("coffee-ground") in color and usually mixed with mucus. May be present in carcinoma, peptic ulcer, acute gastritis, purpura haemorrhagica, acute leukemia, aplastic anemia, agranulocytosis, etc.
Enzymes	Pepsin, rennin and lipase usually present; trypsin frequently present due to regurgitation of duodenal contents. Pepsin may be absent in achlorhydria.	Pepsin and rennin absent in true achylia as in pernicious anemia and subacute combined degeneration of the spinal cord. Diminished in states producing hypochlorhydria.

The Gastric Contents after the One-Hour Ewald Test Meal. Stomach contents removed one hour after the Ewald test meal, oi one of its modifications, usually vary in *amount* from 50 to 100 cc. On standing, an upper layer of almost clear and faintly yellow fluid (due to traces of bile) forms, with a lower layer of partially digested food. A small amount of *mucus* is present and easily recognized by its ropy character when the fluid is passed from one vessel into another.

The reaction is normally acid but the amounts of *free hydrochloric acid* vary considerably in relation to age and sex. Thus, Vansant and her colleagues [19] have observed that free hydrochloric acid increases rapidly from childhood up to the age of 20 years when adult values are obtained. In men this generally varies from 45 to 66 degrees, declining to 30 to 56 degrees after the age of 65 years. In women the average generally varies from 35 to 51 degrees throughout adult life so that in general terms it may be stated that the free hydrochloric acid varies from 25 to 50 degrees or from 0.1 to 0.2 per cent for adults of either sex, being slightly higher in men than women (Fig. 10).

Naturally, *total acidity* also shows similar variations due to age and sex but averages 50 to 100 degrees or 0.2 to 0.3 per cent for adults of either sex. The presence of free hydrochloric acid always presupposes a normal amount of *combined hydrochloric acid*. When, however, free acid is absent, it is important to ascertain whether any acid is secreted, and an estimation of the combined acid or acid deficit then becomes of great value. Normally, the combined hydrochloric acid varies from about 10 to 15 degrees, the quantity depending upon the amount of protein in the test meal, being somewhat higher after the Riegel meal.

In this connection it is to be remembered, therefore, that hydrochloric acid may exist in the stomach not only in a free state and in combination with the proteins of foods, but also as salts of hydrochloric acid (*total chloride*) which are neutral in reaction. For this reason various investigators have considered the curve of total chloride as being more nearly representative of the true state of secretion of hydrochloric acid. During interdigestive periods the total chloride (in terms of Cl) is about 40 per cent higher than that of the blood plasma, averaging about 500 mg. per 100 cc. of residuum. Variable amounts occur in different normal individuals but the variation is not as great as in the case of free hydrochloric acid; furthermore, chloride is more constant than the latter in the same individual at different times. It is slightly increased during digestion, reaching from 550 to 600 mg. per 100 cc., and may continue to rise after the acid curve has begun to fall. This is due to the continued excretion of HCl with neutralization and is particularly common in patients with excessive regurgitation from the duodenum.

The estimation of total chloride in the gastric contents is, therefore, particularly valuable in differentiation between true and false achlorhydria, being low (200 to 300 mg.) in the former and usually normal in the latter.

Lactic acid is absent unless introduced with the food; for this reason an Ewald meal made up with arrowroot cookies or shredded wheat biscuit is preferred to bread. Although lactic acid is often present early in digestion, it disappears when free hydrochloric acid begins to appear. *Blood* is not present unless swallowed or due to trauma from the tube, although the possibility of traces due to its presence in the meat of the Riegel meal must be kept in mind. *Enzymes* (pepsin and rennin) are usually present unless hydrochloric acid is absent; lipase is also normally present.

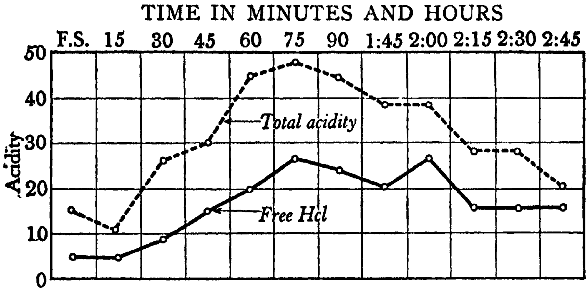

FIG. 11. FREE HYDROCHLORIC ACID AND TOTAL ACIDITY BY THE FRACTIONAL METHOD OF ANALYSIS AFTER THE EWALD TEST MEAL

The Gastric Contents in the Ewald Test Meal by the Fractional Method of Analysis. The results of analysis by this method are likewise influenced by age and sex. In the case of adults of either sex, however, both the free hydrochloric acid and total acidity normally reach their maximum in sixty to ninety minutes, as shown in Figure 11. As previously stated, recent investigations have thrown doubt upon its clinical value because specimens removed from different parts of the stomach at the same time may differ considerably, especially in individuals with gastric disease. For this reason it has been suggested that before the removal of each fifteen-minute specimen the contents be mixed by sucking a portion into the syringe, and forcing it back into the stomach.

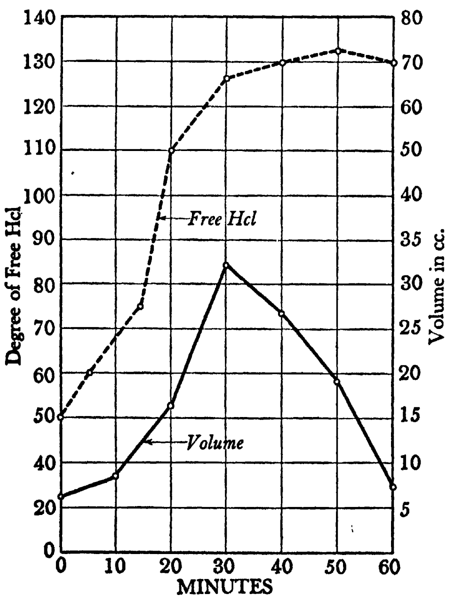

FIG. 12. FREE HYDROCHLORIC ACID AND VOLUME OF GASTRIC JUICE IN THE HISTAMINE TEST WITHOUT A MEAL

The Gastric Contents in the Histamine Test. Widely variable results have been observed in normal individuals.[2, 20] Thus, when conducted without a meal the maximum ten-minute volume of gastric juice may vary from 6 to as much as 35 cc. (Fig. 12) and even reach as high as 70 cc. The free hydrochloric acid generally reaches the maximum in about thirty minutes and usually continues at this level for an hour. Age and sex, however, have considerable influence. Thus, the maximum total acidity in normal adult men averages about 100 degrees at 25 years of age, with a progressive decline to about 67 degrees at the age of 65 years. Women of 25 years show about 82 degrees, declining to about 67 degrees at 65 years of age.

CHANGES IN THE GASTRIC CONTENTS

A great deal of discussion has been devoted to the diagnostic value of gastric analyses. Apparently not a few surgeons are of the opinion that the procedure is hardly worth the time and trouble involved. But this opinion is by no means shared by gastro-enterologists. Probably all agree that the results are seldom pathognomonic of any particular disease; also that gastric analysis should not be resorted to as a routine procedure but employed only when the information to be gained is likely to be of real diagnostic value. But this is apt to be the case in many individuals presenting signs and symptoms of aberration of the digestive functions due to gastric disease as well as in some with extragastric diseases involving these functions.

Undoubtedly the excretion of hydrochloric acid is of most clinical importance. But there is no better example of the vagaries of gastric analyses than in this regard, since the normal may vary all the way from achlorhydria to hyperchlorhydria. For example, from 2 to 3 per cent of healthy men and from 6 to 7 per cent of healthy women between the ages of 20 and 40 years have been found to show achlorhydria, the percentages increasing with age up to 60 years in both sexes.[21, 22] Furthermore, it is commonly thought that hyperacidity is characteristic of gastric ulcer whereas this change is less frequent than in duodenal ulcer; indeed, in gastric ulcer the acidity may be not only within normal but actually below normal (hypo-acidity) in a high percentage of cases (Table 61).

The term *achlorhydria,* therefore, is applied to the absence of free hydrochloric acid and is synonymous with anacidity. It may be "true," in which case there is no secretion of hydrochloric acid even after histamine stimulation, or "false" due to the low excretion of hydrochloric acid with its combination with foods or neutralization by duodenal regurgitation. False achlorhydria, on the other hand, may be (1) relative, in which HCl is not secreted after the Ewald or other meals but is produced by histamine stimulation; (2) transient, when due to fever or other causes holding its secretion in abeyance, or (3) basal, in which no acid is produced under fasting basal conditions but is secreted during a meal. The phrase "*achylia gastrica*" means the complete absence of all gastric juice, including the secretion of pepsinogen and rennin, and is quite rare. For this reason the terms achlorhydria and *anacidity* are to be preferred. The term *hypo-acidity* is applied to acid curves which are lower than normal or lower than the hyposecretory type of curve in fractional analysis.

Recently, however, Winkelstein [23] has advised dropping the terms "anacidity" and "achylia gastrica" and using only "false" or "true" achlorhydria, the latter when it is complete and more or less permanent with a *p*H of 3.5 or over. In his opinion, true achlorhydria is best determined by a combination test employing

TABLE 61. SUMMARY OF THE CLINICAL INTERPRETATION OF ANALYSIS OF THE STOMACH CONTENTS

<table>
<tr><th>Constituents</th><th>Normal</th><th>Abnormal</th></tr>
<tr><td>Amount, Color and Mucus</td><td>(1) One hour after the Ewald test meal: 50 to 100 cc. After standing upper layer almost clear with slight yellow color. Small amount of mucus.
(2) After histamine stimulation without a test meal: maximum ten-minute volume from 6 to 35 cc. but may be as high as 70 cc. (Fig. 11).</td><td>Amount increased with retention of food by deficient motor activity of the stomach as in chronic gastritis with atony, chronic dilatation, pyloric obstruction, in some cases of neuroses (gastro-succorrhea), etc.
Mucus increased in chronic gastritis (very significant).
Large amounts of bile rare; indicates increased intraduodenal tension with a patent pylorus.</td></tr>
<tr><td>Free HCl</td><td>Varies considerably according to age and sex.
General average 25 to 50 degrees (0.1 to 0.2 per cent) in adult men and women (Ewald test meal).
Maximum reached in 60 to 90 minutes by fractional method of analysis (Fig. 10).
After histamine stimulation maximum usually reached in about 30 minutes, continuing at this level for at least an hour (Fig. 11).</td><td rowspan="3">(1) Hyperchlorhydria: May occur in normal individuals.
Duodenal ulcer (usual)
Gastric ulcer (less frequent)
Cholelithiasis (some cases)
Cholecystitis (some cases)
Excessive smoking
(2) Hypochlorhydria and hypoacidity: False hypochlorhydria in 2 to 3 per cent of normal men and 6 to 7 per cent of women between 20 and 40 years of age; percentages increase with age in both sexes. True hypochlorhydria in normal individuals of both sexes and all ages not over 1 to 2 per cent.
Carcinoma of stomach and sometimes in carcinoma elsewhere.
Gastric ulcer (some cases)
Gastric syphilis
Chronic gastritis
Gastrogenous diarrhea; sprue
Duodenitis
Visceroptosis
Spastic and mucous colitis
Chronic constipation
Chronic appendicitis
Cholecystitis (some cases)
Cholelithiasis (some cases)
Pernicious anemia
Severe secondary microcytic anemias
Combined lateral sclerosis
Pregnancy (about 75 per cent)
Chronic alcoholism
Cardiovascular disease (some cases)
Oral sepsis (frequent)</td></tr>
<tr><td>Total Acidity</td><td>Varies considerably according to age and sex.
Averages 50 to 100 degrees (0.2 to 0.3 per cent) in adult men and women (Ewald test meal).
Maximum reached in 60 to 90 minutes by the fractional method of analysis (Fig. 10).
After histamine stimulation maximum in men 67 to 100 degrees and in women 67 to 82 degrees.</td></tr>
<tr><td>Combined HCl</td><td>Variable according to the amount of protein in the test meal. Averages 10 to 15 degrees (Ewald test meal); higher in Riegel test meal.</td></tr>
</table>

TABLE 61. SUMMARY OF THE CLINICAL INTERPRETATION OF ANALYSIS OF THE STOMACH CONTENTS—(Continued)

Constituents	Normal	Abnormal
		Psychoneuroses Hyperthyroidism (some cases) Adrenal insufficiency Diabetes mellitus (some cases) Pulmonary tuberculosis (some cases) Chronic arthritis (some cases) (3) *Anacidity:* Pernicious anemia Severe secondary microcytic anemias Combined lateral sclerosis Chronic gastritis (some cases) Chronic duodenitis (some cases) Spastic colitis and sprue (some cases) Visceroptosis (some cases)
Total Chloride	*Residuum* about 500 mg. per 100 cc. (in terms of Cl). *Height of digestion* about 550 to 600 mg. Varies in different individuals but more constant than free HCl in the same individual at different times.	During digestion reduced in true achylia (200 to 300 mg.); usually normal in false achylia.
Lactic and Other Organic Acids	Absent unless introduced with food in the test meal.	Usually *present* in states of marked stagnation with bacterial fermentation as in carcinoma, pyloric obstruction from other causes, chronic gastritis and chronic dilatation, etc. Practically none if stomach is washed out 12 hours before test.
Blood	Not present unless swallowed, due to trauma by the stomach tube or the presence of blood in meat of the Riegel test meal.	May be *present* in gastric and duodenal ulcers; ulcerating carcinoma; acute gastritis with severe vomiting, etc.
Enzymes	Pepsin and rennin present unless hydrochloric acid is absent; lipase usually present.	Usually *absent* in pernicious anemia and combined lateral sclerosis. Frequently *reduced* in chronic gastritis, chronic dilatation and chronic duodenitis.
Bacteria, Sarcinae and Yeasts	Some bacteria but usually no Boas-Oppler bacilli.	Boas-Oppler bacilli, other bacteria, sarcinae and yeasts usual when lactic and other organic acids are present from stagnation, carcinoma, pyloric obstruction, etc.

oatmeal gruel, histamine, and neutral red; with this procedure the incidence unassociated with organic disease was found to be very low (1.2 per cent) and therefore rare in normal individuals of both sexes, regardless of age.

Furthermore, the large number of gastric and extragastric diseases in which hyperchlorhydria may be observed is another example of the difficulties encountered in clinical interpretation. And this is even more in evidence in the case of achlorhydria and hypo-acidity (Table 61). Under these conditions, the results of gastric analysis should be interpreted in connection with all other data; when this is done in relation to the secretion of hydrochloric acid in addition to the results of other examinations (Table 61), gastric analyses are usually well worth the time and effort involved on the part of the physician as well as the discomfort and inconvenience on the part of the patient.

EXAMINATIONS OF THE DUODENAL CONTENTS

The fasting duodenal contents removed before the instillation of magnesium sulfate usually amount to about 20 cc. and are normally composed of pancreatic and intestinal secretions, a small amount of bile and some gastric contents when the latter has not been effectively excluded. From the clinical standpoint, an analysis of the duodenal contents is ordinarily limited to macroscopic and microscopic examinations, but examinations for the pancreatic enzymes (trypsin, amylase and lipase) are of great clinical value in the diagnosis of chronic pancreatitis (achylia pancreatica) and occlusion of the duct of Wirsung as well as examinations for bile in relation to cholecystitis and particularly cholelithiasis.

Examinations for mucus, pus, crystals, bacteria and parasites likewise possess diagnostic value. For example, the occurrence of considerable mucus and pus is evidence of catarrhal duodenitis or inflammation of the galltract. Further differentiation depends upon the morphology of the epithelial cells, as previously discussed in Chapter 8. Cuboidal or oval cells containing a single nucleus point to acute duodenitis or to chronic duodenitis if they are hyaline or otherwise degenerated. On the other hand, layers of bile-stained, simple, tall, columnar epithelial cells indicate a possible cholecystitis, while bile-stained, short, columnar cells point to cholangitis. *Strongyloides stercoralis, Giardia lamblia, Clonorchis sinensis* or other parasites may be found, sometimes in large numbers, as well as cystic and vegetative forms of *Endamoeba histolytica,* which indicate infestments of the liver or biliary ducts or both.

Examinations for the pancreatic enzymes are probably not as definite as of feces in the detection of achylia pancreatica but are invariably helpful. For this purpose the simplified technic of Lueders [24] may be employed although the methods of Agren and Lagerlöf [25] and Willstatter [26] are recommended for the determination of amylase; the method of Cherry and Crandall [27] for lipase and the methods of Agren and Lagerlöf,[25] McClure [28] and Northrup and Kunitz [29] for trypsin. Most difficulty is experienced with an estimation of amylase because of the chances it was swallowed in saliva (ptyalin) during collection.

The presence of bile in the fasting duodenal contents, however, is clinical evidence against complete obstruction of the common bile duct. Despite repeated stimuli there may be a delay of many hours before the flow of bile is established. This may be due to dislodgment of a stone and overcoming spasm of the sphincter of Oddi, or to a reduction of edema of the ducts sufficient to cause their complete or partial occlusion. However, absence of bile does not mean that it fails to reach the duodenum at any time. Usually there is sufficient unaltered bile pigment to give the duodenal contents a tinge of yellow under normal conditions. A small amount of urobilin (a reduction product of bilirubin) is likewise normal but the chromogen (urobilinogen), is found only when urobilin is present in marked excess. Under the circumstances an excess of either or both has the same significance as their increase in the feces, being indicative of increased blood destruction in the hemolytic anemias or from the other causes.

Complete occlusion of the duct of Wirsung results in no loss of fluid or of electrolytes in the pancreatic juice, with wide variations in ability to digest fats and metabolize sugars and starches; under these conditions, the succus entericus may be capable of carrying on the digestive action of the pancreatic juice. Furthermore, examinations of the duodenal contents may be of great clinical value in differential diagnosis between carcinoma of the ampulla of Vater, lower end of the common bile duct and head of the pancreas and obstruction of the common bile duct by calculi. Thus, in carcinoma of the ampulla of Vater, blood is commonly found along with little or no bile and pancreatic enzymes; in carcinoma of the common bile duct, pancreatic enzymes are present but bile is absent, and in carcinoma of the head of the pancreas, bile and pancreatic enzymes are diminished or absent, while in obstruction of the common bile duct by calculi some bile and cholesterol crystals are commonly found and a reduction of pancreatic enzymes is seldom observed.

An examination of the duodenal contents, therefore, may be of practical value not only in relation to duodenitis, the diagnosis of galltract disease (especially of cholelithiasis) and of increased blood destruction, but likewise in relation to the detection of achylia pancreatica due to pancreatitis, carcinoma or other causes.

FUNCTIONS OF THE PANCREAS

The chief and apparently sole functions of the pancreas are in connection with digestion and metabolism through its internal and external secretions (Table 62). The internal secretion, insulin, produced by the islands of Langerhans and absorbed directly into the blood, plays an important part in the metabolism of carbohydrates, as discussed in Chapter 3. The external secretion provides trypsinogen, rennin, traces of erepsin, amylase, small amounts of maltase and lipase for the digestion of proteins, starches and fats, respectively. It is strongly alkaline in reaction and contains a small amount of coagulable protein as well as inorganic constituents, with special reference to sodium carbonate and chlorides.

The trypsinogen, however, is very feeble in proteolytic activity until converted into trypsin by enterokinase. Trypsin differs from pepsin in being active in an alkaline instead of an acid medium. It carries the digestion of proteins to the stage of peptids, the full cycle being completed by erepsin of the succus entericus; a small amount of erepsin also occurs in pancreatic juice. Pancreatic rennin has an action similar to that of gastric rennin but, as in the case of the latter, it may not be a separate enzyme at all.

TABLE 62. SUMMARY OF THE FUNCTIONS OF THE PANCREAS

Functions	Impairment
The production of insulin.	A deficiency may result in diabetes mellitus. Glycosuria, hyperglycemia and reduced sugar tolerance occur in about 35 per cent of cases of acute pancreatic disease.
The production of trypsinogen, rennin and traces of erepsin.	Creatorrhea or excessive amounts of undigested meat fibers in the feces may occur in chronic pancreatitis, carcinoma of the pancreas, etc. Azotorrhea (25 per cent or more of the nitrogen of food in the feces) is of frequent occurrence in chronic pancreatitis.
The production of amylase and small amounts of maltase. Amylase of the blood is partly of pancreatic origin.	Indigestion of starches may occur in chronic pancreatitis and other diseases producing pancreatic dysfunction. An increase of blood and urine amylase is of frequent occurrence in acute pancreatitis. The production of a glyco-nucleo-protein in the urine, giving the Cammidge reaction, may occur in acute and subacute pancreatitis.
The production of lipase (steapsin).	Indigestion of fats with an increase of total fat, neutral fat, soap fat and fatty acids in the feces (pancreatic steatorrhea) may occur in chronic pancreatitis, carcinoma, etc. An increase of serum lipase is frequent in acute pancreatitis and carcinoma of the pancreas.

Amylase is far more active than ptyalin (salivary amylase) in the hydrolysis of starch, most of which is converted into maltase. Small amounts of glucose are also formed due to traces of maltase in the pancreatic juice. It is important to remember in connection with infant feeding, that amylase is absent from the pancreatic juice during the first few weeks after birth with but very little provision for digestion at this time of any food other than milk. The amylase or diastase of the blood appears to be partly if not entirely due to the absorption of pancreatic amylase from the intestinal tract.

Fat is not digested to any significant degree until it reaches the intestine. There the lipase of the pancreatic juice splits the molecule into fatty acids and glycerol. Some of the fattv acids in turn combine with alkali to form soaps which emulsify fats and thereby expose a larger surface area of them to enzymic activity. The bile salts have a similar and more important effect upon the emulsification process and thereby greatly enhance the fat-splitting action of steapsin.

The complete loss of pancreatic juice in dogs by the establishment of a fistula results in death in from seven to eight days, due in part to loss of plasma chloride, bicarbonate and water with consequent dehydration, azotemia and, possibly, fatty infiltration of the liver. However, death does not appear to be due solely to inanition, since from 70 to 80 per cent of fat, 60 per cent of protein and 20 to 40 per cent of starch is digested; this suggests that the loss of some material essential to life other than salts and water is the cause of death.

In complete occlusion of the pancreatic ducts (edema), however, there is no dehydration or loss of electrolytes. Wide variations in ability to digest fats and metabolize sugars and starches have been observed; under these conditions it is probable that the succus entericus may be capable of carrying on the digestive action of the pancreatic juice.

LABORATORY EXAMINATIONS OF CLINICAL VALUE IN RELATION TO THE PANCREAS

In suspected disease of the pancreas, therefore, examinations of the feces for excessive amounts of undigested meat fibers (*creatorrhea*) is of considerable clinical value (Table 63). The same is true of examinations of the feces for total nitrogen, since the presence of 25 per cent or more of the nitrogen ingested in foods (*azotorrhea*) is of frequent occurrence in chronic pancreatitis. But of greater clinical value is an examination of the feces for the total lipids, since an increase of them (*steatorrhea*) is usual in achylia pancreatica due to chronic pancreatitis, extensive carcinoma of the pancreas, calculus obstruction of the ducts, etc., as described in Chapter 11.

TABLE 63. SUMMARY OF THE PANCREATIC JUICE AND PANCREAS FUNCTION TESTS

Tests	Normal	Impairment
Pancreatic Juice	Produced by the acinar cells; secretin stimulates production of fluids and salt; concentration of proenzymes controlled by the vagi. Colorless; contains a small amount of coagulable protein and various inorganic salts (especially sodium carbonate and chlorides); alkaline (*p*H 7.1–8.2).	Complete loss in dogs results in death; due in part to loss of plasma chloride, bicarbonate and water resulting in dehydration, azotemia and, possibly, fatty infiltration of the liver. In complete pancreatic duct occlusion, however, there is no dehydration or loss of electrolytes; wide variation in ability to digest fats and metabolize sugars and starches; the succus entericus may be capable of carrying on the digestive action of the pancreatic juice.

TABLE 63. SUMMARY OF THE PANCREATIC JUICE AND PANCREAS FUNCTION TESTS (continued)

Tests	Normal	Impairment
Secretion Test	Volume of duodenal drainage increases with paler color and disappearance of bile. 135–250 cc. in one hour. Total bicarbonate: 90–130 M.Eq. Amylase: about 300–1200 units in one hour. Trypsin: about 20–40 units in one hour. Lipase: 7000–14,000 units in one hour. Regurgitation of duodenal contents into the stomach may occur, especially in achylia gastrica.	Deeply bile stained duodenal contents suggest that the gallbladder is absent, diseased or nonfunctioning. Decrease of volume and excretion of amylase and lipase earliest changes followed by decrease of trypsin; bicarbonate less affected. Decrease in volume, bicarbonate and enzymes in obstruction of ducts and extensive destruction of pancreas (necrosis, carcinoma or fibrosis). Acute yellow atrophy of liver. Carcinoma of pancreas. Cholelithiasis (some cases). Cirrhosis of liver (some cases). Diabetes mellitus (some cases). Edema of pancreatic ducts. Hemochromatosis (some cases). Pancreatitis (acute and chronic). Pancreatic cysts. Pancreatic steatorrhea; no changes in sprue (tropical, nontropical and celiac disease). Syphilis (late); some cases.
Schmidt Tests	Based on assumption that cell nuclei are digested only by trypsin. Normally the nuclei are digested in eight to ten hours in standard diet test.	Absence of digestion indicative of pancreatic dysfunction. Nuclei, however, may be digested in thirty hours in the complete absence of trypsin. Indigestion of muscle and fats (steatorrhea) in standard diet test.
Beazell Test	Based on the determination of fecal lipids and nitrogen in standard balanced diet before and after the administration of pancreatin. Daily fecal lipids: 7–10 gm. Daily fecal nitrogen: 1.5–2.3 gm.	In *achylia pancreatica* lipids vary from 44.5 to 94.0 gm. and nitrogen from 4.15 to 13.3 gm. before the administration of pancreatin; during its administration lipids vary from 21.1 to 32.7 gm. and nitrogen from 1.57 to 3.7 gm.

However, a chemical differentiation of the lipids into their various kinds (neutral fat, soap fat and fatty acids) is of limited clinical value.

Furthermore, examinations for insulin deficiency are frequently helpful, since glycosuria, hyperglycemia and diminished sugar tolerance may be found in about 35 per cent of cases with acute pancreatic lesions due to involvement of the islands of Langerhans, although but seldom observed in chronic pancreatic disease. Since the starch-splitting enzyme of the blood (amylase or diastase) appears to be partly of pancreatic origin, the method proposed by Elman [30] for the quantitative determination of this enzyme in the blood has proved of value, particularly in acute pancreatitis. Indeed, a normal blood amylase is stated to be important evidence against the existence of pancreatitis as a cause of acute abdominal symptoms, since an increase has not been found in conditions other than pancreatic disease. Available data, however, are too limited to permit an expression of its value in the diagnosis of chronic pancreatitis, carcinoma or other destructive diseases of the pancreas.[31-33]

A quantitative determination of the amylase (diastase) in the urine is also of value in the recognition of acute pancreatitis. The normal varies from 10 to 25 diastatic units in the total twenty-four hour urine but in acute pancreatitis this may be as high as 100 units or more The cause of increased blood and urine amylase is an open question, although it is thought (on unsatisfactory evidence) that the inflammatory process obstructs the finer pancreatic ducts which forces the enzyme into the blood. Markowitz and Hough [34] have found, for example, that although a reduction in blood amylase occurred after pancreatectomy, it gradually returned to normal after some weeks. Normal values, however, do not invariably exclude pancreatitis, since they may be observed when rapid and extensive necrosis of acinar cells has occurred. Furthermore, while an increase is indicative of pancreatic disease when disease of the salivary glands and, in the case of serum amylase when renal insufficiency are excluded, the increase may be primary or secondary to or associated with stone of the common bile duct, tumor of the duct, stomach or duodenum, hepatic disease or duodenal ulcer.

It may be also stated that a large proportion of patients with acute and subacute pancreatitis show in the urine some substance (believed to be in the nature of a glyco-nucleoprotein) which, upon hydrolysis, yields a pentose capable of forming an osazone (*Cammidge reaction*). However, careful studies reported by many investigators have not substantiated the clinical value of the test, although a strongly positive reaction may aid in establishing the diagnosis of acute or subacute degenerative lesions of the pancreas in the presence of clinical signs and symptoms.

PANCREAS FUNCTION TESTS

Quantitative analysis of the pancreatic juice affords the most accurate method for determining dysfunction of the pancreas (Table 63). Difficulties in obtaining the juice suitable for these purposes have been overcome by the *secretin test,* consisting in the intravenous injection of a purified secretin as a secretory stimulant, and the collection of the juice by duodenal drainage employing a double-lumen gastroduodenal tube.[35] This test may be conducted by passing the long end of the tube into the third portion of the duodenum, in which position the shorter end (10 inches shorter) is in the stomach. Continuous gentle suction is then applied, the negative pressure not exceeding 50 mm. of mercury. The gastric juice and duodenal contents (a mixture principally of pancreatic juice and usually some bile) are collected simultaneously. Usually after about twenty to twenty-five minutes the duodenal juice becomes clear and is no longer contaminated with gastric juice. At that time secretin is injected intravenously in dose of about 0.75 mg. per kilogram of weight, and collection continued for one hour.[35] Subsequent collections may be separated into ten- and twenty-minute samples.

Examinations of the feces for the presence or absence of pancreatic enzymes are of no practical value. Examinations for evidence of inefficient digestion of muscle fibers (especially of their nuclei), lipids and nitrogen (before and after the administration of pancreatin), however, are of clinical value.

The *Schmidt nuclei test* consists in the administration, with a meal, of a 0.5 gm. cube of beef or, better, of thymus, tied in a little gauze bag. The meat must previously have been hardened in alcohol and well washed in water. When the bag appears in the feces it is opened and its contents examined microscopically. If the nuclei are for the most part undigested, pancreatic insufficiency may be assumed, since it is probable that nuclei can be digested only by the trypsin of the pancreatic juice. Normally the nuclei are digested, provided the time of passage through the gastro-intestinal tract is not less than eight or ten hours. However, if the time exceeds thirty hours, nuclei may be partially digested in the complete absence of pancreatic trypsin.

The *standard Schmidt diet* may be employed not only for the determination of the digestion of protein (muscle and nuclei) but for starch and fats as well. The determination of fat by chemical analysis and of muscle for digestion by microscopic examination are of most value. The diet is given for three days with carmine (0.3 gm. in a capsule) at the beginning to identify the feces. The morning after the diet is ended charcoal (15 gm.) is given with a breakfast consisting entirely of milk. After the beginning of the test diet, the first stool colored red is saved and examined, as are all succeeding ones, until the appearance of the first stool colored with charcoal, which is rejected. The diet is as follows:

Mornings: 0.5 liter of milk with 50 gm. zwieback.
Forenoons: Oatmeal gruel (40 gm. rolled oats, 10 gm. butter, 200 cc. milk, 300 cc. water and 1 egg, and salted to taste—strain).
Noons: 125 gm. finely chopped roast beef lightly broiled *so that the interior remains uncooked.* In addition, 250 cc. potato purée (potato 190 gm., milk 100 cc., butter 10 gm., salted to taste).
Afternoons: As mornings.
Evenings: As forenoons.

Excluding cases of steatorrhea due to the absence of bile from the intestine, most of the cases studied by Pratt[36] with this diet were proved to be sprue or pancreatic disease.

Recently, Beazell, Schmidt and Ivy[37] have advised placing the patient on a standard balanced diet supplying 64 gm. of protein (10.3 gm. of nitrogen) and 112 gm. of fat daily for a period of six days. Carmine (5 grains) is given with the first meal, again with breakfast on the fourth day and finally with the first meal after the standard diet has been discontinued. Starting with breakfast on the fourth day, the patient is given with each meal 8.0 gm. of potent pancreatin in the form of enteric-coated tablets. At the time of the appearance of the first dose of carmine in the stool, collection of the total fecal output is started and continued until the last dose of carmine makes its appearance. The carmine given on the fourth day serves to separate the control period from the enzyme period. Immediately after passage, the feces are transferred to an airtight bottle containing 5 cc. of toluene and stored in a refrigerator. Each day the total fecal output for the preceding twenty-four hours is weighed, thoroughly mixed and sampled for chemical determinations. The samples for each period (control and enzyme) are pooled and the determinations are carried out on the composite samples. The samples for the nitrogen determination are suspended in sulfuric acid as recommended by Peters and Van Slyke; samples for the determination of fat are preserved in alcohol, which is then used for the initial lipid extraction. Nitrogen is determined by the Kjeldahl method and fat by a modification of Saxson's method. All determinations are made in duplicate. No information of definite diagnostic value is obtained by determining the ratio of undigested fat (neutral fat) to digested fat (soaps and fatty acids), as it has been shown that even in the absence of pancreatic digestion the ratio may be normal.

Normally, the average daily fecal nitrogen varies from 1.5 to 2.3 gm. and the lipids from 7 to 10 gm. In a series of four cases of achylia pancreatica Beazell and his colleagues found the nitrogen to vary from 4.15 to 13.30 gm. and the lipids from 44.5 to 94.0 gm. during the control period before the administration of pancreatin. During the period in which the enzyme was given, the nitrogen varied from 1.57 to 3.7 gm. and the lipids from 21.1 to 32.7 gm. In other words, this method reveals the excess of both nitrogen and fats in the feces in achylia pancreatica, with increased absorption of both when enzyme therapy is instituted.

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11

THE CLINICAL INTERPRETATION OF EXAMINATIONS OF THE FECES

An examination of the feces is commonly limited to a search for intestinal parasites or their ova, or for pathogenic bacteria. The former is considered in Chapter 12 and the latter in Chapter 15. Much of clinical value can be learned, however, by physical examinations with reference to amount, form and consistency, color, odor and reaction, mucus, etc.; also by chemical examinations for occult blood, bilirubin and urobilinogen, fats and nitrogen, as well as by microscopic examinations for remnants of food, cellular exudates, erythrocytes, etc., discussed herewith.

FORMATION

The feces are normally composed of food residues, material secreted through the wall of the intestine and in the bile, leukocytes, desquamated epithelial cells and bacteria. The contents of the ileum are almost liquid, with about 400 gm. passing into the colon in twenty-four hours. Much of the fluid is absorbed in the cecum and ascending colon, but small amounts are also absorbed along the transverse, descending and pelvic portions of the colon, with the result that the feces evacuated are reduced to about 150 gm. per day in adults under average conditions.

Food residues, however, constitute a much smaller proportion of the bulk than is usually surmised. Indeed, the fat, protein and carbohydrate of the diet is practically all absorbed, with the result that if the food is free from indigestible material, especially cellulose, the feces are composed almost entirely of intestinal secretions, bacteria, etc. During starvation, for example, feces continue to be formed and their composition does not differ materially from that of feces formed after an ample diet. This is of clinical importance, since physicians may assume that the matter of bowel evacuations may be of little or no importance when patients are on a liquid or otherwise highly restricted diet. Even when a segment of the bowel is isolated from the rest of the intestinal tract, in time it becomes packed with a mass of pasty fecal material entirely endogenous in origin due to intestinal secretions. Furthermore, wide variations in the composition of the diet, if the amount of cellulose remains unaltered, exert little or no influence upon the composition of feces. The bulk is reduced, however, during starvation, but the reduction is due to the removal of the stimulating effect of the food upon the secretory activity of the intestine. Indigestible materials, especially cellulose, increase the amount of the feces, not only by adding directly to their bulk, but also by increasing the production of endogenous material.

PHYSICAL EXAMINATIONS

As previously stated, much information of clinical value may be learned from a careful physical examination of the feces for amount, form and consistency, color, odor, mucus, concretions, animal parasites, etc. These physical examinations are especially indicated in most patients with suspected gastro-intestinal disease, and should rarely be omitted in patients who have diarrhea, constipation, jaundice, anemia, or in infants presenting feeding problems. Furthermore, it is frequently better for physicians to make their own inspection of the feces than to rely alone upon the reports of technicians.

Amount. The normal amount of feces for the average adult per day is about 150 to 200 gm. It is increased by a vegetable diet. One or two evacuations in twenty-four hours may be considered normal, yet one every two to four days is not uncommon in healthy persons. The amount is commonly and characteristically increased in states producing steatorrhea due to an increase of fecal lipids; also in sprue and other conditions accompanied by indigestion of carbohydrates, in which the stools are likely to be large and foamy.

Form and Consistency. The normal stool is soft but formed, being about one inch in diameter in adults. Excessively hard feces, sometimes called scybala, are commonly observed in habitual constipation and indicate atomy of the muscular coat of the colon. In spastic constipation the feces are characterized by numerous hard, ball-like masses. Flattened, ribbon-like stools may result from taking mineral oil but otherwise indicate obstruction of the rectum, generally a carcinoma or a stricture from a healed ulcer, most frequently due to syphilis or lymphogranuloma venereum. Soft, mushy, or liquid and voluminous stools may follow the administration of cathartics or result from the many causes of diarrhea. In the dysenteries they are invariably small, numerous and largely composed of mucus and blood with small amounts of fecal material.

Color. Normally, in adults, the feces are of a light to dark brown color, chiefly due to stercobilin (hydrobilirubin). This is derived from sodium bilirubinate in the bile which is reduced to mesobilirubinogen in the colon and finally into stercobilinogen and stercobilin through bacterial activity. On a milk diet, however, the feces are usually of a light yellow color, as in infants, owing partly to their milk diet and partly to the presence of unchanged bilirubin. The ingestion of large amounts of cocoa and chocolate may render them dark gray in color, while large amounts of various fruits may give a reddish or black color, spinach and other chlorophyllic vegetables a green color, beets a red, rhubarb a yellow, etc.

Drugs may likewise alter the color—for example, the green stools due to biliverdin following the administration of calomel; the dark brown or black stools due to iron and bismuth; the red stools due to neoprontosil; the yellow stools due to santonin or senna; the clay-colored stools after a barium meal in connection with x-ray examinations, etc.

Important changes, however, may occur in disease—for example, the "acholic" or clay-colored stools in obstructive jaundice which, however, may be due more to an excess of fecal fat than to a decrease of bile pigments; likewise similar

TABLE 64. SUMMARY OF THE CLINICAL INTERPRETATION OF PHYSICAL EXAMINATIONS OF THE FECES

Constituent	Normal	Abnormal
Formation and Amount	For adults 150 to 200 gm. per day. Increased by vegetable diet and indigestible materials, particularly cellulose, and reduced by meat diet. Composed of food residues, secretions of the intestine and bile, leukocytes, epithelium and bacteria. Feces continue to be formed in starvation states; also in isolated loops of the intestine because of intestinal secretions.	Increased by indigestion. Particularly in steatorrhea due to achylia pancreatica or other diseases due to an increase of fecal fat. Increased in sprue and other conditions accompanied by indigestion of sugars and starches.
Form and Consistency	Soft and in adults about one inch in diameter.	Hard and scybalous in habitual constipation. Small, hard, ball-like masses in spastic constipation. Flattened and ribbon-like after mineral oil or due to obstruction of the rectum (strictures, carcinoma, etc.). Soft, mushy or liquid and voluminous after cathartics and in diarrhea; small and numerous in the dysenteries.
Color	Light to dark-brown in adults largely due to stercobilin. Light yellow on a milk diet. Light yellow in infants due to milk diet and unchanged bilirubin. Alterations may be due to foods.	Alterations due to drugs (calomel, iron, bismuth, neoprontosil, santonin, barium meals, etc.). "Acholic" or clay-colored in obstructive jaundice; also in achylia pancreatica and tuberculous peritonitis. Tarry-black and viscid in extensive hemorrhages of the stomach or upper intestine. May be red if occurring with diarrhea. Dark brown to bright red in bleeding nearer the rectum. Streaks of blood on feces in bleeding from hemorrhoids, fissures, carcinoma, etc., of rectum or anus. Green stools not uncommon in healthy infants; particularly common in the diarrheas of infants and children.

TABLE 64. SUMMARY OF THE CLINICAL INTERPRETATION OF PHYSICAL EXAMINATIONS OF THE FECES—(Continued)

Constituent	Normal	Abnormal
Odor and Reaction	*Odor* due to aromatic substances, particularly indole and skatole from bacterial decomposition of proteins. Increased by meat diet; decreased by vegetable diet. Sour rancid odor may be normal in infants. *Reaction* neutral, slightly acid or slightly alkaline (*p*H 6.9 to 7.2). Influenced by diet.	Increased odor may be due to decomposition of intestinal blood or of tissues from ulcerated lesions. May be sour, rancid, highly acid and gaseous in the diarrheas, due to indigestion of sugars and starches. May be putrid in severe diarrheas. Foul stench may occur in malignant or syphilitic ulcerations of the rectum; also in gangrenous dysenteries, etc. Intestinal indigestion of proteins results in excessive alkalinity; of carbohydrates in excessive acidity with considerable gas. Symptoms of constipation are not due to indole, skatole, histamine, choline or other substances formed by decomposition of intestinal contents by bacteria; apparently reflex rather than toxic in origin.
Mucus	Only small amounts intimately mixed with the stool. Should not be mistaken for mineral oil.	Extensive amounts easily detected by inspection. When intimately mixed with the feces, usually due to inflammation of the small intestine. Large amounts usually indicate colitis. Stools composed almost entirely of blood-streaked mucus characteristic of the acute dysenteries, ileocolitis or intussusception. Large amounts characteristic of "mucus colitis." May occur as "casts," ribbon-like or brownish-black masses relatively free of pus cells.
Concretions	Curds of fat or casein may occur in infants. Also masses of soap and fat in adults after the ingestion of large amounts of olive or other vegetable oils.	So-called "intestinal sand" usually composed of seeds or other vegetable matter. Gallbladder stones may occur and should always be looked for after suspected gallbladder colic. Intestinal concretions (enteroliths) are rare.

stools, largely consisting of fecal lipids and having a greasy appearance, commonly encountered in achylia pancreatica and tuberculous peritonitis.

Large amounts of blood from hemorrhage in the stomach or upper intestine usually produce tarry black and viscid stools because of digestive changes or dark brown to bright red stools when the source of bleeding is nearer the rectum. However, when diarrhea exists the color may be red, even if the source of the blood is higher up. Red streaks of blood on the outside of the feces are due to bleeding from hemorrhoids, fissures, carcinoma or other lesions of the rectum or anus. Blood may be present, however, in amounts too small for recognition by inspection ("occult blood") and require chemical or microscopical tests for detection.

Green stools are not uncommon, especially in the diarrheas of children, due to biliverdin and sometimes to chromogenic bacteria. They are sometimes seen in healthy infants, alternating with normal yellow stools, and have little significance unless accompanied by symptoms.

Odor and Reaction; Intestinal Intoxication. The normal *odor* of feces is due to the presence of aromatic substances, chiefly indole and skatole, derived from the deamination and decarboxylation of tryptophane by putrefactive bacteria in the colon. Therefore, since these substances are products of decomposition of protein, odor depends largely upon the amount of meat in the diet, being much less on a diet of vegetables or milk. The *reaction* is neutral, slightly acid or slightly alkaline, with a pH varying from 6.9 to 7.2. Much depends on the diet, as an excess of protein results in alkalinity while an excess of carbohydrates produces acidity. Pathologically, therefore, variations in reaction may result from intestinal indigestion of the respective foodstuffs.

An increase of odor, however, may be due to other proteins, as in the decomposition of large amounts of blood or of tissues in ulcerated cancers of the sigmoid or rectum. A sour rancid odor due to fatty acids is normal in infants, but may be observed in the diarrheas of older children and adults, along with a highly acid reaction and much gas formation, largely resulting from the fermentation of inadequately digested sugars and starches. In severe diarrheas a putrid odor is common, while feces emitting a foul stench are suggestive of malignant, syphilitic or other ulcerative lesions of the rectum, gangrenous dysentery, etc.

In addition to indole and skatole, other substances, some of which are toxic, are produced in the colon by the bacterial decomposition of proteins, such as histamine, phenol, cresol, ethylamine, etc., including choline formed by the decomposition of lecithin, which in turn gives rise to traces of neurine. Some investigators have suggested that the absorption of these substances is responsible for many ills, particularly anemia and the well-known symptoms of constipation, generally referred to as *intestinal intoxication* or "auto-intoxication." But there is no reliable evidence to support the assumption that these toxic products are responsible for the symptoms of anemia and constipation. Only small amounts of indole and skatole are absorbed into the blood, and indole undergoes conversion in the liver into indoxyl which then for the most part combines with sulfuric acid and potassium to form indoxyl potassium sulfate or indican which is excreted in the urine. In other words, the liver not only detoxifies indole and skatole, but large amounts of indican may occur in the urine of perfectly normal individuals.

Moreover, indole and choline are practically without toxic effects when given orally and the absorption of histamine is, except for traces, prevented by the intestinal wall itself.

Furthermore, a strong argument against the symptoms of constipation being due to the absorption of toxic substances is the almost immediate relief which follows evacuation of the bowels. Another argument is the fact that many individuals with even severe constipation do not always excrete increased amounts of indican in the urine while others, without constipation at all, and in good health, may, for some unknown reason, excrete large amounts. For these reasons, Alvarez has attributed the symptoms of constipation to a reflex rather than a toxic origin, due to afferent impulses set up from the wall of the overloaded rectum.

Mucus. Normally, the feces contain but very small amounts of mucus intimately mixed with the stool. Due care is required against confusing it with mineral oil.

Excessive quantities are easily detected by macroscopic inspection and usually signify irritation or inflammation of the intestine and especially of the colon. When small in amount and intimately mixed with the feces, mucus is usually derived from the small intestine. Stools composed almost entirely of mucus and streaked with blood are the rule in the acute dysenteries, amebic enteritis and ileocolitis; they may also occur in intussusception.

In so-called "mucous colitis" shreds and ribbons of mucus, sometimes representing complete casts of portions of the colon, are passed, especially after an enema. In the ordinary formed stool the mucus may be unrecognized, unless the feces are well mixed with water, when it may appear as firm, irregularly segmented strands resembling segments of the tape worms, or occur as brown or black jelly-like masses. It is distinguished from catarrhal mucus by the absence of pus cells upon microscopic examination. The disease is probably a secretory neurosis, and the name "membranous enteritis" is inappropriate.

Concretions. The stools of infants frequently contain whitish curds, due either to fat or casein or a mixture of both. After the ingestion of considerable amounts of olive or other vegetable oils, and particularly when given for cholelithiasis, the feces of adults may show masses of soap and fat which may be mistaken by individuals for gallstones. So-called "intestinal sand" is now known to occur particularly in neurotic individuals and is composed in most cases of vegetable matter with particular reference to seeds, such as those of berries, bananas, pears, etc.

However, gallbladder stones may occur and should always be looked for in the daily feces of patients over a period of at least four days following obscure colicky abdominal pain suggestive of gallstone colic. They are usually readily recognized by their faceted surfaces; otherwise by chemical examinations for cholesterol and bile pigment. Intestinal concretions (enteroliths) are rare. Animal parasites or segments of them may be found (Chapter 12).

Blood. It is an excellent practice to include chemical tests for occult blood in the routine examination of feces. But since the benzidine and other tests are extremely sensitive, the patient should be on a meat-free diet for at least three or more days before a positive reaction is regarded as significant.[1]

The causes for blood in the feces are very numerous, as bleeding anywhere from the lips to the anus may be responsible. For example, it may be due to that swallowed from bleeding gums, from the nasopharynx, that coughed up from the lower respiratory tract, or by malingerers. Otherwise, however, a persistently positive occult blood reaction is of considerable clinical significance. The most important causes are: (1) peptic ulcer in which bleeding is apt to be intermittent and sometimes severe. Indeed, persistent occult blood in spite of treatment is usually suggestive of malignancy; (2) carcinoma anywhere in the gastro-intestinal

TABLE 65. SUMMARY OF THE CLINICAL INTERPRETATION OF CHEMICAL EXAMINATIONS OF THE FECES

Constituent	Normal	Abnormal
Occult Blood	Absent but traces may be due to meat in the diet. May be swallowed by malingerers.	Bleeding anywhere from the lips to the anus. May be swallowed from the gums, nasopharynx or after being coughed up from the lower respiratory tract. Persistent blood may be due to peptic ulcers, carcinoma, ulcerative colitis, the dysenteries, ruptured esophageal or hemorrhoidal varices in portal cirrhosis, volvulus and intussusception, typhoid fever and tuberculous enteritis, Meckel's diverticulum, regional ileitis, hemorrhoids, the purpuras and hemorrhagic diseases, hemolytic and obstructive jaundice, etc.
Bilirubin and Urobilinogen	Bilirubin absent. Urobilinogen (stercobilin) present in 72 to 320 mg. per 100 gm. by method of Watson; 70 to 600 mg. per 100 gm. by method of Sparkman.	Traces of bilirubin may occur in enteritis with diarrhea and obstructive jaundice. Urobilinogen usually increased in such diseases of the liver as abscess, portal cirrhosis, acute and subacute cholecystitis, etc. Reduced in jaundice of intrahepatic origin. Absent or greatly reduced in obstruction of the common bile duct. Increased in pernicious and hemolytic anemias. Normal in anemia due to hemorrhage, iron deficiency, etc.

TABLE 65. SUMMARY OF THE CLINICAL INTERPRETATION OF CHEMICAL EXAMINATIONS OF THE FECES—(Continued)

Constituent	Normal	Abnormal
Fats	Largely independent of the fat ingested; large portion due to excretion. Closely similar to the blood lipids. Variable according to diet. Total fat in dried feces per day varies from 7.3 to 27.6 per cent (average 17.5 per cent). Of this about 7.3 per cent neutral fat, 5.6 per cent free fatty acids and 4.6 per cent combined fatty acids (soaps).	Abnormal values: total fat exceeding 25 per cent; neutral fat exceeding 11 per cent; soaps (combined fatty acids) exceeding 16 per cent. *Steatorrhea* or excess fecal lipids may occur in: (1) enteritis with diarrhea due to an increase of neutral fat, fatty acids and soaps; (2) extrahepatic jaundice, largely due to free fatty acids and soaps; (3) congenital and acquired achylia pancreatica, largely due to an excess of neutral fat and (4) sprue and idiopathic steatorrhea (celiac disease) largely due to free and combined fatty acids. Fatty diarrheas may result in hypocalcemia, demineralization of bones and tetany.
Nitrogen	Varies according to diet. On an average diet from 5 to 10 per cent of nitrogen of food or from 0.5 to 1.0 gm. per day. An increase is termed *azotorrhea.*	Greatly increased in achylia pancreatica; less so in pancreatogenous fatty diarrhea and idiopathic steatorrhea. Practically normal in obstructive jaundice and sprue ("tropical," "nontropical" and "celiac disease").

tract, with special reference to the stomach, cecum or rectum; (3) ulcerative colitis; (4) amebic, bacillary and balantidic dysentery; (5) ruptured esophageal or hemorrhoidal varices in portal cirrhosis of the liver; (6) blood usually without fecal material in volvulus and intussusception; (7) typhoid and paratyphoid fevers; (8) tuberculosis of the cecum and ileum; (9) Meckel's diverticulum (about 80 per cent of cases); (10) regional ileitis;[2] (11) hemorrhoids; (12) the purpuras, hemophilia and other hemorrhagic diseases, including hemolytic and obstructive jaundice (Table 65).

Bilirubin and Urobilinogen. Normally, bilirubin is never present in the feces of adults. But in enteritis with diarrhea it may be carried through and traces may also be present from the blood in obstructive jaundice. It is probably best detected by the green color observed in the Schmidt test.

When the feces are grossly clay colored a test for urobilinogen or stercobilin is not ordinarily required although sometimes advisable. Owing to constipation and other factors, the amount is subject to marked daily variations. Normally, a twenty-four hour stool gives a dilution value varying from 1:6000 to 1:9000 by the method of Wilbur and Addis, from 72 to 320 mg. per 100 gm. of feces according to the method of Watson [3] and from 70 to 600 mg. per 100 gm. by the method of Sparkman.[4]

Quantitative estimations of stercobilin are of distinct clinical value. For example, this is usually true in detecting and following diseases of the liver and especially in abscess and portal cirrhosis as well as in hepatic damage due to acute and subacute cholecystitis. It is also stated that determinations are of value in differential diagnosis between jaundice of intrahepatic origin, in which stercobilin is reduced, and obstruction of the common bile duct, in which it is usually absent or reduced to less than 5 mg. per day.[4]

Determinations are also of value as an index of the rate of destruction of erythrocytes and as an aid in the differentiation between the anemias due to their increased destruction, in which the output is increased, and those due to their decreased production or loss by hemorrhage in which it is within normal. Thus, in pernicious and the hemolytic anemias, the excretion is high while in secondary anemias resulting from hemorrhage it is usually low or within normal. In hemolytic jaundice with a marked increase in excretion of urobilinogen, there may be a rapid decrease in excretion after splenectomy.

Fat. Even on a fat-free diet there is a daily excretion of about 2 gm. of fat, the composition of which is very similar to that of the blood lipids. Indeed, it has been shown that the amount of fat in the feces and its composition are to a large extent independent of the fat ingested with foods and, consequently, cannot be regarded as representing entirely a residue from the fat of the diet.[5,6] Under the circumstances, it is now generally believed that at least a portion of the fecal lipids is derived from the blood by excretion into the small intestine.

On an unregulated diet considerable variation in the amount of fat in the feces of normal individuals is naturally to be expected and even in the same person from day to day. Thus total fat in dry feces has been found to vary from 7.3 to 27.6 per cent per day (average 17.5 per cent). Of this, neutral fat averages 7.3 per cent; free fatty acids 5.6 per cent, and combined fatty acids (soaps) about 4.6 per cent.[7] Normally, on the Schmidt diet, over 94 per cent of the fat is absorbed so that 6 per cent or less of the dried feces is composed of fat.

A total fat amounting to more than 25 per cent of the dried feces is probably abnormal, as is a neutral fat exceeding 11 per cent (evidence of deficient fat splitting) and more than 16 per cent of fatty acids combined as soap which is regarded as evidence of deficient fat absorption.[7]

An excess of fat in the feces is designated *steatorrhea*. This may occur in enteritis involving the small intestine in which large amounts of undigested and unabsorbed fat are rushed through into the colon with an increase of total fat due to an increase of neutral fat and fatty acids, both free and combined (soaps).

Steatorrhea is likewise common in extrahepatic obstructive jaundice largely because of an excess of free and combined fatty acids (soaps) indicative not only of defective fat digestion and absorption but also of increased excretion of fat. Steatorrhea is also characteristic of congenital pancreatic steatorrhea and that due to chronic pancreatitis or other diseases of this organ producing achylia pancreatica. In these the total fat may be as high as 46 per cent of dried feces due to an increase of neutral fat (29 per cent), free fatty acids (6 per cent) and combined fatty acids or soaps (11 per cent). Indeed, pancreatogenous fatty diarrhea is sometimes so severe that neutral fat separates out as a yellow oil which may be mistaken for mineral oil if the patient is taking it for laxative purposes.

In sprue and idiopathic steatorrhea (celiac disease), the total fat is also increased but here the increase is not due as much to an increase of neutral fats (as it is in achylia pancreatica) as to an increase of free and combined fatty acids.

Fatty diarrheas may result in a loss of calcium with hypocalcemia, demineralization of bones and tetany. The loss of fats may vary from as low as 10 per cent in "good" periods to as high as 50 per cent or more during "bad" periods. It may be due to deficient absorption or abnormal excretion of fat. Diminished lipase activity has also been observed but as a rule it is not as marked as in pancreatogenous steatorrhea. At any rate, the free and combined fatty acids may be as high as 60 to 70 per cent, with only about 30 per cent as neutral fat. However, exceptions may occur in which the latter is the predominating fraction.

Nitrogen. On an average diet from 5–10 per cent of the nitrogen of food is eliminated in the feces, varying in adults from 0.5 to 1.0 gm. per day. An increase is termed *azotorrhea*. For diagnostic purposes it is desirable to analyze feces while the patient is on the Schmidt diet. In achylia pancreatica Pratt [8] has observed that 50.9 per cent of the ingested nitrogen may be recovered from the feces while it is practically normal in obstructive jaundice (8 per cent) and sprue (9.6 per cent). Thaysen [9] states that differential diagnostic significance is to be attached to increased elimination of nitrogen in pancreatogenous fatty diarrhea, since it is seldom increased in sprue. According to Anderson and Lyall,[10] its excretion is characteristically less than 3 gm. per day in idiopathic steatorrhea whereas typically about 3 gm. or more in pancreatogenous fatty diarrhea. These results, as well as the increase of fat and particularly of neutral fat, are probably due to a deficiency or lack of pancreatic secretion and especially of the enzymes, lipase and trypsin.

MICROSCOPIC EXAMINATIONS

Microscopic examinations of the feces are usually limited to a search for animal parasites or their ova and cysts, to be discussed in Chapter 12. But additional information of clinical value is frequently to be obtained, especially by an examination of the feces for the numbers and kinds of cells present, although their differentiation and recognition require special skill and experience. Cytologic examinations of this kind are particularly valuable in distinguishing between amebic and bacillary dysentery.

Remnants of Food. Furthermore, useful information may also be obtained by examinations for remnants of food as a rough index of the state of digestion (Table 66). It is advisable, however, for the patient to be on a standard Schmidt diet for this purpose. In this way some idea may be gained relative to the digestion of *starch* when wet preparations are treated with Lugol's solution, since the granules take a blue color if undigested and a red color if partially digested.

TABLE 66. SUMMARY OF THE CLINICAL INTERPRETATION OF MICROSCOPIC EXAMINATIONS OF THE FECES

Constituent	Normal	Abnormal
Animal Parasites, Ova or Cysts	Not present.	See Chapter 12.
Food Remnants	May be present but well digested. Neutral fat absent or present in but small amounts.	Partially digested starch granules, muscle fibers, connective and elastic tissue. Excess of neutral fat, fatty acids and soaps; especially from excessive ingestion of fats or resulting from faulty digestion or absorption of them.
Crystals	Present but of slight significance. Crystals of bismuth suboxide may occur after the oral administration of bismuth salts.	Excess of calcium oxalate in exclusive vegetable diet. Charcot-Leyden crystals may occur in ulcerative lesions of the colon and particularly in amebic dysentery. Crystals of hematoidin may occur in gastro-intestinal hemorrhage.
Cellular Exudates	Epithelial cells, leukocytes and erythrocytes absent.	*Erythrocytes* present in hemorrhage from the descending colon, rectum or anus. Heavy cellular exudates composed of epithelial cells, polymorphonuclear leukocytes (pus cells), lymphocytes and macrophages (endothelial cells) may occur in bacillary dysentery, chronic nonspecific ulcerative colitis, ulcerating cancers of the colon and sigmoid, lymphogranuloma venereum of women, etc. Light exudates of erythrocytes, degenerated leukocytes and epithelial cells in amebic dysentery. Cellular exudates usually absent in diarrheal states without ulcerating lesions of the intestines.

Muscle fibers appear as short and transversely striated cylinders with rather squarely broken ends and well-preserved nuclei when poorly digested, while presenting rounded ends with no striations at all or but faint ones, with absence of nuclei, when well digested. *Connective tissue* when but partially digested is recognizable as yellowish threads with longitudinal striations and *elastic tissue* as well-defined branching fibers.

Some idea may also be obtained relative to the digestion and absorption of *fats*, although chemical examinations for total fat are always to be preferred when the possibility of indigestion or faulty absorption are of particular clinical interest. Neutral fats, staining strongly with sudan III, are normally absent or present in only very small amounts but are greatly increased in steatorrhea. Fatty acids may occur as flakes staining orange with sudan III or as aggregates of needle-like crystals which do not stain at all. Combined fatty acids or soaps (chiefly calcium soap) appear as yellow amorphous flakes or rounded masses resembling ova which do not stain with sudan III; sometimes they occur as crystals.

Additional kinds of *crystals* may also be found, but few have any clinical significance. These include the characteristic octahedral crystals of calcium oxalate so likely to be in excess on a vegetable diet. Charcot-Leyden crystals are particularly apt to occur in ulcerative diseases of the colon and especially in amebic dysentery. Yellowish or brown, needle-like or rhombic crystals of hematoidin may occur after hemorrhages in the gastro-intestinal tract. However, the dark color of feces after the administration of bismuth is largely due to crystals of bismuth suboxide resembling the crystals of hemin.

Cytology. Under normal conditions epithelial cells, leukocytes and erythrocytes are not present in the feces.[11] When bleeding occurs in the small intestine, erythrocytes can seldom be recognized and the detection of blood depends upon chemical tests. But erythrocytes are usually found in bleeding from the descending colon, rectum, or anus in amebic and bacillary dysenteries (especially the former) as well as in nonspecific ulcerative colitis, ulcerating cancers, hemorrhoids, etc. Furthermore, many investigators have shown that examinations for other cells are of great value in the differential diagnosis between amebic and bacillary dysentery.[12–16] Naturally, this has stimulated inquiry into the relationship of cellular exudates to ulcerative colitis, ulcerating cancers, and other types of intestinal disease.

With formed stools, preparations should be made of the outside of the fecal mass since it comes into closer contact with the intestinal mucosa. But examinations of the watery stools following a saline cathartic are much more valuable, especially the last portions of stools because they may contain cells from pathologic lesions high up in the bowel. But the most valuable material is the terminal mucus evacuated after the last of three saline enemas taken in succession, because gross fecal material is washed away by the first and second enemas.[16] Precautions should be taken against trauma. The new sigmoid cannula of Bercovitz [17] can be used to aspirate the terminal mucus during sigmoidoscopy.

A small amount of the material may be mixed on a slide with a drop of Loeffler's methylene blue and examined microscopically after being covered with a coverslip; or permanent preparations may be stained with Heidenhain's iron hematoxylin.

With these methods it is possible to recognize and differentiate epithelial cells, polymorphonuclear leukocytes or pus cells, lymphocytes and endothelial or macrophage cells.[16] In amebic dysentery the discharge consists largely of clear mucus sometimes streaked with blood, vegetative motile forms of *Endamoeba histolytica,* and a scanty cellular exudate. But in bacillary dysentery, the discharge is characterized not only by the absence of amebae but by the presence of heavy cellular exudates composed of pus cells, epithelial cells, lymphocytes and particularly of endothelial macrophages; the latter must not be mistaken for amebae.

In some types of diarrhea, cellular exudates may not occur but examinations for them are always advisable as aids in the detection of pathologic lesions of the intestinal mucosa; when found, they naturally lead to more thorough examinations relative to the location and character o: the lesions. Thus, heavy cellular exudates have been observed not only in nonspecific chronic ulcerative colitis but likewise in the diarrheas of carcinoma of the colon and sigmoid and in lymphogranuloma venereum of women.[16]

The clinical interpretation of *bacteriologic examination* is discussed in Chapter 15.

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12

THE CLINICAL INTERPRETATION OF PARASITOLOGIC EXAMINATIONS

Only a few parasitic diseases, particularly those which involve the multiplication of the parasite or its larvae in the host, produce characteristic signs and symptoms. The larger group, chiefly of helminthic origin, present but few or indefinite clinical evidences of their presence because so many parasites may live in individuals for years without producing any marked objective or subjective symptoms. Frequently the symptoms, the physical examination, and the history of the patient will be suggestive of a particular infestment, but there is only one certain guarantee of specific diagnosis before specific treatment is undertaken, namely, the detection and identification of the parasite or its products in the blood, feces, bile, urine, sputum or tissues (spleen, liver, lymph nodes, skin or muscles). Successful laboratory diagnosis requires skill and experience in the methods of examination along with familiarity with the diagnostic characteristics of parasites, their ova and larvae for purposes of identification. In some parasitic diseases the presence of anemia and eosinophilia are often suggestive, while in others skin tests (Chapter 19) and serologic examinations are of helpful diagnostic value (Chapter 18).

EXAMINATIONS OF THE FECES

So many diseases caused by animal parasites are due to infestment of the gastro-intestinal tract that examinations of the stools for the worms, their ova or cysts, take first place in importance in laboratory diagnosis (Table 66).

Not all parasites or their products occurring in the feces, however, are pathogenic. For example, several species of free-living amebae, as well as certain species of free-living flagellates, may occur as accidental contaminants. These are known as *coprozoic parasites* and are of no significance except for the fact that they may be mistaken for pathogenic species. Furthermore, various other objects such as intestinal yeasts and fungi, vegetable, epithelial and squamous cells, leukocytes and especially large endothelial macrophages which have ingested erythrocytes, as well as such extraneous materials as air bubbles, partially saponified fat globules, starch granules and even mucus, may be mistaken for intestinal protozoa, cysts or helminthic ova by inexperienced workers.

Collection. In examinations for the *helminths* or their ova, stools should be collected in clean, dry containers and should not be mixed with urine. Most of the ova are identifiable for several days after passage, and usually a small representative sample of feces is suffi-

TABLE 67. SUMMARY OF THE CLINICAL INTERPRETATION OF PARASITOLOGIC EXAMINATIONS OF THE FECES

Diseases	Interpretation
General Considerations	Only a few diseases due to animal parasites produce marked objective or subjective symptoms. The same is true of carriers of *Endamoeba histolytica* and numerous other parasites. There is only one guarantee of specific diagnosis, namely, the detection and identification of parasites or their products in the body excreta, fluids or tissues which requires skill and experience. *Coprozoic parasites,* like free-living amebae and flagellates, are nonpathogenic but may be mistaken for intestinal protozoa, cysts or helminthic ova by inexperienced workers. The same is true of extraneous materials as various cells, leukocytes, endothelial macrophages, fat globules, starch granules and mucus.
Collection	Stools collected in clean, dry containers without mixture with urine are satisfactory for examinations for helminths and their ova. Specimens obtained by enemas or purging are likewise satisfactory. As a check on treatment, all of the feces passed in 24, and preferably 48, hours should be submitted for examination. Repeated examinations are required and especially in strongyloidiasis and clonorchiasis. The cellophane swab method is best for examinations in the diagnosis of enterobiasis (oxyuriasis). In acute amebic enteritis and dysentery stools should be examined immediately after passage. If this is not possible, they should be kept warm in a thermos bottle or some other suitable container until examined. Examinations for *Endamoeba histolytica* are best conducted with bits of mucus or shreds of mucosa obtained directly from ulcerated areas through the sigmoidoscope. Cold stools are satisfactory for examination for cysts up to 3 or 4 days. Protozoa are found more frequently after purging than in normal stools. If contraindicated, enemas may be employed.
Amebiasis	*Endamoeba histolytica* only is definitely pathogenic, producing (1) acute or (2) chronic amebic dysentery; (3) asymptomatic enteritis and (4) the carrier state (contact or convalescent) both of which are subject to exacerbations; also abscesses in the liver, lungs or elsewhere. Laboratory examinations of the feces, tissues or contents of abscesses are essential in diagnosis and differential diagnosis. These include direct microscopic examinations or cultures for *E. histolytica* or complement fixation tests along with cytologic examinations. *E. gingivalis* is probably not pathogenic but may favor secondary bacterial or spirochetal infections in gingivitis. *E. coli, Endolimax nana, Iodamoeba bütschlii* and *Dientamoeba fragilis* are definitely nonpathogenic but important from the standpoint of being possibly mistaken for *E. histolytica* in the laboratory.
Balantidiasis	Balantidiasis or balantidial dysentery is caused by the swallowing of the cysts of *Balantidium coli* in water or vegetables contaminated by the feces of human or hog carriers or flies. Human beings in close contact with infested hogs are especially exposed to infestment. Diagnosis depends upon the finding of motile trophozoites in diarrheic stools or of cysts in semiformed or formed feces.

TABLE 67. SUMMARY OF THE CLINICAL INTERPRETATION OF PARASITOLOGIC EXAMINATIONS OF THE FECES—(Continued)

Diseases	Interpretation
Giardiasis	Giardiasis or lambliasis is caused by *Giardia lamblia* due to the swallowing of cysts. Its pathogenicity is doubtful but heavy infestments may apparently produce an enteritis or giardial dysentery or at least aggravate and prolong infections due to other causes. Laboratory diagnosis is best made by direct microscopic examinations of bile obtained by duodenal drainage. Motile trophozoites may also occur in fresh warm liquid stools. The cysts are found in formed feces.
Ascariasis	Caused by infestment with *Ascaris lumbricoides* due to the consumption of embryonated ova. Children are more commonly infested than adults. The migrating larvae in the blood may produce Ascaris pneumonitis or infestments elsewhere. The adult worms inhabit the small intestine. There may be no subjective or objective symptoms but such may occur, as well as appendicitis, ileus and other complications, especially in children. Laboratory diagnosis is based on the finding of worms in the feces or vomitus but usually on the finding of ova in the feces. Ova are absent when infestment is due only to male parasites.
Oxyuriasis	Oxyuriasis or enterobiasis is caused by infestment of the cecum, colon and lower ileum with the pinworm *Oxyuris vermicularis* (*Enterobius vermicularis*) due to the swallowing of the ova. The disease is most common in children, producing intense pruritus of the perianal and perineal regions with reflex nervous symptoms, loss of sleep, anemia with eosinophilia and emaciation. The migrations of the worms may also cause chronic appendicitis, low-grade peritonitis and chronic salpingitis. Laboratory diagnosis may be based on finding the worms in the feces or in the perianal region. The ova are seldom found in the feces. The collection of material for examination by cellophane swabs is highly recommended. Repeated examinations are sometimes required for diagnosis and should always be made before the patient is regarded as cured. Ova are also frequently obtained from finger nail scrapings.
Uncinariasis	Uncinariasis or hookworm disease is due to infestment with *Necator americanus* or *Ancylostoma duodenale.* Infestment usually occurs with larvae from the soil through the skin of the feet (producing ground-itch or hookworm dermatitis) followed by pulmonary lesions and symptoms and finally by infestment of the intestinal mucosa with adult worms. Light infestments may produce no symptoms, but secondary anemia with eosinophilia, malnutrition, lack of physical and mental energy, intestinal disorders and sexual retardation are usual. Massive infestments may produce more severe symptoms and cardiac failure. Infestment may also occur from the swallowing of the larvae in contaminated water or food. Local cutaneous or creeping eruptions are due to infestment of the skin with soil contaminated with *A. braziliense.* Laboratory diagnosis depends upon the detection of ova in the feces. Hatched larvae in stools must be differentiated from *Strongyloides* and free-living nematodes.

TABLE 67. SUMMARY OF THE CLINICAL INTERPRETATION OF PARASITOLOGIC EXAMINATIONS OF THE FECES—(Continued)

Diseases	Interpretation
Strongyloidiasis	Due to infestment of the small intestine with *Strongyloides stercoralis*. The filariform larvae occurring in the soil penetrate the skin of the feet, producing local irritation with itching, and reach the lungs, producing pulmonary symptoms or mild bronchopneumonia. The larvae occurring in the sputum are swallowed, followed by infestment of the intestine. Symptoms may not occur, but intermittent diarrhea with anorexia, loss of weight and anemia with eosinophilia are not unusual. Laboratory diagnosis is based on finding the characteristic rhabditiform larvae in fresh stools. Embryonate ova may also occur. Occasionally ova and larvae occur in the sputum.
Trichuriasis	Due to infestment of the cecum and sometimes of the colon with *Trichuris trichiura* (*Trichocephalus dispar*). The most common of human intestinal parasites. Infestment results from the swallowing of embryonated ova from the soil and especially in uncooked vegetables and fruits. Symptoms may not occur but are not infrequent and especially in children. Laboratory diagnosis is based on finding the characteristic ova in the feces. Concentration methods may be required.
Bothriocephaliasis	Due to infestment of the intestines with the fish tapeworm *Diphyllobothrium latum*. In North America most cases occur in the Great Lakes region. Infestment results from eating raw or partially cooked fish infested with the parasite. Most persons suffer no ill effects but various symptoms may occur associated with abdominal pain, malnutrition and anemia. The latter (bothriocephalus anemia) resembles pernicious anemia and while common in Finland, apparently does not occur in North America. Laboratory diagnosis is based on finding the characteristic operculate ova or proglottides in the feces.
Dipylidiasis	A rare intestinal infestment of human beings with the dog tapeworm, *Dipylidium caninum*. About 50 per cent of dogs in the United States are infested. Infestment results from accidental swallowing of parasitized fleas and lice from dogs and cats in contaminated food or drink, or from close association with these animals. Most cases occur in infants and young children. Adults seldom show symptoms but these may occur in children. Laboratory diagnosis is based upon finding proglottides or ova in the feces.
Taeniasis	Infestment with *Taenia saginata* is due to the ingestion of raw or partially cooked beef infested with the parasites (*Cysticercus bovis*). Laboratory diagnosis is based on finding the ova or proglottides in the feces. Infestment with *Taenia solium* is far more serious and due to the ingestion of raw or partially cooked pork ("measly pork") infested with *Cysticercus cellulosae* or of contaminated water and uncooked vegetables. Cysticercosis may occur in the skin, brain or eye. Laboratory diagnosis is based on finding the ova or proglottides in the feces.

TABLE 67. SUMMARY OF THE CLINICAL INTERPRETATION OF PARASITOLOGIC EXAMINATIONS OF THE FECES—(Continued)

Diseases	Interpretation
Taeniasis —*Continued*	Infestment with *Hymenolepis nana* (*Taenia nana*) is due to the swallowing of the ova from the hands or of contaminated water or uncooked foods. Most cases occur among children. Laboratory diagnosis is based on finding the ova in the feces. Infestment with *Hymenolepis diminuta* (*Taenia diminuta*) is usually due to the accidental ingestion of intermediate hosts (especially rat fleas) in contaminated water and uncooked foods. The infestment also occurs principally in children but is much rarer than infestments with *H. nana*. Laboratory diagnosis is based on finding the ova or proglottides in the feces.

cient. Specimens obtained by enemas will often be positive for ova or segments of tapeworms when ordinary stools are negative. In feces secured by purging, the whole or fragments of adult worms and ova may be readily obtained. If vermifuges have been employed, all the feces for at least twenty-four, and preferably forty-eight, hours should be preserved for examination as a check on treatment. As a rule, the feces should be examined for ova and larvae at intervals after treatment and over long periods in strongyloidiasis and clonorchiasis.

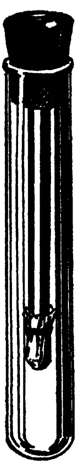

FIG. 13. THE NATIONAL INSTITUTE OF HEALTH CELLOPHANE SWAB FOR COLLECTING *Enterobius* OVA

Enterobiasis or oxyuriasis is best diagnosed by using scrapings of the anal and perianal regions. Although various blunt curets and anal swabs have been employed, the most efficient is the NIH cellophane swab described by Hall. This consists of a small square of cellophane wrapped about the tip of a glass rod and held in place by a wide rubber band (Fig. 13). The prepared swab is inserted in a perforated stopper and placed in a test tube to prevent loss of material after collection and to protect the handler from accidental infestment. The swab is used with a firm stroking motion, directed outward from the anal opening, parallel to and entering the folds of skin of the entire perianal region. The best time for swabbing is early morning before defecation and bathing. Eggs of helminths and sometimes the entire worm are picked up by the swab. At least seven swabs procured on different days should be examined before a negative diagnosis is warranted, although most cases will be detected by the first examination. This method is stated to be far more effective than examinations of the feces.

In examinations for *Endamoeba histolytica* and other intestinal protozoa, stools should also be collected in clean, dry containers without mixture with urine. Specimens from patients to whom barium, bismuth or oil have been given are unsatisfactory.

When the patient is diarrheic, the material should be examined immediately in the fresh warm state because the trophozoites of amebae succumb rapidly outside of the body. When this is not possible the specimen should be kept warm until examined. Thermos bottles are very satisfactory for this purpose. Nondiarrheic stools, however, may be examined after cooling, since cysts in semiformed and formed feces can be identified up to three or four days.

Since protozoa are found more frequently after purging than in normal stools, a saline laxative may be administered and the first or second movement thereafter examined. If purgation is contraindicated, a high enema of warm saline solution may be used. The best materials for examination for *Endamoeba histolytica* are bits of mucus and shreds of mucosa obtained directly from ulcerated areas through the use of the sigmoidoscope.

Amebiasis. At least four genera and six species of amebae occur in man, as well as other species of doubtful authenticity, but only one, *Endamoeba histolytica*, is pathogenic.

Endamoeba gingivalis may be present in healthy mouths and is especially prevalent in dental caries, pyorrhea alveolaris, and other suppurative conditions of the gingivae. While at one time believed to be pathogenic and capable of producing gingivitis, it is now the consensus that it is only a scavenger of the diseased tissues although it is to be admitted that its digging or invasive activities may favor the extension of bacterial or spirochetal infections.

Endamoeba coli, Endolimax nana, Iodamoeba bütschlii and *Dientamoeba fragilis,* however, while commonly occurring in the feces, are definitely known to be nonpathogenic and only of importance in relation to the chances of being mistaken in the laboratory for *Endamoeba histolytica.*

Endamoeba histolytica is the cause of amebic dysentery. Man is the principal host of the parasite and human beings become infested through the ingestion of cysts in raw water, vegetables or other uncooked foods contaminated by human feces, flies or other insects, or by the use of human feces as fertilizer for gardens. There is, however, the possibility of infestment through the ingestion of water or uncooked foods contaminated by carriers among rats, dogs, hogs and monkeys.

In addition to producing acute dysentery of severity ranging from a rare fulminating type with marked toxemia to mild enteritis with spontaneous recovery, *Endamoeba histolytica* may also produce chronic dysentery characterized by acute exacerbations, an asymptomatic type characterized by low-grade infestment with slight discomfort, or no symptoms at all, but likewise subject to exacerbations or a carrier state. In the carrier state about one-half of infested individuals are probably never truly healthy, since a low-grade infestment is present subject to exacerbation and the production of symptoms whenever resistance is lowered. Carriers are both of the contact and convalescent types and are chiefly responsible for the transmission of the parasite. Contrary to common impressions, the incidence of the carrier state in the United States is thought to involve from 8 to 10 per cent of the population and while amebic dysentery is particularly prevalent in tropical and subtropical countries, it is now known to be worldwide in distribution. Unfortunately, both acute and chronic amebic dysentery may be complicated by abscesses in the liver, lungs or elsewhere.

The definite diagnosis of amebiasis depends upon the finding of the parasite in the feces or tissues. At times it is difficult clinically to differentiate amebic dysentery from bacillary, schistosomal or balantidial dysentery, and from other intestinal diseases such as ulcerative or mucous colitis, chronic gastro-enteritis, chronic appendicitis, and food allergy. Consequently, skilful laboratory examinations are of prime importance in relation to diagnosis. Ordinarily, direct microscopic examinations of fresh warm stools for the trophozoites of *E. histolytica* suffice, as likewise examinations for its cysts in the diagnosis of asymptomatic and chronic cases as well as of carriers. As previously stated, bits of mucus and shreds of tissue removed through the proctoscope or sigmoidoscope are particularly valuable in the diagnosis of acute dysentery. The same methods are applicable to the examination of the contents of liver abscesses removed by aspiration during surgical operations. Animal inoculation tests are unnecessary and are not employed.

It is to be emphasized, however, that single negative examinations of the feces never reliably exclude the possibility of amebic enteritis or dysentery. Indeed, positive results may be observed in only one-third of cases subjected to single examinations, whereas positive results occur in practically all cases subjected to six or more examinations because of the cyclic occurrence of cysts. Furthermore, the chances of finding the latter are greatly improved by employing a concentration procedure like the flotation method of Faust. Otherwise, it is advisable to administer magnesium sulfate before the collection of feces, as this increases the chances up to 80–90 per cent of finding cysts upon the first examination.

Careful cytologic examinations are also of great value in differential diagnosis between amebic and bacillary dysenteries. Thus, in amebic dysentery the exudates are characterized by the presence of clumps of erythrocytes, Charcot-Leyden crystals, few pus cells and the presence of cytolyzed leukocytes constituting the highly characteristic "pyknotic bodies"; in bacillary dysentery, however, the exudates are characterized by the presence of erythrocytes occurring singly with no Charcot-Leyden crystals and very large numbers of large mononuclear cells (macrophages), constituting about 90 per cent of the cells, and some of which show characteristic degenerative changes ("ghost cells").

When microscopic examinations fail, cultural methods may be employed which increase the chances of finding the parasite, particularly in old feces or in chronic cases and carriers. The complement fixation test of Craig is also of diagnostic value not only in the detection of carriers and atypical clinical cases, but also as a control on the effectiveness of treatment, since a negative reaction indicates the probable elimination of the infestment, as discussed in Chapter 18.

Balantidiasis. Balantidiasis or balantidial dysentery is caused by *Balantidium coli* and follows the swallowing of cysts transmitted by water or vegetables contaminated by the feces of human or hog carriers or by flies. Man appears to be only an accidental host of the parasites which infest about two-thirds of all hogs. Consequently, human beings frequently derive infestment from hogs, as the dysentery occurs most frequently among those associated with these animals, by the direct transference of hogs' feces to the mouth through soiled hands in the handling of pigs, or in slaughtering operations. The use of hog manure as a fertilizer may be also a source of infestment, as the cysts are highly resistant to destruction.

Clinical balantidiasis varies from a mild colitis with diarrhea to an acute or chronic dysentery. A carrier state similar to that of *Endamoeba histolytica* is relatively common and usually due to an infestation with superficial lesions too mild to produce clinical symptoms.

Diagnosis depends upon the finding of motile trophozoites in fresh warm diarrheic stools and of cysts in semiformed or formed stools; the methods employed are the same as for the detection of *E. histolytica.*

Giardiasis. Giardiasis, or lambliasis, refers to infestment with *Giardia lamblia,* the only and most common intestinal flagellate probably pathogenic for man. The evidence of pathogenicity is inconclusive although it appears that heavy infestments may produce enteritis or dysentery (giardial dysentery) or at least aggravate or prolong infections of the small intestine due to other causes.

Only cysts are capable of producing infestment and are transmitted in the same manner as the cysts of *E. histolytica*. The vegetative forms are only infrequently found in the feces and soon perish. Many species of *Giardia* occur in mice, rats, dogs, cats, etc., but it is impossible to determine whether they are identical with the species found in man or whether these animals serve as reservoir hosts.

Laboratory diagnosis depends upon microscopic examinations of fresh warm liquid or semiliquid stools or of bile obtained by duodenal drainage (the best diagnostic method) for the motile trophozoites. Cysts are found in formed stools.

Ascariasis. This disease is caused by the consumption of *Ascaris lumbricoides* embryos in ova which have been accidentally picked up from the soil contaminated by the same or other human beings, or from food (especially raw or partly cooked vegetables) contaminated by viable embryonated eggs. Young children (especially 5 to 9 years of age) are more commonly infested than adults and more commonly pollute the soil. The use of human feces as fertilizer also favors transmission.

The migrating larvae in the blood may produce infestment of the lungs (*Ascaris pneumonitis*), particularly in children, and also in adults who are heavily infested. The pneumonitis is sometimes fatal. At times there may be a pronounced eosinophilia with urticaria and hemoptysis, with larvae in the sputum. If the larvae reach the general circulation they may produce severe infestments in the brain, spinal cord or the kidneys.

The adult worms which inhabit the small intestine are sometimes discharged by vomiting or regurgitation. In the majority of infested individuals there may be no pronounced objective or subjective symptoms, although children may show reflex nervous manifestations with mental or physical retardation and malnutrition. Vague abdominal discomfort or colic, however, is not unusual and wandering worms may not only produce appendicitis or blockage of the common bile duct with hepatitis, but even acute ileus and peritonitis from perforation of the intestine. Some laboratory workers also develop conjunctivitis, urticaria, asthma and at times even hematuria because of allergic sensitization to the worms.

Laboratory diagnosis is based on finding the parasites in the feces or vomitus but usually depends on the detection of ova in the feces by direct microscopic examination or by any of several methods of concentration. Of course, if only male worms are present (3.34 per cent of cases), ova are not found. In such cases laboratory diagnosis is best conducted by skin tests or precipitin tests employing a polysaccharide antigen.

Oxyuriasis. Oxyuriasis or enterobiasis is caused by infestment of the cecum and nearby appendix, colon and lower ileum with the pinworm *Oxyuris vermicularis* (*Enterobius vermicularis*). Man is the only known natural host of the parasite. The hands, particularly beneath the finger nails, become contaminated with ova through scratching the perianal regions to alleviate the itching produced by the female worms. In this way ova are readily transferred to the same or another person either directly by hand to mouth or indirectly through food and drink.

The ova which adhere to night clothes and bed linens may also be transmitted by the hands to the mouth or foods and swallowed.

The disease is most common in children. The migration of the egg-laying females from the anus causes congestion and irritation with pruritus of the perianal and perineal regions. This leads to scratching, excoriation and pyogenic infections. Intestinal irritation frequently produces reflex nervous symptoms which, with loss of sleep and appetite, frequently result in anemia with eosinophilia and emaciation. The migrations of the worms occasionally may produce chronic appendicitis, low-grade peritonitis and chronic salpingitis.

Often the first evidence of infestment is the discovery of the adult worms in the feces, particularly after enemas, or in the perianal region. In the feces the worms must be differentiated from fly larvae. Since the ova are seldom found in the feces even with concentrative methods, the collection of material with cellophane swabs, as previously described, is highly recommended. Repeated examinations are sometimes required and at least four swabbings should be made in the early morning, on nonconsecutive days, before the patient is considered free from infestment. Ova are also frequently obtained from finger nail scrapings. Intracutaneous tests, using an extract of the worms as an antigen, appear promising, especially negative reactions in eliminating the possibility of the disease.

Uncinariasis. Uncinariasis, ancylostomiasis or hookworm disease is caused by infestment of the small intestine and especially of the duodenum with *Necator americanus* (New World hookworm) or *Ancylostoma duodenale* (Old World hookworm).

Infestment usually occurs through the skin of the feet from contact with infested soil. The filariform larvae enter through the hair follicles or pores, or may bore through the unbroken surface, producing ground itch or hookworm dermatitis. They then enter the lymphatics or venules and are carried to the lungs where they may produce petechial hemorrhages and symptoms resembling strongyloidiasis and ascariasis as well as bronchopneumonia, particularly when accompanied by secondary bacterial infection. From the lungs they ascend the bronchi and trachea and are finally swallowed. After a fourth molt they acquire adult characteristics and adhere to the intestinal mucosa, sucking blood and tearing off bits of macerated mucosa, thus causing chronic hemorrhage and chronic enteritis. They secrete an anticoagulant that facilitates blood sucking, and each time a worm is dislodged it leaves a minute bleeding area. Consequently, secondary anemia with irregular eosinophilia, malnutrition, lack of energy, intestinal disorders, apathy and sexual retardation are commonly produced. Light infestments may produce no symptoms at all, whereas massive ones may produce severe abdominal pain, marked prostration and cardiac failure. *A. duodenale* produces more severe symptoms than *N. americanus*.

Infestment may also occur from the swallowing of the larvae in contaminated water or food. Local cutaneous or so-called creeping eruptions, which are prevalent in the United States along the Gulf of Mexico, are due to *A. braziliense* contracted by contact with soil contaminated by the feces of infested dogs and cats. It requires differentiation from the initial dermatitis produced by schistosomes. Intestinal infections with this parasite are unknown in the United States but have been observed in South America, Africa and the Orient.

Laboratory diagnosis depends upon finding the characteristic ova in the feces by the microscopic examination of ordinary wet preparations. In light infestments, however, concentrative methods may be required. The Stoll dilution ova counting method is also of value in diagnosis and is particularly useful in estimating the intensity of individual infestments in hookworm population surveys. When stools have stood for several hours and the eggs have hatched, the free larvae must be differentiated from those of *Strongyloides* and free-living nematodes.

Strongyloidiasis. Strongyloidiasis is caused by infestment of the upper part of the intestine with *Strongyloides stercoralis* where the females penetrate the villi and lie in the stroma between the glands of Lieberkühn. Here they secure nourishment and deposit ova which hatch into rhabditiform larvae. The latter are discharged in the feces.

In the soil these larvae develop into infective filariform larvae which enter new hosts through the skin of the feet, producing some local reaction with itching at the sites of entrance. From the skin they reach the lungs by way of the venous circulation, producing bronchial irritation and sometimes mild bronchopneumonia. Since the larvae occur in the sputum they are swallowed, causing infestment of the upper intestine. Light infestments may produce no intestinal symptoms, but intermittent diarrhea and urticaria, anorexia, loss of weight, fatigue and anemia with eosinophilia are not unusual. In severe infestments the diarrhea is similar to that of amebic dysentery.

In other words, the mode of transmission and symptoms are similar to those in hookworm disease but of lesser degree since the parasites do not suck blood or produce intestinal bleeding as in the latter.

Laboratory diagnosis consists of finding the characteristic rhabditiform larvae in fresh feces which may either be passed immediately after hatching or grow to two or three times their initial size before leaving the host. Because of small numbers in chronic infestments, concentration methods may be required for their detection. During severe diarrhea, embryonate ova may be found in the stools. Occasionally the ova and larvae may be found in the sputum. There is little risk of confusion with *N. americanus,* since the embryonate ova and rhabditiform larvae of the latter are rarely found in fresh feces.

Trichuriasis. Trichuriasis is caused by infestment of the cecum, and sometimes of the colon, with the whipworm *Trichuris trichiura* (*Trichocephalus dispar*). It is one of the most common of human intestinal parasites, but since pronounced symptoms of the infestment are rare, usually the first intimation of its presence is the finding of ova in the feces.

Infestment results from the ingestion of fully embryonated ova, directly or indirectly from the soil, in contaminated food, especially uncooked vegetables and fruits. Hogs, flies, other insects, and dust may furnish possible means of transmission.

Only occasional cases, particularly children, may present clinical manifestations, such as nutritional disturbances, digestive disorders, nervousness, insomnia, and loss of appetite with anemia and a characteristic eosinophilia up to 25 per cent. Pronounced symptoms are rare and usually associated with secondary bacterial infections.

Laboratory diagnosis is based upon finding the characteristic barrel-shaped ova in the feces. Concentration by centrifugation or brine flotation is recommended.

Bothriocephaliasis. Bothriocephaliasis or diphyllobothriasis is due to infestment of the intestine with the fish tapeworm *Diphyllobothrium latum* (*Dibothriocephalus latus*). In North America most cases occur in the northern Michigan peninsula, northern Minnesota and in the vicinity of Winnipeg, where the parasite has been introduced by immigrants from the Baltic countries.

While various animals (dogs, hogs, foxes, bears, etc.) have been found to be infested, man is primarily responsible for the propagation of the worm in endemic areas. The water is seeded with ova from human feces which mature, hatch and produce infestment of intermediate hosts (copepods), which, in turn, on ingestion of fishes, produce infestment in the fish flesh. Finally persons ingest this infested flesh, raw or inadequately cooked, and thereby acquire the disease. In more recent years infested fish have been refrigerated and shipped to distant communities where persons have become infested hundreds of miles from endemic regions.

Most persons suffer no ill effects, but some may present functional and organic disturbances of the nervous system, digestive disorders and malnutrition, abdominal colic and symptoms of peptic ulcer, stomatitis and anemia. In a small percentage of individuals the anemia (bothriocephalus anemia) may be clinically, hematologically and pathologically indistinguishable from cryptogenic pernicious anemia. However, the incidence of this severe anemia is very small compared with the incidence of infestment. Indeed, it is stated that no cases originating in North America have developed it (Craig) and that about 70 per cent of cases have occurred in Finland, where the population is known to have a predisposition to pernicious anemia.

Laboratory diagnosis is based upon finding the characteristic operculate ova or proglottides of the worm in the feces. In rare instances the ova require differentiation from those of *Diplogonoporus grandis,* occurring in Japan, and from those of *Diphyllobothrium cordatum.* Complement fixation tests may be helpful, although cross-reactions occur in infestments with other cestodes, to be discussed in Chapter 18.

Dipylidiasis. Dipylidiasis is a rare infestment of the intestines of human beings with the dog tapeworm *Dipylidium caninum.* About 50 per cent of dogs in the United States are infested with it.

Most cases in human beings occur in children under eight years of age and about one-third are found in infants under six months. Infestment results from the accidental swallowing of parasitized fleas or lice from dogs and cats, either through contamination of food or drink or by close association with these household pets.

Human beings rarely harbor more than a single parasite. Adults seldom present symptoms, although children may show slight intestinal discomfort, epigastric pain, anal pruritus, and reflex symptoms of the nervous system.

Laboratory diagnosis is based on finding the characteristic, ivory-colored proglottides or, infrequently, clusters of ova in the fecés.

Taeniasis. Taeniasis refers to infestment of the intestine of human beings with the beef tapeworm *Taenia saginata,* the pork tapeworm *Taenia solium,* or the dwarf tapeworms *Hymenolepis nana* (*Taenia nana*) or *Hymenolepis diminuta* (*Taenia diminuta*).

Infestment with *T. saginata* follows the ingestion of raw or partially cooked beef infested with the parasite occurring in the intramuscular connective tissue as *Cysticercus bovis.* The usual symptoms are vague abdominal discomfort and epigastric pain, vertigo (especially when hungry) and mild anemia with eosinophilia in some cases. Laboratory diagnosis is generally based upon finding the typical ova and gravid proglottides in the feces.

Infestment with *Taenia solium* is far more serious and results from the ingestion of raw or partially cooked pork ("measly pork") infested with the parasite occurring in any of the tissues (especially in striated muscles) as the larva, *Cysticercus cellulosae.* Man may also become infested through the ingestion of contaminated water or foods, particularly uncooked vegetables. Auto-infestment may occur through unclean personal habits in the case of an individual who harbors the adult parasite. Unless the infestment is heavy, however, there may be no symptoms, although muscular pains and weakness, fatigue, loss of weight and nervousness may develop. Cysterci, however, may occur in the brain, producing cerebral cysticercosis. Symptoms may not develop for several years until the death of the parasites provokes toxic inflammatory reactions of a severe and frequently fatal character. *T. solium* is likewise the most common larval tapeworm to invade the eye where cysterci occur in the vitreous humor, with infra-orbital pain and disturbances of vision. Cysticercosis may also produce palpable subcutaneous nodules. Laboratory diagnosis is based upon finding the proglottides and ova in the feces although the latter cannot be readily distinguished from those of *T. saginata.* The diagnosis of cysticercosis is made by a biopsy of a subcutaneous nodule and by roentgenologic examinations.

Infestment with *Hymenolepis nana* or *T. nana* is due to the swallowing of the ova and usually is dependent upon immediate contact, since the ova do not long survive outside the body. As a result, they are directly transferred from the hands to the mouth or indirectly by contamination of water and uncooked foods. Because of the unhygienic habits of children, the majority of infestments occur among them. The worms inhabit the upper three-fourths of the ileum and while symptoms are not ordinarily produced, the presence of large numbers of the parasites may produce a catarrhal enteritis in children, with asthenia, vertigo, strabismus, nervous disturbances and even convulsions. Laboratory diagnosis is based on finding the characteristic ova in the feces although several examinations of the feces on different days may be required.

Infestment with *Hymenolepis diminuta* or *Taenia diminuta* occurs much more rarely, the majority of cases also occurring in children under three years of age. Infestment is usually due to the ingestion of water or uncooked foods contaminated by intermediate hosts (especially rat fleas) infested by the larvae of the parasite. Symptoms may not occur but if they do, they are similar to those produced by *H. nana*. Laboratory diagnosis is based on finding the ova and proglottides in the feces.

EXAMINATIONS OF THE BLOOD, URINE AND TISSUES

In many very important diseases due to animal parasites, laboratory diagnoses are based on examinations of the blood, urine, sputum and the tissues (Table 68); also of the spinal fluid in the case of the late stages of African trypanosomiasis. It is true that the laboratory diagnosis of the intestinal types of schistosomiasis, due to infestment with *Schistosoma mansoni* or *S. japonicum*, is based on the finding of ova in the feces but since these diseases are essentially due to the infestment of the mesenteric veins with the worms, they are being considered herewith.

Malaria. Four species of plasmodia are parasitic for man: *Plasmodium vivax*, the cause of benign tertian malaria; *P. malariae*, of quartan malaria; *P. falciparum*, of malignant tertian or estivo-autumnal malaria; and the less known *P. ovale*, which produces a tertian form of the disease. Several other species of questionable status have been reported.

Final proof of malarial infection always depends upon the finding of the parasites or their pigments in the blood. It is the only method of making an accurate diagnosis, although the presence of plasmodia does not exclude the possibility of other superimposed febrile diseases. While in tertian and quartan infections the symptoms may be very characteristic, whenever possible diagnosis should always be confirmed by examinations of the blood. In estivo-autumnal and mixed infections the signs and symptoms are notoriously unreliable from a diagnostic standpoint, and blood examinations should never be neglected. Since any malarial infection severe enough to produce symptoms will show plasmodia in the blood, when the latter is expertly examined, the correctness of diagnosis in which they cannot be found is open to question.

In infections with *P. vivax*, *P. malariae* and *P. ovale*, all stages of the human life cycle of the parasites, as well as the gametocytes, occur in the peripheral blood, but in *P. falciparum* infections only the "rings" or young trophozoites and the young gametocytes are usually present. The plasmodia are most numerous in the peripheral blood during the latter part of the febrile paroxysm, while between the paroxysms, especially in *P. falciparum* infections, they are mostly within the capillaries of the internal organ, so that at this time repeated examinations by the thin and thick smear methods may have to be made before they can be found in the peripheral blood.

The blood may be examined by: (1) Fresh wet preparations; (2) thin films not only for parasites but likewise for morphologic changes in erythrocytes; (3) thick films, especially in chronic and latent malaria, and (4) concentrative methods which, however, are seldom more satisfactory than thorough examinations of thick films. When the latter fail to reveal the parasites, cultures of the blood are occasionally successful, but this procedure is of so little practical value that it is seldom employed. In chronic infections, especially if the patient is under quinine therapy, repeated thick film examinations will usually reveal the parasites

TABLE 68. SUMMARY OF THE CLINICAL INTERPRETATION OF PARASITOLOGIC EXAMINATIONS OF THE BLOOD, URINE AND TISSUES

Disease	Interpretation
Malaria	Due to four species of plasmodia: (1) *P. vivax* (benign tertian); (2) *P. malariae* (quartan); (3) *P. falciparum* (malignant tertian) and (4) *P. ovale* (a tertian form). Final proof of malaria always depends upon finding the plasmodia or their pigments in the blood. In their absence the correctness of the diagnosis of malaria is open to question. The parasites are most numerous in the blood during the latter part of febrile paroxysms. Thin and thick smears of blood should be examined; also by concentration methods. Cultures are of little practical value. Blood examinations are also of great value as criteria of cure; all gametocytes should be removed by treatment.
Leishmaniasis	Kala-azar or visceral leishmaniasis is due to infection with *L. donovani;* leishmania tropica or oriental sore to *L. tropica;* leishmania braziliensis or espundia to *L. braziliensis.* The laboratory diagnosis of kala-azar is based on (1) finding leishmania in the polymorphonuclear and large monocytes of the peripheral blood; (2) the examination of material removed from the liver, spleen or lymphatic glands by puncture; (3) cultures of the spleen and (4) by inoculation of hamsters with blood or puncture material. The laboratory diagnosis of leishmania tropica is based on (1) finding leishmania in material obtained from ulcers, especially by puncture of the margins and (2) by cultures. The laboratory diagnosis of leishmania braziliensis is based on (1) finding the leishmania in material obtained by puncture of the margins of initial lesions or from secondary nodules or ulcers and (2) by cultures.
Trypanosomiasis	African trypanosomiasis or "sleeping sickness" is due to infection with *Trypanosoma gambiense* or *Trypanosoma rhodesiense;* American trypanosomiasis or Chagas' disease is due to infection with *T. cruzi.* Early diagnosis and treatment of African trypanosomiasis is important. Laboratory diagnosis is based on finding the trypanosomes in the blood (especially during febrile periods) and by animal inoculation tests. Diagnosis in the late stages is based on finding the trypanosomes in the spinal fluid and by animal inoculation tests with blood, spinal fluid or material removed from lymphatic glands by puncture. Cultures are not usually successful. The laboratory diagnosis of Chagas' disease is difficult. Trypanosomes may be found in the blood during acute febrile periods in children or adults; guinea-pig inoculation tests and "xenodiagnosis" are helpful.
Filariasis	Filariasis is usually due to infection of the lymphatics by *Wuchereria bancrofti* (*Filaria bancrofti*) but may be caused by many other filarial worms. The laboratory diagnosis of filariasis due to *W. bancrofti* is based on finding the microfilariae in the blood between 10 P.M. and 2 A.M. In infestments due to *W. malayi* the microfilariae are likewise nocturnal

TABLE 68. SUMMARY OF THE CLINICAL INTERPRETATION OF PARASITOLOGIC EXAMINATIONS OF THE BLOOD, URINE AND TISSUES—(Continued)

Disease	Interpretation
	in migration but less absolutely than *W. bancrofti*. In filariasis due to *Acanthocheilonema perstans* and *Mansonella ozzardi* the microfilariae in the blood are nonperiodic.
Onchocerciasis and Loasis	The laboratory diagnosis of onchocerciasis due to *Onchocerca volvulus* is made by the finding of microfilariae or dead worms in material aspirated from suspected tumors or ocular lesions, or of macerated tissue removed by biopsy. The laboratory diagnosis of loasis due to *Loa loa* or the eye worm is based on finding microfilariae in the blood during the day or by the recovery of worms from their tunnels.
Dracunculiasis	Due to infestment with the Guinea worm *Dracunculus medinensis*. Laboratory diagnosis is based on finding the female worms in the subcutaneous tunnels of papulovesicular lesions of the feet or elsewhere, or of larvae in the vesicular fluids.
Trichinosis	Due to infestment with *Trichinella spiralis* from the eating of raw or lean pork or bear tenderloin carrying the cysts. Marked eosinophilia is suggestive. During the early stages adult worms or larvae may occur in diarrheal stools and larvae in the blood or spinal fluid but such examinations are seldom successful in diagnosis. Laboratory diagnosis is usually based on finding unencysted or encysted larvae in excised bits of muscle (deltoid, biceps or gastrocnemius) by press methods, after digestion in artificial gastric juice (preferred) or by the microscopic examination of serial sections.
Echinococcosis	Echinococcosis or hydatid disease is due to the ingestion of the ova of *Echinococcus granulosus*. Man is parasitized only by the larvae or hydatid stage of the worm producing hydatid cysts which are of three types: (1) unilocular occurring principally in the liver or spleen; (2) osseous and (3) alveolar which may be widely distributed throughout the body by the lymphatics or blood. X-ray examinations are particularly useful in locating cysts. Eosinophilia is usual. Laboratory diagnosis is based upon examinations of cyst fluids for scolices, brood capsules or daughter cysts. Preoperative exploratory punctures are inadvisable for diagnostic purposes.
Clonorchiasis	A disease of the bile ducts and liver caused by the fluke *Clonorchis sinensis* due to the eating of infested raw fish. Laboratory diagnosis is based on finding the ova in the feces or in the bile obtained by duodenal drainage.
Opisthorchiasis	Also a disease of the biliary ducts and liver caused by *Opisthorchis felineus* due to the ingestion of raw infested fish. The parasite may also occur in the pancreatic duct and intestine. Laboratory diagnosis is based on finding the ova in the feces or in the bile obtained by duodenal drainage.

TABLE 68. SUMMARY OF THE CLINICAL INTERPRETATION OF PARASITOLOGIC EXAMINATIONS OF THE BLOOD, URINE AND TISSUES—(Continued)

Disease	Interpretation
Paragonimiasis	Usually a disease of the lungs caused by the Oriental lung fluke *Paragonimus westermani* due to the ingestion of infested raw crabs or crayfish. Infestment may also occur in the skin and lymphatic glands (general type), the abdominal or pelvic organs (abdominal type) or the brain (cerebral type). Laboratory diagnosis is based on finding the ova in the sputum, feces or skin lesions. Eosinophilia is common.
Schistosomiasis	*Schistosomiasis* or *bilharziasis* is caused by any of three blood flukes namely, (1) *Schistosoma haematobium* producing the vesical or urinary type; (2) *Schistosoma mansoni* producing the intestinal type and (3) *Schistosoma japonicum* producing the Oriental intestinal type. Due to contact with water contaminated with cercariae discharged by infested snails. Primary infection occurs through the skin with distribution by way of the blood. Laboratory diagnosis is based on finding the ova in the urine or feces.
Trichomoniasis	*T. elongata* of the mouth is apparently nonpathogenic although frequently found in individuals with oral disease or with poor dental hygiene. *T. hominis* of the large and small intestine is found practically always associated with diarrhea. Its pathogenicity is doubtful but very large numbers may aggravate an existing enteritis and especially in young children. *T. vaginalis* is potentially pathogenic and frequently associated with vaginitis. The method of transmission is unknown. Apparently men may become infested through coitus but usually without symptoms. Only about 13 per cent of infested women develop symptoms of leukorrhea, itching, burning and intertrigo of the vulva and neighboring parts. Laboratory diagnosis of oral, intestinal and vaginal trichomoniasis is readily made by the direct microscopic examination of fresh wet preparations supplemented, if necessary, by cultures of the parasites on suitable media.

and especially the resistant gametocytes. Examinations should be made on several successive days before malaria is excluded.

Blood examinations are also important as criteria of cure. It is important that all gametocytes be destroyed by treatment before complete cure is assumed to have occurred in order to prevent the infection of Anopheles mosquitoes and the transmission of the disease. For this reason plasmochin should be administered along with quinine or atabrine or afterward to destroy gametocytes and cure carriers, although atabrine is effective against the gametocytes of *P. vivax* and *P. malariae*.

Various serologic tests have been suggested also in the diagnosis of malaria

(Chapter 17), but thus far none of them has proved as valuable as thorough and skilful examinations of the blood in the diagnosis of the malarial fevers, although complement fixation tests are promising as a diagnostic aid.

Leishmaniasis. *Kala-azar* or visceral leishmaniasis is due to infection with *Leishmania donovani.* The disease should be suspected in a patient from an endemic area who has chronic irregular fever, leukopenia and splenomegaly. It must be differentiated clinically from typhoid and paratyphoid fevers, undulant fever, relapsing fever, malaria and tuberculosis. Final diagnosis depends upon finding the parasites in the blood or in smears, cultures or animal inoculation tests of material from infected tissues. Stained films of the peripheral blood and especially thick films in the hands of skilled workers frequently show the parasites in the polymorphonuclear and large mononuclear leukocytes, but repeated examinations may be required. If the blood is negative, material procured by puncture of the spleen or liver may be stained and examined. Enlarged lymphatic glands may be excised and examined by smears and sections. Cultures of the blood and especially of the spleen are also of diagnostic value. Inoculation of hamsters with blood or material from the liver or spleen are also of value in cases where the parasites cannot be otherwise found. After the fifth month of the disease various serologic tests also possess diagnostic value, to be discussed in Chapter 18.

Cutaneous leishmaniasis, or Oriental sore, is due to infection with *L. tropica.* The local lesion must be distinguished from ulcers due to syphilis, blastomycosis and other infections. Laboratory diagnosis is usually easily made by the microscopic examination of smears of material obtained from the lesions as the flagellates are found in the cells of the granulating tissue, endothelial cells and large mononuclear leukocytes, but not in the peripheral blood. The parasites may not be found in smears of the floor of an ulcer owing to the presence of bacteria with which they cannot live. Consequently, exudative material should be obtained by puncture of the indurated margins of the ulcer. If the smears are negative, cultures should be made on the N.N.N. medium; if these are negative, treatment with antimony may be instituted, for if the suspected lesion is due to infection with *L. tropica* it will respond to such treatment.

American leishmaniasis, or espundia, is due to infection with *L. braziliensis* which produces nasopharyngeal or mucocutaneous lesions. The disease must be differentiated from yaws and syphilis. Laboratory diagnosis is established by the finding of the parasites in stained smears of material obtained by puncture of the edge of the initial ulcer or in material obtained from the secondary nodules or ulcerations in the mucous membrane. Cultures on the N.N.N. medium are also of diagnostic value. Complement fixation tests are of diagnostic value only in infections due to *L. donovani;* of those proposed that employing a nonspecific antigen prepared of the Kedrowsky acid-fast bacillus is to be preferred, to be discussed in Chapter 18.

Trypanosomiasis. African trypanosomiasis, or "sleeping sickness," is due to infection with *Trypanosoma gambiense* or *T. rhodesiense.* American trypanosomiasis, or Chagas' disease, is due to infection with *T. cruzi.*

The clinical diagnosis of African trypanosomiasis in the early stages may be difficult and must be differentiated from relapsing, enteric and undulant fevers, malaria, ancylostomiasis and syphilis. Early diagnosis, however, is essential, as prompt treatment is highly successful. In the late stages the disease must be differentiated from cerebrospinal syphilis, encephalitis lethargica and other diseases of the central nervous system. Final diagnosis depends upon the finding of the trypanosomes by laboratory examinations of the blood (especially during febrile periods), of the lymphatic glands in the early stages, and of the spinal fluid in the late stages. Blood examinations consist of the examination of wet preparations, of stained thick and thin smears and of smears after concentration methods. The same applies to examinations of sediments obtained by the thorough centrifuging of spinal fluids. Smears of material aspirated from enlarged lymphatic glands are also useful, as is the inoculation of white rats, mice or guinea pigs with blood, spinal fluid or gland material. Cultures on the N.N.N. medium may be employed although the parasites are cultivated with some difficulty.

The diagnosis of Chagas' disease is more difficult. Clinically it must be differentiated from leishmanian infections, goiter, hypothyroidism including cretinism, chronic malaria and hookworm disease. Final diagnosis depends upon finding the trypanosomes by the same methods as employed in the diagnosis of African trypanosomiasis. But examination of the blood, even with concentration methods, are usually fruitless, except during the acute febrile stage in children or during febrile exacerbations in the chronic stage in adults. If the blood is negative, the inoculation of guinea pigs should be used as a diagnosis procedure, as this is often successful in the chronic stages of the disease. *Triatoma,* free from *T. cruzi,* may be allowed to feed on the patient. After ten days, their feces are examined for metacyclic forms, which are injected into mice or rats. This method of "xenodiagnosis" of Brumpt is often useful. Complement fixation tests with an antigen made from cultures of *T. cruzi* have also proved useful in the diagnosis of chronic infections, to be discussed in Chapter 18.

Filariasis. Filariasis, or wuchereriasis, is most frequently due to infestment with *Wuchereria bancrofti* (*Filaria bancrofti*) but the disease may be also caused by many other filarial worms like *Onchocerca volvulus, Acanthocheilonema perstans, Mansonella ozzardi, Loa loa* (*Filaria oculi humani*) and *Wuchereria malayi,* as well as other species.

Clinical diagnosis of filariasis due to *Wuchereria bancrofti* is usually based on lymphangitis, lymphadenopathy, hydrocele, chyluria and elephantiasis. Calcified worms may be detected by roentgen ray examinations, which are also of assistance in locating associated living worms in the lymphatics for surgical removal.

Laboratory diagnosis in asymptomatic patients is best made by finding the microfilariae in the peripheral blood or chylous exudates by the thick-film method, using wet or stained preparations. Because of nocturnal periodicity, in most localities the blood should be examined between 10 P.M. and 2 A.M., except where the nonperiodic type prevails. The filariae require differentiation from those of other filarial worms. As stated in Chapter 19, skin tests conducted with filaria antigen are also helpful in diagnosis, although positive reactions, which are of a group character, may not be observed after the forms have become calcified.

Complement fixation tests with aqueous or alcoholic antigens of fresh or dried *Dirofilaria immitis* or *Onchocerca volvulus* are also of some clinical value, especially in active infestments, although negative reactions are of more value in suggesting the absence of filariasis than positive reactions are in establishing its presence (Chapter 18).

The presence of nodules, eosinophilia and ocular lesions is suggestive of *onchocerciasis* in endemic areas. Laboratory diagnosis may be made by the finding of microfilariae in material aspirated from suspected tumors. At times the latter are inhabited only by dead worms, in which case microfilariae are not found. Examination of tissue removed by biopsy and macerated on a slide in saline solution may reveal the presence of embryos. The same method may be employed in the case of ocular lesions. Only exceptionally are microfilariae found in the peripheral blood accompanied by an associated eosinophilia. Complement fixation tests are also helpful in diagnosis.

Examinations of the blood for nonperiodic microfilariae are also of great value in the laboratory diagnosis of filariasis due to *Acanthocheilonema perstans* and *Mansonella ozzardi*, although the former must be differentiated from the latter which are also unsheathed.

The laboratory diagnosis of *loasis* due to infestment with *Loa loa* or the eye worm is made by recovering the adult worms from their tunnels, but more frequently by finding the characteristic microfilariae in the blood during the day. Serologic and intracutaneous tests may be employed in suspected cases, but these tests are only indicative of filarial infestments as a group.

The laboratory diagnosis of filariasis due to *Wuchereria malayi* is also based upon the finding of the microfilariae in the peripheral blood; they are nocturnal in migration but less absolutely than *W. bancrofti*.

Dracunculiasis. Dracunculiasis is due to infestment with the Guinea worm *Dracunculus medinensis* (*Filaria medinensis*) from the swallowing of the parasite in raw water containing parasitized *Cyclops*, the necessary intermediate host.

The incubation period (eight to twelve months) is essentially symptomless. The disease is characterized by the formation of a papulovesicular lesion usually located on the soles of the feet between the metatarsal bones but may occur elsewhere. Beneath the blister the female worm may be found in a subcutaneous tunnel. Secondary bacterial infection is the rule. Laboratory diagnosis is made by finding the worms and larvae in these local lesions. Dead or calcified worms may be located by roentgen ray examination.

Trichinosis. Trichinosis, which is caused by the eating of raw or rare lean pork and, sometimes, tenderloin of the bear infested with *Trichinella spiralis*, is a disease which has apparently increased during the past decade. The seven to fourteen days following ingestion constitutes the first stage or that of invasion during which excysted larvae and adult worms commonly produce a catarrhal or ulcerative enteritis, with such symptoms as nausea, vomiting, colic, diarrhea or dysentery, fever and profuse sweating easily ascribed to other causes. This is followed by the stage of migration of the larvae characterized by a typhoidal type of fever, suborbital edema, sometimes maculopapular rashes, headache and at times delirium. As the larvae encyst in the muscles the third stage is reached with pronounced muscle pains and tendernesss due to myositis along with fever, edema, cachexia, prostration and not infrequently a lemon-yellow chemosis of the conjunctivae. Cardiac symptoms may be produced by larvae in the myocardium as likewise peripheral neuritis, ocular defects, restlessness, hallucinations and other manifestations of encephalitis due to involvement of the central nervous system. Consequently, the disease is characterized by many different objective and subjective symptoms easily misleading the physician. While the prognosis is good in mild cases, it is very grave in severe infestments, many patients succumbing to the toxemia, myocarditis, or to such complications as pneumonia, peritonitis or nephritis.

A marked eosinophilia (up to 50 per cent or more), together with other characteristic signs and symptoms, is suggestive of trichinosis. Recovery of the adult worms and larvae in the feces during the diarrheic stage or of larvae in the blood, spinal fluid or mother's milk during the period of migration constitutes specific laboratory diagnosis, but such examinations are not usually made unless the disease is suspected. Furthermore, adult worms are rarely found in the feces and detection of larvae in dehemoglobinized blood is seldom successful because so few larvae are present, while an examination of spinal fluid is scarcely warranted as a routine measure for the same reason. Under the circumstances, laboratory diagnosis is usually based on the finding of unencysted or encysted larvae in compressed samples of bits of the deltoid, biceps or gastrocnemius muscles obtained by biopsy. The digestion of muscle in artificial gastric juice, however, provides a centrifugate which is stated to be three times more accurate than the press method and four times more accurate than serial sections. As discussed in Chapter 19, skin tests are also very satisfactory diagnostic procedures, as are likewise precipitin tests, especially for the detection of mild and atypical cases.

Echinococcosis. Echinococcosis, or hydatid disease of human beings, results from the ingestion of the ova of *Echinococcus granulosus* in food or drink, or from contact with contaminated utensils or implements, whereby eggs of the worm are conveyed to the mouth and swallowed. The dog is the usual source of the ova, although the wolf, jackal and domestic cat have also been found infested.

Man is parasitized only by the larval or hydatid stage of the worm which produces hydatid cysts. These are usually of the unilocular type, occurring in the liver or spleen but sometimes occurring in the bones (osseous cysts) or widely distributed throughout the body by metastases by direct extension or through the lymphatics or blood stream (alveolar cysts). Some investigators believe this type of cyst is produced by a different species or variety of the parasite, but it is more generally accepted as being due to cramped quarters in which the cyst becomes implanted, with the result that germinal tissue breaks through with metastases of the buds.

Infestment is usually acquired during childhood, but, unless the brain or orbit is involved, symptoms do not usually develop until later in life. They are then comparable to those of a slowly growing tumor and depend upon the location of the hydatid. Clinical diagnosis is based on the presence of cystic tumors. The hydatid thrill, indicative of fluid, is a valuable sign when it can be elicited. Roentgenologic examinations, however, are particularly useful in diagnosis and in locating cysts.

A slight eosinophilia, usually not exceeding 6 per cent, is present in about one-half of cases but becomes more pronounced when seepage of the cyst contents occurs. Otherwise, laboratory diagnosis usually depends upon the skin test described in Chapter 19, supplemented by precipitin and complement fixation tests described in Chapter 18. Of course microscopic examinations of the cyst contents for the scolices, brood capsules or daughter cysts, are highly diagnostic but, unless operation is to be done at once, exploratory puncture for the purpose of obtaining fluid is contraindicated, since leakage may cause secondary echinococcosis and anaphylactic shock.

Clonorchiasis. Clonorchiasis is a disease of the bile ducts and liver occurring principally in China, Japan and neighboring countries, due to a fluke *Clonorchis sinensis*, contracted by the eating of raw infested fish. The lesions are usually localized in the distal biliary ducts, particularly of the left lobe of the liver. The injury to the host depends upon the number of worms, a few parasites producing little damage, but large numbers causing serious and progressive hepatic disease. Mechanical obstruction of the ducts, however, is uncommon; the chief lesions are inflammatory in character and are caused by the parasites and their toxic products.

As a general rule, the disease follows an insidious and chronic course, with periods of jaundice and periods of improvement. Clinical diagnosis is based on hepatic symptoms in patients from endemic areas who give a history of having eaten raw fish. The more advanced cases must be differentiated from malignancies of the liver, hydatid cyst, beriberi, and various forms of hepatic cirrhosis.

Laboratory diagnosis depends upon finding the characteristic ova in the feces or in the bile obtained by duodenal drainage. The ova must be differentiated from those of other heterophyid flukes.

Opisthorchiasis. Opisthorchiasis is also a disease of the biliary ducts and liver, occurring principally in central and eastern Europe and Siberia, caused by *Opisthorchis felineus* and likewise contracted through the eating of raw infested fish. The hepatic lesions and symptoms are similar to those of clonorchiasis, although the parasite may also occur occasionally in the pancreatic duct and in the intestine. Laboratory diagnosis depends upon the finding of ova in the feces or in the bile obtained by duodenal drainage.

Paragonimiasis. Paragonimiasis is usually a disease of the lungs due to the Oriental lung fluke, *Paragonimus westermani.* While it occurs chiefly in the Orient, it has a wide geographic distribution, cases also occurring in South America, although it is a rare disease in North America. Infestment of man usually occurs from the consumption of parasitized raw crabs or crayfish in endemic areas. Thereupon the metacercariae excyst in the duodenum, migrate through the intestinal wall, abdominal cavity, diaphragm and pleural cavity, penetrate into the lungs and finally arrive in the vicinity of the bronchioles, where they develop into adult worms in tissue capsules laid down by the host. This circuitous route of migration also offers a satisfactory explanation for the presence of these worms in atypical foci such as the lymphatic glands and skin (general type), the liver and other abdominal or pelvic organs (abdominal type) and the brain (cerebral type).

The symptomatology, therefore, may be quite varied. The pulmonary type, which occurs most frequently, must be differentiated from pneumonia, bronchiectasis, tuberculosis, pulmonary spirochetosis and pleural effusion. The sputum is usually viscid, frequently blood-tinged and peppered with rusty-brown flecks, consisting of masses of ova. Since the sputum is commonly swallowed, the ova may be found in the feces in about 40 per cent of cases. Eosinophilia is an early sign of the disease. The ova may be likewise found in the skin lesions of the general type. The diagnosis of the abdominal and cerebral types, however, is difficult but in most cases the complement fixation test is of value (Chapter 18).

Schistosomiasis. Schistosomiasis, or bilharziasis, is due to infestment with any of three blood flukes, namely, (1) *Schistosoma haematobium,* producing vesical or urinary tract schistosomiasis; (2) *Schistosoma mansoni,* producing intestinal schistosomiasis and (3) *Schistosoma japonicum,* producing Oriental intestinal schistosomiasis or Katayama disease. Infestment results from contact with water into which the cercariae of the schistosomes have been discharged from parasitized snails. The cercariae penetrate the skin of the feet, ordinarily while the victim stands in contaminated water. The disease is perpetuated by infested individuals who deposit feces in or near water courses containing the appropriate intermediate hosts. Sewage from towns in endemic areas, emptying into streams, may add to the contamination. Domestic animals, like dogs, cats, cattle, horses, and water buffaloes, also serve as reservoir hosts of the adult worms.

After the cercariae pass through the skin they enter the venous circulation. There is probably an impartial distribution of them throughout the body, but after passing through the lungs *Schistosoma haematobium* has a selective affinity for the veins of the hepatic portal system. Here the worms grow to maturity and then migrate into the inferior mesenteric veins where they eventually pass through the hemorrhoidal veins into the vesical and pelvic plexuses. The ova pass from the venules into the bladder wall where they may occlude the blood vessels and produce inflammation with painful or painless hematuria over long periods of time, followed by the objective and subjective symptoms of cystitis.

Mature *Schistosoma mansoni,* however, occur in the ileocolic and colic branches of the superior mesenteric vein and the colic and lower branches of the inferior mesenteric vein where the worms and their ova produce dysenteric symptoms with frequent stools containing mucus and blood.

The same is true of *Schistosoma japonicum* except that those worms which reach the intrahepatic portal circulation by way of the mesenteric artery and capillaries, proceed to feed, grow and migrate out to the branches of the superior mesenteric vein with a deposition of ova into the tissues of the small intestine and the production of enteritis.

In vesical schistosomiasis laboratory diagnosis depends upon finding the typical terminal-spined ova in the urine, usually in the blood and pus discharged at the end of micturition. In intestinal schistosomiasis the lateral-spined eggs are found in the stools. In acute dysenteric cases concentration methods are not usually necessary but are required in chronic cases because the ova become increasingly less frequent. Eosinophilia is suggestive in all cases. Complement fixation and precipitation tests are likewise of helpful diagnostic value, as discussed in Chapter 18, although the reactions are not species-specific or group-specific.

Trichomoniasis. Three trichomonads occur in man, namely, (1) *T. tenax* of the mouth, (2) *T. hominis* of the intestine and (3) *T. vaginalis* of the vagina. The pathogenicity of all three is questionable.

Trichomonas tenax is apparently nonpathogenic although, like *Endamoeba gingivalis,* it is most frequently found in dental caries, pyorrhea alveolaris, diseased tonsils, and in fusospirochetal infections of the gums, throat and lungs due to a favorable environment created by inflammatory conditions. As it is the only parasitic flagellate occurring in the human mouth, laboratory diagnosis is easily made by the microscopic examination of wet preparations or by cultures.

It is very unusual to find *T. hominis* in formed stools, so that the presence of the parasite is practically always in association with diarrhea; for this reason many investigators have regarded it as a pathogenic flagellate. But satisfactory proof of its pathogenicity is lacking although, if present in very large numbers, as is often the case, it apparently may suffice to aggravate an already existing enteritis of the ileum or colon and especially in infants and young children.

Trichomonas vaginalis, however, is potentially pathogenic, since in gynecologic practice it is frequently found associated with vaginitis. The incidence of infestment is high but variable. The method of transmission is not definitely known. If this trichomonad is the same as *T. hominis* it is easy to understand how an infestment of the vagina might occur, and it is thought that men may contract infestment of the urethra, bladder or prostate gland through coitus, although usually without the production of objective or subjective symptoms.

Trichomonas vaginalis is only found in women with abnormal conditions of the vaginal mucosa. However, only about 13 per cent complain of symptoms comprising profuse, frothy, creamy leukorrhea with considerable itching, burning and irritation. Inflammation ranges from a diffuse redness of the vagina and vestibule to an extensive intertrigo of the vulva and neighboring parts.

Laboratory diagnosis is readily made by direct microscopic examinations of fresh wet preparations of the discharges, supplemented if necessary by cultures of the parasite on suitable media.

13

THE CLINICAL INTERPRETATION OF EXAMINATIONS OF TRANSUDATES, EXUDATES AND SEMEN

Bacteriologic examinations of transudates and exudates accumulating in the serous cavities of the body, including the larger joints, are of the greatest clinical interest and are discussed in Chapter 15. However, physical, chemical and cytologic examinations frequently yield additional information of clinical value, especially in relation to differential diagnosis. While semen is neither a transudate nor an exudate, the clinical interpretation of its examination is included in relation to sterility and medicolegal applications.

Collection. Transudates and some types of exudates are readily obtained from the serous cavities for laboratory examination. If for diagnostic purposes only, from 10 to 30 cc. may be obtained by aspiration with a syringe and needle of 16 to 18 gauge. Otherwise, when removed for drainage purposes, larger amounts up to 100 cc. or more should be sent to the laboratory. Since coagulation interferes with some examinations, *it is advisable to add 1 cc.* of a sterile *2.5 per cent solution of sodium citrate to each 10 cc. of effusion* submitted. This is particularly necessary when exudates are suspected.

Rigid aseptic precautions are required not only for the protection of the patient against accidental infection, but for the prevention of contamination of the fluid, since bacteriologic examinations are essential and routinely indicated. Consequently, the skin should be carefully prepared and all instruments and glassware sterilized. The site should be carefully selected and the skin infiltrated with 1 per cent procaine solution.

To obtain fluid from the *pleural cavity* (paracentesis thoracis) the patient sits upright or has the shoulders elevated on pillows, leaning forward with the arms raised and the hands placed on opposite shoulders. The area of greatest dullness is determined. The site usually chosen is the fifth or sixth intercostal space in the midaxillary line. The sixth to the eighth interspaces below the angle of the scapula are chosen if the fluid is small in amount. The puncture should be made close to the upper border of the rib to avoid the intercostal blood vessels. Fluid should not be removed too rapidly, since sudden pulmonary edema or a rapid fall in blood pressure may develop.

In obtaining fluid from the *abdominal cavity* (paracentesis abdominis) the site of puncture is in the midline usually about half-way between the symphysis pubis and the umbilicus. The patient should sit up in bed, most conveniently on the edge, with the back well supported by either an assistant or with pillows. The bladder should have been emptied before making the puncture. When considerable fluid is present and large amounts are withdrawn, it is advisable not to allow the fluid to escape too rapidly and afterward to adjust an abdominal binder. *Great care is required in cases of tuberculous peritonitis,* since the intestines may be adherent to the abdominal wall; indeed, it is frequently safer to make a small incision under local anesthesia.

In obtaining fluid from the *pericardial cavity* (paracentesis pericardii) the patient should be placed in the semirecumbent posture. The usual site is the fifth intercostal space below the left nipple, with the needle directed upward, backward and toward the sternum. Some prefer the fifth or sixth interspace close to the sternal border and others the left costoxiphoid notch close to the ensiform cartilage. In other words, the puncture is neither as simple nor as safe as puncture of the pleural and peritoneal cavities. If the needle or trocar impinges against the heart, the movements of the organ will be imparted to the instrument. It is generally wise to make provisions for pericardotomy if there is a question as to the type of effusion present.

Synovial fluid can be readily obtained with little pain or discomfort. A short needle should be used and care taken not to traumatize the synovial membrane.

FORMATION OF EDEMA FLUIDS AND TRANSUDATES

The excessive accumulation of fluids in the tissue spaces is known as *edema* while excessive accumulations of noninflammatory fluids in the serous cavities are designated as *transudates*. The mechanism of their production is similar and essentially due to the fact that instead of there being a perfect balance between the inward and outward flow of fluid through capillary membranes, absorption is exceeded by transudation (Table 69).

Transudates are termed according to their location, as *hydrothorax* (pleural), *ascites* (peritoneal), *hydrocele* (scrotal), *hydropericardium* (pericardial), *hydroarthrosis* (synovial), etc. When occurring in the subarachnoid space they constitute the so-called "serous meningitis," which is a misnomer, not being due to infection or inflammation at all; for this reason the state is better termed "meningism" or cerebral edema and is discussed in Chapter 14.

The particular factor or factors concerned in the formation of edema and transudates are not always clear but it is evident that they may arise from various causes. Among these (1) a reduction in the colloid osmotic pressure of the plasma, *i.e.*, in protein concentration, ranks high in importance. Normally, fluid leaves the first part of the capillaries because the capillary pressure is greater than the colloid osmotic pressure, and is reabsorbed at a corresponding rate at the venous end of the capillary where the intracapillary pressure is lower than the colloid osmotic pressure. Consequently, anything which lowers the colloid osmotic pressure tends to produce edema and transudation. The colloid osmotic pressure is normally about 30 mm. of mercury and is chiefly due to the albumin fraction of the plasma. Therefore, edema and transudates may develop when the plasma albumin falls below 2.5 gm. per 100 cc. (hypoproteinemia) and account for the fact that there is usually a very low protein content. Such may occur not only in nephrosis but likewise in the anemias and when the diet is deficient in vitamins, fats or proteins.

(2) Increased permeability of the capillaries from dietary deficiencies, toxic agents or excessive heat is also a factor and especially since it facilitates the escape of the plasma proteins. This increased capillary permeability raises extracapillary pressure, with a further reduction in the difference between intracapillary and extracapillary pressures. Under such circumstances, edema fluids and transudates have a relatively high protein content, approaching or equalling that of exudates. But there is a tendency for edema and transudates to reach a certain degree and then become stationary, provided the conditions producing them remain constant, because when the extracapillary pressure reaches a critical level its opposition to intracapillary pressure prevents further transudation.

(3) General or localized increase in intracapillary blood pressure or reduction of tissue pressure may also be involved. The former results from venous stasis

TABLE 69. SUMMARY OF THE CLINICAL INTFRPRETATION OF EXAMINATIONS OF TRANSUDATES AND EDEMA FLUIDS

Constituent	Interpretation
Formation	*Edema* is the excessive accumulation of fluids in the tissue spaces. *Transudates* are accumulations of noninflammatory fluids in the serous cavities and are termed according to location, as hydrothorax (pleural), ascites (peritoneal), hydrocele (scrotal), hydroarthrosis (synovial), etc. May be due to any one or a combination of the following factors: (1) A reduction in the colloid pressure of the plasma due to hypoproteinemia in nephrosis, the anemias, avitaminoses, dietary deficiencies, etc. (2) Increased permeability of the capillaries from dietary deficiencies, toxic agents, excessive heat, anoxemia, etc. (3) General or localized increase of intracapillary pressure from venous stasis or thrombosis, pressure, etc. Also by reduction of tissue pressure. (4) Obstruction of lymphatic channels.
Physical Characteristics	Usually clear or opalescent; light straw or yellowish green in color. Do not coagulate unless considerable blood is present from puncture of a vein during collection or due to malignant tumors. True chylous transudates milky in appearance; may show coagulation. Due to chyle from ruptured or obstructed thoracic duct. Pseudochylous transudates also milky; may be relatively clear when removed and become milky on standing. May occur in lipoid nephrosis, chronic glomerulonephritis with nephrosis, carcinoma of peritoneum, etc. Specific gravity variable in relation to protein content but usually below 1.018.
Chemistry	*Total protein* usually less than 2.5 gm. per 100 cc.; frequently 0.1 to 1.0 gm. per 100 cc. Of diagnostic value. Largely composed of albumin with small amounts of globulin; no fibrinogen. Mucin present in joint transudates. *Chloride* slightly less than that of the blood plasma; usually 720 to 750 mg. per 100 cc. in terms of sodium chloride. *Glucose, uric acid, urea* and *creatinine* present in amounts approximately equal to that of the blood. *Calcium* usually 4.5 to 5.5 mg. per 100 cc. *Inorganic phosphorus* present in amounts approximately that in blood serum. *Sodium, magnesium* and *potassium* present in smaller amounts than in the blood. *Bilirubin* may be present in long-standing hemorrhagic transudates; usually increased in bilirubinemia with jaundice. Determinations of these constituents of little or no clinical value. *Neutral fat* and *fatty acids* absent; *lecithin* present (20 to 100 mg. per 100 cc.). Traces of cholesterol present. True chylous transudates are high in fat (0.05 to 3.85 gm. per 100 cc.). Pseudochylous transudates are high in lecithin and cholesterol with small amounts of fat (derived from fatty degeneration of cells); protein varies from 0.1 to 4.2 cm. per 100 cc.
Cytology	Usually but few cells largely of mesothelial types with an occasional lymphocyte and erythrocyte. Special methods of examination advisable when malignancy is suspected (see method of Papanicolaou). In carcinoma and to a lesser extent sarcoma, cells or fragments of tissue recognizable in many cases.
Bacteriology	Sterile unless contaminated during collection. See Chapter 15.

as in congestive heart failure, thrombosis of veins or obstruction to venous flow by pressure of tumor masses, etc. A certain amount of associated anoxemia, due to impaired blood supply or toxic substances, increases capillary permeability so that the edema fluid or transudate usually contains considerable protein but not as much as occurs in inflammatory edema. If the increased pressure is generalized, as in congestive heart failure, the edema is greatest where the pressure is greatest, namely, in the most dependent parts. But tissue pressure also exercises an influence in the distribution of generalized edema, which is greatest at sites where it is lowest, such as the eyelids or scrotum. This also plays a rôle in the formation of transudates in the pleural, peritoneal and other serous cavities.

(4) Finally, edema and transudates may be due to obstruction of the lymphatic channels even though the veins and capillaries are unaffected, as in elephantiasis due to filariasis. Since the pleural and peritoneal cavities largely depend upon the lymphatics for absorption, hydrothorax and ascites may be due to their obstruction, in which case the transudates are apt to be chylous in nature. Indeed, edema and transudates associated with carcinoma may result from the blocking of the lymphatics with cords of cancer cells, as well as from venous obstruction caused by the pressure of the growth.

The formation of cysts, however, appears to be largely due to the secretory activity of their lining mucous membranes and obstruction to the escape of their secretions. Otherwise, they may be the result of liquefaction necrosis of the tissues caused by infection with bacteria or animal parasites.

EXAMINATIONS OF EDEMA FLUIDS AND TRANSUDATES

Physical Characteristics. In *appearance* transudates and edema fluids are usually clear or opalescent and of a light straw or yellowish-green color unless blood is present. A deeper color may be observed in the presence of jaundice. They do not coagulate unless considerable blood is present from accidental puncture of a vein during collection or from malignant tumors. True chylous transudates (pleural or peritoneal) are milky in appearance owing to the escape of chyle from a ruptured or obstructed thoracic duct (filariasis most commonly) and spontaneous coagulation may occur. Pseudochylous or chyliform transudates have the same appearance. They may be relatively clear when first removed, the turbidity and milky appearance increasing upon cooling; some spontaneous coagulation may occur.

The *specific gravity* of transudates and especially of edema fluids is usually less than that of exudates. When the former are free of blood the specific gravity is usually below 1.018. Specific gravity, however, is subject to considerable variation according to the amount of protein present, which is dependent upon variations in the permeability of the capillaries in different parts of the body. Thus, pleural and peritoneal transudates contain more protein and consequently show higher specific gravities than spinal fluid, and values are as low as 1.005 in the case of subcutaneous transudates or edema fluids.

Chemistry. The total *protein* of transudates is likewise lower than that of inflammatory exudates and usually less than 2.5 gm. per 100 cc. Edema fluids usually show no more than 0.1 gm. or less per 100 cc., while pleural and peritoneal transudates due to congestive heart failure, nephrosis, uncomplicated cirrhosis of the liver, etc., may show no more than 0.1 to 1.0 gm. per 100 cc. However, if transudates are present for some time, water may be reabsorbed more rapidly than solids, with the result that the protein content is increased and eventually approaches that of an inflammatory exudate. Nevertheless, a determination of total protein is usually of value for differentiating transudates from inflammatory exudates. A relatively high protein content may occur in the subcutaneous edema fluid of acute nephritis, suggesting its dependence upon generalized capillary injury rather than upon other factors believed to be responsible for edema in chronic nephritis and myocardial failure. The same has been observed in the fluid of angioneurotic edema.

Albumin usually constitutes the largest part of the protein present in edema fluid and transudates because of the smallness and low viscosity of the molecule which permits its passage through capillary walls. Small amounts of the globulins may be likewise present but only rarely appreciable amounts of fibrinogen. Joint fluids, however, usually contain larger amounts of protein and likewise a substance closely resembling mucin.

The *chloride* content of noninflammatory edema fluid and transudates is somewhat higher than that of the blood plasma, ranging from 720 to 750 mg. per 100 cc. in terms of sodium chloride. The difference is due to the existence of a Donnan equilibrium dependent upon the higher concentration of protein in the plasma as compared with transudates.

Edema fluids and transudates contain *glucose* in practically the same concentration as that of the blood. The same is true of *creatinine, uric acid* and particularly *urea*. The *calcium* usually ranges from 4.5 to 5.5 mg. per 100 cc. and apparently represents the normal diffusible calcium of the serum; it increases with the protein content due to nondiffusible calcium in combination with protein. Transudates also contain approximately the same amounts of *inorganic phosphorus* as the blood serum but somewhat smaller amounts of *sodium, magnesium* and *potassium*. *Bilirubin* may be present in the pleural and peritoneal transudates of patients with congestive heart failure or cirrhosis of the liver without an increase in the blood; of course it is somewhat increased in the presence of bilirubinemia. Since, however, hemoglobin may be converted into bilirubin in the serous cavities, bloody fluids should be centrifuged and tested for bilirubin. A positive van den Bergh reaction indicates that bleeding occurred before paracentesis was conducted. Otherwise, determinations of these chemical constituents possess little or no clinical value.

Lipids (neutral fat and fatty acids) are not usually present in transudates. A small amount of lecithin, however, varying from 20 to 100 mg. per 100 cc., is usually present. Since capillaries appear to be permeable to cholesterol to about the same extent as to protein, only very small amounts are present in transudates. This is true even in nephrosis, in which the concentration of cholesterol in the blood plasma may be enormously increased.

Of course, the milky appearance of chylous transudates is due to the presence of chyle which is rich in finely divided fats; these fats may amount to as much as 0.05 to 3.85 gm. per 100 cc. They cannot be removed by centrifugation but are easily removed by extraction with ether.

The milky appearance of pseudochylous transudates is not due to the presence of chyle but chiefly to lecithin and cholesterol with small amounts of highly emulsified fats derived from fatty degeneration of the cells in the transudate and those lining the pleural or peritoneal cavities. It has also been observed that albumin in a highly dispersed state may impart a milky appearance to such fluids and, indeed, relatively large amounts of protein may be present, varying from 0.1 to 4.2 gm. per 100 cc. with some spontaneous coagulation. These may occur in lipoid nephrosis and in chronic glomerulonephritis with nephrosis; also in carcinoma of the peritoneum and in tuberculous pleurisy and peritonitis.

Cytology. Transudates are characterized by the fact that they contain but few cells in contrast with inflammatory exudates in which they are very numerous. Those commonly formed are mesothelial types comprising large cells, with abundant cytoplasm, and containing one, sometimes two, round or oval, palely staining nuclei. An occasional lymphocyte may be found but only rarely a few polymorphonuclear neutrophils or monocytes. A few erythrocytes are also commonly found, due to puncture, but cytologic examinations are hardly worth while when macroscopic amounts of blood are present, except when malignancy is suspected. In carcinoma, mesothelial cells predominate, but are accompanied by numerous lymphocytes and erythrocytes. Cancer cells are sometimes recognized in stained smears of sediment secured by centrifuging a portion of the transudate. Otherwise, it is advisable to centrifuge large amounts of transudate and to prepare sections of the sediment according to the method of Mandelbaum,[1] described many years ago. Examination of these by expert histopathologists has proved valuable in the diagnosis of malignant tumors involving the pleural and peritoneal cavities.[2,3] Malignant cells or fragments of tissue are found in a little over 50 per cent of cases. Carcinoma is more readily detected than sarcoma. Of course negative findings do not exclude malignant disease.

PAPANICOLAOU'S CYTOLOGY TEST FOR CANCER

While, in 1928, Papanicolaou advocated the examination of vaginal smears as an aid in the early diagnosis of uterine cancer, his method did not become widely known until fifteen years later.[4,5] It is based upon the well-known fact that carcinomas tend to break down superficially and that the exfoliated cells may be recognized in properly prepared smears stained by special methods. Needless to state, such examinations can be reliably made only by expert pathologists.

Various investigators have found the method a distinct aid in the diagnosis of carcinoma of the cervix and endometrium.[6-9] Of course, it fails in cases which do not shed tumor cells. Thus, Wiles and Hellwig [10] have reported falsely negative smears in 20 per cent of cases and state that since it is not possible to differentiate between changes due to irradiation, carcinoma *in situ* and invasive carcinoma, biopsy examinations should never be supplanted by smear examinations in the decisive diagnosis of the disease. Since Fremont-Smith and Graham [11] have found the method successful in the detection of six cases of uterine cancer in the routine examination of 308 unselected women, they regard it as possessing value as a "screening test," even in the detection of carcinoma of the cervix in the early (noninvasive) stage. The value of combining vaginal smear and biopsy examinations, however, is indicated by the fact that Graham observed correct diagnosis in 98 per cent of 181 cases of uterine carcinoma.[11] In 63 proved cases of uterine carcinoma, Scheffey and his colleagues[12] observed correct positive smears in 44 or 70 per cent. In other words, 30 per cent of cases were missed by single-smear examinations. On the other hand, excellent correlation was obtained in 437 patients who did not have carcinoma, since correct negative smears were observed in 430 or 98.4 per cent, while false positive results were observed in 7 or 1.6 per cent of cases. It is to be observed, therefore, that the good results for the entire group of 500 cases—that is, 94.8 per cent correct results—were influenced largely by the high proportion of negative patients. Consequently, while the clinical value of the vaginal smear method is not definitely settled it appears distinctly useful as a diagnostic aid whenever biopsy examinations are not feasible. In cases with accessible lesions, however, biopsy examinations are to be preferred or, at least, should always be employed in conjunction with vaginal smear examinations for the final diagnosis of carcinoma. Certainly this cytology test alone is no substitute for pelvic examinations and single negative smears do not exclude possible carcinoma. Conversely, a positive smear should always be confirmed by biopsy examinations of the cervix and/or of the endometrium obtained by curettage.

Direct smears of the cervix or of material collected on a speculum may be made. They should be thin and even, since thick smears are usually unsatisfactory. Douching should be omitted on the day of examination, since water in the smears interferes with cellular detail. Otherwise, material for the preparation of smears may be collected from the posterior of the cervix by means of a slightly curved pipette fitted with a rubber suction bulb.[10] Cannula are also employed for obtaining material from the endocervix and uterine cavity. The slides should be immediately fixed for at least five minutes in a solution of equal parts of 95 per cent alcohol and ether. Drying of the smears should be avoided. Aspirated materials for cytohistologic examinations also require immediate fixation.

This smear method has also been employed with varying success as an aid in the diagnosis of carcinoma of the bladder, the urine being concentrated by centrifugation; likewise, in the diagnosis of carcinoma of the prostate gland, employing smears of secretions expressed by massage. The method has also been employed in the diagnosis of bronchogenic carcinoma, smears being prepared of sputum or, preferably, of material obtained by bronchoscopic aspiration. According to Graham and her colleagues,[13] it has also proved of value in the detection of gastric cancer, employing gastric washings which must be examined quite promptly before digestion of cells has occurred.

FORMATION AND EXAMINATIONS OF EXUDATES

Exudates are essentially pus in varying degrees of dilution or concentration and usually differ in many important respects from transudates in physical, chemical and cytologic properties. But in some parts of the body, especially in the nose and colon, they must be distinguished from the excessive secretion of mucus due to allergy or other causes.

Formation. Since exudates are inflammatory in origin, they usually occur in acute or chronic infections with pathogenic bacteria or animal parasites. On the other hand, they may be caused by irritation or inflammation by sterile substances. Familiar examples are the sterile abscesses produced by the subcutaneous or

TABLE 70. SUMMARY OF THE CLINICAL INTERPRETATION OF EXAMINATION OF EXUDATES

Constituent	Interpretation
Formation	Caused by inflammation and essentially pus in varying degrees of dilution or concentration. Usually due to infection with bacteria or animal parasites. May be due to irritation or inflammation by trauma, extension or sterile substances without infection.
Physical Characteristics	Vary from serous, serofibrinous, hemorrhagic and seropurulent to purulent according to the acuteness and severity of inflammation and cellular content. Frothy when due to infection with anaerobic bacilli of the gas gangrene group. May show complete or partial coagulation due to the presence of fibrinogen. When free of blood usually light yellow to straw in color. May be greenish or greenish yellow due to *Ps. aeruginosa.* May contain mucin or mucin-like substance (seromucin). May occur as membranous or pseudomembranous exudates on free surfaces. Usually odorless. May be putrid due to putrefactive changes or have a fecal odor due to *Esch. coli.* Specific gravity varies according to protein and cellular content but usually above 1.018.
Chemistry	*Total protein* of the serous portions generally over 3.0 gm. per 100 cc. Particularly increased in severe infections when it may approach that of blood plasma (6.4 to 8.0 gm. per 100 cc.). When due to inflammation of lesser severity may range from 0.1 to 0.5 gm. per 100 cc. as in tuberculous exudates. Composed not only of albumin but of globulins and fibrinogen and is due to greatly increased capillary permeability; fibrinogen with spontaneous coagulation particularly frequent in pneumococcal exudates. *Glucose* usually reduced and may be absent due to glycolysis by bacteria and cells. *Chloride* generally reduced and roughly parallel to the protein content. Particularly low in pleural exudates due to pneumococcal pneumonia. *Creatinine, uric acid* and *urea* present and usually in amounts comparable to that of the blood plasma. *Cholesterol* present but reduced by frequent tapping. *Fat* may be present and particularly in tuberculous exudates. Likewise *bilirubin* in old hemorrhagic exudates. *Calcium* and *magnesium* content usually higher than in transudates due to increase of protein.

TABLE 70. SUMMARY OF THE CLINICAL INTFRPRETATION OF EXAMINATION OF EXUDATES—(Continued)

Constituent	Interpretation
Cytology	*Total cells* always higher than in transudates. May be as low as 200 to 500 per c.mm. in serous exudates and as high as 4000 to 40,000 or more per c.mm. in seropurulent and purulent exudates. Differential cell counts (*cytodiagnosis*) of greater clinical value and should always be included in routine examinations. Well-preserved or degenerated polymorphonuclear leukocytes greatly preponderate (85 to 95 per cent) in exudates in acute pyogenic infection. Many are phagocytic. Lymphocytes, monocytes and eosinophils may occur in late or chronic exudates. Small lymphocytes along with a few monocytes and polymorphonuclear neutrophils characteristic of tuberculous exudates (pleural, peritoneal, etc.). Heavy cellular exudates in the bowel discharges of bacillary dysentery, chronic ulcerative colitis, lymphogranuloma venereum and carcinoma of the sigmoid. Light cellular exudates in amebic dysentery and usually no exudates in simple diarrheas.
Bacteriology	Bacteriologic examinations by smears and cultures extremely important (see Chapter 15).

intramuscular injection of sterile agents—likewise the peritoneal exudates produced through irritation by blood, bile, pancreatic juice and intestinal obstruction without infection, being similar in origin to "aseptic meningitis" produced by intrathecal injections of sterile sera or other substances. Furthermore, sterile peritoneal and pleural exudates may be due to trauma or to irritation by extension from a nearby infectious process, the latter being similar to "meningitis sympathica" secondary to mastoiditis or sinusitis (Table 70).

Exudates are collected preoperatively for examination by aspiration, as previously described, or at the time of surgical intervention. For the diagnosis of peritonitis and especially in children, Steinberg[14] has devised a useful steel capillary pipet for the collection of minute amounts of peritoneal fluid sufficient for bacteriologic and cytologic examinations.

Physical Characteristics. Exudates vary greatly in *appearance* according to their origin and consistency. When low in cells they are serous, serofibrinous or hemorrhagic in character and usually indicative of acute virulent infection. Partial coagulation may occur because of the presence of fibrinogen from intense inflammation. When due to infection with the saccharolytic anerobic bacilli of gas gangrene, they are apt to be frothy in character. When more highly cellular the exudates may be seropurulent or frankly purulent and creamy, the latter constituting pure pus. When free of blood they vary in color from light yellow to straw but may be greenish or greenish-yellow due to the presence of *Ps. aeruginosa.*

Not infrequently exudates occurring in the serous cavities contain a mucin-like substance (seromucin) and those occurring in the stools of amebic dysentery are almost entirely composed of a clear, glairy mucus resembling the white of an egg streaked with blood. Exudates occurring on free surfaces, however, may be membranous or pseudomembranous in character.

Exudates are usually odorless or of a sweetish odor unless long retained with putrefactive changes. Those due to primary or secondary infection with *Esch. coli* frequently possess a characteristic fecal odor.

Since exudates usually contain much more protein and cells than transudates, the *specific gravity* is generally above 1.018 and may be as high as 1.035.

Chemistry. In purulent exudates resulting from severe inflammation, as illustrated by empyema, the *total protein* of the serous portion of the fluid, obtained by centrifugation, is generally over 3.0 gm. per 100 cc. and may be approximately the same as that of the blood plasma (6.4 to 8.0 gm. per 100 cc.). In the case of exudates resulting from inflammatory processes of lesser intensity, such as tuberculous pleurisy and tuberculous peritonitis, the total protein usually ranges from 0.1 to 0.5 gm. per 100 cc. This protein range is due not only to albumin but likewise to globulins and even fibrinogen, because greatly increased capillary permeability permits the passage of these larger molecules. Pneumococcal exudates appear to be particularly rich in fibrinogen, which accounts for the frequency with which they show spontaneous coagulation.

The *glucose* content, however, is usually much lower than that of transudates, owing to the destruction or glycolysis of this sugar by the action of bacteria and cells, the degree of reduction being dependent somewhat upon the intensity of the inflammatory process.

The *chloride* content is likewise usually lower than that of transudates and approaches that of blood plasma, with the degree of reduction varying roughly with the increase of protein in accordance with the laws governing the concentrations of readily diffusible substances on two sides of a semipermeable membrane under such circumstances. The chloride content of pleural exudates in pneumococcal pneumonia is particularly low because of the low chloride concentration of the blood plasma in this disease.

Creatinine, uric acid and particularly *urea* are present in practically the same concentrations as in the blood. *Cholesterol* is likewise practically always present, particularly in exudates of long standing, being probably derived from degenerative changes either in the cells present in the exudates or in those lining serous and abscess cavities. The presence of cholesterol is apparently due to the fact that capillaries have about the same permeability for cholesterol as for protein, so that the lipid content of exudates is roughly parallel to their protein content. The cholesterol content, however, may decrease markedly following repeated tapping, values ranging from 1 to 4.5 gm. per 100 cc. falling to 20 to 50 mg. per 100 cc. In some cases showing large amounts of cholesterol, *fat* is also present, particularly in tuberculous pleural and peritoneal exudates. *Bilirubin* may be present in old hemorrhagic exudates.

The *calcium* content is generally higher than that of transudates because of a nondiffusible fraction, which is probably in combination with protein; the same is likewise true of *magnesium* because of increased protein concentration.

Cytology. The *total cells* of exudates are always higher than that of transudates. They may be counted in noncoagulated fluids by the same technic as employed in counting the leukocytes of the blood. In serous exudates the counts may be as low as 200 to 500 per c.mm. or as high as 4000 to 40,000 or more per c.mm. in the case of seropurulent and purulent exudates.

A differential cell count or *cytodiagnosis,* however, is of far more clinical value and should be included in all routine examinations. These may be made with simple smears of exudates or of sediment stained by the method of Gram or in the same manner as blood smears. Supravital methods usually reveal more details of value in differentiating the cells present.[15]

As previously stated, the cells in transudates are largely composed of exfoliated mesothelial cells occurring singly or in sheets and usually showing some cytoplasmic edema or vacuolization. Only an occasional polymorphonuclear leukocyte is found, with no free bacteria or phagocytosis.

Early or moderately advanced exudates, however, due to infection with the pyogenic micro-organisms, invariably show a great preponderance of polymorphonuclear neutrophilic leukocytes. They are mostly well preserved and many are phagocytic, with usually less than 10 per cent mesothelial cells. In late exudates the polymorphonuclears likewise predominate, with increased phagocytosis, but with varying degrees of degeneration of the cytoplasm and nuclei; about 20 per cent of mesothelial cells are present in varying stages of degeneration. Indeed, the larger the number of well-preserved polymorphonuclears and the larger the percentage of them found phagocytic, the better the defense and prognosis. As shown by Steinberg,[16] in peritonitis following ruptured peptic ulcer, considerable mucin may be present in the exudate during the first twelve hours, along with numerous crystals of cholesterol. In bile peritonitis, cholesterol crystals are likewise numerous with a high percentage of polymorphonuclears and marked phagocytosis, while in intestinal obstruction the exudate shows a fairly large number of mesothelial cells with degenerative changes. As observed by Scott and Finland,[15] the cells in infected pleural exudates in pneumococcal pneumonia are almost exclusively polymorphonuclear neutrophils in various stages of degeneration, while in uninfected fluids they likewise predominate in the beginning with a decrease, along with an increase of monocytes and macrophages sometimes accompanied by eosinophilia, after the first week of the disease.

In tuberculous exudates (pleural, peritoneal, etc.), however, small lymphocytes along with monocytes and but few polymorphonuclear neutrophils greatly predominate, although the latter may be quite numerous in the early stages.

The clinical importance of cellular examinations of exudates in acute and chronic disorders of the intestines, especially of the colon, has been recently emphasized by Bercovitz.[17] Epithelial cells, polymorphonuclear leukocytes, lymphocytes and macrophage cells are the main types and since some of these may resemble the protozoa of dysentery, due care must be exercised in their identification. Heavy cellular exudates were observed by Bercovitz in bacillary dysentery, chronic ulcerative colitis, lymphogranuloma venereum and carcinoma of the sigmoid, but no cellular exudates at all, or but very minor ones, in ordinary diarrheas and acute amebic dysentery, although when the latter is complicated by perforation heavy exudates may occur.

FORMATION AND EXAMINATION OF SEMEN

As previously stated, semen is neither a transudate nor an exudate but is, for convenience, discussed in this place. Its examination for spermatozoa is important in appraising the male partner as a factor in any involuntarily sterile marriage, as well as for determining the effectiveness of sterilization by vasectomy; also in certain medicolegal situations where paternity is disclaimed on the basis of male sterility and in the examination of stains involving the charge of rape.

Formation. Spermatozoa are produced by the spermatogenic cells of the seminiferous tubules of the testicles under stimulation by the follicle-stimulating hormone of the anterior lobe of the pituitary gland. The seminiferous tubules, in their convoluted portions, are lined by several layers of epithelial cells derived originally from the germinal epithelium; these give rise in the mature male to spermatozoa. The youngest cells—the spermatogonia—lie against the basement membrane. These give rise to primary and secondary spermatocytes. The latter divide to form spermatids which are transformed without division into mature spermatozoa. Along with secretions the latter constitute semen which is transported by ducts to the epididymis and thence by the vas deferens of the spermatic cord to the ejaculatory ducts which unite to form the seminal vesicles located on the base of the bladder in front of the rectum.

Collection. *Examination of spermatozoa should be made as soon as possible after the collection of semen.* If a condom is employed it should be thoroughly washed and dried before use. In case of delay in examination it is better to transfer the specimen to a clean glass container to prevent any deleterious effects of the rubber on spermatozoa and particularly since the condom may contain spermicidal substances. Precautions to keep the specimen at 37° C. are unnecessary and may even prove harmful if a higher temperature is used, *i.e.*, by means of a thermos bottle. Furthermore, the motility of spermatozoa is of longer duration at lower temperatures which thereby allows more time for transportation to the laboratory. It is sometimes advisable to collect semen from the vagina for examination when it is thought that the vaginal secretions may be injurious to spermatozoa. Biopsy examinations of the testicles in relation to the etiology of infertility are discussed in Chapter 21.

Physical Characteristics. The *amount* of semen may vary from a few drops to 10 cc. (average 3 to 4 cc.) depending on the period of continence preceding its collection. Amounts less than 1.5 cc. are considered below normal, although sterility cannot be ascribed to this alone unless other deficiencies are found. The *color* is normally opaque and gray or slightly yellowish after extended continence. The *viscosity* of fresh semen is high; a slight viscosity is generally due to a low number of spermatozoa. Self-liquefaction, however, should take place and be completed within an hour. The absence of liquefaction may inhibit the movements of spermatozoa and thereby interfere with fertilization. The *p*H is always on the alkaline side, ranging from 7.2 to 8.9 with an average of about 7.8. Under abnormal conditions it never falls below 7.2.

Microscopic Examinations. A *total count of spermatozoa* may be made in the same manner as a total leukocyte count of the blood, employing a special diluting fluid. Normal semen contains an average of 70 to 150 million per cc. The lower the count below 60 million the less likelihood of fertility. However, this is not alone a cause for sterility unless other abnormalities occur.

Examinations for the presence and motility of spermatozoa are of primary importance. Their complete absence is termed *azoospermia*. Normally from 10 to 15 per cent of spermatozoa may be immobile. Their presence but complete immobility is known as *necrozoospermia* which is a cause for sterility. When present with only a few motile cells, the condition is designated *oligozoospermia* which may be a cause for sterility. Viscosity has an influence on motility; likewise the temperature at which the semen is kept. At 37° C. all spermatozoa are normally rendered immobile after 8 hours. At 4° C. some may remain motile for 4 days or longer. To determine viability it is, therefore, advantageous to keep semen at 4° C. and determine motility after 6, 12 and 24 hours.

It is also advisable to prepare stained smears of the semen for the purpose of detecting *immature and abnormal spermatozoa*. At the same time the examination should include *other cells, i.e.*, epithelium, leukocytes and pus cells and erythrocytes. *Crystals* may be found in abundance and especially in normal semen after standing.

Abnormalities may be referable to the heads of spermatozoa which may be too small or too large, with pointed or ragged edges, or show an atypical distribution of chromatin, the presence of acidophil vacuoles or double refracting heads. They are also referable to the middle portions which may be absent, bifurcated, swollen, etc.; likewise the tails may be double, curled, rudimentary or absent. However, semen containing up to 20 per cent abnormal spermatozoa is still considered fertile. The higher the percentage of abnormal types above this arbitrary standard, however, the more doubtful is fertility.

Medicolegal Examinations. The detection of semen in fresh stains upon clothing is frequently possible by finding spermatozoa (mostly without tails) by the microscopic examination of preparations prepared by soaking and teasing the material in small amounts of physiologic saline solution or dilute alcohol. Their presence is absolute proof that the stain in question is semen, although it is not possible to distinguish human semen from that of the lower animals in this way (Table 71).

Older seminal stains, up to several years, are best detected by the Florence reaction. The suspected material is softened with water, placed upon a slide with a few drops of the reagent (iodine, 2.54 gm.; potassium iodide, 1.65 gm. and distilled water, 30 cc.), and examined microscopically. If the material is semen, dark brown crystals in the form of rhombic plates resembling hemin crystals, or of needles often in clusters, are observed. Similar crystals, however, can also be obtained from stains of crushed insects, watery extracts of various internal organs, and certain other substances, so that they are not absolute proof of the presence of semen. Negative results, on the other hand, are practically conclusive of its absence.

Much more specific tests, not only for semen in general but for human semen

TABLE 71. SUMMARY OF THE CLINICAL INTERPRETATION OF EXAMINATIONS OF THE SEMEN

Constituent	Interpretation
Formation	Spermatozoa are produced by the spermatogenic cells of the seminiferous tubules of the testicles under stimulation by the follicle-stimulating hormone of the anterior lobe of the pituitary gland. Along with secretions they constitute semen which is transported by way of the epididymi and vas deferenia to the seminal vesicles.
Physical Characteristics	*Amount* varies from a few drops to 10 cc. depending on the period of continence preceding collection (average normal 3 to 4 cc.). Less than 1.5 cc. generally abnormal but not necessarily alone a cause of sterility. *Color* normally gray or yellowish. Normally of high *viscosity;* slight viscosity usually due to a low number of spermatozoa. Self-liquefaction should occur within an hour. Normal *p*H about 7.8; never below 7.2.
Microscopic Examinations	Normally semen contains an average of 70 to 150 million spermatozoa per cc. The lower the *total* count below 60 million, the less likelihood of fertility but this is not alone a cause for sterility unless other abnormalities occur. Complete absence of spermatozoa is termed *azoospermia.* Normally from 10 to 15 per cent of spermatozoa may be immobile. Viscosity and temperature have an influence on motility. The presence but complete immobility of spermatozoa is *necrozoospermia* which is a cause of sterility. Their presence with only a few motile cells is *oligozoospermia,* which is likely to be a cause of sterility. Sterility may be also due to immaturity or abnormalities of spermatozoa referable to their heads, middle portions or tails, although semen containing up to 20 per cent abnormal spermatozoa may be fertile. Examinations should also include epithelial cells, leukocytes, pus and erythrocytes. Crystals normally occur, especially after standing.
Medicolegal Examinations	Fresh seminal stains on clothing may be detected by finding spermatozoa in extracts of them. But it is not possible to distinguish human semen from that of a lower animal by this means. Older seminal stains (even up to several years) may be detected by the Florence reaction for crystals. It is not specific for human semen. Furthermore, similar crystals may be produced by stains of crushed insects, watery extracts of various internal organs and other substances, so that they are not absolute proof of the presence of semen. Negative results, however, are practically conclusive of its absence. Precipitation and complement fixation tests are more specific and capable of distinguishing between human semen and that of the lower animals. They do not, however, establish that semen is from a particular individual.

in particular, are those of precipitation and complement fixation, discussed in Chapter 17. However, it is not possible to determine that a stain of semen is that of a particular individual by these serologic procedures.

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THE CLINICAL INTERPRETATION OF CEREBROSPINAL FLUID EXAMINATIONS

Examinations of the cerebrospinal fluid have proved of great value in the diagnosis and differential diagnosis of the acute and chronic meningitides due to infections with bacteria and some of the viruses; also of syphilis of the central nervous system and all cases of the disease in relation to treatment and cure; cerebral hemorrhage or that due to trauma; tumors and abscesses of brain, spine or spinal cord; in the study of coma of unknown etiology, etc. Much information of clinical value is to be obtained by the various physical and chemical examinations discussed in this chapter; also by bacteriologic examinations, discussed in Chapter 15, and serologic examinations, discussed in Chapters 17 and 18.

FORMATION

It is now the consensus that the cerebrospinal fluid is normally produced by the highly vascular choroid plexus in the ventricles of the brain by a process of filtration from the blood plasma through a selectively permeable membrane (Table 72). But since under normal conditions the hydrostatic pressure of the capillary blood is believed to be greater than that of cerebrospinal fluid, it is evident that ultrafiltration alone does not account for its formation and that the cells of the choroid plexus perform work during the process.[1]

Furthermore, there is some anatomic evidence indicating that the cerebrospinal fluid may be, at least in part, a secretion of the cells of the choroid plexus; likewise that the perivascular spaces and the ependymal cells of the ventricles and the spinal canal may participate to some degree in its production. But there is no evidence of any secretory activity of the arachnoid villi.

TABLE 72. SUMMARY OF THE FORMATION, ABSORPTION AND COLLECTION OF THE CEREBROSPINAL FLUID

Subject	Mechanism
Formation	(1) Largely by a process of filtration of blood plasma by the choroid plexus. (2) Small amounts produced by the secretions of the choroid plexus and the ependymal cells of the ventricles and spinal canal; also from the perivascular spaces. Rate of formation not definitely known. Probably one or more liters per day with frequent renewal. Increased production in acute and chronic congestion of the meninges by transudation of plasma; also by inflammatory exudates in meningitis.

TABLE 72. SUMMARY OF THE FORMATION, ABSORPTION AND COLLECTION OF THE CEREBROSPINAL FLUID—(Continued)

Subject	Mechanism
Permeability of the Choroid Plexus	Highly permeable to chlorides, magnesium, sodium, and CO_2. Less permeable to proteins, urea, glucose, and other organic and inorganic substances normally present in the plasma. Very slightly, if at all, permeable to bilirubin, fibrinogen, complement, natural and acquired antibodies, and cholesterol in the plasma. Freely permeable to alcohol; less so to chloroform, urethane and hexamethylenamine. Practically impermeable to toxins and other chemical agents under normal conditions. Permeability may be increased (1) by an increase of substances in the plasma; (2) by meningitis or other factors breaking down the barrier of the choroid plexus; or (3) by increasing permeability of the capillaries of the meninges.
Circulation and Absorption	Circulation downward from the ventricles followed by circulation upward to the base of the brain. Mainly absorbed through the arachnoid villi into the great dural sinuses. True solutions readily absorbed; colloids more slowly; particulate matter not at all. Absorption reduced or retarded by meningitis or states producing a prolonged rise in venous pressure. Rapidly absorbed after death.
Functions	Chiefly mechanical by forming a water bed protecting the cord, brain and nerves. Aids in keeping blood volume constant in the brain, cord and meninges. Provides a medium for the exchanges of metabolic substances. May aid in internal respiration and the regulation of alkali reserve in the tissues of the central nervous system. Apparently does not convey hormones or enzymes. Affords no protection against infection or toxins.
Collection	By spinal (lumbar) or cisternal puncture; without danger when properly conducted. Examinations *indicated* (1) as aids in the etiologic diagnosis of all cases of known or suspected meningitis including "serous meningitis" and aseptic meningitis; (2) for the detection of asymptomatic and symptomatic neurosyphilis and in relation to the treatment and cure of all cases of syphilis; (3) as aids in the diagnosis of cerebral edema, multiple sclerosis, poliomyelitis, encephalitis, tumors and abscesses of the brain, tumors of the spine or spinal cord, cerebral hemorrhage, hemorrhage due to trauma and cases of coma of unknown etiology. *Contraindicated,* or conducted with precautions, in suspected tumors of the posterior fossa. Not contraindicated in hemorrhage and papilledema if *slowly* withdrawn with careful watch for respiratory distress. Not contraindicated in septicemia or localized meningitis. Headache may follow, especially in ambulatory patients.

The rate of its formation is not well established, but when continuous drainage occurs, as through the nose or ears from fractures of the base of the skull, as much as several liters per day may drain away. Experimental investigations have indicated that the fluid is renewed every three or four hours but this calculated rate of formation is probably too high.

In acute and chronic congestion of the meninges, however, the volume of cerebrospinal fluid is increased, not only by increased permeability of the choroid plexus, but from increased transudation of plasma through the capillaries. Likewise, in acute and chronic meningitis it is increased by the production of inflammatory exudates.

Permeability of the Choroid Plexus and Meninges. Filtration by the choroid plexus and the meninges is highly selective. As shortly to be discussed, the chemical constitution of normal cerebrospinal fluid is essentially that of Locke's solution plus small amounts of glucose and plasma proteins. However, chlorides, magnesium, sodium and carbon dioxide of the plasma pass very freely.

Albumin, globulins, urea, creatinine, amino acids, uric acid, lactic acid, glucose, acetone, lipase, amylase, and such inorganic substances as calcium, potassium, phosphates, sulfates and iron pass to some extent owing to partial permeability. However, when some of these are greatly increased in the plasma, increased amounts are commonly found in the cerebrospinal fluid with special reference to glucose, urea and chlorides.

On the other hand, bilirubin, fibrinogen, complement, natural antibodies (antitoxins, agglutinins, opsonins, etc.) and cholesterol do not pass at all or but in minute amounts. Even when these are greatly increased in the plasma only traces, or none at all, occur in the cerebrospinal fluid, provided the choroid plexus and meninges are not involved.

As far as foreign substances are concerned, only alcohol passes freely although chloroform, urethane and hexamethylenamine pass to a considerable extent. Arsenic, lead, mercury, bromides, iodides, salicylates, strychnine, the sulfonamides and toxins do not pass at all or only in very minute amounts. But permeability is commonly increased to some extent in acute and chronic meningitis and especially through a breakdown in the barrier of the choroid plexus. Thus, in neurosyphilis, the pentavalent arsenical compounds and bismuth may pass to a considerable extent, as may some of the sulfonamides in acute meningitis with special reference to sulfanilamide. Naturally, this has an important bearing upon the treatment of acute and chronic infections of the central nervous system.

Circulation and Absorption. The fluid formed in the lateral ventricles passes through the foramen of Monro to join the fluid produced in the third ventricle and thence through the aqueduct of Sylvius to the fourth ventricle. From the latter it escapes by way of the foramen of Magendie and the foramina of Luschka into the subarachnoid space and the cisterna magna. From there it passes slowly down the spinal subarachnoid space and then, with some loss due to absorption and some gain due to formation by the cells lining the channel, it returns to the cerebral subarachnoid space. The circulation upward from the cisterna magna is somewhat more rapid and the fluid bathes the base of the brain, the cerebral hemispheres, and indeed the whole central nervous system.

Normally, the fluid is mainly absorbed through the arachnoid villi into the great dural sinuses with, possibly, a small part escaping into the true lymphatic vessels. Key and Retzius postulated that it was absorbed through the pacchionian granulations but these are absent from the brains of infants and are now regarded as pathologic enlargements of a few of the arachnoid villi. In other words, it has been established that absorption occurs through the numerous microscopic arachnoid villi instead of the pacchionian bodies or granulations.

Since the hydrostatic pressure in the subarachnoid space is always greater than that in the dural sinuses, filtration is apparently adequate to account for the absorption of cerebrospinal fluid into the venous blood. True solutions readily pass through the subarachnoid villi, colloids more slowly, the rate depending upon the size of the molecule, and particulate matter not at all. Absorption is reduced or retarded by prolonged rise in venous pressure. Cerebrospinal fluid is rapidly absorbed after death.

FUNCTIONS

The cerebrospinal fluid serves as a protective covering for the brain and spinal cord. In other words, its main function is a mechanical one of forming a water bed and thus preventing jarring as well as equalizing pressure in the subarachnoid space and the ventricles of the brain. Furthermore, by changes in its volume, compensation for changes in the amount of blood is effected and the contents of the cranium and spinal canal are thereby kept quite constant.

In addition, there is probably considerable exchange of metabolic substances between the nerve cells and the fluid. Thus, according to Mott, it conveys oxygen and glucose to the tissues and returns carbon dioxide to the blood. Furthermore, it may also aid in the regulation of the alkali reserve of the nervous system through the presence of carbonates. Aside, however, from the possibility of serving these metabolic and respiratory functions, there is no support for the claims that it may be a carrier of internal secretions and enzymes. Certainly it is not capable of destroying toxins and, indeed, affords little or no immunologic resistance to infection of the meninges, brain or spinal cord. As a matter of fact, it is a good culture medium, which adds to the necessity of exercising aseptic precautions during its collection by spinal or cisternal puncture.

COLLECTION

Cerebrospinal fluid is usually collected by spinal puncture. Cisternal puncture is advocated by some because of its technical ease and greater freedom from pain and puncture headache, but it is not recommended except in cases of subarachnoid block due to purulent exudates or other causes.

Indications and Contraindications. Aside from the use of spinal puncture and drainage for therapeutic purposes, examinations of the cerebrospinal fluid are indicated as indispensable aids in establishing etiologic diagnosis not only in all cases of known or suspected acute and chronic meningitis due to infection, but in the diagnosis of so-called "serous meningitis" or "meningismus" and aseptic meningitis as well; also in all cases of chronic syphilis for the detection of asymptomatic or symptomatic infection of the central nervous system as well as in relation to the treatment of the disease. Indeed, no case of syphilis can be regarded as cured unless at least one examination of the cerebrospinal fluid has shown a normal pressure, normal total cells and negative protein, Wassermann and colloidal gold reactions, as discussed in Chapter 18.

Spinal fluid examinations are also indicated as aids in the diagnosis of cerebral edema, multiple sclerosis, acute anterior poliomyelitis, lethargic and other types of encephalitis, tumors and abscesses of the brain, tumors of the spine or spinal cord, cerebral hemorrhage, hemorrhage due to trauma and in cases of coma of unknown etiology.

Many authors list a considerable number of contraindications to spinal puncture with special reference to cerebral hemorrhage and tumors in the posterior fossa. But the danger of increasing hemorrhage by reducing spinal fluid pressure is so slight, when the minimal amount of fluid is *slowly* withdrawn, that the operation is seldom contraindicated. The same is true in all cases of increased intracranial pressure with papilledema, provided the fluid is removed very slowly, with a careful watch for respiratory distress which may be due to herniation of the medulla or cerebellum in the foramen magnum. For this reason a spinal or cisternal puncture should not be done at all or only with extraordinary precautions in suspected tumors of the posterior fossa of the skull.

It is sometimes stated that spinal puncture should not be done in the presence of septicemia because of the danger of favoring the localization of micro-organisms from the blood in the meninges. Experimental and clinical investigations, however, have not shown this to occur unless, possibly, when some substance is injected at the same time capable of producing an aseptic meningitis with a breakdown of the barrier of the choroid plexus. The same is true in suspected localized meningitis, provided only small amounts of fluid are slowly removed.

Accidents have happened in connection with spinal puncture, due to injury of the cord, infection, or other causes, but clinical experience has amply proved that the operation is so safe, when properly conducted, that it should never be omitted when examinations of cerebrospinal fluid are required for diagnostic or, by drainage, for therapeutic purposes. It is true that spinal puncture headache may follow in a small percentage of cases, even under the best of conditions and especially in ambulatory cases, but the information to be gained usually far outweighs the temporary incapacity and discomfort with no after effects at all.

Technic. 1. Spinal puncture for the collection of cerebrospinal fluid may be conducted in an office or laboratory, but is better done in a hospital or the home of the patient, since it is advisable for the patient to rest in bed for at least eighteen hours immediately after the puncture as a safeguard against spinal puncture headache.

2. The *needle* should not be too large, in order to reduce pain to a minimum and to inflict the minimum of damage to the meninges. Gage No. 19 is about right, unless acute suppurative meningitis is suspected, in which case No. 15 may be used if a purulent and thick fluid is present. The needle should be sterilized just before use and should be perfectly straight and sharp with a short bevel. Crooked, rusty, dull and unnecessarily large needles are the usual causes of failure and the infliction of unnecessary pain.

FIG. 14. SPINAL PUNCTURE IN THE SITTING POSITION

The patient is sitting on the edge of a chair and is bent forward. The crests of the ilia are indicated by black lines, and are on a level with the spinous process of the fourth lumbar vertebra. The "soft spot" is found just above. (From Kolmer, *Chemotherapy with Special Reference to the Treatment of Syphilis,* W. B. Saunders Co.)

3. The *sitting position* may be used in the puncture of ambulatory adults, as shown in Figure 14, but the reclining posture with the patient lying on his right side (Fig. 15) is recommended, especially if the spinal fluid pressure is to be taken. The latter is required in the case of children and sick adults. It is important that the back be well arched with no slumping forward of the body (Fig. 16).

4. The skin should be carefully disinfected with tincture of iodine followed by alcohol. The hands of the operator should be likewise carefully cleansed and the use of sterile rubber gloves is recommended. The operative field should be protected with sterile sheets and towels.

5. With adults the puncture can usually be made without a general anesthetic. The skin may be infiltrated with sterile 1 per cent procaine or butyn solution (Fig. 17). Struggling children and adults may require a few drops of chloroform, as it is dangerous to conduct the puncture under such conditions since the needle may be broken.

6. Puncture is best conducted between the fourth and fifth or between the third and fourth lumbar vertebrae.

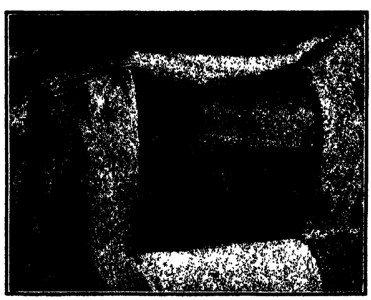

FIG. 15. SPINAL PUNCTURE IN THE PRONE POSITION WITH THE BACK WELL ARCHED AND PERPENDICULAR TO THE TABLE

(From Kolmer, *Chemotherapy with Special Reference to the Treatment of Syphilis,* W. B. Saunders Co.)

7. The "soft spot" between the spinous processes is located and the needle *gently and slowly* passed in the middle line. The distance varies according to the age and weight. A peculiar "give-way" sensation to the needle denotes entrance into the subarachnoid space. or during its passage the stylet may be removed from time to time to determine whether or not it has entered sufficiently as shown by flow of fluid.

FIG. 16. IMPROPER POSITION FOR SPINAL PUNCTURE IN THE PRONE POSITION

The back is not arched and has slumped forward. (From Kolmer, *Chemotherapy with Special Reference to the Treatment of Syphilis*, W. B. Saunders Co.)

8. If pure blood is obtained, the needle should be withdrawn and the needle cleansed or the puncture repeated with a fresh needle.

9. If there is no flow of fluid the needle may be greatly turned or slightly withdrawn or entered a little farther. "Dry taps" are usually due to the fact that the needle has not entered the subarachnoid space.

10. The pressure should be taken before the escape of fluid (Fig. 18).

11. *Fluid should be collected in two sterile tubes*, one of which (No. 2) may contain a trace of powdered potassium oxalate to prevent coagulation. From 3 to 5 cc. may be collected in No. 1 to be used for culture and the Wassermann test even if it is slightly blood-tinged. A similar amount may be collected in No. 2 to be used for the total and differential cell counts, protein and sugar determinations and the colloidal tests (gold, mastic or benzoin). The fluid should be free of blood.

FIG. 17. PRODUCING LOCAL ANESTHESIA

(From Keen's *Surgery*, W. B. Saunders Co.)

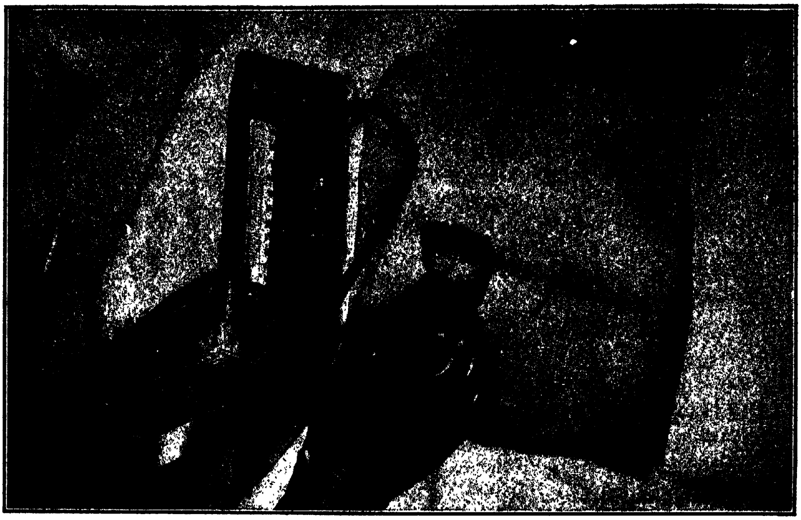

FIG. 18. MEASURING SPINAL FLUID PRESSURE WITH A MERCURY MANOMETER
(From Kolmer, *Chemotherapy with Special Reference to the Treatment of Syphilis*, W. B. Saunders Co.)

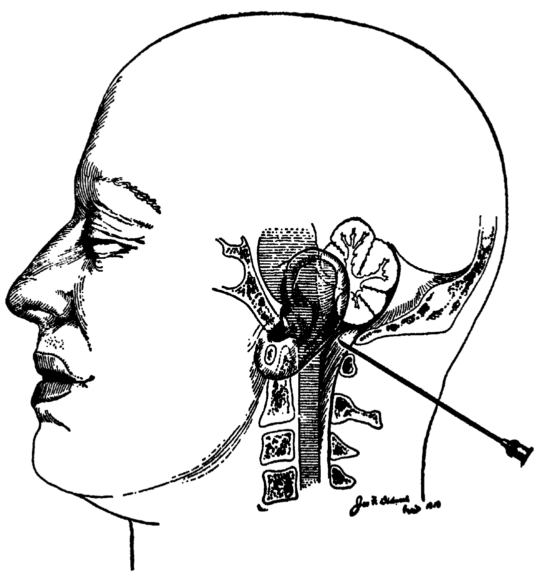

FIG. 19. LANDMARKS USED IN PERFORMING CISTERNAL PUNCTURE
(From Wegeforth, Ayer and Essick in Kolmer, *Chemotherapy with Special Reference to the Treatment of Syphilis*, W. B. Saunders Co.)

12. The needle is now quickly withdrawn, the iodine removed from the skin and the puncture sealed with flexible colloidin or with an aseptic dressing. The patient should rest on the back for at least half an hour and preferably stay in bed for at least eighteen hours to reduce the chances of developing spinal puncture headache, which is believed to be due to the continued leakage of spinal fluid into the epidural space (hence the advisability of using as small a needle as possible and of reaching the fluid at the first puncture).

13. Puncture of the cisterna magna is sometimes necessary or advisable for the collection of cerebrospinal fluid or purposes of drainage of the subarachnoid space. The landmarks are shown in Figure 19.

PHYSICAL CHANGES

Considerable information of diagnostic value is to be gained at the bedside by manometric determinations of pressure and the appearance of the fluid.

Amount and Pressure; The Queckenstedt Test. The total *amount* of cerebrospinal fluid has been variously estimated, but satisfactory data in relation to age and body weight are not available. For the adult it is thought to vary from 100 to 150 cc. which roughly corresponds to about 1 cc. per pound of weight (Table 73). Under the circumstances the withdrawal of 5 to 10 cc. for diagnostic purposes is harmless and especially since it is rapidly replaced.

The *pressure* of the cerebrospinal fluid under normal conditions has been stated to vary from 60 to 200 mm. of water or from 0 to 8 mm. of mercury when the individual is in the recumbent position and perfectly quiet. The average is commonly placed at 100 to 200 mm. of water. Pressures between 200 and 250 mm. are suspiciously increased and are definitely abnormal over 250 mm. It is not possible to define the limits of abnormally low pressure except to state that 90 mm. or less may be so regarded. There are no differences in relation to sex and apparently no great variation in relation to age; it is to be noted, however, that pressure readings in infants and young children are notoriously unreliable because complete physical and emotional relaxation are most difficult to attain. The fluid usually flows from the needle at the rate of about one drop per second but calculations made on this basis are very inaccurate. The pressure is almost doubled in the sitting position and averages about 200 mm. of water. Crying, coughing, sneezing, excitement, emotional states and general anesthetics raise the pressure, presumably by increasing the size of the capillary bed in the brain and meninges. Recent investigations have indicated a reciprocal relationship between blood and spinal fluid volumes with an "elastic" factor which takes into consideration a component depending on the ease of vascular adjustments as well as the distensibility and collapsibility of the meningeal sac.[2]

As a general rule, it is advisable to measure the pressure before the withdrawal of fluid; again after the withdrawal of 5 cc., with a final reading after the withdrawal of 10 cc. Normally the pressure falls 30 to 50 mm. of water. A drop of less than 20 mm. is suggestive of a large reservoir, as in hydrocephalus or "serous meningitis," while an excessive drop is suggestive of a small reservoir, as in loculation of the fluid below a cord tumor. The *Ayala quotient* [3] is determined by taking the initial pressure, withdrawing 10 cc. of fluid, and taking the final pressure expressed as follows: $\frac{\text{10 cc.} \times \text{final pressure}}{\text{initial pressure}}$. Normal values are 5.5 to 6.5. A value of

TABLE 73. SUMMARY OF THE CLINICAL INTERPRETATION OF MACROSCOPIC AND MICROSCOPIC CHANGES IN THE CEREBROSPINAL FLUID

Constituent	Normal	Abnormal
Amount	Not definitely known; probably from 100 to 150 cc. in the adult or roughly about 1 cc. per pound of weight.	Pressure from 200 to 250 mm. of water slight increase; above 250 definite increase.
Pressure	In the recumbent posture usually 100 to 200 mm. of water or 0 to 8 mm. of Hg. Almost doubled in the sitting position. Bears a relationship to blood volume with an "elastic" factor. Increased (1) by crying, coughing, etc.; (2) by the administration of large amounts of isotonic solutions; (3) by compression of the jugular veins. *Decreased* by the intravenous injection of hypertonic solutions. Pressure calculated on rate of flow from the needle very inaccurate. Advisable to take initial pressure and again after the withdrawal of 5 and 10 cc.	*Increased pressure* in (1) acute and chronic meningitis of all types; (2) meningisms and cerebral edema; (3) brain tumors, especially those situated subtentorially; (4) hydrocephalus; (5) cerebral and meningeal hemorrhage; (6) brain abscess; (7) cerebral thrombosis; (8) congestive heart failure, uremia, polycythemia, etc. *Decreased pressure* in (1) fainting and shock; (2) during spinal puncture headache; (3) in long-standing degenerative disease of the central nervous system; (4) below lesions producing complete or partial block of the spinal subarachnoid space; (5) brain tumors blocking the foramen magnum; (6) in obstructive hydrocephalus; (7) in states of dehydration. May be below average normal in some apparently healthy individuals.
Ayala Quotient	A rough estimate of the cerebrospinal fluid reservoirs; normally 5.5 to 6.5.	A quotient of 7 or higher suggestive of hydrocephalus or meningitis (especially "serous meningitis"). A quotient of 5 or less suggestive of brain tumor.
Queckenstedt Test	Conducted by compression of the jugular veins. Normally causes an immediate rise of pressure.	Rise of pressure does not occur in complete subarachnoid block; delayed or slight rise in partial block. Rise of pressure reduced or absent by pressure upon the jugular vein on the affected side in lateral sinus thrombosis; normal rise on compression of the jugular vein on the unaffected side.

TABLE 73. SUMMARY OF THE CLINICAL INTERPRETATION OF MACROSCOPIC AND MICROSCOPIC CHANGES IN THE CEREBROSPINAL FLUID—(Continued)

Constituent	Normal	Abnormal
Appearance	Perfectly clear, transparent and colorless. May be rendered opalescent or cloudy by accidental mixture with blood during collection.	Perfectly clear fluids may be pathologic, as in syphilis, tuberculous meningitis, poliomyelitis, encephalitis, etc. Opalescent and turbid fluids are always pathologic if accidental contamination with blood is excluded. Usually due to excess of leukocytes, with or without bacteria, as in the acute and chronic meningitides, severe poliomyelitis, etc.
Coagula and Sediments	Coagula, pellicles or sediments do not form upon standing.	Pathologic fluids may not form coagula, pellicles or sediments on standing but their production is usual and frequent. Due to fibrinogen and if accidental contamination with blood is excluded are always pathologic. Vary in size, appearance and rate of formation in different diseases.
Color	Colorless.	May be grayish or yellowish-green in suppurative meningitis. Sometimes reddish due to hemolysis in hemorrhages communicating with the ventricles or subarachnoid space. Xanthochromia or yellow color most characteristic. Of two kinds: (1) hemorrhagic due to bleeding in the ventricles, or subarachnoid space, or the diapedesis of erythrocytes in severe meningitis, from passive congestion, etc.; or (2) the syndrome of Froin observed in fluid removed from below lesions producing compression of the spinal subarachnoid space (tumors, localized pachymeningitis, etc.), due to venous stasis and transudation. Xanthochromia may be also due to bile pigment in jaundice, carotenemia, acriflavine, etc.

TABLE 73. SUMMARY OF THE CLINICAL INTERPRETATION OF MACROSCOPIC AND MICROSCOPIC CHANGES IN THE CEREBROSPINAL FLUID—(Continued)

Constituent	Normal	Abnormal
Blood	Absent unless due to the accidental puncture of a vein during collection. Traces may be present in the fluids of the newborn; increased by birth injuries.	Pure blood indicative of accidental puncture of a vein. Blood mixed with spinal fluid indicative of hemorrhage communicating with the ventricles or subarachnoid space of the brain or spinal cord, provided accidental puncture of a vein may be excluded. Absence of blood does not exclude cerebral hemorrhage.
Cytology	Total cells of lumbar or cisternal fluids 0 to 8 per c.mm. Slightly less in ventricular fluids. Composed of lymphocytes. Counts should be made as soon as possible after collection. Total cells increased by even traces of blood.	*Pleocytosis:* 9 to 12 borderline; 13 to 30 slight; 31 to 100 moderate; 200 to 500 marked; 1000 or higher very marked. Slight to moderate pleocytosis largely due to lymphocytes; usual in tuberculous meningitis, poliomyelitis, encephalitis and encephalomyelitis, meningismus or "serous meningitis," tabes dorsalis, paresis, multiple sclerosis, herpes zoster, radiculitis, polyneuritis, fracture of skull without hemorrhage, brain abscess without rupture, etc. Moderate to marked pleocytosis due to lymphocytes or lymphocytes and some polymorphonuclears, usual in lymphocytic choriomeningitis, tuberculous meningitis, aseptic meningitis, localized meningitis, syphilitic meningitis, Weil's disease, etc. Marked pleocytosis largely due to polymorphonuclears usual in all the acute diffuse meningitides, aseptic meningitis, ruptured brain abscess, etc.

7 or over indicates a large reservoir, as in hydrocephalus or "serous meningitis," while values below 5 indicate a small reservoir.

The intravenous injection of large amounts of isotonic solutions causes a temporary rise in pressure; oral administration may have the same effect. The intravenous injection of a hypertonic solution, as a 30 per cent solution of sodium chloride, produces a reduction in pressure persisting for long periods; this has been found to facilitate operations on the brain by causing its shrinkage and thereby preventing the extrusion of the brain through trephine openings.

It has long been known that constriction of the neck increases spinal fluid pressure, provided there is no block of the subarachnoid space. But it was Queckenstedt [4] who first analyzed the significance of jugular compression in causing increased intracranial pressure which may be detected immediately by an increase of spinal fluid pressure. This test should always be done whenever complete or partial subarachnoid block or lateral sinus thrombosis is suspected. In complete subarachnoid block the normal rise in pressure after compression of the jugular veins fails to occur; in partial block it is delayed and partial. In thrombosis of a lateral sinus, compression of the jugular on the affected side causes no rise in pressure, while compression of the unaffected side alone gives the same rise as one would expect from compression of both jugular veins. A useful refinement of the procedure has been suggested by Grant and Cone,[5] employing a blood pressure cuff about the neck of the patient.

It is apparent, therefore, that many factors influence the pressure of the cerebrospinal fluid under normal and abnormal conditions. Any irritation of the meninges increases both the amount and pressure in relation to the acuteness of the process, not only by increased production through the addition of inflammatory products, but by decreased absorption as well. Apparently healthy individuals may occasionally show unexplainable low pressure but under abnormal conditions low pressure may be encountered in fainting, shock, during headache following a previous spinal puncture, in long-standing degenerative diseases of the central nervous system, in brain tumors with blocking of the foramen magnum, obstructive hydrocephalus and below space-constricting lesions of the spine or spinal cord in which the pressure, however, may be increased above the level of the lesion.

Appearance. The normal cerebrospinal fluid is transparent, colorless and of crystalline clarity, with a specific gravity of 1.006 to 1.008 and a freezing point of —0.551° to —0.558° C.

As previously stated, it is advisable to collect the fluid in two test tubes, as the first flow may contain a small amount of blood from the puncture which renders the fluid hazy or cloudy. Otherwise, the normal fluid has the appearance of distilled water. The physician should always inspect it very carefully at the bedside and preferably against a dark background. If the presence of blood may be excluded, any departure from perfect clarity is to be regarded as abnormal. On the other hand, *perfectly clear fluids may be pathologic,* as is almost invariably true in syphilis and of frequent occurrence in tuberculous meningitis, poliomyelitis, lymphocytic choriomeningitis, and encephalitis.

When distinctly hazy or of a ground-glass appearance, the total cell count is increased to at least 300 to 700 leukocytes per c.mm. (pleocytosis) and if meningitis is suspected clinically, the physician is justified in immediately instituting treatment on this basis until further diagnostic data are supplied by the laboratory. Higher total cell counts, along with the presence of bacteria, render fluids faintly turbid, markedly turbid or frankly purulent.

Coagula and Sediments. Normal spinal fluid does not form coagula, pellicles or sediments upon standing. Under abnormal conditions, however, coagula and pellicles may form due to the presence of fibrinogen, although *their absence does not exclude the possibility of fluids being otherwise pathologic.* The time required for their formation varies in different diseases. Thus, in suppurative meningitis a coagulum may form in a very short time, while in tuberculous meningitis twelve to twenty-four hours may be required.

Coagula may occur as numerous, small flocculi without pellicles, as in syphilitic fluids, as "cobwebs" or "pine-tree" coagula with pellicles suspended from the surface of the fluid in tuberculous meningitis, or as heavy coagula with sediments sinking to the bottom of the test tube, as seen in acute purulent meningitis. The presence of small amounts of fresh blood intimately mixed with the fluid does not produce coagulation. Under the conditions, if blood is absent, coagulation is always abnormal.

Color; Syndrome of Froin. As previously stated, normal spinal fluid is colorless. In acute purulent meningitis it may be grayish, as in pneumococcal and streptococcal meningitis, or yellowish-green, as in meningococcal meningitis.

The most characteristic color change is that designated as *xanthochromia* (yellow) which may be due to the presence of various pigments. For example, a frequent cause is the presence of altered hemoglobin (hemorrhagic xanthochromia) from hemorrhage or the diapedesis of erythrocytes in severe tuberculous or suppurative meningitis, brain abscess, etc. It may also occur from venous stasis with transudation in cerebral tumors and especially tumors or other lesions producing compression of the spinal subarachnoid space. On the other hand, xanthochromia may be due to the presence of bilirubin in jaundice, or that produced from hemoglobin in old hemorrhages, to carotenemia, or the administration of such drugs as neutral acriflavine.

A frequent cause is hemorrhage into the ventricles or subarachnoid space of the brain or spinal cord. When occurring without coagulation, it is suggestive of subdural hemorrhage. Under these conditions, xanthochromia usually develops within 4 to 8 hours and increases in intensity while the number of erythrocytes decreases during the ensuing 4 to 8 days. It then decreases, unless there is a recurrence of bleeding, and usually disappears by the third week. On the other hand, the fluid may show a reddish color due to spontaneous hemolysis. The xanthochromia of the newborn is ascribed to the presence of blood and especially after birth injuries. Of course, the color is best detected after the fluid has been centrifuged. Fresh blood from a very recent hemorrhage or the accidental puncture of a vein does not impart any color to the supernatant fluid, except at times a slight reddish tinge from hemolysis.

The most pronounced examples of xanthochromia are observed in cerebrospinal fluids removed from *below* space-constricting lesions of the spinal subarachnoid space and especially tumors of the spine or spinal cord. Under these circumstances the fluid is usually a clear yellow color with a great excess of protein and fibrinogen producing almost immediate coagulation which is commonly designated "xanthochromia with massive coagulation" or the *syndrome of Froin.* Originally thought to be pathognomonic of spinal tumors, the syndrome is now known to be produced by any lesion compressing the spinal subarachnoid space with stagnation of the fluid and passive congestion such as adhesions, localized pachymeningitis, or any other space-constricting process.

Various observers have found the benzidine occult blood test of value in differentiating between the xanthochromia of the syndrome of Froin and that due to hemorrhage.[6,7] In the former it is stated to give a negative reaction, with the yellow color ascribed to venous stasis and transudation. In the latter it is stated to be positive due to hemorrhage or inflammatory diapedesis of erythrocytes, for which the term "erythrochromia" has been proposed.[7]

Blood. In obtaining cerebrospinal fluid, a vein is sometimes punctured with the collection of pure blood and no fluid at all. This blood undergoes coagulation with the separation of serum in the usual manner.

However, blood evenly mixed with spinal fluid does not coagulate or but incompletely. This may occur when the lumen of the needle is partly in a vein and partly in the subarachnoid space. But if the physician believes that the needle is solely in the latter, the presence of blood indicates a recent hemorrhage communicating with the ventricles or the subarachnoid space of the brain or spinal cord. The absence of blood, however, does not exclude cerebral hemorrhage. Erythrocytes may be present in the cerebrospinal fluids of apparently healthy newborn infants [8] as well as in those with birth injuries.[9]

Physicochemical Changes. Minor changes have been observed in the specific gravity of cerebrospinal fluids in disease, but since the normal is subject to considerable variation, they are of no clinical significance. The freezing point may be reduced in tuberculous and suppurative meningitis, and increased in diabetes mellitus and uremia. Minor changes have been observed in viscosity, conductivity, *p*H, refractometric indices, and crystallography, but while these examinations are worthy of further investigation, they possess no clinical value at the present time and consequently are not included in routine examinations.

CYTOLOGY

One of the most valuable routine examinations of cerebrospinal fluid is a determination of the *total cells* per c.mm. Various methods have been described but that employing the Fuchs-Rosenthal counting chamber is the most accurate and is commonly employed. Needless to state, the counts must be accurately made when the total cells are but slightly increased, as in the case of clear or but faintly hazy fluids. When distinctly turbid or purulent, the counts are so high that slight errors make no difference from the diagnostic standpoint. But when a slight or moderate increase of cells is of diagnostic importance, as in tuberculous meningitis, meningisms (serous meningitis), syphilis of the central nervous system, poliomyelitis, etc., accuracy is of paramount importance. Furthermore, since the total cells are reduced by the formation of flocculi and coagula, it is necessary to *make the counts as promptly as possible after collection of fluid* and always within twenty-four hours, since the cells tend to undergo autolysis. If counts cannot be made immediately when coagulation is expected to occur, a portion of the fluid should be collected in a tube carrying a small amount of potassium oxalate.

Spinal fluids containing visible amounts of blood are unsuited for total cell counts because of the presence of leukocytes resulting in counts that are too high. Even traces of blood too small for macroscopic detection increase the total cells, which may result in diagnostic errors in diseases in which an increase of total cells is ordinarily between 20 to 100 per c.mm.

The total cells of normal cerebrospinal fluid obtained by lumbar or cisternal puncture vary from 0 to 8 per c.mm. in terms of undiluted fluid. In children the upper limit of normal is about 10 per c.mm. These cells are composed of small lymphocytes and for this reason are commonly regarded as being hematogenous in origin. Slight differences, however, have been observed with fluids obtained from other loci; ventricular fluids, for example, may contain fewer cells than lumbar fluids (Table 73).

An increase of cells is designated as *pleocytosis*. Under acceptable technical conditions a total cell count in adults of 9 to 12 per c.mm. is regarded as borderline; 13 to 30 represents a slight increase; 31 to 100 a moderate increase; 200 to 500 a marked increase, while 1000 or more represents a very marked increase.

A differential count is frequently of clinical value and is known as *cytodiagnosis*. When fluids contain less than 300 cells per c.mm. they are, however, scarcely worth while because aside from the difficulty of securing sufficient sediment for making the examination, experience has shown that under these conditions the cells are essentially small lymphocytes.

With higher total cell counts, however, differential counts are usually of additional clinical value. An excess of polymorphonuclear neutrophilic leukocytes (pus cells) is indicative of the acute purulent meningitides including aseptic meningitis. Endothelial cells from the meninges may be likewise present in meningisms or "serous meningitis" and are frequently classified as large lymphocytes. Elaborate classifications of the cells have been proposed, based on sections of the fixed and hardened sediments, but possess no clinical value since differentiation into lymphocytes, polymorphonuclears and endothelial cells serves all useful purposes. Tumor cells, however, are sometimes recognized by expert histopathologists, especially in cases of medulloblastoma.

The extent of pleocytosis is in relation to the degree of irritation or inflammation of the meninges. Consequently, the highest counts, largely composed of polymorphonuclear leukocytes, are observed in the acute diffuse meningitides due to meningococcal, pneumococcal, streptococcal, influenzal or other pyogenic infections. In localized and aseptic meningitis, lower counts are observed, composed of polymorphonuclears and lymphocytes, while the total cell count is usually normal in lateral sinus thrombosis.

In tuberculous meningitis the changes are usually slight or moderate, being likewise in relation to the degree and extent of the disease. The same is true of viral diseases, like poliomyelitis, lymphocytic choriomeningitis and the encephalitides, as well as of asymptomatic and symptomatic syphilis of the central nervous system. Consequently, in systemic and diffuse degenerative diseases involving the nervous system, like multiple sclerosis, herpes zoster, cerebral and spinal tumors, the total cells may or may not be slightly increased, while in epilepsy, amyotrophic lateral sclerosis, subacute combined degeneration of the cord, syringomyelia, chorea, paralysis agitans, spastic spinal paralysis and other degenerative diseases, the total and differential cell counts are almost invariably within normal.

Cytologic examinations and especially total cell counts are also of clinical value in relation to prognosis and treatment. Increased changes usually indicate progression or exacerbation of the disease, while decreased changes usually indicate a favorable course. The total cells, however, are usually among the last of the abnormal changes to disappear and it should be remembered that intraspinal therapy, especially the injection of immune sera, may maintain high total leukocyte counts with a preponderance of polymorphonuclears due to irritation of the meninges and along with the so-called "meningitis sympathica" designated "aseptic meningitis." For this reason, a bacteriologic examination of smears and cultures of the cerebrospinal fluid is always of greater value in relation to the prognosis and treatment of the infective meningitides than cytologic and chemical examinations. These aspects are discussed in Chapter 15.

CHEMICAL EXAMINATIONS

The chemical composition of normal cerebrospinal fluid varies slightly in different loci but is essentially similar to Locke's saline solution plus small amounts of protein and glucose. It is composed of 98.23 to 99.17 parts water and 0.83 to 1.77 parts total solids. A great deal of investigation has been devoted to chemical changes in relation to disease but, in general terms, only those referable to protein, glucose and sodium chloride have been found of sufficient diagnostic value to be included in routine examinations (Table 74).

Reaction. The reaction of the normal cerebrospinal fluid is alkaline, with a pH of 7.35 to 7.40, when determined immediately after collection. As shown by Levinson, it tends to become slightly more alkaline on standing, due to the loss of CO_2. The alkaline reserve, as measured by the CO_2 combining power, is also practically identical with that of blood plasma (55 to 75 volumes per cent).

The pH of the fluid is usually within normal in tuberculous meningitis but in the suppurative meningitides it is sometimes less alkaline (pH 7.2 to 7.5), the increase in acidity being due, in all probability, to an increase of lactic or other organic acids. On standing, the acidity shows much less tendency to decrease than in the case of normal fluids (Levinson). This is perhaps due either to an increased rate of glycolysis by bacteria, or to increased production of CO_2 by the cells present in the fluid, balancing the loss of CO_2 which occurs on standing exposed to air. These changes, however, are of minor degree so that a determination of the pH of cerebrospinal fluids possesses little or no diagnostic value.

Protein. The protein content of the normal cerebrospinal fluid obtained by lumbar puncture is lower than that of any other normal body fluid with the exception of the aqueous humor of the eye, which it closely resembles in chemical composition, varying from 15 to 45 mg. (average 30 mg.) per 100 cc. Cisternal and ventricular fluids usually contain from 10 to 35 mg. per 100 cc. There is as yet no agreement concerning the type of protein present but it is apparently largely made up of albumin (23 mg. per 100 cc.), which is the most diffusible of the

TABLE 74. SUMMARY OF THE CLINICAL INTERPRETATION OF CHEMICAL CHANGES IN THE CEREBROSPINAL FLUID

Constituent	Normal	Abnormal
Reaction	Alkaline with a *p*H 7.35 to 7.40; alkalinity slightly increased upon standing exposed to air through loss of CO_2. Alkaline reserve practically identical with that of the blood plasma.	Usually normal in tuberculous meningitis. Sometimes slightly less alkaline (*p*H 7.2 to 7.5) in the suppurative meningitides due to lactic or other organic acids. Of no diagnostic value.
Protein	Total protein 15 to 45 (average 30) mg. per 100 cc. Less in cisternal and ventricular fluids. Largely composed of albumin with small amount of globulin; ratio about 6:1. No fibrinogen. Increased by the presence of blood. $KMnO_4$ index (a rough measure of total organic matter) varies from 1.3 to 1.8.	Albumin and globulins increased. Usually determined qualitatively by the "globulin tests" and expressed as +, ++, +++ and ++++. Quantitative tests for total protein preferred. *Slight increase* usual or frequent in tabes dorsalis; multiple sclerosis; paralysis agitans and progressive myelitides; poliomyelitis; epidemic encephalitis; meningism of uremia; pneumonia; typhoid fever; etc.; after convulsions; alcoholic psychopathies; unruptured brain abscess; localized meningitis; cerebral tumors; fracture of skull with hemorrhage; cerebral thrombosis and hemorrhage, etc. *Moderate or marked increase* usual in the suppurative meningitides; tuberculous meningitis; aseptic meningitis; ruptured brain abscess; paresis; syphilitic meningitis; syndrome of Froin (spinal tumors, Pott's disease, etc.). The Levinson test of corroborative value in tuberculous meningitis.
Glucose	Derived from the blood glucose. Best determined in the fasting state. A blood sugar determination should be made at the same time; also an estimation of the chloride. Varies from 40 to 70 mg. per 100 cc. in fasting adults. In children up to ten years from 70 to 90 mg. per 100 cc. Slightly higher in cisternal and ventricular fluids. Approximately 60 to 70 per cent of the blood sugar.	An increase is designated as *hyperglycorachia.* Occurs in diabetes mellitus and other states which produce hyperglycemia. A slight increase of little or no diagnostic value may occur in some cases of "serous meningitis," epidemic encephalitis, poliomyelitis, cerebral tumor, cerebral abscess without meningitis, and functional mental diseases (dementia praecox).

TABLE 74. SUMMARY OF THE CLINICAL INTERPRETATION OF CHEMICAL CHANGES IN THE CEREBROSPINAL FLUID—(Continued)

Constituent	Normal	Abnormal
Glucose (*continued*)	Increased by the presence of blood in the fluid. Cerebrospinal fluid also contains nonglucose reducing substances amounting to about 4.0 mg. per 100 cc. or about 10 per cent of the total.	A decrease is designated as *hypoglycorachia.* May occur in states which produce hypoglycemia. Characteristic of all the acute purulent meningitides; also occurs in tuberculous meningitis, especially after the first week of the disease; may also occur in severe meningovascular syphilis with marked pleocytosis. Normal or but slightly reduced in paresis and tabes dorsalis.
Other Organic Constituents	Urea: 5 to 38 mg. per 100 cc. Urea nitrogen: 6 to 15 mg. Creatinine: 0.45 to 1.5 mg. Total nonprotein nitrogen: 12.5 to 30 mg. Residual nitrogen: 2 to 6 mg. Amino-acids: 1.5 to 3.0 mg. Uric acid: 0.4 to 2.8 mg. Lactic acid: 8 to 27 mg. (probably largely formed on standing by the glycolysis of sugar).	Determinations of little or no diagnostic or prognostic value. Urea, urea nitrogen, creatinine and total nonprotein nitrogen increased in nephritis and uremia with increased blood retention. Urea nitrogen particularly likely to be increased. Residual nitrogen likewise likely to be increased out of proportion to that in the plasma. Uric acid frequently increased in all types of meningitis. Lactic acid increased in all forms of suppurative meningitides; less so in tuberculous meningitis. Due to the glycolysis of sugar.
Chloride	720 to 760 mg. per 100 cc. in terms of sodium chloride in lumbar fluid; same in cisternal and ventricular fluids. Slightly less in infants and children (625 to 720 mg. per 100 cc.). Derived from the blood plasma by filtration through the choroid plexus. Fluctuates to some extent with changes in plasma chloride.	In the absence of meningitis increased in hyperchloremia (nephritis) and reduced in hypochloremia (lobar pneumonia, pyloric obstruction, etc.). Characteristically reduced in tuberculous meningitis (below 620 mg.). Also reduced in the acute purulent meningitides (630 to 680 mg.). May be slightly reduced in some cases of anterior poliomyelitis (670 to 710 mg.). Usually normal in localized meningitis, encephalitis, lymphocytic choriomeningitis, cerebral tumor, cerebral abscess without septicemia or meningitis, and in all types of syphilis of the central nervous system.

TABLE 74. SUMMARY OF THE CLINICAL INTERPRETATION OF CHEMICAL CHANGES IN THE CEREBROSPINAL FLUID—(Continued)

Constituent	Normal	Abnormal
Other Inorganic Constituents	Inorganic phosphate: 1.25 to 2.0 mg. per 100 cc. In children 1.5 to 3.5 mg. Sodium: 300 to 343 mg. Potassium: 8.5–11.5 mg. (av. 9.8) Calcium: 4.5 to 5.5 mg. Magnesium: 1.0 to 3.6 mg. Cholesterol: 0 to 0.22 mg.	Estimations of little or no diagnostic and prognostic significance but sometimes of corroborative value. Inorganic phosphate increased in nephritis with uremia. An increase may occur in the suppurative meningitides but especially in tuberculous meningitis, hydrocephalus, cerebral tumors, and some degenerative diseases of the brain or cord. Usually no changes in calcium in relation to hypercalcemia and hypocalcemia. May be reduced in uremia. Frequently increased in the suppurative meningitides and especially in tuberculous meningitis. Also in many cases of hydrocephalus and cerebral tumors. Cholesterol may be increased in meningitis, cerebral tumor, cerebral hemorrhage and various mental diseases. No changes in syphilis of the central nervous system.

plasma proteins, with about 5 mg. of globulin per 100 cc., giving a ratio of about 6:1. Needless to state, even traces of blood increase the protein content so that it is useless to conduct qualitative or quantitative tests when macroscopic amounts of blood are present.

In inflammatory conditions of the meninges, brain and spinal cord, the capillaries of the choroid plexus and meninges become more permeable and, depending upon the degree of inflammation or congestion, increasing amounts of albumin, pseudoglobulin, euglobulin and fibrinogen pass into the cerebrospinal fluid, the last two appearing only in severe meningitis or complete compression of the cord. Of course, the amount of protein is in relation to the degree and extent of meningitis, including aseptic meningitis, being highest in the acute purulent forms and less in the localized and chronic forms (Table 74) although it may not be increased in some cases of early meningitis. Protein is not generally increased in meningismus or "serous meningitis" but an increase may occur to a very slight extent in isolated cases with otherwise normal chemical and cytologic findings, although protein is not usually detectable by the ordinary routine qualitative or so-called "globulin tests."

An increase of protein may also occur in other disease states in which capillary and cell permeability are increased. For example, during and shortly after convulsions in epilepsy and spasmophilia of children; in such toxic states as uremia, pneumonia and typhoid fever, and in conditions causing a generalized or localized increase of subarachnoid tension, as brain and spinal cord tumors. While normal in about 75 per cent of cases of delirium tremens,[10] it has been found increased in about 71.6 per cent of the alcoholic psychopathies, which has been ascribed to a direct toxic effect of alcohol upon the central nervous system, with part of the protein due to increased permeability of the choroid plexus and part to a pathologic catabolism of the tissues of the central nervous system.[11]

Protein is also commonly increased in many of the organic diseases of the brain and spinal cord, usually with associated pathologic changes in the meninges. Familiar examples are paresis, tabes dorsalis, cerebrospinal syphilis, poliomyelitis, epidemic encephalitis, abscess of the brain and cerebral hemorrhage, thrombosis or embolism. The highest protein values, however, are found in the "syndrome of Froin" in fluids removed from below lesions causing complete compression of the spinal subarachnoid space, in which there may be as much as 2 gm. per 100 cc.

Since globulin is present in abnormally large amounts in most pathologic fluids containing an increased quantity of protein, so-called "globulin tests" are commonly employed as a means for roughly detecting and estimating the protein content of cerebrospinal fluid. The most popular of these tests are those of Pandy, Noguchi, Nonne-Apelt and Ross-Jones, the results being reported as —, +, ++, +++ or ++++, depending upon the amount of globulin precipitated. More exact quantitative determinations, however, are readily made with the results expressed in milligrams per 100 cc. of fluid. A modification of the Dennis-Ayer method is commonly employed[12] but the technic of Johnston and Gibson[13] for determining protein by the tyrosine equivalent method is considered more sensitive. By this method the upper limit of normal has been found to be about 46 mg. per 100 cc.,[14] which is slightly higher than ordinarily regarded as normal.

According to some observers, the determination of qualitative differences in the albumin and globulin of the cerebrospinal fluid may be of value in differentiating between tuberculous and other forms of meningitis. The *Levinson test* consists essentially in the comparison of the amount of precipitation produced in the fluid by mercuric chloride and sulfosalicylic acid. A precipitate ratio of 2 to 1 or higher (bichloride precipitate: sulfosalicylic acid precipitate) is regarded as being highly suggestive of tuberculous meningitis, although the readings are sometimes difficult with borderline ratios difficult to interpret. Furthermore, ratios similar to those occurring in tuberculous meningitis occur in other conditions so that the test is by no means specific for the disease, although of definite corroborative value. The same is true of the *tryptophane test* which, however, yields positive reactions with spinal fluids containing blood as well as those markedly xanthochromic or from cases of purulent meningitis.

The *Takata-Ara reaction* is regarded as possessing a relation to the albumin-globulin ratio. Szanto and Burack[15] have recently found it of value in the diagnosis of syphilis of the central nervous system and bearing a close parallelism to the colloidal gold reaction.

The *potassium permanganate test* of Mayerhofer is expressed in terms of an index expressing the total amount of organic matter measured by the reduction of a solution of potassium permanganate of known strength. The normal index of fluid collected by spinal puncture varies from 1.3 to 1.8 with an index of 1.1 to 1.5 in the case of cisternal and ventricular fluids. Higher indices are observed in meningitis and other disorders accompanied by pleocytosis and increase of protein. Under the circumstances, the test adds little or nothing to the information gained by other and simpler procedures.

Glucose. It is now generally agreed that the glucose of the cerebrospinal fluid is derived from that of the blood. Consequently, it varies not only (1) according to the blood sugar concentration, but also according to (2) the permeability of the choroid plexus and possibly of the capillaries surrounded by prolongations of the subarachnoid space, as well as to (3) the rate of glycolysis in the fluid. Furthermore, it is possible that the cells of the choroid plexus may utilize some of the sugar.

Determinations of cerebrospinal fluid sugar are best made with fluid collected after a period of fasting, preferably overnight, with a blood sugar estimation at the same time. Of course, the fluid should be free of macroscopic amounts of blood.

In view of the variable factors concerned in cerebrospinal fluid glucose, the normal cannot be accurately stated, but is generally regarded as varying from 40 to 70 mg. per 100 cc. in adults with slightly larger amounts in cisternal and ventricular fluids. In children up to ten years of age the normal has been found somewhat higher, varying from 70 to 90 mg. per 100 cc. Cerebrospinal fluid also contains nonglucose-reducing substances which disappear on hydrolysis, amounting to about 4.0 mg. per 100 cc. or about 10 per cent of the total. The ratio of fluid to blood glucose likewise cannot be accurately stated but in normal individuals under fasting conditions the cerebrospinal fluid glucose is thought to be approximately 60 to 70 per cent of the blood glucose.

An increase of cerebrospinal fluid glucose is designated as *hyperglycorachia.* Naturally this occurs in diabetes mellitus as well as in other states producing marked hyperglycemia because of increased passage of plasma glucose through the choroid plexus. Consequently, determinations of spinal fluid glucose are of value in unexplained coma for establishing or excluding diabetes mellitus as the cause. Acetone and even diacetic acid may be present.

As in the case of protein, however, any condition associated with increased permeability of the choroid plexus may result in a slight increase of spinal fluid glucose, provided the blood glucose is within a normal range and the fluid glucose is not destroyed by glycolysis due to the presence of carbohydrate-splitting bacteria. For this reason a slight increase may be observed in some cases of "serous meningitis," especially those due to uremia, as well as in some cases of epidemic encephalitis and poliomyelitis. However, in these states the glucose is usually within normal or so slightly increased as to be without diagnostic value.

A slight increase may also occur in diseases producing an increase of cerebrospinal fluid pressure like cerebral tumor, cerebral abscess without an associated meningitis, and convulsive states; likewise in some of the functional mental disorders, especially dementia praecox, but the increase, if any, is usually so slight that it possesses little or no diagnostic value. Under these conditions hyperglycorachia is so slight and inconstant in diseases of the central nervous system that it possesses but little clinical importance.

A decrease of cerebrospinal fluid glucose is known as *hypoglycorachia*. This is of far more diagnostic value and especially when both glucose and chlorides are determined. Naturally a decrease may occur in states of hypoglycemia and for this reason a blood sugar determination is frequently advisable at the same time.

Hypoglycorachia is characteristic of all of the purulent meningitides in which the glucose falls rapidly, usually to under 20 mg. per 100 cc. within the first twenty-four to forty-eight hours, although usually much slower in meningitis which is at first localized. Indeed, during the height of acute diffuse purulent meningitis there may be no glucose at all in the fluid. This hypoglycorachia is due to the glycolysis of the glucose by the infecting microorganisms plus the possibility of some being absorbed by the pus cells as claimed by Mestrezat.

In tuberculous meningitis the glucose, which may be normal or even somewhat elevated at the onset, likewise steadily decreases, nearly always to below 35 mg. per 100 cc. during the first week of the disease, and may drop to 15 mg. or even lower. Consequently, a normal or slightly elevated glucose content on two or more occasions at intervals of two or more days, with a normal or but slightly diminished chloride value, points strongly against acute purulent and tuberculous meningitis. Furthermore, since tuberculous meningitis in the early stage may be clinically indistinguishable from epidemic encephalitis and anterior poliomyelitis in which the glucose is either normal or slightly increased, a decrease of glucose along with a marked decrease of chlorides points strongly to tuberculous meningitis, although a slight decrease of sodium chloride (670 to 710 mg. per 100 cc.) may occur in acute anterior poliomyelitis.

Slight or moderate hypochlorachia may also occur in severe meningovascular syphilis with marked pleocytosis. But in these cases the total cell counts are much higher than in tuberculous meningitis while the chlorides are but slightly reduced. In paresis and tabes dorsalis, however, the glucose is usually within normal or but very slightly reduced.

Other Organic Constituents. As shown in Table 74, practically all of the known nonprotein nitrogenous and other organic substances of the blood plasma also occur in the normal cerebrospinal fluid, although the amounts differ according to their degree of diffusibility. The determination of these substances, however, is in most instances of little or no clinical value, either diagnostically or prognostically, although the nonprotein nitrogenous constituents of the fluid are usually increased in the fluid in nephritis and uremia with nitrogen retention and especially urea nitrogen and the undetermined or residual nitrogen.

Uric acid may be increased in all forms of meningitis. The same is true of lactic acid in the suppurative meningitides and, to a lesser extent, in tuberculous meningitis, since in all conditions in which the glucose is decreased the lactic acid is found to be correspondingly increased because of glycolysis of the sugar. It is also formed by glycolysis when cerebrospinal fluid is permitted to stand at room temperature, which probably accounts for the fact that some observers state that lactic acid is absent from normal cerebrospinal fluid while others assert that it is normally present.

Chlorides. The chlorides of the cerebrospinal fluid are expressed in terms of sodium chloride. The normal varies from 720 to 760 mg. per 100 cc., being the same for lumbar, cisternal and ventricular fluids. Values in infants and children exhibit slightly more variation, ranging from 625 to 720 mg. per 100 cc. Normal cerebrospinal fluid, therefore, contains more chloride than the normal blood plasma (570 to 620 mg. per 100 cc.) from which it is derived through the free permeability of the choroid plexus, and very much more than the blood serum (352 to 383 mg. per 100 cc.). This is explained in part on the basis of the Donnan equilibrium governing the concentration of ions on either side of a semipermeable membrane (the capillary walls of the choroid plexus) when the fluid on one side (plasma) contains molecules which are diffusible (protein). Consequently, the quantity of cerebrospinal fluid chloride varies directly with the plasma chloride, although the quantitative distribution between plasma and fluid is influenced not only by the protein content of both plasma and cerebrospinal fluid, but likewise by the concentration of cells in the blood and the failure of the blood and spinal fluid to reach an equilibrium when the fluid and salt content of the body are rapidly changing.[16] In general terms, therefore, the greater the difference in protein between the plasma and fluid, in favor of the former, the greater will be the difference in the chlorides in favor of the latter.

In the absence of meningitis the chloride of the fluid naturally varies to some extent with that of the plasma. For example, it may be increased in some cases of nephritis with hyperchloremia while decreased in states of hypochloremia, as occurs in lobar pneumonia, pyloric obstruction, etc. Consequently, the plasma chloride concentration must be considered in interpreting reduced amounts of chloride in the cerebrospinal fluid and particularly in acute infections, like pneumonia, in which the development of symptoms of meningism may arouse a suspicion of meningitis.

From the diagnostic standpoint a determination of the chloride of cerebrospinal fluid is of most value in tuberculous meningitis. In this disease it is characteristically below 620 mg. and usually from 450 to 580 mg. per 100 cc. It is also usually reduced (630 to 680 mg.) in the acute purulent meningitides. These changes are largely due to an increase of protein in the cerebrospinal fluid and a more equal distribution of protein between the plasma and fluid, which results in a more equal distribution of the chloride. This, however, only partly accounts for the reduction of the chloride in the meningitides, suggesting that the hypochloremia occurring at times in these diseases may be also involved in the mechanism. When chloride values are within normal limits, or, if initially low, subsequently increase, the meningitis is likely to run a mild course, and conversely. The chances of a fatal outcome, however, are not significantly greater if the curve falls during the first few days.[17]

A slight decrease (670 to 710 mg. per 100 cc.) may also occur in some cases of anterior poliomyelitis, but in other diseases of the central nervous system the chloride of the cerebrospinal fluid is normal or but so slightly reduced as to possess no diagnostic value. Such include localized meningitis, epidemic encephalitis, lymphocytic choriomeningitis, cerebral tumors, cerebral abscess without septicemia or meningitis and all types of syphilis of the central nervous system.

Other Inorganic Constituents. As shown in Table 74, the normal cerebrospinal fluid also contains various other inorganic constituents all of which (except magnesium) are much less than those occurring in the blood plasma from which they are derived. With but few exceptions, however, their estimation possesses little or no diagnostic and prognostic value except in the case of phosphorus and calcium, which possess corroborative value in tuberculous meningitis, hydrocephalus and brain tumors.

Inorganic phosphate is slightly increased in nephritis with uremia and phosphate retention. A slight increase may also occur in occasional cases of acute purulent meningitis but especially in tuberculous meningitis, in which it may be of corroborative value in diagnosis.[18] A slight increase may also occur in degenerative diseases of the central nervous system, such as tabes dorsalis and paresis, but especially in hydrocephalus and cerebral tumors due, probably, to increased meningeal permeability supplemented by the destruction of brain tissue.[18]

All of the calcium is apparently in the ionized form and, under normal conditions, is quantitatively identical with the diffusible calcium of the serum. However, in both hypercalcemia and hypocalcemia there is usually no change in the cerebrospinal fluid calcium, although some observers believe that there may be some relationship between the fluid calcium and the muscular twitchings or convulsions of uremia, in which the fluid calcium may be less than 4 mg. per 100 cc.[19] Calcium is occasionally increased in the suppurative meningitides but consistently increased in tuberculous meningitis; also in many cases of hydrocephalus and brain tumors.[18]

The magnesium is usually higher in normal cerebrospinal fluid than in the serum, being the only inorganic constituent apparently independent of variations in concentration in the latter. Increased, normal and decreased amounts in the fluid have been reported in the various forms of meningitis but possess no diagnostic value.

An increase of cholesterol may occur in meningitis (trace to 12 mg.), brain tumor and abscess (5 to 15 mg.), cerebral hemorrhage (5 to 20 mg.), and various mental diseases (0.2 to 0.7 mg.) but determinations are not usually employed for diagnostic purposes. In most cases of syphilis of the central nervous system the cholesterol is not appreciably increased.

COLLOIDAL GOLD, MASTIC AND BENZOIN TESTS

It is now well known that the cerebrospinal fluid in various diseases of the brain, cord and meninges may cause the precipitation of gold (Lange), mastic (Emanuel) and benzoin (Guillan, Laroche and Léchelle) in colloidal suspensions under proper technical conditions and especially in paresis, tabes dorsalis and other types of syphilis of the central nervous system. The reactions are physicochemical in nature and ascribed to increased amounts of globulin with different curves of precipitation depending on the albuminglobulin ratios. Consequently, *none of these tests are applicable to fluids containing blood because falsely positive and variable reactions may be produced.*

TABLE 75. SUMMARY OF THE CLINICAL INTERPRETATION OF COLLOIDAL REACTIONS WITH THE CEREBROSPINAL FLUID

<table>
<tr><th>Test</th><th>Normal</th><th>Abnormal</th></tr>
<tr><td>Colloidal Gold</td><td>The most sensitive and preferred.
But the reagent is difficult to prepare and the test is more subject to technical errors.
Normal or negative reaction: 00000-00000. But reactions like 111000-0000 or 0011000000 may occur due to traces of blood or too sensitive reagent.</td><td rowspan="3">Tests cannot be done with cerebrospinal fluids containing macroscopic amounts of blood.
Three characteristic curves of reactions may occur designated as Zone I (paretic curve) with maximum precipitation in the first two to four tubes; Zone II (tabetic or luetic curve) with maximum precipitation toward the middle; Zone III (meningitic curve) with maximum precipitation toward the end. All curves of precipitation subject to considerable variation.
Zone I reactions occur in paresis, taboparesis, meningovascular syphilis, and multiple sclerosis.
Zone II reactions occur in tabes dorsalis, other types of symptomatic and asymptomatic syphilis of the central nervous system, multiple sclerosis, the nonsyphilitic meningitides, anterior poliomyelitis, the encephalitides, tumors of the brain and cord and other disorders of the central nervous system with meningeal irritation.
Zone III reactions occur in acute purulent meningitis, tuberculous meningitis, aseptic meningitis, etc. Of little or no diagnostic value.
All reactions modified or reduced by effective therapy. In syphilis they are generally the last of the spinal fluid changes to disappear under treatment.</td></tr>
<tr><td>Colloidal Mastic</td><td>Reagent easy to prepare and less subject to technical error. But not as sensitive as the colloidal gold and benzoin reactions.
Normal or negative reaction: 00000.</td></tr>
<tr><td>Colloidal Benzoin</td><td>Reagent easy to prepare and less subject to technical error. But not as sensitive as the colloidal gold reaction.
Normal or negative reaction: 00000-0000000000.</td></tr>
</table>

Colloidal Gold Test. Undoubtedly the colloidal gold test is the most sensitive and the best of the three, provided the reagent is properly prepared with the gold in such suspension that it is neither too sensitive nor too unsensitive to precipitation in relation to the test. Indeed, difficulties experienced in the preparation of the reagent have been the main reasons for seeking a substitute for it (Table 75).

When properly prepared the reagent is of a brilliant orange-red color. The test is conducted in ten *chemically clean* test tubes with 1 cc. amounts of spinal fluid diluted with 0.4 per cent solution of sodium chloride, ranging from a 1:10 dilution in tube No. 1 to 1:5120 in tube No. 10. Five cc. of the reagent is then added to each tube and a saline solution control, and the reaction read after standing at room temperature overnight. Complete discoloration of the reagent is designated by the numeral 5, with intermediate colors as pale blue 4, blue 3, lilac or purple 2, red-blue 1 and no precipitation or orange-red 0 (Fig. 20). The results are recorded and reported by these numerals with three principal curves or reactions of precipitation, shown in Figure 21. The Zone I reaction (5555432000) is commonly designated *the "paretic curve"* because so frequently observed in paresis; the Zone II reaction (1123210000) is commonly designated *the tabetic* or *luetic curve* because so frequently observed in tabes dorsalis and other types of symptomatic and asymptomatic syphilis of the central nervous system, while *the meningitic curve* (0001234530) is characteristic of acute purulent meningitis. All three are type curves only, subject to considerable variation according to the degree of change in the cerebrospinal fluid in disease as well as to the degree of "protection" of the gold in colloidal suspension in the reagent. However, if the reagent is unduly sensitive to precipitation or if a trace of blood is present, *normal* cerebrospinal fluid may give a reaction like 1110000000 or 0011000000 instead of 0000000000. Consequently, very weak reactions of these kinds should be ignored, insofar as syphilis is concerned, and especially if complement fixation or flocculation tests have given completely negative reactions.

Otherwise, however, weakly positive reactions like 2210000000 or 0012211000 should not be ignored in relation to the possibility of neurosyphilis even though the serologic reactions are negative with both spinal fluid and serum. This is particularly true if some increase of protein and pleocytosis are present because, not infrequently, such changes are the first laboratory evidences of syphilitic infection of the central nervous system and not infrequently the only evidences of chronic syphilis at a time of favorable therapeutic opportunity. Under the conditions, the proper conduct of the colloidal gold test places a heavy responsibility on the laboratory, which is equally true in relation to serologic tests.

As previously stated, Zone I curves showing maximum precipitation in the first two to five tubes, are generally indicative of paresis when syphilis is known to be present. Therefore, it is the most significant change occurring in the spinal fluid in this disease and one calling for energetic treatment. Furthermore, a curve of this kind may occur before clinical manifestations become apparent, which should always warn the physician and the family of the patient of the possibility of impending mental changes. A curve of this kind is also of usual or frequent occurrence in taboparesis. Zone I or paretic reactions, however, may also occur in meningovascular syphilis and occasionally in other types of syphilis of the central nervous system; likewise, and for reasons as yet unknown, in at least 50 per cent or more of cases of multiple sclerosis, in which disease, however, the serologic tests give negative Wassermann and flocculation reactions in the absence of coincident syphilitic infection.

While Zone II reactions commonly occur in tabes dorsalis they may be likewise observed in both asymptomatic and other types of symptomatic syphilis of the central nervous system. They may also occur in many nonsyphilitic diseases

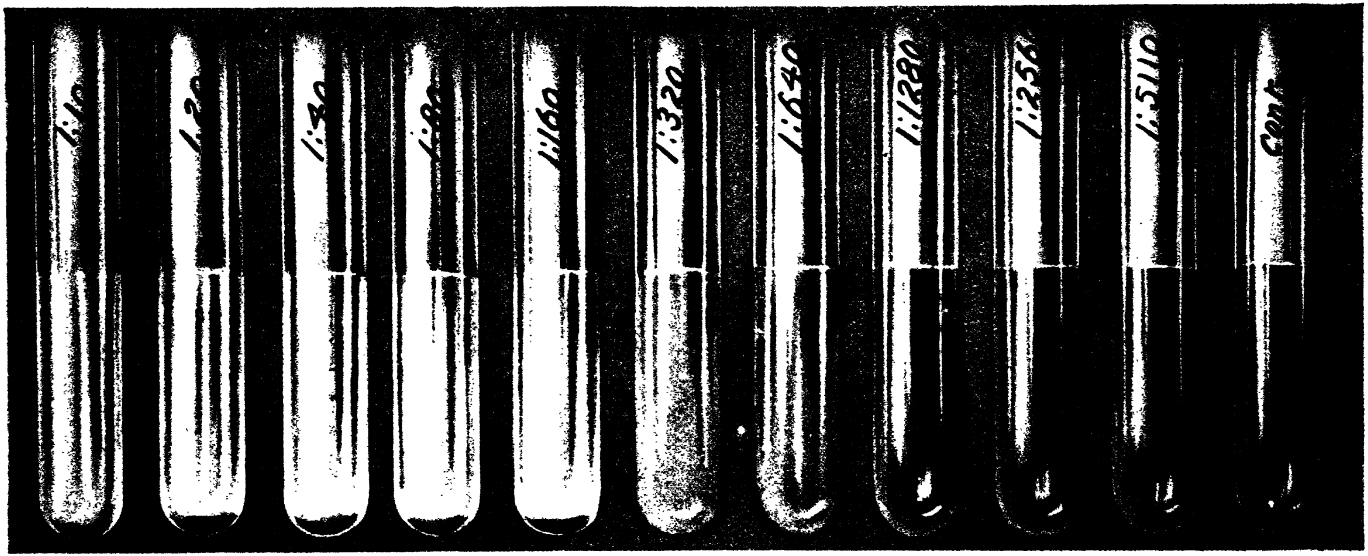

FIG. 20. COLLOIDAL GOLD REACTION (PARETIC OR ZONE I CURVE)

(From Kolmer, *Infection, Immunity and Biologic Therapy*, W. B. Saunders Co.)

as the result of any cause for meningeal irritation. These include not only multiple sclerosis but likewise the bacterial and aseptic meningitides, anterior poliomyelitis, the encephalitides, tumors of the brain and cord, hypertensive cardiorenal disease, convulsive states, trigeminal neuralgia, transverse myelitis, etc. Fortunately, in these diseases such reactions do not usually prove confusing or misleading in clinical interpretation since the serologic reactions are negative in the absence of syphilis, along with the diagnostic aid given by signs and symptoms.

Not infrequently the colloidal gold reactions due to syphilis are changed in character and reduced in degree by treatment, but they are usually the last of the spinal fluid changes to disappear completely.

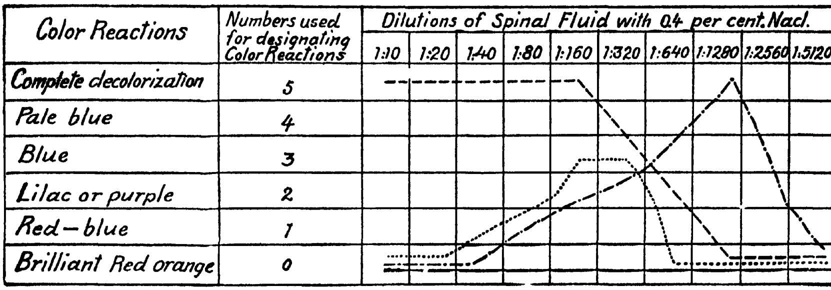

FIG. 21. THE FOUR COMMON TYPES OF COLLOIDAL GOLD REACTIONS

— — — — — , Paretic or Zone I curve; , Luetic or Zone II curve; . . — . — . — . — . . Meningitic or Zone III curve; ————, Normal or Negative curve. (From Kolmer, *Infection, Immunity and Biologic Therapy*, W. B. Saunders Co.)

The so-called "meningitic curve," characterized by maximum precipitation of gold toward the end of the series of tubes, occurs with great regularity not only in the acute purulent meningitides but in aseptic and tuberculous meningitis as well. It is, however, the least in clinical significance and importance, so that the colloidal gold test is not required as a routine procedure in these conditions since laboratory diagnosis is so readily made by other tests with special reference to bacteriologic examinations. This type of curve may also occur in anterior poliomyelitis and epidemic encephalitis but all attempts to establish a characteristic or diagnostic curve for the former have proved unsuccessful.

Mastic and Benzoin Tests. Considerable difference of opinion exists relative to the comparative merits of the mastic and benzoin colloidal reactions with cerebrospinal fluid. Both reagents are easier to prepare than the colloidal gold reagents. Consequently, both are less likely to produce falsely positive or confusing reactions. But both are likewise less sensitive than colloidal gold; at least minor degrees of precipitation are more difficult to read because color changes are not involved. However, the readings are no more difficult than those of macroscopic agglutination tests employing bacterial suspensions.

Of the two it appears that the mastic test according to the technic of Cutting [20] is generally preferred, although the benzoin test is likewise strongly recommended.[21] In the colloidal mastic test, five tubes are employed carrying 1 cc. of a 1:4 dilution of fluid in alkaline-saline solution in No. 1 to 1 cc. of 1:64 dilution in No. 5, with an extra tube as a control. To each tube is added 1 cc. of the reagent and the reading made after standing at room temperature for twenty-four hours. The benzoin test requires fifteen tubes carrying a 3:4 dilution of spinal fluid in the first to 1:16,384 dilution in the last, with an extra tube as a control.

In both tests the reactions are recorded according to the degree of precipitation of the reagents, with the numeral 3 designating complete precipitation, 2 about half precipitation, 1 slight precipitation and 0 no precipitation.

Three types of reactions are observed corresponding to the curves or zones of the colloidal gold reaction (I, II and III). These are similar to those observed in the latter test in syphilis, multiple sclerosis, meningitis, anterior poliomyelitis and other diseases previously discussed.

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THE CLINICAL INTERPRETATION OF BACTERIOLOGIC EXAMINATIONS

Bacteriologic examinations, when properly conducted, are among the most valuable of laboratory procedures in the diagnosis of diseases. But reliable results usually require the close cooperation of physicians and surgeons in the collection of materials and frequently include the preparation of smears and cultures. Furthermore, the clinical interpretation of bacteriologic examinations is sometimes difficult and especially in the case of those of exposed mucous membranes, feces, sputum, etc., by reason of possible confusion with normal bacterial floras. Consequently, clinicians require considerable knowledge of the practical or clinical aspects of bacteriology, as the mere presence of microorganisms, sometimes including those which are potentially pathogenic, does not necessarily mean that they are producing infection.

For the sake of convenience, the pathogenic spirochetes are included herein; likewise, the rickettsiae and filtrable viruses, although methods for the laboratory diagnosis of diseases due to these agents are of limited value and confined to animal inoculation tests, except in the case of rabies in which examinations of smears of the brains of dogs or other animals for Negri bodies are of diagnostic value.

GENERAL PRINCIPLES

Quite commonly the results of bacteriologic examinations are pathognomonic and of specific diagnostic value. Not infrequently, however, they may yield falsely negative results due to errors on the part of the physician in collecting material or in making smears and cultures; likewise to faulty laboratory technic. Falsely positive results may also occur because of contamination in the collection of materials or in the laboratory. Needless to state, the clinical value of bacteriologic examinations is in strict relationship to the skill with which they are conducted. As with all procedures, the results cannot be better than the laboratory conducting them, since special methods are frequently required (Table 76).

Interpretation may also be complicated by the carrier state. For example, the presence of diphtheria bacilli in a culture of the nose or throat does not necessarily mean the presence of diphtheria. Furthermore, infection may be only of secondary instead of primary importance as, for example, an excess of spirochetes and fusiform bacilli in smears of the gingival secretions due to latent scurvy from vitamin C deficiency. Under such conditions the results of bacteriologic examinations are not always a short-cut to diagnosis or a substitute for clinical skill and judgment.

As previously stated, interpretation is particularly difficult in chronic diseases due to mixed infections and especially those of open mucous membranes and

TABLE 76. SUMMARY OF THE PRINCIPLES OF THE CLINICAL INTERPRETATION OF BACTERIOLOGIC EXAMINATIONS

Subject	Interpretation
General Principles	Results frequently pathognomonic and of specific diagnostic value. But may yield falsely negative results due to errors on the part of physician in collecting materials or in making smears and cultures; likewise to poor or faulty laboratory technic. Falsely positive results may be due to contamination in collecting materials or occur in the laboratory. The clinical value of a bacteriologic examination can be no better than the laboratory conducting it. Interpretation may be complicated by the carrier state. Infection may be only of secondary instead of primary importance. Interpretation is particularly difficult in the case of examinations of open mucous membranes, chronic mixed infections, feces, sputums, etc., on account of confusion with normal bacterial floras. For this reason physicians and surgeons need to know considerable of the practical or clinical aspects of bacteriology. Differentiation between clinically important and unimportant microorganisms is frequently difficult or impossible. Animal inoculation and *in vitro* tests are helpful; likewise serologic examinations for acquired antibodies and allergic skin tests.
Collection of Materials	(1) Should be as free as possible from contamination. (2) Should be as far as possible exactly what is to be examined. (3) Cultures should be made on fresh media of the proper kinds and especially in the case of those bacteria requiring special growth principles. (4) Smears should be neither too thick nor too thin. (5) Material (especially swabs and cultures) should be delivered to the laboratory as soon as possible. (6) The request should specify the examination required or give the suspected clinical diagnosis for aiding the selection of laboratory methods.

wounds. Likewise, interpretation is difficult in the case of feces, sputums, etc., in which so many different kinds of bacteria are present. Many attempts have been made to develop methods for differentiating between bacteria possessing clinical and no clinical importance under these conditions. In the final analysis, animal inoculation tests are of most value in this condition; but they are not always applicable aside from the possibility of errors due to bacterial dissociation and the fact that they are both time-consuming and expensive. Otherwise, *in vitro* tests are sometimes of value with special reference to the production of pigments, hemolysins and electrophoresis;[1] also serologic and allergic skin tests. For example, whenever specific antibodies like agglutinins, complement-fixing and opsonins are found above normal levels for a particular bacterium, it is always good presumptive evidence that it is playing a rôle in the production of disease, provided previous infections and active immunization may be excluded. The same is generally true if allergic sensitization is found. Unfortunately, the "pathogen selective method" of Solis-Cohen and his colleagues, based upon the assumption that only bacteria capable of surviving in the whole coagulated blood of individuals are to be regarded as pathogenic, has not solved the problem and particularly not in relation to chronic mixed local infections. Furthermore, the method is not applicable for the spore-forming bacilli and those microorganisms owing all or a part of their pathogenicity to exogenous toxins which include hemolytic streptococci and many staphylococci.[2,3]

COLLECTION OF MATERIALS

As previously stated, bacteriologic examinations may be rendered entirely valueless and the results very misleading by faulty methods on the part of the physician in the collection and handling of material. Under these circumstances, it is advisable for the laboratory to reject their examination since misleading results are worse than none at all. The subject, therefore, is one of considerable importance in which physicians may require the advice and guidance of bacteriologists. The important principles may be briefly summarized as follows:

1. To obtain the material *as free as possible from contamination* by exercising due care in collection, including the use of sterile containers.

2. To obtain as far as possible *exactly what is desired for examination.* For example, pus or secretions obtained from the nasal accessory sinuses by a rhinologist are always to be preferred to those obtained by merely swabbing the nose. Likewise, with exudates in the fauces a light hasty swabbing may result in a falsely negative culture in the case of diphtheria because the physician has failed to secure the bacilli. In making smears and cultures of ulcers, wounds, pus, etc., small sterile cotton swabs are generally better than platinum or other wires because material can be secured more thoroughly and in larger amounts.

3. To *use fresh media of the proper kind for making cultures.* This is especially important in the case of suspected infections with streptococci, pneumococci, gonococci, *Hemophilus pertussis* and similar micro-organisms requiring special growth principles. Otherwise, it is far better to deliver the material or swabs to the laboratory for the inoculations of media, especially if mixed infection is suspected, in which case cultures on plates of blood agar or other appropriate solid media are preferred to those on slants.

4. To *deliver material to the laboratory as promptly as possible after collection.* This is particularly important in relation to swabs because drying may result in the destruction of delicate micro-organisms. In the case of unavoidable delay material should be kept in a refrigerator, since micro-organisms will survive considerable period of time under these conditions. Cultures may be left overnight at room temperature without harm before incubation.

5. To make *smears that are neither too thin nor too thick.* If only small amounts of material are available, one or two smears about the size of a dime are much better than larger ones spread out so thinly that it is difficult to decide on which side of the slide they have been made. The common practice of covering a large amount of material on one slide with a second is mentioned only to be condemned as filthy, potentially dangerous for laboratory workers, and usually entirely unsatisfactory for examination.

6. *To specify whenever possible the kind of examination to be made.* This is especially important in the case of feces and sputum. For example, if the former are submitted solely for examination for typhoid or dysentery bacilli, the request should be so stated in order that the laboratory may employ the special methods required. At least, the laboratory should be informed of the suspected infection in order to avoid unnecessary, expensive and time-consuming examinations.

EXAMINATIONS OF THE BLOOD

The detection of bacteria in the blood depends entirely upon blood cultures, since they are too few for detection by the examination of stained smears. *Treponema pallidum* occurs in the blood during the primary and secondary stages of syphilis but in too few numbers for detection by darkfield or other direct methods of examination. Furthermore, since *T. pallidum* cannot be readily cultivated, detection depends entirely upon the inoculation of the testicles of rabbits with blood but is not employed for diagnostic purposes. Some of the other pathogenic spirochetes, however, like those producing relapsing fever and rat bite fever, may be detected by direct microscopic examinations of the blood along with animal inoculation tests. Blood cultures and animal inoculation tests have also proved of value in the diagnosis of infectious jaundice due to *Leptospira icterohaemorrhagiae*. None of the rickettsiae or viruses, however, are ordinarily detectable by blood examinations employing cultural methods.

Blood Cultures. The clinical value of blood cultures depends a great deal upon the technic employed (Table 77). It is particularly important to *avoid contamination* in the collection of blood, by the medium or in the laboratory. *Staphylococcus albus, Bacillus subtilis* and diphtheroid bacilli are the most frequent contaminants as are, occasionally, nonhemolytic streptococci.

TABLE 77. SUMMARY OF THE CLINICAL INTERPRETATION OF BACTERIOLOGIC EXAMINATIONS OF THE BLOOD

Subject	Interpretation
Blood Cultures	Clinical value depends a great deal upon the technic employed. It is particularly important to avoid contamination. Anaerobic methods should be employed more frequently than customary and especially in puerperal sepsis and postoperative septicemias. Para-aminobenzoic acid should be added to the medium in all blood cultures in cases receiving sulfonamide therapy. Appropriate fluid media preferred to plating methods using solid media. The latter give a high percentage of falsely negative results; should be employed only under special conditions in relation to therapy and not as a routine diagnostic procedure. Massive blood cultures indicated when the number of micro-organisms are likely to be small. All blood cultures should be studied for at least ten days before being reported as sterile. Blood should be collected from a vein draining a focus of infection whenever possible. Arterial (femoral) blood cultures worthy of trial.
Bacteremia	Detectable only by blood cultures. Defined as a temporary invasion of the blood without clinical evidences of infection of this tissue. Probably of frequent occurrence and especially in focal infections, after the extraction of infected teeth, tonsillectomies, operations for osteomyelitis, etc. The bacteria are usually rapidly removed by the clearing mechanism although infections of the fixed tissues may occur.

TABLE 77. SUMMARY OF THE CLINICAL INTERPRETATION OF BACTERIOLOGIC EXAMINATIONS OF THE BLOOD—(Continued)

Subject	Interpretation
Septicemia	Detectable only by blood cultures since severe local infections may produce similar signs and symptoms. Defined as an infection of the blood. Secondary infections of the fixed tissues are frequent and especially in the surgical septicemias due to staphylococci and hemolytic streptococci. Usually secondary to primary infections of the fixed tissues. These may be trivial and even crytogenic when resistance is low. Theoretically possible with all pathogenic bacteria but most frequently due to streptococci, staphylococci, pneumococci, meningococci, *S. typhosa, Br. abortus, Br. melitensis* and *B. anthracis;* frequently due to gonococci, *Esch. coli, Ps. aeruginosa, K. pneumoniae, H. influenzae* and Bacteroides. Rarely caused by the toxin-producing anaerobic bacilli (*C. diphtheriae* and *Cl. tetani*) but may be caused by the bacilli of the gas gangrene group and especially *Cl. perfringens.* Not necessarily in relation to the virulence of organisms as septicemia may be due to *Staph. albus, P. vulgaris* and the like when resistance is unusually low.
Spirochetemia	Occurs in the early stages of *syphilis* but is detectable only by the inoculation of the testicles of rabbits with blood which is not a practical diagnostic procedure. Occurs early and frequently in *relapsing fever;* direct examinations of the blood for the spirochetes and inoculation of mice are valuable diagnostic procedures. Frequent in *rat-bite fever.* Darkfield examinations subject to error due to artefacts. Stained smears for *Sp. minus* preferred but inferior in diagnostic value to animal inoculation tests. May occur in *fusospirochetosis* with positive blood cultures and especially in cases with metastatic lesions. Occurs in *leptospirosis* or *infectious jaundice* (Weil's disease). Darkfield examinations inadvisable because subject to error from artefacts. Cultures of the blood and animal inoculation preferred for diagnostic purposes along with serum agglutination tests.

Aerobic methods are generally employed but undoubtedly many falsely negative results occur because of failure to use *anaerobic methods.* It is true that in the deeper parts of broth cultures there is sufficient reduction of oxygen tension for the cultivation of micro-aerophilic organisms, but strictly anaerobic methods should be employed alone or in conjunction with aerobic methods in blood cultures on all suspected cases of puerperal sepsis, with special reference to hemolytic streptococci and *Clostridium perfringens* (*Cl. welchii*), as well as in all suspected cases of the postoperative septicemias in relation to hemolytic streptococci and organisms of the genus *Bacteroides.*

Furthermore, it is particularly important to add 5 mg. of *para-aminobenzoic acid* (P.A.B.) to each 100 cc. of broth medium in all blood cultures of individuals who are receiving sulfonamide therapy. Otherwise, streptococci and other micro-organisms actually present may fail to proliferate with falsely negative results which can be not only misleading but actually disastrous in relation to sulfonamide therapy.

Blood cultures are best made in fluid media although it is true that the results are not quantitative in the sense of showing the number of microorganisms per cubic centimeter of blood. For this purpose plating 1 or 2 cc. of blood with agar-agar at the bedside may be employed; or 5 cc. of citrated blood may be delivered to the laboratory for plating purposes. But only 5 to 10 per cent of positive blood cultures employing a broth medium may prove positive by these plating methods. Consequently, the clinical value of the latter is sharply limited and never to be relied upon alone for diagnostic purposes; indeed, they are advisable only on special occasions in relation to sulfonamide or serum therapy.

Needless to state, the *kind of medium* employed is of great importance. The optimum *p*H is ordinarily from 7.4 to 7.6. Many media have been employed and especially hormone broth with 0.2 per cent glucose, but tryptose phosphate broth and the heart-brain broth of Kracke are particularly useful. Ordinarily, flasks carrying 75 cc. inoculated with 5 to 6 cc. of blood are satisfactory. As a general rule it is advisable to collect blood at the height of fever whenever possible. *Massive blood cultures* are indicated when small numbers of bacteria are likely to be present;[4] for this purpose flasks carrying 150 cc. of medium inoculated with 15 to 20 cc. of blood ordinarily suffice.

Preliminary reports should be rendered by the laboratory at the end of 24 to 72 hours but no blood culture should be accepted as sterile short of at least ten to twenty-one days of incubation and study.

Finally, it would appear that more attention should be given the matter of *choice of vein* for securing blood for culture purposes. As a general rule a vein in the arm is employed but apparently this is not always a good routine procedure. For example, it has been shown that in septicemia due to thrombophlebitis, blood taken from a vein directly draining the focus contains more organisms than venous blood removed from the arm because of less dispersion.[5] It would seem advisable, therefore, to secure blood from a vein draining an infected area whenever feasible. Several investigators have reported that cultures of blood removed from the femoral artery have shown a higher incidence of positive results than duplicate cultures of venous blood.[6,7] Whether or not the procedure is justified for ordinary diagnostic purposes, in view of its greater technical difficulties, cannot be stated although it appears worthy of further trial.

Bacteremia. Undoubtedly the blood is frequently invaded by microorganisms which, under normal conditions, are rapidly removed by the clearing mechanism with special reference to phagocytosis by the cells of the reticulo-endothelial system. The incidence is unknown but there are reasons for surmising that it is particularly frequent in local infections. Thus streptococci often are found by blood cultures in rheumatoid arthritis; likewise streptococci and staphylococci in dental infections after chewing[7] and in as high as 60 to 70 per cent of cases after the extraction of teeth under general anesthesia[8] with about 17 per cent after the extractions under local anesthesia.[9] Similar results have been observed before and after tonsillectomies,[10] operations in osteomyelitis and the like. In such instances there are no clinical evidences of infection although infection of the fixed tissues may follow. Consequently, it appears that this state constitutes *bacteremia* which may be defined as *a temporary invasion of the blood with bacteria without evidences of infection of this tissue.*

Septicemia. When the immunologic resistance of the blood is broken down by unusual numbers of virulence of microorganisms, or by failure of the clearing mechanism, permitting the latter to multiply in the blood along with the production of toxins and the signs and symptoms of infection, the state may be defined as *septicemia* or an *infection of the blood.* Secondary infections of the fixed tissues, and particularly of the lungs, frequenly occur and especially in the surgical septicemias due to staphylococci and hemolytic streptococci.

The terms *bacteremia* and *septicemia*, however, are commonly employed synonymously because it is frequently difficult to draw a sharp distinction between them. For example, the occurrence of positive blood cultures in the early stage of typhoid fever may be only a bacteremia, since it is usually temporary and occurs at a time when the patient is not severely ill, but if it persists, or becomes more pronounced, it may become an overwhelming septicemia.

Septicemia is usually secondary to a primary infection of the fixed tissues. The latter, however, may be relatively trivial and, indeed, may be cryptogenic without being discovered at all and especially when resistance is low. Furthermore, it may develop very rapidly after an initial infection of a mucous membrane and particularly of the respiratory tract as, for example, the septicemias in the early stages of meningococcal meningitis and pneumococcal pneumonia, which may prove rapidly fatal. Furthermore, a diagnosis of septicemia is not usually justified without the evidence of one or more positive blood cultures since a severe local infection may produce all of the signs and symptoms suggestive of this state.

While, theoretically, all pathogenic bacteria may cause septicemia, they are not all equally likely to do so. The pyogenic bacteria, especially streptococci, staphylococci, pneumococci and meningococci, belong to the group most likely to produce septicemia, which also includes *Salmonella typhosa, Brucella abortus, Brucella melitensis, Escherichia coli, Salmonella choleraesuis, Bacillus anthracis, Streptobacillus moniliformis, Actinomyces necrophorus* and the gonococcus. Apparently, *Mycobacterium tuberculosis* may also occur in the blood in pulmonary tuberculosis according to the technic of Loewenstein but not as frequently as he has reported.[11] The toxin-producing bacilli, like *Corynebacterium diphtheriae* and *Clostridium tetani* and those of the gas gangrene group, occur but rarely in the blood, except *Clostridium perfringens,* although this may be due in part to the infrequency with which anaerobic methods of blood culture are used, while septicemias due to the *Esch. coli, Pseudomonas aeruginosa, Alcaligenes faecalis, Hemophilus influenzae, Klebsiella pneumoniae* and the bacteroides are more uncommon. In other words, bacteremia and septicemia are largely in relation to the aggressiveness of microorganisms but otherwise not in relation to their pathogenicity, since organisms of such low virulence as *Staphylococcus albus* and *Proteus vulgaris* [12] may invade the blood when the resistance of individuals is unusually low.

Spirochetemia. As previously stated, spirochetemia occurs in *syphilis* and especially during the primary and secondary stages, but is not detectable except by the inoculation of the testicles of rabbits with blood, which is not a practical diagnostic procedure. It also occurs so frequently and early in *relapsing fever* that examinations of fresh blood for the spirochetes by darkfield microscopy or of stained smears (preferred), as well as by the inoculation of mice, are valuable diagnostic aids. Likewise in *rat-bite fever* darkfield examinations of fresh blood may reveal the presence of *Sp. minus* but since errors may occur due to artefacts, examinations of stained smears are preferred although for diagnostic purposes they are inferior to intraperitoneal inoculation of mice or guinea pigs with blood, exudate or tissue removed from the primary lesion or material aspirated from lymphatic glands. While infection of the blood with the fusiform bacilli and spirochetes associated with Vincent's angina, gingivitis and gingivostomatitis (*fusospirochetosis*) is rare, nevertheless metastatic lesions are not infrequent and positive blood cultures have been reported [13] although not usually required for diagnostic purposes unless metastatic infections are suspected. Strictly anaerobic methods are required.

Likewise in *leptospirosis* or *infectious jaundice* (Weil's disease), *Lept. icterohaemorrhagiae* may be found by darkfield examinations of the blood but the chances of error due to artefacts are so great that the procedure cannot be recommended for diagnostic purposes. Cultures of the blood have proved more valuable and especially inoculation of guinea pigs with blood and urine along with serum agglutination tests. The latter procedures are strongly recommended for diagnostic purposes, especially for the detection of moderately severe icteric or nonicteric types of the disease, which are frequently unsuspected, and particularly in the case of individuals exposed to occupational hazards or circumstances from contact with the excreta of rats or, rarely, with those of dogs.[14]

EXAMINATIONS OF THE CEREBROSPINAL FLUID

Bacteriologic examinations of the cerebrospinal fluid are valuable aids in the diagnosis of the acute and chronic infectious meningitides (Table 78). In fact, the clinical signs and symptoms of acute purulent meningitis due to meningococci, *H. influenzae,* streptococci, pneumococci, etc., are so similar that they are usually required for etiologic diagnosis and specific therapy with the sulfonamide and antibiotic compounds. They are likewise extremely valuable aids in differentiating "serous meningitis" or meningismus and aseptic meningitis from meningitis due to infection; also in the detection of tuberculous meningitis and its differentiation from encephalitis which it may resemble clinically. Bacteriologic examinations, however, are of no value in the diagnosis of viral infections of the central nervous system like acute anterior poliomyelitis, the encephalitides, choriomeningitis, etc.

Normal cerebrospinal fluid is sterile but contaminations during its collection are not infrequent. If promptly examined, contaminating bacteria are too few to be found in direct examinations of stained smears but occur in cultures. In case of one or more days of delay in bacteriologic examinations, however, contaminating organisms may proliferate sufficiently to be found in both smears and cultures. Consequently, great care is required in the clinical interpretation of bacteriologic examinations of cerebrospinal fluid and the possibility of contamination is always to be kept in mind, with special referencc to staphylococci, unless other changes are present indicative of meningitis, like pleocytosis, increase of protein, reduction in glucose, etc.

The fluid is also sterile in *meningismus* or "serous meningitis" unless contaminated during its collection; likewise in *aseptic meningitis* due to the intrathecal injection of sterile substances. But in "*meningitis sympathica*" as well as in *localized meningitis,* infecting micro-organisms are frequently present in cultures although usually too few for detection in stained smears of the fluid or its sediment.

TABLE 78. SUMMARY OF THE CLINICAL INTERPRETATION OF CEREBROSPINAL FLUID EXAMINATIONS

Disease	Interpretation
General Consider-ations	Of great value in the etiologic diagnosis of the acute and chronic meningitides due to bacterial infection and for their differentiation from "serous meningitis" (meningismus) and aseptic meningitis. Also of value in relation to specific therapy with the sulfonamide and antibiotic compounds. Treatment should be continued until the fluid has become sterile as a safeguard against residual infections and their recrudescence. Acute *primary* meningitis usually due to the meningococcus and *H. influenzae.* Less frequently due to hemolytic streptococci, pneumococci, staphylococci, *Esch. coli* and other organisms. *Secondary* meningitis usually due to hemolytic streptococci, pneumococci, staphylococci, *Myco. tuberculosis* and *K. pneumoniae.* Of no value in the diagnosis of infections of the central nervous system due to the filtrable viruses (acute anterior poliomyelitis, lymphocytic choriomeningitis and the encephalitides). Of no value in relation to the diagnosis of syphilis of the central nervous system.
Normal	Sterile unless contaminated during collection or in the laboratory.
"Serous Menin-gitis"	
Aseptic Meningitis	
Localized Meningitis	Cultures usually positive although smears of the fluid or its sediment may be negative due to few micro-organisms.
Meningo-coccal Meningitis	The most frequent type of acute primary meningitis. Fluid should be examined as promptly as possible after collection. Large amounts should be used for cultures. Smears and cultures may be negative in the early stages due to the presence of but few organisms. The diplococci may be difficult to decolorize in the gram stain and be mistaken for pneumococci or staphylococci.

TABLE 78. SUMMARY OF THE CLINICAL INTERPRETATION OF CEREBROSPINAL FLUID EXAMINATIONS—(Continued)

Disease	Interpretation
Influenzal Meningitis	Usually an acute primary type of meningitis. Bacilli may be overlooked in smears due to their smallness and faint staining. Long filamentous forms may occur.
Streptococcal, Pneumococcal and Staphylococcal Meningitis	Usually acute types of meningitis secondary to sinusitis, mastoiditis or otitis media. Staphylococcal meningitis uncommon. Organisms usually numerous in smears and cultures. Identification usually requires cultural examinations.
Tuberculous Meningitis	Usually a chronic secondary type of meningitis. May be acute. Mortality very high. Usually but few bacilli present in the fluid. Smears of sediment and coagula frequently negative. Examination of specially stained smears by fluorescent microscopy helpful. Cultures on special media required. Guinea-pig inoculation tests advisable as a routine procedure but requires a month or more for results.

Primary acute diffuse meningitis is usually due to the meningococcus or *H. influenzae*. The latter is a misnomer as it is not the primary cause of influenza, which is due to a filtrable virus, but is frequently an important organism of secondary bacterial infection in this disease with special reference to bronchopneumonia. Under the circumstances its name should be changed to *Hemophilus pfeifferi* in honor of its discoverer. Primary acute diffuse meningitis may be also due to hemolytic streptococci, pneumococci, staphylococci, *Esch. coli, S. choleraesuis, Alcaligenes faecalis, B. anthracis* and the gonococcus but much less frequently. While the etiologic relationship of *Listerella monocytogenes* is uncertain, nevertheless this bacterium is known to cause meningocephalitis, with a mortality of about 70 per cent.[15] Under the circumstances, the physician is usually justified in treating cases of acute primary meningitis on the basis of meningococcal infections until or unless proved otherwise by bacteriologic examinations of acceptable accuracy.

In this connection emphasis should be placed upon the promptness of bacteriologic examinations. This is particularly important if meningococcal meningitis is suspected, since in the early stages of the disease the gram-negative diplococci are seldom numerous and quickly die or disappear due to autolytic changes. Indeed, under these conditions smears of the fluid or its sediment secured by centrifugation may show no diplococci at all. But, if the fluid is cloudy, with a high total cell count due to polymorphonuclear neutrophil leukocytes, along with an increase of protein, a tentative diagnosis of meningococcal meningitis is justified and especially since cultures of the fluid in the early stages may be negative; this is particularly likely to occur if only small amounts are cultured or inappropriate media employed.

In influenzal meningitis, however, the gram-negative bacilli are usually quite numerous in the early stages of the disease although easily overlooked in direct smears because of their smallness and faint staining. Quite frequently, however, they occur in long filamentous forms which may be erroneously interpreted as indicative of a mycotic infection. Needless to state, special culture media must be employed and especially those containing blood.

Secondary acute diffuse and localized meningitis is usually due to pneumococci, streptococci, staphylococci or *Ps. aeruginosa* by extension of infection from the nasal accessory sinuses, mastoid cells or middle ear. As a general rule, the organisms are quite numerous, even in the early stages, but differentiation between streptococci and pneumococci usually requires cultural studies. They are all gram-positive but since the meningococcus is more difficult to decolorize than usual, grievous and even disastrous errors may occur by mistaking it for a gram-positive diplococcus (pneumococcus). Furthermore, staphylococci in smears stained with methylene blue may assume a diplococcus grouping and be mistaken for the meningococcus. Good gram staining, therefore, is technically very important.

Typhoid meningitis is likewise usually a secondary infection but is not infrequently of the toxic type or meningismus with sterile spinal fluids and not a true meningitis at all. The same may also occur in pneumonia, scarlet fever and other acute infectious diseases.

Tuberculous meningitis, which is the most dreadful of all because of its high mortality, is also of the secondary type, since primary lesions are usually discovered at autopsy if not during life. As a general rule, so few bacilli are present in the spinal fluid that prolonged search of stained smears of sediment or coagula is required for their detection. Even then, none may be found, so that negative smears by no means exclude the possibility of the disease. In this connection the examination of smears stained with auramine O and examined without counterstain with a simple fluorescent microscope after the method of Richards and Miller,[16] may improve the chances of finding the bacilli as it has in the case of tuberculous sputums. Cultures on special media, especially that of Petragnini, are also helpful; likewise and particularly guinea-pig inoculation tests, although a month or more is required before the results are available.

While *T. pallidum* has been found in the cerebrospinal fluid by inoculation into the testicles of rabbits, the method is not employed for diagnostic purposes. Darkfield examinations are worthless not only because of the infrequency of the presence of spirochetes in the fluid, but likewise because of their extremely small numbers.

Finally, bacteriologic examinations of the cerebrospinal fluid are extremely valuable in relation to the specific therapy of meningococcal, influenzal, streptococcal, pneumococcal, staphylococcal and tuberculous meningitis. This is true not only in etiologic diagnosis in relation to a selection of sulfonamide and antibiotic compounds with or without immune serums for treatment purposes, but likewise in relation to dosage and duration of treatment. In general terms, treatment should be continued until two or more specimens of fluid are found free of microorganisms in both smears and cultures as a safeguard against residual infections and their recrudescence.

EXAMINATIONS OF THE EARS, NOSE, THROAT AND ADNEXA

Infections of the nose and throat are invariably air-borne and usually by droplet transmission through contact with carriers or infected individuals. The accessory sinuses, however, are usually infected by progression of infection from the nasal mucosa while the middle ear is commonly infected by extension from the throat by way of the eustachian tubes. The mastoid cells are commonly infected by extension from the middle ear with the possibility of further progression to the lateral sinuses or meninges.

Unfortunately, bacteriologic examinations of the nasal or faucial mucosa are complicated insofar as interpretation is concerned, by a normal flora carrying various types of streptococci, pneumococci and staphylococci; also *Neisseria catarrhalis* and other gram-negative diplococci, as well as by pseudodiphtheria bacilli, *H. influenzae* and *K. pneumoniae*. However, they are not without clinical value, especially in the acute infections in relation to specific therapy, as well as in the detection of diphtheria, Plaut-Vincent angina, pertussis, meningococcus carriers, etc.; also in the case of chronic infections of the sinuses and tonsils in relation to the diseases of focal infection.

Ears and Mastoids. *Furunculosis* of the external auditory canal is almost invariably due to *Staph. aureus* or *albus*. The etiology of otomycosis (otitis externa mycotica) with ulcerations is not definitely known but is apparently due to the molds of the genera *Penicillium* or *Aspergillus* and sometimes to the Saccharomyces or yeasts (Table 79).

Acute suppurative otitis media is usually due to pure infections with a hemolytic streptococcus, *Staph. aureus* or a pneumococcus but may be due to *C. diphtheriae, H. influenzae, K. pneumoniae* or *Borrelia vincentii* in association with *B. fusiformis*. As a general rule it is advisable to make cultures upon incision of the tympanum, or soon after its spontaneous rupture, and especially if autogenous vaccines are to be used in treatment in case the disease becomes chronic.

Chronic suppurative otitis media is invariably a mixed infection due to one or more of the same micro-organisms with *Ps. aeruginosa* along with *C. pseudodiphtheriticum* or *P. vulgaris* as saprophytic contaminants. It may be also caused by *Myco tuberculosis*, usually in mixed infections with staphylococci or other micro-organisms, but since it is particularly difficult to find the bacilli in smears and cultures, negative results do not exclude the possibility of the infection.

Acute and chronic mastoiditis as well as *lateral sinus phlebitis* are usually due to infection with the same micro-organisms producing suppurative otitis media with special reference to hemolytic streptococci, pneumococci, *Staph. aureus* and *K. pneumoniae*. The same is true of *acute and chronic labyrinthitis*.

Nose and Accessory Sinuses. *Furunculosis* of the atrium is due to infection of hair follicles with *Staph. aureus* or *albus* while *vestibulitis* or dermatitis of the nasal vestibule may be due to staphylococci, streptococci or pneumococci. *Septal abscesses* are likewise usually due to infection with *Staph. aureus*.

TABLE 79. SUMMARY OF THE CLINICAL INTERPRETATION OF BACTERIOLOGIC EXAMINATIONS OF THE EARS, NOSE, THROAT AND ADNEXA

Organ	Interpretation
General Considerations	Infections of the nose and throat are air-borne and usually by droplet transmission. Infections of the accessory sinuses are usually by extension from the nasal mucosa and of the middle ear from the throat by way of the eustachian tubes. The normal mucosa of the nose and throat commonly shows the presence of any of the following micro-organisms: various types of streptococci, staphylococci and pneumococci as well as *N. catarrhalis* and other gram-negative diplococci, pseudodiphtheria bacilli. *H. influenzae* and *K. pneumoniae*. The presence of these may complicate the clinical interpretation of bacteriologic examinations.
Ears and Mastoids	*Furunculosis* of the external auditory canal is due to staphylococcus infection. Etiology of *otomycosis* with ulceration is not definitely known; probably caused by the molds Penicillium or Aspergillus and sometimes by the yeasts Saccharomyces. *Acute suppurative otitis media* usually due to pure infections with hemolytic streptococci, staphylococci and pneumococci; less frequently due to the diphtheria, influenza or Friedländer bacilli or fusospirochetal infection. *Chronic suppurative otitis media* usually due to one or more of the same micro-organisms; *Ps. aeruginosa* frequent; pseudodiphtheria bacilli or *P. vulgaris* common saprophytes. May be due to the tubercle bacillus. *Acute* and *chronic mastoiditis* and *lateral sinus phlebitis* usually due to hemolytic streptococci, staphylococci, pneumococci or *K. pneumoniae*. The same is true of *acute* and *chronic labyrinthitis*.
Nose and Sinuses	*Furunculosis* of the atrium and *septal abscess* due to staphylococcus infection; *vestibulitis* usually a dermatitis due to staphylococci, streptococci or pneumococci. The common cold is primarily due to a filtrable virus followed by mixed bacterial infection; the latter may produce sinusitis or infection of the lower respiratory tract. *Acute membranous rhinitis* is due to the diphtheria bacillus (nasal diphtheria). Diagnosis may be complicated by presence of *C. pseudodiphtheriticum* frequently present and nonpathogenic. *Acute bacterial rhinitis* is usually due to exacerbation of chronic sinusitis due to mixed infection. *Chronic rhinitis* is due to mixed infection. The etiology of ozena and rhinoscleroma are unknown. Chancres may occur in the nose; diagnosis greatly aided by darkfield examinations for *T. pallidum*. Leprosy bacilli commonly present in the nose and nasopharynx; examination of smears for acid-fast bacilli of diagnostic value. *Chronic sinusitis* usually due to pure or mixed infections with staphylococci, streptococci, pneumococci, *K. pneumoniae* or influenza bacilli; *N. catarrhalis*, pseudodiphtheria bacilli and *P. vulgaris* common.

TABLE 79. SUMMARY OF THE CLINICAL INTERPRETATION OF BACTERIOLOGIC EXAMINATIONS OF THE EARS, NOSE, THROAT AND ADNEXA—(Continued)

Organ	Interpretation
Throat	Bacteriologic examinations valuable in the diagnosis of *diphtheria*. Cultures preferred to smears. One or two primary negative cultures may not exclude the disease. Guinea-pig virulence tests valuable for differentiating between diphtheria and pseudodiphtheria bacilli in prolonged quarantine. Bacteriologic examinations also valuable in the diagnosis of *Plaut-Vincent angina;* also for the detection of *meningococcus carriers* and the diagnosis of *pertussis* in the catarrhal stage by the cough-plate method. *Acute follicular tonsillitis* and anginas usually due to hemolytic streptococci, staphylococci or pneumococci. *Peritonsillar abscess* and *retropharyngeal abscess* are usually due to hemolytic streptococci or staphylococci. *Chronic tonsillitis* usually due to mixed infections with streptococci, staphylococci and pneumococci; bacteriologic examinations are of limited value in diagnosis. *Acute septic sore throat* is due to hemolytic streptococci; usually a milk-borne infection occurring in epidemics. *Acute laryngitis* usually due to infection with hemolytic streptococci, staphylococci, pneumococci, *N. catarrhalis* or the influenza bacillus. *Chronic laryngitis* is usually due to mixed infections with these microorganisms. May also be due to the tubercle bacillus or *T. pallidum*. *Chronic pharyngitis* is usually due to infection with streptococci, staphylococci or pneumococci secondary to chronic sinusitis.

The *common cold* or acute infectious rhinitis is now commonly thought to be due primarily to a filtrable virus followed by an inevitable secondary bacterial infection with streptococci, staphylococci, pneumococci, *N. catarrhalis* or *K. pneumoniae,* singly or in combinations, due to lowered resistance. Some infection of the accessory sinuses occurs, especially of the ethmoid, sphenoid or frontal cells, which may result in chronic sinusitis when common colds frequently occur. This secondary bacterial infection may also produce laryngitis, pharyngitis, tracheitis and bronchitis.

Acute membranous rhinitis is usually due to infection with *C. diphtheriae* with the production of nasal diphtheria. Bacteriologic diagnosis may be complicated by the frequency of *C. pseudodiphtheriticum* in the normal nasal secretions but differentiation from *C. diphtheriae* is usually readily made on the basis of morphologic characteristics, aided when necessary by sugar fermentation tests. Not infrequently quarantine is needlessly prolonged after recovery from nasal diphtheria by cultures showing *C. pseudodiphtheriticum* or pseudodiphtheria bacilli. These can be differentiated from *C. diphtheriae* by cultural characteristics and especially by guinea-pig inoculation tests for virulence, which are very reliable when properly conducted.

Acute rhinitis, closely resembling the common cold in its clinical manifestations, may be also due to exacerbations of chronic nasal accessory sinusitis with special reference to ethmoiditis and sphenoiditis. This constitutes the so-called *acute bacterial rhinitis* and is always to be suspected in individuals known to have chronic sinusitis. It may be also due to *Malleomyces mallei* although glanders in human beings is fortunately rare.

Chronic rhinitis may be due to mixed bacterial infection, with or without sinusitis, and assume the hypertrophic or hyperplastic types. The etiology of the atrophic type is unknown although the odor is generally due to the presence of *K. ozaenae* or other micro-organisms of secondary infection.

Seasonal rhinitis or coryza is due to allergy to various pollens (hay fever) while vasomotor rhinitis occurring the year round and erroneously called "perennial hay fever," is generally due to allergy to dusts or other air-borne allergens as well as to food allergies in some cases. The presence of polypi is usually indicative of allergic rhinitis.

In this connection it should be stated that *chancres* may also occur on the nasal mucosa near the atrium due to accidental infection by the fingers contaminated with *T. pallidum*. Darkfield examinations possess great diagnostic value, since contaminating spirochetes, so commonly occurring in the mouth (*T. macrodentium* and *T. microdentium*) do not ordinarily occur in the normal nose.

Furthermore, *Myco. leprae* occurs on the nasal mucosa of a large percentage of individuals with *leprosy* without the presence of demonstrable lesions. For this reason the examination of smears of nasal secretions stained by the acid-fast method possess diagnostic value in this disease.

The etiology of *rhinoscleroma* is still uncertain.[17] The bacillus associated with this disease by Frisch is now believed to be *K. pneumoniae* belonging to Type C.[18]

Chronic nasal accessory sinusitis is usually due to pure or mixed infections with *Staph. aureus*, hemolytic streptococci, pneumococci, *K. pneumoniae* or *H. influenzae*. Other micro-organisms of lesser importance may also be observed, such as *Ps. aeruginosa* and *N. catarrhalis* as well as such saprophytes as pseudodiphtheria bacilli and *P. vulgaris*. Postnasal catarrh or chronic pharyngitis is usually present and apparently due to the same mixed infections. As previously stated, cultures of the exudates from the sinuses are always best when made by an experienced rhinologist, especially if autogenous vaccines are to be prepared, in order to reduce the chances of contamination to a minimum.

Throat. Bacteriologic examinations of the throat, and especially of the tonsils, are especially valuable in the diagnosis of *diphtheria*. It is particularly necessary to prepare the cultures with care and thoroughness. Otherwise, they may fail to reveal the presence of *C. diphtheriae* and thereby prove misleading. The prompt administration of antitoxin is always justified on clinical grounds alone without awaiting the results of bacteriologic examination or when the primary culture is negative which is not infrequently the case. The examination of direct smears may suffice for early diagnosis but requires the services of experienced bacteriologists or technicians; when negative, smears do not exclude the disease as this frequently happens in cases showing positive cultures. Needless to state, fresh culture media should be used; the Loeffler medium is that commonly employed. As in the case of nasal diphtheria, quarantine may be needlessly prolonged following recovery due to the presence in cultures of *C. pseudodiphtheriticum* or nonvirulent *C. diphtheriae*. Guinea-pig inoculation tests for virulence are reliable if properly conducted and, when proving negative, quarantine may be safely lifted. Acute anginas resembling diphtheria may be also due to pneumococci or hemolytic streptococci, the latter especially in the early stages of scarlet fever.

Bacteriologic examinations of stained smears are also invaluable in the diagnosis of *Plaut-Vincent angina* due to infection with *Bor. vincentii* and *B. fusiformis* which are present in large numbers in this disease and especially the spirochetes. Total and differential leukocyte counts should always be made at the same time for possible primary agranulocytic angina.

Acute follicular tonsillitis is usually due to *Staph. aureus* but may be caused by hemolytic streptococci or pneumococci. *Peritonsillar abscess* or quinsy as well as *retropharyngeal abscesses* are usually due to hemolytic streptococci and less frequently to *Staph. aureus,* pneumococci or *H. influenzae.*

Chronic tonsillitis is invariably due to mixed infections and especially with streptococci, including *Str. viridans, Staph. aureus* and *albus* and pneumococci. *N. catarrhalis* and other gram-negative diplococci as well as pseudodiphtheria bacilli are likewise commonly present. But the diagnosis of chronic tonsillitis depends far more on clinical than on bacteriologic examinations because all of these micro-organisms may occur in the normal bacterial flora of the fauces and tonsils. The presence of numerous hemolytic streptococci is more significant and alone responsible for epidemics of *septic sore throat* usually due to milk-borne infection.

Acute specific laryngitis is generally due to pure or mixed infections with hemolytic streptococci, staphylococci, pneumococci, *N. catarrhalis* or *H. influenzae.* Of course, membranous croup or laryngeal diphtheria is due to *C. diphtheriae.* Cultures are particularly apt to be negative so that two or more may be required for confirmation of clinical diagnosis.

Chronic specific laryngitis is generally due to mixed infections with the same micro-organisms; also to *Myco. tuberculosis* (tuberculous laryngitis) secondary to pulmonary tuberculosis in which diagnosis depends almost entirely upon the clinical manifestations supplemented by the presence of tubercle bacilli in the sputum. Chronic laryngitis may be also due to tertiary syphilis but under the circumstances bacteriologic examinations by darkfield microscopy are of no value in diagnosis.

Chronic pharyngitis is commonly due to hemolytic streptococci, *Staph. aureus* or pneumococci, with infection of the solitary lymph follicles usually secondary to chronic nasal sinusitis. Acute exacerbations are of frequent occurrence especially when resistance is lowered by local or general factors.

Bacteriologic examinations of the fauces and especially of the nasopharynx, constitute the only means for the detection of *meningococcus carriers* and are of invaluable assistance in tracing sources of infection responsible for epidemics of meningococcal meningitis as well as in relation to quarantine in the disease. This is likewise true of streptococcus carriers.

Cough plates are likewise the most valuable means for the diagnosis of *pertussis* and especially during the catarrhal stage when clinical diagnosis is difficult or impossible.

EXAMINATIONS OF THE SPUTUM AND EXUDATES OBTAINED BY BRONCHOSCOPIC ASPIRATION

The clinical value of bacteriologic examinations of the sputum is confined to the etiologic diagnosis of infectious diseases of the larynx, trachea, bronchi and lungs with special reference to tuberculosis and the pneumonias (Table 80). Unless precautions are taken during collection, sputums are likely to be contaminated with saliva and postnasal secretions, with the result that various unimportant micro-organisms may be found, especially in cultures, which are always likely to be confusing in relation to clinical interpretation. In acute infections of the lower respiratory tract, particularly in the pneumonias, the infecting organisms usually predominate so greatly in both smears and cultures that their etiologic significance is readily apparent. In chronic infections, however, like the chronic bronchitides and bronchiectasis, mixed bacterial floras are the rule. Under the circumstances, it is frequently difficult to decide upon the micro-organisms of clinical importance, especially in view of the chances of contamination incident to the collection of specimens. Therefore, bacteriologic examinations of secretions and exudates secured by bronchoscopic aspiration or drainage are always preferred whenever possible for etiologic diagnosis and the preparation of autogenous vaccines.

Tuberculosis. Tubercle bacilli do not occur in the sputum until pulmonary lesions break down into the bronchi. Consequently, they are usually absent in the early stages of the disease. Frequent examinations may be required before they are found. For this reason a single negative examination never reliably excludes the possibility of tuberculosis of the lungs. Even in advanced tuberculosis with copious sputum they may not be found in smears due to small numbers or their irregular elimination. Examinations of specially stained smears by the method of fluorescent microscopy [16] are advisable under these circumstances since they sometimes reveal the presence of tubercle bacilli which escape detection by ordinary examinations. Concentration methods (antiformin or substitutes) are always advisable in the case of copious sputums when bacilli are not otherwise found. The detection of one or two acid-fast bacilli in smears is always presumptive evidence of tuberculous infection but should not be accepted clinically until confirmed. Mistakes may occur due to scratches in slides resisting decolorization; also to the presence of saprophytic acid-fast bacilli occurring in stale distilled water used in staining methods; likewise from the presence of acid-fast saprophytic organisms of the thiobacilli group (timothy bacillus, butter bacillus, etc.) as well as *Nocardia asteroides,* all of which may occur in the mouth and gingival secretions. Cultures and guinea-pig inoculation tests are frequently required for diagnostic and confirmatory purposes. Reports on the average number of tubercle bacilli per field in stained smears of sputum, according to the method of Gaffky, are clinically helpful; these vary from 1 to 4 in the whole preparation (No. 1) to enormous numbers in each field (No. 10).

Pneumococcal Pneumonia. This disease is usually of the lobar or croupous type but may occur as a bronchopneumonia, especially in children and the aged. When sputum is procurable, bacteriologic diagnosis is usually quickly and readily made by the examination of stained smears and cultures, supplemented if necessary by mouse inoculation tests. When sputum is not procurable bronchial secretions can usually be obtained in children and adults by swabbing the throat after

TABLE 80. SUMMARY OF THE CLINICAL INTERPRETATION OF BACTERIOLOGIC EXAMINATIONS OF THE SPUTUM AND EXUDATES OBTAINED BY BRONCHOSCOPIC ASPIRATION

Disease	Interpretation
General Considerations	Sputums may be contaminated with saliva or postnasal secretions during collection which may complicate the clinical interpretation of bacteriologic examinations. These factors, however, are not likely to be confusing in the acute infections and contaminations are usual in the chronic bronchitides, bronchiectasis and abscess of the lung. Consequently, bacteriologic examinations of exudates secured by bronchoscopic aspiration are preferred in these diseases.
Tuberculosis	Tubercle bacilli may not occur in the sputum in the early stages of tuberculosis. A single negative examination never reliably excludes the disease. Repeated examinations are required. Tubercle bacilli may not be found in the sputum in advanced tuberculosis due to small numbers or intermittent elimination. Repeated examinations may be required; also concentration methods, cultures and guinea-pig inoculation tests. Errors in bacteriologic diagnosis based upon the examination of stained smears may occur and especially if acid-fast saprophytic thiobacilli or *N. asteroides* are present as contaminants. Diagnosis based upon smear examinations is greatly facilitated by special staining and fluorescent microscopy. The numbers of tubercle bacilli present in smears, reported according to the method of Gaffky, are usually of some clinical value.
Pneumococcal Pneumonia	Pneumococci are responsible for about 96 per cent of cases of lobar pneumonia and about 50 per cent of cases of bronchopneumonia. Bacteriologic examinations are best conducted with sputum. Throat swabs after coughing or throat cultures may be employed; also exudates secured by lung puncture. Typing is not employed as frequently at present as formerly because of the efficacy of penicillin and the sulfonamide compounds in the treatment of all types. Typing is always advisable, however, and is required for the serum treatment of the disease. The method of Neufeld is recommended. Laboratories should always report the average number of pneumococci per field as this is frequently of clinical value in relation to prognosis and treatment.
Streptococcal and Other Pneumonias	About 4 per cent of cases of lobar pneumonia are due to hemolytic streptococci, *K. pneumoniae, H. influenzae,* staphylococci or mixed infections. About 50 per cent of cases of bronchopneumonia are due to pneumococci; about 30 per cent to streptococci and about 20 per cent to miscellaneous micro-organisms. Etiologic diagnosis is usually based upon the micro-organism predominating in smears and *plate* cultures. For this reason laboratories should always report the predominating micro-organism whenever possible. Pneumonia may also be due to *Past. pestis* (pneumonic plague), *B. anthracis* (pulmonary anthrax) and rarely to *Past. tularensis* (tularemia).

TABLE 80. SUMMARY OF THE CLINICAL INTERPRETATION OF BACTERIOLOGIC EXAMINATIONS OF THE SPUTUM AND EXUDATES OBTAINED BY BRONCHOSCOPIC ASPIRATION—(Continued)

Disease	Interpretation
Abscess and Gangrene	The etiologic diagnosis of *abscess* is best determined by the bacteriologic examination of pus secured by bronchoscopic aspiration. Early cases may be due to pure infections with streptococci or staphylococci but in late cases mixed infections are generally found. *Gangrene* is usually due to mixed infections in which spirochetes alone or in association with fusiform bacilli are of frequent occurrence.
Bronchitis, Infectious Asthma, Bronchiectasis and Pulmonary Spirochetosis	*Acute primary bronchitis* is frequently due to hemolytic streptococci, pneumococci, *Staph. aureus* or *H. influenzae.* Usually due, however, to mixed infections with these as well as with *K. pneumoniae* and *N. catarrhalis* and especially when secondary to the "common cold" or sinusitis. *Chronic bronchitis* is usually a mixed infection. Etiologic diagnosis is best made by bacteriologic examination of secretions obtained by bronchoscopic aspiration. Likewise in *infectious asthma.* *Bronchiectasis* is always a mixed infection. Spirochetes alone or in association with fusiform bacilli are frequently present along with other micro-organisms and are possibly of etiologic importance. *Pulmonary spirochetosis* is due primarily to infection with spirochetes alone or in association with fusiform bacilli.

coughing for the preparation of smears and cultures. Otherwise, cultures of the throat may be examined although these may show the presence of pneumococci occurring normally in the saliva and fauces in addition to the pneumococcus producing infection. If necessary, material may be obtained for the preparation of smears and cultures by the simple and apparently safe method of lung puncture.[19]

Determinations of the serologically specific types of pneumococci present are not now as frequently conducted as formerly, since penicillin, sulfadiazine and sulfamerazine have been found therapeutically effective in the treatment of pneumonias due to all types. When indicated, however, the Neufeld method is now generally employed because of its speed, simplicity, accuracy and inexpensiveness. Furthermore, there appears to be a significant correlation between prognosis and the number of pneumococci per field;[20] indeed, it appears advisable for the laboratory to report on this phase of examinations for the assistance given in relation to treatment.

Streptococcal and Other Pneumonias. About 4 per cent of lobar pneumonias are due to beta hemolytic streptococci, *K. pneumoniae, H. influenziae,* staphylococci or mixed infections. While about 50 per cent of cases of bronchopneumonia are due to the less virulent types of pneumococci, about 30 per cent are due to streptococci and about 20 per cent to miscellaneous micro-organisms. Because of the possibility of contamination with saliva, the clinical interpretation of bacteriologic examinations is frequently difficult. As a general rule, this is based upon the micro-organism predominating in smears and *plate* cultures. Laboratories should always give this information when two or more micro-organisms are present instead of merely listing those found. Of course, pneumonia is characteristic of the pneumonic type of plague due to *Pasteurella pestis* and of pulmonary anthrax due to *B. anthracis*, in both of which the respective micro-organisms occur in the sputum in practically pure cultures. Apparently, pneumonia due to *Past. tularensis* may also occur as a rare complication of tularemia [21] but diagnosis largely depends upon positive serum agglutination reactions instead of upon bacteriologic examinations.

Pulmonary Abscess and Gangrene. Whenever possible, the etiologic diagnosis of pulmonary abscess is best made by the bacteriologic examination of pus obtained by bronchoscopic aspiration. Not infrequently pure cultures of hemolytic streptococci or staphylococci are obtained in early cases but mixed infections with these as well as with *K. pneumoniae*, the colon bacillus, *H. influenzae, Ps. aeruginosa*, spirochetes and fusiform bacilli are not infrequent, especially in late cases. The same is true in pulmonary gangrene in which mixed infection, especially with spirochetes and fusiform bacilli, is the rule. Since the latter micro-organisms are strictly anaerobic, their detection depends upon the examination of stained smears supplemented sometimes by darkfield examinations.

Bronchitis; Infectious Asthma; Bronchiectasis; Pulmonary Spirochetosis. Bacteriologic examinations are not ordinarily required for the etiologic diagnosis of *acute bronchitis.* When occurring as a primary infection it is commonly due to a hemolytic streptococcus, pneumococci, *Staph. aureus, H. influenzae* or *A. necrophorus.*[62] Mixed infections with these, as well as with *K. pneumoniae, N. catarrhalis* and other micro-organisms, however, are of frequent occurrence, particularly in bronchitis secondary to the "common cold" or chronic sinusitis.

Mixed infections with these and other micro-organisms are also responsible for *chronic bronchitis* which is always so likely to be due to excessive smoking, air-borne irritants or secondary to infection of the upper respiratory tract, with special reference to nasal accessory sinusitis. Here again bacteriologic examinations of secretions obtained by bronchoscopic aspiration are always to be preferred to those of sputum. The same is true in *infectious asthma,* so likely to be associated with allergic sensitization to streptococci and staphylococci, and especially if bacterial skin tests are to be conducted or autogenous vaccines prepared.

Of course, *bronchiectasis* is always due to mixed infection, since the acquired type generally results from chronic bronchitis and the latter in turn is frequently secondary to upper respiratory tract infection with special reference to chronic sinusitis. Spirochetes and fusiform bacilli are usually present and sometimes in such large numbers as to indicate an etiologic relationship to the disease. Indeed, it has been suggested that spirochetes may play an important rôle in the weakening of the bronchial walls analogous to the production of aortic aneurysms secondary to aortitis due to *T. pallidum.*

Furthermore, there can be no doubt that fusospirochetal infections are responsible for the *pulmonary spirochetosis* of Castellani characterized by unusual hematemesis and frequently associated with expectoration. Whether or not the disease is due to spirochetes normally occurring in the mouth (*T. microdentium, T. macrodentium,* etc.) cannot be stated but available evidence indicates that it may be due to infection of the bronchi with *Bor. vincentii* or *Bor. buccale* alone or in association with *B. fusiformis,* which are regarded as the etiologic agents of Vincent's angina and fusospirochetal gingivitis or "trench mouth."

EXAMINATIONS OF PLEURAL AND PERICARDIAL FLUIDS

All pleural and pericardial fluids should be subjected routinely to bacteriologic examinations. Consequently, they should be collected with rigid precautions against contamination, as described in Chapter 13. When contamination occurs it is usually due to *Staph. albus, B. subtilis, Proteus vulgaris, Esch. coli* or diphtheroid bacilli.

Pleural Fluids. Pleural *transudates* in congestive heart failure, chronic nephritis with anasarca or mediastinal pressure, are invariably sterile unless contaminated during collection or in the laboratory (Table 81). The same is usually true of chylous transudates (chylothorax). Effusions due to blood (hemothorax), however, are frequently contaminated with micro-organisms when due to gunshot or stab wounds of the chest and especially in ruptures of the lungs communicating with the larger bronchi as in pulmonary tuberculosis. Staphylococci, streptococci or pneumococci occur most frequently.

Pleural *exudates* invariably show the presence of micro-organisms in cultures and frequently in smears because they are always inflammatory in origin. Primary pleurisy, with serofibrinous exudates, is due to tuberculous infection in at least 80 per cent of cases. The balance occur as secondary infections in chronic nephritis, acute rheumatic fever, gout, leukemia, etc., and are usually due to hemolytic or other types of streptococci, pneumococci or *Staph. aureus.* The same micro-organisms are usually responsible for the primary pleuritis with empyema due to stab and gunshot wounds of the chest.

In the exudates of tuberculous pleurisy *Myco. tuberculosis* is not usually found in more than 30 per cent by direct smear examinations. A higher percentage of the bacilli may be found by the fluorescent microscopic method.[16] When smears are negative, cultures and guinea-pig inoculation tests should always be done if the disease is suspected.

Otherwise, pleurisy with effusion (pleuritis serofibrinosa) and empyema (pleuritis purulenta) are usually secondary to the lobar pneumonias, bronchopneumonias, pulmonary tuberculosis, pulmonary abscesses or gangrene due to pure or mixed infections of the pleurae with pneumococci, hemolytic streptococci (aerobic or anaerobic), *Staph. aureus, K. pneumoniae,* tubercle bacilli or *Bor. vincentii* with *B. fusiformis,* according to the nature of the primary acute or chronic pneumonitis. They may be also due to infection of the pleurae by hemolytic streptococci, pneumococci or *Staph. aureus* by extension from pericarditis or mediastinitis; likewise to the rupture of subphrenic abscesses into the pleural cavities, in which case the pleuritis may be due not only to any of these pyogenic micro-organisms but to *Esch. coli* or *Cl. perfringens* as well. In open empyemas secondary infection or contamination with *Ps. aeruginosa* or *P. vulgaris* are quite frequent and especially with the former organism.

Pericardial Fluids. Pericardial *transudates* (hydropericardium) are invariably sterile unless contaminated during collection or in the laboratory; they are less

TABLE 81. SUMMARY OF THE CLINICAL INTERPRETATION OF BACTERIOLOGIC EXAMINATIONS OF PLEURAL AND PERICARDIAL FLUIDS

Source	Interpretation
General Considerations	Bacteriologic examinations should be made routinely with all pleural and pericardial fluids. They should be collected and examined with rigid precautions against contamination which is usually due to *Staph. albus, B. subtilis, P. vulgaris* or diphtheroid bacilli.
Pleural	*Transudates* (hydrothorax and chylothorax) are usually sterile unless contaminated during collection or in the laboratory. Effusions of blood (hemothorax) due to trauma or rupture of a lung are frequently contaminated with staphylococci, streptococci or pneumococci. Pleuritis may follow with or without empyema, due to infection with any of these or other micro-organisms. *Exudates* (pleuritis serofibrinosa) and empyema (pleuritis purulenta) are always due to infection. Primary pleuritis is usually due to *Myco. tuberculosis*. Smears are positive in about 30 per cent of cases; the percentage may be higher by fluorescent microscopic examinations. Cultures and guinea-pig inoculation tests are always required when the disease is suspected if smears are negative. Primary pleuritis due to infection with hemolytic streptococci, pneumococci or staphylococci may also occur in chronic nephritis, acute rheumatic fever, gout, leukemia, etc. Secondary pleuritis is a frequent complication by extension of infection from the lungs in the pneumonias, tuberculosis, abscess or gangrene. Depending on the nature of the primary pneumonitis it is usually due to pneumococci, hemolytic streptococci, *Staph. aureus, K. pneumoniae, Myco. tuberculosis* or *Bor. vincentii* with *B. fusiformis*. Secondary pleuritis may be also due to extension of infection with streptococci, pneumococci, or staphylococci in pericarditis or mediastinitis; likewise to these micro-organisms and to *Esch. coli* or *Cl. perfringens* from the rupture of subphrenic abscesses into the pleural cavities. In open empyemas secondary infections or contaminations with *Ps. aeruginosa* or *P. vulgaris* are of frequent occurrence.
Pericardial	*Transudates* (hydropericardium) are invariably sterile unless contaminated during collection or in the laboratory. *Exudates* (pericarditis with effusion) are always due to infection. Empyema (pyopericardium) is relatively uncommon. Acute serofibrinous pericarditis is most frequently secondary to acute rheumatic fever. It may also occur as a secondary infection in the pneumonias, scarlet fever, the surgical septicemias and focal infections of dental, tonsillar or other origin. Likewise from trauma or by extension of infection in empyema, tuberculous pleurisy and mediastinal adenitis or acute mediastinitis; also from the rupture of subphrenic abscess into the pericardial sac. The bacteriologic findings depend upon the primary sources of infection. Hemolytic streptococci, staphylococci and pneumococci are most frequent; *K. pneumoniae, Ps. aeruginosa, H. influenzae, Cl. perfringens, Esch. coli* and *S. typhosa* are less frequent.

common than pleural transudates but are not infrequently overlooked clinically with the discovery of their presence during postmortem examinations.

Pericardial *exudates* (pericarditis with effusion) are rarely due to primary infection of the pericardium, and empyema of the sac (pyopericardium) is relatively uncommon. The most frequent cause of secondary pericarditis is acute rheumatic fever in which the exudate frequently shows infection due to hemolytic streptococci or pneumococci. Secondary pericarditis may also be a complication of lobar or bronchopneumonia (due to pneumococci, hemolytic streptococci or *K. pneumoniae*), scarlet fever (due to hemolytic streptococcus or *Staph. aureus*), the surgical septicemias including postabortal and puerperal sepsis (usually due to hemolytic streptococci or *Staph. aureus*) and not infrequently to focal infection of dental, tonsillar or other origin (due to streptococci, staphylococci or pneumococci). It may also result from trauma as well as by extension of infection from empyema, tuberculous pleurisy and mediastinal adenitis, acute mediastinitis or the rupture of a subphrenic abscess into the pericardial sac. Pericarditis may be also due to infection with *Ps. aeruginosa* and more rarely to *S. typhosa, H. influenzae, Cl. perfringens* or *Esch. coli.*

EXAMINATIONS OF THE MOUTH, GINGIVAE AND TEETH

From the clinical standpoint, bacteriologic examinations of the oral cavity are largely confined to the gingivae and buccal mucosa in the etiologic diagnosis of those types of gingivitis and stomatitis due to bacterial or spirochetal infections, and to the teeth in relation to dental sepsis (Table 82). Examinations for mycotic diseases are discussed in Chapter 16 but none are available for the etiologic diagnosis of those due to the filtrable viruses.

The *normal saliva,* however, usually contains so many different micro-organisms that great care is required in the interpretation of results because of the chances of contamination in the preparation of both smears and cultures. This normal flora is especially likely to contain *Str. viridans* and other streptococci, staphylococci, pneumococci, *N. catarrhalis, M. tetragenus, L. acidophilus* and other lactobacilli, pseudodiphtheria bacilli, *B. fusiformis, P. vulgaris* and various spirochetes with special reference to *T. microdentium, T. macrodentium* and *T. mucosum.*

Cheilitis. As far as the lips are concerned, bacteriologic examinations are largely confined to darkfield examinations of lesions for *T. pallidum* when *primary syphilis* is suspected. Great care is required in making preparations to avoid contamination with saliva, since *T. microdentium* is morphologically indistinguishable from *T. pallidum* by microscopic examinations.

Cracks and fissures involving the corners of the mouth are frequently due to riboflavin deficiency. Perlèche of children may be likewise due to vitamin deficiency, although some have thought it may be the result of infection with *Candida albicans.*

Stomatitis. There are many causes for stomatitis. Some are not due primarily to infection at all as, for example, *allergic stomatitis, stomatitis medicamentosa* caused by chemical agents and drugs (mercury and bismuth) including that pro-

TABLE 82. SUMMARY OF THE CLINICAL INTERPRETATION OF BACTERIOLOGIC EXAMINATIONS OF THE MOUTH, GINGIVAE AND TEETH

Disease	Interpretation
General Consider-ations	Bacteriologic examinations may be of aid in the etiologic diagnosis of some types of stomatitis, gingivitis and dental sepsis. Of no value in the diagnosis of diseases due to viruses except for secondary infection. The normal saliva contains many different micro-organisms which may complicate bacteriologic examinations due to contamination of smears and cultures.
Cheilitis	Darkfield examinations for *T. pallidum* are of great value in the diagnosis of chancres of the lips. Errors may occur due to contamination with saliva containing *T. microdentium* which cannot be differentiated from T. *pallidum* by microscopic examinations. Cracks and fissures of the corners of the mouth may be due to riboflavin deficiency. Perlèche is of unknown etiology but may be due to vitamin deficiency or infection with *Candida albicans*.
Stomatitis	Bacteriologic examinations are of no value except for superimposed infections in stomatitis due to allergy, chemical irritants, nicotine, diabetes, menstruation, pregnancy and leukemia. These superimposed infections may be due to *Bor. vincentii* with *B. fusiformis*, streptococci, staphylococci or pneumococci. Bacteriologic examinations are not ordinarily required for the etiologic diagnosis of catarrhal stomatitis (*N. catarrhalis* or pneumococcal) or for stomatitis scarlatina (streptococcal). Fordyce's disease is not due to infection but to the presence of anomalous sebaceous glands in the buccal mucosa. The etiology of habitual aphthosis or Mikulicz stomatitis (canker sores) is unknown; bacteriologic examinations are of no value. Bednar's aphthae of children is due primarily to trauma with secondary infection. Bacteriologic examinations are of no value in the diagnosis of herpetic stomatitis, foot and mouth disease or pemphigus vulgaris. Ulcerative stomatitis is generally due to infection with hemolytic streptococci or *Staph. aureus*. Nonspecific membranous stomatitis is generally a streptococcus infection. Vincent's stomatitis is due to infection with *Bor. vincentii* and *B. fusiformis*. The etiology of gangrenous stomatitis (noma) is unknown but apparently due to mixed infection with *Bor. vincentii, B. fusiformis*, streptococci and staphylococci. *Gonococcal stomatitis* is rare. Etiologic diagnosis is based upon positive cultures. Direct smears are unreliable. Darkfield examinations for *T. pallidum* are of value in the diagnosis of syphilitic stomatitis (mucous patches) but should never be relied upon alone because of the chances of error due to the possible presence of *T. microdentium*.

TABLE 82. SUMMARY OF THE CLINICAL INTERPRETATION OF BACTERIOLOGIC EXAMINATIONS OF THE MOUTH, GINGIVAE AND TEETH—(Continued)

Disease	Interpretation
Gingivitis	Gingivitis is usually due to local or systemic factors with secondary bacterial infection. *Marginal gingivitis* is due primarily to food impactions, tartar deposits, overhanging fillings, ill-fitting crowns, malhygiene, etc. *Hypertrophic gingivitis* may be due to similar local causes including malocclusion or to menstruation, pregnancy, allergy, diabetes, advanced nephritis, scurvy, leukemia, purpura, etc. Gingivitis may be also due to the administration of mercury or bismuth and especially to drugs producing agranulocytosis (barbiturates, sulfonamides, etc.). Secondary infections with streptococci, staphylococci, pneumococci and especially with *Bor. vincentii* and *B. fusiformis* occur with the production of *ulcerative gingivitis.* Vincent's or fusospirochetal gingivitis ("trench mouth") may be primary but is usually a secondary infection. Bacteriologic examinations and especially of smears for *Bor. vincentii* and *B. fusiformis* are indicated and usually helpful but the etiologic diagnosis and treatment of most types of gingivitis require the close cooperation of dentists and physicians.
Dental Caries	Affects eventually about 100 per cent of individuals. Occurs in both the deciduous and permanent teeth. Prophylaxis demands the closest cooperation of physicians and dentists. Caries of the deciduous teeth should receive dental care as well as caries of the permanent teeth of children, adolescents and adults. Exact etiology still unknown. But apparently due to the destruction of enamel by organic acids produced by aciduric bacteria (especially *L. acidophilus* and other lactobacilli) as well as by acids produced by streptococci and staphylococci in mucinous or bacterial plaques. Greatly influenced by the chemistry of the saliva with special reference to the buffering salts and the mucin content. Progression is mainly due to infection with streptococci, staphylococci and other micro-organisms of the mouth. Susceptibility is greatly influenced by the resistance or defensive mechanism of the tooth which is largely dependent upon heredity, general health, diet and vitamin intake and the state of calcium-phosphorus metabolism. May be confined to the enamel if resistance is high.
Pulpitis and the so-called "Dead Tooth"	Pulpitis is usually due to infection from caries of the dentine with streptococci (especially *Str. viridans*), *Staph. aureus* or mixed infections with these and *B. fusiformis, Cl. perfringens, B. ramosus, Esch. coli,* etc. Results in pulp death or the so-called "dead tooth" but the dentine may be still vital due to intercommunication with the periodontal membrane. Pulp death may be also due to blows of the teeth or their fracture by cutting off blood supply at their apices. Since the majority of "dead teeth" are infected they may, therefore, be potentially or actually sources of focal infection. Root canal therapy should be checked by bacteriologic examinations and especially before permanent fillings are made.

TABLE 82. SUMMARY OF THE CLINICAL INTERPRETATION OF BACTERIOLOGIC EXAMINATIONS OF THE MOUTH, GINGIVAE AND TEETH—(Continued)

Disease	Interpretation
Periodontitis and Dento-Alveolar Abscesses	Acute suppurative periodontitis may be initiated by chemical agents used in the disinfection of cavities and root canals but is usually due to infection with special reference to streptococci. Chronic periodontitis of the simplex type is due to gingivitis. The complex type is due to atrophy of the tooth socket. Mixed infection occurs in both with the production of pockets (pyorrhea alveolaris). Infection especially due to *Str. viridans* alone or in association with other streptococci, staphylococci, *N. catarrhalis, B. fusiformis,* etc. Dento-alveolar abscesses (periapical abscesses or dental granulomas) are most frequently due to *Str. viridans.* Parodontal abscesses are usually due to infection with streptococci or staphylococci. Bacteriologic examinations of whole teeth are worthless in relation to the etiology of periodontitis. Only amputated roots and sockets should be cultured with extraordinary precautions against contamination.

duced by nicotine, *diabetic stomatitis,* the *stomatitis of menstruation* and *pregnancy,* apparently due to endocrine dysfunctions and *leukemic stomatitis.* In these, bacteriologic examinations are generally of no value, since they are likely to show only those micro-organisms occurring normally in the saliva, although it cannot be denied that superimposed infections with *Bor. vincentii* in association with *B. fusiformis,* streptococci, staphylococci or pneumococci are sometimes of clinical importance.

Catarrhal stomatitis may be due to infection with *N. catarrhalis* or pneumococci but bacteriologic examinations are not ordinarily required for diagnostic purposes. The same is true of the *stomatitis scarlatina.* Not infrequently bacteriologic examinations are requested in *Fordyce's disease* but they show nothing more than the usual bacteria of the normal saliva, since the condition is due to the presence of anomalous sebaceous glands in the buccal mucosa.

One of the most frequent types of stomatitis subjected to bacteriologic examinations is that of Mikulicz or *habitual aphthosis* popularly designated as "canker sores." Since the cause is unknown, although suspected as being due to food allergy, gastro-intestinal derangements or endocrine dysfunctions, smears and cultures show nothing more than the usual micro-organisms of the saliva which are apparently without any etiologic relationship to the disease. *Bednar's aphthae* is a special form due to trauma and occurs in the form of ulcers on the palates of young children, the infection being only of secondary importance. In children herpes simplex of the lips may also extend to the cheeks, gingivae, tongue and tonsils, constituting *herpetic stomatitis.* It is apparently due to the herpes virus in which bacteriologic examinations are of no value. The same is true of herpes zoster attacking the skin and buccal mucosa as well as of *foot and mouth disease* and the highly fatal *pemphigus vulgaris* of unknown etiology.

Bacteriologic examinations, however, are of clinical value in the diagnosis of *ulcerative stomatitis* generally due to infection with hemolytic streptococci or *Staph. aureus*; *nonspecific membranous stomatitis* generally due to infection with streptococci, and *Vincent's stomatitis* which is generally acute and due to infection with *Bor. vincentii* and *B. fusiformis*. Recently Black [22] has claimed that Vincent's angina, gingivitis and stomatitis as well as ulcerative stomatitis are due primarily to the herpes virus, since herpetic keratoconjunctivitis was produced experimentally in rabbits by inoculation of the cornea with materials secured in the early stages of these diseases as well as fatal herpetic encephalitis by intracerebral inoculation of mice and rabbits.

The etiology of *gangrenous stomatitis* or noma is uncertain but the disease is apparently due to mixed infections with *Bor. vincentii* and *B. fusiformis*, streptococci and staphylococci in infants and children of greatly reduced resistance.

Gonococcal stomatitis is rare but diagnosis largely depends upon bacteriologic examinations by means of cultures; smears are unreliable because of the difficulty in differentiating among gonococcus, *N. catarrhalis* and other gram-negative diplococci by morphology alone.

Syphilitic stomatitis is characteristic of the secondary stage of the disease with the lesions occurring as highly infectious mucous patches. Darkfield examinations for *T. pallidum* may be made but, if positive, should never be relied upon alone for diagnosis because of the chances of error due to the possible presence of *T. microdentium* of the saliva from which it cannot be differentiated by microscopic examinations.

Gingivitis. In many respects the causes of gingivitis are the same as those of stomatitis. In fact, the two commonly occur together with the production of gingivostomatitis.

Gingivitis occurs much more frequently than stomatitis. This is because the gums are not only far more subject to local injuries predisposing them to infection, but likewise because they are peculiarly susceptible to the effects of general or systemic intoxications and disease. In fact, the gingivae have a high natural resistance to infection in the absence of local or systemic factors predisposing them to it. For this reason infection is generally only of secondary importance in relation to the etiology of gingivitis. In other words, every case of the disease requires a thorough search for local factors producing trauma and also general or systemic factors primarily responsible for it.

Thus, *marginal gingivitis* is usually due primarily to local irritation by food impactions, tartar deposits, ill-fitting crowns, over-hanging fillings, etc. Infections with streptococci, staphylococci or other micro-organisms of the saliva are purely secondary although they may be primary by extension from caries involving the necks of the teeth. *Cotton roll gingivitis* is caused by traumatic desquamation of the epithelium. Thanks to the care exercised by dentists, however, it is of infrequent occurrence and secondary infection does not usually occur.

Types of *hypertrophic gingivitis* may be due primarily not only to such local factors as dental sepsis, malocclusion, food impactions, mouth breathing, hereditary elephantiasis of the gums, etc., but also to such systemic causes as menstruation, pregnancy, allergy, diabetes, advanced chronic nephritis, latent or manifest scurvy, leukemia, purpura haemorrhagica, etc.

Furthermore, the gingivae are peculiarly susceptible not only to irritation by such drugs as mercury and bismuth administered in the treatment of syphilis, particularly if previously damaged by necrotic stumps of teeth and bad oral hygiene, but especially by such drugs as aminopyrine, the barbiturates, and sometimes the sulfonamide compounds, which are capable of depressing the bone marrow with the production of agranulocytosis. In these types of gingivitis due to drug intoxications as well as those due to scurvy, leukemia, agranulocytosis and other blood dyscrasias, secondary bacterial infection inevitably occurs not only with streptococci, staphylococci and pneumococci, but especially with *Bor. vincentii* and *B. fusiformis*, resulting in the production of *ulcerative gingivitis.*

Ulcerative gingivitis, however, may be due primarily to infection with *Bor. vincentii* and *B. fusiformis* with the production of what is designated clinically as Vincent's or fusospirochetal gingivitis or "trench mouth." But apparently it is most frequently due primarily to local trauma, scurvy, agranulocytosis or other systemic factors as well as, possibly, to primary virus or herpetic infection,[22] with secondary infection with these micro-organisms.

Under the circumstances, bacteriologic examinations of the gingivae are frequently indicated or required and especially of smears for *Bor. vincentii* and *B. fusiformis* for the aid given in diagnosis and especially in relation to treatment. But the point of special clinical or practical importance is the fact that infection is usually only a secondary factor in the production of gingivitis. Consequently, the etiologic diagnosis and treatment of the disease frequently requires the close co-operation of the dentist and physician.

Dental Caries, Pulpitis and the "Dead Tooth." Dental caries affects practically 100 per cent of individuals, since it involves both the deciduous and the permanent dentitions. Measures for its prophylaxis place a heavy responsibility not only upon dentists, but upon physicians as well, and not only merits but demands the closest co-operation between the two professions. Indeed, insofar at least as the deciduous teeth are concerned, prophylaxis begins during prenatal life, with the responsibility resting solely upon physicians in relation to the diet and health of pregnant women. As a general rule, physicians need to give more attention to the deciduous as well as the permanent teeth, in relation to the diet, vitamin intake (especially C and D), and calcium-phosphorus metabolism of both children and adults. Altogether too frequently the deciduous teeth are neglected leading to their premature loss through caries which may affect general health and nutrition and the eruption of the permanent teeth as well, with the production of malocclusion, changes in the palate, etc. In other words, physicians can do a great deal in helping to solve the great problem of caries by insisting upon periodic dental care not only in relation to adults, but especially in the case of children and adolescents. Furthermore, physicians cannot escape sharing in the responsibility when it is remembered that caries, with the almost inevitable pulpitis and periodontitis with or without dento-alveolar abscesses, may involve the general health of individuals with special reference to the diseases due to focal infection.

Practically all dental schools now include instruction in clinical medicine[23] because dentists need to know much more than the purely mechanistic phases of their great profession. But if this is right and proper, it is equally important for medical schools to teach more about dentistry in relation to medicine than is commonly the case.[24]

Unfortunately, the etiology of dental caries is not yet definitely known in spite of a great deal of meritorious research devoted to it by the dental profession. Indeed, it would appear that there is no one specific cause but that several etiologic factors may be involved of varying importance according to circumstances. But since caries first affects the exposed surfaces of the teeth, it appears to be due primarily to the concentrated effects of organic acids produced by the aciduric bacteria upon the enamel. The latter are chiefly composed of the lactobacilli with special reference to *L. acidophilus,* but in this connection it must not be overlooked that the initial injury may be produced by other acid-producing micro-organisms, including streptococci and staphylococci, which so commonly occur in mucinous or bacterial plaques. The amount and concentration of these organic acids are greatly influenced by the chemistry of the saliva with special reference to its buffering salts and mucin content which in turn may be altered by metabolic factors. This initial injury is soon followed by secondary infection with other bacteria occurring in the mouth and these are largely responsible for the progressive necrosis of the dentine with eventual involvement of the pulp. If the resistance or defensive mechanism of the tooth is high, the progression of caries may be inhibited by zones of sclerotic dentine or secondary deposits of it. In other words, the aciduric bacteria initiate caries but streptococci (especially *Str. viridans*), staphylococci, *Neisseria flava, N. perflava, B. fusiformis* and other micro-organisms of the saliva are then responsible for its progression if resistance or the defensive mechanism is low. Consequently, caries is in relationship to the resistance of teeth to infection, just as in the case of all other tissues of the body. However, the resistance of the teeth is largely dependent upon heredity, general health, diet and adequate vitamin intake and the state of calcium-phosphorus metabolism, since the walling off of necrotic enamel and dentine by leukocytes and the destruction of bacteria by phagocytosis and humoral antibodies plays little or no rôle in view of their structure. In this manner endogenous or systemic factors play an important part in the etiology of caries, not only in relation to the saliva, but in relation to the resistance to infection of the enamel and dentine of the tooth itself.

Even when caries is still superficial, ***pulpitis*** may be present (pulpitis clausa) due to infection and especially with *Str. viridans* or staphylococci. When caries reaches the pulp (pulpitis aperta) mixed infection occurs due to any of the mouth bacteria, with special reference not only to *Str. viridans* and *Staph. aureus,* but to *B. fusiformis, B. ramosus* and even *Cl. perfringens* and perhaps *Esch. coli* as well, with the production of suppurative pulpitis. Of course, this results in the destruction of the pulp, commonly designated as a "dead tooth," but the tooth may be still vital in spite of the death of its pulp because of the intercommunications existing between the cells of the cementum and the dentinal tubuli on the one hand, with the cemental cells in communication with the periodontal membrane on the other. Pulp death may be also due to blows on the teeth and especially their fracture by cutting off blood supply at the apices.

For this reason the so-called "dead tooth" may be a factor in the etiology of the diseases due to focal infection, since *Str. viridans* or other organisms in the dentinal tubuli may still have access to the general circulation of the blood. It is for this reason that physicians are so greatly interested in the results of root canal therapy. Undoubtedly, dentists skilled in this work are able to sterilize pulp canals before permanent fillings are made but physicians not infrequently wonder why they do not more generally make cultures before doing so. With proper precautions in technic against contamination, these are readily made with paper points dropped into tubes of glucose hormone broth. After 48 hours' incubation the results are available. If the medium remains perfectly clear, it may be assumed to be sterile. If cloudy, bacteria are present and the microscopic examination of simple smears stained by the method of Gram are sufficient for gaining an adequate idea of the organism or organisms present. In other words, the technic is so simple that every dentist should be able to make the examinations without engaging the services of a bacteriologist. Physicians can only hope that at least two or three sterile cultures in succession will be required before permanent fillings are made. Even under these circumstances, however, the dentist has no way of checking on the presence or absence of infection of the dentinal tubuli and must rely upon the possibility of the cement used in bringing about their disinfection. Of course, complete filling of the root canal without an overflow of cement into the periapical tissues is required and this should be routinely checked by x-ray examinations, since well-filled root canals are much less likely to retain infection than poorly filled ones.

At all events, the status of pulpless teeth in relation to general health has been extensively discussed pro and con by physicians and dentists. If x-ray examinations reveal periodontitis, apparently 75 to 90 per cent are found infected upon bacteriologic examination after extraction. If no x-ray evidences of periodontitis are found, the incidence is likely to be slightly lower but undoubtedly the majority of pulpless teeth are at least potential if not actual sources of focal infection. Furthermore, the periapical tissues are always likely to become eventually infected because of their lowered resistance.

Periodontitis and Dento-Alveolar Abscess. Acute suppurative periodontitis (acute alveolar abscess) may be initiated by chemical agents employed for the disinfection of cavities and root canals, but is usually due to mixed infection with aerobic or anaerobic streptococci, staphylococci, *B. ramosus, Cl. perfringens,* and the streptococci being by all odds the most frequent and important.

Chronic periodontitis or periodontoclasia of the simplex type, which begins at the bases of the gingival crests, is usually initiated by gingivitis due to local factors (food impactions, over-hanging fillings, ill-fitting crowns, occlusial strain, etc.) as well as by the systemic causes for gingivitis and periodontosis (pregnancy, allergy, improper diet, diabetes mellitus, and other endocrine dysfunctions, nephritis, blood dyscrasias, "intestinal auto-intoxication," disturbances of acid-base equilibrium, etc.). But in this type, as well as in chronic periodontitis of the complex type, which begins as a noninfective atrophy of the tooth socket (rarefying pericementitis fibrosa), the pockets become infected, with the production of so-called "pyorrhea alveolaris" in either case. Undoubtedly, *Str. viridans* is the most frequent and important micro-organism of infection and may be found in pure culture, but mixed infections with hemolytic and nonhemolytic streptococci are frequent as well as, sometimes, with *Staph. aureus* and *albus*, *N. catarrhalis* and even *B. fusiformis* or other micro-organisms. Rarer forms are due to *Actinomyces bovis* and the tubercle bacillus, the latter being regarded as a hematogenous infection.

Dento-alveolar abscesses (periapical abscesses or dental granulomas) are usually due to streptococcus infection with the chances of pure cultures of *Str. viridans* in over 50 per cent of cases. Parodontal abscesses, however, may be due to infection with streptococci or staphylococci. They occur between the roots of multirooted teeth and are different from periapical abscesses because of their location and the fact that they may occur on teeth with normal pulps and especially in diabetes mellitus.

It is to be emphasized that if cultures are to be made of extracted teeth for etiologic diagnosis with or without the preparation of autogenous vaccines, it is useless for the dentist to culture the whole tooth or send it to the laboratory for this purpose. As a matter of fact, extraordinary precautions are required against contamination and only the amputated apices, with or without cultures of the socket, should be submitted. The best medium is glucose hormone broth because it is more likely to develop *Str. viridans* and other streptococci than slants or plates of blood agar, while plain agar should never be used.

EXAMINATIONS OF THE STOMACH AND DUODENUM

Stomach. There is probably no other single internal organ of the body more subject to bacterial contamination than the stomach, considering the frequency with which saliva, sputum, and secretions of the upper respiratory tract are swallowed, in addition to the direct ingestion of organisms in foods and beverages. Even in a healthy state of the mouth and respiratory tract, enormous numbers of various bacteria, yeasts and molds are constantly swallowed, while in infections of the nose, accessory sinuses, postnasal space, gingivae, tonsils and the lower respiratory tract, the swallowing of bacteria protected in pus or mucoid secretions must sometimes reach appalling proportions. Fortunately, however, bacterial contamination of the stomach does not necessarily mean infection of this organ because of various protective factors with special reference to the bacteriostatic and bactericidal properties of the free hydrochloric acid of its secretions.

For example, the investigations of Bartle and Harkins[25] have shown that gastric juice with a free hydrochloric acid content of 20 to 40 degrees was completely bactericidal *in vitro* for streptococci and *Esch. coli* but not for staphylococci or *L. acidophilus,* while gastric juice with a free hydrochloric acid of 60 to 100 degrees was bactericidal for all organisms tested except *L. acidophilus*. On the other hand, however, gastric juice with a free hydrochloric acid of 10 to 15 degrees was bactericidal for streptococci only, while those with 0 to 5 degrees were not bactericidal at all. It will be observed, therefore, that gastric juice is most bactericidal for streptococci, next for bacilli of the colon group and staphylococci, while without destructive effects upon lactobacilli. Undoubtedly the spore-forming bacilli, like *B. anthracis*, are likewise highly resistant as well as tubercle bacilli. Indeed, as reported by Stiehm,[26] tubercle bacilli have been found in 71.4 per cent of gastric contents of patients with pulmonary tuberculosis in which the sputums were negative, with the suggestion that their absence in the stomach, as determined by repeated examinations, may be accepted as one of the criteria of healing.

Consequently, the *normal flora* of the fasting stomach varies greatly according to the acidity of its secretions with special reference to free hydrochloric acid, since combined hydrochloric acid is much less bactericidal. Under normal conditions, when the swallowing of excessive amounts of saliva is avoided in the collection of the residuum, bacteriologic examinations usually show nothing more than a few *Staph. albus, B. subtilis,* lactobacilli (with special reference to *L. acidophilus*) and various yeasts and molds which are likewise highly resistant to destruction. In hypoacidity and especially in anacidity, however, additional micro-organisms may be present, including streptococci, *Esch. coli,* pseudodiphtheria bacilli and *Ps. aeruginosa* (Table 83).

Special precautions are required in the *collection of gastric contents for bacteriologic examination,* as follows:

1. The patient should fast for at least eighteen hours and the teeth and gums should be carefully cleansed several times during this interval.

2. Beginning one hour before the test-meal is given, the mouth and throat should be gargled several times with a bactericidal solution like 1:10,000 Zephiran or 1:2 dilution of cepacol, followed by sterile water.

3. If the nose and postnasal space are infected, these parts should be cleansed to remove the secretions.

4. The test-meal should be sterilized.

5. For at least one-half hour or, better, one hour prior to the time set for removal of the stomach contents, the patient should be cautioned against the swallowing of saliva; a dental suction apparatus may be of aid.

6. The stomach-tube should be sterilized and protected as much as possible against contamination during its passage. The tube should be pushed down rather than left entirely to the swallowing efforts of the patient, in order to reduce the chances of swallowing saliva. Specimens for bacteriologic examination are always best secured from "tube-broken" individuals.

7. The specimen should be collected in a sterile container and examined as soon as possible, being kept on ice if a delay is unavoidable.

Direct smears of the material as secured or after centrifuging should be made promptly because the organisms found are almost sure to be those actually in the stomach, since time is not afforded for the proliferation of those from the mouth that may have gained access through contamination with sputum. Stained and unstained specimens generally give a very good idea of the bacteriology; indeed, they are frequently to be preferred to cultures, since dead or devitalized organisms are found as well as such yeasts and molds as are not readily cultivated. The Gram stain is especially serviceable for the differentiation and detection of the

TABLE 83. SUMMARY OF THE CLINICAL INTERPRETATION OF BACTERIOLOGIC EXAMINATIONS OF THE STOMACH AND DUODENUM

Organ	Interpretation
Stomach	The stomach is more subject to bacterial contamination than any other single internal organ of the body. It is, however, greatly protected against infection by the free hydrochloric acid of its secretions which is particularly destructive for streptococci and colon bacilli while without destructive effects on lactobacilli, spore-forming bacilli and *Myco. tuberculosis.* Consequently, the *normal bacterial flora* of the gastric residuum may contain only a few staphylococci, *B. subtilis, lactobacilli* and various yeasts and molds. In hypoacidity and anacidity, however, streptococci, *Esch. coli* and other micro-organisms may be present. Special precautions are required in the collection of the stomach contents for bacteriologic examinations. Tubercle bacilli may be present in pulmonary tuberculosis when absent in the sputum. The Boas-Oppler bacillus is not pathognomonic for gastric carcinoma and may be present in any disease producing retention, including atony and chronic dilatation. Infection may play a rôle in the etiology of *acute gastritis,* especially in individuals with hypoacidity or anacidity. Infection with streptococci alone or in combination with other micro-organisms is apparently the cause of *phlegmonous gastritis.* Diphtheritic or *membranous gastritis* is due to infection with the diphtheria bacillus. Gastritis occurring during the acute infectious diseases is apparently due to the excretion of bacterial toxins (*gastritis by elimination*). Infection may also play a rôle in the etiology of some types of *chronic gastritis.* Hematogenous infection and especially with streptococci derived from the teeth, tonsils or elsewhere may be primarily responsible for the production or persistence of peptic ulcers of the stomach and duodenum.
Duodenum	The normal bacterial flora is heavier than in the stomach because of more favorable cultural conditions with special reference to *Staphylococcus albus,* nonhemolytic streptococci, *Esch. coli* or other intestinal organisms. Bacteriologic examinations are chiefly of interest in relation to the possibility of contamination of bile secured by duodenal-biliary drainage. Infection may play a rôle in the etiology of *suppurative duodenitis, ulcerative duodenitis* and *chronic duodenitis.* *Intestinal anthrax* may be due to direct infection with *B. anthracis.*

various organisms present. Cultures are best made in a fluid medium because the hydrochloric acid should be diluted below its bacteriostatic threshold.

Bacteriologic examinations of the stomach, however, are seldom made for diagnostic purposes except for lactobacilli in relation to diseases producing retention with fermentation with special reference to the Boas-Oppler bacillus which appears to be identical with *L. bulgaricus.* This organism, as well as other lactobacilli, is commonly found not only in carcinoma of the stomach with pyloric obstruction, but also in atony and chronic dilatation due to other causes.

Whether or not the swallowing of streptococci and staphylococci in mucopurulent secretions from the mouth and upper respiratory tract plays an important rôle in the production of *acute gastritis* cannot be stated, but the possibility is to be admitted and especially in individuals with hypoacidity or anacidity. Undoubtedly, however, streptococci alone or in association with staphylococci, pneumococci or *Esch. coli,* are primarily responsible for the rare *phlegmonous gastritis* due to direct or hematogenous infection of the gastric mucosa. Furthermore, *C. diphtheriae* may produce *diphtheritic or membranous gastritis* by direct infection, although it is likewise rare and usually discovered only at autopsy. It may be also stated that acute gastritis is not uncommon during the course of many of the acute infectious diseases. This is thought to be largely due to the effects of toxins excreted by the gastric mucosa and designated by Rehfuss as so-called *gastritis by elimination.* It is also likely that infection may play some rôle in the production of *chronic gastritis* and especially that form known as "achlorhydria hemorrhagica gastrica" in which foci of infection may also occur elsewhere, as in the gallbladder, liver, colon or appendix.

Whether or not infection plays an important rôle in the production of *peptic ulcer* of the stomach and duodenum has been debated pro and con with no unanimity of opinion. However, there can be no doubt that ulcers may be produced experimentally in rabbits and particularly by intravenous injection of streptococci recovered from the teeth, tonsils or other foci of infection. According to Rehfuss, it appears altogether probable that micro-organisms, and especially streptococci, may play a primary rôle in the production or persistence of the disease by producing a lessening in resistance of areas of the gastric or duodenal mucosa either by direct or hematogenous infection.

Duodenum. Micro-organisms escaping destruction in the stomach find very favorable conditions for their survival and proliferation in the duodenum and the balance of the intestinal tract because of the alkalinity and favorable pabulum of its contents. Consequently, the duodenal contents under normal conditions are apt to show the presence of more bacteria than the stomach (especially *Staph. albus,* nonhemolytic streptococci and *Esch. coli*) with the flora becoming increasingly heavy in the ileum, jejunum and colon (Table 83).

Bacteriologic examinations may be made with contents removed by duodenal drainage but clinical interpretation of the results is always difficult because of a *normal bacterial flora* which may comprise not only staphylococci but streptococci (especially nonhemolytic types), *Esch. coli,* and other bacteria of the small intestine, including *Cl. perfringens* and *Bacteroides* if anaerobic cultures are employed.

Apparently, the duodenum is singularly free of diseases due primarily to infection. *Suppurative duodenitis* may occur but is very rare. Trauma is an important predisposing cause. *Ulcerative duodenitis,* however, usually associated with ulcers elsewhere in the small intestine, is by no means uncommon during some of the acute infectious diseases, biliary tract infections, surgical septicemias, etc. Whether or not they are due to direct infection of the duodenal mucosa or to the effects of toxins eliminated by it (as in Curling's ulcer due to peripheral burns) cannot be stated. Direct infection with *B. anthracis*, however, may occur as a form of *intestinal anthrax* and infection may play some rôle in the production of *chronic duodenitis* as believed to be possible in the etiology of some cases of chronic gastritis, as previously discussed.

EXAMINATIONS OF THE GALLBLADDER AND BILE

From the clinical standpoint bacteriologic examinations of bile obtained by duodenal-biliary drainage are of potential value in relation to the detection of infections of the gallbladder and the balance of the biliary tract. Unfortunately, bile collected in this manner is subject to contamination not only by bacteria in the duodenum, but likewise by those in the saliva and stomach. Consequently extraordinary precautions are required in collection as described in Chapter 8. In case of unusual difficulties increasing the chances of contamination, it is scarcely worth while to prepare cultures. But examinations of stained smears of the bile or its sediment as soon as possible after collection are always indicated because the effects of contamination are much less likely to be misleading. Even under the most favorable circumstances the results of cultural examinations must be interpreted with great caution (Table 84).

Under the conditions, bacteriologic examinations of gallbladders and bile removed during operations are far more reliable for determining the rôle of bacterial infection in the production of cholecystitis, cholangitis and hepatitis. As reported by Andrews and Henry,[27] about 37 per cent of normal gallbladders were found to be invaded with *Staph. albus* or *Cl. perfringens* without evidences of infection. But specimens of bile removed from such gallbladders were almost invariably sterile.

In cholelithiasis about 50 per cent of gallbladders and 33 per cent of specimens of bile removed from them showed the presence of *Staph. albus, Str. viridans, Esch. coli, Cl. perfringens* or diphtheroid bacilli, while all gallbladders and specimens of bile removed from patients with obstruction of the common bile duct showed the presence of *Esch. coli* or *Cl. perfringens*. Cultures of gallbladders removed from patients during the quiescent stages of cholecystitis and cholelithiasis were positive in 33 per cent of cases with positive bile cultures in 25 per cent; cultures of both removed during the active stages were positive in 42 per cent. The bacteria found were staphylococci, streptococci, *Esch. coli, Cl. perfringens* or diphtheroid bacilli in pure or mixed cultures.

Undoubtedly, infection may be of primary importance in the etiology of cholecystitis and cholangitis but apparently mechanical, vascular, toxic, and chemical factors are important predisposing causes, with the possibility of infection having a secondary rôle.[27] Needless to state hepatitis with an associated cholangitis may result not only from ascending infection with streptococci, staphylococci or *Esch. coli* due to obstruction of the extrahepatic bile ducts, but likewise and more frequently with these bacteria as well as with *Lept. icterohaemorrhagiae* (infectious jaundice) and *T. pallidum* by hematogenous infection. Furthermore, hepatitis with an associated cholangitis may be produced by bacterial toxins alone as the result of the excretory activities of the liver, which apparently accounts for the frequency of jaundice during many of the acute infectious diseases.

TABLE 84. SUMMARY OF THE CLINICAL INTERPRETATION OF BACTERIOLOGIC EXAMINATIONS OF THE GALLBLADDER AND BILE

Subject	Interpretation
Gallbladder	The normal gallbladder may be invaded with staphylococci or *Cl. perfringens* without evidences of infection. In cholelithiasis and especially in obstruction of the common bile duct, the gallbladder is frequently invaded or infected with these micro-organisms, streptococci or *Esch. coli.* Cultures of gallbladders showing the presence of cholecystitis may be sterile in 30 to 50 per cent of cases, indicating that infection may be of but secondary importance to mechanical, vascular or chemical factors in the etiology of many cases.
Bile	Normally the bile is almost invariably sterile. Bacteriologic examinations of bile obtained by duodenal-biliary drainage are of potential value in the detection of infection of the galltract but are subject to possible contamination by the saliva, stomach or duodenum during collection. Consequently, the results, especially of cultures, must be interpreted with great caution. They are of particular value in the detection of typhoid carriers. From 60 to 70 per cent of cases of cholelithiasis show sterile bile cultures but in common bile duct obstruction they are usually positive. The bile may be also sterile in cholecystitis and cholangitis. Positive cultures are usually due to pure or mixed infections with staphylococci, streptococci, *Esch. coli* or *Cl. perfringens.* Hepatitis with an associated cholangitis may be due to bacterial toxins excreted by the liver with sterile bile cultures or to ascending infections with staphylococci, streptococci or *Esch. coli* due to extrahepatic duct obstruction; also to hematogenous infections with these and other micro-organisms including *Lept. icterohaemorrhagiae* and *T. pallidum.*

It will be observed, therefore, that cholecystitis, cholangitis and hepatitis due primarily or secondarily to bacterial infection, may be present without the infecting micro-organisms occurring in the bile. Consequently, sterile cultures of bile obtained by duodenal-biliary drainage do not alone exclude the possible presence of these diseases. Also because of the chances of contamination during collection, positive cultures must be interpreted with great caution as previously stated. Incidentally, however, bile cultures by this method are acceptable and of value in the detection of typhoid carriers due to residual infection of the gallbladder with *S. typhosa.*

EXAMINATIONS OF THE FECES

About one-third of the weight of dried feces is composed of bacteria, mostly dead, constituting about 9 per cent of the total solids. It is stated that more than fifty different species have been found although many are closely related and difficult to differentiate from each other.

In a general manner the *normal flora* of the feces may be divided into two main groups: (1) those micro-organisms characterized by their ability to ferment sugars (saccharolytic) like *Esch. coli, K. pneumoniae, Cl. perfringens, S. aertrycke, L. acidophilus* and other lactobacilli, and (2) those characterized by their proteolytic activities like *Ps. aeruginosa, Cl. septicum, Cl. oedematiens* and *Cl. histolyticum*. Some, however, possess both properties like staphylococci, streptococci and pneumococci while others possess neither, like the diphtheroid and pseudodiphtheria bacilli.

The normal flora may also be roughly classified into those cocci and bacilli which are gram-positive and those which are gram-negative. A rough estimate may be gained by examination of thin smears of feces stained by the method of Gram although the proportions of the two groups are so greatly influenced by diet that they possess but little clinical value.

Furthermore, while most of the normal bacteria of the feces are aerobic, some are anaerobic, including not only streptococci but especially the gram-positive spore-forming bacilli like *Cl. tetani, Cl. perfringens* and other bacilli of the gas gangrene group, as well as *Bacteroides funduliformis* and others of the genus Bacteroides.

Physicians and surgeons should understand, therefore, that the normal bacterial flora of the feces is the richest in numbers and varieties of any part of the body. For this reason *it is always advisable to specify the kind of bacteriologic examinations desired* or to at least furnish the laboratory with sufficient clinical data for guiding the kind of examinations to be made. This is particularly true since special methods are required for the isolation and identification of many of the fecal micro-organisms producing infection like typhoid, paratyphoid, dysentery and cholera bacilli, the brucella, tubercle bacilli and micro-organisms producing the food infections (Table 85).

Collection. In order to avoid contamination as much as possible certain precautions in the collection of specimens are advisable as follows:

1. The feces should be passed directly into a quart-size mason jar previously sterilized, or some other suitable container. Or, the patient may pass a stool into a basin, previously sterilized, and a portion (especially feces with mucus) removed with a sterilized spatula or spoon to a sterile wide-mouthed bottle or vial.

2. Swabs of the rectum for typhoid, dysentery or cholera bacilli in suspected carriers may be made by cleansing the skin about the anus with soap, water and alcohol, followed by the introduction of a sterile cotton swab previously moistened with sterile saline solution; or sterile vaseline may be applied to the anus and the finger inserted, covered with a sterile rubber cot, and swabs prepared from the cot. The swabs should be delivered promptly to the laboratory before drying occurs.

In ulcerative colitis, or diseases involving the anus and rectum, swabs or cultures are best made of the lesions direct instead of submitting feces for examinations. For this reason bacteriologic examinations of diseases of the anus, rectum and sigmoid colon are considered separately.

TABLE 85. SUMMARY OF THE CLINICAL INTERPRETATION OF BACTERIOLOGIC EXAMINATIONS OF THE FECES

Disease	Interpretation
General Considerations	The *normal flora* is the richest in numbers and kinds of bacteria of any part of the body. About fifty different varieties of micro-organisms have been described; about one-third of the weight of dried feces is composed of bacteria which are mostly dead. The micro-organisms of the normal feces are divisible into those which are chiefly saccharolytic or proteolytic, those possessing both properties and those possessing neither. Also into gram-positive and gram-negative species although classification on this basis has but little clinical value since the proportions are greatly influenced by diet. The micro-organisms are mostly aerobic but some are anaerobic with special reference to streptococci, the bacilli of the gas gangrene group and those belonging to the genus Bacteroides. *Requests for examinations should always specify the kind of bacteriologic examination to be made;* otherwise the laboratory should be furnished with sufficient clinical data to guide the methods to be employed. Precautions in the collection of feces are advisable in order to guard against contamination as much as possible. Swabs of the rectum may be employed and especially for the detection of carriers.
Typhoid and Paratyphoid Fevers	Of limited value in diagnosis during the first ten days of the disease. Thereafter of increasing value and especially in the diagnosis of suspected or atypical cases with negative blood cultures and doubtful agglutination reactions. Extremely valuable for the detection of carriers. About 11 per cent of cases of typhoid fever show the bacilli for eight to ten weeks (convalescent carriers) and 2 to 4 per cent for a year or an indefinite period of time (chronic carriers). Women are more likely to become carriers than men, and children less likely than either. Generally due to invasion or infection of the gallbladder or the intrahepatic bile ducts. Passive carriers (no history of typhoid fever) are rare.
Bacillary Dysentery	Due to any of several types of dysentery bacilli. In the United States bacillary dysentery is more frequently caused by the Flexner than the Shiga bacillus. Bacteriologic examinations of the stools are of great value in the diagnosis of acute, chronic and atypical cases. Reliable differentiation from the dysenteries due to *End. histolytica* and *Balant. coli* only possible by this means. The carrier state after recovery is not as frequent as after typhoid fever. Chronic carriers occur more frequently after Shiga than after Flexner infections. They may occur after clinically unrecognized bacillary dysentery. Passive carriers among healthy persons with no history of dysentery occur but are rare. Carriers are extremely important in relation to endemic and epidemic bacillary dysentery.

TABLE 85. SUMMARY OF THE CLINICAL INTERPRETATION OF BACTERIOLOGIC EXAMINATIONS OF THE FECES—(Continued)

Disease	Interpretation
Infectious Diarrhea	*Epidemic diarrhea of adults* is apparently due to infection with a virus transmitted by way of the upper respiratory tract. The disease has a predilection for older children and young adults. The virus has not been successfully cultivated. *Epidemic diarrhea of the newborn* is now thought to be due to infection with a virus or a group of related viruses. The disease is characterized by high morbidity and mortality rates. The virus is infective for newborn calves but has not been cultivated. The etiology of *summer diarrhea* is obscure. Apparently may be due to infection with dysentery bacilli, Salmonella, *Ps. aeruginosa,* staphylococci or other organisms. The clinical interpretation of bacteriologic examinations of the stools is both difficult and uncertain.
Brucellosis	Due to infection with *Br. abortus* of cows, *Br. melitensis* of goats or *Br. suis* of hogs. Infection due to the ingestion of contaminated raw milks as well as of raw or partially cooked meats of infected animals. May also occur through the skin from contact with infected animals or their raw meats as well as from the handling of cultures. Brucellosis, however, is not primarily an enteric disease. Brucella are not usually found in the feces and bacteriologic examinations are not commonly employed for diagnostic purposes. Guinea-pig inoculation tests, however, are apparently better than cultures. Convalescent or chronic carriers (fecal) are known to occur; also healthy carriers not known to have had the disease. Apparently human carriers may be important sources of infection and especially among food handlers.
Asiatic Cholera	An enteric disease due to infection with *V. cholerae* transmitted by the ingestion of contaminated water, raw foods contaminated by water or flies or by direct contact with the dejecta of patients or carriers. Bacteriologic examinations of the stools and vomitus are of great diagnostic value. Bacteriologic examinations of the feces or rectal swabs are also of great value in the detection of carriers. As a general rule, the vibrios disappear in four to fourteen days but may persist for one to two months (convalescent carriers). Chronic and passive carriers may constitute as high as 6 to 7 per cent of the populations of epidemic areas; sometimes due to invasion or infection of the gallbladder or biliary ducts.
Food Infections and Intoxications	Food *infections* are due to the ingestion of Salmonella, especially *S. typhimurium, S. enteritidis,* and *S. choleraesuis.* Bacteriologic examinations of the stools may possess clinical value but are usually made of the suspected food or foods. Food *intoxications* are due to the ingestion of preformed toxins. The most notable example is botulism due to the ingestion of the preformed exotoxins of *Cl. botulinum* in canned vegetables. Bacteriologic examinations of the feces, therefore, possess no clinical value. It is likewise frequently due to the ingestion of enterotoxins produced by *Staph. aureus.* *"Ptomaine poisoning"* is due to the ingestion of toxic nitrogenous products resulting from the decomposition of meats, cheeses, etc. *"Auto-*

TABLE 85. SUMMARY OF THE CLINICAL INTERPRETATION OF BACTERIOLOGIC EXAMINATIONS OF THE FECES—(Continued)

Disease	Interpretation
	intoxication" is assumed to be due to the absorption of toxic products largely produced by proteolytic bacteria in the colon. Bacteriologic examinations may be of value and especially for implantations of *L. acidophilus* administered in treatment for alteration in the flora through the production of lactic and other organic acids.
Tuberculous Enteritis	Frequently of value in diagnosis and especially by the detection of tubercle bacilli in cultures and by guinea-pig inoculation tests. Positive results, however, may be due to the swallowing of tubercle bacilli in sputum in pulmonary tuberculosis. Negative results do not necessarily exclude the possible presence of the disease.
Intestinal Anthrax	Due to the ingestion of *B. anthracis* or its spores in raw or partially cooked meats of infected animals; also, possibly, to the swallowing of spores in dusts. The disease is rare and usually rapidly fatal. Bacteriologic examinations of the stools possess a high degree of diagnostic value.

Typhoid and Paratyphoid Fevers. As previously stated, blood cultures are the best laboratory procedures for the diagnosis of typhoid fever during the early stages, since about 90 per cent of cases show the presence of a bacteremia during the first and about 75 per cent during the second weeks of the disease. On the other hand, only 10 to 15 per cent of cases are likely to show typhoid bacilli in the feces during the first ten days of the disease; thereafter the percentage rapidly increases, reaching about 50 per cent by the third week and from 80 to 90 per cent during convalescence. Under the conditions, bacteriologic examinations of the feces possess diagnostic value and especially in suspected or atypical cases of typhoid and paratyphoid fever with negative blood cultures and doubtful agglutination reactions.

Bacteriologic examinations of the feces, however, are extremely valuable for the detection of typhoid carriers. About 11 per cent of patients show the presence of typhoid bacilli for eight to ten weeks (convalescent carriers) and from 2 to 4 per cent for a year or an indefinite period (chronic carriers). Passive carriers, or those without clinical history of the disease, are rare; Rosenau reports finding but three in a bacteriologic survey of 1040 individuals. Women are more likely to become chronic carriers than men and children less likely than either. The carrier state appears to be due in most cases to an invasion or chronic infection of the gallbladder or the biliary ducts in the liver; consequently, cholecystectomy may not remove the carrier state. Therefore, the carrier state is frequently detected by cultures of the bile obtained by duodenal drainage, as previously stated.

Bacillary Dysentery. Bacillary dysentery may be due to infection with any of several types of *Shigella dysenteriae*. In the United States more cases are apparently caused by the Flexner than the Shiga bacilli but epidemics due to the Sonne bacillus have also occurred. Furthermore, it appears that some cases of sporadic summer diarrheas of children are due to infection with *S. dysenteriae*, although the finding of the bacilli in the feces does not necessarily establish the presence of infection since children may be carriers of them.

Bacteriologic examinations of the stools are extremely valuable in the diagnosis of bacillary dysentery, since the bacilli are almost invariably present even in the earliest stages of the disease. In fact, differentiation among bacillary, amebic and *Balantidium coli* dysenteries can be only reliably made by laboratory examinations.

Convalescent carriers are not as frequent as typhoid carriers, since the bacilli are generally absent from the feces within two to six weeks after recovery. On the other hand, however, chronic carriers occur and more frequently after recovery from dysentery due to the Shiga than to the Flexner bacilli. Passive carriers occurring among healthy persons with no clinical history of dysentery are apparently uncommon or rare although likewise known to occur. It is commonly thought that many such carriers are individuals who have actually had the disease in clinically unrecognized forms, as mild or sporadic diarrheas. At all events, Shiga and other investigators have laid great stress upon carriers in relation to the endemic and epidemic types of bacillary dysentery. Certainly in institutional or other outbreaks of the disease it is always necessary or advisable to make bacteriologic examinations of the feces of contacts, especially of cooks and food handlers, for the possible detection of carriers.

Infectious Diarrheas. In 1945 Reimann and his colleagues described a type of *epidemic diarrhea of adults* apparently due to infection with a virus transmitted by way of the upper respiratory tract. This disease, which occurs most frequently in the autumn and winter, appears to have a predilection for older children and young adults. The active agent is noninfective for mice and has not been cultivated.

Epidemic diarrhea of the newborn usually occurs within the first month after birth. This highly contagious disease, with its high morbidity and mortality rates, is characterized by profuse watery diarrhea, loss of weight, inanition, dehydration and acidosis. Various bacteria have been suspected as the cause but a virus is now thought to be the etiologic agent, at least in certain outbreaks of the disease. The virus, however, has not been cultivated and, among the lower animals, has proved infective only for newborn calves. It is possible that different viruses are responsible for different epidemics. Whether or not the virus is the same as that producing epidemic diarrhea of adults cannot be stated but this may be possible.

Many investigators have found dysentery bacilli in the stools of infants and young children with acute gastro-enteritis or *summer diarrhea*. But the etiologic relationship of the bacillus is obscure as such infants may not appear to differ clinically from those in whose feces the bacilli are not found. It is to be admitted, however, that dysentery bacilli may produce the disease. On the other hand, it appears that members of the Salmonella group of bacilli may likewise produce the disease as well as *Ps. aeruginosa*, staphylococci or other micro-organisms.

Therefore the clinical interpretation of bacteriologic examination of the stools is difficult but is always advisable and especially in institutional outbreaks.

Brucellosis. Brucellosis is of increasing frequency in the United States. The disease is caused by *Br. abortus* of cows, *Br. melitensis* of goats or *Br. suis* of hogs. Infection is due to the ingestion of contaminated raw milks as well as of raw or partially cooked meats of infected animals. Infection may also occur through the skin by contact with infected animals or their raw meats, as well as from the handling of cultures in the laboratory. Subtypes also occur. *Br. melitensis* and *Br. suis* are more virulent for human beings than *Br. abortus,* but most cases of the disease are due to the latter type.

Since infection so frequently occurs from the ingestion of contaminated raw milk, brucellosis is being considered in this place although it is not by any means to be classed as an enteric disease. At least it is exceedingly difficult to find the Brucella in the feces although this may be due, at least partly, to technical difficulties. Consequently, bacteriologic examinations of the feces are not commonly employed for diagnostic purposes although guinea-pig inoculation tests may prove more helpful in this connection than cultures. Blood cultures, agglutination, opsonocytophagic and brucellergen skin tests are of far more diagnostic value.

On the other hand, Brucella have been found to persist in the feces of chronic and recurring types of undulant fever over long periods of time. The incidence of convalescent or chronic carriers, however, is unknown. Furthermore, Goldstein and his colleagues,[28] employing a guinea-pig inoculation test, found 2 carriers among 219 apparently normal healthy individuals never known or suspected of having had the disease. It is likely that carriers also occur among individuals with the ambulatory and atypical chronic types of the disease and certainly no known carrier should be permitted to handle or prepare foods.

Asiatic Cholera. Asiatic cholera is due to infection of the intestinal tract, principally the small intestine, with *Vibrio cholerae* contracted by the ingestion of unboiled water, raw milk, vegetables or other foods contaminated by water or flies or by direct contact through contamination of the hands by the dejecta of cases of the disease or carriers of the micro-organism.

Enormous numbers of the vibrios or bacilli occur in the stools and vomitus where they are readily and easily found by bacteriologic examinations. Consequently, the latter possess great diagnostic value and especially in the diagnosis of mild or atypical types of the disease escaping clinical detection.

As a general rule, the vibrios disappear from the feces in from four to fourteen days but may persist for one or two months, constituting convalescent carriers. Chronic or passive healthy carriers may occur in as high as 6 to 7 per cent of the populations in epidemic areas. As in the case of chronic typhoid carriers, many of these are due to invasion or infection of the gallbladder or intrahepatic biliary ducts with the vibrios. Carriers are readily detected by bacteriologic examinations of the feces or of rectal swabs and, needless to state, are of tremendous importance in relation to the endemic and epidemic occurrence of the disease.

Food Infections. Food-borne bacterial infections are of two general types. One consists of those diseases like typhoid and paratyphoid fevers, bacillary dysentery, cholera and other enteric infections of which food is a vector of transmission. The second type is that due to infection with bacilli of the Salmonella group, in which the incubation period is short and the symptoms those of an acute gastroenteritis typical of food poisoning. Of the Salmonella infections, one of four species is generally involved, *S. typhimurium* (*S. aertrycke*) and its varieties, which are most frequently observed, *S. enteritidis*, *S. choleraesuis* and *S. give*. No enterotoxins have been shown to be produced by these bacilli, and it is probable that actual infection occurs. Bacteriologic examinations of the feces and of the suspected food or foods possess diagnostic value; such examinations of the feces are also indispensable for the detection of carriers of the salmonellae responsible for the contamination of foods.

Food Intoxications. Food intoxications are those due to the ingestion of preformed exogenous toxins. The most outstanding example is botulism due to ingestion of the toxin produced by *Cl. botulinum*, which is an anaerobic spore-bearing bacillus commonly occurring in the soil. Consequently, botulism usually occurs from the ingestion of contaminated canned or preserved vegetables (especially those canned in homes) in which the spores escape destruction and germinate, producing extremely powerful toxins resistant to heat and causing little or no change in the color or odor of the food. Fortunately, botulism is rare and, being an intoxication rather than an infection, bacteriologic examinations of the feces possess no diagnostic value.

It is now definitely proven that the ingestion of enterotoxins produced by hemolytic strains of *Staph. aureus* in foods containing starch thickening, such as eclairs, cream puffs, cake fillings, salad dressings and the like, is also capable of producing a common type of food intoxication. It is likely that similar intoxications may be produced by the ingestion of the toxins of other organisms like those of streptococci, *A. aerogenes* and *P. vulgaris*. Bacteriologic examinations of the feces are of limited value in diagnosis but such examinations of a suspected food or foods are of definite value.

Food intoxications may be due also to ptomaines or nitrogenous preformed toxic substances due to the decomposition of foods, especially meats and cheeses, by various saprophytic micro-organisms. True ptomaine poisoning, however, is rare and bacteriologic examinations of the feces possess no diagnostic value.

Related to this subject is that of the much discussed "auto-intoxication" which is assumed to be a clinical state due to the absorption of toxic substances from the colon produced by an abnormal bacterial flora particularly involving the proteolytic micro-organisms, since abnormal indicanuria is regarded as laboratory evidence of its presence. Bacteriologic examinations of the feces are of value, provided the diet is controlled. They are likewise of value for the detection of successful implantations of *L. acidophilus*, which is commonly administered in treatment with the hope that its capacity for producing lactic and other organic acids, especially from lactose, may alter the intestinal flora sufficiently for therapeutic purposes.

Tuberculous Enteritis. Bacteriologic examinations of the stools are frequently of value in the diagnosis of tuberculous enteritis and especially by the detection of tubercle bacilli in cultures and by guinea-pig inoculation tests. Positive results, however, may be due to the swallowing of tubercle bacilli in sputum in pulmonary tuberculosis, since the bacilli readily escape destruction in the stomach. For this reason positive findings do not necessarily indicate the presence of tuberculous enteritis. Furthermore, one or two negative examinations do not exclude the possible presence of the disease, being in these respects similar to the limited value of negative sputum examinations unless frequently observed.

Anthrax. Intestinal anthrax follows the ingestion of *B. anthracis* or its spores in raw or partially cooked meats of infected animals. It is also possible that infection may occur from the swallowing of spores in dusts during the handling of contaminated dried wools and hairs, the spores escaping destruction in the stomach. The disease is rare and usually rapidly fatal. Bacteriologic examinations of the feces, however, possess a high degree of clinical value, since *B. anthracis* is easily detected in cultures and readily differentiated from *B. subtilis* and other spore-forming aerobic bacilli occurring in the normal intestinal flora.

EXAMINATIONS OF THE ANUS, RECTUM AND SIGMOID

Bacteriologic examinations of the anus, rectum and sigmoid colon are being separately considered because they are subject to the preparation of smears and cultures by proctoscopic or sigmoidoscopic procedures. Following trauma or other predisposing factors, the tissues of these parts are greatly exposed to infections by micro-organisms occurring in the feces. For this reason contamination with fecal micro-organisms not infrequently adds to the difficulties of clinical interpretation of the results of bacteriologic examinations. Consequently, due care should be exercised in the preparation of smears and cultures of pus and secretions of abscesses, fistulae and ulcers to avoid contamination as much as possible (Table 86).

Anus. While infection with *Esch. coli,* staphylococci, or streptococci is important in relation to the etiology of *external hemorrhoids* it is always secondary to some type of primary trauma producing congestion and varicosity of the veins. *Internal hemorrhoids* are much less subject to infection although this occurs in some cases. *Cryptitis* is due to infection of the crypts of Morgagni with *Esch. coli,* staphylococci, streptococci, gonococci, *S. dysenteriae* or the tubercle bacillus and is a frequent cause for *pruritus ani.* Not infrequently *Enterobius vermicularis* is found in the crypts, as are likewise segments of *Taenia saginata* or the ova of these and other helminths. Apparently, however, they do not alone produce cryptitis but predispose to bacterial infection. Pruritus ani may be due also to scabies or infection of the skin with molds and yeasts. Whether or not infection with streptococci from the feces may produce this frequent and troublesome condition cannot be stated although this has been suggested.

Anorectal abscesses are usually due to infection with *Staph. aureus, Esch. coli,* or streptococci, with the tubercle bacillus producing the disease in about 2 to 5 per cent of cases. *Ps. aeruginosa* and *P. vulgaris* may also occur in cultures but are usually regarded as micro-organisms of secondary infection or contamination. *Anorectal fistulae* are invariably due to mixed infections with staphylococci, streptococci, *Esch. coli,* or *Ps. aeruginosa;* also, but much less frequently, to pneumococci, gonococci, *S. dysenteriae, S. typhosa* and the tubercle bacillus. Tuberculous abscesses and fistulae are due to the human tubercle bacillus in about 98 per cent of cases. It is commonly thought that infection occurs through the swallowing of tuberculous sputum but undoubtedly it is due in some cases to hematogenous infection or the result of direct lymphogenous extension from tuberculous foci in neighboring parts.

TABLE 86. SUMMARY OF THE CLINICAL INTERPRETATION OF BACTERIOLOGIC EXAMINATIONS OF THE ANUS, RECTUM AND SIGMOID

Organ	Interpretation
General Considerations	Following trauma or other predisposing causes the tissues are greatly exposed to infections by micro-organisms occurring in the feces. Contamination with micro-organisms of the normal fecal flora may complicate the clinical interpretation of bacteriologic examinations. Care is required in the preparation of smears and cultures to avoid contamination as much as possible.
Anus	Infection secondary to some type of primary trauma plays an important rôle in the etiology of *external hemorrhoids*. Secondary infection may also occur in *internal hemorrhoids*. *Cryptitis* is due to infection with colon bacilli, staphylococci, streptococci or other micro-organisms. Helminths or their ova in the crypts predispose to bacterial infection.. *Pruritus ani* may be due to cryptitis or infection of the skin with yeasts, molds or scabies but may be due to other causes. *Anorectal abscesses* are commonly due to infection with staphylococci, colon bacilli, streptococci or the tubercle bacillus. *Anorectal fistulas* are commonly due to mixed infections with the same micro-organisms as well as with *Ps. aeruginosa* or other micro-organisms. *Chancroids* not infrequently occur about the anus; likewise *anal chancres* and *condyloma latum,* in which darkfield examinations for T. *pallidum* possess diagnostic value.
Rectum and Sigmoid	Apparently nonspecific infection may be a secondary if not a primary factor in the etiology of acute catarrhal and chronic *proctitis*. Proctitis may be due to specific infection with the gonococcus, *T. pallidum* or the virus of lymphogranuloma venereum. The latter commonly produces strictures of the rectum in women and less frequently in men. Specific *sigmoiditis* or proctosigmoiditis may be due to *S. dysenteriae* or *E. histolytica* in connection with the respective dysenteries. Undoubtedly infection plays a rôle in the etiology of *chronic ulcerative colitis*. It appears, however, to be nonspecific with micro-organisms occurring normally in the feces and secondary to other primary etiologic factors. At least, no one micro-organism has as yet been proven to be the specific cause of the disease. Food allergy is of etiologic importance in some cases.

It is also to be stated that tetanus has occurred in some cases following anorectal operations, like hemorrhoidectomies, due to contamination of the wounds with *Cl. tetani* contained in the feces of the patients who were carriers.

Chancroids, due to infection with *H. ducreyi*, occur not infrequently about the anus. Anal *chancres* are likewise observed as well as *condyloma latum*; darkfield examinations for *T. pallidum* are valuable aids in diagnosis and especially in the former.

Rectum and Sigmoid. Just what rôle, if any, infection plays in the etiology of simple acute catarrhal and chronic *proctitis* (atrophic and hypertrophic) cannot be stated. While not usually regarded as the primary cause, it is apparently a factor of secondary importance. Gonorrheal proctitis occurs as a specific infection, especially in women, but is uncommon. Proctitis may also be due to syphilis. Darkfield examinations for *T. pallidum* are not commonly employed for diagnostic purposes but complement fixation or Wassermann and the flocculation tests are extremely valuable in this connection. Lymphogranuloma venereum, likewise a venereal disease due to a filtrable virus, commonly produces *strictures of the rectum* in women but much less frequently in men.

Sigmoiditis or proctosigmoiditis may be due to specific infection with *S. dysenteriae* in bacillary dysentery; also to infestment with *E. histolytica* as part of amebic enteritis or dysentery.

The rôle of infection in the etiology of *chronic ulcerative colitis* (thrombo-ulcerative proctocolitis) is difficult to define. The subject has been extensively investigated and discussed but with no unanimity of opinion. Undoubtedly, it plays an important rôle in the etiology of the disease but whether as the primary cause or as a secondary factor, cannot be definitely stated at the present time.

Various micro-organisms have been claimed the specific agents with special reference to the diplostreptococcus of Bargen which is now regarded as being *S. bovis*. At least, this micro-organism occurs in cultures of the lesions of some cases but is difficult to differentiate from the nonhemolytic streptococci of the normal fecal flora or to identify it as a micro-organism in specific etiologic relationship to the disease. Other investigators have regarded chronic infection with *S. dysenteriae*, *Esch. coli* or pneumococci as the specific causes. More recently Dragstedt and his colleagues [29] have claimed *Spherophorus necrophorus* as the specific etiologic agent. Since it is an anaerobe of the genus Bacteroides, which occur normally in the intestinal tract, the matter is of particular interest with the chances of the micro-organism being *Bacteroides funduliformis*.

In some respects, the etiology of chronic ulcerative colitis is apparently similar to that of peptic ulcer in which nonspecific infection with streptococci or other micro-organisms of focal origin appears to play a secondary rôle of importance even if not to be regarded as the primary cause of the disease. At least, chronic ulcerative colitis, like peptic ulcer, occurs more frequently in men than in women and especially between the ages of twenty and forty years. Available evidence indicates that food allergy,[30] metabolic disturbances and vitamin deficiencies (especially vitamin B complex), as well as the neuroses, may be among the primary causes of the disease, with inevitable infection as a secondary factor materially contributing to ulceration of the tissues. If this is true, most any of the micro-organisms of the normal feces may play this secondary rôle which, in individual cases, may be streptococci, *Esch. coli*, *Bacteroides*, *S. dysenteriae* or other micro-organisms in pure or mixed infections. In other words, there are many kinds of ulcerative colitis from the standpoint of etiology. Thus, it may be streptococcal, tuberculous, amebic, due to the virus of lymphogranuloma venereum, a late phase of bacillary dysentery, a condition associated with vitamin deficiency, due to food allergy or finally occur as a group of bizarre ulcerative colitides in which the etiology is uncertain.

EXAMINATIONS OF PERITONEAL FLUIDS

The peritoneal cavity is the largest serous sac of the body, since the total area of its surface is somewhat greater than that of the skin. It possesses great absorptive powers, not only for fluids injected into it, but likewise for bacterial toxins, so that profound intoxication may develop with great rapidity and with but few obvious clinical warnings in spreading or diffuse peritonitis. Fortunately, however, it is also endowed with a remarkable capacity for quickly walling off infections largely through the activities of the great omentum.

The *collection* of peritoneal fluids for cytologic, bacteriologic and other examinations has been described in Chapter 13. Exudates in peritonitis are easily obtained at operation but preoperative specimens are likewise readily and safely secured by peritoneal puncture. Examinations of stained smears for bacteria and cells, including phagocytes, as well as of cultures, are of diagnostic value not only in relation to the etiology of peritonitis, but in relation to its treatment, especially with the antibiotic and sulfonamide compounds, and in prognosis as well.

Transudates (ascites) due to congestive heart failure, chronic nephritis or other noninflammatory causes are invariably sterile unless contaminated with staphylococci or other micro-organisms during collection or in the laboratory (Table 87).

Exudates are due to acute or chronic inflammation (peritonitis) which may be localized or spreading and diffuse. Peritonitis, however, may be (1) aseptic (noninfective) due to irritation and far too little emphasized, or (2) septic due to infection.

Aseptic Peritonitis. This is due to inflammation excited by the escape of sterile fluids into the peritoneal cavity like blood, urine, the contents of tumors or cysts, circulatory disturbances like intestinal obstructions and twisted ovarian cysts, or it may result from intraperitoneal injections of sterile saline or glucose solutions, citrated or defibrinated blood, etc. Smears and cultures are sterile, although cytologic examinations reveal large numbers of polymorphonuclear neutrophils or pus cells. Peritonitis may also result from the escape of gastric contents into the peritoneal cavity although infection usually follows; also from the escape of bile but this, likewise, is usually followed by infection, since ruptured gallbladders are invariably infected unless a normal gallbladder is ruptured by trauma.

Primary Peritonitis. Primary or idiopathic peritonitis is uncommon but occurs in infants and children and especially females. Infection is commonly believed to be hematogenous in origin. Most cases are due to infection with pneumococci, especially Type I; it may be also caused by hemolytic streptococci. The mortality varies from 70 to 90 per cent in the absence of surgical drainage

TABLE 87. SUMMARY OF THE CLINICAL INTERPRETATION OF BACTERIOLOGIC EXAMINATIONS OF PERITONEAL FLUIDS

Disease	Interpretation
General Considerations	The total area of the peritoneal surface is somewhat greater than that of the skin. It possesses great absorptive powers not only for fluids but for bacterial toxins as well. Peritoneal fluids for diagnostic, cytologic, bacteriologic and other examinations are readily and safely obtained preoperatively by peritoneal puncture.
Transudates and Exudates	*Transudates* (ascites) are invariably sterile unless contaminated during collection or in the laboratory. *Exudates* are due to acute or chronic inflammation producing peritonitis which may be (1) aseptic or (2) septic. Either may be acute, subacute or chronic; likewise local or diffuse.
Aseptic Peritonitis	Due to inflammation excited by the escape of sterile blood, urine, contents of tumors or cysts into the peritoneal cavity; also by circulatory disturbances and intraperitoneal injections of saline or glucose solutions, blood, etc. Smears and cultures are sterile. May also result from the escape of gastric contents or bile into the peritoneal cavity but infection usually follows.
Primary Peritonitis	Uncommon but may occur in infants and children and especially females. Due to pneumococci (especially Type I) or hemolytic streptococci. Infection believed to be hematogenous in origin. Etiologic diagnosis readily made preoperatively by the examination of smears or cultures of exudates removed by peritoneal puncture.
Septic Peritonitis	May be local or diffuse and spreading. Preoperative bacteriologic examinations are not usually attempted in localized peritonitis but smears and cultures should be made routinely of exudates obtained during operations. Preoperative bacteriologic examinations of exudates obtained by peritoneal puncture possible in diffuse peritonitis. Smears and cultures should be made routinely during operations. The examination of smears for numbers and kinds of micro-organisms, as well as for the degree of phagocytosis, is helpful in connection with cultures in relation to prognosis. Aside from gonococcal peritonitis (rare) and tuberculous peritonitis, mixed infections are the rule. *Anaerobic as well as aerobic cultures of exudates* should be made. The common infecting micro-organisms are colon bacilli, hemolytic and nonhemolytic streptococci, staphylococci, pneumococci, *Cl. perfringens*, *Ps. aeruginosa* and Bacteroides.
Tuberculous Peritonitis	Always secondary to a primary infection of the lungs, mesenteric lymph nodes or elsewhere. Usually occurs as a chronic peritonitis. Smears of exudates usually fail to show tubercle bacilli. Smears stained and examined by the method of fluorescent microscopy advisable. Concentration methods required. Cultures and guinea-pig inoculation tests are particularly valuable in bacteriologic diagnosis.

and specific therapy. Etiologic diagnosis is readily made preoperatively by the examination of smears and cultures of exudates secured bv peritoneal puncture.

Septic Peritonitis. Septic peritonitis may be *localized* when due to the rupture of a suppurative appendix, gallbladder or fallopian tube; also after the rupture of peptic, typhoid, tuberculous, dysenteric or other ulcers, as well as following stab and gunshot wounds of the gastro-intestinal tract. Localized peritoneal abscesses may develop. Not infrequently, however, it occurs as a *diffuse* peritonitis from the outset or as one spreading from an initial localized peritonitis.

Preoperative bacteriologic diagnosis is not usually attempted in localized peritonitis. Smears and cultures of the exudates, however, should be made routinely at the time of operation for drainage, as the information gained is of value from the standpoint of chemotherapy in case diffuse or spreading peritonitis should follow.

Bacteriologic diagnosis, however, is readily made preoperatively in diffuse peritonitis by the examination of smears and cultures of exudates obtained by peritoneal puncture; also by the examination of smears and cultures obtained during operations. The results of examinations of smears stained by the method of Gram should be available in ten minutes or less. The results of these may be very helpful to the surgeon in arranging a therapeutic program as well as in relation to prognosis. As shown by Steinberg,[31] from 0 to 6 micro-organisms per high-power field of a stained smear is indicative of early peritonitis, from 1 to 10 of moderately advanced, and larger numbers of severe peritonitis. Furthermore, the more phagocytic polymorphonuclear neutrophil pus cells found, along with their preservation, the better the prognosis, since marked phagocytosis is indicative of resistance.

The bacteriologic findings are quite diverse. Aside from gonococcal peritonitis (rare) and tuberculosis, mixed infections are the rule. Colon bacilli, hemolytic and nonhemolytic streptococci, pneumococci, staphylococci, *Cl. perfringens, Ps. aeruginosa* and micro-organisms of the genus Bacteroides are the common etiologic agents. *Cultures should be made anaerobically as well as aerobically as a matter of routine* for the detection of anaerobic streptococci, *Cl. perfringens* and Bacteroides.

Tuberculous Peritonitis. Tuberculous peritonitis is always secondary to a primary infection in the lungs, mesenteric lymph nodes or elsewhere. It usually occurs as a chronic peritonitis. The exudates are characterized by containing large numbers of lymphocytes although polymorphonuclear neutrophils frequently predominate in acute exacerbations of the disease. Smears of the exudate usually fail to show tubercle bacilli. Concentration methods are always advisable. Smears stained and examined by the method of fluorescent microscopy[16] may give a higher percentage of positive findings. Cultures are particularly valuable; likewise guinea-pig inoculation tests.

EXAMINATIONS OF THE URINE

Bacteriologic examinations of the urine are of great value in the etiologic diagnosis of infectious diseases of the kidneys and bladder. They frequently yield early evidence of renal tuberculosis; also the presence of low-grade and clinically "silent" infections of the kidneys or bladder without pyuria or other urinary changes. They are likewise of value in relation to the choice of compounds for the treatment of infections of the kidneys and bladder with special reference to the sulfonamides, mandelic acid and other urinary tract disinfectants. They are also of aid in the diagnosis of typhoid fever and the detection of typhoid carriers.

Collection. Urine collected by voiding, however, is generally unsatisfactory for bacteriologic examinations except for the detection of tubercle bacilli, since the possibility of errors due to contamination with *Mycobacterium smegmatis* has been greatly overemphasized. Otherwise, however, urine is unavoidably and invariably contaminated with staphylococci, colon bacilli and other micro-organisms. Consequently, reports on bacteria detected in urinary sediments in the course of ordinary routine microscopic examinations are of no clinical value.

Therefore, urine for bacteriologic examinations should be collected by catheterization whenever possible. Great care is required in the technic, not only for the purpose of avoiding contamination but likewise for the prevention of accidental infection of the bladder. When this is not possible and especially in the case of infants, the voided urine should be examined *immediately* after collection before contaminating micro-organisms have greatly multiplied. Smears of sediment obtained by centrifuging and stained by the method of Gram will usually yield valuable information. Cultures in broth are of no value but plate cultures frequently show a preponderance of the infecting micro-organisms.

Even when urine is collected by catheterization of the bladder, however, contamination may occur with micro-organisms in the meatus and first portions of the urethra. Consequently, the clinical significance of such micro-organisms as *Staph. albus, Esch. coli* and *P. vulgaris* is frequently difficult to determine. Contamination by these sources is much less likely to occur in the collection of urine from each kidney separately by ureteral catheterization, which is always advisable in the etiologic diagnosis of infections of the kidneys whenever possible.

Catheters should be very carefully sterilized. Glass catheters are preferred for the removal of urine from the bladder of women. Patients should be instructed to retain urine for several hours at least so that the first ounce or two may be discarded before a specimen is collected in a sterile test tube or vial.

The meatus and neighboring parts should be carefully cleansed with tincture of green soap and water followed by the application of solutions of metaphen, merthiolate or some other suitable disinfectant.

In the case of children, small catheters must be used. In infants sterile test tubes may be fastened with adhesive tape over the penis or over the urethral meatus in girls. Contamination, however, is unavoidable. For this reason the urine should be examined bacteriologically just as soon as possible after collection; 2 to 5 cc. are ordinarily sufficient.

Bacteriuria. The presence of bacteria in the urine is designated as *bacteriuria.* In the absence of an excess of leukocytes or pus (pyuria) it is suggestive of contamination. On the other hand, it may be due to latent, lowgrade and clinically "silent" infections of the urinary tract with or without pyuria and especially in cystitis of women and pyelitis of children (Table 88).

TABLE 88. SUMMARY OF THE CLINICAL INTERPRETATION OF BACTERIOLOGIC EXAMINATIONS OF THE URINE

Disease	Interpretation
General Considerations	Of great value for the detection and etiologic diagnosis of infections of the kidneys and bladder. They frequently furnish the earliest evidence of renal tuberculosis and the presence of low-grade clinically "silent" infections with or without pyuria. They are also of value in the diagnosis of typhoid fever and the detection of carriers. Likewise in relation to the choice of urinary disinfectants for chemotherapeutic purposes.
Collection	Voided urine is generally unsatisfactory because of contamination except in the case of examinations for tubercle bacilli. Voided urine, however, must be used in the case of infants. Voided urine should be examined *immediately* after collection before contaminating micro-organisms have greatly multiplied. Stained smears and plate cultures are of diagnostic value. Bacteria detected in urinary sediments by ordinary routine microscopic examinations possess no diagnostic value. Urine collected by catheterization of the bladder or ureters is always preferred; the latter is frequently required for the detection of infections of the kidneys. Due precautions are required in collection by catheterization to avoid contamination as much as possible and for the prevention of accidental infections.
Bacteriuria	Bacteriuria is the presence of bacteria in the urine. It is frequently due to contamination in the collection or examination of urine. When associated with pyuria it is usually indicative of infection of the urinary tract. It may occur, however, in low-grade clinically "silent" infections of the kidneys or bladder in the absence of pyuria. *Normal kidneys* apparently do not pass tubercle bacilli or other micro-organisms into the urine. In pulmonary tuberculosis, however, tubercle bacilli may occur in the urine without inevitably resulting in progressive renal tuberculosis. In nephritis, however, bacteria in the blood may be apparently passed by the glomeruli into the urine without detectable evidences of infection of the kidneys. Bacteriuria due to infections of the kidneys or bladder is usually due to the presence of colon bacilli, *A. aerogenes*, *P. vulgaris*, staphylococci, streptococci, typhoid or tubercle bacilli. With the exception of typhoid and tubercle bacilli any of these may be due to contamination which adds greatly to the difficulties of clinical interpretation. Infections due to gonococci and pneumococci are uncommon. *Ps. aeruginosa* and *N. catarrhalis* are usually due to contamination but, presumably, they may also produce infection.

TABLE 88. SUMMARY OF THE CLINICAL INTERPRETATION OF BACTERIOLOGIC EXAMINATIONS OF THE URINE—(Continued)

Disease	Interpretation
Cystitis	May be primary or occur secondarily to pyelonephritis. Tuberculous cystitis is always secondary to renal tuberculosis. Acute primary cystitis is most frequently due to infection with colon bacilli. May be also due to staphylococci (especially *Staph. albus*), hemolytic streptococci. *Str. viridans* or *Str. faecalis* (enterococcus). Infections with gonococci are rare and with pneumococci uncommon. Mixed infections are common in chronic cystitis which is frequently secondary to pyelonephritis. Usually due to the same micro-organisms; also to *P. vulgaris* and paracolon bacilli and less commonly to *Ps. aeruginosa* and *N. catarrhalis; A. aerogenes* and *G. tetragena* are usually secondary invaders.
Pyelonephritis	Pyelitis is usually a type of pyelonephritis. Pyelonephritis may be due to hematogenous infections; also to ascending urogenous and lymphogenous infections. Multiple or embolic abscesses and carbuncles of the kidneys are usually due to hematogenous infections with staphylococci or streptococci. Chronic pyelonephritis, including clinically "silent" types with or without pyuria, is usually due to infections with *E. coli*, paracolon bacilli, *Staph. albus*, hemolytic types of streptococci, *Str. viridans* or *Str. faecalis*. Mixed infections are frequent. Pyonephrosis may result from pyelonephritis or develop as a secondary infection of a hydronephrosis with any of these micro-organisms.
Tuberculosis	Tuberculosis of the kidneys is always secondary to tuberculosis of the lungs or some other focus. Tuberculosis of the bladder is secondary to tuberculosis of the kidneys. Bacteriologic examinations of the urine are extremely valuable diagnostic aids although negative results do not exclude the possibility of tuberculosis unless repeatedly negative over long periods of time. Smears of sediment are usually positive in 50 to 80 per cent of cases. Smears stained and examined by the method of fluorescent microscopy give a higher percentage of positive findings. Cultures also give a higher percentage of positive results. Guinea-pig inoculation tests are positive in over 80 per cent of cases.
Typhoid Fever and Carriers	Typhoid bacilli occur in the urine in about 25 to 30 per cent of cases of typhoid fever but not usually until after the second week of the disease. Bacteriologic examinations, therefore, are not usually employed for diagnostic purposes except in atypical or doubtful cases of the disease. About 12 per cent of cases show the bacilli in the urine during early convalescence. Consequently, the urine as well as the feces should be repeatedly examined bacteriologically before the lifting of quarantine. A small percentage of chronic carriers may show the bacilli in the urine over months or years of time. The bacteriuria is usually intermittent and associated with albuminuria. Obstinate chronic cystitis due to *S. typhosa* may occur; pyelonephritis is much less frequent.

TABLE 88. SUMMARY OF THE CLINICAL INTERPRETATION OF BACTERIOLOGIC EXAMINATIONS OF THE URINE—(Continued)

Disease	Interpretation
Dysentery and Undulant Fever	*S. dysenteriae* may occur in the urine during bacillary dysentery and sometimes in carriers. *Br. abortus* and *Br. melitensis* may also occur in the urine in undulant fever; the incidence of urinary carriers is unknown.

Whether or not bacteriuria may occur in the absence of urinary tract infections by the passage of bacteria through *normal kidneys* brought to them by the blood has been extensively discussed and especially in relation to tubercle bacilli. It is now the consensus, however, that the presence of tubercle bacilli in the urine is usually indicative of renal tuberculosis, providing errors in examination are excluded, and especially if pus and blood are present in the urine. On the other hand, Ordway and Medlar [32] have recently reported that the urine of 22 (7.7 per cent) of a group of 287 cases of pulmonary tuberculosis showed tubercle bacilli in the urine. Of these, 17 (77 per cent) had no clinical symptoms suggestive of renal tuberculosis. Consequently, in their opinion the presence of tubercle bacilli in the urine does not indicate that progressive renal tuberculosis will inevitably follow and that surgical intervention should therefore be delayed until such time as progressive renal destruction has been proved. As previously stated, the possibility of mistakes due to possible contamination with *Myco. smegmatis* has been greatly overemphasized.

Bacteriuria due to the presence of *S. typhosa* during typhoid fever, and in carriers after recovery, is now believed to be the result of renal damage. During typhoid fever it is apparently due to infection of the kidneys with the bacilli since they are not ordinarily found in the urine until after the second week of the disease. The same may be true in carriers, since the presence of the bacilli in the urine is almost invariably associated with albuminuria and thereby analogous to persistent chronic infection of the gallbladder as the cause of fecal carriers. However, it is both possible and probable that the elimination of typhoid bacilli in the urine is due to a nephritis, without actual infection of the kidneys, permitting their passage through the kidneys in periods of temporary bacteremia.

At all events, it appears reasonably certain that bacteria in the blood are not passed by *normal kidneys* into the urine. If contamination can be excluded with reasonable accuracy, their presence in urine secured by catheterization is indicative of either (1) manifest or clinically "silent" infections of the kidneys or bladder, or (2) the presence of sufficient nephritis without infection to permit the passage of bacteria through the glomeruli brought to them by the blood. For this reason bacteriuria during the course of the septicemias is not always indicative of infection of the kidneys although this is usually the case. In the intermittent bacteremias of the chronic focal infections, however, it is likely that bacteriurias may occur at the same time without actual infection of the kidneys if the filterability of the glomeruli has been increased by chronic nephritis.

In infections of the kidneys and bladder bacteriuria is usually due to the presence of colon bacilli, staphylococci, streptococci, *P. vulgaris,* typhoid or tubercle bacilli. These may occur singly or in combinations. With the exception of *Myco. tuberculosis* and *S. typhosa,* however, any or all of them may be due to contamination which adds greatly to the difficulties of clinical interpretation. Infections due to gonococci and pneumococci are uncommon. The presence of *A. aerogenes, Ps. aeruginosa* and *N. catarrhalis* is usually due to contamination although it is stated that they may produce infections of the urinary tract.

Cystitis. Acute primary cystitis is usually due to infection with colon bacilli, staphylococci or streptococci. Infection with *Staph. albus* apparently occurs more frequently than with *Staph. aureus*. The streptococci include not only hemolytic types but *Str. viridans* and those of the enterococcus group with special reference to *Str. faecalis* which belongs to Lancefield's Group D. Infections with colon bacilli, however, are by all odds most frequently observed; infections due to the gonococcus are rare and those due to pneumococci quite uncommon.

Chronic cystitis, including trigonal cystitis, may follow or develop secondarily to primary pyelonephritis. Tuberculous cystitis is always a secondary infection. The same micro-organisms are usually responsible; mixed infections are common. The infection may occur predominantly in the mucosa or in the other layers of the bladder wall. *P. vulgaris* and paracolon bacilli are also frequently found in the urine and may be a factor in the production of chronic cystitis; likewise, but much less frequently, *Ps. aeruginosa* and *N. catarrhalis. A. aerogenes,* however, as well as *Gaffyka tetragena,* are usually to be regarded as saprophytic invaders.

Pericystitis or infection of the loose areolar tissue in front and around the bladder is usually due to hematogenous infection with colon bacilli, staphylococci or streptococci.

Pyelonephritis. Pyelitis may occur as an infection confined to the pelves of the kidneys but is commonly regarded as a pyelonephritis and is almost invariably present.

Acute pyelonephritis is frequently due to hematogenous infection resulting in the production of multiple or embolic abscesses, as well as carbuncle of the kidneys, and especially in the septicemias due to staphylococci or the hemolytic streptococci. It may also result from ascending (urogenous) or lymphogenous infections. Pyelonephritis may be caused by the typhoid bacillus producing so-called "renal typhoid fever." It is thought to occur more frequently than surmised with no symptoms referable to the intestinal tract. Needless to state, such individuals may be dangerous sources of infection. Some cases due to *Salmonella paradysenteriae* have also been reported [33] and infections are frequently due to paracolon bacilli.[34]

Chronic pyelonephritis may follow the same routes of infection and result in pyonephrosis. The latter may also occur as a secondary infection in hydronephrosis.

Embolic abscesses and carbuncle of the kidneys are usually due to infection with staphylococci or hemolytic streptococci. Otherwise, acute pyelitis and pyelonephritis are most frequently due to infections with colon bacilli, staphylococci (especially *Staph. albus*) or streptococci. Chronic pyelonephritis, including those types which are clinically "silent" with or without pyuria, is likewise most frequently due to infections with colon bacilli, *Staph. albus,* hemolytic streptococci, *Str. viridans* or apparently, sometimes, to the enterococcus (*Str. faecalis*). Mixed infections are commonly observed due to these micro-organisms as well as to *P. vulgaris* or others.

Tuberculosis. Tuberculosis of the kidneys is apparently always secondary to a primary tuberculosis of the lungs or some other organ of the body. As previously stated, tuberculosis of the bladder is always secondary to tuberculosis of the kidneys.

In the bacteriologic diagnosis of tuberculosis of the kidneys, urine obtained by catheterization of the ureters is preferred for examination. This is necessary for determining whether one or both kidneys are infected. Otherwise, however, bladder urine is preferred because of the larger amounts available for examination and especially since the bacilli may be so few as to escape detection in the small amounts ordinarily obtained by ureteral catheterization.

The presence of tubercle bacilli is always indicative of tuberculosis infection, provided sources of error due to scratches in slides and other technical factors are excluded. The possibility of error due to contamination with *Myco. smegmatis* has been greatly overemphasized and probably does not occur; this is certainly true when urine is collected by ureteral or bladder catheterization.

Since but few tubercle bacilli ordinarily occur in the urine in the absence of "showers," careful and prolonged examinations of stained smears of sediment are ordinarily required for their detection. The bacilli are apt to be found, however, in from 50 to 80 per cent of cases. Smears stained and examined by the method of fluorescent microscopy are likely to yield a higher percentage of positive results. Cultures on appropriate media, however, give a higher percentage of positive findings than direct smears, although they ordinarily require several days before the results are available. Guinea-pig inoculation tests are positive in over 80 per cent of cases but require four to six weeks of time for their conduct. Under these conditions, positive smears are indicative of tuberculous infection and especially if repeated positive. It is always advisable to have the results confirmed by culture or guinea-pig inoculation and preferably by both whenever possible. Negative smears, cultures and guinea-pig inoculation tests, however, do not exclude the possibility of tuberculosis of the kidneys unless repeatedly negative over a long period of time.

Typhoid Fever and Carriers. Typhoid bacilli do not ordinarily occur in the urine in typhoid fever until after the second week of the disease when only about 25 to 30 per cent of cases show their presence. Bacteriologic examinations of the urine, therefore, are not ordinarily indicated or required for diagnostic purposes except in atypical or doubtful cases of the disease.

During the early period of convalescence about 12 per cent of cases show the presence of the bacilli in the urine. Consequently, repeated bacteriologic examinations of both urine and feces are indicated before quarantine is lifted to guard against the possible discharge of carriers. In a small percentage of cases chronic carriers continue to discharge typhoid bacilli in the urine over months or years of time but the incidence is not as high as in the feces. They usually occur intermittently in the urine and commonly with albuminuria. An obstinate type of chronic cystitis is sometimes due to *S. typhosa* but pyelonephritis is much less frequent.

Dysentery and Undulant Fever. *Shigella dysenteriae* may likewise occur in the urine during bacillary dysentery but not regularly enough ordinarily to render bacteriologic examinations of diagnostic value. Urinary carriers, however, may occur after recovery although not as frequently as in typhoid fever.

Brucella abortus or *Br. melitensis* may also occur in the urine during undulant fever but bacteriologic examinations are not usually conducted for diagnostic purposes. It is likely that urinary carriers may occur but their incidence is unknown.

EXAMINATIONS OF THE GENITAL ORGANS

Owing to the frequency of venereal infections of both sexes with the gonococcus, *T. pallidum, H. ducreyi* and the "Donovan bodies" of granuloma inguinale, bacteriologic examinations have proved of great clinical value in their detection and differentiation, although they are not applicable to the diagnosis of viral diseases, like herpes genitalis and lymphogranuloma venereum, except by exclusion. While this is the most important field for bacteriologic examinations in relation to the diseases of the genital organs, these examinations are not without additional value in relation to the etiologic diagnosis of many of the nonvenereal diseases of these parts due to acute and chronic infections.

As is true of other external parts of the body, however, bacteriologic examinations of the genital organs are likewise subject to errors because of normal bacterial floras, which not infrequently result in difficulties in the clinical interpretation of results. For example, the meatus and first portion of the urethra of males commonly harbor not only staphylococci but sometimes various gram-negative diplococci which may be morphologically mistaken for gonococci. The same is true of the urethrae of women, while the vaginal flora of both adults and children commonly show not only staphylococci, *N. catarrhalis* and other diplococci, but likewise various streptococci, pneumococci, diphtheroid bacilli, *P. vulgaris,* the Döderlein and other lactobacilli, etc. Furthermore, *T. refringens,* which is a saprophytic spirochete occurring about the external genitalia of both sexes, is readily mistaken for *T. pallidum* by the inexperienced in the bacteriologic diagnosis of chancres and other early lesions of syphilis by darkfield examinations. Since tuberculous infections of the external genitalia are rare, the saprophytic acid-fast *Myco. smegmatis,* however, is not usually confusing except occasionally in bacteriologic examinations of chronic indolent progressive ulcers of the penis.

Acute Gonorrhea and Its Complications. Acute gonorrhea of males occurs as a urethritis. In the adult females it also occurs as a urethritis frequently associated with infection of the Bartholin glands and the vagina, with special reference to the vaginal vault around the cervix where the mucosa is more susceptible to infection. In female infants and children it occurs as an acute and highly contagious vaginitis because the mucosa is far more susceptible to infection than that of the adult vagina.

The examination of well-prepared and properly stained smears of the pus usually suffice for bacteriologic diagnosis. Small amounts of discharge should not

TABLE 89. SUMMARY OF THE CLINICAL INTERPRETATION OF BACTERIOLOGIC EXAMINATIONS OF THE GENITAL ORGANS

Disease	Interpretation
General Considerations	Bacteriologic examinations are of great value in the diagnosis and differential diagnosis of venereal diseases due to bacterial infections as well as in the diagnosis of nonvenereal infections. Bacteriologic diagnosis is frequently complicated and subject to errors due to the normal bacterial flora of the genital organs. Well-prepared and properly stained smears are essential; cultures employing selective and enriched media are frequently required.
Acute Gonorrhea and Its Complications	Smears stained by the method of Gram are usually sufficient for diagnostic purposes. Numerous leukocytes or pus cells without intracellular diplococci are suspicious but never alone sufficient for positive diagnosis and especially in the case of women and children. The absence of numerous pus cells does not exclude the possibility of gonococcus infection. *Acute meatitis* in men is frequently due to staphylococcus infection. *Periurethritis* with or without abscesses in men may be due to the gonococcus alone but are usually mixed infections. The same is true of *acute prostatitis* with or without abscess, *acute epididymitis* and *acute vesiculitis.* Bacteriologic examinations are indispensable as criteria of recovery and cure. Smears alone are insufficient; cultures are also required.
Chronic Gonorrhea and Its Complications	Usually due to mixed infections with gonococci, staphylococci, streptococci, *Esch. coli* or other micro-organisms. The detection of gonococci is more difficult. Smears alone are frequently insufficient; cultures are frequently required. The presence of numerous pus cells without intracellular diplococci is suspicious but not alone sufficient for diagnosis and especially in the case of women and children. Smears and cultures are likely to agree in about 85 per cent of cases. Negative smears alone do not exclude chronic gonorrhea; cultures are frequently required. Negative cultures are particularly required as criteria of recovery and cure. Bacteriologic examinations are of value in differential diagnosis between *chronic gonorrhea* and *urethrorrhea, prostatorrhea, chronic prostatovesiculitis, chronic prostatitis* due to infestment with *T. hominis* and chronic anterior urethritis of men with stricture.
Syphilis	Darkfield examinations for *T. pallidum* are of great value in the diagnosis of chancres when properly and skillfully conducted. Stained smears and cultures are of no diagnostic value. Due care is required for guarding against *T. refringens* being mistaken for *T. pallidum.* Darkfield examinations for *T. pallidum* may be negative in chancres with advanced healing. They are of no value in the examination of gummata or of the vaginal secretions of women with late syphilis. Darkfield examinations are indicated in all abrasions and ulcerative lesions of the genital organs. They are of great value in excluding syphilis in the diagnosis of chancroid, lymphogranuloma venereum, granuloma inguinale, balanoposthitis, herpes genitalis, erosive and gangrenous balanitis, chronic phagedenic ulcers and carcinoma.

TABLE 89. SUMMARY OF THE CLINICAL INTERPRETATION OF BACTERIOLOGIC EXAMINATIONS OF THE GENITAL ORGANS— (Continued)

Disease	Interpretation
Chancroid	All cases should be examined one or more times for *T. pallidum* by darkfield microscopy for the exclusion of syphilis. Bacteriologic examinations of the pus of the lesions or buboes for *H. ducreyi* are positive in about 90 per cent of cases. Smears and cultures (in sterile whole rabbit blood) are required. Of value in differentiating chancroid from lymphogranuloma venereum and granuloma inguinale. An intradermal test with chancroidal vaccine is likewise of clinical value.
Lympho-granuloma Inguinale	Occurs almost exclusively among Negro men and women. Due to infection with *D. granulomatis* occurring within monocytes ("Donovan bodies"). Stained smears of pus from buboes for intracellular "Donovan bodies" and complement fixation tests possess diagnostic value. Cultures are not usually employed. The disease must be differentiated from lymphogranuloma venereum.
Lympho-granuloma Venereum	Also occurs most frequently among Negroes and especially in the South. Transmitted by sexual contact. Due to a filtrable virus. Bacteriologic examinations, therefore, possess no diagnostic value and are not employed. The Frei intradermal test is of helpful diagnostic value; also the complement fixation test.
Gangrenous Balanitis	Also known as erosive balanitis; phimosis with infection due to staphylococci or streptococci is the usual cause. May be also due to infection with *Bor. vincentii* and *B. fusiformis* of the saliva, constituting the so-called "fifth venereal disease." *Chronic phagedenic ulcers* of the penis may be due to the same infection but in most cases the etiology is unknown.
Prostatitis	*Acute prostatitis* may occur as a complication of acute gonorrhea due to infection with the gonococcus. Abscesses are generally due to mixed infection with staphylococci or streptococci. *Chronic prostatitis* is frequently a complication of chronic gonorrhea due to mixed infections with gonococci, staphylococci, streptococci, *Esch. coli* or other micro-organisms. It may be also due to focal infection of dental, tonsillar or other origin. Secretions obtained by massage for bacteriologic examinations are almost invariably subjected to contamination; the same also applies to semen. Chronic prostatitis may be also due to tuberculous infection; bacteriologic examinations of secretions or semen by cultures or guinea-pig inoculation tests possess diagnostic value. It may be also due to syphilis but darkfield examinations for *T. pallidum* possess no clinical value.
Seminal Vesiculitis	*Acute vesiculitis* is usually a complication of acute gonorrhea. Pelvic cellulitis and abscess as well as infection of the vas deferens or all structures of the spermatic cord (*funiculitis*) may follow. *Chronic vesiculitis* is a frequent complication of chronic gonorrhea due to mixed infections. It may, however, be due to focal infection. *Tuberculous vesiculitis* is usually secondary to tuberculous epididymitis.

TABLE 89. SUMMARY OF THE CLINICAL INTERPRETATION OF BACTERIOLOGIC EXAMINATIONS OF THE GENITAL ORGANS—(Continued)

Disease	Interpretation
Epididymitis	*Acute epididymitis* is usually due to gonococcus infection secondary to seminal vesiculitis and occurs more frequently than acute orchitis. *Chronic epididymitis* is generally due to mixed infections. *Tuberculous epididymitis* is always secondary to tuberculosis of the lungs or other primary foci.
Orchitis	*Acute orchitis* is commonly due to hematogenous infections with the virus of mumps; also, but much less frequently, to infections with staphylococci, streptococci or other micro-organisms in the course of the septicemias. Abscesses of the testicles are generally due to staphylococcus or streptococcus infections. *Chronic orchitis* is usually secondary to chronic epididymitis or periorchitis and generally due to mixed infections. *Syphilitic orchitis* is a tertiary or late lesion and very common, ranking next in frequency to syphilis of the cardiovascular and central nervous systems.

be spread out too thinly or with too much rubbing in order to avoid the breaking up of pus cells as much as possible. Very thick smea s are likewise unsatisfactory. In women, smears should be prepared of urethral discharges, after the expression of Skene's glands, and likewise of the cervix uteri. It is also important that smears be properly stained by the method of Gram, since staphylococci in diplococcus grouping may be readily mistaken for gonococci when stained with methylene blue.

In the very early stages the serous discharges are apt to show but few pus cells and gonococci. The latter are likely to be mostly or entirely extracellular, frequently resulting in a doubtful bacteriologic diagnosis. But within a day or two, the smears usually show so many pus cells with numerous typical intracellular gonococci that positive diagnosis is both easy and the rule (Table 89).

The presence of numerous pus cells without intracellular diplococci is suspicious but never alone sufficient for making a positive diagnosis. This is particularly true in the case of women and female children. For example, smears of the vaginal discharges of women, including those from around the cervix, may show large numbers of pus cells without gonococcus infection as the result of lacerations or erosions of the cervix, the use of irritating douches, menstruation, etc. Furthermore, as shown by Steer,[35] vaginal smears of 40 to 50 per cent of children from six to ten years of age may show large numbers of leukocytes without gonococcus infection, and after the age of ten these cells undergo a sharp drop due to the influence of the estrogenic hormone in replacing them with epithelial cells. On the other hand, acute urethritis of women is apt to be a transient infection in which smears may show but few pus cells with extracellular gonococci.

The examination of well-prepared and properly stained smears is. therefore, of great value in the bacteriologic diagnosis of acute gonococcus infections of adults of both sexes and of vaginitis of infants and children and especially if intracellular gram-negative diplococci are found. Consequently, they should always be employed for diagnosis and differential diagnosis. In men, for example, an *acute meatitis* may not be due to gonorrhea at all but the result of infection with *Staph. albus*. Furthermore, they are of great value as aids in differential diagnosis between acute gonorrhea and intra-urethral chancres as well as between acute gonorrhea and chancroid with phimosis. *Periurethritis* and *periurethral abscesses* of men may be due to the gonococcus alone but are usually mixed infections with *Staph. albus* or *aureus*. *Acute prostatitis* with or without abscess, *acute epididymitis* and *acute seminal vesiculitis* may be due to gonococcus infection alone but are frequently mixed infections with staphylococci or streptococci.

Bacteriologic examinations are likewise indispensable as criteria of recovery and cure. For this purpose, however, smears alone are not sufficient. Cultures are also required because it is so well known that smears may be negative for pus cells and intracellular gonococci while the identification of extracellular gonococci by staining and morphology alone is risky and frequently impossible.

Chronic Gonorrhea and Its Complications. In chronic gonorrhea of adults of both sexes, including chronic vaginitis of infants and children, bacteriologic diagnosis, however, is usually more difficult. Mixed infections with gonococci, staphylococci, streptococci, *Esch. coli* or other micro-organisms are the rule. Cultures are usually required in addition to the examination of smears; the same is true in the case of bacteriologic examinations as criteria of recovery and cure.

As a general rule, the gonococci are less numerous, except during acute exacerbations, and are so frequently extracellular along with morphologic changes that diagnosis by smear examinations alone may be impossible or the results doubtful. Apparently smears are positive in only about 75 per cent of cases of chronic gonorrhea of men and even a smaller percentage of women, unless smears are prepared of the exudates around the cervix uteri, in which case about 85 per cent are likely to yield positive results. Persistent infection, however, is especially likely to be within the cervical canal. The reappearance of Döderlein or other lactobacilli is generally indicative of receding gonococcal infection.

As in acute gonorrhea, diagnosis is not greatly aided by the presence or absence of pus cells. Their presence is always suspicious but not alone sufficient for positive diagnosis as in both men and women, as well as in female children, an excess of leukocytes or pus cells may be due to nonspecific infections or other causes.

As shown by Gronau,[36] smears and cultures agree in about 88 per cent of cases. In the remaining 12 per cent, cultures alone are more frequently positive than smears alone. For example, in his series cultures were positive in about 13 per cent of cases with negative smears, while in about 7 per cent of cases the cultures were negative with positive smears. It is apparent, therefore, that cultures should be employed in all cases in which negative smears are observed. This is especially important when bacteriologic examinations are employed as criteria of cure, particularly if sulfonamide compounds or penicillin have been employed in treatment, in order to avoid the error of dismissing individuals as "cured" who may be carriers of gonococci without discharges or other clinical evidences of infection. In men, cultures of discharges obtained by massage of the prostate gland and seminal vesicles, as well as of the shreds and sediment of the first urine passed in the morning, are particularly indicated.

Furthermore, bacteriologic examinations by smears and cultures possess a high degree of value in differential diagnosis. For example, the serous secretions of the urethral and Cowper's glands in men (*urethrorrhea*), as well as the discharge in *prostatorrhea* and *spermatorrhea,* may be readily mistaken for chronic gonorrhea. The same is true in cases of *chronic prostatovesiculitis* as well as in chronic anterior urethritis of men with stricture in which the discharges may be due to nonspecific infections with staphylococci alone or in association with streptococci, *Esch. coli* or other micro-organisms instead of to gonococci. Furthermore, chronic prostatitis may be due to infestment with *Trichomonas hominis,* but usually without discharge unless associated with infection by staphylococci, streptococci or colon bacilli which is frequently the case

Syphilis. Undoubtedly, darkfield examinations for *T. pallidum* should be conducted routinely and as soon as possible in the diagnosis and differential diagnosis of all ulcerative lesions of the genitalia of both men and women before any local measures have been applied. Otherwise, all local treatment should be stopped for a day or two when this extremely valuable examination should be made. Secretions of the lesion are required as free as possible from blood and pus. Dried smears stained for *T. pallidum* are worthless because of technical difficulties; the same is true of cultures because the spirochetes cannot be cultivated for diagnostic purposes. If the physician is unable to conduct the examination in his office the patient should be sent to a laboratory. Otherwise, the physician may place a drop of sterile saline solution on a slide, add a small amount of secretion obtained with a suitable sterile platinum wire or other instrument, cover with a cover glass and deliver to a laboratory as quickly as possible before drying has occurred. Chilling will not interfere with the examination, as the motility of the spirochetes is maintained over long periods of time. Otherwise, the physician may collect a drop or two of secretion in a capillary tube which, after sealing, may be mailed or otherwise delivered (Fig. 32).

Primary chancres, including those occasionally resistant to treatment, as well as secondary chancres or those recurring at the same or other sites during or after treatment as recrudescent infections, are positive for *T. pallidum* in over 95 per cent of cases. Indeed, it is now well known that darkfield examinations constitute the earliest and most reliable means for the diagnosis of primary syphilis, since serologic examinations are not likely to yield positive reactions until chancres have been present for over a week or ten days. More than one examination may be required at daily intervals and, needless to state, should be entrusted only to those with adequate skill and experience in order to avoid the error of mistaking *T. refringens* for *T. pallidum.* When advanced healing has occurred, darkfield examinations are not generally applicable and are apt to yield negative results largely due to the difficulty of securing proper material.

As previously stated, darkfield examinations should be made routinely in the case of all ulcerative lesions of the genitalia. Regardless of clinical experience, diagnostic errors may otherwise occur. Wholly insignificant and trivial fleeting chancres may resemble only abrasions. Indeed, *T. pallidum* has been found in completely healed penile abrasions not suspected as being syphilitic. Painless meatal or urethral chancres may occur in about 1.4 per cent of acute gonorrheas of men and about 3.0 per cent of women [37] and are always likely to show clinical evidences of their presence. Darkfield examinations are usually advisable for the exclusion of syphilis before the diagnosis of chancroid, lymphogranuloma venereum, granuloma inguinale, or balanoposthitis is finally made. The same is frequently true in cases of herpes genitalis, erosive and gangrenous balanitis, chronic phagedenic ulcers and carcinoma of the penis. On the other hand, darkfield examinations are of little or no value in the diagnosis of late syphilis with ulcerating gummas because of the presence of so few *T. pallidum;* nor for the examination of the vaginal or cervical secretions of women with late syphilis, since many attempts to find *T. pallidum* in this stage of the disease have been fruitless.

Chancroid. Chancroid is a venereal disease due to infection with *H. ducreyi.* In the male the lesions commonly occur in the coronary sulcus, the inner surface of the prepuce or on the glans penis. In the female they occur about the vulva. Owing to the short incubation period and clinical characteristics, bacteriologic examinations are not frequently required for diagnostic purposes. But, as previously stated, the lesions should always be examined one or more times by darkfield for *T. pallidum* for the exclusion of syphilis.

Otherwise, bacteriologic examinations may be required for differentiating chancroid with buboes from lymphogranuloma venereum and granuloma inguinale. In this connection, intradermal skin tests with chancroid vaccine and the virus of lymphogranuloma venereum (the Frei test or one of its modifications) are very helpful, as discussed in Chapter 19.

Bacteriologic examinations, however, probably detect about 90 per cent of cases of chancroid. Smears and cultures are prepared of pus picked up from the lesion with the bent end of sterilized wire, or of pus removed from buboes preferably by aspiration. The best culture medium is apparently the whole sterile and coagulated blood of rabbits in test tubes heated for five minutes at 55° C. After 24 to 48 hours' incubation, stained smears of the serum around the clots usually show the characteristic chains of small gram-negative bacilli in pure or mixed culture. Stained smears of pus show numerous extremely small bacilli occurring in short chains or in parallel rows ("shoals") close to pus cells.

Lymphogranuloma Inguinale. This disease, which is also known as granuloma inguinale, occurs almost exclusively in Negro men and women, being comparatively uncommon among whites. The etiologic agent is known as *Donovania granulomatis* which is found in smears of pus from buboes and mostly within mononuclear and polymorphonuclear leukocytes ("Donovan bodies").

Bacteriologic diagnosis is usually made by an examination of smears of pus from buboes for "Donovan bodies." The organisms can be cultivated [38] but cultures are not ordinarily employed. The disease must be differentiated from lymphogranuloma venereum in which the Frei intradermal test, or one of its modifications as well as the complement fixation test, are usually of helpful diagnostic value. Biopsy examinations also possess diagnostic value.[39]

Lymphogranuloma Venereum. This disease, which is also known as lymphopathia venereum, climatic bubo and by many other names,[40] is now known to be due to a filtrable virus. Consequently, bacteriologic examinations are of no value in diagnosis. The intradermal Frei test, however, or one of its modifications, is of helpful clinical value in diagnosis, as discussed in Chapter 19, as is likewise the complement fixation test (Chapter 17).

The disease is far more prevalent than lymphogranuloma inguinale and, like the latter, also occurs most frequently among Negroes and especially in the Southern states. Its venereal transmission makes it more widespread in the lower classes of society, prostitutes in some localities showing a high morbidity.

Since the primary lesion occurring on the genitalia is usually small, it is often overlooked by the patient. Extension into the lymphatics is characteristic, producing inguinal buboes in the male, while in the female lymph nodes within the pelvis are involved. The chronic inflammation in this locality frequently results in rectal strictures. Extensive ulceration in the perianal region, and hypertrophic lesions of the external genitalia, including elephantiasis of the labia majora, are of frequent occurrence.

Gangrenous Balanitis. This disease, which is also known as erosive balanitis, is not usually due to venereal infection. Phimosis with infection due to staphylococci or streptococci is the usual cause. *Myco smegmatis* is commonly found in smears but it is a nonvirulent saprophytic micro-organism easily mistaken for *Myco. tuberculosis.*

Erosive and gangrenous balanitis, however, is sometimes due to infection with *Bor. vincentii* and *B. fusiformis* which are found in stained smears or by darkfield examinations. The infection is usually caused by saliva carrying these micro-organisms in sexual relationships. For this reason it is frequently referred to as the "fifth venereal disease," gonorrhea, syphilis, chancroid and lymphogranuloma venereum being the other four.

Chronic phagedenic ulcers of the penis may be due to the same infection but in most cases the etiology is unknown.

Prostatitis. As previously stated, *acute prostatitis,* with or without prostatic abscess, may occur as a complication of acute gonorrhea due to infection of the gland with the gonococcus. Mixed infections with staphylococci or streptococci, however, are frequent and especially in the production of abscesses.

Chronic prostatitis frequently occurs as a complication of chronic gonorrhea due to mixed infections with gonococci, staphylococci, streptococci, *Esch. coli,* diphtheroid bacilli or other micro-organisms. Bacteriologic diagnosis generally depends upon cultures of prostatic secretions obtained by massage of the gland. Gonococci, however, are frequently reported as absent in cultures perhaps because they are overgrown by the micro-organisms of the secondary infection and thereby overlooked.

Furthermore, chronic prostatitis may be due to focal infection secondary to primary foci or dental, tonsillar or other origin without a preceding gonococcus infection. Needless to state, the collection of prostatic secretions for bacteriologic examinations almost inevitably entails contamination which adds greatly to difficulties in the clinical interpretation of the results.

Chronic prostatitis is sometimes, although but rarely, due to tuberculous infection in which tubercle bacilli may be found in the secretions or semen and especially by cultures and guinea-pig inoculation tests. It is likewise sometimes due to syphilis in which darkfield examinations of the secretions or semen, however, possess no diagnostic value.

Seminal Vesiculitis. *Acute vesiculitis* is likewise a frequent complication of acute gonorrhea. It may be followed by acute pelvic cellulitis and abscess presumably also due to gonococcus infection. Infection of the vas deferens may also occur as well as *funiculitis* or involvement of all structures of the spermatic cord.

Chronic vesiculitis, like chronic prostatitis, is a frequent complication of chronic gonorrhea due to a mixed infection with gonococci, staphylococci, streptococci or other micro-organisms. Like chronic gonorrhea, however, it may be also due to focal infection without a preceding gonococcus infection. *Tuberculous vesiculitis* is usually secondary to tuberculous epididymitis.

Epididymitis and Orchitis. *Acute* and *chronic epididymitis* occurs more frequently than orchitis and is usually secondary to seminal vesiculitis. Like the latter, infection with the gonococcus is the most frequent cause, with secondary infections due to staphylococci, streptococci, *Esch. coli* or other micro-organisms the general rule in chronic epididymitis. Hematogenous infections produce orchitis more frequently than epididymitis. *Tuberculous epididymitis* is always secondary to a primary tuberculosis of the lungs or elsewhere in the body.

Acute orchitis is most commonly due to hematogenous infections with the virus of mumps; it may also occur by hematogenous infections with staphylococci, streptococci, pneumococci or meningococci in the course of the septicemias as well as, more rarely, with *S. typhosa, M. mallei, H. influenzae* or the Brucella of undulant fever. Abscesses of the testicles are generally due to staphylococcus or streptococcus infections.

Chronic orchitis is usually secondary to chronic epididymitis or periorchitis and, like the former, is generally due to mixed infections.

Syphilitic orchitis is almost invariably a tertiary lesion and very common; indeed, it ranks next in frequency to syphilis of the cardiovascular and central nervous systems.

EXAMINATIONS OF THE EYES

Bacteriologic examinations of the eyes are usually confined to those of the lids, lacrimal apparatus, conjunctivae, sclerae, cornea and anterior chamber, in view of the ease with which smears and cultures of these parts may be prepared. They possess clinical value not only in diagnosis and differential diagnosis but likewise in relation to preparation for intra-ocular operations.

Bacteriologic examinations may be made also of bits of the iris removed by iridectomy as well as of the vitreous humor, the uveal tract and orbital tissues during operations, following injuries, or after enucleation.

Various mycotic infections of the lids, lacrimal system and conjunctivae also occur and are discussed in the following pages. Likewise various infections of the conjunctivae may be due to filtrable viruses, some of which may be detected by smear examinations for inclusion bodies or by animal inoculation.

Fortunately, the external parts of the eye are normally well protected against infection through mechanical cleansing by the tears; also to some extent because the latter are regarded as containing a lysozyme which is believed to possess antiseptic and feeble bactericidal properties for staphylococci, streptococci, gonococci, pneumococci, meningococci and other micro-organisms. Lysozyme, however, is believed to be reduced by excessive tearing and vitamin A deficiency; it does not occur in the aqueous or vitreous humors.

The epithelium of the conjunctivas and cornea is highly impermeable to bacteria under normal conditions. Moreover, the conjunctival epithelium proliferates during infection, permitting the superficial layers to be cast off. These epithelial cells and polymorphonuclear leukocytes are also phagocytic and thereby afford additional resistance to infection.

Normal Bacterial Flora; Preoperative Disinfection; Postoperative Infections. A small percentage of normal conjunctivae are sterile but the majority contain small numbers of various micro-organisms like *Staph. albus, Str. viridans,* hemolytic streptococci, pneumococci, diphtheroid bacilli, *B. subtilis, Esch. coli, Ps. aeruginosa, P. vulgaris,* sarcinae and various yeasts and molds. This normal flora frequently adds greatly to the difficulties of clinical interpretation of bacteriologic examinations (Table 90).

Unless surgeons employ preoperative disinfection, micro-organisms may be introduced into the deeper portions of the eye where resistance to infection is very low due to the absence of lysozyme and antibodies. Serious infections may occur by the introduction of micro-organisms from the conjunctivae like staphylococci, streptococci, pneumococci, *B. subtilis, P. vulgaris, Ps. aeruginosa, Esch. coli, A.*

TABLE 90. SUMMARY OF THE CLINICAL INTERPRETATION OF BACTERIOLOGIC EXAMINATIONS OF THE EYES

Diseases	Interpretation
General Considerations	Especially of value in the etiologic diagnosis of infections of the lids, lacrimal system, conjunctivae, sclerae cornea and anterior chamber. Also of value in the diagnosis of mycotic and some viral diseases of the eye. The external parts are protected against infections by mechanical cleansing by the tears and the lysozyme contained in them. Methods for the preparation of smears and cultures are very important in relation to bacteriologic and mycologic infections.
Normal Flora	A small percentage of conjunctivae are sterile. The majority, however, harbor staphylococci and diphtheroid bacilli. Streptococci, pneumococci, colon bacilli, *B. subtilis, Ps. aeruginosa, A. faecalis,* etc., may be present. Their presence sometimes renders the interpretation of bacteriologic examinations difficult.
Postoperative Infections	Preoperative disinfection is frequently indicated and advisable. The internal parts of the eye have very low natural resistance to infection. The introduction of any of the micro-organisms present on the conjunctivae may produce severe postoperative infections.

TABLE 90. SUMMARY OF THE CLINICAL INTERPRETATION OF BACTERIOLOGIC EXAMINATIONS OF THE EYES—(Continued)

Diseases	Interpretation
Ophthalmia Neonatorum	Gonorrheal infections occur only exceptionally. "Aseptic conjunctivitis" may be due to irritation by chemical disinfectants used for prophylactic purposes. Most infections are due to staphylococci, pneumococci, streptococci, *N. catarrhalis, Esch. coli, H. influenzae,* etc. Sometimes due to a virus producing inclusion conjunctivitis or "inclusion blenorrhea of the newborn."
Blepharitis and Dacryocystitis	Marginal blepharitis and hordeola are usually due to staphylococci. Chancres of the lids may occur in which *T. pallidum* may be found by darkfield examination. Dacryocystitis is sometimes due to mycotic infection but usually to staphylococci, streptococci, pneumococci, *N. catarrhalis, Ps. aeruginosa, K. pneumoniae,* etc.
Conjunctivitis	A large number of various bacteria are capable of producing conjunctivitis; bacteriologic examinations are usually required for etiologic diagnosis. Acute and chronic catarrhal conjunctivitis are usually due to pneumococci, stapyhlococci, streptococci, *N. catarrhalis, A. faecalis* or *C. xerose.* Angular conjunctivitis is usually due to the Morax-Axenfeld diplo-bacillus (*H. duplex*). Acute purulent conjunctivitis due to the gonococcus is uncommon. It is more likely to be due to *Staph. aureus,* streptococci, pneumococci, *Ps. aeruginosa, Esch. coli, K. pneumoniae* or the Koch-Week's bacillus. Uncommon forms are due to *Past. tularensis, Lept. icterohaemorrhagiae, T. pallidum* or spirofusillar infections.
	Membranous conjunctivitis may be due to the diphtheria bacillus, hemolytic streptococci or pneumococci. Phlyctenular conjunctivitis is of unknown etiology but may be due to tuberculosis. Allergic conjunctivitis may be due to sensitization to the pollens, cosmetics, drugs, etc. Secondary low-grade bacterial infections may be present. The etiology of *vernal* conjunctivitis is unknown but apparently is due to allergic sensitization to dusts or other air-borne allergens. Epidemic conjunctivitis and keratoconjunctivitis may be due to the Koch-Week's bacillus, pneumococci, *A. faecalis,* gonococcus or the viruses of inclusion conjunctivitis and epidemic keratoconjunctivitis.
Keratitis	Acute and chronic ulcerative keratitis are generally due to pneumococci, hemolytic streptococci or *Staph. aureus.* Hypopyons are due to the same infections. Acute keratitis may also be due to *Ps. aeruginosa, Esch. coli, C. diphtheriae* or *T. pallidum.* Chronic keratitis may be due to any of these micro-organisms or to the tubercle bacillus, leprosy bacillus or Morax-Axenfeld diplo-bacillus. Interstitial keratitis is generally due to congenital syphilis.

TABLE 90. SUMMARY OF THE CLINICAL INTERPRETATION OF BACTERIOLOGIC EXAMINATIONS OF THE EYES—(Continued)	
Diseases	Interpretation
Episcleritis and Scleritis	Etiology variable. May be due to menstrual disturbances, rheumatic diathesis, allergy, tuberculosis, syphilis or infections with streptococci or pneumococci.
Iritis and Iridocyclitis	May be due to trauma with secondary bacterial infections, focal infections due to streptococci or gonococci, syphilis or tuberculosis. May be due also to embolic infections in the course of the acute septicemias. In some cases the etiology is unknown (sympathetic and idiopathic types).
Choroiditis	Exudative or nonsuppurative type usually due to *Str. viridans* or other micro-organisms of focal infection.
Uveitis and Panophthalmitis	Purulent uveitis and panophthalmitis are usually due to penetrating wounds followed by infection with staphylococci, streptococci or pneumococci. May be due also to endogenous infections in the course of the septicemias.
Orbital Cellulitis and Periostitis	Orbital cellulitis is usually due to streptococci, staphylococci or pneumococci secondary to trauma, sinusitis, focal infections, the septicemias or influenza. Orbital periostitis may be due to the same causes, tuberculosis or syphilis.
Optic Neuritis	Intra-ocular neuritis usually due to syphilis. Less frequently due to the viruses of the encephalitides, meningococci, streptococci of focal infections or lead poisoning. Optic atrophy is usually due to syphilis (especially tabes dorsalis and paresis) and sometimes to multiple sclerosis. Retrobulbar neuritis is usually due to multiple sclerosis but sometimes to syphilis, chronic local infections and acute infections, especially influenza.
Mycotic Infections	Many different fungi and yeasts have been reported as occurring on the normal conjunctivae and cornea. They may produce chronic infections.
Virus Infections	Many of the viruses produce infections of the eye such as inclusion conjunctivitis or "inclusion blenorrhea of the newborn," epidemic keratoconjunctivitis, trachoma and ocular infections in lymphogranuloma venereum. Ocular infections may also occur in herpes zoster, vaccinia, measles, mumps, molluscum contagiosum, etc.

faecalis and *H. influenzae*. Even infections with *Cl. tetani* and *Cl. perfringens* have been reported, although these are usually due to local injuries with the introduction of dirt or foreign bodies.

Such postoperative infections are one of the causes of failure following operations for the removal of cataracts. It is generally impossible to disinfect completely the conjunctivas before operations by local irrigation with solutions of sulfanilamide, sulfathiazole or other chemical agents but reduction of the bacterial flora is always advisable before intra-ocular operations are conducted.

Collection of Material. 1. It is advisable to prepare smears and cultures at a suitable stage of the disease. As a general rule, this is during the period in which the disease is developing, or is at its height. The actual causal agent can disappear rapidly, but the discharge lessens more slowly. In the stage of regression the primary agent may not be found but only staphylococci, diphtheroid bacilli, etc.

2. In conjunctivitis, an effort should be made to avoid the collection of secretions in contact with the angles or margins of the lids unless angular conjunctivitis or blepharoconjunctivitis are present. In dacryocystitis, an effort should be made to secure fresh pus by expression.

3. In making smears and cultures, it is generally advisable first to remove excessive exudates with saline solution or sterile gauze in order to secure micro-organisms located in the epithelium. For this purpose it is necessary first to anesthetize the eye by the local instillation of sterile 4 per cent solution of cocaine or a 1 per cent solution of pontocaine followed by gentle scraping of the conjunctiva or cornea with a sterilized platinum spatula or von Graefe knife. Otherwise, a small, sterile cotton swab may be used after it has been dipped in broth medium. Swabs, however, are not suitable for the preparation of smears to be examined for the intracellular inclusion bodies of the viral infections.

4. In corneal infections, great care is required for avoiding injury of the tissues with the spread of infection. The cornea should be anesthetized and kept perfectly quiet. Superficial swabbings may be unsatisfactory. The point of a sterile von Graefe knife, platinum spatula or needle is generally preferred for obtaining material, as the causal micro-organism is likely to be deeply located.

5. Material from the anterior chamber may be aspirated with a small sterile syringe and needle.

6. Portions of the iris removed by iridectomy should be placed at once in glucose hormone broth suitable for the cultivation of streptococci and pneumococci (*p*H 7.4 to 7.6).

7. *Properly prepared smears are always of great value in all eye examinations* as they may show the presence of micro-organisms failing to grow in culture media. At least two should be made. Avoid making smears too thin or too thick; smears the size of a dime are large enough.

8. In cultures of styes, plain agar may be used because they are always caused by staphylococci. Otherwise, however, enriched media like blood agar may be required. In suspected tularemia, brucellosis, tuberculosis, etc., special media are required.

9. Smears and cultures should not be made within four hours after irrigation or instillation of disinfectant solutions. Indeed, it is better to wait for twelve to twenty-four hours if conditions permit.

10. Enucleated eyes should be seared or dipped momentarily in boiling water or a disinfectant solution for surface disinfection before being opened with a sterile knife or scissors for the purpose of preparing smears and cultures of the iris, lens, humors or uveal tract.

11. Darkfield examinations are of great value in the diagnosis of chancres, spirofusillar infections, the detection of *Spirillum minus* in ocular infections of

rat-bite fever and for the detection of *Lept. icterohaemorrhagiae* and *Lept. canicola* in infectious jaundice or Weil's disease.

12. The inoculation of guinea-pigs with bits of tissue or secretions is indicated when oculoglandular tularemia, brucellosis or tuberculosis is suspected; also the corneal inoculation of rabbits in suspected virus infections. Rabbits may be inoculated intratesticularly as an additional means for the detection of *T. pallidum.*

13. Smears for examinations of intracellular inclusion bodies in suspected virus infections should be stained by the Giemsa method.

14. Serologic examinations for syphilis are indicated in suspected syphilitic keratitis, iritis, choroiditis, etc.; also complement fixation tests in suspected gonococcal iritis and iridocyclitis. Agglutination tests are indicated in suspected brucellosis and especially recurrent iritis, iridocyclitis or neuroretinitis sometimes due to *Br. melitensis.* Agglutination tests are also of diagnostic value in suspected infections with *Lept. icterohaemorrhagiae* or *Lept. canicola.*

Ophthalmia Neonatorum. Gonorrheal conjunctivitis of the newborn occurs only exceptionally at the present time. Conjunctivitis, however, may occur as the result of chemical disinfection after birth, producing "aseptic conjunctivitis," in which smears and cultures are sterile. Otherwise, conjunctivitis of the newborn is likely to be due to infection with any of a number of micro-organisms like staphylococci, pneumococci, streptococci, *N. catarrhalis, Esch. coli, H. influenzae* or diphtheroid bacilli.

It is also important to remember that conjunctivitis neonatorum may be due to infection with the virus of inclusion conjunctivitis or so-called "inclusion blenorrhea of infants" in which initial and elementary inclusion bodies are likely to be found within cells in smears stained by the Giemsa method.

Blepharitis and Dacryocystitis. Marginal blepharitis is usually due to infection of the sebaceous follicles with staphylococci. *Hordeola,* or styes, are likewise due to staphylococcus infections of the hair follicles. Primary *syphilis* or chancres of the lids sometimes occur in which darkfield examinations for *T. pallidum* possess great diagnostic value.

Dacryocystitis is rarely a primary infection because of the downward flow of the tears. As a general rule, it is due to extension of infection from the nasal accessory sinuses, obstruction or a foreign body. Lacrimal conjunctivitis may occur secondarily. Dacryocystitis is sometimes due to mycotic infection but is usually due to micro-organisms derived from the respiratory or intestinal tracts like staphylococci, streptococci, pneumococci, *N. catarrhalis, Ps. aeruginosa, K. pneumoniae* or *H. influenzae.* Infection with *T. vincentii* and *B. fusiformis* may be responsible in some cases.

Conjunctivitis; Epidemic Conjunctivitis and Keratoconjunctivitis. A large number of various micro-organisms are capable of producing conjunctivitis. Under the circumstances, bacteriologic examinations are of great clinical value in diagnosis and differential diagnosis since etiologic diagnosis is not always possible by signs and symptoms. *Acute catarrhal conjunctivitis* is usually due to pneumococci, staphylococci or *N. catarrhalis. Chronic catarrhal conjunctivitis* may be due to the same micro-organisms as well as to streptococci, *A. faecalis* or *C. xerose.*

Mixed infections are the rule. *Angular conjunctivitis* is frequently due to the Morax-Axenfeld diplobacillus (*H. duplex*).

Acute purulent conjunctivitis of adults due to the gonococcus is comparatively uncommon. *Staph. aureus* is the most frequent cause but purulent conjunctivitis may be due to hemolytic streptococci, *Str. viridans*, pneumococci, *Ps. aeruginosa, Esch. coli, A. faecalis, K. pneumoniae* or the Koch-Week's bacillus. Uncommon forms are caused by *Past. tularensis, Lept. icterohaemorrhagiae,* spirofusillar infections and *T. pallidum.*

Membranous conjunctivitis is usually due to virulent diphtheria bacilli which produce a very severe and dangerous type of infection. In smears and cultures the bacilli are not apt to be confused with *C. xerose* which may produce a low-grade and chronic type of infection. Membranous conjunctivitis, however, is likewise sometimes due to severe infections with highly virulent pneumococci or hemolytic streptococci.

Phlyctenular conjunctivitis or phlyctenular keratoconjunctivitis is believed to be due to some constitutional cause and especially tuberculous infection of the lungs or elsewhere in the body with tuberculin hypersensitiveness. However, infection with the herpetic viruses may be primarily responsible but in either case bacteriologic examinations possess no diagnostic value. The same is true of *vernal conjunctivitis* which is regarded by some investigators as being due to allergic sensitization to dusts or other air-borne allergens. While this has not been proven, there can be no doubt that *allergic conjunctivitis* occurs, not only in acute forms due to sensitization to pollens, cosmetics and drugs such as atropine, pilocarpine and yellow oxide of mercury, but also as a chronic conjunctivitis in which frequently repeated attacks of allergic conjunctivitis are followed by low-grade bacterial infections with staphylococci, streptococci, pneumococci or *N. catarrhalis.*

Epidemic conjunctivitis and *keratoconjunctivitis* are frequently due to infection with the Koch-Week's bacillus which is now thought to be closely related to *H. influenzae.* Bacteriologic diagnosis is greatly aided by the examination of smears showing large numbers of very small, slender, gram-negative bacilli occurring in "shoals" close to pus cells and some of which may be intracellular. Cultures are best prepared on potato-blood agar supplying both the X and V growth factors.

Epidemic conjunctivitis is also frequently due to infection with pneumococci. Outbreaks have also been reported due to *A. faecalis,* the Morax-Axenfeld diplobacillus and the gonococcus, the latter being nonvenereal in origin and transmitted by droppers, etc. Epidemics have been reported also in which the etiologic agents were the viruses of inclusion conjunctivitis of infants or epidemic keratoconjunctivitis of adults ("shipyard conjunctivitis").

Keratitis. Acute and chronic serpiginous *ulcerative keratitis* are usually due to infection with pneumococci although sometimes caused by hemolytic streptococci or *Staph. aureus.* Hypopyons, which very often follow, are usually due to the same micro-organisms responsible for the keratitis, especially pneumococci.

Acute keratitis, however, may also be due to infection with *Ps. aeruginosa, Esch. coli, B. subtilis, C. diphtheriae* or *T. pallidum.* Chronic keratitis may follow any of these infections or result from infection with *Myco. tuberculosis, Myco. leprae* or the Morax-Axenfeld diplobacillus.

Interstitial keratitis is due almost invariably to congenital syphilis in which bacteriologic examinations for *T. pallidum* possess no practical diagnostic value but in which the serologic tests, along with clinical and x-ray examinations for other evidences of the disease, are of great value. Isolated cases of corneal ulcers have been reported to be due apparently to allergic sensitization to orris root or other air-borne allergens.

Iritis and Iridocyclitis. Acute and chronic iritis and iridocyclitis may be due not only to staphylococci, streptococci, pneumococci, meningococci, *Ps. aeruginosa, Esch. coli, S. typhosa, Br. melitensis*, or other micro-organisms, but likewise to syphilis, tuberculosis, diabetes or the rheumatic diathesis. The latter probably represents an iritis or iridocyclitis due either to actual infection of these tissues with *Str. viridans* or other streptococci or to the effects of their toxins. The same is true of the iritis and iridocyclitis due to primary focal infections of dental, tonsillar or other locations. Without doubt both may likewise be due to infection with the gonococcus secondary to chronic primary gonococcus infections of the genital organs of both sexes and especially males. Acute iritis and iridocyclitis may also occur during the course of some of the septicemias, especially those caused by streptococci, staphylococci, pneumococci and meningococci. On the other hand, some cases are sympathetic or idiopathic in origin in which the causes cannot be ascertained. Cases have also been attributed to the toxin of *S. dysenteriae*, spiro-fusillar infections, *T. pallidum, Past. tularensis, Lept. icterohaemorrhagiae* and the virus of *lymphogranuloma venereum*. Trauma is not infrequently a predisposing factor and under these circumstances *Cl. perfringens* may also be responsible for infection.

Choroiditis. Exudative or nonsuppurative choroiditis is likewise frequently due to infection with *Str. viridans* or other streptococci secondary to primary focal infections of dental, tonsillar, prostatic or other origin. Some cases, however, are apparently due to tuberculous or syphilitic infections.

Uveitis and Panophthalmitis. Purulent uveitis (suppurative endophthalmitis) and panophthalmitis may be due to penetrating wounds of the eye followed by infection with staphylococci, streptococci, pneumococci or mixed infections with these or other micro-organisms. They may also be endogenous in origin resulting from septic embolism in the course of the septicemias due to hemolytic streptococci, staphylococci or meningococci (especially in connection with meningococcal meningitis).

Episcleritis and Scleritis. Episcleritis and scleritis occur especially among women. The etiology is not always apparent, as either or both may be due to menstrual disturbances or the rheumatic and gouty diatheses. Tuberculous and syphilitic infections are likewise sometimes responsible as also allergic sensitization to air-borne allergens in some cases. On the other hand, it appears that acute episcleritis and scleritis may be due also to bacterial infections and especially with streptococci or pneumococci.

Orbital Cellulitis and Periostitis. *Orbital cellulitis* may be due to infection by extension from the nasal accessory sinuses and especially the ethmoid cells, to focal infection of dental origin, or infection following injuries by foreign bodies. It also sometimes occurs during the course of the septicemias as likewise during some of the acute infectious diseases, especially influenza. Infections with hemolytic streptococci are most frequently responsible while some cases are due to infection with *Staph. aureus* or pneumococci.

Orbital periostitis of the acute type is usually due to injuries followed by infection with *Staph. aureus* although some cases are due to hemolytic streptococci or pneumococci. Chronic types occurring without preceding injuries are usually due to infection with *Myco. tuberculosis* but may also occur as a form of late syphilis in which the serologic tests are of great diagnostic value.

Optic Neuritis. Intra-ocular optic neuritis is most frequently due to syphilis. However, it may be due less frequently to infection with the viruses of the encephalitides, meningococci as a sequelum of meningococcal meningitis, to other micro-organisms in the course of some of the acute infectious diseases, to focal infections or lead poisoning. *Optic atrophy* may result and is particularly a manifestation of tabes dorsalis although it may also occur in paresis as likewise in multiple sclerosis.

About one-half of cases of *retrobulbar neuritis* are due to multiple sclerosis and are therefore of unknown etiology; but some cases are likewise due to syphilis, the rheumatic diathesis and focal infections (presumably due to *Str. viridans*) as well as to some of the acute infectious diseases, especially influenza.

Mycotic Infections. Since the conjunctivas and cornea are freely exposed, it is not surprising that many different fungi and yeasts have been reported as responsible for infections resulting from malnutrition, injuries, or the extension of mycotic infections from the scalp, face or eyelids.

Reference has been made to the possibility of dacryocystitis being due to infection with fungi and especially actinomyces (streptothrix) of zur Nedden, leptothrices, *Streptothrix foersteri*, etc. These and additional fungi have been isolated from the normal conjunctivae, cornea and eyelids and sometimes held responsible for low-grade or chronic infections like *A. schoenleinii, Aspergillus fumigatus, C. albicans, Penicillium glaucum,* various trichophyta, etc.

Virus Infections. As previously stated, inclusion conjunctivitis or "inclusion blenorrhea of the newborn" is ascribed to infection with a virus. Trachoma is now commonly regarded as also due to a virus infection. Epidemic keratoconjunctivitis or "shipyard conjunctivitis" is likewise due to a virus which may be also the cause of "swimming pool conjunctivitis"; reference has been also made to ocular infections with the virus of lymphogranuloma venereum.

Infections of the conjunctivae or cornea, or both, have also been reported as due to the viruses of herpes zoster and varicella, vaccinia, rabies, measles, mumps, acute anterior poliomyelitis, encephalitis, molluscum contagiosum, yellow fever, etc.

EXAMINATIONS OF WOUNDS AND OTHER SURGICAL INFECTIONS

All wounds are *contaminated* with one or more varieties of micro-organisms but infection does not necessarily follow. Bacteriologic examinations are of value as controls on methods of local disinfection before surgical procedures are employed but if sulfanilamide crystals or sulfathiazole have been used for this purpose, it is advisable for the culture medium to carry 5 mg. of para-aminobenzoic acid per 100 cc., as otherwise falsely sterile cultures may be observed. The area should be selected with great care with particular reference to the deepest parts, necrosed areas of fascia and the surfaces of damaged bone and cul-de-sacs where secretions can accumulate protected from the disinfectant. Bleeding areas should be avoided, as both smears and cultures from these are likely to be unsatisfactory.

Bacteriologic examinations are also of great value in etiologic diagnosis. Simple smears are useful for showing the approximate numbers and kinds of bacteria present but cultures are required. The latter should be not only aerobic but anaerobic as well not only for the detection of micro-aerophilic streptococci, but especially for the anaerobic bacilli of the gangrene group. Enriched media like blood agar plates or one of its substitutes are required. Otherwise, the surgeon may collect pus or secretions on sterile swabs which should be promptly sent to the laboratory for the preparation of smears and cultures before drying has occurred. It is always advisable to prepare the swabs of those areas in which bacteria are most likely to be present in large numbers, as around foreign bodies, necrotic bone, and from the deeper parts and crevices of the wound. Not infrequently "sterile pus" from surgically drained abscesses and similar affections may show the presence of nonspore-forming anaerobic bacilli belonging to the genus *Bacteroides;*[41] being anaerobic they are usually overlooked unless anaerobic cultures are employed.

Postoperative Wounds. Contrary to widespread impressions, practically every operative wound is contaminated with micro-organisms, as shown by the results of bacteriologic examinations employing aerobic and anaerobic cultures (Table 91). Staphylococci have been found in 60 to 70 per cent and streptococci in from 8 to 10 per cent with various other bacteria in the balance including *Esch. coli, P. vulgaris, B. subtilis,* diphtheroid bacilli, etc. Evidently these contaminations are air-borne in transmission while some of the micro-organisms, like *Staph. aureus* and hemolytic streptococci, are potentially virulent. But, nevertheless, clinically recognizable postoperative infections are very infrequent because modern surgical technic reduces to a minimum trauma and other factors favorable to the survival and proliferation of these contaminating bacteria.

Stitch abscesses may occur due to infection with staphylococci; likewise, infections of wounds and *fistulas* and particularly after operations upon the colon or other parts of the intestinal tract, with special reference to mixed infections with *Esch. coli,* staphylococci and streptococci.

However, delayed healing and the disruption of postoperative wounds may not be due to infection but to hypoproteinemia or protein depletion, especially after gastro-enterostomy,[42] as well as vitamin C deficiency.[43] At least, both hypoproteinemia and C avitaminosis have been shown experimentally to delay wound healing or cause wound disruption by interfering with normal fibrosis.[44,45,46] Clinically, hypoproteinemia appears to be more frequently responsible and important than vitamin C deficiency.[46]

Traumatic Wounds. Needless to state, all traumatic wounds are likewise contaminated and unless the sulfonamide compounds or penicillin are promptly used by local application, supplemented by oral or parenteral administration, the majority become infected and especially with *Staph. aureus* or hemolytic strepto-

TABLE 91. SUMMARY OF THE CLINICAL INTERPRETATION OF BACTERIOLOGIC EXAMINATIONS OF WOUNDS AND OTHER SURGICAL INFECTIONS

Disease	Interpretation
General Considerations	All wounds are *contaminated* with bacteria but infection does not necessarily follow. Bacteriologic examinations are of value as controls on local disinfection. If the sulfonamide compounds have been used locally the culture medium should contain para-aminobenzoic acid in order to avoid falsely negative results. They are also of great value in the etiologic diagnosis of infections of wounds. Due care is required in the preparation of smears and cultures. Anaerobic as well as aerobic cultures on enriched media should be employed routinely.
Postoperative Wounds	Practically all are *contaminated* by air-borne transmission of bacteria and especially with staphylococci and streptococci. *Infections,* however, are comparatively uncommon. *Stitch abscesses* are usually due to staphylococci. *Fistulas* are usually due to mixed infections. Delayed healing and wound disruption are not usually due to infection but to hypoproteinemia or vitamin C deficiency.
Traumatic Wounds	All are contaminated and unless penicillin or the sulfonamides are used locally, supplemented by their oral or parenteral administration, the majority become infected, especially with the various types of staphylococci and streptococci. Deep lacerated wounds and compound fractures contaminated with dirt or other foreign material are dangerous from the standpoint of possible infection with the anaerobic bacilli of tetanus and gangrene. Chronic suppurative wounds are usually due to mixed infections with staphylococci and streptococci; *Esch. coli* and *Ps. aeruginosa* are common, as likewise such contaminants as *P. vulgaris, B. subtilis* and diphtheroid bacilli.
Tetanus	Very difficult to find spores and bacilli in fresh wounds because so few are present. Cultures and guinea-pig inoculation tests are required. It is likewise difficult to find the bacilli in wounds after tetanus has developed. Consequently, bacteriologic examinations are not ordinarily employed for diagnostic purposes. Smears are worthless. Strictly anaerobic cultures and guinea-pig inoculation tests are required.
Gangrene	May be predominantly gaseous or phlegmonous. Usually due to mixed infections with *Cl. perfringens, Cl. novyi, Cl. septicum* and other anaerobic bacilli of lesser importance. Secondary infections with staphylococci, streptococci, *Esch. coli* or other aerobic micro-organisms are quite common. Gangrene may be likewise due to acute infections with hemolytic streptococci or with *Bor. vincentii* and *B. fusiformis.* Chronic gangrene sometimes occurs and especially after thoracic and abdominal operations as the result of progressive synergistic mixed infections. Bacteriologic examinations of fresh wounds for possible contamination with the bacilli of the gangrene group possess clinical value. They are especially valuable, however, in the etiologic diagnosis of gangrene.

TABLE 91. SUMMARY OF THE CLINICAL INTERPPETATION OF BACTERIOLOGIC EXAMINATIONS OF WOUNDS AND OTHER SURGICAL INFECTIONS—(Continued)

Disease	Interpretation
Cutaneous Anthrax	Bacteriologic examinations are of great diagnostic value. Routinely advisable in the case of pustules occurring among those especially exposed to the risks of anthrax infection. Smears and cultures of the lesions are employed. Blood cultures are advisable in all cases.
Furuncles and Carbuncles	Small and superficial *furuncles* are generally caused by *Staph. albus;* larger and more severe ones by *Staph. aureus.* *Felons* and *paronychia* are usually due to staphylococcal infections. *Carbuncles* may be caused by infections with *Staph. aureus* or hemolytic streptococci. Blood sugar tests are advisable in recurrent furunculosis and carbunculosis.
Burns	Always likely to become infected with staphylococci or hemolytic streptococci unless preventive measures are promptly applied. Otherwise pure or mixed infections with these as well as with *Esch. coli* or *Ps. aeruginosa* may occur. *P. vulgaris*, diphtheroid bacilli and other micro-organisms are usually contaminants.
Erysipelas and Erysipeloid	*Erysipelas* is due to infection of the skin with any hemolytic streptococcus of Group A. *Str. erysipelatis* is no longer regarded as a specific member of this group. Susceptibility is especially marked in postpartum women, after injuries, burns or operations. Bacteriologic examinations are not ordinarily required for diagnostic purposes but may be employed. *Erysipeloid* is due to infection of the skin with *Erysipelothrix rhusiopathiae.* It occurs particularly among handlers of foods and fish and after crab bites. Cultures of excised skin possess diagnostic value.
Tularemia	A disease of rodents and particularly of wild rabbits due to *Past. tularensis* transmissible to man. Infection may be caused by the handling of infected animals, the bites of blood sucking insects, the ingestion of partially cooked flesh of infected rabbits, or through the unbroken skin or conjunctivae. Ulcers on the fingers or hands should be examined by darkfield for *T. pallidum* for the exclusion of primary syphilis. Bacteriologic diagnosis is difficult but of clinical value by cultures on special media and guinea-pig inoculation tests. Blood cultures are advisable in all cases for septicemia which has a mortality of about 50 per cent with a general mortality of about 5 per cent for all types of the disease.
Osteomyelitis	*Acute osteomyelitis* is usually caused by *Staph. aureus* and less frequently by hemolytic streptococci, pneumococci, *Esch. coli* or the typhoid bacillus. *Chronic osteomyelitis* may be due to any of these micro-organisms but is frequently due to the tubercle bacillus.

TABLE 91. SUMMARY OF THE CLINICAL INTERPRETATION OF BACTERIOLOGIC EXAMINATIONS OF WOUNDS AND OTHER SURGICAL INFECTIONS—(Continued)

Disease	Interpretation
	Bacteriologic examinations are only possible at operations or after spontaneous opening but possess clinical value in etiologic diagnosis. *Ps. aeruginosa, P. vulgaris,* diphtheroid bacilli or other micro-organisms may be present in the pus as contaminants or secondary invaders. Tubercle bacilli are seldom found in smears or pus but more frequently in cultures and by guinea-pig inoculation tests.
Suppurative Arthritis	*Acute suppurative arthritis* is usually due to infections with *Staph. aureus,* hemolytic streptococci, gonococci, pneumococci, meningococci or the typhoid bacillus. Bacteriologic examinations of pus removed by aspiration or at operation possess great value in etiologic diagnosis. *Chronic suppurative arthritis* may be due to any of the same micro-organisms or to the tubercle bacillus. The latter is seldom found in smears; cultures and guinea-pig inoculation tests are preferred. Secondary infections due to staphylococci, streptococci, *Esch. coli* or *Ps. aeruginosa* are common. *P. vulgaris,* diphtheroid bacilli and other micro-organisms are usually unimportant contaminants.

cocci in pure or mixed cultures. Those contaminated with dirt or other foreign material are particularly dangerous from the standpoint of infection with the anaerobic bacilli of tetanus and gangrene and especially in the case of deep lacerated wounds and compound fractures.

Bacteriologic examinations, especially by well-prepared cultures on plates of blood agar or in glucose hormone broth, are of great value in etiologic diagnosis. As previously stated, both aerobic and anaerobic cultures should be employed routinely. Chronic suppurative wounds are usually due to mixed infections not only with various types of staphylococci and streptococci, but sometimes with *Esch. coli* and *Ps. aeruginosa;* not infrequently *P. vulgaris, B. subtilis,* diphtheroid bacilli or other micro-organisms are likewise present as unimportant contaminants.

Tetanus. Usually so few tetanus spores are introduced into wounds by dirt or other foreign material that cultures for the bacilli usually fail to reveal their presence. If employed it is advisable to prepare anaerobic cultures in a broth medium of foreign matter and bits of lacerated tissues or splinters of bone. Not infrequently a better method is to inoculate young guinea-pigs with 2 or 3 cc. of emulsions of such material in sterile saline solution and likewise additional animals with 0.5 cc. of ten-day broth cultures.

Once signs and symptoms of tetanus have developed it is likewise quite difficult to find tetanus bacilli in wounds because so few are apt to be present. Consequently, bacteriologic examinations are not ordinarily employed for diagnostic purposes. Direct smears are worthless. Only strictly anaerobic cultures and guinea-pig inoculations possess any value at all.

Gangrene. Gangrene due to infected wounds or, following amputations due to vascular occlusions, as may happen in Buerger's disease, diabetes mellitus, etc., may be predominantly gaseous or phlegmonous. About 72 per cent of cases occurring in both military and civilian populations are due to the infection of wounds with *Cl. welchii* (*Cl. perfringens*) although pure infections are rare and mixed infections with other anaerobic spore-bearing bacilli of the gangrene group the general rule. Thus, about 26 per cent of infected wounds show the presence of *Cl. novyi* (*Cl. oedematiens*) and about 10 per cent *Cl. septicum* (*Vibrion septique*). *Cl. sporogenes, Cl. fallax, Cl. putrificus* and *Cl. bifermentans* (*sordellii*) may be likewise found in cultures but because of their low incidence and doubtful pathogenicity are of much less importance. As a general rule, secondary infections with the various types of staphylococci, streptococci, *Esch. coli* or other aerobic micro-organisms are generally present.

As in the case of tetanus, it may be difficult to find these anaerobic, gram-positive, spore-bearing bacilli in fresh wounds, although anaerobic cultures are more likely to reveal their presence than is true of tetanus bacilli because they are always likely to be more numerous. On the development of gangrene, however, bacteriologic examinations of smears, and particularly of cultures, are far more likely to reveal the presence of members of the gangrene group of bacilli than is true of tetanus bacilli. Consequently, they possess a high degree of diagnostic value although the identification of the different species is usually difficult, requiring the use of iron-milk, sugar fermentation tests and other special media. The intravenous injection of rabbits with 1 to 3 cc. of saline suspensions of material from lesions, however, usually affords a rapid method for the detection of *Cl. perfringens*. Likewise, for rapid diagnostic purposes, the subcutaneous injection of guinea-pigs with 1 cc. amounts of saline suspensions of material mixed in test tubes with 0.5 to 1.0 cc. amounts of the respective antitoxins for *Cl. tetani, Cl. perfringens, Cl. novyi* and other bacilli of the gangrene group.

Gangrene may be likewise due to acute infections with hemolytic streptococci of group A, or to mixed infections with *Bor. vincentii* and *B. fusiformis*. Chronic gangrene sometimes occurs and especially after thoracic and abdominal operations as the result of progressive synergistic mixed bacterial infections.

Tropical Ulcer. The etiology of tropical ulcer is still unsettled but apparently infection with *C. diphtheriae* plays an important rôle in many cases, along with infections by hemolytic *Staph. aureus, P. vulgaris* and *Ps. aeruginosa*.[47,48]

Cutaneous Anthrax. Bacteriologic examinations are extremely valuable in the diagnosis of cutaneous anthrax or the "malignant pustule." Since the lesions may occur as small pustules and thereby readily escape clinical diagnosis, it is a good routine practice to culture all furuncles occurring among those whose occupation particularly exposes them to anthrax infection with special reference to veterinarians, butchers, shepherds and the handlers of hides, hairs and wools.

Smears frequently show sufficient numbers of the large, gram-positive bacilli for immediate or presumptive diagnosis but cultures are ordinarily required. Since *B. anthracis* grows rapidly and abundantly on ordinary media, cultures may be prepared on slants of agar-agar, the Loeffler blood serum medium or in tubes of broth. Of course, the bacilli in cultures must be differentiated from *B. subtilis* which they closely resemble. If in doubt, guinea-pig inoculation tests should be employed, as *B. anthracis* is highly virulent for these animals.

Owing to the relatively high resistance of man, the lesions often heal spontaneously but may progress into a septicemia. For this reason one or more blood cultures are always advisable not only in the case of large lesions but in the case of small ones as well.

Furuncles and Carbuncles. *Furuncles* are always due to infection with staphylococci. *Staph. albus* is usually responsible for those which are small and superficial, while *Staph. aureus* produces those which are larger and more severe. *Felons* and *paronychia* are likewise usually due to staphylococcal infections.

Carbuncles are frequently due to *Staph. aureus* but not infrequently to hemolytic streptococci of Group A or to mixed infections with *Staph. aureus*. Recurrent furunculosis and carbunculosis are frequently associated with or due to hyperglycemia (diabetes mellitus); for this reason the fasting blood sugar should be determined routinely in these cases.

Burns. Burns, from any cause, are always likely to become infected with staphylococci or hemolytic streptococci unless preventive measures are promptly applied. Otherwise, pure or mixed infections with these as well as with *Esch. coli* or *Ps. aeruginosa* may occur and especially in the case of deep and extensive burns. Not infrequently cultures also reveal the presence of *P. vulgaris*, diphtheroid bacilli or other micro-organisms although these are usually to be regarded as contaminants.

Erysipelas and Erysipeloid. Surgical *erysipelas* is invariably due to infection of the skin with any of the hemolytic streptococci of Group A. *Str. erysipelatis* is no longer regarded as a specific member of this group in the etiology of the disease. Susceptibility is especially marked in postpartum women, after injuries, burns or operations. Owing to the clinical characteristics of the disease, bacteriologic examinations are not ordinarily required for diagnostic purposes although sometimes helpful in doubtful cases. Cultures are best prepared of the serous exudates obtained by skin puncture after very careful preliminary disinfection to avoid contamination. It is important to use enriched media like blood agar and especially glucose hormone broth.

Erysipeloid, which may be mistaken clinically for erysipelas with the lesions occurring most frequently on the fingers or hands, is caused by the *Ery. rhusiopathiae*. It occurs most frequently among cooks, kitchen workers and butchers due to wounds contaminated by dead animal matter of diverse nature. It is common after crab bites and a particularly severe form has been described by Klauder and Harkins [49] among fish handlers. Bacteriologic examinations are very valuable for diagnostic purposes. Usually fragments of excised skin, obtained after very careful disinfection against contamination, must be employed for the preparation of cultures.

Tularemia. Tularemia is a disease of rodents, and especially of wild rabbits, transmissible to human beings. It is due to *Past. tularensis* and transmissible to man not only through the handling of infected animals but likewise by some of the blood-sucking insects. Since the organism is very small and highly invasive, it may also penetrate the unbroken skin. These methods of inoculation usually produce the ulceroglandular and glandular types of the disease. Infection may also occur through the unbroken conjunctiva, producing oculoglandular tularemia, or by the ingestion of incompletely cooked rabbit meat. A persistent septicemia may occur (typhoid type) with a mortality of 50 per cent although the general mortality for all types is about 5 per cent.

The local lesions occur as ulcers and when these are on the fingers or hands generally require darkfield examinations for *T. pallidum* to exclude primary syphilis. Otherwise, bacteriologic diagnosis is usually quite difficult but frequently helpful in diagnosis by the preparation of cultures of the secretions and pus, preferably of excised tissue, or material secured by the aspiration of infected glands on tubes or plates of glucose-cystine agar or glucose-blood-cystine agar. Inoculation of the abraded abdominal skin of guinea-pigs with secretions or pus from the lesions or glands is also helpful, as the animals usually succumb in five to ten days. Blood cultures in glucose-cystine broth are valuable and should be included routinely in all cases for the detection of a possible septicemia.

Osteomyelitis. Acute osteomyelitis from trauma or the result of endogenous infections by way of the blood is usually due to infection with *Staph. aureus* (about 75 per cent) with the balance due to hemolytic streptococci, pneumococci, *Esch. coli* or the typhoid bacillus. Infections due to gonococci are rare. *Chronic osteomyelitis* may be likewise due to any of these micro-organisms but is frequently caused by the tubercle bacillus. *Ps. aeruginosa* is frequently present in the pus of discharging fistulas as likewise *P. vulgaris*, diphtheroid bacilli and other micro-organisms as contaminants or secondary invaders.

Of course, bacteriologic examinations are not possible except at operations or after spontaneous opening, but possess clinical value in etiologic diagnosis. Tubercle bacilli, however, are not usually found in direct smears although it is likely that examinations by the method of fluorescent microscopy will increase the incidence of positive findings in the future. Cultures for tubercle bacilli on appropriate media are much better. Guinea-pig inoculation tests are also of value, although so many cases of open tuberculous osteomyelitis are due to mixed infections with staphylococci or streptococci that the animals not infrequently succumb to infections with these soon after inoculation.

Arthritis. *Acute suppurative arthritis* due to trauma or endogenous infections by way of the blood are usually due to infections with *Staph. aureus*, hemolytic streptococci, gonococci, pneumococci, meningococci or the typhoid bacillus. Bacteriologic examinations made preoperatively by means of smears and cultures of pus removed by aspiration or during operations for drainage possess great value in etiologic diagnosis, especially in relation to treatment with the antibiotic and sulfonamide compounds.

Chronic suppurative arthritis may be due to any of these micro-organisms but is more frequently the result of infection with the tubercle bacillus. The latter is seldom found in direct smears but, as in the case of tuberculous osteomyelitis, it is likely that direct examinations by fluorescent microscopy will increase the percentage of positive results in the future. Cultures on appropriate media and guinea-pig inoculation tests are more likely to be of diagnostic value than direct smears. Once fistulas have formed the pus almost invariably shows the presence of staphylococci, streptococci or *Esch. coli* as important micro-organisms of secondary infection. *Ps. aeruginosa* is likewise frequently found and probably also plays some rôle in the production of secondary infection, although *P. vulgaris,* diphtheroid bacilli and other micro-organisms are usually only unimportant contaminants.

EXAMINATIONS FOR RICKETTSIAL AND VIRAL INFECTIONS

While several of the pathogenic rickettsiae are cultivable in media containing living tissue cells, cultural methods and animal inoculation tests are not usually employed at present in the diagnosis of epidemic and murine typhus fevers, Rocky Mountain spotted fever, boutonneuse fever, South African tick-bite fever, rickettsialpox or other rickettsial diseases. Laboratory diagnosis is usually based on the results of Weil-Felix agglutination and complement fixation tests employing OX_{19}, OX_2 and OXK strains of *P. vulgaris* or rickettsial antigens, as discussed in Chapter 17.

Some of the viruses are also cultivable, but again cultural methods are not commonly employed for diagnostic purposes. However, examinations for inclusion bodies, serum neutralization and animal inoculation tests are available for diagnostic purposes (Table 92).

Smallpox. The Paul test is frequently of clinical value in the diagnosis of doubtful cases of pox-like disease, like varicella, resembling smallpox. It is conducted by scarifying the cornea of the rabbit and inoculating the scratch with material from the suspected lesion. In about 50 per cent of cases of smallpox a positive reaction is observed, characterized by the development of keratitis with a small umbilicated papule in which Guarnieri bodies are demonstrable by microscopic examination.

The intradermal inoculation of rabbits may be also employed. A normal rabbit and one previously immunized by vaccination are employed. Each is given an intradermal injection with material from lesions of the patient. In the case of smallpox, local papular and vesicular lesions develop at the site of inoculation in the normal rabbit in about four days, while the immune rabbit does not form any lesion, or shows an accelerated and transient reaction.

Material from vesicular or pustular lesions may be also collected on a sterile swab, washed with 2 cc. of saline solution containing 200 units of penicillin and 0.1 amounts inoculated on the chorio-allantoic membranes of ten-day embryonated eggs. After three days, the eggs are examined, material is taken from those showing lesions and a 10 per cent suspension of the infected membranes is prepared which is centrifuged and the supernatant fluid employed in a complement fixation test, using rabbit antivaccinal serum (Army Medical Department Research and Graduate School).

Rabies. Rabies is a disease of carnivora, especially of dogs, cats, wolves and coyotes, due to a filtrable virus occurring in the saliva and transmissible to human beings usually by bites. Deep bites, especially when multiple, and badly lacerated ones, are the most dangerous, especially those about the face, head, neck, or hands. Bites on other parts of the body, especially if they are inflicted through clothing, are less dangerous. While rabies unquestionably can be occasionally

TABLE 92. SUMMARY OF THE CLINICAL INTERPRETATION OF LABORATORY EXAMINATIONS IN THE RICKETTSIAL AND VIRAL DISEASES

Disease	Interpretation
General Considerations	Cultural methods are not usually available for the diagnosis of Rocky Mountain spotted fever, typhus fever or other diseases due to the pathogenic rickettsiae. Weil-Felix and complement fixation tests with antigens of *P. vulgaris* or rickettsia are employed. Cultural methods are not available for the diagnosis of diseases due to the filtrable viruses; complement fixation and serum neutralization tests are usually employed.
Smallpox	The Paul corneal and the intradermal tests employing rabbits possess some clinical value in differentiating between smallpox and other pox-like diseases; also the complement fixation test with antigen of cultivated smallpox virus.
Rabies	A disease of carnivora and especially of dogs, cats and wolves due to a filtrable virus present in the saliva. Transmissible to man usually through bites. Deep and lacerated bites are especially dangerous, particularly about the face, head, neck, or hands. Bites on other parts of the body, especially through clothing, are less dangerous. Infection may occur from the contamination of recent open wounds with saliva. There is little or no danger of infection through the intact skin contaminated with saliva. No danger of infection from the ingestion of milk or other foods possibly contaminated with saliva. All animal bites should be thoroughly cauterized with fuming nitric acid as soon as possible. Suspicious animals should not be killed but kept in secure confinement for observation for at least two weeks. Obviously rabid animals should be killed and the head sent to a laboratory for an examination of the brain for Negri bodies. If direct stained smears are negative a mouse or rabbit inoculation test should be conducted. Requires 8 to 16 days to 3 weeks or longer for positive results; negative results require a longer period of observation. Rabies of human beings is invariably fatal. Negri bodies are found in the brain by direct smears or mouse or rabbit inoculation tests.
Hodgkin's Disease	Of unknown etiology. The intracerebral inoculation of rabbits with suspensions of lymph nodes frequently produces an encephalitic syndrome (Gordon test). Apparently of clinical value, although falsely positive reactions with the lymph nodes of other diseases have been reported. The mechanism of the reaction is unknown.
Trachoma	Cytoplasmic inclusion bodies and cytologic changes in expressed follicular material. Inclusion bodies morphologically identical with those in inclusion conjunctivitis but much more numerous on the upper tarsal conjunctiva. Follicular material shows the presence of cell debris, pale-staining cells, and numerous macrophages containing cell fragments.

TABLE 92. SUMMARY OF THE CLINICAL INTERPRETATION OF LABORATORY EXAMINATIONS IN THE RICKETTSIAL AND VIRAL DISEASES—(Continued)

Disease	Interpretation
Inclusion Conjunctivitis	Cytoplasmic inclusion bodies which are particularly numerous on the lower tarsal conjunctiva. Expressed follicular material shows no changes.
Epidemic Keratoconjunctivitis	Must be differentiated from herpetic keratoconjunctivitis. Nonpurulent conjunctival secretions. Virus transmissible to mice and rabbits. Development of a specific serum-neutralizing antibody during convalescence.
Herpes and Varicella	Virus of herpes simplex transmissible to rabbits by corneal inoculation. Virus of herpes zoster not transmissible to rabbits; related to the virus of varicella. No conclusive evidence that the virus of varicella is transmissible to the lower animals.

acquired through the contamination of minor wounds, like scratches, with the virus and particularly when they are open and of recent origin, yet there is apparently little or no danger of contracting the disease through the intact skin contaminated with the virus in the saliva of rabid animals. Furthermore, there is no reason to fear infection through the ingestion of food, possibly contaminated by an individual who has unwittingly handled a rabid animal. The same applies to the occasional use of raw or pasteurized milk from a cow which later develops the disease.

Animal bites are extremely common and all should be routinely cauterized with fuming nitric acid as soon as possible, a glass rod or capillary pipet being employed. The acid should be carefully and thoroughly applied and not neutralized. Such cauterization not only gives protection against pyogenic infections common after bites but, if done thoroughly within forty-eight hours, is an important factor in the prevention of rabies.[50]

The animal should be always obtained whenever possible and subjected to clinical examination by a veterinarian. In case of doubt it should never be killed but securely confined under observation for at least two weeks. If rabid, it usually develops rabies or dies in seven days, in which case the head should be severed and sent to a laboratory for an examination of the brain for Negri bodies by stained smears. If negative, mouse or rabbit intracerebral inoculation tests should be conducted. Of course, if the animal is obviously rabid it should be killed at once, preferably by shooting through the heart, and the brain examined for Negri bodies.

To kill a dog or other animal in the early stages of rabies usually results in failure to find Negri bodies in the brain by the examination of stained smears. The disease may be revealed by mouse or rabbit inoculation tests but these usually require a minimum of eight to sixteen days and sometimes three weeks or longer for positive results, while negative results require keeping the rabbits under observation for sixty to ninety days before being reported.

Unfortunately, rabies in human beings is invariably fatal. Negri bodies are to be found in the brain by the examination of stained smears and likewise by mouse or rabbit inoculation tests.

Hodgkin's Disease. While the etiology of Hodgkin's disease is unknown, it has been suggested by Gordon [51] that it may be due to a filtrable virus. This is based upon the observation that the intracerebral inoculation of rabbits with suspensions of the lymph nodes produces an encephalitic syndrome in a large percentage of cases. But the reaction appears to be due to some other factor because of the absence of intracellular inclusions and the fact that the encephalitis cannot be transmitted from rabbit to rabbit.[52] False positive reactions have been reported following inoculation with tuberculous lymph nodes and various normal tissues [53,54] although Goldstein [55] observed positive reactions with the nodes of 7 of 9 cases of Hodgkin's disease, with no false positive reactions due to inoculation with the nodes of 20 controls, including 9 tuberculous nodes and 2 from cases of infectious mononucleosis. It would appear, therefore, that the Gordon test might serve as a helpful laboratory test for Hodgkin's disease even though the etiology of the disease and the mechanism of the reaction are unknown.

EXAMINATIONS IN RELATION TO THE SULFONAMIDE AND ANTIBIOTIC COMPOUNDS

Mechanism of Activity. Although considerable information and numerous theories have accumulated bearing on the mechanism of the antimicrobial activity of the sulfonamide and antibiotic compounds, the precise mechanism is still unknown (Table 93). It is believed, however, that they are more active against young cells and those about to divide than against resting cells, with the suggestion that their antibacterial activity depends to a large extent upon genestasis. Available evidence also indicates that their activity depends upon an interference with essential metabolites or metabolic processes on the part of susceptible organisms. This may result only in bacteriostasis with the assumption that *in vivo* the crippled micro-organisms are finally disposed of by immunologic processes with special reference to phagocytosis by the cells of the lymphoid-reticulo-endothelial system. On the other hand, higher concentrations of the compounds are bactericidal with complete destruction of the cells through lysis or disintegration. Curiously enough, however, mutants of various bacteria have been found in cultures which apparently depended upon the presence of streptomycin or penicillin in culture media as essential metabolites or growth factors, designated as "dependent" strains,[56,57,58] but this phenomenon has not been observed with the sulfonamide compounds.

Bacteriologic Examinations. In view of the fact that the sulfonamide and antibiotic compounds possess a high degree of specificity in their antimicrobial activities, it must be emphasized that their choice in treatment should largely depend upon accurate bacteriologic diagnosis, since the administration of these compounds is indicated only in the treatment of those diseases due to highly and moderately susceptible micro-organisms (Table 94). Furthermore, bacteriologic

TABLE 93. SUMMARY OF EXAMINATIONS IN RELATION TO THE SULFONAMIDE AND ANTIBIOTIC COMPOUNDS

Examinations	Interpretation
Mechanism of Anti-microbial Activity	The exact mechanism still unknown but apparently depends upon an interference with essential metabolites or metabolic processes on the part of susceptible micro-organisms. More active against young cells and those about to divide than against resting cells (genestatic activity). This bacteriostasis *in vivo* probably results in removal of crippled organisms by immunologic processes with special reference to phagocytosis. Bactericidal effects, however, may be produced.
Bacteriologic Examinations	The sulfonamide and antibiotic compounds possess a high degree of specificity in their antimicrobial activities (see Table 94). Consequently, their choice in treatment should depend largely upon the results of accurate bacteriologic examinations. Para-aminobenzoic acid (a growth-stimulating substance) should be added to all culture media employed in bacteriologic examinations of individuals under sulfonamide therapy in order to avoid falsely negative results. This is particularly advisable when cultures are employed as criteria of cure. The optimum concentration in fluid and solid media is 5 mg. per 100 cc.
Susceptibility Tests	Susceptibility tests of micro-organisms to the sulfonamide and antibiotic compounds are frequently indicated, particularly in cases where a satisfactory response to treatment is not being observed. Their purpose is to detect naturally resistant mutants and micro-organisms of acquired resistance. They may be, however, of limited value because of many variable factors and technical conditions.
Assays	Assays of the blood and other body fluids for the sulfonamide compounds are frequently required and of great value in relation to dosage and administration. The same is true in relation to antibiotic therapy. Effective blood concentrations of penicillin vary from 0.02–0.2 units per cc. serum or plasma; streptomycin 0.005–0.02 mg. (5–20 units) or higher per cc.
Acquired Resistance	Pathogenic micro-organisms may acquire resistance to the sulfonamide and antibiotic compounds, especially to streptomycin. Acquired resistance to the sulfonamide compounds is apparently due to bacteria acquiring the ability to synthesize para-aminobenzoic acid. According to Demerec, acquired resistance to penicillin consists of the selection of naturally resistant variants which undergo further increase in resistance. The same may be the mechanism involved in acquired resistance to streptomycin.

examinations are always indicated when mixed infections are suspected. This is true not only in relation to prognosis but to treatment as well, because it is sometimes advantageous to combine the sulfonamide compounds with penicillin or streptomycin or penicillin with streptomycin in synergistic and additive chemotherapy.[59]

In many instances, however, the physician or surgeon is justified in making a presumptive diagnosis and proceeding with sulfonamide or antibiotic therapy without further delay. Indeed, in many of the severe infections treatment should be instituted as promptly as possible as it can be changed, if and when necessary, on the basis of accurate bacteriologic examinations.

TABLE 94. SUSCEPTIBILITY OF THE LIVING AGENTS OF DISEASE

Sulfonamides	Penicillin	Streptomycin
A. bovis	*A. bovis*	*A. aerogenes*
Brucellae	*B. anthracis*	*P. vulgaris*
Clostridia of gangrene	Borrelia of relapsing fevers	Brucellae
D. pneumoniae	*C. diphtheriae*	*Esch. coli*
H. ducreyi	Clostridia of gangrene	*S. typhosa*
H. influenzae	*D. pneumoniae*	*H. influenzae*
K. pneumoniae	*Ery. rhusiopathia*	*H. pertussis*
N. gonorrheae	*H. ducreyi*	*K. pneumoniae*
N. meningitidis	*H. influenzae*	*Myco. tuberculosis*
Past. tularensis	*Lept. icterohaemorrhagiae*	*Past. pestis*
S. dysenteriae	*N. catarrhalis*	*Ps. aeruginosa*
Staphylococci	*N. gonorrheae*	Salmonellae
Streptococci	*N. meningitidis*	*S. dysenteriae*
Virus lymphogranuloma venereum	*S. minus*	Staphylococci
Virus trachoma	Staphylococci	*Str. pyogenes*
	S. moniliformis	
	Streptococci	
	T. pallidum	
	T. pertenue	
	Virus of ornithosis	
	Virus of psittacosis	

Since the sulfonamide compounds are capable of injuring the proliferative activities of many of the pathogenic bacteria it is always advisable to incorporate the growth-stimulating para-aminobenzoic acid in all culture media employed for bacteriologic examinations in individuals under treatment with sulfonamides by local, oral or parenteral administration. Otherwise, blood cultures as well as cultures of wounds, peritoneal exudates, prostatic secretions or urethral pus for gonococci, etc., may give falsely negative results not because the micro-organisms are absent, but because they are too greatly devitalized to survive or proliferate. Consequently, this is particularly important when cultures are employed as criteria of recovery and cure.

Para-aminobenzoic acid is not only readily soluble but heat-stable, so that it escapes destruction in the sterilization of media. The optimum concentration is 5 mg. per 100 cc. of medium. This concentration should be employed in all fluid media employed for blood cultures, as previously stated. Likewise, in all broths used in the preparation of the solid or semisolid media. Indeed, the addition of para-aminobenzoic acid to all media is now a matter of routine in many laboratories for its growth-stimulating properties, especially since bacteriologic examinations are so frequently conducted in individuals under sulfonamide therapy. Para-aminobenzoic acid also protects susceptible bacteria against the bacteriostatic and bactericidal activities of the sulfonamide compounds *in vitro*.[60]

Susceptibility Tests. Since gonococci and other micro-organisms are sometimes unusually resistant to sulfonamide therapy,[61,62] the question frequently arises whether or not *in vitro* tests may be of clinical value in aiding the selection of a sulfonamide compound for therapeutic purposes. Keefer and his colleagues [63] have developed such a test employing citrated blood and have demonstrated that gonococci resistant to sulfathiazole therapy were likewise resistant to the compound *in vitro*. Cohn and his co-workers [64] have observed similar results with a modification of this technic. Poston and Orgain,[65] however, found great variation in the bacteriostatic effects of the sulfonamide compounds on *Str. viridans,* with sodium sulfapyridine exhibiting the most marked effectiveness against the largest number of strains. There are, however, so many factors influencing the bactericidal properties of the sulfonamide compounds *in vitro* that no technic has been evolved at present for the purpose of choosing a compound for treatment purposes on the basis of maximum bactericidal activity for the micro-organism recovered in cultures of the individual patient. But such tests are highly desirable and especially since it is probable that susceptibility to the sulfonamides *in vitro* is a reliable index of susceptibility *in vivo*.

Certainly it is frequently desirable to determine the degree of susceptibility of infecting micro-organisms to penicillin, streptomycin, bacitracin and aureomycin *in vitro,* particularly in cases where a satisfactory response to treatment is not being observed. Unfortunately, however, these tests may be of but limited clinical value because of so many variable factors and technical conditions. Consequently, tests conducted in different laboratories, with the same culture, may give widely varying and confusing results. In other words, there are many factors influencing susceptibility tests of bacteria to penicillin and especially to streptomycin.[66] It is particularly important that tests be capable of detecting the presence of naturally resistant mutants and micro-organisms of acquired resistance. Furthermore, as ordinarily conducted, susceptibility tests show only the bacteriostatic activities of penicillin or streptomycin, whereas it is advisable to determine susceptibility to the bactericidal effects of these agents as a more reliable guide to dosage and administration.

Assays. It is now well established that assays of the blood and other body fluids for the sulfonamide compounds are frequently required and of great value in gauging dosage and frequency of administration, especially in the treatment of severe infections.

The same is true in relation to antibiotic therapy. At present there is no unanimity of opinion on what constitutes an effective blood concentration of penicillin but it appears that it should be at least within the limits of 0.02 and 0.2 units per cubic centimeter of serum or plasma, with a general average of 0.15 units. The same is true of streptomycin but apparently this should be within the limits of 0.005 to 0.02 mg. (5–20 units) or more per cc. It is true that the technic of these assays is more difficult than in the case of the sulfonamide compounds, but present methods[66] are readily conducted in most laboratories and should be available to physicians and surgeons in all hospitals.

Acquired Resistance. As is well known, pathogenic micro-organisms may acquire resistance to the sulfonamide and antibiotic compounds not only *in vitro* but *in vivo* as well. This is particularly true of streptomycin. Whether or not resistance is also acquired to tyrothricin, bacitracin, subtilin, polymyxin (aerosporin), aureomycin, chloromycetin, etc., cannot be stated but, theoretically at least, this appears to be both possible and probable.

The exact mechanism is unknown but, according to Demerec, consists in the elimination of susceptible micro-organisms, leaving naturally resistant mutants which undergo further enhancement of resistance on repeated exposures to the compounds. In other words, according to this hypothesis, acquired resistance consists of the selection of naturally resistant mutants which undergo a further increase of resistance through "training" *in vitro* or *in vivo;* from this viewpoint the mechanism is essentially one of bacterial genetics. At all events, it is practically certain that both natural and acquired resistance of bacteria to penicillin is not solely due to the production of penicillinase. Furthermore, bacteria apparently do not produce a similar inhibitor for streptomycin to account for their natural or acquired resistance to this compound. Acquired resistance of bacteria to the sulfonamide compounds, however, is apparently due to the fact that they acquire the ability to synthesize more para-aminobenzoic acid than is required in their enzyme systems for survival and growth.

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THE CLINICAL INTERPRETATION OF MYCOLOGIC EXAMINATIONS

The majority of diseases due to the pathogenic fungi and yeasts with which the clinician comes in contact are those which produce superficial lesions of the skin and mucous membranes. The former are caused for the most part by members of the genera *Achorion, Microsporum, Trichophyton,* and *Epidermophyton.* The most important order consists of the *Hyphomycetales,* including the *fungi imperfecti,* which embrace practically all the fungi pathogenic for human beings.

In Sabouraud's classification, fungi having small spores in which the elements are found in mosaic arrangement and in profusion on the surfaces of hairs, are known as *Microspora.* The next group consists of the *Trichophyta,* divided into endothrix, which invades the hair shaft with the formation of large spores in linear arrangement, and ectothrix, which forms chains of spores external to the hair. The endothrix micro-organisms are usually not inoculable into laboratory animals, whereas the ectothrix fungi are often pathogenic for them. The genus Achorion includes only one common pathogen, namely, *Achorion schoenleini,* the cause of favus. The term "Epidermophyton" indicates lack of invasion of a hair follicle.

Laboratory diagnosis can usually be made by direct microscopic examinations of hairs or scrapings from the lesions. Not infrequently, however, cultures are required as in differentiation between *Microsporum audouini* and *Microsporum lanosum.* Clinical differentiation is based upon the more inflammatory type of lesion produced by *Microsporum lanosum,* but noninflammatory lesions may be produced by this fungus. In such cases identification of species is necessary for correct therapeutic measures. Thus, lesions due to *Microsporum lanosum* usually respond readily to the local application of fungicides, whereas those due to *Microsporum audouini* can be treated successfully only after epilation.

Less readily recognized clinically are those mycotic diseases which either occur less frequently or produce infection without an initial local lesion. Examples are pulmonary and intestinal actinomycosis, chromoblastomycosis, systemic histoplasmosis and Torula meningitis. Indeed, diagnosis of such infections is often delayed, or, in the case of a fatal termination, discovered only at autopsy.

Laboratory diagnosis is based on various examinations. Direct microscopic examinations of specimens of skin scrapings, hairs, nail scrapings, pus or exudates are the simplest and the first step in establishing diagnosis but rarely permit one to identify species. Cultural methods, including hanging-drop or slide cultures, are frequently the only means of identification. The phenomenon of fluorescence is helpful in determining the presence of tinea capitis or of tinea versicolor. Animal inoculation tests are sometimes helpful, especially in the case of deep fungous infections if negative results are observed with direct examinations or cultures.

Allergic skin tests employing trichophytin are particularly helpful in diagnosis, although tests employing oidiomycin are practically useless, as will be discussed in Chapter 19. Agglutination tests may be helpful in the identification of the monilias, although complement fixation and precipitation tests are practically valueless because so few or no antibodies are produced in the course of the superficial ringworms, as will be discussed in Chapter 17.

Collection of Material. The selection of suitable materials for examination is very important. If there are different types of lesions, specimens should be obtained from all. An abundance of material is usually desirable, but a small amount of good material is better than a large amount of unselected material. Specimens should be collected in sterile containers for delivery to the laboratory but it is frequently better to send the patient for the best possible selection of material and to reduce the incidence of contaminations.

Since treatment may affect the abundance and the stage of development of a fungus, material from untreated areas, especially recent ones, is preferred. Thus, the components of a medicament may obscure a fungus or confuse the examiner by their similarity to fungi, *e.g.*, oil droplets may resemble yeast cells. With ringworm of the scalp the infected hairs should be selected while the patient is observed under filtered ultraviolet rays, since these reveal the sites of tinea versicolor which are not readily discerned in daylight.

Removal of accidental saprophytic fungi from the surface of a lesion may usually be accomplished by cleansing with 70 per cent alcohol.

When infection produces a porous condition of the nails, the deeper parts are preferred for examination. Small portions of crumbly material are better than large clippings or even the entire nail. Horny or scaly material is practically useless for direct examinations but suitable for cultures. Fungi are frequently found at the sites of apparently healed lesions which are sometimes responsible for recurring infections unless treatment is continued.

In suspected mycosis of the lungs, a specimen of sputum or a fragment of tissue obtained bronchoscopically should be examined. It is frequently difficult to decide by direct or cultural examinations whether Monilias, Actinomycetes or other micro-organisms are actually producing infection of the lungs or are merely present in the mouth as saprophytes.

EXAMINATIONS IN THE SUPERFICIAL MYCOSES

There are two large groups of the superficial mycoses: (1) the ringworms, or tineas, in which the site of invasion and propagation of the fungi is keratin (stratum corneum, hairs, nails) and in which, while there may be dissemination by the blood, involvement of the internal organs does not occur, and (2) moniliasis, in which *Candida albicans* produces infections of the oral cavity, other mucous membranes, the intertriginous skin and sometimes of the internal organs. Included also are erythrasma, otomycosis and several tropical mycoses. All are due to pathogenic or potentially pathogenic fungi even though some produce little cellular reaction and little or no immunologic response. Classifications are best made on the basis of etiology but time and custom have sanctioned their division into clinical varieties.

Tinea Capitis. Tinea capitis is a superficial fungous infection occurring mainly in children before the age of puberty. In certain forms, the disease may persist into adult life, but it is unusual for it to appear then for the first time. About 80 per cent of all cases are due to *M. lanosum* and *M. audouini*, the balance being due to *Trichophyton violaceum*, *T. crateriform*, *M. fulvum* or other species (Table 95).

TABLE 95. SUMMARY OF THE CLINICAL INTERPRETATION OF EXAMINATIONS IN THE SUPERFICIAL MYCOSES

Disease	Interpretation
General Considerations	The majority of the mycotic diseases are those producing superficial lesions of the skin or mucous membranes. Some, occurring less frequently, produce systemic infections in which diagnosis is frequently delayed or not made at all. Diagnosis and identification of species by laboratory examinations are of clinical value and especially in relation to treatment. The proper selection and collection of material suitable for laboratory examinations is very important.
Tinea Capitis	Occurs mainly in children before the age of puberty. About 80 per cent of cases are due to infection with *M. lanosum* and *M. audouini;* balance due to *T. violaceum, T. crateriform* or other species.
Favus	A disease of the scalp usually occurring after puberty due to *A. schoenleini.* Material from scutula is best for microscopic diagnosis. One type of the disease may resemble seborrheic eczema; laboratory examinations are valuable in differential diagnosis.
Tinea Barbae	Occurs as a ringworm of the bearded region; uncommon in the United States. The inflammatory type is usually caused by *T. gypseum* or *M. lanosum.* The sycosis type is usually caused by *T. violaceum* or *T. purpureum.*
Tinea Glabrosa	Ringworm of the smooth skin occurs as scaly lesions, circinate patches, solid plaques or lesions with gyrate configurations. May resemble eczema. Lesions may also occur in the scalp, nails, bearded region, inguinal region, or the feet. Usual cause in children is *M. lanosum.* May be also due to infection with *A. schoenleini, T. gypseum* or *T. purpureum.*
Tinea Cruris	Ringworm of the crural region or upper parts of the thighs. May involve the skin of other parts of the body. Usually due to infection with *E. inguinale.* Some cases due to *T. gypseum* or *T. purpureum.*
Tinea Pedis and Tinea Manuum	*Tinea pedis* or "athlete's foot" is very common. Secondary lesions may be acquired by contact on the hands (*tinea manuum*). Acute infections are usually caused by *T. gypseum* and chronic infections by *T. purpureum.* Intertrigo of the toes may be also due to infections with *C. albicans, M. lanosum* or other dermatophytes. Laboratory examinations possess clinical value in etiologic diagnosis in relation to treatment and especially in differentiating tinea pedis from many conditions with which it may be confused clinically.
Tinea Unguium	Tinea unguium or onychomycosis is due to infection of the nails of the fingers or toes with *T. gypseum* or *T. purpureum; A. schoenleini* and *C. albicans* or other fungi are occasionally responsible.

TABLE 95. SUMMARY OF THE CLINICAL INTERPRETATION OF EXAMINATIONS IN THE SUPERFICIAL MYCOSES—(Continued)

Disease	Interpretation
	Usually due secondarily to interdigital or other primary infections. Chronic paronychia usually occurs in infections due to *C. albicans*. Laboratory examinations are of value in etiologic diagnosis, especially for differentiating tinea unguium from affections of the nails due to psoriasis, syphilis, tuberculosis and leprosy as well as from paronychia due to pyogenic infections.
Tinea Versicolor	A common superficial mycosis of the skin caused by *Malassezia furfur* (*Microsporon furfur*). Occurs especially in young adults but may affect children and the aged. Mycologic examinations are of value in diagnosis and especially in differential diagnosis.
Tinea Imbricata	A disease occurring in tropical and subtropical countries. Caused by any of four species of Endodermophyton (*E. concentricum, E. indicum, E. tropicale* and *E. mansoni*).
Erythrasma	A superficial mycosis resembling tinea versicolor. Caused by *M. minutissimum* (*N. minutissima*). Usually occurs among young adults; men more commonly than women. High magnification advisable in the examination of material.
Dermatophytid	Dermatophytids are skin rashes due to allergic sensitization to pathogenic fungi in localized areas of infection. Rigid criteria must be fulfilled before the diagnosis is justified. Laboratory examinations are of value in the detection of the primary foci. The trichophytin skin test should give a positive reaction. Dermatophytid-like rashes may be due to natural or acquired allergies to various drugs. Skin tests are indicated for diagnostic purposes.
Moniliasis	Due to infection with *C. albicans*. The micro-organism is commonly present in the gastro-intestinal tract without the production of lesions or symptoms but may produce allergic sensitization resulting in *moniliids*. It is also sometimes present on the skin and in the mouth, throat and vagina without evidences of infection. *C. albicans* produces *thrush* in infants and children, sometimes associated with glossitis and stomatitis; also *perlèche*, generalized infections of the scalp and glabrous skin and possible infantile eczema. In adults it may produce a *dermatitis, intertrigo, vaginitis* and *pruritus ani*—likewise *bronchitis, meningitis* and fatal systemic infections. Resistance is especially lowered by diabetes; also by obesity and profuse perspiration.
Otomycosis	Characterized by an exudative inflammation with pruritus involving the external auditory canal and tympanum. May extend to the tympanic cavity and mastoid cells. Usually occurs among young adults. The etiology is uncertain. Under the special conditions of the canal due to cerumen, it may be caused by Monilia, Aspergillus, Penicillium or Achorion. Streptococcal infection has been also suggested as the cause.

TABLE 95. SUMMARY OF THE CLINICAL INTERPRETATION OF EXAMINATIONS IN THE SUPERFICIAL MYCOSES—(Continued)

Disease	Interpretation
Trichomycosis Axillaris	A clinically silent disorder involving the hairs of the axillae and pubis due to the formation of firmly attached yellowish or reddish concretions caused by *A. tenuis* (*N. tenuis*). Microscopic examinations readily distinguish them from nits.
Chromoblastomycosis	A rare disease in the United States characterized by the formation of reddish verrucous nodules on the legs or other exposed parts, producing cauliflower-like masses which break down into ulcers with foul discharges. Apparently caused by *H. pedrosoi, H. compactum, H. langeroni* or *P. verrucosa*. The disease must be differentiated from tuberculosis verrucosa cutis, blastomycosis, Madura foot and coccidioidal granuloma.

The infecting fungus first invades the stratum corneum, later enters the hair follicle, and finally attacks either the superficial or the deep parts of the hair. The chief lesions are partial loss of hair in patches, breaking off and lack of luster of the infected hair and varying degrees of inflammation. Atrophy and scarring may follow certain types of infection. Kerion is a painful, elevated, boggy, erythematous, localized tumefaction.

Favus. Favus is a disease of the scalp usually occurring after puberty, due to infection with *A. schoenleini* which is essentially an endothrix Trichophyton. The lesions are usually characterized by sulfur-colored or yellow friable crusts or scutula pierced with hairs. The skin in the affected areas becomes atrophic. After several years spontaneous recovery may occur but permanent alopecia may result.

A second type of favus occurs as a diffuse superficial but adherent scaling with little, if any, alopecia or evidence of follicular involvement. It closely resembles seborrheic eczema. Consequently, laboratory examinations are valuable in differential diagnosis and especially in relation to treatment.

Piedra. Piedra is a term applied to two types of fungous infections of the hair, common in Brazil. Black piedra is caused by *Piedraia hortai* [1] and is characterized by hard black nodules which are adherent to the hair. White piedra is caused by *Trichosporon beigelii* [2] and is characterized by soft and light-colored nodules which are easily detached from the hair. Both organisms may be identified by microscopic examinations of the nodules and both may be cultivated on Sabouraud's glucose agar.

Tinea Barbae. Tinea barbae is rare in the United States. It usually occurs as a ringworm of the bearded regions of men, the infection being frequently contracted in barber shops. In some instances the infection is contracted from another person, not uncommonly from a child with infection of the scalp or of the glabrous skin.

The majority of cases are due to infection with *T. gypseum* or *M. lanosum* which produce the inflammatory type of the disease. The sycosis type, characterized by pustular (crusted) folliculitis with the breaking off of the invaded hairs, is usually due to *T. violaceum* or similarly without the formation of hair stumps to *T. purpureum*.

Tinea Glabrosa. Ringworm of the skin (Trichophytosis corporis) usually occurs as a superficial scaly lesion, a circinate patch, a solid plaque, or as a lesion with gyrate configurations. It may resemble eczema, or deep granulomatous lesions may develop. In many instances, concomitant lesions occur in the scalp, the nails, the bearded region, the inguinal region or the feet. It does not include the intertriginous infections like tinea cruris or the secondary allergic dermatophytids.

The infection may be contracted from a cat or other pet, a playmate or some other member of a household. The usual cause is *M. lanosum*, particularly in children. Some cases, however, are due to infection with *A. schoenleini* secondary to infection of the scalp; to *T. gypseum* by infection from an animal, from a focus on the patient's feet or from another person, or to *T. purpureum* in which case the disease is almost always part of a syndrome involving the nails and feet.

Tinea Cruris. This disease, which is also known as "eczema marginatum," occurs as a superficial infection of the skin of the upper parts of the thighs, or crural region, usually due to *Epidermaphyton inguinale* (floccosum) which has an affinity for intertriginous areas; *T. gypseum* or *T. purpureum*, however, are the etiologic agents in some cases. There may be contiguous spreading, or other parts of the skin may become infected. It may be spread by infected articles of clothing, especially suspensories, and epidemics have occurred.

Tinea Pedis and Tinea Manuum. Tinea pedis, popularly known as "athlete's foot," is one of the most common of the superficial fungous infections of the intertriginous areas of the skin, occurring at one time or another in from 60 to 90 per cent of individuals.

Secondary lesions may be acquired by contact on the hands (*tinea manuum*). Both, including the dermatophytids, constitute the dermatophytoses or dermatomycoses; the terms epidermophytoses, epidermomycoses and trichophytoses are also used as synonyms.

Tinea pedis and tinea manuum are usually due to infections with *T. gypseum* or *T. purpureum*. *C. albicans* and *E. inguinale* may also cause intertrigo of the toes, and in rare instances *M. lanosum* or other dermatophytes may be responsible.

Tinea pedis occurs much more frequently in men than in women and is comparatively uncommon in children. The most vulnerable period appears to be from the sixteenth to the twenty-fifth years of age. Males not only appear to be more vulnerable than females but are likewise more exposed to infection in camps, clubs, locker rooms, shower baths, etc.

The disease is more prevalent in the summer; at least, the lesions are then usually more acute. Hyperhidrosis is undoubtedly a predisposing cause to infection as well as factors lowering general health and vitality. The chief sources of infection are foci on the feet of carriers, who are unaware of the disease or are careless in treating it. Undoubtedly, the fungi, and especially their spores, may remain viable in a dry state for six months to a year and have been found in cultures of material from shoes, stockings, floors, mats, gymnasium apparatus and various fabrics.

The inflammatory type of tinea pedis and tinea manuum is caused by *T. gypseum*, while the chronic type is usually due to *T. purpureum*. Since there is a wide difference in the clinical manifestations, course and response to treatment of each, etiologic diagnosis by laboratory examinations is always advisable. If the infection is present on the interdigital webs of the feet alone, it may be impossible to determine the species of infection by clinical observation. If all the webs are affected, infection with *C. albicans* is probable. Scaling between the toes due to the use of strong chemical agents may lead the physician astray. Maceration of the interdigital webs may be caused by perspiration or by lack of drying after washing of feet. Syphilis may produce lesions difficult to distinguish from those of acute tinea pedis. Soft corns at the bases of webs may, on superficial examination, resemble the disease. The same is true of dermatitis venenata due to allergic sensitization to shoe leather, the dyes in stockings, foot powders, "corn cures" and the like. Orbicular eczema involving the feet or ankles may also mislead the unwary physician, as likewise "pustular psoriasis," acrodermatitis perstans and streptococcal infections of the skin of the feet.

So-called aberrant types of psoriasis involving the feet are also usually due to *T. purpureum*. Pruritus, the lack of involvement of the scalp and the localization to areas not commonly the sites of psoriasis, such as the palms, the soles or the inner surfaces of the thighs, favors the diagnosis of dermatophytosis. Neurodermatitis or atopic eczema may be likewise confusing but careful inspection of the history usually shows that pruritus and scratching preceded the rash along with a history of sensitization to foods or inhalants.

Tinea Unguium. Tinea unguium (Trichophytosis unguium) or *onychomycosis* refers to fungous infections of the nails of the fingers or toes. In the majority of cases the disease is caused by *T. gypseum* or *T. purpureum; A. schoenleini, C. albicans* or other fungi are occasionally responsible. While the toenails are usually infected secondarily to an interdigital infection of the feet, the fingernails may or may not be involved after infection in another site. In some instances the evidence points to a primary infection of the fingernails due to poor hygiene during a manicure. The incidence is about 1 to 500 of the population.

Infection with *T. gypseum* may produce only a white patch on the surface or in the substance of the affected nail (leukomychia trichophytica). In some instances, however, the lesions are inflammatory and destructive, in which case the nail becomes yellowish, opaque, lusterless and finally friable. Separation of the nail from its bed may occur. Subungual hyperkeratosis and uneven dystrophic changes in the nail are frequent although paronychia is rare. Infection of a fingernail should always lead to an examination of the toenails, since the two sites are frequently simultaneously affected. In onychomycosis caused by *T. gypseum* or *T. purpureum* the toenails are involved more frequently than the fingernails in the ratio of 11:1.

In onychomycosis due to *C. albicans* the edges of the nail become yellow and eroded, but the nail substance is frequently firm and translucent. Uneven ridges and grooves are common, probably due to interference with nutrition. Chronic paronychia is practically always present.

Laboratory examinations, therefore, are of value in establishing etiologic diagnosis. They are likewise of value in differentiating tinea unguium from pitting of the nails in psoriasis, paronychia due to pyogenic infection and affections of the nails due to syphilis, tuberculosis and leprosy.

Tinea Versicolor. This disease, also known as pityriasis versicolor, and chromophytosis, is a common superficial mycosis of the skin caused by *Malassezia furfur* (*M. furfur*). It affects young adults by preference but may occur in children and in the aged. Lack of personal hygiene and hyperhidrosis appear to be predisposing factors. Contrary to a common impression, the disease is apparently not more common in individuals with pulmonary tuberculosis.

The disease is characterized by scaly macules and patches starting from barely visible lesions in single or multiple areas. The color varies from that of the skin to dark brown but is usually yellowish fawn to light brown, being darker during the summer months. The sites of predilection are the chest, abdomen and back, but the disease may also involve the scalp and the palms and soles.

Examinations of the scales for the fungus are not usually required for diagnostic purposes except when necessary for differentiating the disease from erythrasma, pseudo-achromia, chloasma, syphilitic leukoderma of women, vitiligo, posteruptional depigmentations in syphilitic, psoriatic and other cutaneous diseases.

Tinea Imbricata. This disease is rarely seen except in the tropics or subtropics and especially in places where the cocoanut tree grows. The cause has been ascribed by Castellani to any of four species of Endodermophyton (*E. concentricum, E. indicum, E. tropicale* and *E. mansoni*).

The disease occurs especially among young adults, men being more susceptible than women. It begins as one or more brownish spots, which slowly increase in size. The central portion of the superficial epidermis finally becomes detached, the epidermis cracks, and there is an opening from the center toward the border. This process is repeated until numerous rings are formed. Finally large areas of skin are affected; the nails may become infected. Scaling may be profuse and itching is usually intense.

Erythrasma. This superficial mycosis resembles tinea versicolor but is usually confined to the axillae, the groins, the intergluteal cleft or other intertriginous areas, with involvement of one or more regions.

The causative fungus is *M. minutissimum* which is also known as *Nocardia minutissima*. Because it occurs as a very small thread-like micro-organism, examinations of material usually require high magnification with the oil-immersion objective. Mycologic diagnosis should be made in all cases.

The disease usually occurs among young adults, more commonly in men than in women. It begins as small scaly macules which gradually enlarge to form patches which are well circumscribed, of a yellowish brown to reddish brown color, with margins accentuated by a reddened border.

Dermatophytid. Dermatophytid is the term commonly employed for designating widespread or localized rashes commonly ascribed to allergic sensitization to pathogenic fungi in localized areas of infection. The term is preferred to trichophytid because allergic rashes due to Microsporum may be clinically indistinguishable from those due to Trichophyton. Not infrequently, however, the term dermatophytid is used too loosely without proof that the erythematous, vesicular and eczematous eruptions so commonly affecting the hands are due to allergic sensitization to fungi. In all cases possible sensitization to contactants should be carefully considered with the conduct of intradermal trichophytin (see Chapter 19), patch or other skin tests. Dermatophytid-like rashes may be due to natural or acquired allergies to drugs. The diagnosis of pompholyx is made by excluding dermatophytid and dermatitis venenata.

Indeed, the diagnosis of dermatophytid is justified only when a focus of fungous infection is found, but usually not in the dermatophytid itself, along with positive intracutaneous reactions to trichophytin. The rash should occur upon irritation of the primary focus by treatment or spontaneous exacerbation and disappear when the focus is eradicated. When the primary focus occurs on the feet it is usually due to infection with *T. gypseum*. Furthermore, the fact should not be overlooked that many lesions at points removed from the initial infection may be due to the external dissemination of fungi; these are not dermatophytids and should not be classed as such.

The same type of vesicular dermatophytid which appears on the hands may also be found on the feet, particularly the soles. Here, as on the hands, fungi are not likely to be found. Keratolysis exfoliativa may be a dermatophytid as likewise erysipelas-like rashes on the legs which, however, may be also due to streptococcal infections.

Moniliasis. This disease syndrome is due to infection with a yeast-like organism, *C. albicans*. It is occasionally found on the skin and not infrequently in the throats of adults without lesions. In these locations carriers among nurses and mothers may be sometimes responsible for the infection of infants with the production of thrush. The organism may also occur in the adult vagina and is a common inhabitant of the gastro-intestinal tract of adults, where it usually produces no lesions or symptoms. While it was formerly considered, mainly through the investigations of Ashford, to be of etiologic significance in tropical sprue, later investigations do not appear to establish this relationship.

Intra-oral *thrush* most commonly occurs in infants and sometimes in babies only a few days old. Superficial glossitis and stomatitis sometimes occur. The disease is highly infectious and cultures may show the presence of the organism several days before the development of lesions. It is particularly apt to occur in bottle-fed infants and those kept in hospitals over long periods of time. Local trauma, debility and prematurity are stated not to be predisposing causes but it is quite likely that they may be factors favoring infection. Pooled breast milk is regarded as being sometimes a source of infection as likewise overcrowding and failure in the early diagnosis and isolation of infected infants. Air-borne infection has been stated to be of little significance [3] but in my laboratory the investigations of Dr. Earle Spaulding in co-operation with Dr. Nina A. Anderson and Miss Dorothy Sage, have indicated that air-borne infections, as well as contaminations of nipples, bed clothing, etc., may be far more important sources of infection with *C. albicans* than the vagina of the mother or the throats and hands of nurses insofar as hospital outbreaks of the disease are concerned. At least, cultures of the air, dusts, furniture and bed clothing very often show the presence of *C. albicans* due to the high resistance of the spores to destruction by drying. In private homes the feces, vagina or throat of the mother appear to be the principal sources of infection.

The bacteriologic diagnosis of thrush is comparatively easy by direct microscopic examinations of wet preparations of the lesions and especially by cultures. The latter should be always made with negative results before quarantine is lifted because not infrequently they are positive after complete recovery has occurred. *C. albicans* can be readily isolated from the feces and skin of the hands of a large percentage of infants with the disease.

Perlèche of children is likewise frequently due to infection with *C. albicans*. Generalized infections of the scalp and glabrous skin are fortunately uncommon because they are extremely resistant to therapy. It is also thought that the micro-organism may produce a type of infantile eczema.

In adults *dermatitis* due to continuous baths, prolonged wetting or occlusive dressing, with infection of the macerated skin by *C. albicans* or other yeast-like micro-organisms, may occur although the organisms may be present only as saprophytes. Likewise, monilial *intertrigo* and especially in the axillae, inframammary folds, the groins, the umbilicus, the interdigital webs of the fingers (erosio interdigitalis blastomycetica) and the intergluteal fold. The presence of *C. albicans* in the vagina does not necessarily indicate infection although it may produce a low-grade *vaginitis* accompanied by a thin discharge with troublesome pruritus. It may also produce *pruritus ani*. Indeed, instances of fatal *systemic infections* in children and adults have been reported; likewise cases of *meningitis* and *bronchitis*.

The incidence of these infections increases with age. Resistance is especially lowered by diabetes, and potential diabetes is always to be considered. Obesity also predisposes to cutaneous moniliasis as likewise profuse perspiration. The micro-organism is apparently of low virulence but once infection has occurred it is apt to persist indefinitely. *Moniliids* or levurids may also occur as sterile vesicular lesions on the hands or elsewhere, generally ascribed to allergic sensitization to *C. albicans* in the gastro-intestinal tract.

Otomycosis. Otomycosis or myringomycosis is characterized by an exudative inflammation accompanied by pruritus (worse at night) involving the external auditory canal and tympanum. If the latter is perforated, it may extend to the tympanic cavity and even involve the mastoid cells. No age is exempt, although the majority of cases occur among young adults. Season and climate have little effect, but swimming in pools has been suggested as a possible source of some infections.

The etiology is uncertain. Monilia, Aspergillus, Penicillium and Achorion have been suspected as the cause and especially the first two. However, these micro-organisms are common saprophytes, although they may cause the disease under the special conditions of the external auditory canal, with special reference to retained cerumen. Fungi of recognized pathogenicity have not been found. Streptococcal infection has been considered a possible cause.

The disease must be differentiated from seborrheic eczema and allergic dermatitis or eczema.

Trichomycosis Axillaris. This condition, which is also known as trichomycosis, is an infection of axillary or pubic hairs characterized by the presence of yellowish, reddish or black concretions firmly attached to the hairs. According to Castellani, this silent disorder is caused by *Actinomyces tenuis* (*N. tenuis*). If a hair is examined under the microscope the concretions are found and nits readily excluded, but it is difficult to demonstrate the presence of the fungus. Cultures require the use of special media, such as those employed for the isolation of *Actinomyces bovis.*

Chromoblastomycosis. This disease, which is also known as dermatitis verrucosa, is relatively rare in the United States but occurs in South America, Puerto Rico, Cuba and Panama.[4] The lesions usually develop as verrucous reddish nodules or ulcers on the feet, legs, buttocks or hands. After many months or years cauliflower-like masses may develop, with secondary bacterial infections, resulting in ulcers and foul discharges. Adenopathy is infrequent; metastasis to the internal organs does not occur.

The disease bears a superficial resemblance to tuberculosis verrucosa cutis, blastomycosis, and at times to Madura foot and coccidioidal granuloma. Apparently it may be caused by any of three closely related fungi, namely, *Hormodendrum pedrosoi, H. compactum* or *Phialophora verrucosa. H. langeroni* has also been reported as causing it. Microscopic examinations of scrapings mounted in sodium hydroxide show large, double-walled, brown, spherical cells.[5] They must be distinguished from *Blastomyces dermatitidis* and *Coccidioides immitis.* On Saubouraud's dextrose agar, growth takes place slowly with the production of black colonies.

EXAMINATIONS IN THE DEEP MYCOSES

Fortunately, the deep or potentially systemic mycotic diseases occur much less frequently, but they are vitally important because a delay in their detection and early treatment may result fatally. Patients with these infections may consult the general practitioner, the dermatologist or some other specialist. As a general rule, diagnosis is not difficult provided they are kept in mind as possibilities (Table 96).

Actinomycosis. This is probably the most frequent type of the deep mycotic diseases, since it occurs in all parts of the world. It is caused by *Actinomyces bovis* which may occur in the normal mouth and saliva as well as in the throat, with special reference to the tonsillar crypts.[6] Wild and domestic animals (particularly cattle) are susceptible to the disease. Adults are affected more commonly than children and men much more frequently than women; nearly half of infected individuals occur among those engaged in agricultural pursuits. Trauma is undoubtedly a frequent factor in infection; cases have developed following the extraction of teeth as well as after bites. There is also a possibility that the saliva and nasal discharges may be responsible for its transmission.

The infection may be spread by continuity, or by means of the blood, and may involve any part of the body. About 50 per cent of cases occur about the head and neck (buccal mucosa, gingivae, jaws, subcutaneous tissues, lymph nodes); 20 per cent in the abdominal organs (appendix or cecum, fallopian tubes, gallbladder, liver, kidneys); 15 per cent in the lungs where it may be mistaken clinically for

TABLE 96. SUMMARY OF THE CLINICAL INTERPRETATION OF EXAMINATIONS OF THE DEEP MYCOSES

Disease	Interpretation
Actinomycosis	The most frequent type of the deep mycotic diseases. World wide in distribution. Also occurs in wild and domestic animals (particularly cattle). Caused by the *Actinomyces bovis* which may occur as a saprophyte in the oral cavity and tonsillar crypts. Adults affected more frequently than children and particularly males engaged in agricultural pursuits. Trauma is frequently responsible for infection. Infection spread by continuity; also by the blood. May involve any part of the body. Microscopic examinations of the granules in pus, sputum, etc., are extremely valuable in diagnosis; cultivation of the fungus is difficult.
Mycetoma	Mycetoma, maduromycosis or Madura foot is rare in the United States but common in India. Caused by various species of Actinomyces. Maduromycosis is a second type caused by a variety of fungi. Microscopic examinations of the granules are valuable for diagnostic purposes.
Nocardiosis	A disease, which is also rare in the United States, characterized by skin lesions resembling sporotrichosis, or as gangrenous ulcers resembling pyoderma gangrenosum. May also produce pulmonary infections resembling those of tuberculosis; also brain abscesses. Believed to be due to infections with *Nocardia*, a species of *Actinomycetaceae*. The term *Cladothrix* is no longer employed. Readily detected by direct microscopic examinations of material; granules are not ordinarily produced.
Sporotrichosis	In the United States usually due to infection with *S. schenckii*. Local injuries by thorns, cacti, briars, etc., contaminated with the microorganism appear to be largely responsible for the disease. Infection may also occur through the eating of contaminated raw fruits or vegetables as well as through bites by wild rats or diseased individuals and by accidental inoculation with cultures. The localized lymphangitic type is most common in the United States; systemic infections may occur. Laboratory examinations are of great value in diagnosis and differential diagnosis and particularly cultures and animal inoculation tests.
Blastomycosis	The American form is chiefly encountered in the Middle West, particularly around Chicago. Due to infection with *Blastomyces dermatitidis*. Most cases occur among men. Trauma is an important predisposing factor. Usually begins as a local lesion on the exposed parts of the body; sometimes on the tongue. Systemic infections may involve any organ or tissue of the body, especially the lungs, and closely resemble granuloma coccidioides. Microscopic examinations of wet preparations of pus for budding cells valuable in diagnosis; also guinea-pig inoculation tests for the differentiation of systemic blastomycosis from granuloma coccidioides.

TABLE 96. SUMMARY OF THE CLINICAL INTERPRETATION OF EXAMINATIONS IN THE DEEP MYCOSES—(Continued)

Disease	Interpretation
Coccidioido-mycosis	Due to infection with *C. immitis*. The disease is practically confined to the San Joaquin Valley or persons living in California. Usually due to the inhalation of the spores of the fungus, with a primary infection of the lungs. May be also due to a primary infection of the skin in which trauma occasionally provides a portal of entry for the fungus. Systemic or generalized infection may occur by way of the blood in which almost any tissue or organ may become involved. Four types of the disease may occur: (1) primary pulmonary alone with good prognosis; (2) primary pulmonary followed by generalized infection; (3) primary pulmonary followed by secondary lesions of the skin and (4) primary cutaneous with later generalization (*granuloma coccidioides*). Microscopic examinations and cultures of pus, sputum, spinal fluid and other material are of diagnostic value; also guinea-pig inoculation and histologic examinations. Thorough clinical and mycological examinations are required for differential diagnosis between granuloma coccidioides and *South American blastomycosis* or paracoccidioidal granuloma due to infection with *B. braziliensis*.
Torulosis	Due to infection with *C. neoformans* or *T. histolytica.* The portal of entry is believed to be the upper respiratory tract. Produces *torula meningitis* which usually runs a prolonged course and is invariably fatal. *Cutaneous torulosis* may occur primarily followed by meningitis or secondarily to meningitis. Examinations of the spinal fluid for the micro-organisms and associated changes are extremely valuable in diagnosis.
Histoplas-mosis	A disease due to infection of the reticulo-endothelial system with *H. capsulatum.* Laboratory examinations are essential in diagnosis. These include biopsy, blood and sternal bone-marrow smears, cultures, histoplasmin skin tests, complement fixation and guinea-pig inoculation tests.
Aspergil-losis	Due to an infection of the lungs with *A. fumigatus* or *A. niger* and especially the former. The acute infection resembles bronchopneumonia and the chronic infection pulmonary tuberculosis. Examinations of sputum by smears and cultures are valuable diagnostic aids. But since Aspergilli are widespread in nature and may occur normally in sputums, laboratory diagnosis is only warranted upon finding large numbers in repeated examinations and by the careful exclusion of tubercle bacilli and *C. albicans.*

tuberculosis, and about 15 per cent elsewhere in the body (meningitis, vertebrae, skin) although primary actinomycosis of the skin is rare.

Microscopic examinations are extremely valuable; diagnosis is usually based upon finding the characteristic yellowish-white granules or flakes of the ray fungus in pus, sputum, etc., by direct microscopic examination. Cultures are difficult and not always successful; dextrose-veal infusion agar is recommended. Anaerobic methods are generally preferred. Cultures of the sputum showing Actinomyces may be misleading, as the micro-organism is frequently present in the oral cavity as a saprophyte. Animal inoculation tests with cultures are advisable as temporary lesions usually develop in about 50 per cent. Secondary bacterial infections are common in the later stages of the disease.

Mycetoma. This disease, which is also known as "Madura foot," is comparatively rare in the United States but common in India, especially in Madura. It develops as a subcutaneous swelling or tumor which becomes progressively larger with sinus tracts and the discharge of seropurulent exudates containing small, rounded and brittle granules which may be white, yellowish, reddish or blackish in color. The deeper tissues of the foot are frequently involved, accompanied by swelling and distortion. On the surface are numerous pea-sized eminences, with the orifice of a sinus tract in the center of each. Rarely, the hand or some other part of the body may be involved, but never the internal organs.

Mycetoma is caused by various species of Actinomyces; *maduromycosis* may be caused by a variety of fungi.[7] Many of these described as pathogenic may merely be growing saprophytically in the diseased tissues.

Microscopic examinations of the granules possess diagnostic value. Cultures are frequently successful but animal inoculation tests have so far been unsuccessful.

Rhinosporidiosis. This disease, which is caused by *Rhinosporidium seeberi,* is characterized by the formation of friable, sessile, or pedunculated polyps on the mucous membranes of the nose, nasopharynx, soft palate, conjunctiva, and rarely in the ear, vagina, on the penis, or on the skin. Twelve cases have been reported in the United States by Caldwell and Roberts[8] and other investigators. The organism occurs in polypoid masses as round, thick-walled sporangia filled with hundreds or thousands of spores. The mechanism of transmission is unknown.

Nocardiosis. This disease, which is also rare in the United States, is believed to be caused by Nocardia, a species of Actinomycetaceae which is a common saprophyte of the soil.

The lesions affecting the skin may resemble those of sporotrichosis or occur as gangrenous sloughing ulcers resembling pyoderma gangrenosum.[9] Metastatic lesions in the skin and brain may also occur[10] as well as pulmonary infections (pseudotuberculosis) ascribed to *Nocardia asteroides* which may be acid-fast but somewhat more readily decolorized than tubercle bacilli. Eppinger cultivated this micro-organism from a brain abscess about fifty years ago and called it *Cladothrix asteroides* but this nomenclature is no longer used.

The Actinomyces producing the disease do not usually produce granules[10] but are readily detected by microscopic examinations as rods and filaments with well-marked branching sometimes with bulbous or club-shaped ends.

Sporotrichosis. This disease is especially prevalent in France but occurs in the United States (particularly in the Mississippi Valley) and other parts of the world. While several members of the genus Sporotrichum have been considered the cause in this country, *S. schenckii* is apparently responsible for most infections.

It is a common saprophyte on vegetation and, according to Foerster,[11] particularly the barberry shrub as well as sphagnum moss, which Gastinau and his colleagues[12] have recently reported as responsible for an epidemic of sporotrichosis affecting six florists. The disease, therefore, may occur as an occupational dermatosis. Local injuries by thorns, cacti or briars contaminated with Sporotrichum appear to be largely responsible for the disease. De Beurmann, however, has shown that the micro-organism may penetrate the intestinal mucosa, and that infection may be contracted through the eating of contaminated raw fruits or vegetables. In isolated instances the disease has also been acquired from the bites of wild rats, from patients with the disea e and from cultures of the micro-organisms as laboratory infections.

Most of the reported cases in the United States have been of the localized lymphangitic type in which an indurated primary lesion occurs on the hands, arms or other exposed parts of the body, followed by abscess formation and indolent ulcers. Infection of the regional lymphatics occurs with secondary nodules, although the regional lymph nodes are not commonly involved, which helps to differentiate the disease clinically from tularemia.

The disseminated subcutaneous types, with or without ulceration, occur more commonly in France. An epidermal type has also been described as well as a systemic type involving the bones (particularly the tibia), joints, muscles, lungs and rarely the epididymis, meninges and gastro-intestinal tract. Allergic lesions or sporotrichids have been described by de Beurmann.

Differential diagnosis must exclude cancer, syphilis, tuberculosis, tularemia and other infections. Laboratory examinations, and especially cultures, are of great value. The micro-organism is difficult to find in wet preparations by direct microscopic examinations, although spores may be found in the pus. Cultures of pus on dextrose agar are particularly valuable, as the micro-organism is easily cultivated at room temperature. Most laboratory animals are susceptible to inoculation, particularly the white rat, in which animal the testes are especially vulnerable to infection.

Blastomycosis. The American form of blastomycosis is chiefly encountered in the Middle West, particularly around Chicago, but may appear sporadically in any section of the country. Most cases occur among men. The disease is due to infection with *Blastomyces dermatitidis*. The European type of blastomycosis (torulosis) is probably a separate entity.[13]

The micro-organism may have a saprophytic existence on plants, since numerous blastomyces are widespread in nature. Scratches, puncture wounds, bruises and the like are important predisposing factors to infection.

In the majority of cases the disease begins as a local lesion on the skin of the face, hands, wrists and forearms although any part of the body may be involved. In some instances it first involves the tongue. In systemic infections any organ or tissue may be attacked and especially the lungs (90 per cent of cases) characterized by acute infection with considerable pain followed by a syndrome resembling tuberculosis but with less tendency to cavitation. The kidneys, bones, joints, central nervous system, larynx and abdominal organs have all been reported as occasional sites of the disease.[14]

Microscopic examinations of pus expressed from small abscesses are valuable diagnostic aids, as budding cells are usually readily observed at once or after wet preparations have been ringed with vaseline and allowed to stand for a few hours. Examinations are more difficult and less satisfactory in the case of sputums or the secondary ulcers on the skin. The systemic form so closely resembles granuloma coccidioides that differential diagnosis is only possible by laboratory methods and especially by inoculation of guinea-pigs which are highly resistant to *B. dermatitidis* but highly susceptible to *Coccidioides immitis*.

Geotrichosis. This disease, which is caused by *Geotrichum candidum*, is characterized by infection of the bronchi with persistent cough.[15,16] The sputum is white, mucoid, and contains grayish flakes which occasionally are streaked with blood; x-ray examination usually shows a diffuse, peribronchial thickening. There is little systemic reaction, and the general health of the patient is good. The disease may resemble tuberculosis, since smooth dense areas of infiltration with or without thin-walled cavities may occur. Lesions may also occur in the mouth (oral geotrichosis) and of the skin, resembling those due to infection with *C. albicans*.

The organism may be identified by microscopic examinations of material removed from lesions or occurring in sputum; it grows quickly on Sabouraud's glucose agar.

Coccidioidomycosis. This term has been proposed by Dickson[17] to include all types of infection with *C. immitis*. The fungus has been isolated from soil, from plants and from the internal organs of slaughtered sheep and cattle. No instance has been recorded of transmission of any type of the disease from one human being to another. It is practically confined to the San Joaquin Valley of California. Scattered cases have occurred elsewhere but in most instances the patients have lived in California.

The most frequent type of the disease occurs as an acute primary infection of the lungs resembling influenza or bronchopneumonia in which roentgenograms show parenchymatous involvement. It is due to the inhalation of the chlamydospores of the micro-organism.[18] This acute infection has been reported only from the San Joaquin Valley of California where the disease has long been known as "valley fever" or "desert fever." A person of any age and either sex may be affected. Commonly, from eight to fifteen days after the onset nodules of erythema nodosum develop on the shins or in other areas, disappearing spontaneously in four or five days. Recovery from this acute infection usually occurs in three to six weeks. In 354 cases Dickson has reported only a single death from coccidioidal meningitis.

A second type of the disease also begins with primary infection of the lungs followed by general infection by way of the blood in which almost any tissue or organ may become involved except the skin. A third type also begins with primary infection of the lungs followed by secondary lesions of the skin. A fourth and well-known type begins with primary infection of the skin producing the form known as *granuloma coccidioides* which is likewise usually followed by a generalized infection. In this type an injury of the skin, *e.g.*, from a puncture by a cactus spine, or abrasions from picking walnuts, may occasionally provide a portal of entry for the fungus. Most of the patients are laboring men, usually farmers, a high percentage being Mexicans.

The more superficial cutaneous lesions develop as granulomas, which eventually ulcerate. Healing may occur, or granulomas and papillomatous lesions result. The pus present in active lesions is thick, yellowish gray and ropy. Temporary healing results from formation of scar tissue. Disfigurement and limitation of movement may be produced.

When generalization occurs the bones and joints are particularly involved with a type of destructive osteomyelitis accompanied by slight pain and tenderness. Pus may burrow to the surface of the skin and fistulas may form. The meninges, liver, kidneys, spleen and heart may become involved although the stomach and intestines escape infection. As previously stated, the prognosis is favorable in cases of acute primary infections of the lungs but remissions may occur, and relapse after four or more years is not unknown. Otherwise, however, many cases terminate fatally and especially in the case of persons newly arrived in the endemic territory. It has been stated that when patients develop cutaneous lesions on the face the outlook is hopeless.

Microscopic examinations of pus, sputum, spinal fluid and other material usually show the presence of the micro-organism and have proved valuable in diagnosis; likewise cultures and guinea-pig inoculation tests. Differential diagnosis requires the exclusion of tuberculosis, syphilis, and pyogenic infections. An early cutaneous lesion of blastomycosis is not likely to be confused with granuloma coccidioides, but when the process becomes extensive, particularly when involvement of the internal organs occurs, clinical differentiation of the two mycotic diseases may be impossible without cultural and histologic examinations.

South American blastomycosis or paracoccidioidal granuloma (Almeida's disease) is a similar disease [19] caused by infection with *Blastomyces braziliensis* encountered only in South America. In some respects it resembles granuloma coccidioides so that thorough clinical and mycologic examinations are required for differential diagnosis.[20]

Torulosis. This disease, which is also known as cryptococcosis or European blastomycosis, is due to infection with a yeast-like micro-organism, *Cryptococcus neoformans* or *Torula histolytica*. It is commonly found as a saprophyte on the skin and also in the throat and gastro-intestinal tract; likewise on many plants. It is probable that some strains acquire virulence.

Infection of human beings apparently occurs through the upper respiratory tract. Men are affected twice as frequently as women.[21] The symptoms are usually referable to the central nervous system with the production of *torula meningitis* which usually runs a protracted course with progressive loss of weight. After several weeks, months, or even years, the patient becomes comatose and dies of respiratory failure. In other words, the meningitis is invariably fatal which may be partly due to the fact that in most cases diagnosis is not made until after death and suitable treatment has not been instituted. A total of 74 cases have been reported.[22]

Examinations of the spinal fluid are required for diagnosis and usually show not only the yeast cells but an increase of pressure, increase of albumin and globulin, pleocytosis due to an increase of lymphocytes and a colloidal gold curve of the meningitic type with a negative Wassermann reaction. The micro-organisms are best seen in smears of sediment stained by the method of Gram or treated with India ink.[5] Cultures of the sediment on Sabouraud's dextrose agar and the inoculation of mice and rats are likewise valuable diagnostic procedures.

Cutaneous lesions alone or associated with the meningitis (*cutaneous torulosis*), are very seldom noted. These consist of granulomas with enormous numbers of giant cells of the foreign body type, very little inflammatory reaction, and a peculiar form of very rapid caseation which may lead to ulceration.[23] In some cases subcutaneous and deep-seated nodules develop resembling ecchymoses, ranging from small plaques to lesions the size of the hand and having no tendency to ulceration.[24] The lesions may regress spontaneously in four to six weeks. In all instances cutaneous torulosis results in the meningitis.

Generalization, with particular involvement of the lungs, is a rare possibility. Widespread visceral involvement with Hodgkin's disease has been reported.[25]

Histoplasmosis. Systemic histoplasmosis is due to infection of the cells of the reticulo-endothelial system with *Histoplasma capsulatum*. When the disease was first described by Darling [26] in 1908 the organism found in tissue sections was thought to be a protozoan parasite. Da Rocha-Lima subsequently suggested that it was a yeast-like fungus; this was proved by De Monbreun [27] in 1934 by the successful cultivation of the fungus from the blood and spleen of an infant. Its habitat in nature and modes of transmission are still unknown. Primary infection of the skin and adjacent mucous membranes apparently occurs; likewise systemic infections through the respiratory and gastro-intestinal tracts with the production of secondary cutaneous and mucosal lesions.[28] Clinical histoplasmosis is relatively rare but if it is true that positive histoplasmin skin reactions in pulmonary calcifications are due to subclinical infections with *H. capsulatum*, the disease is by no means uncommon. In the United States the endemic area is largely confined to the central states.[29]

The onset is insidious with weakness and loss of weight followed by severe sweats (particularly at night), splenomegaly, hepatomegaly, lymphadenopathy, irregular fever, secondary anemia and leukopenia. About 50 per cent of cases show cutaneous and mucosal lesions, especially about the face and neck. The disease must be differentiated from syphilis, tuberculosis cutis, basal-cell epithelioma, blastomycosis, chromoblastomycosis, lymphogranuloma inguinale, leishmaniasis, etc. It is refractory to treatment and almost invariably fatal. Some cases have occurred in association with leukemia, Hodgkin's disease and disseminated miliary tuberculosis.

Laboratory examinations are essential for diagnosis. Biopsy examinations for the presence of the fungus in sections of tissue are of most value; portions of the tissue should be kept in sterile saline solution at a low temperature for cultures if negative results are observed.[30] Examinations of the blood for parasites within the monocytes and of sternal bone marrow for their presence in macrophages are also helpful. When stained by Wright's method the parasites appear as bluish or bluish-red, oval cells, from 2 to 4 microns in diameter, surrounded by a clear capsular-like substance; they must be differentiated from the leishmania and toxoplasmosis.[5] *H. capsulatum* is readily cultivated on Sabouraud's dextrose agar, blood agar, or semisolid veal infusion blood agar. Blood cultures may be of diagnostic value; likewise complement fixation tests.[31] Interperitoneal inoculation of guinea-pigs usually produces a chronic, afebrile, systemic disease with pathologic changes resembling those occurring in human beings.

In clinical cases histoplasmin skin tests also possess diagnostic value, as discussed in Chapter 19. Unfortunately, however, positive reactions may be observed in healthy individuals with pulmonary calcifications and negative tuberculin reactions, as first reported by Smith [32] and confirmed by Palmer [33] and other investigators. Some believe that these positive reactions are due to previous subclinical or unsuspected infections with *H. capsulatum;* cross positive histoplasmin skin reactions have been observed in guinea-pigs infected with Blastomyces, Coccidioides, Haplosporangium and Candida.[34]

Aspergillosis. Aspergillosis is an uncommon disease of the lungs due to infection with *Aspergillus fumigatus* or *A. niger* and especially the former. Pigeons, parrots and other birds are susceptible to the infection and may be carriers. At least, bird fanciers and grain handlers are said to be among the most frequent victims of the disease.

Pulmonary aspergillosis may be acute and resemble a bronchopneumonia, or chronic, resembling pulmonary tuberculosis.[35,36] Hemoptysis is common. Aspergilli may also cause onychomycosis, osseous infections, dacrocystitis, blepharitis and adenitis; asymptomatic infections may also occur.

Diagnosis is usually made by a history of exposure, roentgenologic studies and laboratory examinations of the sputum for Aspergilli by direct and cultural methods. Since Aspergilli are widespread in nature, with at least 350 different species, they may occur normally in the sputum. Consequently, laboratory diagnosis is only warranted by finding large numbers on repeated examinations and by the careful exclusion of tubercle bacilli and *C. albicans.*

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THE CLINICAL INTERPRETATION OF SEROLOGIC EXAMINATIONS

Serologic examinations consist of various antigen-antibody tests conducted with serum, plasma, spinal or other body fluids. Under proper technical conditions they are based upon the specificity of reactions *in vitro* between antigens and their antibodies and have proved of clinical value not only in the diagnosis of some bacterial, spirochetal, rickettsial and viral diseases, but likewise in some of the diseases due to animal parasites. Furthermore, at least in syphilis, serologic examinations are of clinical value in relation to treatment on the assumption that the reduction and disappearance of antibodies from the body fluids are indicative of recovery from infection.

METHODS FOR THE COLLECTION OF BLOOD

Unless otherwise stated, blood for the preparation of serum may be collected from a vein or a finger, as it appears that the antibody content is practically the same in both. However, venous blood is preferred because it may be collected aseptically and in larger amounts, yielding an excess of serum, which is always advisable in case serologic tests require repetition. Precautions should be taken against bacterial contamination, especially if the tests are delayed four days or longer, since serums clouded by bacteria are always apt to be anticomplementary in complement fixation tests, as well as sometimes unsatisfactory in agglutination and precipitation or flocculation tests. For the same reasons, blood should not be taken immediately after a meal because chylous serums are also apt to be unsatisfactory, although this is not as important as in the collection of blood for chemical examinations.

If the specimen of blood is subject to agitation, as in the mails, the container should be of such size as to be filled with blood, or almost so, in order to reduce to a minimum the amount of agitation and consequent hemolysis. Otherwise, it is better to separate the serum before mailing although whole blood is always preferred for complement fixation tests, since serums left on their clots are not as likely to acquire anticomplementary properties (provided marked hemolysis has not occurred) as separated serums.

Blood may also be collected in sodium citrate, as plasma is generally satisfactory for serologic tests. Potassium oxalate, however, should not be used as it frequently renders plasma anticomplementary in complement fixation tests. In other words, blood collected for the preparation of plasma or for preservation in "banks" is generally satisfactory.

Technic of Venipuncture. In the great majority of cases the veins of the forearm and especially those about the elbow, are employed, *i.e.*, the median cephalic, median basilic, common ulnar and radial veins (Fig. 22). The median, ulnar and cephalic veins are also employed; indeed, any prominent, *well-supported* vein may be used, but the median basilic is probably the least desirable for the inexperienced because of the proximity of the internal cutaneous nerve and because it is separated from the brachial artery by the biceps fascia which may be pierced by clumsy technic.

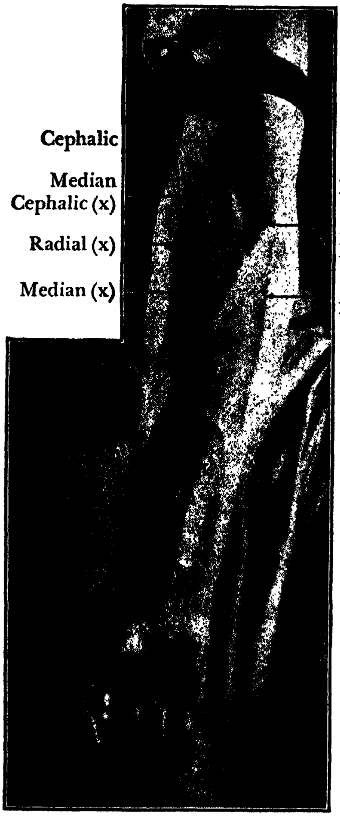

Fig. 22. Choice of Veins for Securing Blood

The sites of choice are marked with an x. It is always advisable to avoid punctures near the crease of the elbow in order to avoid subsequent discomfort. (From Kolmer, *Chemotherapy with Special Reference to the Treatment of Syphilis,* W. B. Saunders Co.)

The most prominent vein is not always the easiest to enter because it may roll under the needle; under such circumstances it is especially advisable to use a short and very sharp needle. The best veins are those which are full but well supported by the subcutaneous tissues to prevent rolling and dimpling while the needle is being introduced.

In some cases the veins about the wrists or on the back of the hand may be employed; however, never by choice but only by necessity, since they are small, very thin, easily pierced and likely to give subcutaneous hemorrhages. The same is true of veins about the ankle. When the veins are employed the needle must be short, very sharp and not larger than gauge No. 20 or 22.

The practical importance of a proper tourniquet is not to be overlooked. It should be at least an inch wide in order to produce the minimum of discomfort and should be applied in such a manner that it may be released instantly and with no commotion.

In many instances a tourniquet may be improvised by twisting a towel, a piece of bandage or the shirt sleeve, which is held by an assistant or the patient, but these procedures are inferior to a piece of ordinary garter elastic or a strip of rubber sheeting held by a slip knot. Sometimes an assistant, or even the patient, may firmly grasp the arm and distend the vein sufficiently. The ordinary surgical tourniquet is too clumsy and generally unsatisfactory; the tourniquet of rubber tubing held by a hemostat is only a makeshift, apt to wrinkle and pinch the skin. The cuff of a sphygmomanometer makes a most excellent tourniquet, being broad and comfortable and readily applied and released. A satisfactory tourniquet consists of 15 inches of good garter elastic, about an inch wide, applied with one firm turn and held by a slip knot (Fig. 23) which is released instantly by a slight pull without disturbance. The tourniquet should be applied flat without wrinkling or pinching the skin.

After applying the tourniquet the patient should open and close the hand vigorously for a few seconds and keep the hand clenched during the introduction of the needle; this aids in distending the superficial veins.

The tourniquet should not be applied too soon; all should be in readiness, as the most comfortable tourniquet becomes very uncomfortable when venous stasis is maintained over several minutes.

For simple venous puncture it is sufficient to prepare the skin by cleansing with 1:1000 mercurophen or bichloride of mercury in alcohol; tincture of iodine (5 per cent) or a solu-

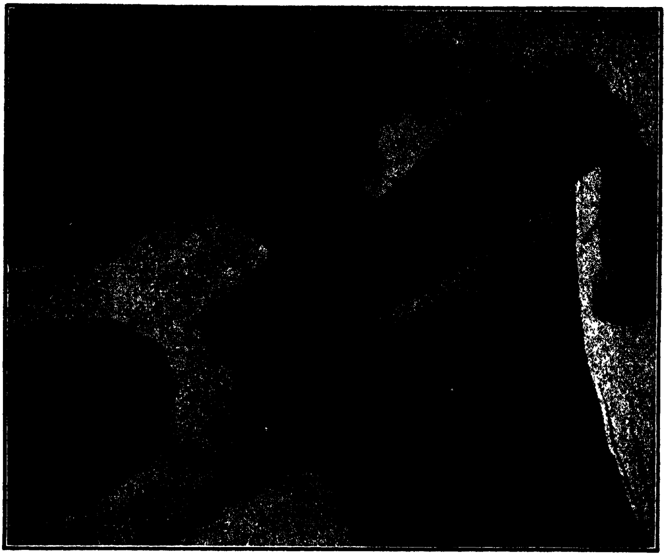

Fig. 23. Showing the Details of a Satisfactory Slip-Knot Tourniquet of Ordinary Garter Elastic

(From Kolmer, *Chemotherapy with Special Reference to the Treatment of Syphilis,* W. B. Saunders Co.)

tion of picric acid (5 per cent) may be applied but, if so, they should be wiped away with alcohol so as not to hide the vein.

If it is necessary to incise the skin there should be thorough preliminary cleansing with soap and water, followed by iodine and a final cleansing with alcohol in order to guard against infection of the skin to prevent scar formation as much as possible.

The vein should be steadied with the thumb and index finger; some physicians prefer to stretch the skin slightly over the vein to steady it although this tends to flatten the vein and increase the chances of transfixion. Various clamps have been devised for grasping and steadying the vein through the skin but there is no better means than the finger and thumb of the disengaged hand.

The needle should be held at an acute angle to the arm (Fig. 24); it is a mistake to attempt entering the vein at a right angle because of the danger of passing through the vessel. It is also good practice to puncture the vein obliquely, that is, with the needle directed to the side of the vein rather than directly over its top and especially if the vessel is very prominent, sclerotic and freely movable. When an attempt is made to puncture directly over the vein the needle sometimes slips over its top and passes into the peri-

FIG. 24. CORRECT ANGLE FOR HOLDING A SYRINGE FOR ENTERING A VEIN
(From Kolmer, *Chemotherapy with Special Reference to the Treatment of Syphilis*, W. B. Saunders Co.)

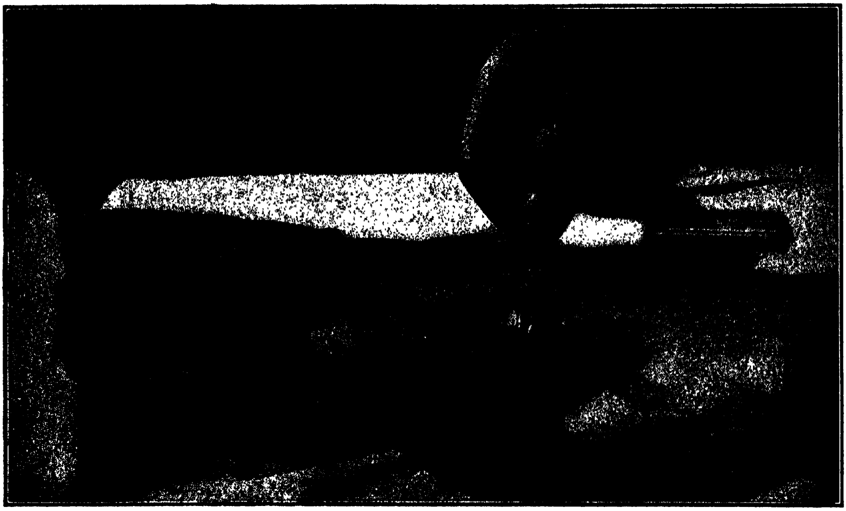

FIG. 25. SECURING BLOOD WITH THE KEIDEL TUBE; METHOD OF PASSING THE NEEDLE
(From Kolmer, *Chemotherapy with Special Reference to the Treatment of Syphilis*, W. B. Saunders Co.)

vascular tissues. However, when the vein is not prominent but is felt full and elastic in the subcutaneous tissues, one may enter the needle directly over the vein as the vessel is well supported and unable to roll.

With the Keidel Tube. One of the most satisfactory methods of obtaining blood from a vein quickly, aseptically and with the least pain, is by means of the Keidel tube. As made at present, this very useful apparatus is available with a glass window permitting one to see a flow of blood, and thereby indicating that the vein has been entered, before the neck of the ampule is broken. The apparatus is dispensed sterilized and ready for use as follows:

1. The patient may sit beside a table or desk with the arm extended and elbow supported.
2. The tourniquet is applied above the elbow and the patient requested to open and close the hand vigorously.

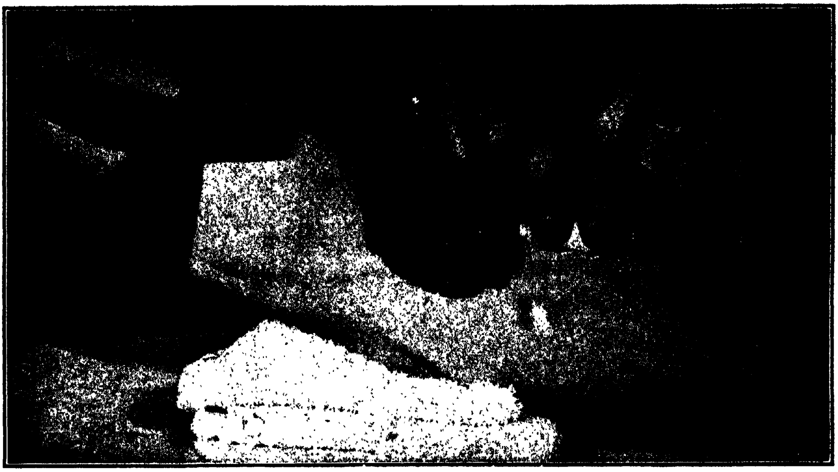

FIG. 26. SECOND STEP IN SECURING BLOOD WITH THE KEIDEL TUBE

Blood is seen in the "window" indicating that the needle is in the lumen of the vein; the neck of the ampule is being broken to release the vacuum followed by suction of blood. (From Kolmer, *Chemotherapy with Special Reference to the Treatment of Syphilis*, W. B. Saunders Co.)

3. The skin is wiped with cotton and alcohol.
4. The wire is removed and the hub of the needle grasped (avoiding contamination of the shaft) between the thumb and first finger (Fig. 25) and entered through the skin into the vein at an acute angle to the arm.
5. As the vein is entered there is a peculiar "give-away" sensation readily appreciated by experience; if the "window" type of apparatus is employed, a flow of blood is seen.
6. The long stem of the ampule in the rubber tubing is now crushed with the fingers or by a hemostat (Fig. 26) and the ampule filled with blood by suction owing to the release of the vacuum.
7. If blood does not flow the ampule may be rotated, in case the lumen of the needle is in contact with the inner wall of the vein. If it is apparent that the needle is not in proper position it may be partly withdrawn and another attempt made, due care being taken not to withdraw the eye of the needle from beneath the skin, which permits the entrance of air, in case the neck of the ampule has been broken.
8. The tourniquet should be released before the needle is withdrawn to prevent subcutaneous hemorrhage.

9. The site of puncture is compressed for a few seconds and cleansed with alcohol; a dressing is not required.

10. If the blood is to be mailed it is necessary to bend down the rubber tubing and hold it in this position by a rubber band or tie it with a piece of string, to prevent leakage of blood or serum in transit.

With a Syringe. Blood may also be obtained from a vein in the same manner by means of a sterilized 5 or 10 cc. Luer or Record syringe fitted with a No. 20 to 22 needle (Fig. 24); it is not necessary to use a larger needle which inflicts unnecessary pain. The needle should be short and not over 1¼ inches; long needles interfere with the flow of blood and are

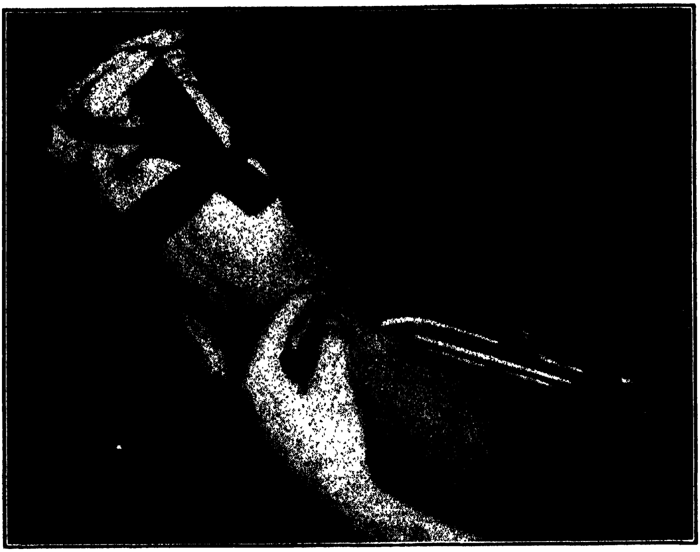

FIG. 27. METHOD OF WITHDRAWING BLOOD WITH A NEEDLE

(From Kolmer, *Chemotherapy with Special Reference to the Treatment of Syphilis*, W. B. Saunders Co.)

more difficult to enter into a vein. The bevel must be short rather than long but not too stumpy; it is almost imperative to use a sharp and rust-free needle to conduct the operation with the minimum of pain and without leaving a stained puncture site in the skin.

With a Needle. Blood may be secured from a vein by merely entering a sufficiently large sterilized needle and allowing it to flow into a prepared test tube or vial. For this purpose the needle should be gauge 18 to 20 since suction is not applied, and the shaft should not be too long as otherwise coagulation may occur. It is of advantage to grasp the needle with a hemostat as shown in Figure 27. This method, however, is apt to be "messy" and is inferior to the Keidel tube and syringe methods.

By Puncture of a Finger. From 3 to 5 cc. of blood is readily obtained from a finger. In obese individuals with difficult veins or in nervous individuals who are almost sure to faint if venous puncture is attempted, this method is satisfactory although it requires a little more time and patience. The technic is as follows:

1. The hand should be warm. If cold and clammy, have the patient immerse the hand in hot water for a few minutes, followed by brisk drying.

2. Select the middle or ring finger; cleanse with alcohol and dry.

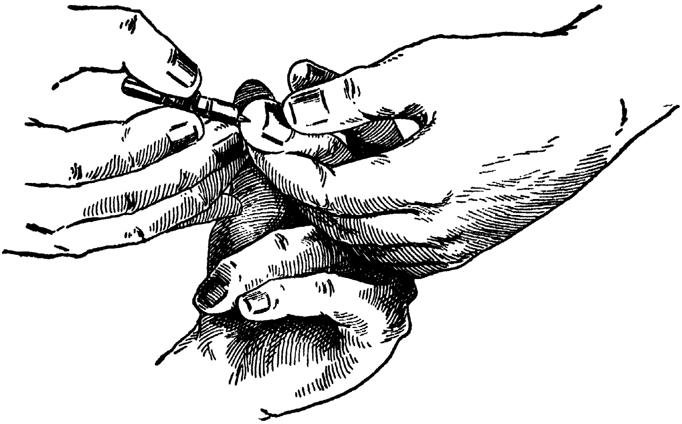

FIG. 28. METHOD OF PRICKING A FINGER

(From Kolmer, *Infection, Immunity and Biologic Therapy,* W. B. Saunders Co.)

3. Cleanse the broad blade of a Daland blood lancet with alcohol and puncture deeply across the lines of the tip of the finger (Fig. 28); there is very little pain.
4. Massage the blood into a small test tube or vial; it is a mistake to use a large receptacle as blood is wasted on its walls (Fig. 29).

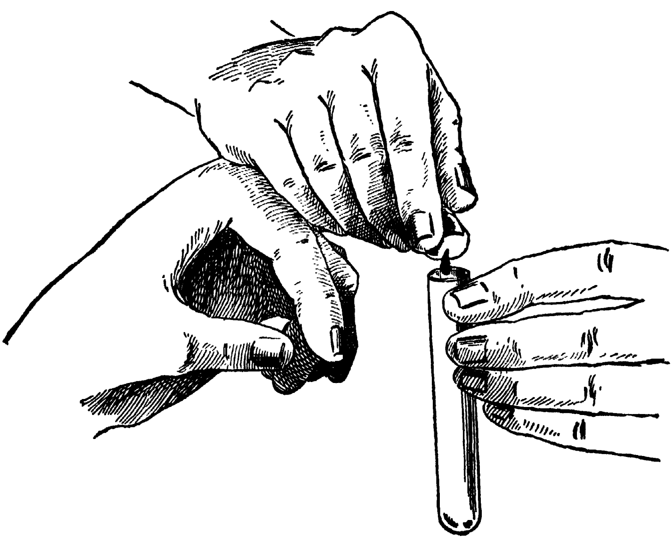

FIG. 29. METHOD OF SECURING A SMALL AMOUNT OF BLOOD FROM A FINGER

By pricking the finger deeply *across* the lines of the skin with a *broad* lancet 2 cc. or more of blood is easily collected in a small test tube. Do not use a large tube, as blood may be lost on its sides. (From Kolmer, *Infection, Immunity and Biologic Therapy,* W. B. Saunders Co.)

5. Cleanse the finger and apply compression for a few seconds. A dressing is not ordinarily required unless bleeding continues.

Securing Blood from Infants and Children. A few cubic centimeters of blood may be readily obtained from infants by puncturing one of the great toes with a Daland lancet and massaging the blood into a small test tube, as described above for securing blood from the finger.

In the case of children from 1 to 6 years of age sufficient blood may be obtained by puncture of one or several fingers. In children over 6 years of age, a vein may be entered and blood drawn with a Keidel tube or syringe, as above described.

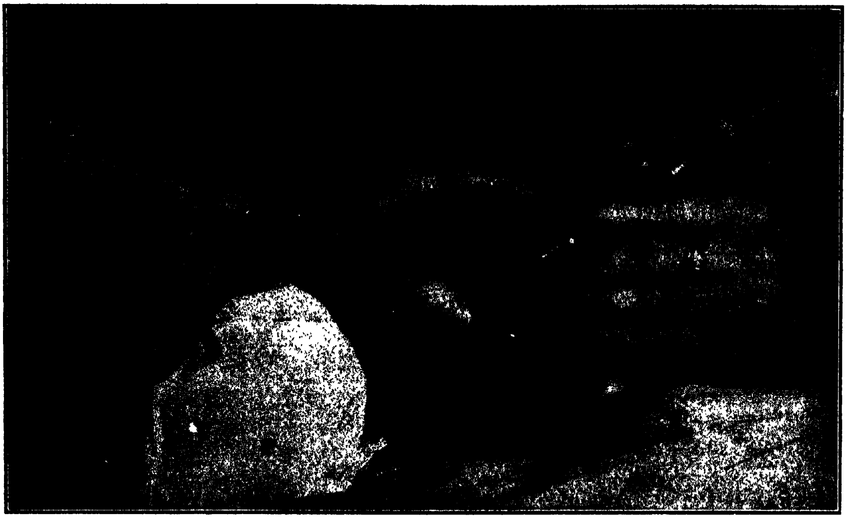

FIG. 30. METHOD OF OBTAINING BLOOD FROM A TEMPORAL VEIN

(From Kolmer, *Chemotherapy with Special Reference to the Treatment of Syphilis*, W. B. Saunders Co.)

Blood may be obtained with a syringe from one of the external jugular veins or from a temporal vein, as shown in Figure 30.

From infants under one year of age blood may be secured by puncture of the superior longitudinal sinus as follows:

1. The infant is wrapped in a blanket and the head is steadied by an assistant.

2. The puncture is made on the median line of the posterior angle of the anterior fontanelle.

3. The skin is carefully cleansed. The needle, gauge No. 18, with a short bevel, sterilized and attached to a sterile 5 cc. Record or Luer syringe, is passed inward at a right angle for a distance of about 4 mm. and suction made; if blood does not flow, the needle should be passed about 2 mm. further, which suffices for the majority of children up to fifteen months of age.

4. At least 3 to 5 cc. of blood may be safely withdrawn and discharged into a vial. The puncture site is then cleansed and may be sealed with a touch of collodion.

5. Blood may also be secured from children by cupping (Fig. 31).

A ***method for the collection of cerebrospinal fluid*** is described in Chapter 14.

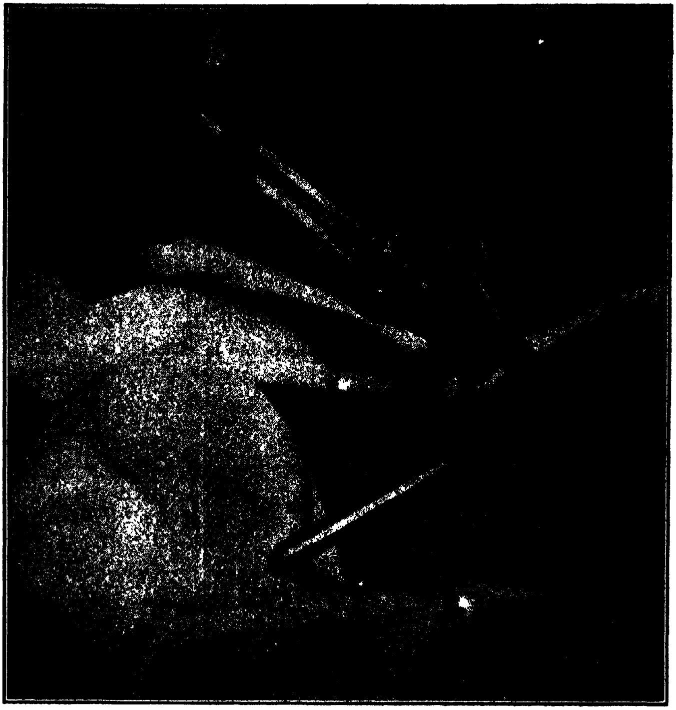

FIG. 31. METHOD OF OBTAINING BLOOD BY CUPPING

The child is seven years of age; the Blackfan apparatus is being used and blood collected from a scarified area into a small test tube. (From Keen's *Surgery*, W. B. Saunders Co.)

ANTIBODIES IN RELATION TO SEROLOGIC EXAMINATIONS

In general terms, antibodies are substances produced by the body cells in response to stimulation by antigens and reacting specifically with them *in vivo* or *in vitro,* although natural antibodies, occurring normally in the blood but not in the cerebrospinal fluid, may be of genetic origin with no ascertainable connection with antigenic stimulation. They are either globulins or are associated so closely with them that their behavior is in large part determined by that of the globulin molecule. As far as acquired antibodies are concerned, it is possible that they may be produced by various body cells but available evidence indicates that they are largely produced by the cells of the reticulo-endothelial system.

Kinds of Antibodies. Knowing little or nothing of their real nature when discovered, it was natural to adopt the plan of naming each antibody according to its action when mixed with its antigen. Thus, the antibody capable of neutralizing a toxin was an *antitoxin;* that which caused a clumping or agglutination of bacterial or other cells, an *agglutinin;* that which gave a precipitate with an antigen in solution, a *precipitin;* that sensitizing a cell to the effects of complement and resulting in its lysis or solution, a *lysin*—a *hemolysin* if it acted upon erythrocytes, a *bacteriolysin* if upon bacteria or a *cytolysin* or *cytotoxin* if on some other kind of cell; that which rendered a bacterial or other cell more susceptible to phagocytosis an *opsonin* or *bacteriotropin;* and that which produced anaphylactic or allergic sensitization an anaphylactin or *allergin* (Table 97). It is now generally believed, however, that these are the same antibody doing different things with its antigen, according to the physical state of the latter, the nature of the co-operative substances (complement, leukocytes, tissue cells), and the environmental conditions under which the tests are made. This *unitarian hypothesis,* however, does not in any way modify the assumption that a multiplicity of antibodies may be produced corresponding to a multiplicity of antigens. For example, a complex antigen like the typhoid bacillus may produce one antibody against the major antigenic constituent and additional antibodies against other constituents enclosed in the same cell. In other words, it simply implies that a chemically pure antigen produces one antibody, and one only.

TABLE 97. SUMMARY OF ANTIBODIES AND ANTIGENS IN RELATION TO SEROLOGIC EXAMINATIONS

Subject	Serologic Applications
Antibodies	Serologic tests are only applicable in case antibodies are present in serums and spinal or other body fluids. Antibodies are named according to their behavior *in vitro* or *in vivo*. Natural antibodies do not occur in the normal cerebrospinal fluid. None are utilized in serum diagnosis except isohemagglutinins. But many are concerned in the specificity of agglutination, complement fixation and other serologic tests. Since varying periods of time are required for the production of acquired antibodies, serologic tests are usually inferior to bacteriologic, mycologic and parasitologic examinations in the *early* diagnosis of many diseases. The living agents of disease vary greatly in their capacity for producing antibodies in the blood. Antibodies may be produced by nonspecific stimuli and lead to diagnostic errors (the *anamnestic reaction*). Group antibodies occur which may lead to errors in serologic diagnosis. Agglutinin and hemolysin for sheep corpuscles occur normally or naturally in human serums. Their origin is unknown but they are usually increased in infectious mononucleosis and serum disease (*heterophil antibodies*). Serologic examinations for diagnostic purposes are of two kinds: (1) tests for suspected antibodies in the presence of known antigens and (2) tests for suspected antigens in the presence of known antibodies.
Antitoxins	Antitoxins may be detected by serum-neutralization tests in the lower animals but are not ordinarily employed for diagnostic purposes. Schick and Dick skin tests for natural or acquired antitoxins in diphtheria and scarlet fever are preferred.

TABLE 97. SUMMARY OF ANTIBODIES AND ANTIGENS IN RELATION TO SEROLOGIC EXAMINATIONS—(Continued)

Subject	Serologic Applications
Virucidins	Protective or virus-neutralizing antibodies are produced in the blood in many of the diseases of man due to the filtrable viruses. They may be detected by serum-neutralization tests in the lower animals.
Agglutinins	Agglutination tests are very valuable in the serum diagnosis of typhoid, paratyphoid and undulant fevers, glanders, tularemia and leptospirosis while helpful in the diagnosis of some other bacterial diseases. They are also of value in the detection of typhoid, paratyphoid and brucella carriers as well as for the identification of typhoid and paratyphoid bacilli, pneumococci, streptococci, meningococci, etc., recovered in cultures. Bacterial conglutination tests are not employed. However, they are of but limited or doubtful clinical value in the diagnosis of mycotic diseases and those due to viruses and protozoa. Agglutination tests are extremely valuable for the determination of blood groups in relation to blood transfusion, the administration of plasma and in medicolegal applications.
Precipitins	Precipitin tests are very valuable in the serum diagnosis of syphilis although they are properly designated flocculation tests. Precipitin tests may be employed in the serum diagnosis of various bacterial diseases by the detection of precipitinogens in sputum, urine, spinal fluid, pus and tissues but are of limited clinical value. They have not proved of value in the serum diagnosis of mycotic diseases except, possibly, in some of the deeper mycoses. Nor have they proved of value in the diagnosis of diseases due to the rickettsiae and filtrable viruses. Precipitin tests, however, are of clinical value in the diagnosis of trichinosis and hydatid cyst disease but of little or doubtful value as aids in the serum diagnosis of other diseases due to animal parasites. Precipitin tests are very valuable for the detection and species identification of blood stains and meat adulteration.
Lysins	The Pfeiffer bacteriolysis test is of value in the identification of *Vibrio cholerae* recovered in cultures of the stools of Asiatic cholera and carriers. Tests for bacteriolysins or bactericidins in whole coagulated blood may be of value in the selection of some bacteria for incorporation in autogenous vaccines ("pathogen-selective" method of Cohen). Immune hemolysins are employed in complement fixation tests. Cytotoxins in human serums are without value in the serum diagnosis of malignant disease.

TABLE 97. SUMMARY OF ANTIBODIES AND ANTIGENS IN RELATION TO SEROLOGIC EXAMINATIONS—(Continued)

Subject	Serologic Applications
Complement Fixation	Apparently any antibody like agglutinin, precipitin and lysin may produce complement fixation and especially precipitins. The origin and nature of the antibody or reagin concerned in the Wassermann reaction, however, are unknown. Complement fixation reactions are highly specific and very sensitive when properly conducted with special reference to antigens. The Wassermann and flocculation reactions in syphilis, however, are biologically nonspecific, since the antigen is composed of alcohol-soluble lipids of beef heart. Complement fixation reactions have been found of great clinical value not only in serum diagnosis of spirochetal diseases (syphilis, yaws, etc.) but likewise in some of the bacterial, rickettsial, and viral diseases and those due to animal parasites as well as in the detection and identification of blood stains, the detection of meat adulteration, etc.
Opsonins	Opsonins are antibodies which render bacteria, other cells and foreign substances more susceptible to phagocytosis. They are, therefore, of great importance in relation to natural and acquired immunity. They were formerly employed for diagnostic purposes and in relation to vaccine therapy (opsonic and phagocytic indices) but owing to technical difficulties and many possible sources of error, are not used at present for clinical purposes except the opsonocytophagic test of Huddleson in the serum diagnosis of brucellosis.
Anaphylactins, Allergins and Reagins	*Anaphylactin* is the antibody producing anaphylaxis in the lower animals by active or passive sensitization of the body cells. It is identical with precipitin or closely related to it. *Allergin* is the name frequently given to the antibody producing allergy in human beings. *Reagin* is the term commonly employed for designating the antibody involved in those allergic diseases of human beings influenced by heredity (hay fever, asthma, eczema). It is characterized by its capacity to sensitize the skin. Reagins can be demonstrated in the blood by the passive method of Prausnitz and Küstner which is sometimes employed in the etiologic diagnosis of allergy.
Antigens	In the usual sense antigens are any substances capable of stimulating the production of antibodies. The term is also applied, however, to substances reacting with antibodies *in vitro*. Antigens may be complete or partial (haptens). Complete antigens are usually foreign proteins of large molecular size; complex polysaccharides may also be complete antigens. Protein-free lipids are not capable of producing antibodies but may react with them *in vitro* as in the serum tests for syphilis. A single bacterial cell may contain several antigens which may be important in relation to serum diagnosis. Methods employed for the preparation of antigens for serologic examinations are important in relation to both the sensitivity and specificity of the reactions.

Antibodies occurring normally in the blood are called *natural antibodies*. They may also occur in transudates, exudates, colostrum or milk but, as previously stated, they do not occur normally in the cerebrospinal fluid or other secretions and excretions like the bile, urine, saliva or tears. Antibodies produced during clinically recognizable and unrecognizable infections, as well as by active immunization with vaccines, are known as *acquired antibodies*. They likewise occur in large amounts in the serum or plasma as well as in the lymph and cerebrospinal fluids. Undoubtedly, both kinds of antibodies play an important rôle in natural and acquired immunity to disease and recovery therefrom (*humoral immunity*) but, on the other hand, it is a reasonable assumption that they may also be sessile or cellular in situation (*tissue immunity*). For this reason immunity may be present without antibodies in the blood or other body fluids detectable by serologic examinations. In other words, only humoral or circulating antibodies are ordinarily available for serological diagnosis by methods commonly employed.

Natural Antibodies. A surprisingly large number of natural antibodies to various bacteria and other antigens, however, may occur normally in serums. None are employed in serologic diagnosis except the agglutinins (isohemagglutinins) occurring in the serum of one individual for the erythrocytes of another in relation to blood transfusions and the administration of plasma as well as in relation to medicolegal applications, with special reference to the determination of paternity.

The source of these natural antibodies is unknown. Undoubtedly, however, the isohemagglutinins and isohemolysins are genetic in origin, since their occurrence and transmission follow the mendelian laws of heredity. Others, and especially those for the various bacteria and other living agents of disease, are undoubtedly acquired in some instances at least by reason of clinically unrecognizable or unsuspected infections. It is also possible that natural antibodies may be produced through antigenic stimulation by group antigens in bacteria as well as in air-borne and ingested substances. Be that as it may, however, their occurrence in serums is always to be reckoned with in serologic tests from the standpoint of both sensitivity and specificity, since only a definite increase of antibody over and above the normal is of diagnostic significance.

Acquired Antibodies. Otherwise, serologic examinations are based on the detection of acquired antibodies, the result of clinically recognizable or unrecognizable infections with the living agents of disease, or the result of stimulation of the reticulo-endothelium of the body by other antigens.

Unfortunately, however, varying periods of time are required for the production of detectable amounts of serologic methods. Consequently, serologic examinations are always inferior to bacteriologic, mycologic and parasitologic examinations in the early diagnosis of acute infections. For example, blood cultures are of greater value in the early diagnosis of typhoid fever than the Widal test, and darkfield examinations for *T. pallidum* are of far greater value in the diagnosis of chancres than the complement fixation or flocculation tests.

Furthermore, and very importantly, the living agents of disease vary greatly in their capacity to produce circulating or humoral antibodies in the blood and other body fluids. Some, like the exotoxins and their toxoids, spirochetes, viruses and the rickettsiae, are highly antigenic, as well as many of the gram-negative bacilli (typhoid, paratyphoid, glanders, tularemia, brucella, etc.), while others, like the tubercle bacillus and other gram-positive bacilli, are but poorly antigenic. The same is true of many of the pathogenic fungi and animal parasites although, in some instances, this is apparently due to the superficial or evanescent character of the infections produced. At all events, serologic examinations for diagnostic purposes are limited to those diseases in which the living agents producing them are capable of causing the production of relatively large amounts of antibodies in the blood or spinal fluid.

It also appears that once the body cells have been sensitized with the production or storage of either natural or acquired antibodies, they may produce them anew, or in greater amounts, in response to some nonspecific stimulus which increases the activity of the antibody-producing tissues. In fact, this is believed to happen and is called the *anamnestic reaction* (anamnesis = recollection) in designating the fresh production of antibody in response to stimulation by a new and unrelated antigen. For example, it is thought that the natural or acquired agglutinin for *S. typhosa* may undergo a temporary increase during an attack of influenza, or some other unrelated acute infectious disease, which may lead to the false serologic diagnosis of typhoid fever.

Group Antibodies. It is also to be pointed out that many of the living agents of disease capable of producing humoral antibodies are related biologically by reason of sharing common antigenic constituents. As a result, group antibodies may be produced which may complicate serologic examinations and lead to diagnostic errors. For example, in typhoid fever, agglutinins and other antibodies are commonly produced for the paratyphoid bacilli although in smaller amounts than for the typhoid bacillus itself with differentiation usually possible by serologic methods; also, in syphilis, an increase of spirochetal antibody produces agglutination and complement fixation reactions with the saprophytic spirochetes of the mouth almost as well as with antigens prepared of cultures of *T. pallidum,* to which further reference will be made.

Another and mysterious phenomenon possibly related to this subject of group antibodies is the agglutinin and complement-fixing antibody, produced by rickettsiae in typhus and Rocky Mountain spotted fevers for certain strains of the common saprophyte, *P. vulgaris* (Weil-Felix reaction). Certainly there can be no etiologic relationship between the rickettsia and this micro-organism but the latter may share with the rickettsia a common antigenic constituent capable of producing these antibodies.

Furthermore, the occurrence of biologic falsely positive Wassermann and flocculation reactions in leprosy, malaria, infectious mononucleosis, vaccinia, etc., suggests that the living agents of these diseases may share with *T. pallidum* a common antigenic constituent capable of producing a reagin similar or identical with that produced in syphilis and which will be discussed later in more detail.

Heterophil Antibody. As shown by Forssman and subsequently by others, the tissues of guinea-pigs, horses, mice, chickens and several kinds of fish, as well as human erythrocytes of Groups A and AB, contain a protein in combination with lipid and polysaccharide haptens capable of producing agglutinin and hemolysin for sheep corpuscles when injected into rabbits. Certain bacteria (pneumococci, paratyphoid and dysentery bacilli) also contain carbohydrate haptens possessing the same antigenic capacity. Since the production of these antibodies does not follow the usual laws of species specificity, they have been designated heterophil antigens and heterophil antibodies.

Practically all serums of human beings contain natural antisheep agglutinin and hemolysin of unknown origin but both are increased in serum disease and acute infectious mononucleosis. Indeed, the increase is so characteristic and constant in the latter that a quantitative agglutination test employing suspensions of washed sheep corpuscles has proved a valuable diagnostic procedure. According to Davidsohn,[1] however, the heterophil antibody occurring in serum disease is of a different type from that occurring in infectious mononucleosis. For example, in the former the antibodies are almost completely absorbed by guinea-pig kidney but only partly by rabbit kidney, while in the latter, absorption by both tissues is only partial and about equal in degree. Heterophil antibody may also occur in viral hepatitis.[2,3]

Diagnostic Serologic Tests. The value of serologic tests in diagnosis depends entirely on the principle that antigens react specifically *in vitro* with their corresponding antibodies to produce recognizable and controllable reactions. Such tests are of two main kinds:

1. *Tests for Suspected Antibody in the Presence of a Known Antigen.* These may be conducted *in vivo* as in the *Schick* and *Dick* tests when the toxins of the diphtheria bacillus and hemolytic streptococcus are injected intracutaneously, on the basis that if their respective antitoxins are present in the blood in sufficient amounts the injected toxins are neutralized with observable negative reactions; or various allergens may be applied to the skin or injected intracutaneously on the basis that the individual will develop observable positive reactions to any to which hypersensitiveness exists.

Tests, on the other hand, may be conducted *in vitro* with serum or some other body fluid and a suitable preparation of a known antigen, as in the conduct of *agglutination, precipitin, complement fixation* and *opsonic* reactions. These reactions are based on the assumption that the presence of antibody for a known antigen indicates that at the time of the test the patient is or has been actively immunized with the antigen in the nature of overt or unrecognized infection or by a vaccine. Success in obtaining a positive reaction here depends largely on the capacity of the antigen to produce antibody and, in the case of infection, on the degree and duration of the disease itself. For example, in very acute infections like bubonic plague and cholera, death may occur before humoral antibodies appear, whereas in more protracted diseases like typhoid and undulant fever, antibody formation takes place early enough to be of service in serologic diagnosis.

2. *Tests for a Suspected Antigen in the Presence of a Known Antibody.* In tests of this kind the antibody is usually furnished by the serum of an individual known to have had a certain disease, or preferably by that of an animal in which the antibody has been produced by active immunization. Such immune serum, when used according to appropriate methods, serves to detect or identify antigens. For example, a known antityphoid serum may be used in an agglutination test

for the final identification of a typhoid bacillus cultivated from the blood, feces, or urine in a suspected case of typhoid fever or a carrier; a known antihuman serum may be used in the precipitin or complement fixation test for the detection and identification of a human blood stain, etc.

As previously stated, however, serologic methods are not intended to supplant the various bacteriologic and other laboratory tests employed to detect the specific micro-organisms responsible for infection.

Antitoxins. Only those antibodies detectable by *in vitro* tests and reactions are properly included in the category of serologic examinations. For this reason, Schick and Dick tests for natural or acquired antitoxins in diphtheria and scarlet fever are not included. Furthermore, the tests possess no diagnostic value in these diseases except that positive reactions indicate that they may occur because of susceptibility to them. Negative reactions, however, do not necessarily exclude diphtheria or scarlet fever, since light infections with the diphtheria bacillus may occur in spite of negative Schick reactions, as well as scarlet fever without exanthems in spite of negative Dick reactions, as discussed in Chapter 19. It is true that diphtheria antitoxin may be detected in serum by the toxin-antitoxin neutralization test conducted in guinea-pigs, which is thereby a serologic examination, but this procedure is not ordinarily employed, since the Schick test has been found much simpler and more satisfactory. Furthermore, diphtheria antitoxin may be detected *in vitro* by the Ramon precipitation or flocculation test, but this is employed only in the standardization of antitoxin, without any application at present to the detection of natural or acquired diphtheria antitoxin in the serums of human beings.

In this connection it is also to be stated that an antibody may be detected in the serums of some cases of anaphylaxis and allergy by the passive transfer method of Prausnitz and Küstner. This test is frequently of diagnostic value, as discussed in Chapter 19, but the procedure is not ordinarily included under the category of serologic examinations, since it is likewise essentially an *in vivo* test.

Virucidins. Complement-fixing antibody may occur in the serums in some of the diseases due to filtrable viruses with special reference to smallpox, vaccinia and lymphogranuloma venereum, but a protective or virus-neutralizing antibody is best known. It occurs in so many of the viral diseases of man like smallpox, varicella, influenza, measles, poliomyelitis, equine encephalomyelitis and yellow fever that there can be no doubt of its importance in relation to both natural and acquired immunity to them. This antibody is closely similar to the antitoxins in the sense that it may neutralize virus without actually destroying it, but in some instances, or under certain technical conditions, it is destructive or virucidal.

The antibody may be detected by serum-virus neutralization tests conducted in the lower animals. These, however, are not of practical or clinical value at the present time, except for the detection of immunity as in the case of yellow fever in which a mouse-protection or serum-neutralization test has been widely used in studies bearing upon the incidence of natural or acquired immunity to this disease.

Agglutinins. Owing to their simplicity, agglutination tests have proved extremely valuable in the serum diagnosis of many bacterial diseases with special reference to typhoid, paratyphoid and undulant fevers, tularemia and leptospirosis, and are sometimes helpful in the diagnosis of bacillary dysentery, bubonic plague, cholera, etc. They are likewise of value in the detection of bacterial carriers with special reference to typhoid and paratyphoid bacilli and brucella. Needless to state, bacterial antigens for agglutination tests require the careful selection of agglutinable strains. Unfortunately, freshly isolated micro-organisms, like the typhoid bacillus, may be temporarily nonagglutinable, while other micro-organisms, like streptococci and meningococci, may undergo spontaneous agglutination leading to possible errors in the reading and interpretation of reactions. Prozone or agglutinoid reactions may also occur with the possibility of diagnostic errors. Agglutination tests are also of value in the diagnosis of primary atypical pneumonia employing antigens of group O human erythrocytes and MG streptococci.

In the laboratory, agglutinins occurring in specific immune serums are likewise extremely valuable in agglutination tests for the identification of pneumococci, meningococci, streptococci, typhoid bacilli, etc., recovered in cultures. Group agglutinins are easily detected by properly conducted absorption tests.

Agglutination tests, however, are only of limited practical value in the diagnosis of some of the mycotic diseases like actinomycosis, sporotrichosis and moniliasis (*mycoagglutinins*) as likewise in viral and rickettsial diseases and those due to protozoa, like leishmaniasis and trypanosomiasis.

As previously stated they are, however, of great value in determining blood groups (*isohemagglutinins*) in relation to blood transfusions and the administration of plasma as well as from medicolegal applications. This subject will be discussed later in more detail.

Conglutination. In 1906, Bordet and Gay [4] discovered that a thermostabile substance occurring in bovine serum was capable of agglutinating erythrocytes which had been previously treated with specific hemolysin and complement. This substance was called "bovine colloid" but was later renamed "conglutinin" by Bordet and Streng, while the reaction became known as the "conglutination reaction." Streng also discovered that bacteria underwent conglutination when previously treated with immune serum from which specific agglutinins had been removed by absorption, bovine conglutinin and complement. Lucas and his colleagues,[5] in 1910, utilized this test for the serum diagnosis of bacillary dysentery of infants, but it has never been employed as a practical diagnostic procedure.

Recently Wiener and his associates [6] have adopted the term "conglutination reaction" in explanation of the well-known fact that many Rh-negative mothers with clinical evidence of Rh sensitization had no demonstrable Rh antibodies in their serums. According to Wiener *et al.*, human plasma and serums may contain a colloidal aggregate of albumin, globulin and phospholipid (so-called "X-protein") which is absorbed by human erythrocytes, after they have been sensitized by "blocking" antibodies, which causes the cells to stick together. "X-protein" does not occur in fetal blood but is rapidly acquired after birth, occurring in the plasma and serum of older children and adults. Plasma, however, contains more than serum due to the presence of fibrinogen.[7] The application of the term "conglutination" in this connection, however, may not be justified, since there is no evidence that "X-protein" is essential. Indeed, certain concentrations of acacia, pectin and numerous other nonprotein substances may be used, although these are not as satisfactory as bovine conglutinin because of their tendency to form rouleaux formation.

Precipitins. Precipitins are apparently identical with agglutinins except that they produce precipitates when brought in contact with solutions of their antigens. The latter are called *precipitinogens* and the precipitates formed in mixtures with the precipitins in serum are largely composed of the proteins in the serum.

Almost any foreign protein can call forth a precipitin, but the latter may react *in vitro* with haptens. Indeed, a large portion of our knowledge of the chemistry of antigens, including the haptens, has been acquired as a result of studies with precipitins and precipitation reactions.

Precipitins are highly specific but the technic of precipitation tests is exacting, since they require a close adjustment of the amounts of antigen and serum employed in order to prevent falsely negative or prozone reactions and to secure reactions of maximum sensitivity.

Syphilis is practically the only bacterial disease in which so-called "precipitation tests," are conducted with serum and spinal fluid for diagnostic purposes. However, it is a mistake to regard the antibody or reagin involved a precipitin, since it causes a flocculation or agglutination of lipids in colloidal suspension in saline dilutions of alcoholic extracts of beef heart or other mammalian tissues (antigen). The flocculi may be of macroscopic or microscopic size, depending upon the amounts and proportions of syphilitic serum and tissue extract employed. Under the circumstances, these serum tests in syphilis are properly designated flocculation reactions.

A similar macroflocculation reaction has been described by Landau and German [8] in the serodiagnosis of malignant disease, employing alcoholic extracts of malignant tumors as antigen. Positive reactions have also occurred in syphilis, tuberculosis and pregnancy and the test has not provided a means for the serum diagnosis of malignant disease. Indeed, it may be stated that during the past thirty years many different serologic tests have been proposed for this purpose but none have proved of acceptable clinical or diagnostic value.

Precipitation tests, however, may be employed in the serum diagnosis of some bacterial diseases by employing precipitins in specific immune serum, prepared by the immunization of the lower animals, and using them in tests for the detection of precipitinogens in sputum, urine, exudates, tissues, etc. For example, in pneumonia, type-specific precipitinogens of pneumococci regularly occur in the sputum and frequently in the urine, although type-diagnosis is better determined by agglutination tests and especially by the capsular swelling or "quellung" reaction of Neufeld, in which the capsular changes may be due to precipitation reactions involving the polysaccharide haptens.

Similar tests for precipitinogens have also been employed in the serum diagnosis of meningococcal meningitis employing spinal fluid and antimeningococcal serum, in the diagnosis of bacillary dysentery using filtrates of the stools and antidysentery serum, in the diagnosis of gonorrhea using filtrates of pus and antigonococcal serum and in the diagnosis of diphtheria using filtrates prepared of swabs and diphtheria antitoxin. None of these tests, however, are generally employed at the present time, as laboratory diagnosis is better served by bacteriologic examinations. However, thermoprecipitin tests conducted with extracts of the flesh of putrid rats and potent antiplague serum possess some value in the detection of *Past. pestis* infections of these animals, as likewise similar tests with extracts of the flesh of animals conducted with potent antianthrax serum (Ascoli reaction) in the detection of *B. anthracis* infections.

Precipitin tests, however, have not proved of value in the diagnosis of mycotic diseases except, possibly, in some of the deeper and rarer infections like coccidioidal granuloma.[9] Nor have they been found of clinical or diagnostic value in the serum diagnosis of diseases due to the rickettsiae and filtrable viruses.

Precipitins may occur in the serums of human beings infested with some of the animal parasites. For example, precipitin tests have proved of practical value in the serum diagnosis of trichinosis and hydatid cyst disease. They have also been advocated as aids in the diagnosis of amebiasis, malaria, ascariasis, taeniasis, leishmaniasis and schistosomiasis but ar inferior in diagnostic value to microscopic examinations for the parasites and to skin and complement fixation tests.

Precipitin tests have been found extremely valuable in the detection and species identification of blood stains and meat adulteration to which further reference will be made.

Lysins. The chief practical application of the bacteriolysins in serum diagnosis is the Pfeiffer phenomenon for the identification of *V. cholerae* recovered in cultures of the stools in Asiatic cholera and carriers. Solis-Cohen and his colleagues,[10,11] have advocated a "pathogen selective method" for aid in deciding on the importance of micro-organisms in cultures for incorporation in autogenous vaccines. It is based on the assumption that only those bacteria capable of surviving in the whole coagulated blood of the patient, due to the absence of bacteriolysins and bactericidans, are pathogenic or potentially pathogenic. The method, however, is not applicable for the spore-forming bacilli and those micro-organisms owing all or a part of their pathogenicity to exogenous toxins.

As previously stated, antisheep hemolysin occurs normally or naturally in human serums and is increased in infectious mononucleosis and serum disease (heterophil) but serum diagnosis is based on agglutination tests with suspensions of washed sheep erythrocytes. Otherwise, however, immune hemolysins, especially antisheep hemolysin prepared by the immunization of rabbits, is commonly employed in the hemolytic system for the conduct of complement fixation tests.

Many years ago Freund and Kaminer thought the serums of cancerous individuals might contain a *cytotoxin* capable of destroying cancer cells *in vitro,* as a test for the serum diagnosis of carcinoma, but it is now known that this procedure is without clinical value. Indeed, none of the cytotoxins are employed for diagnostic or therapeutic purposes at the present time.

Complement Fixation. There is no reason for believing that a special kind of antibody is involved in the phenomenon of complement fixation discovered by Bordet and Gengou in 1901. Apparently any antibody capable of sensitizing its antigen like precipitin, agglutinin and lysin may produce the reaction although precipitins are undoubtedly most active in this regard. There has been considerable speculation, however, on the source of the antibody or *reagin* concerned in the Wassermann reaction. Indeed, its exact nature and origin are still unknown.

This subject is discussed in more detail under *Complement Fixation in Syphilis* (Chapter 18).

Complement fixation reactions are very sensitive when properly conducted with suitable antigens. They are likewise highly specific under these conditions although, as in agglutination and precipitin tests, they may give group reactions with biologically related antigens. A notable exception, however, is the Wassermann reaction in syphilis, which, like the flocculation reactions in this disease, is biologically nonspecific, since the antigens are not prepared of *T. pallidum* but of alcoholic extracts of beef heart containing lipids with which the reagin reacts, resulting in the fixation of complement. In other words, the biologically nonspecific character of the reaction is similar to that of the Weil-Felix reaction in which certain strains of *P. vulgaris* are agglutinated by the antibody produced in typhus fever and other rickettsial diseases.

In view of the sensitivity and specificity of complement fixation reactions, they have been proved of clinical value not only in relation to the serum diagnosis and treatment of spirochetal diseases like syphilis, yaws, pinta, bejel, relapsing and rat bite fevers, but in many of the bacterial diseases, with special reference to typhoid, paratyphoid and undulant fevers, glanders, chancroid, gonorrhea and tuberculosis—likewise in the diagnosis of many viral and rickettsial diseases like lymphogranuloma venereum, foot and mouth disease, psittacosis, ornithosis, encephalitis, yellow fever, mumps, smallpox, typhus fever, Rocky Mountain spotted fever, etc. They are, however, of but little or no value in the serum diagnosis of the mycotic diseases except, sometimes, in the more severe infections like sporotrichosis and blastomycosis. They are also of aid in the diagnosis of some of the diseases due to animal parasites, with special reference to amebic dysentery, trichinosis, hydatid cyst disease and some of the helminthic infestments while being extremely valuable in the detection and species identification of blood stains, the detection of meat adulteration, etc.

Opsonins. Opsonins are antibodies which render bacteria, other cells and foreign substances more susceptible to phagocytosis. Many confine this term to the antibodies found normally or naturally in the blood while using the term "bacteriotropins" when they are produced by infection or by the administration of vaccines; but there does not appear to be any valid reason for this practice.

As in the case of the agglutinins, a surprisingly large number of natural opsonins occur normally in the blood. Since phagocytosis is one of the most important mechanisms involved in both natural and acquired immunity, there can be no doubt of the great importance of natural and acquired opsonins in resistance to infection and recovery therefrom.

Largely due to the investigations of Wright, opsonins were formerly determined quantitatively in serums by means of the opsonic and phagocytic indexes for diagnostic purposes in some of the bacterial diseases and especially in relation to vaccine therapy. However, owing to technical difficulties and many possible sources of error, such tests are no longer employed, except for the opsonocytophagic test of Huddleson which is an aid in the serum diagnosis of brucellosis or undulant fever.

Anaphylactins, Allergins and Reagins. *Anaphylactin* is the term commonly employed for designating the antibody producing active or passive sensitization of the body cells of guinea-pigs or other of the lower animals resulting in that type of hypersensitiveness known as anaphylaxis. It is identical to precipitin or closely related to it.

Allergin is the term frequently applied to the antibody producing active or passive sensitization of human beings resulting in that kind of hypersensitiveness designated as allergy.

Reagin is the term commonly applied to the antibody producing that type of allergy in human beings called "atopy" by Coca, characterized by the influence of heredity with special reference to hay fever, asthma and eczema. As far as is known, reagins do not occur in the lower animals but have been found also in the blood of individuals hypersensitive to trichophytin, diphtheria toxin and *Ascaris lumbricoides*. This is the type of antibody commonly occurring in human allergies and is characterized by its capacity to sensitize the skin. Indeed, in no instance, either in allergic or nonallergic individuals, have the reagins been demonstrated in the blood in the absence of positive skin reactions.

The presence of allergic antibody or reagin in the blood was first successfully demonstrated by Prausnitz and Küstner [12] by passively sensitizing an area of skin of a nonallergic individual by the intradermal infection of the serum of an allergic individual. For this reason it is known as the passive method of Prausnitz and Küstner and is sometimes employed as a means for determining the substance or substances to which an individual is allergic, as more fully discussed in Chapter 19.

ANTIGENS IN RELATION TO SEROLOGIC EXAMINATIONS

In the usual sense, antigens are any substances capable of stimulating the production of antibodies. But the term is also applied to substances reacting with a given antibody *in vitro* without being able to produce it (Table 97). For example, the alcoholic extracts of beef heart employed in complement fixation and flocculation tests for syphilis are commonly designated "antigens" although they are not capable of producing the reagin or antibody by injection into the lower animals. The same is true of the suspensions of *P. vulgaris* used in agglutination and complement fixation tests in the diagnosis of typhus fever and other rickettsial diseases.

A *complete antigen* is composed of one component producing antibody and a second component on which immunologic specificity depends. A *partial antigen* or *hapten* is one which ordinarily cannot produce antibody but is capable of specifically reacting with it *in vivo* or *in vitro*. The antigens producing anaphylactic sensitization in the lower animals are known as *anaphylactogens* while those producing allergic sensitization in human beings are known as *allergens* or *atopens*.

Complete antigens are usually foreign proteins of large molecular size and soluble in the body fluids. Their remarkable antigenicity and specificity are determined by chemical constitution rather than by their origin. Consequently, their specificity may be altered by heat or by the introduction of new chemical atoms or groups. The complex polysaccharides of certain bacteria may be complete antigens in some animals but not in others; in most instances they are partial antigens or haptens in combination with proteins. Purified lipids free of proteins do not produce antibodies but may react with them *in vitro,* as in the complement fixation and flocculation reactions in syphilis.

Exogenous toxins and toxoids are proteins and complete antigens; the same is probably likewise true of the rickettsiae and viruses. Endotoxins are proteins or protein complexes in combination with carbohydrates or lipids; they are probably likewise complete antigens but are less antigenic than the exogenous toxins.

A single bacterial cell may contain several antigens, some being in the flagella (flagellar antigen) or capsules when these are present, while others are on the surface (surface antigens) and still others superficially or deeply beneath the surface (somatic antigens). Each may act as a complete antigen when attached to the cell but many become haptens or partial antigens when separated and submitted to chemical purification. Surface antigens are particularly important not only in relation to antibody production, but also in relation to antibody reactions *in vitro* concerned in various serologic diagnostic tests.

As far as the serologic examinations of agglutination, precipitation and complement fixation are concerned it is apparent, therefore, that the methods employed in the preparation of the antigens are very important. For example, the agglutination test for typhoid fever is best conducted by using both flagellar (formolized) and somatic (alcoholic) antigens and especially for the detection of typhoid fever in an individual previously immunized by injections of typhoid-paratyphoid vaccine. Furthermore, a long step toward the standardization of the complement fixation and flocculation tests for syphilis, as well as further improvement in their sensitivity and specificity, could be expected by the identification of the exact lipid or lipids involved in the extracts used as antigens along with the possibility of producing them synthetically.

EXAMINATIONS IN RELATION TO BLOOD TRANSFUSION

The discovery by Landsteiner in 1900 that the agglutinins for human corpuscles in blood made it possible to divide the latter into three groups was one of the most important steps in the history of blood transfusion. A year later von Decastello and Sturli added a fourth group. Since then, when donors of the same blood group are used along with other precautions, transfusions (and especially first transfusions) are practically free from danger. Theoretically they should be perfect tissue grafts but even under the best of conditions, a certain small percentage is followed by untoward reactions, inconsequential as a rule, but at times due to serious hemolytic reactions which are sometimes fatal. While it has been known for many years that repeated transfusions of the same patient with the blood of the same donor belonging to the same blood group may produce serious reactions, the reasons for these have only recently been discovered as, likewise, the cause for hemolytic reactions sometimes occurring in pregnant women and during the puerperium. Under these conditions, the careful selection of a donor belonging to the same blood group as the patient, checked whenever pos-

TABLE 98. SUMMARY OF THE CLINICAL INTERPRETATION OF EXAMINATIONS AND HAZARDS IN RELATION TO BLOOD AND PLASMA TRANSFUSIONS

Subject	Interpretation
Major Blood Groups	The Landsteiner or International classification of human corpuscles into Groups O, A, B and AB is preferred to the classifications of Jansky and Moss. Grouping tests should be conducted with sterile serums and fresh corpuscles to avoid errors. The corpuscles of the donor and recipient should belong to the same group, as the transfusion of incompatible blood may produce untoward reactions, due to intravascular agglutination and hemolysis, which are sometimes serious and ever fatal.
Blood Sub-groups	Subgroups of A and AB corpuscles may occur classified as A_1, A_2, A_3, A_2B and A_3B. Agglutinins for these subgroups are normally rarely present in the blood. They are of the "cold" type producing agglutination *in vitro* at 0 to 5° C. Consequently, their presence in the blood of donors and recipients does not produce reactions. Agglutinins for these subgroups, however, may be increased by multiple transfusions, especially with the blood of the same donor. They may also be produced during pregnancy through iso-immunization by the fetus. Since they may agglutinate their homologous corpuscles at body temperature ("atypical warm agglutinins"), their presence in the blood of recipients may produce reactions. Human corpuscles may also contain the agglutinogens M, N and P. Agglutinins for these do not occur normally in the blood. Therefore, they do not produce reactions. They are likewise of very low antigenicity. Consequently, agglutinins for them are but rarely produced by multiple transfusions. The corpuscles of about 85 per cent of human beings also contain the Rh agglutinogen (RH+); types Rh′ and Rh″ also occur. Agglutinins for these do not occur normally in the blood. Consequently, Rh+ corpuscles do not produce reactions in first transfusions. However, multiple transfusions with Rh+ corpuscles may produce agglutinins. The latter may be also produced during pregnancy through iso-immunization by Rh in the fetus. The agglutinins are readily detected by typing tests; the presence of large amounts in the blood of recipients may produce hemolytic reactions upon the transfusion of Rh+ corpuscles. They may also produce erythroblastosis fetalis. Consequently, it is advisable to select compatible donors whose corpuscles are Rh— for the transfusion of individuals who have had previous transfusions, as likewise for the first and subsequent transfusions of women in pregnancy and the puerperium. Small amounts of auto-agglutinins also occur normally in the blood of a large percentage of human beings. They are of the "cold" type and therefore do not usually produce reactions. In paroxysmal hemoglobinuria, syphilitic cirrhosis of the liver, severe anemias, Raynaud's disease and other conditions, however, they may be increased, but transfusions with compatible blood warmed to body temperature are usually well borne.

TABLE 98. SUMMARY OF THE CLINICAL INTERPRETATION OF EXAMINATIONS AND HAZARDS IN RELATION TO BLOOD AND PLASMA TRANSFUSIONS—(Continued)

Subject	Interpretation
Universal Donors	The corpuscles of universal donors belong to Group O. Their plasma, however, sometimes contains sufficiently large amounts of agglutinins for A, B or AB corpuscles to produce marked and even serious hemolytic reactions. Consequently, the plasma of Group O donors should be examined for these agglutinins before transfusion. Otherwise, they should be removed by the addition of the polysaccharide substances A and B to citrated blood. Since multiple transfusions with Group O blood may produce agglutinin for the Rh factor, the corpuscles of Group O donors should be RH— in multiple transfusions; the same is also advisable in the case of first and subsequent transfusions of women in pregnancy or the puerperium with Group O blood.
Transfusion Hazards	The majority of reactions and hazards are avoidable. Most reactions are without danger. The incidence of reactions has been greatly reduced by the selection of donors of the same blood group as the recipients, by cross-matching tests and improved transfusion technic. Reactions may occur as (1) mild fever with no objective symptoms; (2) fever with brief chilliness; (3) high fever with chills and (4) high fever, chills, and symptoms due to severe intravascular agglutination and hemolysis (hemolytic reaction). Reactions are divisible on the etiologic basis into (1) nonspecific; (2) specific; (3) those due to speed of injection of blood and circulatory disturbances and (4) allergic. Additional hazards mainly refer to the possible transmission of syphilis, malaria and homologous serum jaundice.
Stored Blood	Alsever's solution (ACD) is preferred to sodium citrate in the collection of blood since it is a better preservative for erythrocytes. Satisfactory methods for the preservation of the unstable granulocytes and platelets have not been discovered. The incidence of reactions following the administration of sterile blood stored for periods up to two or three weeks is ordinarily no higher than that of fresh citrated blood. Contaminating bacteria in stored blood, however, may produce an increased percentage of the nonspecific protein shock type of reaction. The administration of old stored blood may produce the hemolytic type of reaction. Stored blood is satisfactory for blood-volume restoration in the treatment of hemorrhage and shock. However, it should not be used after 3 to 5 days in the treatment of the anemias, prothrombin deficiencies, hemorrhagic dyscrasias or the septicemias. There is no danger of the transmission of syphilis or malaria after storage for 2 or 3 days. The stored blood of universal donors (Group O) should not contain an excess of agglutinins for the corpuscles of Groups A, B or AB recipients. Rh— stored blood should be used in the transfusion of individuals who have had previous transfusions as well as in the transfusion of women in pregnancy and the puerperium.

TABLE 98. SUMMARY OF THE CLINICAL INTERPRETATION OF EXAMINATIONS AND HAZARDS IN RELATION TO BLOOD AND PLASMA TRANSFUSIONS—(Continued)

Subject	Interpretation
Plasma	Pooled plasma may be administered intravenously without the need for determining the blood group of the recipient or cross-matching tests. Reactions are very infrequent and usually mild when they do occur. Pooling and filtration greatly reduce the incidence of reactions due to allergy. Plasma has proved of great value in the treatment of hemorrhage through the restoration of blood-volume; also in the treatment of surgical shock and conditions producing hypoproteinemia and dehydration. It is probably inferior to transfusions of fresh blood in the treatment of the septicemias and other infections. There is no danger of the transmission of syphilis or malaria by plasma, although homologous serum jaundice may be transmitted and especially by pooled plasma.
Other Blood Derivatives	*Erythrocytes* obtained from blood collected in Alsever's solution may be resuspended in sterile 1 per cent sodium chloride solution and employed in the treatment of some of the anemias. Solutions of *dried hemoglobin* are effective in the treatment of surgical shock but may produce temporary impairment of renal function. *Fibrin foam* with thrombin on absorbable sponges is effective in the control of bleeding and for the attachment of skin grafts. *Fraction I globulin* is effective in the treatment of hemorrhage; when prepared of fresh plasma it also contains the "antihemophilic globulin." May transmit the virus of homologous serum jaundice. *Gamma globulin* (fraction II) is effective in the prevention and modification of measles; also effective in the prevention of rubella and infectious or viral hepatitis. Low sodium chloride and mercurial-free *serum albumin* (stabilized by heating at 60° C. for ten hours) is effective in the treatment of shock due to hemorrhage and hypoalbuminemia due to nephrosis, severe infections, burns, peritonitis, cirrhosis of the liver, etc.

sible by direct cross-matching tests, is the chief means for avoiding severe reactions, but it is now evident that additional precautions are required and that pretransfusion examinations should be entrusted to experienced and skilful workers.

Major Blood Groups. As previously stated, the corpuscles of human beings are divisible into four main or major groups on the basis that they contain two major agglutinogens (A and B) with two major agglutinins occurring in the plasma or serum (a and b). Consequently, Landsteiner divided the blood of human beings into four major groups designated O, A, B and AB, according to the antigenic structures of the respective erythrocytes (Table 98). In 1907, however, Jansky divided them into groups I, II, III and IV. In 1910 Moss, unaware of this classification, also divided them into groups I, II, III and IV but reversed Jansky's groups I and IV so that group I of Jansky corresponds to group IV of Moss and group IV of Jansky to group I of Moss. In view of possible confusion it is recommended, therefore, that all laboratories adopt the Landsteiner classification which is also known as the International classification. Although the percentages of these groups in adults vary according to race, Wiener [13] has given those shown in Table 99 on the basis of 38,000 examinations in the United States:

TABLE 99

Landsteiner (International)	Jansky	Moss	Agglutinogen in Corpuscles	Agglutinin in Serums	Percentages
O	I	IV	...	ab	45
A	II	II	A	b	39
B	III	III	B	a	12
AB	IV	I	AB		4

Blood Subgroups. However, it is now known that two subgroups exist within each of the groups, A and AB, of human blood based on differences in the agglutinogen A.[14,15,16] Landsteiner and Levine,[17] who pointed out that the difference is a qualitative one, suggested their designations A_1 and A_2. Recently Wiener and Silverman [18] have discovered an additional one, A_3, in the blood of a mother and her one-year-old child. Consequently, the groups A and AB are now divided into the subgroups A_1, A_2, A_3, A_1B, A_2B and A_3B, respectively. A_1 is about four times as common as A_2, and A_1B about one and one-half times as common as A_2B. The A_2 is also relatively intensive to agglutination and especially so when in combination with B in subgroup A_2B. A_2 and A_2B occur much more frequently in Negroes than in whites and the latter may be mistaken for group B. A_3 and A_3B are extremely rare but may be mistaken for group O.

Landsteiner and Levine [19] have found that about 3 per cent of individuals belonging to these subgroups have in their plasma anomalous agglutinins acting on the corpuscles of the opposite subgroup at 20° C. but not at body temperature. Under the circumstances, these anomalous agglutinins may lead to confusion and error in pretransfusion agglutination tests conducted at room temperature.

Consequently, while some investigators have reported that these differences in the subgroups can cause hemolytic reactions with the warning that only donors of the homologous subgroups should be used for transfusions, no case has yet been reported where they were conclusively proved to be responsible for serious reactions in first transfusions.[20] However, it has been observed that repeated transfusions of a patient with the blood of the same donor carrying a subgroup agglutinogen may result in the gradual production of increased amounts of agglutinin (iso-immunization) responsible for reactions.[21] Furthermore, it would appear that women, during pregnancy, may develop these agglutinins through iso-immunization by the fetuses if the latter inherit from the fathers certain dominant agglutinogens which are lacking in the mother.[22] Unfortunately, this adds one more hazard to pregnancy should such a mother during this state, parturition or the puerperium, require blood transfusion. Curiously enough, these agglutinins produced by iso-immunization have been found to react strongly *in vitro* at 37° C. while practically inactive at 25° C.; for this reason they have been called *"atypical warm agglutinins."* [21] At 20° C. or lower, however, the serums may also show strong reactions of a different specificity indicating the coexistence of *"cold agglutinins."* In other words, the "cold agglutinins" react best at 0 to 5° C. Two varieties may occur: one showing a certain degree of specificity and the other entirely nonspecific. The latter are referred to as *auto-agglutinins* because they may agglutinate and hemolyze the individual's own corpuscles when local or general temperature is reduced. For this reason they are believed to be abnormally increased in paroxysmal hemoglobinuria, cirrhosis of the liver (especially syphilitic cirrhosis), severe anemias including hemolytic jaundice, Raynaud's disease, streptococcus and staphylococcus infections and other conditions. Wiener,[23] however, has reported a fatal case of hemolytic anemia occurring in a child, due to autoantibodies of unknown origin, which not only agglutinated the patient's own corpuscles at 37° C., but the corpuscles of all other groups including even those of O. As expected under the circumstances, a blood transfusion was ineffective.

But in addition to these subgroups it is now known that human erythrocytes may also contain three additional minor agglutinogens designated M, N and P. They are likewise transmitted by heredity and therefore are highly important in agglutination tests for the determination of paternity, shortly to be discussed in more detail. Fortunately, however, corresponding agglutinins for them do not exist in the plasma or serums. Consequently, they are of no clinical importance as far as first transfusions are concerned. Furthermore, they are very low in antigenic activity so that when the bloods of donors carrying these minor agglutinogens are repeatedly administered to the same individuals there is but little or no danger of the latter developing antibodies to them, capable of producing serious reactions of the hemolytic type. For example, up to the present time only three such instances have occurred in repeated transfusions of patients with the bloods of donors carrying M, only one in the case of P, and none in the case of N,[20] despite the fact that thousands of transfusions are given yearly without regard to these agglutinogens in the corpuscles of the donors.

Rh Agglutinogens and Agglutinins. Up to this point it may be stated, therefore, that agglutinins for the subgroups A_1, A_2, A_3, A_1B, A_2B and A_3B agglutinogens are but rarely present in the human serums although sometimes developed through iso-immunization by repeated blood transfusions, as well as during pregnancy, and that agglutinins for the minor agglutinogens M, N and P in human corpuscles are unknown and very rarely developed by repeated transfusions but not during pregnancy. Landsteiner and Wiener,[24] however, have discovered that the erythrocytes of about 85 per cent of human beings contain another agglutinable factor designated Rh because it is elicited by the serums of guinea-pigs immunized with the corpuscles of rhesus monkeys. Curiously enough, the corpuscles of most group O individuals (so-called universal donors) contain this factor, *i.e.,* are Rh+.

After this discovery, human erythrocytes were divided into two types, namely, Rh+ and Rh—. Since then Wiener and his colleagues [25] have identified two additional Rh agglutinogens designated Rh′ which is present in the blood of 70 per cent of Caucasians, and RH″ which occurs in 30 per cent of Caucasians, while the original Rh agglutinogen has been designated Rh_0. With the aid of three human testing serums, anti-Rh_0, anti-Rh′ and anti-Rh″, eight types of human blood can be identified. All of these agglutinogens are transmitted according to the mendelian laws of heredity so that their determination is of medicolegal value in disputed parentage, as shortly to be discussed in more detail. In 1940, Wiener and Peters [20] calculated that with the agglutinogens A_1, A_2, B, M, N, P and Rh_0 alone, as many as 72 different types of human blood may be identified, while the number is now known to be even larger following the discovery of the different types of Rh agglutinogens.

However, agglutinins for these Rh agglutinogens do not occur in the serums of individuals who have never received injections of human blood and in women who have never been pregnant. Consequently, first transfusions of patients with the bloods of donors containing the agglutinogens usually do not produce hemolytic reactions. The agglutinogens, however, are antigenic with the result that agglutinins may be produced by repeated transfusions of Rh— individuals with Rh+ blood which, on reaching a high titer, are responsible for approximately 90 per cent of posttransfusion reactions of the hemolytic type. Scott and Conant [26] have reported such an instance occurring in a woman who developed Rh agglutinin following transfusions with group O blood carrying an Rh agglutinogen. Fortunately, however, only about 4 per cent of Rh— individuals transfused with Rh+ blood develop Rh agglutinins.

Rh— women may also develop the agglutinins as the result of pregnancy and especially multiple pregnancies with Rh+ fetuses through iso-immunization. This may occur when Rh— women are impregnated by Rh+ men with transmission of the Rh agglutinogens to the fetuses. This situation involves about 10 per cent of marriages. Fortunately, however, only about 4 to 5 per cent of women in such marriages produce agglutinins as the result of pregnancy. Of course, this danger does not occur when the wife is Rh+, when both husband and wife are Rh+, or when both are Rh—. But whenever an Rh— woman becomes pregnant to an Rh+ man and she requires transfusions during pregnancy or the puerperium, compatible Rh— blood should be administered as a safeguard against possible posttransfusion reactions of the hemolytic type. Just how Rh— women produce Rh agglutinins during pregnancies with Rh+ fetuses is unknown. Certainly the Rh agglutinogens do not occur in solution in the plasma, with passage through the placenta, but during the first and subsequent labors Rh+ corpuscles from the fetus may gain access to the maternal blood through the placental villi with the production of agglutinins.

However, since the Rh agglutinins developed during pregnancy occur in the maternal plasma they may, upon reaching a sufficiently high concentration, traverse the placenta into the blood of the fetus, with the production of stillbirths or the birth of living infants who present immediately, or within a few hours or days, the manifestations of acute hemolytic anemia (erythroblastosis fetalis), icterus gravis neonatorum, hydrops fetalis, etc. This rarely occurs, however, among first-born children. If and when it does, the history of the mother usually reveals that she had transfusions or intramuscular injections of blood prior to marriage, with the result that agglutinins were produced by Rh+ corpuscles. In this connection it is to be stated, however, that erythroblastosis fetalis may also be due to transfusions during pregnancy with A and B corpuscles (agglutinogens) which are capable of producing agglutinins in the mother with placental transmission to the fetus.[27]

Ideally, all Rh— patients requiring transfusion should be given compatible Rh— blood. In all individuals receiving repeated transfusions in whom reactions have occurred, tests for the Rh agglutinogens should be conducted before each transfusion. If the patient is Rh+ the transfusion of compatible Rh+ blood is permissible but if the patient is Rh—, only compatible Rh— blood should be administered. The same applies to transfusions during pregnancy and the puerperium and to all women whose obstetric histories show habitual abortions, stillbirths or the birth of infants with erythroblastosis fetalis. Indeed, it is always advisable to determine if females of any age are Rh+ or Rh— and if found Rh— to administer only Rh— blood.[28]

Furthermore, Rh— negative women should choose Rh— men in marriage. Obstetricians should determine the Rh status of husbands and wives during pregnancies and especially after the first pregnancy. If the husband is Rh+ and the wife is Rh—, the obstetrician is duly warned of potential danger due to the development of Rh agglutinins in the mother. After the first pregnancy, and especially in women with histories of habitual abortion, stillbirths or erythroblastosis fetalis, it is advisable to determine whether or not Rh agglutinins are present in the blood. This involves the possibility of falsely negative reactions due to the presence of "blocking" antibody in the blood which prevents the agglutination of Rh+ corpuscles by Rh agglutinins. Consequently, all laboratories conducting tests for Rh agglutinins should include the "blocking" test according to the technic of Diamond and Abelson[29] by which Rh agglutinins may be detected in the serum. Indeed, "blocking" tests are the most reliable means for determining the presence or absence of Rh agglutinins.

Universal Donors. In 1911 Ottenberg[30] suggested that in case of necessity group O individuals may be used as universal donors because of the absence of natural group O agglutinins and on the assumption that the agglutinins present in their blood for A, B or AB corpuscles do not usually produce reactions.

But it is now known that if group O blood contains large amounts of agglutinins for the corpuscles of groups A, B and AB, they may not be sufficiently diluted by the blood of recipients and produce not only an increased incidence of minor reactions, but sometimes severe ones as well.[30,31] Gesse[33] has reported 46 cases of hemolytic shock following the transfusion of universal or group O blood, 20 of which were fatal, and emphasized the necessity for first titrating the plasma for agglutinins for the corpuscles of the patient. If no higher than 1:8 to 1:16, he thought the blood might be administered in amounts not exceeding 100 to 200 cc. to individuals having 2000 million or more erythrocytes per c.mm. of blood. But with these precautions Gesse warned against the use of the blood of universal donors even under wartime conditions unless, in case of emergencies, homologous blood should not be available. It is likely, however, that these severe hemolytic reactions were due to Rh sensitization with the administration of Rh+ blood.

Under the conditions, in 1940 the Public Health Council adopted an amendment to the Sanitary Code of the State of New York requiring the titration of group O universal blood donors for agglutinins for A and B corpuscles in order to exclude those with high titers. Moreover, it appears that the same precautions should apply to plasma for intravenous administration although the pooling of a large number of group O plasmas may reduce the risk of reactions by reason of dilution of any containing large amounts of agglutinin, to be discussed shortly in more detail.

Otherwise, it would appear that the removal of agglutinins for A and B corpuscles from universal or group O blood is highly desirable. Indeed, this has been found possible by Witebsky, Klendshoj and Swanson [34] and other investigators [35, 36] who found that the addition of a complex polysaccharide (substance A) derived from commercial pepsin, mucin or peptone [37, 38] to citrated blood removed the agglutinin for A corpuscles, while the addition of a polysaccharide (substance B) derived from the gastric juice of human beings belonging to group B removed the agglutinin for B corpuscles.[34] Witebsky and his colleagues have reported that the addition of 25 cc. of a 1:1000 stock solution of substance A and 10 cc. of a similar stock solution of substance B to 500 cc. of citrated blood of group O donors, at least five minutes before administration, effectually reduces or completely removes the agglutinins for A and B corpuscles and since both substances are protein-free, they have not produced reactions in forty transfusions. Of course, if preliminary tests of group O blood show the presence of only A agglutinin, it is not necessary to add substance B, and vice versa. Consequently, universal or group O blood conditioned in this manner is believed by these investigators to be safe for administration to any patient independently of the blood group to which he or she belongs, without the necessity of determining the blood group and, in emergencies, even without cross-matching tests.

This, however, should probably apply only to men and nonpregnant women receiving first transfusions because it is true that the plasma of human beings belonging to group A, B and AB rarely contains agglutinin for group O corpuscles which has been found in only a small percentage of individuals belonging to the subgroups A_1 and A_1B. But since repeated transfusions with group O blood may result in the production of O agglutinin and especially agglutinin for the Rh factor, the method may not be equally safe under these conditions. The same may be true in the case of women in pregnancy, parturition and the puerperium if they have developed agglutinins for the Rh factor from iso-immunization by fetuses. Consequently, when universal or group O blood is to be given individuals who have had previous transfusions with group O blood, it would appear advisable to select donors whose corpuscles are Rh negative and whose plasmas are low in agglutinins for A and B corpuscles. The same also appears advisable in the case of women in pregnancy and parturition receiving first as well as subsequent transfusions.

Hazards. The hazards of blood transfusion refer not only to nonspecific reactions of the pyrexial type mainly due to technical factors, and specific reactions due to intravascular agglutination and hemolysis, but likewise to speed reactions and circulatory disturbances, allergic reactions and the possibility of disease being transmitted from the donor.

Fortunately, the majority of these hazards are avoidable. Reactions occur not infrequently but are mostly without danger. It is important, however, to prevent them as much as possible, especially in the case of severely ill recipients. Much has been learned of the causes but, unfortunately, they continue to occur in a small percentage of individuals for unknown reasons and in spite of all known precautions.

Their incidence varies considerably. When all known precautions are taken, apparently reactions vary in the case of transfusions of fresh citrated blood from 2 to about 5 per cent. Formerly, transfusions of blood by direct methods gave a lower incidence of reactions than transfusions by the indirect or citrate method but presentday precautions in the preparation of apparatus and the crystalloid solutions used in the latter have greatly reduced the incidence of reactions.

Nonspecific Reactions. Reactions may consist of nothing more than a mild fever of about 100° F. with no objective symptoms. Others may be accompanied by chilliness, while more severe ones are characterized by high fever and chills coming on in thirty to sixty minutes after transfusion and lasting for thirty minutes or longer. These latter closely resemble the reactions due to intravascular hemolysis following transfusions of incompatible or overaged stored blood except that intense lumbar pain is generally absent.

Apparently these reactions are of the nonspecific protein shock type, largely due to the use of stale distilled water carrying dead bacteria for the preparation of sodium citrate and sodium chloride solutions, or dirty apparatus. Merely by using freshly distilled water (with precautions against carrying over foreign matter) along with the scrupulous cleansing of all glassware and apparatus, Lewisohn and Rosenthal[39] have reduced the incidence of citrate transfusion reactions from 12 to 1.2 per cent. Apparently the reactions are not due to sodium citrate when a chemically pure product is used in a total concentration not exceeding 0.35 per cent in the blood. Fortunately these reactions do not detract from the therapeutic efficacy of transfusions because the erythrocytes of the donor survive in the blood of the recipient for the usual period of three to four months.

As is well known, the plasma of some sick patients contains an increased amount of fibrinogen or other proteins capable of increasing its viscosity and causing pseudo-agglutination or marked rouleaux formation of a donor's corpuscles in pretransfusion tests. It does not appear, however, that this quality of a patient's plasma constitutes a hazard or produces transfusion reactions.

The same is apparently also true in the case of those patients whose plasmas show the presence of nonspecific "cold agglutinins" which agglutinate not only the corpuscles of all groups at 0 to 5° C. but the patient's own corpuscles as well, and therefore are known as auto-agglutinins. As shown by Landsteiner and Levine,[17] they are commonly present in the serums of normal human beings but, as previously stated, they may be so greatly increased in some individuals with paroxysmal hemoglobinuria, syphilitic cirrhosis of the liver, severe anemias and especially hemolytic jaundice, Raynaud's disease and other conditions, that they may agglutinate the corpuscles of the patient at room temperature. However, if such auto-agglutinins are present in the plasma of the patient, it appears that transfusion is without hazard although it is advisable to keep the donor's blood at body temperature.

In this connection it should be emphasized, however, that injudicious attempts to warm the blood as a routine procedure in citrate transfusions are inadvisable and dangerous as warming may lead to both nonspecific and specific hemolytic reactions.

Furthermore, the administration of preserved citrated blood which has been stored too long may produce nonspecific reactions due to the hemolysis of unduly fragile erythrocytes, to which further reference will be made shortly.

Specific Reactions. These refer to reactions due to intravascular agglutination and hemolysis. They vary greatly in severity. Fortunately, the majority are not serious, being characterized by fever, chills, hemoglobinemia and possibly hemoglobinuria and jaundice. But severe reactions due to gross incompatibility of the bloods of the donors and recipient are serious and commonly designated "hemolytic shock reactions"; however, they are not necessarily fatal even when large amounts of blood are transfused. The symptoms include violent pains in the back, chills, respiratory embarrassment, circulatory collapse, hematuria and hemoglobinuria, jaundice, urticaria and symptoms due to small hemorrhages or emboli in the brain, mesentery, endocardium and the gastro-intestinal mucosa. The terminal stages of those who survive the immediate effects are characterized by renal failure.

Fortunately, these severe reactions are uncommon because of the care exercised in the typing of both patient and donor. But mistakes may occur in the conduct of these tests as well as clerical errors in reports and labelling. As a general rule, the administration of blood of the same group as that of the patient is safe for first transfusions but checking by direct cross-matching tests in addition is always advisable, especially in the transfusion of women in pregnancy or the puerperium, as well as in the case of all individuals who have received two or more transfusions on former occasions.

As previously stated, the conduct of pretransfusion compatibility tests requires skill and experience. The use of weak or impotent serums for grouping tests may lead to errors with serious results, including the possibility of labelling A_2 bloods as group O, and A_2B bloods as group B. Furthermore, serums or corpuscle suspensions heavily contaminated with bacteria may give panagglutination (the *Hübener-Thomsen phenomenon*) and lead to the error of labelling blood as group AB. Typing, therefore, should be done only with sterile group serums and fresh suspensions of corpuscles.

The administration of the sulfonamide compounds does not appear to produce difficulties in blood-grouping or cross-matching tests as the troubles sometimes experienced appear to be due to the diseases under treatment rather than to the compounds.[40]

Special care is required in the selection of donors for patients who have been given two or more transfusions because of the possibility of their having developed agglutinins for the subgroups of A and B corpuscles, as well as for the Rh factor, and especially in the case of the latter if group O blood has been previously administered. The same is true in the case of first and multiple transfusions to women in pregnancy and the puerperium who sometimes become Rh+ by reason of iso-immunization by fetuses. Under these conditions, direct cross-matching tests for subgroups and tests for Rh agglutinins, especially "blocking" tests, are especially desirable. Indeed, as recently reported by Wiener,[41] Hr sensitization may also cause hemolytic transfusion reactions in Rh+ individuals, although if Hr sensitization occurs at all it is usually mild in degree. Consequently, when hemolytic reactions occur Hr tests should be done and if the patient is Hr—, only Hr— blood of a compatible blood group should be used for subsequent transfusions.

It is true that the administration of incompatible blood does not always produce serious reactions and especially if less than 500 cc. is administered. Indeed, reactions from the first transfusion of subgroups may not be apparent at first but produce marked reactions if repeated. But such transfusions are of little or no therapeutic benefit, as the transfused incompatible erythrocytes are rapidly removed from the blood of recipients.

Undoubtedly, there is far more danger of serious reactions by transfusing corpuscles incompatible with the plasma of the recipient than transfusing plasma incompatible with the corpuscles of the recipient. But since the latter may result in intravascular agglutination and hemolysis, reactions may occur although recipients have considerable protection by reason of the rarity of large amounts of agglutinins in their plasma, the dilution of them by their blood, and by reason of the fact that they may be absorbed by the body cells. In this connection reference has already been made to the serious and even fatal hemolytic reactions which may occur due to the transfusion of group O or so-called universal blood if the plasma happens to contain unusually large amounts of agglutinins for the corpuscles of the recipient.

Under the conditions it appears advisable to conduct the pretransfusion blood examinations with both the corpuscles and the plasma of donor and recipient and always to include direct cross-matching tests if time and conditions permit. In the case of women in pregnancy and the puerperium it is also advisable to select Rh negative compatible donors. This is likewise true of all patients receiving multiple transfusions. Certainly the old dictum "once compatible always compatible" is both fallacious and dangerous. Both A and B agglutinogens given by transfusion or occurring in fetuses are antigenic and capable of producing agglutinins in recipients who are lacking in them. Fortunately O agglutinogen present in O and A_2 corpuscles is but feebly antigenic while N is without antigenic activity, although M and P are antigenic on rare occasions. But the Rh factor may be antigenic, especially after repeated transfusions of O blood and sometimes in pregnant women.

An important practical problem is how to differentiate severe reactions due to nonspecific causes from those due to incompatibility with intravascular hemolysis. According to Wiener[23,42] "the first step is to repeat the grouping and cross-matching tests on the samples of blood taken before transfusion, and also on fresh blood in order to determine whether there has been an error in grouping. The sample taken from the patient after the transfusion should also be examined for hemoglobinemia and its icterus index, and at the same time the urine should be examined. When the patient and donor are of the same blood group, tests for M-N should be made. If the M-N types of patient and donor are different, tests on the patient's blood with anti-M and anti-N serums will demonstrate whether the donor's blood is still present in the patient's circulation. If it is, a hemolytic reaction is excluded. If the M-N types are the same, this test cannot be used and one may then have to depend on the hemoglobin determinations. In an adult, for example, a 500 cc. transfusion should produce a rise of about 8 to 10 per cent in hemoglobin concentration. If this rise does not occur or is followed by a drop back to the original level within a day or two, the probability of latent hemolysis is increased. Finally, 5 to 10 days after the transfusion, the patient's serum should be matched against the donor's cells to detect any immune iso-antibodies which may have formed."

Speed Reactions and Circulatory Disturbances. As shown by Hyman and Hirshfeld,[43] the rapid introduction into the blood of various solutions may produce a type of reaction, designated "speed shock," which is characterized by a rapid fall of blood pressure, irregularities of respiration and lack of coagulability of the blood. For this reason they have recommended the drip method of transfusion, in which the flow of citrated blood is reduced to from 30 to 35 drops per minute, in transfusions in cases of surgical shock, uremia, septicemia, etc., requiring the administration of large amounts of blood; also in the transfusion of individuals with cardiac weakness. In other words, the rate should not exceed 1 cc. per pound of body weight per hour in the average case, while in those with an initial hemoglobin below 25 per cent with cachexia, cardiac disorders or respiratory embarrassment, the rate should be reduced to 0.5 cc.[44] In these, too, small transfusions at intervals are usually better than single large ones. If severe anemia in individuals with myocardial weakness is the chief indication for transfusion it is sometimes advisable to administer only concentrated suspensions of compatible erythrocytes.[45,46]

However, while blood transfusion is absolutely contraindicated in pulmonary edema and cardiac decompensation, in cases of pneumonia and myocardial weakness without decompensation blood may be usually administered in small amounts and by slow injection without the danger of producing pulmonary edema. Slow injections are also advisable in transfusions to individuals with thrombophlebitis or advanced bacterial endocarditis in order to minimize the danger of dislodging thrombi or vegetations. Otherwise, in the absence of marked myocardial weakness, large transfusions of citrated blood at the usual speed appear to be without danger.

The rapid administration of blood by direct methods of transfusion, however, may produce reactions. Not infrequently, by the time the first 200 or 300 cc. have been given, patients may begin to complain of pain in the arm due to distention of the vein, accompanied by coughing, complaints of fullness in the head and vertigo. If these symptoms are not heeded and the transfusion continued, acute cardiac dilatation with collapse may result, especially in individuals with valvular heart disease and marked anemia. Vascular accidents such as retinal hemorrhages are not uncommon and especially in individuals with advanced chronic nephritis or with the blood dyscrasias.[47]

Allergic Reactions. About one per cent of transfusions are followed by reactions of an allergic nature, the most common manifestation being a generalized urticaria, sometimes associated with angioneurotic edema, responding to treatment with adrenalin chloride.[42] This may be due to the transfer of reagin in the blood of a donor allergic to foods, reacting with the allergenic foods in the blood of the recipient.[48] For this reason the use of fasting donors has been recommended.[49] But urticaria frequently occurs after transfusions in which neither the donor nor the recipient is known to be allergic. Urticaria and severe reactions of a shock-like allergic nature are especially likely to occur in patients who have had repeated transfusions of the bloods of the same donors and for this reason it has been claimed, although without adequate proof, that they may be due to the presence of acquired isoprecipitins for plasma proteins in the blood of the recipients. However, whatever the causes may be, it is apparent that if the blood of a particular donor produces urticaria it should not be used for subsequent transfusions of the same individual, since more severe reactions may be produced, whereas transfusion with the blood of another donor is usually satisfactory.

Transmission of Disease. The possibility of the transmission of syphilis, malaria and homologous serum jaundice also constitute additional hazards in blood transfusion. Needless to state, donors should be in good general health. Usually those with active tuberculosis are not employed but, to the best of my knowledge, there have been no instances of proved transmission of this disease by transfusion and only one recorded instance of transmission of a virus which was that of varicella, producing encephalitis in the recipient. The transmission of streptococci, staphylococci, *S. typhosa* or other micro-organisms present in the blood of donors with low-grade septicemias or bacteremias is a possibility although a remote one. In former times, however, some cases of accidental infections of donors with streptococci or staphylococci were reported due to the back flow of the blood of patients with septicemia during transfusions, by artery-to-vein anastomosis or other direct but obsolete methods.

In view of the enormous number of transfusions given yearly throughout the world, the number of reported and unreported instances of *transfusion syphilis* is small although there can be no denial of the hazard. This is particularly true in the case of donors in the incubation, primary and secondary stages of syphilis during which *T. pallidum* is especially likely to be in the blood although Klauder and Butterworth [50] believe that the hazard also exists to some extent in untreated and inadequately treated syphilitic donors within ten years of infection, and especially within five years.

For this reason, donors are now usually subjected to a serologic test for syphilis, with the rejection of those giving positive reactions regardless of the duration of the disease and whether or not treatment has been given. This is right and proper although it is to be admitted that the risks are very slight in chronic syphilis and especially in treated cases. Unfortunately, however, serologic tests fail to detect the disease in donors during the late incubation and early primary stages of the disease when the risks of transmission by transfusion are greatest. Consequently, a physical examination of donors for primary and secondary lesions of syphilis is highly advisable whenever possible and particularly in the case of strangers. Negative histories are of but limited value for various and obvious reasons.

In case of doubt, or when time does not permit an investigation of the donor for syphilis, and especially when donors known to have the disease must be used (donors of necessity) it would appear that the blood may be rendered safe for the recipient by the simple method of Kast, Peterson and Kolmer [51] consisting of the addition of 1 cc. of a solution of neoarsphenamine (prepared by dissolving 0.1 gm. in 10 cc. of sterile distilled water) to each 100 cc. of citrated blood about 15 minutes before transfusion. This gives a final concentration of 1:10,000 solution of neoarsphenamine which is treponemicidal and well-borne. Eichenlaub and his associates [52] have reported similar results with mapharsen and advise adding 0.01 gm. to the citrate solution required for 500 cc. of blood.

However, in this connection it is to be stated that *T. pallidum* dies out spontaneously in preserved citrated blood kept for at least three days in the refrigerator.[53, 54]

The situation in relation to the hazard of *malaria* is more difficult and especially in malarial endemic areas. Filtration of plasma or serum removes the danger, but in the case of blood, no one should be used as a donor who has ever had the disease,[55] since transmission of malaria has been frequently reported by donors apparently free of the disease. Ironically enough the donor may suffer more than the recipient, due to exacerbation of chronic malarial infection. Otherwise, some protection may be afforded by the cinchonization of both donor and recipient for several days prior to transfusion.[56]

The accidental transmission of the virus of homologous serum jaundice is now known to be an additional and important hazard, as will be discussed in more detail in Chapter 26. *This hazard is particularly important in transfusions of pooled stored blood and especially pooled plasma.* No cases of transmission have been reported following transfusions of fresh blood or fresh serum from single donors. In 2443 transfusions of plasma or blood, Scheinberg and his associates [57] have reported 11 cases of transmission of the virus of homologous serum jaundice or an incidence of about 0.5 per cent; it is highly probable, however, that these cases were due to the transfusion of pooled plasma. Brightman and Korns [58] have reported an incidence of 4.5 per cent of the disease in follow-up studies of 649 cases transfused with pooled plasma. The virus is apparently the same as that producing infectious or viral hepatitis. Unfortunately, the latter disease may occur in an asymptomatic form without jaundice or laboratory changes. Needless to state, all donors with jaundice or a history of recent jaundice should be excluded, but there are no clinical or laboratory tests for the disease in its asymptomatic form, during which the virus may be present in the blood.

The incubation period of homologous serum jaundice is from 40 to 60 to as long as 120 or more days following transfusions of blood or plasma. Consequently, the disease is not an immediate or early but a late hazard.

In the United States, human blood is commonly stored in "banks" by the addition of 14 cc. of a sterile 2.5 per cent solution of chemically pure sodium citrate in physiologic saline solution to each 100 cc. of blood, with preservation at 4 to 6° C. in a refrigerator. This gives a 0.35 per cent concentration of sodium citrate for the prevention of coagulation, and after typing, sterility, and Wassermann tests, the blood is ordinarily available for transfusion over a period of ten days to several weeks. The addition of dextrose or dextrin apparently aids in the preservation of the erythrocytes by decreasing sodium penetration and hemolysis of these cells.[59–62] For this reason the collection and preservation of blood in Alsever's solution (ACD) or a modification of it is to be preferred, since 70 per cent of erythrocytes remain viable for at least twenty-one days, whereas in sodium citrate solution 70 per cent remain viable for only seven to ten days. Satisfactory methods for the preservation of the unstable granulocytes and platelets, however, have not yet been discovered. Alsever's solution is prepared by dissolving 0.55 gm. citric acid, 8.0 gm. sodium citrate, 4.2 gm. sodium chloride and 20.5 gm. dextrose in 1000 cc. of distilled water followed by autoclaving for sterilization which produces little or no caramel; 70 cc. may be used for the collection and preservation of 500 cc. of blood.

Ordinarily, the administration of *sterile* blood stored for periods up to two or three weeks produces no more reaction than fresh citrated blood. But the incidence of contamination with bacteria tends to be high. Consequently, unless the blood is immediately chilled and constantly stored at a low temperature until used, multiplication of the contaminating bacteria occurs, with an increased incidence of the nonspecific protein shock type of reaction. Furthermore, with increasing storage the erythrocytes become increasingly fragile and there is an increased tendency to more severe reactions of the hemolytic type. At least one death from such a cause has been reported.[63]

Indeed, the erythrocytes of preserved citrated blood have shown evidences of dehemoglobinization as early as forty-eight hours after collection with progressive degenerative changes and increasing fragility up to fourteen days when a large percentage were mere shadows, swollen and fragile. As previously stated, however, these cells are better preservd in Alsever's solution. Rapid degenerative changes in the polymorphonuclear neutrophilic leukocytes and platelets have also been observed, beginning within forty-eight hours, with a diminution in the phagocytic activities of the former and destruction of complement and natural antibodies beginning within a few days.[62,64,65] A progressive decrease in prothrombin also occurs, so that stored blood is believed inferior to fresh blood for controlling hemorrhage in jaundiced individuals.[66,67,68,69]

Under the conditions, it appears that stored blood is satisfactory for blood-volume restoration in the treatment of hemorrhage and surgical shock up to two or three weeks after collection. But it is inferior to fresh blood in the treatment of the anemias, prothrombin deficiencies, hemorrhagic dyscrasias and the acute and chronic infections, especially the septicemias; for this purpose it should not be used when more than three to five days old. Furthermore, the plasma of the stored blood of universal donors (group O) should not contain an excess of agglutinins for the corpuscles of groups A, B and AB recipients in order to avoid reactions.[70] And since stored erythrocytes carrying the Rh factor may produce reactions in individuals who have had previous transfusions, and likewise in women during pregnancy and the puerperium, it would appear advisable to have compatible Rh– blood available for these purposes.

In passing it may be stated, however, that the use of stored blood reduces the hazard of transmission of syphilis since, as previously stated, *T. pallidum* is destroyed within the first 48 to 72 hours of storage.[53,54] Apparently the plasmodia of malaria are also destroyed by storage over the same period of time. In this connection I have also observed that *P. vivax* is killed during the process of preserving malarial blood by the lyophile and cryochem processes. As previously stated, however, stored blood, especially pooled stored blood, may transmit the virus of homologous serum jaundice.

PLASMA

Since individuals with severe hemorrhage do not suffer from the loss of erythrocytes as much as from reduction in blood-volume, the intravenous administration of properly prepared sterile plasma has proved valuable in its treatment. Furthermore, it appears preferable to blood transfusion in the treatment of surgical shock because it does not add to the degree of hemoconcentration but rather relieves that already present.

Plasma has also proved of value in the treatment of hypoproteinemia due not only to severe protein loss in nephritis and ascites, but to trauma and severe burns as well; also in the treatment of severe dehydration (dehydration shock) due to excessive vomiting, diarrhea, intestinal obstruction, etc. Various methods have been described for its preparation in the fluid and dried states.[71,72]

Fresh plasma is probably also of value in the treatment of the septicemias, since complement and natural antibodies are present, but its value after storage for varying periods of time cannot be stated; it is likely, however, that gradual deterioration of complement and natural antibodies occurs, as observed in the case of stored blood (as previously discussed). At all events, it is highly probable that transfusions of fresh blood are to be preferred in the treatment of the acute and chronic infections. *Fresh* plasma also contains fibrinogen and prothrombin substances but fibrinogen is precipitated upon storage and requires filtration of the plasma for its removal before administration.

Since plasma is pooled, which lowers its content of agglutinins for the corpuscles of blood groups A, B and AB, it is considered unnecessary to determine the group of the patient; even cross-matching tests are regarded as unnecessary because the small amounts of agglutinins administered are quickly diluted in the blood of the recipient with the chances of some being absorbed by the body cells. Consequently, reactions are infrequent and usually of the thermal or allergic types.[73] Since corpuscles are not injected, the question of the presence or absence of agglutinin for the Rh factor in the blood of individuals who have had previous transfusions is unimportant; the same is true of women in pregnancy or the puerperium. Pooling and filtration also greatly reduce the incidence of reactions due to allergy.

It is possible that the fresh plasma of syphilitic individuals may contain *T. pallidum,* with the hazard of transmission of syphilis. But this is highly improbable in view of the technical procedures employed in its preparation, including that of filtration. Indeed, it is almost certain that *T. pallidum* is destroyed within 48 to 72 hours as has been found true in the case of stored citrated blood, as previously discussed. At least, no cases of syphilis due to the administration of plasma have been reported to the best of my knowledge. Furthermore, the filtration of plasma removes the hazard of the transmission of malaria. As will be pointed out on page 779, however, the administration of *pooled* plasma especially entails the hazard of transmission of the virus of infectious or viral hepatitis, producing homologous serum jaundice. Plasma from single donors, however, entails much less risk as is true of transfusions of blood from single donors. While pooled *fluid* plasma may produce an incidence of about 7 per cent homologous serum jaundice, the incidence following the administration of pooled *dried* plasma has been reduced to about 3 to 4.5 per cent probably because the methods of preparation may result in the destruction of the virus. Under the circumstances, pools of plasma should be furnished by not more than two donors and especially if fresh fluid plasma is employed. Ultraviolet irradiation of plasma has been proposed for the destruction of the virus and is well borne on intravenous injection[57,74] although its value in the prevention of homologous serum jaundice cannot be stated at the present time. For the prophylaxis of this disease, however, 10 cc. of gamma globulin may be administered intramuscularly on two occasions one month apart, starting a month after pooled plasma has been given.

OTHER BLOOD DERIVATIVES

Erythrocytes. When blood is collected in Alsever's solution and allowed to sediment at 4° C. overnight, the erythrocytes may be collected after withdrawal of the supernatant plasma and resuspended in sufficient sterile 1 per cent sodium chloride solution to give a hematocrit reading of about 65 per cent, followed by filtration through sterile gauze. The cells keep about as well as in whole blood. Such suspensions may be given intravenously in the treatment of all anemias except those due to acute hemorrhage or severe infection, not only because they supply more hemoglobin with less osmotic activity of the plasma proteins and less sodium chloride, but because the iron of nonviable cells can be reutilized for the synthesis of hemoglobin.

Hemoglobin. The administration of solutions of dried hemoglobin have proved highly effective in the treatment of experimental shock in the lower animals. However, since renal function may be temporarily depressed, all investigators have been cautious in relation to its administration to human beings. A derivative of hemoglobin, modified globin, has been developed as a plasma protein substitute but its therapeutic value has not been evaluated.

Plasma Fractions. *Fibrin foam* with thrombin on absorbable sponges has proved successful in the control of bleeding by local application and for the attachment of skin grafts.

Fraction I globulin not only provides a means for supplying more fibrinogen than whole blood in the treatment of bleeding but when prepared of *fresh* plasma also contains the so-called "antihemophilic globulin" which has proved effective in the control of bleeding in hemophilia when administered in doses of 200 to 600 mg. dissolved in 5 to 30 cc. of sterile saline solution. It may, however, transmit the virus of infectious hepatitis with the production of homologous serum jaundice.

Gamma Globulin. Because gamma globulin (fraction II) contains antibodies, especially when prepared of the plasma of convalescents, it has been found effective in the prevention and modification of measles and also in the prevention of rubella and infectious or viral hepatitis.

Serum Albumin. Serum albumin was originally developed as a substitute for blood in the emergency treatment of surgical shock due to hemorrhage. In this form it was dissolved in 1.6 per cent sodium chloride solution containing a mercurial bacteriostatic preservative. Needless to state, it has no oxygen-carrying power and is therefore without value in the treatment of the resulting anemia. Furthermore, the administration of this type of serum albumin has been found to produce homologous serum jaundice in about 1.5 per cent of cases.

Subsequently the use of serum albumin in the treatment of hypoproteinemic edema and nephrosis led to the preparation of a salt-poor albumin solution in which the sodium chloride content is one-seventh that of an osmotically equivalent volume of plasma and in which stabilization has made possible heating at 60° C. for ten hours. This procedure not only permits the omission of a mercurial preservative but also inactivates or destroys the virus of infectious hepatitis responsible for homologous serum jaundice. Consequently, all albumin being produced now conforms to the specifications for low sodium chloride and mercurial-free serum albumin. It has proved effective not only in the treatment of surgical shock due to hemorrhage but in the treatment of hypoalbuminemia due to nephrosis, severe infections, burns, peritonitis, cirrhosis of the liver, surgical operations, etc.

EXAMINATIONS IN RELATION TO DISPUTED PARENTAGE

In most instances, disputed parentage occurs in cases where a man, named by the mother as the father of a child born out of lawful wedlock, denies such paternity, or, a husband denies paternity of a child born in lawful wedlock. Disputes involving maternity are much less common and usually involve a woman who has secretly secured a child (of which she claims to be the mother) for the purpose of compelling an alleged father to marry her, or to obtain dower rights in her dead husband's estate (in jurisdictions where birth of issue is a prerequisite to the right of dower), or in order that the child may become the heir to her husband's estate. Disputes also arise occasionally in relation to the parentage of infants born in hospitals and accidentally interchanged as well in the case of wet-nurses who wilfully substitute their own infants for those placed in their charge, for the purpose of having their own secure the benefits of better homes.

Since the agglutinogens of erythrocytes belonging to groups A, B, M, and N are inherited in accordance with definite laws, determinations of the groups to which the man, woman and child belong are usually of great value as aids in

TABLE 100. SUMMARY OF THE CLINICAL INTERPRETATION OF EXAMINATIONS IN RELATION TO DISPUTED PARENTAGE

Subject	Interpretation
General Considerations	The agglutinogens, A, B, M, N, and Rh are transmitted by heredity. Examinations in relation to disputed parentage are mainly based upon determinations of the major blood groups (O, A, B, AB) of the man, woman and child. Examinations for the subgroups of A and AB corpuscles are not employed but supplementary examinations for the agglutinogens M, N and Rh are of additional value and sometimes essential. In view of the medicolegal aspects, the tests must be conducted with care and skill and especially those for M, N and Rh. Small amounts of blood collected in sodium citrate solution are required for erythrocytes; also blood in dry sterile test tubes if cross-matching tests are to be conducted. Agglutinogens A and B cannot be present in the corpuscles of a child unless present in the corpuscles of either or both parents. A child of Group O cannot have a parent belonging to Group AB. A child of Group AB cannot have a parent belonging to Group O.
Paternity	The tests may prove the nonpaternity of an accused man. However, they cannot establish his paternity because some other man of the same blood group may be the father. If the man is AB and the child O, or vice versa, nonpaternity is established regardless of the blood group of the mother. If both father and child are A, nonpaternity cannot be established. Men belonging to MN cannot prove nonpaternity. If the child is M or N and these are absent in one or both parents, nonpaternity is established; also if the man is M and the child N, or the reverse.
Maternity	The chances of establishing nonmaternity by the major blood groups alone are slight on account of the rare occurrence of AB. Maternity is disproven if an alleged mother is AB and the child O, or the reverse; also if the alleged mother is M and the child N, or the reverse.
Identity of Infants	In cases involving the parentage of infants accidentally interchanged in a hospital, or purposefully exchanged by wet nurses, tests for agglutinogens M and N alone solve 40 per cent and tests for agglutinogens A, B, M and N about 70 per cent.

settling disputes (Table 100). Needless to state, however, the tests must be conducted with care and skill; indeed, when they require examinations for the agglutinogens M and N, they should be entrusted only to experts since it is necessary to employ anti-M serum prepared by the immunization of rabbits with washed erythrocytes which are M+N− and anti-N serum by immunization with those which are M−N+; in both instances the erythrocytes should be preferably those of group O so that no agglutinins are produced for A and B corpuscles.[75]

Examinations require the submission of small amounts of blood collected in sodium citrate solution from the mother, child and alleged father or fathers; also small amounts collected from each in sterile test tubes if cross-matching tests with serums are to be done. If only the blood groups are to be determined, the corpuscles of infants at birth or any subsequent age are satisfactory but if cross-matching tests are to be conducted, it is better to wait until the child is at least a month old at which time more agglutinins are present in the serum. Means for the positive identification of an accused man by photograph, or otherwise, are advisable in order to prevent him from substituting an accommodating friend for the tests.

As far as the major blood groups are concerned, Table 101 shows what the groups of the children must be when the groups of the alleged parents are known.

TABLE 101

Groups of Parents	Groups of Children Possible	Groups of Children Not Possible
O × O	O	A, B, AB
O × A	O, A	B, AB
O × B	O, B	A, AB
O × AB	A, B	O, AB
A × A	O, A	B, AB
A × B	O, A, B, AB	—
A × AB	A, B, AB	O
B × B	O, B	A, AB
B × AB	A, B, AB	O
AB × AB	A, B, AB	O

It will be observed (1) that agglutinogens A and B cannot appear in the blood of a child unless present in the blood of one or both parents; (2) that a parent belonging to group AB cannot produce a child belonging to group O and (3) that a group O parent cannot produce a child belonging to group AB. Of course, these tests cannot prove that an accused man *is* the father of a child because any other man belonging to his group could be the actual father. His chances of proving nonpaternity, however, are between 16 to 19 per cent. These routine blood-grouping tests, therefore, not only establish the innocence of a large number of men falsely accused of paternity, but also serve to diminish the incidence of fake accusations by women who may fear the charge of perjury in court. Incidentally, whenever a woman refuses to submit to the tests, false accusation on her part may be reasonably suspected; likewise the refusal of a man creates the impression that he may be the actual father even though he has everything to gain and nothing to lose by submitting to the tests. In other words, blood-grouping tests can only be used to exclude but not to prove paternity. However, if two men are involved as possible fathers of a child, the tests may readily enough prove that one of them could not be the actual father.

Occasionally, however, it may only be possible to make the grouping tests with the bloods of a man and child as, for example, when a husband, suspecting his wife of infidelity, desires to obtain further evidence before making any charges. If both the man and child are found to belong to group A, it is useless to go further, since irrespective of the group of the mother, nonpaternity cannot be proved. On the other hand, if the man belongs to group AB and the child to group O, or vice versa, nonpaternity is established, regardless of the group of the mother, although the chances of such proof are only about 5 per cent.

TABLE 102

Groups of Parents	Groups of Children Possible	Groups of Children Not Possible
MN × MN	MN, M, N	—
MN × N	MN, N	M
MN × M	MN, M	N
M × N	MN	M, N
M × M	M	MN, N
N × N	N	MN, M

The subgroups of A and AB corpuscles cannot be used for excluding paternity or maternity, but tests for the agglutinogens M and N give a man increased chances of proving nonpaternity. Men belonging to type MN, however, have no chance at all, whereas those belonging to M have one in three, and in the case of N, two in five. When the major blood groups and the subgroups M and N are determined at the same time, the chances of proving nonpaternity are significantly increased in most cases. The chances of proving nonmaternity by determinations of the major blood groups alone are slight on account of the rare occurrence of AB, but maternity is disproved by any of the following four combinations: (1) alleged mother AB and child O; (2) alleged mother O and child AB; (3) alleged mother M and child N; (4) alleged mother N and child M.

If a child possesses agglutinogen M or N which are not present in the corpuscles of one or both parents, nonpaternity is established. If either of the combinations, type M man with type N child or type N man with type M child are found, nonpaternity is likewise proved. This type of exclusion is of particular importance when it is impossible to examine the mother's blood. Furthermore, in cases involving the parentage of infants accidentally interchanged, tests for agglutinogens

M and N alone solve 40 per cent whereas tests for all four agglutinogens A, B, M, and N solve almost 70 per cent (Table 102).

The Rh-Hr blood types, however, have considerably enhanced the usefulness of blood tests in cases of disputed parentage, since these have increased the varieties of human blood that may be differentiated from 36 to 360. Under these conditions, an innocent man now has approximately a 50 per cent chance of being excluded when the tests are properly and skilfully conducted.[76] Two Rh— parents can only have Rh— children. If one parent is Rh— and the other is Rh+, the children will all be Rh+ if the Rh+ parent is homozygous, or half the children will be Rh+ and half Rh— if the Rh+ parent is heterozygous. When both parents are Rh+, all the children will be Rh+ except when the parents are both heterozygous, in which case one-fourth of the children will be Rh—.

EXAMINATIONS IN RELATION TO THE DETECTION OF BLOOD, SEMEN, SALIVA AND OTHER SUBSTANCES

When properly and skilfully conducted, serologic examinations have proved of great value not only in the identification of human blood, semen and saliva but likewise of bones and other tissues as well as in the detection of meat and milk adulterations (Table 103).

Blood Stains. Examinations of blood stains are usually confined to forensic cases for the primary purpose of determining whether or not they are of human origin and, if so, whether they are composed of the blood of the victim or some other human being. Chemical or spectroscopic tests for determining whether or not stains are of blood, also precipitin and complement fixation tests for determining whether or not they are of human or other origin, can be successfully conducted with material many years old. But blood-grouping tests for aid in determining whether or not the blood is that of the victim or some other individual are best conducted with relatively fresh material. Consequently, police officers should be instructed to bring promptly to the laboratory all blood stains found at the scene of a crime, and blood-grouping tests, including those for M and N types, should be done routinely in all necropsies on cases of death by violence, whether or not there is suspicion of murder at the time.

The first step is to determine whether or not a stain or material scraped from an implement is composed of blood by means of one of the chemical tests for occult blood, the Teichmann hematin crystal test, or by spectroscopic examination. If positive results are observed, the next step consists in conducting precipitin tests with properly prepared extracts of the stain or other material and potent antihuman serum, although the complement fixation test is usually more sensitive and decisive.[77] Needless to state, either or both of these tests should be conducted by skilful and experienced serologists with all precautions against sources of error. When properly conducted, negative reactions usually indicate that the stain or other material is not human blood. In this case, additional tests may be conducted with the antiserums of the domestic animals such as those for the bloods of cattle, sheep, dogs, cats, chickens, etc. With stains containing mixtures of the blood of two species, tests with both of the respective antiserums will give positive reactions.

TABLE 103. SUMMARY OF THE CLINICAL INTERPRETATION OF EXAMINATIONS IN RELATION TO THE DETECTION OF BLOOD, SEMEN, SALIVA AND OTHER SUBSTANCES

Substance	Interpretation
Blood	Blood occurring in stains or other material may be detected by chemical or other tests but they do not determine whether it is human blood or that of the lower animals. However, human blood may be differentiated from that of the lower animals by precipitin or complement fixation tests but these tests cannot differentiate the blood of one human being from that of another. Tests for iso-agglutinins and iso-agglutinogens in stains or other material, however, possess positive but no negative value in determining the source of human blood.
Seminal and Other Stains	Stains of human semen may be detected by precipitin or complement fixation tests but they cannot differentiate the semen of one individual from that of another. Extracts of dried semen, however, may contain the major blood agglutinogens which may aid in determining the source of a stain. Stains of normal and albuminous urine and saliva give negative complement fixation reactions with prepared antisemen serum. Urine and extracts of dried saliva may contain the major agglutinogens corresponding to those in the erythrocytes of individuals.
Other Substances	Precipitin tests are of value in the identification of fragments of human bones in medicolegal cases. Precipitin and especially complement fixation tests are of value in the detection of the flesh of horses, cats, dogs, etc., in sausages and similar foods. Special immune serums are required for the detection of adulterated meats which have been cooked or smoked. Precipitin and especially complement fixation tests are of value in the detection of human milk adulterated with cow's milk; they are unable, however, to detect the adulteration of goat's milk with cow's milk.

If the results show that the stain is composed of human blood, tests for iso-agglutinins and iso-agglutinogens may then be employed. But almost forty years ago Landsteiner and Richter [78] showed it was possible to prove that a blood stain could not have come from a given individual without first determining the group of the stain. Thus, if an extract of a stain is found to agglutinate the erythrocytes of the individual, it could not have been derived from that person's blood. It is always best, however, to combine this method with the actual determination of the blood group of the stain, since the two tests serve as mutual checks. Needless to state, the technic is complicated and exacting; [13] consequently, precautions must be exercised, especially against errors due to pseudo-agglutination.

In tests for iso-agglutinins, extracts are employed instead of serums against standard suspensions of erythrocytes belonging to groups O, A_1 and B, A_1 cells being preferred to A because of being more sensitive. Only positive results, however, are of significance. Agglutination of either or both A_1 and B corpuscles proves that the corresponding iso-agglutinin is present in the stain. But the absence of agglutination does not necessarily mean that the agglutinins were originally absent, as they may have deteriorated. If both A_1 and B cells are agglutinated but not O cells, the stain belongs to group O. If the A_1 cells are agglutinated but not the B cells, the stain may belong to group B but it could also belong to group O if the iso-agglutinin *b* had deteriorated; at any rate, the stain could not belong to groups A or AB.

If the stain, or other material, is not completely dry, it may be possible to prepare suspensions of the corpuscles for grouping tests with standard A and B serums for agglutinogens. If, however, the stain is dry, extracts must be prepared and an indirect method of testing for agglutinogens employed by absorption tests.[13] Here again only positive findings are of significance. In other words, failure to find iso-agglutinogens does not necessarily mean that the stain belongs to group O because the agglutinogens originally present may have deteriorated, the amount of material to be tested may be inadequate, or the technic of the test may not have been sufficiently sensitive. However, if with proper technic the stain is found to absorb anti-B agglutinins but not anti-A, it cannot belong to groups O or A although it is not possible to assert with certainty whether it belongs to group B or to subgroup A_2B. Attempts to identify group O have not yet proved completely successful, since anti-O serum of sufficient potency is difficult to secure and the reactions are not decisive.

Individual diagnosis of stains by means of determining the presence of agglutinogens M and N is possible in the case of stains several weeks old and especially in the case of M. The anti-M and anti-N serums, however, should be of sufficient potency to give consistent and reliable results with stains of known types. Blood stains containing the agglutinogens A_1 and A_2 are distinguished by a difference in their ability to absorb anti-A agglutinins but this is not sufficiently pronounced for medicolegal purposes.

Seminal and Other Stains. Precipitin and complement fixation tests are also of value in the detection of seminal stains of human origin although, as in the case of blood stains, they cannot determine whether a stain is composed of semen of a particular individual.

Both tests are conducted with a potent antiserum prepared by the immunization of rabbits with human semen. Antibodies are also present for human serum which must be removed by mixing the antiserum with an equal part of 1:200 dilution of human serum, followed by centrifugation for the removal of precipitate.[79] In complement fixation tests it is also advisable to absorb the antiserum with vaginal secretions free of spermatozoa.[77]

Dried semen also frequently contains the major blood agglutinogens, which may be detected by special tests more readily than in the case of dried blood[13] and aid in determining the source of a stain.

In some instances stains in clothing are claimed to be of urine or saliva instead of semen. Extracts of stains of normal and albuminous urine, as well as extracts of dried saliva, give negative complement fixation reactions with prepared antisemen serum.[77] Normal urine, however, may contain the major blood agglutinogens although not all individuals carrying them in their corpuscles excrete them in their urine. The iso-agglutinins do not occur in normal urine but may be present in urine containing large amounts of protein. Extracts of dried saliva also frequently contain large amounts of the major blood agglutinogens, which may be detected by special tests,[13] although not all persons excrete them. Their absence in extracts of stains may be due to the fact that the saliva is derived from a group O individual, or from a "nonsecretor" or because the agglutinogens originally present have undergone marked deterioration.

Other Substances. Precipitin tests conducted with antihuman serum and extracts of fragments of bones may be employed for the identification of those of human origin in medicolegal cases.

Precipitin tests may be also employed for the detection of adulteration of sausages and the like with the flesh of horses, cats, dogs, etc., by testing extracts with known antiserums. Complement fixation tests, however, are more sensitive and equally specific.[77] The detection of cooked or smoked adulterating meats requires the use of antiserums containing coctoprecipitins prepared by the immunization of rabbits with the serums of horses, cats, dogs, etc., heated at 70° C. for thirty minutes.[80]

Precipitin and, preferably, complement fixation tests[77] are also capable of differentiating between human and cow milk; consequently, they have proved of value in the detection of the adulteration of human milk with that of the cow, which is sometimes to be suspected when human milk is being purchased for the feeding of infants. Unfortunately, however, the tests do not serve for the detection of adulteration of goat's milk with cow's milk which is sometimes suspected in those localities where goat's milk is produced on a commercial scale.

EXAMINATIONS IN THE BACTERIAL DISEASES

Typhoid Fever. Since time is required for the production of antibodies in typhoid fever, bacteriologic examinations of the blood and feces during the first week after the onset of symptoms are usually of greater value in the diagnosis of the disease than serologic tests. Thus, blood cultures are likely to be positive in about 80 per cent of cases, with only about 20 per cent or less positive serologic reactions. After the first seven to ten days, however, the incidence of positive blood cultures progressively decreases, while the incidence of positive serologic reactions progressively increases. When blood is collected in sterile Keidel or test tubes with all precautions against contamination, the laboratory should routinely make cultures of the clots after removal of the serums. The average of positive clot cultures during the first three weeks is about 35 per cent; positive agglutination reactions, however, occur sometime during the disease in well over 90 per cent. Needless to state, a positive blood or feces culture, or both, is more conclusive evidence of typhoid fever than a positive agglutination reaction alone.

It is to be emphasized, however, that both bacteriologic and serologic examinations may give negative results during the first week or ten days of the disease, in which case the serologic tests at least should be repeated every three to five days until a diagnosis of typhoid fever has been established or excluded.

Two serologic procedures are available for diagnostic purposes, namely, the agglutination or Widal test and the complement fixation test. The former is usually employed because of greater technical simplicity. Its sensitivity, however, varies considerably in different laboratories so that standardization of technic is highly desirable. Indeed, it is highly probable that serologic surveys similar to those employed in syphilis with so much profit, would appreciably improve the diagnostic value of agglutination tests in both typhoid and paratyphoid fevers (Table 104).

Agglutination Tests. The microscopic agglutination or Widal test, conducted with living cultures of the typhoid bacillus, was commonly employed until the last few years. Even when dried blood was used the results were of great value. When a sensitive, motile, smooth culture is employed with final dilutions of serum of 1:40 and 1:80, the test is almost as valuable as present-day macroscopic tests employing H and O antigens and especially when conducted by experienced workers. Since the readings can be made one hour later it is an excellent "screen test" because serums giving negative reactions do not usually require retesting by macroscopic tests employing H and O antigens. Agglutination at 1:40 is suspiciously positive, while agglutination at 1:80 is definitely positive. Agglutinins above the normal range of 1:20 to 1:30 may occur as early as the third or fourth days of typhoid fever but this is very exceptional. As a general rule, they are first increased between the seventh and tenth days with 60 to 70 per cent positive reactions, reaching the maximum in three to four weeks (80 to 95 per cent positive reactions), and are but rarely absent after the disease has been present for five weeks.

There has been a tendency in recent years, however, to conduct macroscopic agglutination tests with H and O antigens of typhoid bacilli. The H antigen is prepared by treating a culture of motile bacilli with formalin which yields a flagellar antigen giving large flaky flocculi in positive reactions. The O antigen is prepared by treating a culture with alcohol and is regarded as yielding a somatic antigen giving small flocculi in positive reactions. It is extremely important to prepare the antigens of sensitive strains and particularly in the case of O antigen. The tests may be conducted with one or two dilutions of serum with each antigen, but a series of at least six dilutions with each is preferred. The temperature and duration of incubation varies in different laboratories, but 18 to 24 hours at 52 to 55° C. appears to be satisfactory.

Normal serums agglutinate H antigen in a final dilution of about 1:20; agglutination at 1:40 is suspiciously positive while agglutination at 1:80 to 1:100 is definitely positive. Normal serums may agglutinate O antigen at 1:80 or 1:100; agglutination at 1:160 or higher is definitely positive.

Agglutination of H antigen may occur somewhat earlier in typhoid fever than agglutination of O antigen but some laboratories believe that the O antigen is the more sensitive of the two as well as being more specific and much less likely to yield falsely positive or anamnestic reactions due to other acute infections.[81] Agglutination of H antigen at 1:80 or higher indicates that the individual has

TABLE 104. THE CLINICAL INTERPRETATION OF SEROLOGIC EXAMINATIONS IN TYPHOID AND PARATYPHOID FEVERS

Disease	Interpretation
Typhoid Fever	Agglutination (Widal) and complement fixation tests are available for serologic diagnosis but the former is generally employed. Bacteriologic examinations of the blood and feces are preferred for diagnostic purposes during the early stages of the disease. During the first week or ten days both bacteriologic and serologic examinations may give negative results; under these conditions the serologic tests should be repeated every three to five days. Positive agglutination reactions occur in about 60 to 70 per cent of cases between the first and second weeks and in about 80 to 95 per cent between the third and fourth weeks of the disease. Normal serums in the microscopic test agglutinate living bacilli at 1:20 to 1:30; agglutination at 1:40 is suspicious and at 1:80 definitely indicative of typhoid fever. Normal serums agglutinate H antigen at about 1:20; agglutination at 1:40 is suspicious and at 1:80 or 1:100 or higher definitely indicative of typhoid fever. Normal serums agglutinate O antigen at 1:80 to 1:100; agglutination at 1:160 or higher is definitely indicative of typhoid fever. Both antigens should be employed routinely. Agglutination tests with both antigens and especially with O are helpful in the detection of typhoid carriers. Tests employing Vi antigen are of lesser value. In individuals previously vaccinated against typhoid fever a high titer of O agglutinin with a low titer of H agglutinin is indicative of typhoid fever. In vaccinated individuals a drop of H and especially of O agglutinins to normal levels may be indicative of the advisability of reimmunization. Complement fixation tests are apparently more specific and more sensitive than agglutination tests. They are also of value for the detection of typhoid fever in previously vaccinated individuals as well as for the detection of carriers.
Paratyphoid Fever	Due to infection with *S. paratyphi* A or B. Uncommon in the United States; most cases are due to *S. paratyphi* B. Diagnosis and differential diagnosis from mild typhoid fever is purely a laboratory problem. Bacteriologic examinations of the feces, urine and blood and agglutination tests are required. The latter usually require repetition one or more times at intervals of three to five days. Agglutination tests should be conducted with H and O antigens of both of the paratyphoid bacilli and the typhoid bacillus. Absorption tests may be required. In suspected paratyphoid fever occurring in a previously immunized individual a progressive increase of agglutinin for O antigen is of diagnostic value.

typhoid fever, has had the disease or has been immunized against it with typhoid-paratyphoid vaccine. Agglutination of O antigen at 1:160 or higher, however, almost invariably indicates the presence of typhoid fever. At any rate, both antigens should be employed routinely as well as an O antigen of the paratyphoid bacillus B. Indeed, as shortly to be discussed, the agglutination tests for brucellosis should also be conducted routinely with all specimens of blood submitted for the typhoid or paratyphoid tests, in view of the frequency with which brucellosis is thereby detected in clinically unsuspected cases of the disease. Furthermore, the agglutination tests always detect more cases of typhoid fever than are clinically suspected or reported by physicians.

Because of the close relationship of the typhoid bacillus to the paratyphoid bacilli, and especially *S paratyphi* B, agglutinins for the latter are also increased in typhoid fever. However, the agglutinin titer is considerably lower for the paratyphoid bacilli than for the typhoid bacillus, so that little difficulty is experienced in detecting typhoid fever, particularly since paratyphoid fever is uncommon in this country.

An increase of H and especially of O agglutinins is also of aid in the detection of typhoid carriers among nonvaccinated individuals. To be significant, the titer of H agglutinin must be above 1:20 and of O agglutinin above 1:100. According to several investigators,[82,83] agglutination of Vi antigen is also useful for the detection of carriers, but if employed the antigen should be prepared according to the method of Felix and his colleagues.[82] Horgan and Drysdale [84] found agglutination of this antigen by the serums of some healthy contacts to vary from less than 1:50 to as high as 1:250. In typhoid convalescents it varied from less than 1:25 to 1:500. Under the circumstances, they could not confirm the suggestion that patients convalescent from typhoid fever were carriers on the basis of VI agglutination tests. Indeed, two carriers were observed whose serums remained negative for Vi agglutination. Gunther, however,[85] has found the Vi agglutination test of distinct value in the detection of typhoid carriers. She has advised "screening" by the glycerin-slide method in a 1:2 dilution with confirmation of the positive serums by the H rapid antigen in a 1:2 dilution. This procedure is stated to have given 100 per cent sensitivity with an incidence of 6 per cent falsely positive reactions.

Active immunization with typhoid-paratyphoid vaccine produces agglutinins and especially for H antigen, but contrary to the original statements of Felix, agglutinin for O antigen is also produced although to a lesser degree.[86] Consequently, the presence of O agglutinin in high titer, with H agglutinin in low titer, strongly suggests the disease in a previously vaccinated individual. On the other hand, a high titer of the H agglutinin with a low titer of O agglutinin suggests that the illness is due to some infection other than typhoid fever giving an anamnestic reaction. However, a very high titer of H agglutinin as, for example, 1:1280 or 1:2560, may be indicative of typhoid fever in a previously vaccinated individual, since it is rather unusual for H agglutinin to persist in titers of more than 1:640 for longer than six months after immunization. Consequently, when the disease is suspected in a previously vaccinated individual the tests should be repeated every three to five days with the same antigens. If the titers progressively increase, and especially for O antigen, typhoid fever is most likely present.

Whether or not the agglutination tests are of value in aiding a decision on reimmunization against the disease cannot be stated, since it is likely that a persisting tissue immunity may be present in spite of the fact that both H and O agglutinins have dropped back to normal levels. After two years or longer the H agglutinin is almost sure to have decreased to the normal titer of about 1:20. However, if the O agglutinin has likewise fallen to the normal of 1:80 or 1:100, it may be accepted that humoral immunity at least has practically disappeared. In this case the intracutaneous injection of a single dose of 0.1 cc. of the triple vaccine is probably sufficient for reimmunization.

The Complement Fixation Test. Available data indicates that the complement fixation test for typhoid fever may be superior to the agglutination test in specificity and sensitivity. Owing to the early popularity and greater simplicity of the latter, however, it has never received much attention in the serum diagnosis of the disease.

Garbat,[87] who has studied the complement fixation test very extensively, emphasizes the importance of using a polyvalent antigen. Apparently the test is less likely to give falsely positive or anamnestic reactions than the agglutination test.

About 50 per cent of cases of typhoid fever give positive reactions between the first and second weeks of the disease and between 90 to 95 per cent after the second week. Frequently, positive reactions are observed several days or even a week before positive agglutination reactions occur.[77] Garbat has found the test the only means of serum diagnosis in as high as 9 to 15 per cent of clinically doubtful cases of the disease.

Complement-fixing antibody is produced by active immunization with typhoid-paratyphoid vaccine but it generally disappears within six months. Consequently, if typhoid fever is suspected in an individual one or more years after vaccination, positive complement fixation reactions are usually indicative of typhoid fever; this is especially true when the tests are conducted at intervals of three to five days by a quantitative method with progressively stronger reactions.[77]

Garbat has also observed that the serums of about 85 per cent of carriers, six months or longer after recovery, give positive reactions, so that the complement fixation test is apparently of value in aiding the detection of these individuals.

Paratyphoid Fever. Paratyphoid fever, which is due to infection with *Salmonella paratyphi A* or *S. paratyphi B* (*S. schottmülleri*) is distinctly uncommon in the United States; most infections are due to paratyphoid bacillus B which likewise usually produces more severe infections than paratyphoid bacillus A.

Since the disease closely resembles mild typhoid fever, diagnosis is purely a laboratory problem based on bacteriologic examinations of the blood, feces and urine and agglutination tests.

Normal serums in microscopic tests agglutinate cultures of both living bacilli at about 1:20; agglutination at 1:80 or higher is indicative of paratyphoid fever. Normal serums in macroscopic tests agglutinate H antigens of the paratyphoid bacilli at about 1:20; agglutination at 1:80 or higher is indicative of the disease. Normal serums agglutinate O antigens of the paratyphoid bacilli at about 1:80;

agglutination at 1:160 or higher is also indicative of paratyphoid fever (Table 104).

In typhoid fever agglutinins for the paratyphoid bacilli also undergo an increase, especially for B, but not nearly to the degree of the increase of agglutinin for the typhoid bacillus; this is due to the biologic interrelationships of the group. In paratyphoid fever the agglutinins are especially increased for the antigens of the A or B bacilli, depending on the one producing infection; some increase of agglutinin for the typhoid bacillus also occurs. Consequently, serum diagnosis requires quantitative agglutination tests with the H and O antigens of both of the paratyphoid bacilli as well as of the typhoid bacillus. Usually two or three tests at intervals of three to five days are required before diagnosis is established on the basis of increasing agglutinin for the particular paratyphoid bacillus producing infection. The agglutinin absorption test of Castellani may be required.

As in typhoid fever, the diagnosis of paratyphoid fever in an individual previously immunized with typhoid-paratyphoid vaccine largely depends on observing a progressive increase of agglutinin for the O antigens of the paratyphoid bacilli A or B.

Brucellosis. The only method by which the diagnosis of brucellosis may be completely established is by the cultivation and identification of Brucella in the blood, feces or tissues (Table 105). Blood cultures and the inoculation of guinea-pigs with blood according to the methods of Poston [88] usually require two weeks or longer for their conduct but should be made in all cases and especially when acute brucellosis is suspected. But the incidence of positive cultures is so low that negative results do not by any means exclude the disease. This also applies to bacteriologic examinations of the feces by the method of Amoss and Poston [89] or by other methods.

Furthermore, as discussed in Chapter 19, the skin test is of limited diagnostic value. Positive reactions are frequently observed in acute brucellosis but may not occur until late in the disease because of the time required for the acquisition of allergic sensitization. Indeed, the reactions may be persistently negative even in cases with positive blood cultures. On the other hand, positive reactions may be due to previous minor and clinically undetectable infections with Brucella and when occurring in individuals with typhoid fever, subacute bacterial endocarditis, malaria, acute tuberculosis, influenza or other diseases, may readily lead to a mistaken diagnosis of brucellosis. In other words, a negative skin reaction is of value in excluding present or past infection with Brucella but a positive reaction does not necessarily mean that the patient has acute or chronic active brucellosis at the time the test is conducted.

Unfortunately, agglutination and opsonocytophagic tests are likewise of limited diagnostic value although capable of yielding very helpful information when the results are properly interpreted in relation to the clinical status of the patient. *Since agglutinins and opsonins may be produced by the intradermal injection of heat-killed brucella, blood for agglutination and opsonic tests should always be taken before skin tests are conducted.*

Agglutination Tests. Agglutination tests may be conducted by the usual macroscopic technic or by the rapid slide method of Huddleson. While a polyvalent

TABLE 105. SUMMARY OF THE CLINICAL INTERPRETATION OF SEROLOGIC EXAMINATIONS IN BRUCELLOSIS, INFECTIOUS MONONUCLEOSIS AND TULAREMIA

Disease	Interpretation
Brucellosis	The detection of Brucella in the blood, feces or tissues by bacteriologic examinations is of most diagnostic value but negative results do not exclude the disease. A negative skin reaction is of value in excluding past or present infection with Brucella but a positive reaction does not necessarily mean that acute or chronic brucellosis is present at the time the test is conducted. Blood for agglutination and opsonic tests should be collected before skin tests are conducted. About 98 per cent of the serums of normal individuals with *negative skin reactions* do not agglutinate Brucella in dilutions higher than 1:10. Agglutination at 1:30 to 1:50 or higher constitutes a positive reaction under these circumstances. About 30 per cent of the serums of normal individuals with *positive skin reactions* may agglutinate Brucella in dilutions up to 1:100. Consequently, agglutination in dilutions higher than 1:100 is usually required for a positive reaction in this group. Positive agglutination reactions are usually indicative of brucellosis, especially if they become progressively stronger when repeated at intervals. Positive reactions, however, may be due to previous unsuspected infections with Brucella and result in diagnostic errors when occurring in individuals with other illnesses. Negative agglutination reactions alone or with negative skin and opsonic reactions do not necessarily exclude brucellosis. About 70 per cent of normal individuals with negative skin reactions have negative opsonic indices but about 98 per cent with positive skin reactions show positive opsonic indices due to previous minor infections with Brucella. Individuals with positive opsonic indices may contract clinical brucellosis. Positive skin and agglutination reactions along with a negative or weakly positive opsonic index are usually indicative of brucellosis.
Tularemia	The detection of *Past. tularensis* in cultures or by guinea-pig inoculation tests (usually required) constitutes the most definite diagnostic procedure; however, these examinations not only require considerable time but are dangerous to laboratory workers. The antigen skin test is of great diagnostic value. Positive agglutination tests at 1:80 almost invariably occur by the second or third weeks of the disease. If necessary, the tests should be repeated at intervals until diagnosis is established or the disease excluded. The agglutinins persist in the blood for years or the balance of life. Cross-agglutination with *Br. abortus* or *P. vulgaris* OX_{19} may occur. Positive opsonocytophagic reactions usually occur after the second or third weeks of the disease. The test is sometimes of value in conjunction with skin and agglutination tests in questionable cases.

TABLE 105. SUMMARY OF THE CLINICAL INTERPRETATION OF SEROLOGIC EXAMINATIONS IN BRUCELLOSIS, INFECTIOUS MONONUCLEOSIS AND TULAREMIA—(Continued)

Disease	Interpretation
Infectious Mononucleosis	Heterophil agglutinin for sheep corpuscles, which is not of the Forssmann type, is usually and characteristically produced in infectious mononucleosis. Heterophil agglutinin is also produced by injections of horse serum and by various bacteria; it may be distinguished from the antibody occurring in infectious mononucleosis by differential absorption tests employing guinea-pig and rabbit kidney. The serums of normal human beings contain heterophil agglutinin. In the absence of recent injections of horse serum and serum sickness the titer is not higher than 1:8. Under these conditions agglutination at 1:16 to 1:32 is suggestive of infectious mononucleosis; about 90 per cent of cases show agglutination at 1:28 or higher. Agglutination in final dilutions of serum 1:224 or higher is diagnostic of infectious mononucleosis, provided serum sickness can be excluded even though injections of horse serum have been given. Positive reactions do not occur in the leukemias, Hodgkin's disease, etc. Transient or temporarily positive Wassermann and flocculation reactions may occur.

antigen composed of all three varieties of Brucella may be employed, monovalent antigens or an antigen prepared of multiple strains of *Br. abortus* are preferred. As a general rule, tests conducted with antigens prepared of *smooth* strains of *Br. abortus* and *Br. melitensis* are sufficient, as their use reduces or eliminates the possibility of falsely negative reactions due to so-called "para" types. Unfortunately, the results of agglutination tests vary in different laboratories according to the antigens employed so that standardization of technic is highly desirable.[90,91]

The serums of normal individuals with negative skin reactions do not agglutinate Brucella in final dilutions higher than 1:25; indeed, about 98 per cent are negative at 1:10.[92] Consequently, agglutination at 1:30 to 1:50 may be regarded as positive and clinically significant in individuals with suspected brucellosis who give negative skin reactions. Small amounts of agglutinin may occur as early as the fifth day after the onset of symptoms but not usually until about the tenth day or even the second or third week. The titer usually increases as the disease progresses so that the tests should be repeated at intervals of five days until diagnosis is definitely established. Agglutinins may occur during the initial stage and then disappear or persist in low titers. In other cases they occur intermittently and at unpredictable intervals. Cross agglutination may occur with antigens of *Past. tularensis* but usually this presents no difficulties. However, fever produced by streptococci or occurring in other illnesses may sometimes produce a slight increase of agglutinins for Brucella [92,93] resulting in falsely positive or anamnestic reactions. Positive reactions have also been reported in individuals given cholera vaccine.[94]

Negative agglutination reactions, however, do not exclude brucellosis. Indeed, they may be consistently negative even in 6 to 10 per cent of cases of acute brucellosis with positive blood cultures. As in typhoid fever, single agglutination tests may give negative reactions in about 40 per cent of cases showing positive blood cultures although, curiously enough, when agglutinins are produced the incidence of positive blood cultures tends progressively to increase.[95] Even in chronic brucellosis Evans and her colleagues [96] have observed negative reactions in as high as 46 per cent of cases of the disease.

Unfortunately, however, positive agglutination reactions in cases of suspected acute or chronic brucellosis are frequently difficult to interpret because previous minor and clinically undetectable infections with Brucella may produce them. This is especially likely to occur in the case of individuals who have partaken of raw milk over long periods of time or whose occupation has particularly exposed them to infection. While the serums of about 70 per cent of such individuals may react negatively at 1:10, about 30 per cent may give positive reactions in higher dilutions up to 1:100 and especially in the case of those whose previous minor infections with Brucella have produced allergic sensitization with positive skin reactions.[92] Consequently, when the physician suspects acute or chronic brucellosis in individuals known to have been exposed to minor infections with Brucella, as the result of occupation or the habitual use of raw milk, and especially in endemic areas, it would appear advisable to regard agglutination only in dilutions higher than 1:100 as possessing possible diagnostic significance. This is particularly true in individuals given skin tests with or without positive reactions.[97] Under these circumstances, it is especially advisable, therefore, to repeat the agglutination tests at intervals, as a progressive increase of agglutinins almost surely indicates the presence of acute or chronic brucellosis. Otherwise, accepting positive agglutination reactions in final dilutions at 1:50 to 1:100 along with positive skin reactions, may result in an erroneous diagnosis in such individuals who may have typhoid fever, malaria, acute tuberculosis, subacute bacterial endocarditis, rheumatic fever, influenza or other illnesses clinically resembling acute or chronic brucellosis.

In other words, as stated by Simpson,[98] "while the agglutination test is undoubtedly of great value, its limitations must be recognized. Otherwise, errors will be made in two directions: first, the correct diagnosis of brucellosis may not be made because too much reliance is placed in a negative reaction; or second, an incorrect diagnosis of brucellosis may be made in a person who has a residual agglutinin titer from previous infection with Brucella, but who is suffering from some other disease when the test is made."

Opsonocytophagic Test. In view of the fact, therefore, that skin and agglutination tests may not distinguish between present and past infection with Brucella, Huddleson and his colleagues [99] turned to opsonic tests for additional assistance. These are conducted with living or killed cultures of Brucella (killed cultures preferred in order to reduce the danger of laboratory infections) and the patient's citrated blood to furnished opsonin and polymorphonuclear leukocytes. The average number of Brucella engulfed by twenty-five leukocytes is determined. The absence of phagocytosis constitutes a negative reaction, from 1 to 20 Brucella per cell slightly positive, 21 to 40 moderately positive, and over 40 strongly positive.

Negative or slightly positive reactions (0 to 20 due to normal opsonins), along with negative skin and agglutination reactions, are regarded as indicative of susceptibility. Negative, slightly or moderately positive reactions (0 to 40) with positive skin and with or without positive agglutination reactions, are regarded as indicative of infection or brucellosis. Strongly positive reactions with positive skin and with or without positive agglutination reactions, are regarded as indicative of immunity.

About 70 per cent of normal individuals with negative skin and agglutination reactions have shown negative opsonocytophagic reactions but 30 per cent have shown weakly to strongly positive opsonic reactions.[92] The latter are apparently due to opsonins acquired by clinically unrecognized and unsuspected infections with Brucella even though insufficient to produce allergic sensitization. In normal individuals giving positive skin reactions indicative of previous minor infections with Brucella resulting in allergic sensitization, about 76 per cent have given negative agglutination but about 98 per cent weakly to strongly positive opsonic indexes.[92] In other words, positive skin and opsonic reactions are apparently more indicative of previous infections with Brucella than positive agglutination reactions.

In general terms, strongly positive opsonic with positive skin reactions may be accepted as evidence of acquired immunity to Brucella because the rôle of opsonins in antibacterial immunity is well recognized, with evidence indicating that allergic sensitization to Brucella may also play a rôle in its mechanism just as tuberculin hypersensitiveness appears to contribute to resistance and immunity in tuberculosis. However, to surmise that an individual under these circumstances has acquired sufficient immunity to give protection against acute or chronic symptomatic brucellosis is open to question and especially since Evans and her colleagues[96] have reported strongly positive opsonic reactions in four individuals from whom Brucella were cultivated. Furthermore, others have observed individuals who have apparently recovered from brucellosis with negative opsonic indexes or indexes fluctuating from weakly to strongly positive over months of time.

Under the circumstances, it does not appear that the results of opsonocytophagic tests materially contribute to the laboratory diagnosis of brucellosis. At least, this is apparently true of acute brucellosis since individuals usually give negative opsonic indexes which, however, are to be expected since varying periods of time are required for the development of opsonins. Indeed, individuals not only with negative opsonic, but with negative agglutination and negative skin reactions as well, may nevertheless have acute brucellosis. This is especially true in the early stages of the disease in view of the time required or because of a delay in the production of both agglutinins and opsonins and of allergic sensitization. Moreover, individuals with moderately to strongly positive opsonic indexes may nevertheless have brucellosis, as previously stated. Furthermore, a positive skin reaction and a weakly positive agglutination, with moderately positive opsonic reactions, do not necessarily prove that brucellosis is present, although it may be stated that a negative to weakly positive opsonic index, a strongly positive agglutination and a positive skin reaction constitute a triad usually indicative of the disease.

Tularemia. The isolation of *Past. tularensis* from cases of tularemia constitutes the most definite diagnostic procedure in this disease (Table 105). Bacteriologic examinations, however, not only require considerable time but are not always possible. Furthermore, they usually require guinea-pig inoculation tests and, as reported by Francis,[100] a large number of laboratory workers have become accidentally infected from the conduct of necropsies on infected guinea-pigs or rabbits, or from the handling of infected ticks. Fortunately, serologic methods have proved so satisfactory for diagnostic purposes that bacteriologic examinations are rarely necessary.

It is true that the physician may not require laboratory aid in the diagnosis of the ulceroglandular type of the disease, although this is sometimes required for the differentiation of the lesions from those of syphilis or other infections producing local ulcers with regional lymphangitis and adenitis. In other varieties of tularemia, however, and particularly in the typhoidal, glandular, and pulmonic types, about the most the clinician can do is to suspect the possibility of tularemic infection.

The diagnostic value of skin tests conducted by the intracutaneous injection of heat-killed micro-organisms (antigen skin test) or of immune serum (antiserum skin test) of Foshay is discussed in Chapter 19. The former is of great clinical value, as positive reactions usually occur as early as the first week of the disease. Occasionally, however, normal individuals may show doubtful or slightly positive nonspecific reactions. Skin tests conducted with goat antitularense serum have not proved as satisfactory because of the difficulty experienced in finding suitable control serums, but they have been found of aid in the diagnosis of about 30 to 40 per cent of cases.[101]

Agglutination Test. Agglutination of formalinized suspensions of washed tularense micro-organisms in final dilutions of serum 1:80 or higher, constitutes a positive reaction but lower values may be significant if there has been a previous negative reaction or if positive skin and opsonocytophagic reactions are observed. Positive reactions do not usually occur until the second week of the disease, so that in suspected tularemia several tests may be required before diagnosis is established or the disease excluded. Cross-agglutination with *Br. abortus* or *P. vulgaris* OX_{19} may occur but usually only in lower dilutions of serum. As in brucellosis, the acquired agglutinin tends to persist in the serum for years and even for the balance of life.

Opsonocytophagic Test. This test is conducted with a formalin-killed suspension of *Past. tularensis* in the same way as the opsonocytophagic test with Brucella. Positive reactions usually develop between the second and third weeks of the disease but their interpretation is quite difficult in some cases since many weakly positive and occasionally even strongly positive reactions have been observed in conditions other than tularemia.[101] The test, however, may be of aid in conjunction with skin and agglutination tests in questionable cases of tularemia and particularly when there is cross-agglutination between *Past. tularensis* and Brucella.

TABLE 106. SUMMARY OF THE CLINICAL INTERPRETATION OF SEROLOGIC EXAMINATIONS IN GONOCOCCUS INFECTIONS, TUBERCULOSIS AND OTHER BACTERIAL DISEASES

Disease	Interpretation
Gonococcus Infections	The complement fixation tests is of clinical value not only in the diagnosis of chronic gonococcus infections of both sexes when bacteriologic methods are impossible or of doubtful value, but also as a criterion of cure. The technic, with special reference to the preparation of antigen, is extremely important. Under proper technical conditions the test is highly specific. The administration of gonococcus vaccine may produce positive reactions.
Tuberculosis	The complement fixation test yields positive reactions in from 60 to 70 per cent of cases of tuberculosis of the lungs but is not ordinarily required for diagnostic purposes. It is also of value in the diagnosis of tuberculosis of the bones and joints and of the kidneys as well as of tuberculous pleurisy and meningitis. Falsely positive reactions may occur with the serums of 10 to 16 per cent of nontuberculous syphilitic individuals. A negative reaction does not exclude the possibility of tuberculous infection; consequently, the test possesses more positive than negative value.
Other Bacterial Diseases	Complement fixation and agglutination tests are of no value in the diagnosis of *chancroid.* Complement fixation and agglutination reactions are of no value in the diagnosis of *pertussis* except, possibly, in atypical cases. Agglutination tests are sometimes of value in aiding the diagnosis of *bacillary dysentery.* Agglutination and complement fixation tests are of no value in the diagnosis of *Asiatic cholera.* Agglutination tests are sometimes of value in the diagnosis of *bubonic plague.* The value of agglutination and complement fixation tests in the diagnosis of *human glanders* cannot be stated. Complement fixation, agglutination and precipitin tests with antigens of streptococci may be of some value in the etiologic diagnosis of *chronic arthritis.*

Infectious Mononucleosis. While infection with *Bacterium monocytogenes* (*Listerella monocytogenes*) has been suggested as the possible cause of infectious mononucleosis or glandular fever,[102] the evidence is so weak and inconclusive that the etiology of the disease is still unknown. Its clinical and epidemiologic features, however, conclusively indicate that it is due to some living agent transmissible to human beings. For the sake of convenience, therefore, serologic examinations in the diagnosis of this disease are being considered at this point (Table 105).

Certainly, the serums of cases of infectious mononucleosis may not contain increased amounts of agglutinins or other antibodies for *Bact. monocytogenes.* Furthermore, while cases of the disease may show temporarily positive Wassermann or flocculation reactions, the immunization of rabbits with living and dead cultures of the micro-organism by Kolmer and Howard [103] have not shown the production of the Wassermann reagin or heterophil agglutinins for sheep corpuscles.

As originally discovered by Paul and Bunnell,[104] however, the serum of individuals with infectious mononucleosis so frequently and characteristically show an increase of heterophil agglutinin for washed sheep erythrocytes that an agglutination test employing these cells as antigen has proved of great value in diagnosis and in differential diagnosis from the leukemias, Hodgkin's disease, etc. Agglutinin and hemolysin for sheep corpuscles occur normally in the serum of human beings. Apparently their increase in infectious mononucleosis is due to the fact that the living agent of the disease contains heterophilic antigen although, on the other hand, they may be antibodies produced by abnormal cells in the blood or elsewhere during the active stage of the disease. Certainly it is well known that *S. dysenteriae*, pneumococci, paratyphoid bacilli and other micro-organisms may contain the antigen capable of producing antisheep agglutinin in rabbits and the production of a large amount in a case of septicemia due to *Esch. coli* has been recently reported.[105]

As shown by Davidsohn and Walker,[106] the heterophil antisheep antibody produced in infectious mononucleosis is not of the Forssmann type. They have found that sheep corpuscles contain two heterophil antigens, namely, one of the Forssmann type and another reacting with the heterophil antibody produced in infectious mononucleosis. For example, by absorbing one portion of serum with guinea-pig kidney and another with rabbit kidney, they have found that the heterophil antibody produced by injections of horse serum, or occurring in serum sickness, and in diseases other than infectious diseases, is almost completely removed by guinea-pig kidney but only partly by rabbit kidney. Absorption of serums from cases of infectious mononucleosis with guinea-pig and rabbit kidney, however, only partially removes the heterophil antibody and in about equal degree by both tissues. Consequently, this "differential absorption test" is being widely employed for distinguishing between the heterophil antibody produced in infectious mononucleosis and that produced by injections of horse serum or occurring in serum sickness or other diseases. It has been found especially valuable in the diagnosis of those cases of infectious mononucleosis with borderline titers of heterophil agglutinin and in cases complicated by a history of injections of normal horse serum or the horse immune serums; however, in cases with low titers, the test may not exclude infectious mononucleosis.[107]

Unfortunately, the technic of the test for infectious mononucleosis has not been standardized. Opinions differ widely on the titers suggestive or diagnostic of the disease. Apparently the serum of normal human beings who have not received injections of horse serum do not agglutinate washed sheep corpuscles in dilutions above 1:4 to 1:8. Some investigators regard agglutination in final dilutions of serum of 1:16 to 1:32 or higher as indicative of infectious mononucleosis, in the absence of serum sickness or recent injections of horse serum. Others state that agglutination up to 1:128 or 1:224 is required for diagnostic purposes under such circumstances. Davidsohn is of the opinion that agglutination at 1:224

or higher is diagnostic providing the patient does not have serum sickness at the time, or shortly before, even though horse immune serum has been given. In a series of 75 cases reported by Straus and Bernstein [107] about 10 per cent showed agglutination in final dilutions of serum 1:8 to 1:64; about 40 per cent at 1:128 to 1:512 and about 50 per cent at 1:1024 or higher. Agglutination at 1:112 or higher has been reported in 62 per cent of cases; repeat tests with rising titers are of great aid in diagnosis.[108]

As previously stated, positive reactions do not occur in the leukemias [109] although Kent [110] has reported a positive reaction in one case which may have been due to coincident infectious mononucleosis. Negative reactions have also been observed in Hodgkin's disease, lymphosarcoma, syphilis, tuberculosis, typhoid fever, agranulocytic angina and other diseases.[109] During infectious mononucleosis, however, agglutinins may be produced for typhoid bacilli and the Salmonellae which may lead to errors in diagnosis.[108]

Another peculiar feature of infectious mononucleosis is the frequency with which temporarily positive Wassermann and flocculation reactions may occur. Several tests at intervals may be required for their detection. The incidence of these has varied greatly in different reports all the way from negative reactions in some series of cases to as high as 100 per cent in others, with a general average of about 21 per cent.[111] In a series of 19 cases the Kolmer complement fixation test gave negative reactions in all but one.[111] The reactions are characterized by their transient nature. It does not appear that they are due to a reaction between heterophil antibody in the serums and heterophil antigen in the alcoholic extracts of beef heart reenforced with cholesterol as antigens; rather it has been suggested that reactions may be due to the production of reagin by the living agent of infectious mononucleosis similar to that produced by *T. pallidum*, *Myco. leprae* and the plasmodia of malaria.[111]

Gonococcus Infections. Within recent years newer methods for the cultivation of gonococci have greatly improved the bacteriologic diagnosis of acute and chronic gonorrhea in both sexes. They have also proved of value as criteria of cure and especially in relation to treatment with penicillin and the sulfonamide compounds. However, when gonococci have penetrated into the tissues and surrounding structures, completely disappearing from discharges, or with no discharges at all, it is obvious that examinations of smears and cultures are of little or no value for diagnostic purposes or as criteria of cure. Under these conditions, the complement fixation test properly commands consideration.

Undoubtedly, the gonococcus is highly antigenic and capable of producing complement-fixing antibody in the course of both acute and chronic infections (Table 106). Needless to state, however, time is required for its production in detectable amounts in the serum. Furthermore, the amount produced is in relation to the extent of infection and the virulence of the organism. For these reasons, positive reactions are of infrequent occurrence within the first one or two weeks of acute gonorrhea in adults of both sexes as well as in vaginitis of children. But even were it otherwise, it is obvious that examinations of smears and cultures are to be preferred for diagnostic purposes under these conditions. But once foci of infection have become established in the glands of Cowper, the prostate gland, seminal vesicles and epididymis of the male, or deeply in the tissues of the cervix, the fallopian tubes and ovaries of the female, or in the joints of either sex, the complement fixation test is of far more clinical value in their detection than commonly surmised.

Unfortunately, however, the test is difficult, not only because the technic must be as sensitive as consistent with specificity in order to detect small amounts of antibody in the serum, but especially in relation to the preparation of antigen.[77] As shown by Torrey,[112] not all strains are suitable for the preparation of antigen aside from the fact that the efficacy of various methods employed in its preparation vary according to the strain or strains employed. No wonder, therefore, that clinicians have become dissatisfied with the gonococcus complement fixation test as it is ordinarily conducted, not only because of a high incidence of falsely negative reactions but because of falsely positive ones as well. Indeed, it is far better not to employ the test at all unless it can be conducted with a proper antigen and a proper technic. According to Torrey,[112] a "dissolved" antigen prepared by a modified Price method of two special strains of gonococci is to be recommended.

It is necessary to render the gonococcus complement fixation test as sensitive as possible but not to the extent of producing nonspecific or falsely positive reactions. This is a common error. With a good antigen and technic the test can be rendered highly specific with no falsely positive reactions due to syphilis, tuberculosis or other infections. In this connection, it is to be remembered, however, that positive reactions may occur in individuals to whom gonococcus vaccine has been given since it is capable of producing complement-fixing antibody which may persist for varying periods of time up to three months.[113]

Under proper conditions, therefore, the test is not too sensitive; indeed, it is not sensitive enough. Consequently, negative reactions alone never exclude the possibility of infection although positive reactions are indicative of its presence even in the absence of discharge or other clinical manifestations. In other words, *the test has far more positive than negative value.* When the test is properly conducted, weakly positive reactions should be regarded as significant.

In general terms, positive reactions occur in from 10 to 20 per cent of cases of acute urethritis, 60 to 90 per cent of chronic urethritis with prostatitis and seminal vesiculitis and 90 to 100 per cent of cases of chronic epididymitis; also in about 50 per cent of cases of cervicitis and 70 to 90 per cent of chronic salpingo-oophoritis. In arthritis the incidence varies from 70 to 100 per cent, in iritis about 80 per cent, and in endocarditis 90 to 100 per cent. Consequently, the test is of value in the diagnosis and differential diagnosis of these although it is to be remembered that positive reactions may be due to foci of gonococcus infection in the genital tract. In chronic gonorrheal vaginitis of children the incidence of positive reactions is not usually higher than about 60 per cent.

The test is also of value in the determination of cure. It has been calculated that at least two to four months are required for the disappearance of antibody; consequently, positive reactions persisting longer than one year after clinical and bacteriologic recovery are a strong indication that latent foci of infection are present.[113] In other words, the gonococcus complement fixation test is not intended to replace clinical and bacteriologic methods of diagnosis, but when properly conducted is to be regarded as a very helpful supplementary procedure not only in diagnosis but as one of the criteria of cure.

Tuberculosis. While complement-fixing antibody may be produced in the course of tuberculosis (Table 106), the complement fixation test is not ordinarily employed for diagnostic purposes in view of the highly satisfactory state of clinical diagnosis supplemented by roentgenologic and bacteriologic examinations and tuberculin skin tests.

As in the case of the gonococcus complement fixation test, various methods have been proposed for the preparation of antigens.[77] Even when the lipids of the bacilli have been largely removed, falsely positive reactions may occur with the serums of 10 to 16 per cent of nontuberculous syphilitic individuals due to cross complement fixation by the lipophilic reagin with the fats and waxes of the tubercle bacillus.[77,114,115] Consequently, the Wassermann test should always be conducted at the same time and in the case of positive reactions positive tuberculosis reactions should be interpreted with caution.

Since but small amounts of complement-fixing antibody are apt to be produced in tuberculosis, the technic of the test should be as sensitive as is consistent with specificity. Under these circumstances, positive reactions occur in about 60 per cent of cases of incipient tuberculosis of the lungs and in 60 to 70 per cent of moderate or advanced cases of this disease. For example, in a recent investigation by Kolmer and Mahoney,[115] 63.7 per cent of the serums of 408 tuberculous individuals gave positive reactions. The presence or absence of fever had little or no influence on the results since 63 per cent of febrile and 64.1 per cent of afebrile cases gave positive reactions. Four or 16 per cent of 25 serums from syphilitic nontuberculous donors gave positive tuberculosis complement fixation reactions.

When syphilis can be excluded, a positive reaction is, therefore, confirmatory evidence of the presence of pulmonary tuberculosis, but obviously the test is not usually required in diagnosis except in a small percentage of clinically doubtful cases where actinomycosis, nontuberculous abscesses, malignancy, etc., require consideration. The test is also of value in the diagnosis of tuberculosis of the bones and joints and of the kidneys, as well as of tuberculous pleurisy. In the latter the test may be conducted with the exudate as well as with serum.[116] Likewise in suspected tuberculous meningitis the test may be conducted with cerebrospinal fluid.[117]

Negative reactions, however, never reliably exclude the possibility of tuberculous infection; in other words, the test has far more positive than negative value.

Other Bacterial Diseases. While positive complement fixation reactions employing antigens prepared of the bacillus of Ducrey have been reported as occurring in *chancroid,* earlier observations have not been confirmed. Recently Sanderson and his colleagues[118] observed negative reactions with the serums of all cases tested with an antigen of the bacillus cultivated in human blood media; an antigen prepared of bacilli cultivated in rabbit blood media, however, gave positive reactions in 26 out of 28 cases but these were regarded as falsely positive. Agglutination tests are likewise of no diagnostic value (Table 106).

Normal serums may agglutinate the whooping cough bacillus in final dilutions of 1:100 but agglutination at 1:200 or higher has been reported in the paroxysmal or later stages of *pertussis;* likewise, positive complement fixation reactions in about 50 per cent of cases. But since antibody is not produced until rather late in the disease, and since bacteriologic examinations by the "cough plate" method have proved highly satisfactory in its early diagnosis, agglutination and complement fixation tests are of no clinical value except, possibly, as aids in the detection of atypical cases.

Agglutination tests, however, may be of some value in the diagnosis of *bacillary dysentery.* At least two antigens should be employed routinely prepared of the Shiga and Flexner types of the bacilli. Agglutination of the former in final dilutions of serum 1:64 or higher and of the latter at 1:128 or higher, are regarded as diagnostic.[119] The tests may be of some assistance in diagnosis along with bacteriologic examinations of the stools. [20] Agglutination tests, however, have not proved of any value in the diagnosis of *Asiatic cholera* because of the fulminant nature of the disease; the same applies to complement fixation tests. Normal serums agglutinate the cholera bacillus at about 1:10.

Positive agglutination reactions are stated to occur in *bubonic plague* by about the ninth day of the disease, but the test is not of much practical value in diagnosis except, occasionally, for the assistance rendered in deciding whether or not a recovered individual has really suffered from the disease. Normal serums are stated to agglutinate the bacillus in final dilutions of serum about 1:10.

While agglutination and complement fixation tests have proved of value in the diagnosis of *glanders* among the lower animals, in the diagnosis of the disease in human beings they have not been employed frequently enough to warrant an expression of their value. Normal human serums are stated to agglutinate the bacillus in final dilutions of serum up to 1:100, so that agglutination in higher dilutions would be required for diagnostic purposes in glanders of human beings among whom the disease, fortunately, is comparatively rare.

Complement fixation tests have indicated that streptococci and especially *Str. viridans* apparently play an important rôle in the etiology of *chronic arthritis* and especially of the rheumatoid or atrophic type.[121] Clawson and his colleagues [122] found that normal human serums agglutinated a chronic arthritis strain of streptococcus in final dilutions up to 1:1600 although 28 per cent of the serums of 81 individuals did not agglutinate above 1:400. In chronic arthritis, agglutination occurred up to 1:6400 with 59.5 per cent of the serums of 60 cases agglutinating in dilutions above 1:400. Bruce and Caswell [123] observed no positive precipitin reactions with the serums of 10 normal persons and an antigen prepared of *Str. hemolyticus* although the serums of 22 of a group of 32 cases of rheumatoid arthritis gave positive reactions. Complement fixation, agglutination and precipitin tests, however, are not ordinarily employed for the diagnosis of chronic arthritis, although when positive reactions are observed, they may aid in differentiation between the atrophic or rheumatoid and hypertrophic types of the disease.

EXAMINATIONS IN THE SPIROCHETAL DISEASES

Serologic examinations are so important in relation to the diagnosis and treatment of syphilis that the subject is discussed separately in Chapter 18 while other spirochetal diseases are for purposes of convenience, considered herewith (Table 107).

TABLE 107. SUMMARY OF THE CLINICAL INTERPRETATION OF SEROLOGIC EXAMINATIONS IN SPIROCHETAL DISEASES OTHER THAN SYPHILIS

Disease	Interpretation
Yaws, Pinta and Bejel	Positive Wassermann and flocculation reactions occur in 90 to 100 per cent of cases of *yaws* with well-defined lesions. Positive Wassermann reactions do not usually occur in tests with spinal fluid. Positive Wassermann and flocculation reactions are stated to occur almost invariably in *pinta*. Positive Wassermann reactions may occur in tests with spinal fluids. Positive Wassermann reactions also occur in *bejel.*
Lepto-spirosis	Agglutination tests are of diagnostic or clinical value in the diagnosis of infectious jaundice or Weil's disease. Weakly positive reactions commonly occur on or about the fifth day of illness followed by strongly positive reactions, Positive reactions may continue to occur for several years after recovery. Negative reactions or only temporarily weakly positive reactions, however, may occur in mild cases. The agglutination-lysis test is stated to be a quicker and more satisfactory test after the tenth day of the disease than guinea-pig inoculation tests. A mouse protection test is also employed for diagnostic purposes but its value cannot be stated at the present time. Temporarily positive Wassermann and flocculation reactions may occur in infectious jaundice.
Other Spiro-chetal Diseases	Temporarily positive Wassermann and flocculation reactions may occur in that variety of *rat-bite fever* due to infection with *S. minus.* Temporarily positive Wassermann and flocculation reactions may also occur in *relapsing fever* due to *S. minus.*

Yaws, Pinta and Bejel. Yaws, or "framboesia tropica," is generally considered due to infection with *T. pertenue* which is morphologically and culturally indistinguishable from *T. pallidum* of syphilis; indeed, some investigators regard yaws as only a tropical form of syphilis. Be that as it may, positive Wassermann and flocculation reactions are observed with serums in from 90 to 100 per cent of cases with well-defined cutaneous lesions. As a general rule, however, the spinal fluid does not give positive reactions because infections of the central nervous system with *T. pertenue* do not usually occur.

Whether or not *pinta* is a form of yaws or syphilis, or a separate disease, cannot be stated at the present time. It occurs commonly in Mexico and Colombia as well as in Venezuela, Brazil, Peru, Central America and the West Indies and is considered due to infection with a spirochete.[124] It is stated that the Wassermann and flocculation reactions with serums are almost invariably positive; spinal fluid reactions have been observed in about 10 per cent of cases. Positive reactions also occur in a high percentage of cases of *bejel.*

Leptospirosis. Leptospirosis of human beings may be due to infection with *Lept. icterohaemorrhagiae* of wild rats, transmissible to man, *Lept. canicola* of dogs, transmissible to man, *Lept. grippotyphosa* producing a syndrome known in different places as "summer influenza," "harvest fever" or "swamp fever" or *Lept. hebdomidis* producing "seven day fever" in Japan. Infections with the last two have not been reported in the United States where most infections are believed to be caused by *Lept. icterohaemorrhagiae* (producing infectious jaundice or Weil's disease) and sometimes by *Lept. canicola.*[125]

As stated in Chapter 15, the diagnosis of infectious jaundice or Weil's disease may be made by darkfield examinations of the blood, although the chances of error due to artefacts are so great that cultures of the blood, and especially the inoculation of blood and urine into guinea-pigs, are greatly preferred.

However, agglutination tests conducted with the serums of patients are stated to yield highly specific reactions of diagnostic value. The serums of normal human beings may agglutinate cultures of *Lept. icterohaemorrhagiae* in final dilutions up to 1:40. Agglutinins are stated to appear about the fifth day after the onset of illness with the production of weakly positive reactions (1:100). Subsequently, they rapidly increase, reaching a maximum by about the fifteenth day when the serum titer may be as high as 1:100,000. Strongly positive reactions continue to occur for about seven weeks after which the agglutinins decrease (1:300 to 1:900) but usually persist for several years after recovery.[126] Indeed, it has been stated that in the United States the incidence of spirochetal jaundice would probably be found higher than is now surmised if agglutination tests were conducted routinely in all cases of jaundice of obscure origin. According to Rimpau,[127] however, agglutinins may not develop at all in mild cases or increase to a slight extent and then disappear within a few weeks.

It is also stated that the agglutination-lysis test conducted with patient's serum, beginning within the tenth day of the disease, is probably a quicker means of diagnosis than the guinea-pig inoculation test since guinea-pigs rarely show evidence of the disease, develop jaundice or succumb to infection sooner than a week or ten days after inoculation.[128] A mouse protection test has also been proposed but its value cannot be stated at the present time. It consists in inoculating mice intraperitoneally with a lethal dose of leptospira along with patient's serum. Mice between four and six weeks of age must be used. Temporarily positive Wassermann reactions are also stated to occur in some cases of infectious jaundice.

Other Spirochetal Diseases. Otherwise, serologic tests have little or no clinical or diagnostic value in spirochetal diseases except that temporarily positive Wassermann and flocculation reactions are stated to occur in some cases of that variety of rat-bite fever due to infection with *Spirillum minus* as well as in *relapsing fever.*

EXAMINATIONS IN THE RICKETTSIAL AND VIRAL DISEASES

Typhus Fever. In 1916, Weil and Felix[129] isolated from the urine of typhus fever patients two strains of *P. vulgaris* which were agglutinated by the serums of individuals suffering from this disease. These strains were of the so-called X_2 variety but a little later a strain, known as X_{19} was isolated which proved more susceptible to agglutination for diagnostic purposes.

TABLE 108. SUMMARY OF THE CLINICAL INTERPRETATION OF SEROLOGIC EXAMINATION IN THE RICKETTSIAL AND VIRAL DISEASES

Disease	Interpretation
Typhus Fever	The Weil-Felix and rickettsial agglutination tests are highly diagnostic. Positive reactions may occur early in the disease. Negative reactions usually occur within five or more months after recovery but positive reactions may persist for several years. Complement fixation tests with rickettsial antigens are of additional diagnostic value in typhus fever.
Rocky Mountain Spotted Fever	Weil-Felix agglutination reactions conducted with antigens prepared of X_2 and X_{19} strains of *P. vulgaris* are of diagnostic value. It is not possible, however, to differentiate between typhus and Rocky Mountain spotted fevers by the agglutination test. Cross-immunity or serum neutralization tests as well as inoculation tests employing guinea-pigs. however, are of value in this connection. Complement fixation tests employing specific rickettsial antigens may also be employed as aids in the diagnosis of Rocky Mountain spotted fever and rickettsialpox.
Lympho-granuloma Venereum	Complement fixation tests conducted with antigens prepared of yolk sac cultures of the virus have yielded positive reactions in about 100 per cent of known cases. Positive reactions, however, have occurred in 50 to 75 per cent of cases of syphilis, gonorrhea and chancroid. Positive reactions have also occurred with the serums of about 7.6 per cent control cases. Positive reactions, therefore, may indicate (1) the disease is clinically present; (2) that it is present but not clinically apparent, or (3) that unsuspected infection with the virus has occurred in the past.
Human Encephalitides	Specific complement fixation tests have been described for the diagnosis of the Eastern and Western types of equine encephalomyelitis, St. Louis and Japanese B encephalitis, and lymphocytic choriomeningitis.
Other Viral Diseases	Specific complement fixation reactions occur in *smallpox;* also specific complement fixation and precipitin reactions in *chickenpox*. These tests, however, are not ordinarily employed for diagnostic purposes. They may, however, be of value in differentiating between smallpox and chickenpox. Serologic methods are also of value in the diagnosis of *influenza, psittacosis, ornithosis, mumps* and *yellow fever.*

Since then the Weil-Felix reaction has been found of great value in the diagnosis of the different types of typhus fever as well as of Rocky Mountain spotted fever (Table 108). It is thought that *P. vulgaris* and the rickettsia share a common soluble specific substance or hapten in the nature of a polysaccharide.

The test is carried out by the macroscopic method in the same manner as other agglutination tests employing smooth cultures of *P. vulgaris* X_{19} and, whenever possible, X_2 as antigens and using the O antigens in preference to the H. Proper controls, consisting of a known positive human or rabbit immune serum and a known normal human serum, should always be included. The occurrence of agglutination in a dilution of 1:50 or more should be considered positive, since agglutinins are not ordinarily present in normal serum in a titer of more than 1:25 to 1:50.

TABLE 109

Disease*	Locality	Agglutination			Main Antigen
		OX_{19}	OX_2	OXK	
Classical typhus	Old and New Worlds	+++	+	–	OX_{19}
Tabardilla; Brill's disease	Mexico; U. S.	+++	?		OX_{19}
Endemic typhus	Australia	+++	+		OX_{19}
Tropical typhus (Shop)	Malaya; Dutch East Indies	+++	+ ?		OX_{19}
Tropical typhus (type K)	Malaya; Dutch East Indies	–	–	+++	OXK
Tsutsugamushi	Japan	–	–	+++ ?	OXK (?)
Rocky Mountain spotted fever	U. S.	+	+	+	Unknown
Tick-bite fever	South Africa	+	+	+	Unknown
São Paulo typhus	Brazil	+++	+	+	OX_{19}

+++ = main agglutination. + = group agglutination. ? = not tested adequately.

In repeat tests, the presence of agglutinins in a titer of 1:50 or more in a previously negative serum may be regarded with significance, particularly if corroborated by suspicious clinical findings. As a rule, in most typhus fever patients the agglutinin titer is 1:25 on the fourth day and 1:50 or higher by the eighth day. By the end of the second week it may climb to several thousands (even as high as 50,000), after which it declines rapidly during convalescence. The reaction may be negative within five or more months after recovery or may persist for several years. Prozone reactions with no agglutinations in the lower dilutions may occur. Some difficulty and confusion in relation to this reaction arises at times in the differentiation of typhus fever from certain typhus-like infections. Table 109, after Felix,[130] may be helpful in this differentiation. Incidentally, the serums of pregnant women may contain agglutinins for *Proteus* OX_{19} which may interfere with the usefulness of this agglutination test in the diagnosis of rickettsial diseases during pregnancy.[131,132,133]

Castaneda [134] has also found that typhus-immune serum contains complement-fixing antibodies for *Rickettsia prowazeki* (Mexican type), but not for *P. vulgaris* X_2 or X_{19}. The serums from rabbits immunized against *P. vulgaris* (X_{19}) gave complement fixation in the presence of *Rickettsia prowazeki* and also the homologous antigens. On the basis of complement-fixation reactions with the serum of typhus patients and convalescents, as well as serum from typhus-immune guinea-pigs, Castaneda believes these reactions may prove to be useful specific tests for the detection of typhus fever and, in some cases, for the detection of a past typhus infection. Bengtson [135] found the complement fixation test useful in detecting recent and also past endemic typhus fever.

It is apparent, therefore, that both the Weil-Felix and rickettsial agglutination and rickettsial complement fixation tests are of value as aids in the diagnosis of both epidemic and murine typhus fever. A high percentage of positive reactions has been observed in endemic areas, probably due to unrecognized or subclinical infections.[136] Clear differentiation between epidemic and endemic typhus fever has not been obtained by the complement fixation test [137] and there has been a higher percentage of cross reactions among typhus vaccinated than among non-vaccinated individuals.[138] In murine typhus fever, Nelson [139] has reported that Weil-Felix and rickettsial agglutination tests usually yield positive reactions earlier in the disease than rickettsial complement fixation tests.

Rocky Mountain Spotted Fever. As in typhus fever, agglutination tests with antigens of *P. vulgaris* X_2 and X_{19} possess diagnostic value in Rocky Mountain spotted fever (Table 108).

The Weil-Felix reaction, however, does not serve to differentiate between Rocky Mountain spotted fever and typhus fever. But since epidemic typhus does not occur in the same areas as spotted fever, there is little occasion for this differentiation, except for those rather rare instances in which endemic typhus may likewise be present, as in the eastern states or in Mexico. Under such circumstances, differential diagnosis may be extremely difficult, since the clinical features of both diseases are closely similar if not identical and both give a positive Weil-Felix reaction. Definite differentiation can then be made only by means of animal inoculation tests. Spotted fever is especially virulent for the guinea-pig and injection of infected blood into that animal produces extremely severe scrotal lesions accompanied by ulceration and necrosis of the skin; on the other hand, typhus fever, especially the endemic form, is less virulent for the guinea-pig and although it may cause some swelling and congestion of the scrotal tissues, this never goes on to the stage of necrosis or ulceration. Both typhus and spotted fever produce febrile reactions in guinea-pigs as well as characteristic histologic lesions in the brain that distinguish them, not only from other febrile diseases, but even from one another.

Differentiation may also be accomplished by means of cross-immunity or neutralization tests in guinea-pigs. The cross-immunity test is carried out by injecting the patient's blood into guinea-pigs which have recovered from infection with a known strain (*e.g.*, typhus); if no infection ensues, then the infection is of the same type (in this case typhus). In the neutralization test, the convalescent serum of the patient is mixed with a known strain (*e.g.*, spotted fever) and injected into a guinea-pig. If no infection ensues, the patient may be regarded as having at the time or having had in the past an infection with that particular strain (in the latter instance it would be spotted fever).

These animal inoculation tests are not easy to carry out, and especially since the occasion for their use is very infrequent. Fortunately, they are made readily available to every practitioner by the laboratories of the National Institute of Health in Bethesda, Md., where these differential studies will be carried out, without charge, on any blood specimen submitted for examination.

Recently, however, Plotz and Wertman [140] have described a complement fixation test conducted with antigens prepared of *R. dermacentroxenus* which apparently yielded specific reactions with the serum of nine cases of Rocky Mountain spotted fever. Negative reactions were observed in Brill's disease, "Q" fever, various other febrile diseases and syphilis, as well as with the serums of normal individuals. If these results are confirmed it would appear, therefore, that this test may prove of value in the diagnosis of Rocky Mountain spotted fever.

Rickettsialpox. Rickettsialpox is a new rickettsial disease caused by *R. akari* isolated by Huebner and his associates in 1946.[141] During this disease, negative Weil-Felix agglutination reactions with antigens OX_{19}, OX_2 and OXK have been usually observed or the titers have been low. Positive complement fixation reactions employing the MK rickettsialpox antigen, however, have been observed in all cases; weekly positive reactions may occur during the first week of the disease, with rising titers and strongly positive reactions during the second and third weeks. About 80 per cent of serums have also given weakly positive reactions with Rocky Mountain spotted fever antigens.

Lymphogranuloma Venereum. Complement fixation tests employing antigens prepared of human bubo pus or virus-infected mouse brain tissue have not proved satisfactory in the diagnosis of lymphogranuloma venereum (Table 108). Apparently, however, antigens prepared of virus-infected mouse lung tissue and especially of yolk sac cultures of the virus have proved more satisfactory.[142]

The latter antigen (lygranum) has been found by Rake and his colleagues [143] satisfactory not only for the conduct of the Frei skin test but also in complement fixation tests employing a primary incubation of seventy-five minutes at 37° C. Indeed, these investigators believe that the test may be more sensitive than the Frei test, with the possibility of positive reactions occurring as early as within a week after the onset of symptoms. For example, in two series of cases of lymphogranuloma venereum, positive reactions were observed in 161 of a total group of 163 cases. Positive reactions were also observed in 145 of a group of 146 additional cases, with or without clinical manifestations of lymphogranuloma venereum, showing positive Frei skin reactions. However, positive reactions were also observed in 7 or 7.6 per cent of a group of 92 control cases comprising 69 normal individuals and 23 with various acute and chronic infections or organic diseases. Whether or not these were nonspecific reactions, or due to previous clinically unsuspected infections with the virus, cannot be stated. Furthermore, positive reactions have been observed in 20 of a group of 39 cases of syphilis, in 8 of a group of 17 cases of gonorrhea and in 3 of a group of 4 cases of chancroid. This high incidence of positive reactions in other venereal diseases, however, may indicate that clinically unsuspected infection with the virus of lymphogranuloma venereum may be more prevalent among venereally exposed individuals than hitherto surmised. This is also suggested by an incidence of 81 per cent positive complement fixation and 55 per cent positive Frei reactions in a group of 53 cases of venereal diseases (mostly syphilitic) among whom signs of lymphogranuloma venereum were found in 18 cases.

In a separate series of 149 cases, Shaffer and Rake [144] have observed 98 per cent positive reactions along with a few weakly positive cross reactions in individuals known to have had infections with psittacosis and related viruses. Similar results have been reported by other investigators, some of whom found the complement fixation test more sensitive than the skin test as an aid in diagnosis.[145-148] Positive reactions have also been reported in cases of chancroid in which they have been interpreted as indicative of dual infections or chancroid in individuals previously infected with the virus of lymphogranuloma venereum with persistence of complement fixing antibody.

It would appear, therefore, that complement fixation tests conducted with yolk sac antigen of the virus may prove of clinical value in the diagnosis of lymphogranuloma venereum and especially in conjunction with the skin test. If employed, however, it is apparent that positive reactions are to be expected in individuals without clinical manifestations of lymphogranuloma venereum. When such occur in individuals either with venereal disease or known to have been venereally exposed, it is a reasonable assumption that clinically unsuspected infections with the virus may be responsible in at least some of them. If positive reactions occurring in normal individuals are nonspecific, it is likely that improvements in technic may prevent them. Whether or not these, as well as some of the positive reactions observed among those with syphilis, gonorrhea and chancroid are due to clinically unsuspected infections with the viruses of psittacosis or meningopneumonitis cannot be stated, although it is known that these two viruses share a common antigenic factor with the virus of lymphogranuloma venereum capable of yielding cross-complement fixation reactions.[149]

Lymphogranuloma Inguinale. Positive complement fixation reactions have also been observed in about 85 per cent of cases of lymphogranuloma inguinale employing antigens prepared of cultures of *Donovania granulomatis,* the presumed causative agent of this disease.[150,151] Positive reactions have also been observed in syphilis, gonorrhea, lymphogranuloma venereum, chancroid and other chronic ulcers of the genitalia but it is likely that these were dual infections or occurred in cases previously infected with lymphogranuloma inguinale.

Influenza. In 1941, Hirst [152] discovered that type A and type B influenza viruses, as contained in the allantoic fluid of infected eggs, have the property of agglutinating chicken erythrocytes. Provided the strains are fully virulent, the agglutination titers parallel the results of mouse serum neutralization tests. Hirst also discovered that agglutination is specifically inhibited by immune serum, the degree of inhibition affording an index to the ability of the serum to neutralize virus.

These observations have been amply confirmed, with the result that the test can be used diagnostically for detecting the presence either of virus or of antibodies.[153-158] The latter is now known as the *agglutination inhibition test* (A-I) or the "*Hirst phenomenon.*" Several modifications of the test have been described employing chicken erythrocytes but it is now known that group O human erythrocytes may be employed with satisfactory results. In the conduct of the test the virus combines with the antibody and thereby prevents the virus from agglutinating the erythrocytes. About 90 per cent of cases of influenza show the presence of this agglutination-inhibiting antibody in the serums during the course of the disease or during convalescence. Antibody for type A virus tends to develop earlier than antibody for type B virus.[156]

This agglutination-inhibition test has proved valuable as a practical diagnostic aid in respiratory tract infections. A specimen of blood should be taken as soon as possible and the serum kept at a low temperature. If diagnosis remains in doubt a second specimen should be collected four to ten days later and both serums tested simultaneously for inhibition of agglutination of type O human erythrocytes by type A (PR8) and type B (Lee) viruses. A four- or five-fold increase of antibody in the second specimen of serum is regarded as indicative of infection with influenza virus.

The Human Encephalitides. Since the etiologic diagnosis of the human encephalitides is difficult, diagnostic serologic tests are greatly needed. Hitherto, only serum neutralization tests not adapted for routine use have been available, in addition to the fact that neutralizing antibodies are not generally produced until convalescence is well under way. Recently, however, Casals and Palacios [159] have reported specific complement fixation reactions not only in some of the viral diseases of the lower animals but in some of those of human beings as well, and especially in equine encephalomyelitis. For example, specific reactions were observed in seven persons 2½ years after attacks of Western and Eastern equine encephalomyelitis and in two persons 8 years after an attack of louping ill. In cases of St. Louis encephalitis and lymphocytic choriomeningitis, complement-fixing antibodies have been found shortly following infection but not after long periods. In a subsequent investigation Casals [160] reported that of 83 serums tested with antigens of the Eastern and Western types of equine encephalomyelitis and St. Louis encephalitis, 44 or 53 per cent gave positive reactions with the antigen of Western equine encephalomyelitis while 39 reacted negatively with all antigens. If these observations are confirmed and if positive reactions are found to occur early in these viral diseases, it would appear that complement fixation tests may prove of practical or clinical value in diagnosis and differential diagnosis. The antigens are prepared of the brains of mice infected with the respective viruses and the tests conducted with human serums after inactivation at 60° C. for 20 minutes. According to Sabin,[161] complement fixation tests conducted with properly standardized antigens have proved the method of choice in the serum diagnosis of encephalitis due to the Japanese B type of virus, even though the necessary rise in titer or even the first appearance of positive reactions may be delayed for as long as five weeks after the onset of the disease.

Other Viral Diseases. Although the serums of about 60 per cent of cases of *variola* have been observed to yield positive complement fixation reactions in tests employing antigens of variolous and cowpox viruses,[162] yet the complement fixation test is not employed in the diagnosis of the disease except with an antigen of the virus and rabbit-antivaccinal serum, as discussed in Chapter 15. Positive reactions have been observed also in 30 to 39 per cent of cases of *varicella* [162] which were specific in that positive reactions were not observed with the serums of smallpox or vaccinia patients. A specific precipitin test has also been described [163] so that these serologic procedures as well as the Paul reaction may be useful at times in differentiating between smallpox and chickenpox. The complement fixation reaction has also proved of value in the diagnosis of *psittacosis* and *ornithosis*.[164] Likewise in the serum diagnosis of *mumps* employing antigens prepared of infected monkey parotid gland, positive reactions having been observed during an attack of the disease or during convalescence.[165,166] Perlowagora and Hughes [167] have also reported the complement fixation reaction, employing "globulin antigen," as being both sensitive and specific in clinical *yellow fever* regardless of the severity of the disease; since positive reactions also occur in inapparent and endemic yellow fever the test is regarded as being of value in epidemiologic surveys. Otherwise, however, complement fixation tests have not been found helpful or of clinical value in the diagnosis of acute anterior poliomyelitis [168] or other viral diseases of human beings.

EXAMINATIONS IN THE MYCOTIC DISEASES

Serologic examinations are not generally employed in the diagnosis of diseases due to the pathogenic fungi. In the superficial infections, like the various ringworms, only few or no antibodies are detectable in the blood by the complement fixation test.[169] In some of the deeper mycotic diseases, however, which are essentially or potentially systemic infections, antibodies may occur in the blood like agglutinins and complement-fixing antibodies in actinomycosis and sporotrichosis,[170,171] precipitins in coccidioidal granuloma,[172] etc. In suspected infections antigens may be prepared of stock cultures of the organisms and used in tests with patient's serum for possible diagnostic aid, but mycologic examinations, supplemented in some instances by skin tests, are usually sufficient.

Tenenburg and Howell [173] have developed a complement fixation test for *histoplasmosis*, using histoplasmin as antigen, which has given positive reactions in cases of the disease as well as in a number of persons with active lung lesions associated with histoplasmin sensitivity. Individuals with healed or no lung lesions gave negative reactions even though histoplasmin sensitive, as did also histoplasmin-negative individuals. Saslaw and Campbell [174] have also found antigens prepared of the yeast phase of *H. capsulatum* satisfactory in the complement fixation test.

EXAMINATIONS IN THE DISEASES DUE TO ANIMAL PARASITES

Antibodies are present in the blood in many of the diseases due to animal parasites and especially those producing infestments of the deeper tissues as in amebic dysentery and liver abscess, hydatid disease, trichinosis and malaria, in which complement fixation or precipitation tests have proved of diagnostic value

(Table 110). The practical applications of serologic examinations, however, are limited because of the difficulties experienced in preparing or obtaining efficient antigens. Fortunately, they are not usually required for diagnostic purposes in view of the efficiency of parasitologic examinations.

Amebiasis. According to Craig,[175] the complement fixation test conducted with an alcoholic extract of cultures of *E. histolytica* as antigen is highly sensitive, being capable of yielding positive reactions in about 90 per cent of cases of amebiasis. Craig also regards the test as highly specific, as the serums of normal individuals and those suffering from other diseases have not given positive reactions unless there was a coincident infestment with *E. histolytica*. The test is stated to be of value in the diagnosis of amebic abscess of the liver unaccompanied by intestinal symptoms; also in the diagnosis of apparently healthy "carriers" of *E. histolytica* and of those presenting atypical or mild symptoms of infection. Kiefer[176] has reported positive reactions in 15 out of 19 cases of chronic ulcerative colitis, with the suggestion that this disease may be a pyogenic infection of the colon superimposed upon an original amebic ulceration. Paulson and Andrews,[177] however,

TABLE 110. SUMMARY OF THE CLINICAL INTERPRETATION OF SEROLOGIC EXAMINATIONS IN AMEBIASIS, TRICHINOSIS, HYDATID DISEASE AND OTHER DISEASES DUE TO ANIMAL PARASITES

Diseases	Interpretation
Amebiasis	Complement fixation tests are sometimes of supplementary value, especially in the diagnosis of amebiasis of the liver and other infestments involving the deeper tissues. Positive reactions are of more clinical value than negative reactions. The complement fixation test also is of value in the detection of carriers of *E. histolytica* and in the diagnosis of atypical cases of amebiasis; also in relation to anti-amebic treatment. The test is not required when the parasite or its cysts are detectable by parasitologic examinations.
Trichinosis	The precipitin test is stated to yield positive reactions in over 90 per cent of cases of active trichinosis of human beings. When employed with the skin test it is particularly valuable as an aid in the detection of mild, sporadic and chronic cases. Positive reactions are not usually observed until the fourth week of the disease. Nonspecific reactions may occur.
Hydatid Cyst	Precipitin and complement fixation tests are not as valuable in diagnosis as skin tests. Of the two serologic procedures the complement fixation test is preferred. Cross-precipitin and complement fixation reactions occur with antigens of other taenial worms. Positive complement fixation reactions are stated to occur in 84 to 90 per cent of cases; positive precipitin reactions in about 50 per cent. The former is especially valuable for the postoperative detection of residual cysts.

TABLE 110. SUMMARY OF THE CLINICAL INTERPRETATION OF SEROLOGIC EXAMINATIONS IN AMEBIASIS, TRICHINOSIS, HYDATID DISEASE AND OTHER DISEASES DUE TO ANIMAL PARASITES—(Continued)

Diseases	Interpretation
Other Parasitic Diseases	Highly specific precipitin and complement fixation reactions may occur with polysaccharide antigens in *ascariasis* but the tests have not proved of practical value. Positive group complement fixation reactions occur in *filariasis* and especially in active infestments. Negative reactions are particularly valuable in excluding the disease. Positive complement fixation reactions may occur in infestments with *D. latum* but cross-reactions may occur with antigens of other cestodes. Complement fixation tests are of practical value in the diagnosis of *paragonimiasis* and especially for the detection of nonpulmonary infestments. Group precipitin and complement fixation reactions occur in 71 to 82 per cent of cases of *schistosomiasis*. A complement fixation test has proved of value in the diagnosis of Chagas' disease due to infestment with *T. cruzi*. Complement fixation, the Napier aldehyde and Sia precipitin tests have proved of value in the diagnosis of *kala-azar*. A group-specific complement fixation test conducted with antigens prepared of *P. knowlesi* has been found of value in the diagnosis of *malaria* when parasites are difficult to find in the blood and especially in epidemiologic surveys, but is of no value in the detection of latent malaria. Precipitin tests possess no practical value. Nonspecific serologic tests based on the detection of an increase of euglobulin in the serum also possess diagnostic value.

regard the test as being only of supplementary value in the diagnosis of amebiasis and believe that because of falsely positive as well as of falsely negative reactions, it may be unreliable in individual cases. Meleney and Frye [178] state that positive reactions are presumptive evidence of the presence of amebae in the tissues, and that most infested individuals giving a negative reaction (except those in the early stage) harbor the parasite only in the intestine without tissue invasion.

According to Craig, the test is also of value as a control on antiamebic treatment, as repeatedly negative reactions over a period of several weeks are stated to be indicative of cure. The practical application of the test is limited by the difficulty of preparing efficient antigen; fortunately, it is not generally required for diagnostic purposes, since *E. histolytica* can almost always be found in the stools in acute and chronic amebiasis if careful and repeated examinations are made by skilful and experienced workers.

Trichinosis. Precipitin tests conducted with antigen of *Trichinella spiralis* are stated to yield positive reactions with the serums of over 90 per cent of individuals with trichinosis.[178,179] Positive reactions may occur as early as the second week of the disease but not usually until the fourth week. This test, along with the skin test, is particularly valuable in the diagnosis of mild, sporadic and chronic cases of the disease. Nonspecific reactions may occur when the antigen is used in 1:100 or 1:1000 dilutions but positive reactions occurring in dilutions of 1:2500 or higher are specific in 90 per cent of trichinous patients.[180] It is stated that falsely positive reactions may occur in malarial patients under quinine treatment.

Hydatid Cyst. In general terms, the precipitin and complement fixation tests in hydatid cyst disease due to infestment with *Echinococcus granulosus* have not proved as satisfactory as the skin test for diagnostic purposes. The chief drawback to their use is the difficulty of obtaining fresh antigen for their conduct. Antigens prepared of purified hydatid fluids are recommended. The complement fixation test is usually to be preferred to the precipitin test; it is also the test of choice in old complicated cases because of greater sensitivity.

Positive complement fixation reactions in human echinococcosis are stated to occur in 84 to 90 per cent of cases. The test is especially valuable in the detection of residual cysts in patients after operation.

The precipitin reaction does not show as good correlation with clinical echinococcosis as the complement fixation and skin reactions. Most investigators consider the test unsatisfactory for diagnostic purposes because of nonspecific reactions and failure to give more than 50 per cent positive reactions. With few exceptions investigators consider the precipitin, complement fixation and skin reactions as nonspecific because of group reactions for other taenial worms. Thus, cross complement fixation and precipitin reactions have been reported with both echinococcal and cysticercal fluid antigens in echinococcosis and intestinal taeniasis.

Other Parasitic Diseases. Precipitin and complement fixation tests have not proved of practical value in the diagnosis of *ascariasis* although highly specific precipitin reactions have been observed with polysaccharide antigens.[181]

Positive group complement fixation reactions have also been observed in *filariasis* with aqueous and alcoholic antigens prepared of fresh or dried *Dirofilaria immitis* or *Onchocerca volvulus*. Active infestments give the highest percentage of positive reactions. Positive reactions, however, are not of diagnostic importance although negative reactions are of value in excluding filarial infestments.

Positive complement fixation reactions may occur also in infestments with *Diphyllobothrium latum* but the tests are not of practical value since cross reactions occur with antigens of other cestodes.

Complement fixation reactions, however, are of clinical value in the diagnosis of *paragonimiasis* due to infestment with *Paragonimus westermani* and especially in the detection of nonpulmonary infestments when the worms are lodged in deep foci and when the ova are not present in the subcutaneous tissues or in the excreta. The antigen should be a saline extract of macerated adult worms from mammalian hosts.

Precipitin and complement fixation reactions are also of value in the diagnosis of *schistosomiasis*. The reactions are of a group character as the antibodies produced by the three human schistosomes give cross reactions with each other as well as with antigens prepared from the livers of snails infested with *S. spindale* and *S. bovis* of the lower animals. Positive precipitin reactions have been reported in 82 per cent of human beings infested with *S. mansoni* and in 71 per cent of cases infested with *S. japonicum*. Recently infested individuals are more apt to show positive reactions than those with infestments of over two years' duration. Falsely positive reactions may occur with the serums of noninfested syphilitic individuals and especially if alcoholic extracts are employed as antigens.

While efforts have been made to develop complement fixation tests of clinical value in the diagnosis of *trypanosomiasis,* no method has been found of practical value in African sleeping sickness due to infestment with *T. gambiense* or *T. rhodesiense.* In Chagas' disease due to infestment with *T. cruzi,* however, a test recently developed by Kelser [182] has given very promising results.

In *kala-azar,* due to infestment with *L. donovani,* the complement fixation test has proved useful in diagnosis when the parasites cannot be found. After the fifth month of the disease, however, when there is an increase in the globulin of the serum, the Napier aldehyde test (formol-gel), the Sia precipitin test for euglobulin and the Chopra antimony test are more valuable since positive reactions are observed in a high percentage of patients. Cold hemagglutinins are stated to occur in Chinese kala-azar.[183]

The presence of precipitins and complement-fixing antibodies has been demonstrated in the serums in *malaria* by many investigators. The results indicate group reactions with plasmodia rather than species-specific reactions. These group reactions are not confined to the human plasmodia, since antigens prepared of the simian species, *P. knowlesi,* give positive complement fixation reactions one to twelve months after the onset of symptoms due to infestment with *P. vivax* and *P. falciparum.*[182,184] Highly specific reactions in human malaria, however, have been observed in tests conducted with antigens of *P. knowlesi.* Nonspecific reactions may occur occasionally with the serums of nonmalarial syphilitic individuals. Syphilitic individuals inoculated with *P. vivax* develop complement-fixing antibody for malarial antigen.

Positive complement fixation reactions have been found to parallel more closely the presence of parasites in the blood than the occurrence of chills or fever. The reactions tend to become more strongly positive as the parasites increase in the blood. As the parasites decrease, the reactions become weaker. The complement fixation test, therefore, while not capable of detecting all cases of malaria, appears to be highly specific for human infestments and of value when parasites are difficult to detect in the blood and especially in epidemiologic surveys, although it is stated to be of but limited value in the detection of latent malaria.[186] Precipitin tests, however, have not been found of practical value in diagnosis.

Nonspecific serologic reactions based upon an increase of euglobulin in the serum are likewise of diagnostic value, negative reactions being particularly significant. The melanoflocculation test of Henry,[187] which is largely used in Europe, gives a high percentage of positive reactions. Unfortunately, it also yields from 2 to 3 per cent of positive reactions in nonmalarial individuals, falsely positive reactions being observed not only in syphilis but in tuberculosis, the leptospiroses, leukemia, eclampsia, hepatic and other diseases causing a disturbance in the equilibrium of the plasma proteins. Proske and Watson [188] have developed a simpler protein-tyrosin test for determining changes in the euglobulin which they state gives 97.4 per cent positive reactions in malaria and which they recommend for the detection of chronic malaria and as a guide in treatment, but it is subject to the same objections as apply to the Henry test in respcct to falsely positive reactions in syphilis and other diseases.

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18

THE CLINICAL INTERPRETATION OF SEROLOGIC EXAMINATIONS IN SYPHILIS

GENERAL CONSIDERATIONS

In no other disease have serologic tests been as widely and usefully employed as aids in diagnosis as in syphilis. The original Wassermann test and many of its earlier modifications are no longer employed but, fortunately, its intrinsic merit enabled it to survive all the abuses committed in its name. Newer and superior methods in both complement fixation and flocculation procedures have been developed during the past twenty-nine years with the result that those commonly and widely employed at the present time have been proved to possess a high degree of sensitivity and practical specificity *when properly conducted.* Great credit is due the Committee on Evaluation of Serodiagnostic Tests for Syphilis for the serologic surveys and educational campaign conducted by the United States Public Health Service, in cooperation with the American Society of Clinical Pathologists, among serologists during the past fourteen years in the need for conducting the present approved methods by experienced and careful workers *exactly as described by their authors.* Even under the best conditions errors can occur but their incidence is to a large extent in relation to the skill and experience of those who conduct them, as well as to the reagents employed, with special reference to the preparation and standardization of the antigens employed. *No test can be better than the laboratory conducting it.* There is still room for improvement in the sensitivity of serologic tests but not at the expense of specificity. I am of the opinion that it is far better to miss the serum diagnosis of occasional cases of chronic latent syphilis than to incur unnecessary risks of falsely positive reactions with all that these may mean to the individual concerned. But the need is not so much for additional methods as for the proper conduct of those now available and approved (Table 111).

At the present time no one can deny that the serologic tests, when properly and skilfully conducted, are of inestimable value as aids in the diagnosis and treatment of syphilis. But common sense in their use and interpretation is equally essential. They do not always provide an easy or royal road to diagnosis; after all, they are usually only additional diagnostic aids although in chronic latent syphilis they may be and frequently are the sole means for detecting the disease. Final judgment and responsibility in their interpretation is properly the function of the clinician and he should not attempt to "pass the buck" to the serologist or technologist who is doing his or her full duty when seeing to it that the tests are conducted exactly as they should be, using every known precaution against error and reporting the reactions exactly as observed and not as the physician expects or may desire.

TABLE 111. SUMMARY OF GENERAL CONSIDERATIONS IN THE SEROLOGY OF SYPHILIS
Serologic tests are of two kinds: (1) complement fixation (Wassermann) and (2) flocculation tests (macroscopic and microscopic). The tests employed should possess *the maximum of sensitivity consistent with specificity.*
Serologic Surveys of State laboratories by the Committee on the Evaluation of the Serodiagnostic Tests for Syphilis and intrastate evaluation surveys conducted by State laboratories have resulted in a progressive improvement in the serodiagnosis of syphilis.
There is no one best test. *No test can be better than the laboratory conducting it.* No test is so simple that it can be properly and reliably conducted by untrained and inexperienced workers. *All tests should be conducted exactly as described by their authors.* All tests approved by the U. S. Public Health Service are good and acceptable when properly conducted. *Accuracy should not be sacrificed for mere speed in conducting the tests.*
The serum diagnosis of syphilis is best served by using two or three approved tests routinely.
The final interpretation of serologic reactions in relation to the diagnosis and treatment of syphilis is primarily and fundamentally the duty and function of the clinician.

In view of the wide prevalence of syphilis in both sexes and all races, the frequency with which it is responsible for chronic disease when not suspected clinically, and the fact that a negative history and respectability are unreliable in excluding the possibility of its presence, it is advisable to use the tests routinely in the majority of patients in both clinic and private practice. Indeed, physicians as a whole need to be more "syphilis conscious" and to suspect the possibility of the great "masquerader" among both the high and the low.

In private practice it is very easy to conduct these tests without the knowledge of the patient when offense is feared. In other words, blood can be taken for any of the numerous blood chemistry determinations and a portion placed surreptitiously in a plain sterile test tube for the serologic tests.

Kinds of Serologic Tests. *The serologic tests for syphilis should possess the maximum of sensitivity consistent with specificity.* They are divisible into two kinds: (1) complement fixation (Wassermann) and (2) flocculation tests. The latter are subdivided into macroscopic and microscopic procedures.

Complement fixation tests are technically more difficult but the reactions are easier to read. They are likewise readily adapted to quantitative methods which are of particular value as serologic guides in relation to the treatment of syphilis. Furthermore, they are highly satisfactory in the examination of spinal fluids.

Flocculation tests are technically simpler and quicker but the reactions are more difficult to read. Their proper conduct requires just as much skill and experience on the part of serologists and technologists as complement fixation tests.

There is no one best test. Indeed, the results of all of the serologic surveys conducted by the Committee on the Evaluation of the Serodiagnostic Tests for

Syphilis have shown that the serum diagnosis of syphilis is best served by using at least two or three approved tests routinely whenever possible.

No other laboratory test carries as much responsibility as the serologic tests for syphilis. No test is so simple that it can be reliably conducted by untrained and inexperienced workers. All of the tests approved by the U. S. Public Health Service are good when properly performed. As previously stated, great credit is due the Committee on the Evaluation of Serodiagnostic Tests for Syphilis in conducting yearly surveys of the methods employed by state laboratories. Many of the latter now conduct surveys of the various county, municipal, hospital and private laboratories within their own domains. The results have shown a progressive and very gratifying improvement in the serum diagnosis of syphilis. Every laboratory should constantly check its results and especially in cooperation with a good syphilis clinic. Every physician should exercise great care in the choice of a laboratory entrusted with this important work. Since accuracy should never be sacrificed for mere speed, physicians should not bring undue pressure on laboratories for quick reports except under special circumstances.

Recently, complement fixation tests conducted with antigens prepared of cultures of alleged *Treponema pallidum* have also commanded considerable interest. Their value in the serum diagnosis of syphilis will be discussed later.

THE MECHANISM OF SEROLOGIC REACTIONS

It was originally thought that the Wassermann reaction was biologically specific and due to the fixation of complement by syphilis antibody interacting with *T. pallidum* in antigens prepared of fetal syphilitic liver or other syphilitic tissues. It was soon discovered, however, that efficient antigens could be prepared of nonsyphilitic tissues, like beef heart, and that the reaction was due to the fixation of complement by an antibody-like substance occurring in syphilitic serum or spinal fluid reacting with the alcohol-soluble lipids of normal tissues and especially lipids sensitized by cholesterol (Table 112). On this basis it is now thought that the antibody-like substance occurring in syphilis produces a flocculation of the lipids followed by the fixation of complement by the flocculi. Later it was discovered that by changes in the amounts of syphilitic serum and lipids employed, the flocculi of the latter could be seen microscopically or macroscopically which, after improvements in technic, constitute the various flocculation tests used today in the serum diagnosis of syphilis. Consequently, since both complement fixation (Wassermann) and flocculation tests are conducted with antigens prepared of the alcohol-soluble lipids of normal mammalian tissues (usually beef heart) the reactions are biologically nonspecific although they possess a remarkable degree of what may be called "practical specificity" in the serum diagnosis of syphilis. In other words, the serum tests for syphilis are not based on a specific antigen-antibody reaction since the antigen is not prepared of *T. pallidum*. Under the circumstances the situation is similar to the biologic nonspecific agglutination of strains of *P. vulgaris* in the Weil-Felix reaction by the serums of individuals with rickettsial diseases like typhus and Rocky Mountain spotted fevers.

TABLE 112. SUMMARY OF THE MECHANISM OF SEROLOGIC REACTIONS IN SYPHILIS
Complement fixation (Wassermann) and flocculation reactions are due to the flocculation of the alcohol-soluble lipids of normal tissues by antibody-like substance occurring in serum and spinal fluid called *reagin.* Consequently they are biologically nonspecific but nevertheless possess a remarkable degree of practical specificity in the serum diagnosis of syphilis.
The identity of the tissue lipid or lipoids reacting with reagin is unknown.
The reagin is produced not only in syphilis, yaws and to some extent in other spirochetal diseases, but also sometimes in other diseases as well. It may rarely occur in normal human serums but frequently in the serums of normal rabbits and other lower animals.
The reagin is not a true antibody in the sense of being destructive for *T. pallidum.* It appears to be entirely separate and distinct from natural spirochetal antibodies occurring in normal human serums as well as from acquired spirochetal antibodies in syphilis. The mechanism of its production is unknown.
Natural group agglutinin for *T. pallidum* and other spirochetes occur in the serums of normal nonsyphilitic individuals. They are increased in syphilis and especially for virulent tissue *T. pallidum* but not sufficiently for rendering agglutination tests of practical diagnostic value.
Natural group complement-fixing antibody for *T. pallidum* and other spirochetes also occurs in a small percentage of the serums of normal nonsyphilitic human beings. It may yield falsely positive or nonspecific serum reactions with spirochetal antigens. It does not occur in the spinal fluid of normal nonsyphilitic individuals. Spirochetal complement-fixing antibody is increased in syphilis, leprosy and malaria. Spirochetal antigens have been advocated in the complement fixation tests for syphilis.

Lipoidal or Tissue Antigens; Cardiolipin Antigen. Unfortunately, the exact identity of the antigenically active lipids occurring in alcoholic extracts of mammalian tissues has never been established. When this has been accomplished a long step will have been taken toward increasing the sensitivity and specificity of the complement fixation and flocculation tests for syphilis and especially if the lipid or lipids particularly involved are capable of being produced synthetically in a state of chemical purity. At the present time all that can be stated is that the most active lipids are those which are soluble in alcohol but insoluble in acetone and largely composed of phosphatids although, as shown by Brown and Kolmer,[1] antigenic activity is apparently not as much a property of lecithin or cephalin as of an unknown substance as yet unidentified containing phosphorus but no nitrogen. Recently Pangborn[2] has isolated and purified a phospholipid from beef heart which, upon hydrolysis, yielded fatty acids and a phosphorylated polysaccharide called *cardiolipin,* which is also nitrogen free. Since its discovery Pangborn[3] has described improved methods for its isolation and purification. These methods, however, are very complicated so that the preparation of cardiolipin should be entrusted to chemists experienced in its preparation. Under these circumstances cardiolipin has the advantage of chemical reproducibility which has never been possible hitherto with the lipoidal or tissue antigens commonly employed.

Cardiolipin alone cannot be used in the complement fixation or flocculation tests for syphilis because of being too low in antigenic activity. For this reason it requires the addition of lecithin (also derived from beef heart) and, likewise, sensitization with cholesterol in some tests. Furthermore, the proportions of these ingredients must be determined for each complement fixation or flocculation test employed in order to obtain reactions of maximum sensitivity consistent with specificity in the serum diagnosis of syphilis. In the Kolmer complement fixation test a mixture composed of 0.03 per cent cardiolipin, 0.05 per cent lecithin and 0.6 per cent cholesterol, in dose of 0.5 cc. of 1:150 dilution, has been proposed in the examination of serums and cerebrospinal fluids.[4]

The Antibody or Reagin. Some investigators have suggested that an antibody or antibody-like substance is not involved at all in complement fixation and flocculation tests in syphilis but that positive reactions are due to some vague and unknown colloidal lability of the serum proteins. This hypothesis, however, is not tenable since a definite antibody-like substance occurs in the serum and spinal fluid of this disease in the nature of a globulin which can be removed by absorption with tissue lipids. On the other hand, it is to be admitted that both complement fixation and flocculation in syphilis are colloidal reactions greatly influenced by physical factors. Indeed, it may be that falsely positive or nonspecific reactions sometimes observed in nonsyphilitic individuals with febrile diseases, jaundice, uremia, etc., may be due to colloidal factors of unknown nature and mechanism instead of involving the presence and activity of an antibody-like substance.

As previously stated, however, an antibody or antibody-like substance (reagin) is produced in syphilis capable of sensitizing and flocculating suitable tissue lipids *in vitro* although its exact nature and origin are as yet unknown. Because of this capacity it has been called "lipoidotropic" or "lipoidophilic." Whatever its nature may be, it occurs in the blood not only in syphilis and other spirochetal diseases (especially yaws) but frequently in leprosy and malaria and sometimes in other diseases and even occasionally in normal individuals. Furthermore, the mystery is increased by its occurrence in the serums of normal rabbits and other of the lower animals.[5,6,7]

Some investigators[8] regard this substance as an antibody to the host's own lipids, liberated in the course of tissue destruction in foci of infection by *T. pallidum,* which are rendered antigenic by combination with spirochetal protein. But since various bacterial proteins should be equally capable of activating the lipoidal tissue hapten and thus inducing antibody production, the hypothesis fails to explain the extraordinary specificity of complement fixation and flocculation reactions in syphilis.

Undoubtedly, however, the substance is to be regarded as a kind of antibody. It does not appear, however, to be a true antibody in the sense of being destructive for *T. pallidum,* although its persistence in chronic syphilis may be of immunologic significance since it may indicate the coexistence of sufficient tissue or humoral immunity to protect syphilitic individuals against progression or relapse of infection.[9] Furthermore, there is no reason for doubting that it is produced not only by *T. pallidum* and other pathogenic spirochetes but by *Myco. leprae,* the plasmodia of malaria, and possibly by other micro-organisms as well.

True antibodies for *T. pallidum* are produced in syphilis. The question at issue is whether they are the reagin or whether the latter is an entirely separate and distinct substance. According to Gaehtgens,[10] Beck,[11] and Kolmer, Kast and Lynch,[12] the reagin is entirely separate and distinct from the true spirochetal antibodies producing agglutination and complement fixation with antigens of *T. pallidum*. This is based upon the results of experiments showing that the absorption of syphilitic serums with tissue lipids removes the reagin but not the spirochetal antibodies, while absorption with *T. pallidum* (Reiter strain) removes the latter but not the reagin. Furthermore, the immunization of rabbits with various strains of *T. pallidum* produces large amounts of spirochetal antibodies but not the reagin.[9] According to Eagle and Hogen,[13] however, the Wassermann and flocculation reactions with tissue lipids on the one hand and complement fixation with spirochetal antigens on the other, are due to spirochetal antibodies because in their experiments the absorption of syphilitic serum with tissue lipids removed not only the latter but the reagin as well.

I believe, however, that the weight of evidence at present is in favor of regarding the reagin as separate and distinct from spirochetal antibodies although, as previously stated, the manner of its production is unknown. At all events, the reagin is concerned in the mechanism of both the Wassermann and flocculation reactions in syphilis. This is indicated not only by reason of the fact that the reactions agree in over 90 per cent of syphilitic serums, but likewise because absorption of syphilitic serum with tissue lipids removes the reagin producing both positive Wassermann and flocculation reactions.

SYPHILIS ANTIBODIES

While tissue immunity is undoubtedly of primary and fundamental importance in syphilis it is now known that specific antibodies for *T. pallidum* may be produced and play a rôle in the acquired immunity of the disease.[14]

Agglutinins for T. pallidum. About thirty years ago I discovered agglutinins for Noguchi's cultures of *T. pallidum*[15] and recent investigations have shown their presence in syphilitic serums for cultures of various nonvirulent strains of the micro-organisms in final dilutions varying from 1:10 to as high as 1:1000.[11,12,16] However, the serums of normal human beings usually contain almost as much natural agglutinin not only for cultures of nonvirulent *T. pallidum* but for *T. macrodentium* and *T. microdentium* as well.[12] But while syphilitic human serums have been found to agglutinate the cultivated nonvirulent spirochetes of the Nichols-Hough strain at 1:10 to 1:20, they have agglutinated the virulent spirochetes of this strain (secured from acute testicular syphilomas of rabbits) in dilutions as high as 1:500 to 1:1000.[17] This suggests that the cultivated nonvirulent spirochetes may have undergone dissociation with a change in antigenic structure similar to the S → R dissociation occurring with many of the pathogenic bacteria. Similar results have been observed with the serums of syphilitic rabbits.[12] Under the circumstances it may be stated, therefore, that while agglutinin for cultivated nonvirulent *T. pallidum* may undergo some increase in syphilis it is not sufficient to render an agglutination test of practical value in the serum diagnosis of syphilis. Agglutinin, however, is far more definitely increased for virulent *T. pallidum* obtained from the testicles of syphilitic rabbits but, owing to technical difficulties in their preparation, agglutination tests employing them have no practical diagnostic applications at the present time.

Complement-Fixing Antibody for T. pallidum. In 1912 Noguchi [18] reported that the serums of about 45 per cent of human beings gave positive complement fixation reactions with antigens prepared of saline suspensions of his cultures of *T. pallidum.* Subsequent investigations [19,20] showed, however, that in general terms the tests were inferior in sensitivity to those conducted with antigens of alcoholic extracts of beef heart reenforced with cholesterol. Nevertheless, these pioneer investigations indicated that either a true antibody was producd in syphilis reacting specifically with spirochetes in addition to the reagin reacting with tissue lipids in the Wassermann reaction, or that the reactions occurring with antigens of cultivated spirochetes were partly due to the fixation of complement by the reagin reacting with the lipids of spirochetes, as suggested by my colleagues and myself.[20]

At all events, the idea of possible specific complement fixation in syphilis by specific antibody and antigens of *T. pallidum* was never completely abandoned in the following years, and in 1929 Gaehtgens and Otto [21] greatly renewed interest in the subject by reporting that a phenolized saline suspension of the whole spirochetes of the Reiter strain of alleged *T. pallidum* yielded specific complement fixation reactions in syphilis which were apparently separate and independent of the Wassermann and flocculation reactions as well as being more sensitive than the latter reactions and especially in treated syphilis.

Since then a very large literature has accumulated on the subject and especially in relation to the use of antigens prepared of the Reiter strain produced commercially in Germany and marketed under the name of "palligen." [16] Its practical value in the serum diagnosis of syphilis, as well as that of antigens prepared of cultures of other strains (Noguchi, Kasan, Kroò and Nichols-Hough) will be discussed shortly but here it may be stated that their sensitivity varies considerably, with available evidence indicating that the Reiter and Kasan strains have proved most sensitive, with antigens of the Kroò and Noguchi strains ranking next in this property.

Various investigators [11,22,23,24] have found that the serums of nonsyphilitic human beings may yield falsely positive or nonspecific reactions with "palligen" as well as with antigens prepared of cultures of other strains of alleged *T. pallidum* and other spirochetes. Evidently these are due to the same natural group spirochetal antibody previously discussed in relation to agglutination. In this connection it should be stated, however, that antigens prepared of virulent *T. pallidum* (Nichols-Hough strain) have given a lower percentage of falsely positive or nonspecific complement fixation reactions than antigens prepared of nonvirulent cultures.[17] Furthermore, since none of the natural antibodies occur normally in the spinal fluid, complement fixation tests conducted with them and spirochetal antigens do not give these falsely positive reactions.[24]

Under the circumstances, it appears that cultivated nonvirulent *T. pallidum* are more susceptible to both agglutination and complement fixation by *natural* spirochetal antibody than virulent tissue *T. pallidum* but that in syphilis the *acquired* spirochetal antibody is more active against the virulent tissue spirochetes. Consequently, antigens prepared of virulent *T. pallidum* appear preferable to those prepared of nonvirulent cultures in complement fixation tests but owing to technical difficulties in their preparation and the small yield they probably cannot be employed.

THE SENSITIVITY AND SPECIFICITY OF SEROLOGIC TESTS

As previously stated, serologic tests should aim to possess the maximum of sensitivity consistent with specificity for syphilis. Ideally, a test should be sufficiently sensitive to detect every case of the disease with no falsely positive or nonspecific reactions with the serums of normal individuals, or those with diseases other than syphilis or yaws. It is highly improbable, however, that this ideal will ever be realized. Every complement fixation and flocculation test now employed can be made more sensitive by technical modifications but the margin between specific reactions due to the presence of very small amounts of syphilis reagin and falsely positive or nonspecific reactions occurring with the serums of normal individuals is so narrow that there is a limit to the sensitivity of every test in relation to its specificity. With spinal fluids, however, it is easier to increase sensitivity with less jeopardy of specificity than is true of serums.

TABLE 113. SUMMARY OF SENSITIVITY AND SPECIFICITY OF SEROLOGIC TESTS FOR SYPHILIS

All complement fixation and flocculation tests of proved value now employed possess a marked degree of sensitivity *when properly conducted* although none is sufficiently sensitive.
Different complement fixation and different flocculation tests vary considerably in sensitivity.
In general terms, the serologic tests yield positive reactions in about 70 per cent of cases of primary syphilis, in about 99.5 per cent of secondary syphilis, in over 90 per cent of untreated late syphilis, in 50 to 90 per cent of treated late syphilis, in over 90 per cent of early congenital syphilis and in a large percentage of late congenital syphilis.
Properly conducted complement fixation and flocculation tests of proved value possess a high degree of practical specificity (99 to 100 per cent) in syphilis when leprosy, malaria, infectious mononucleosis, viral pneumonia, vaccinia, etc., are excluded.
Since *all* tests usually give falsely negative reactions in the early stages of both genital and extragenital chancres, darkfield examinations for *T. pallidum* should *always* be employed for diagnostic purposes when syphilis is suspected.
Spirochetal complement fixation tests with serums yield a higher percentage of fasely positive or nonspecific reactions than Wassermann and flocculation tests. Nonspecific reactions, however, do not occur in tests employing spinal fluids. Their value in the serum diagnosis of syphilis cannot be stated at the present time.

The present serologic tests for syphilis are not too sensitive when specificity is preserved; in truth they are not sensitive enough (Table 113). There is, therefore, plenty of room for improvement but I doubt that much progress will be made in this direction until we acquire more knowledge of their mechanism, with special reference to improvements in the preparation of antigen and the optimum amounts to employ.

Nevertheless, the serologic tests of proved value now employed possess a remarkable degree of sensitivity and specificity *when properly conducted.* Doubtless improvements and newer methods of superior sensitivity and specificity will be evolved in the future. In the meantime the more urgent need is for the proper conduct of the tests of *proved value* now available. Hazen [25] states that their sensitivity is as follows:

1. In primary syphilis, 70 per cent positive. It is usually believed that positive reactions may occur between the tenth and fifteenth days.
2. In untreated secondary syphilis, positive reactions occur in over 99.5 per cent of cases.
3. In untreated late syphilis, positive reactions occur in over 90 per cent of cases.
4. In treated late syphilis, positive reactions are observed in from 50 to 90 per cent of cases, depending on the amount of treatment.
5. In congenital syphilis, positive reactions almost always occur in early cases and usually in late cases.

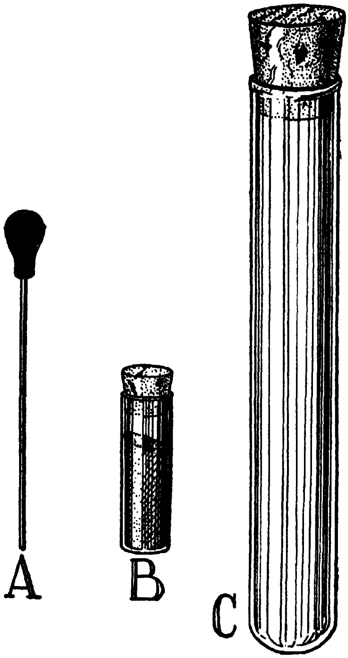

FIG. 32. OUTFIT FOR THE COLLECTION OF CHANCRE MATERIAL FOR DARKFIELD EXAMINATION FOR *T. Pallidum*

A, capillary tube with bulb for suction; *B,* vaseline for sealing capillary tube; *C,* test tube for carrying capillary tube.

It is important for physicians to remember that *in primary syphilis a minimum amount of time is required for the production of sufficient amounts of reagin for its detection by serologic tests.* Consequently, darkfield examinations for *T. pallidum* should always be made in suspected genital or extragenital chancres, as they are far and away the best means for laboratory diagnosis since positive results are observed in at least 90 per cent of cases. It is not always necessary to bring the patient to a laboratory for the examination. Exudate may be collected in a capillary tube (as free as possible of blood), sealed with vaseline and delivered or even mailed in accordance with postal regulations (Fig. 32). But as the chancre becomes older—three to five weeks—the percentage of positive results decreases while the percentage of positive serologic reactions increases. In secondary syphilis, darkfield examinations are of great value in the diagnosis of condylomas and mucous patches although not usually required since the serologic tests are likely to yield positive reactions in over 99 per cent of cases. Darkfield examinations, however, are of very limited value in the diagnosis of intra-oral lesions because it is impossible to distinguish between *T. microdentium* and *T. pallidum*. But darkfield examinations are sometimes of value in the examination of material aspirated from enlarged lymph nodes. At least five examinations on consecutive days should be made before negative results are acceptable. But if syphilis is still suspected, the patient should be subjected to follow-up serologic tests for at least four months before a lesion is definitely considered to be non-syphilitic.

Probably the sensitivity and specificity of present day complement fixation and flocculation tests can best be expressed according to the results of the 1948 National Serologic Evaluation Survey since various author serologists and state laboratories employed cardiolipin-lecithin-cholesterol antigens in complement fixation and flocculation tests. The sensitivity and specificity ratings are shown in Table 114. I have selected the results of this survey not only because it is reasonably expected that the various tests were conducted in the laboratories of author serologists with scrupulous attention to all technical details, but because this was the first survey in which cardiolipin antigens were subjected to an extensive comparison with the antigens of various author serologists.

It will be observed that the sensitivity ratings of complement fixation tests with the serums of 237 treated and untreated syphilitic donors varied from 60.1 per cent (Eagle) to 76.5 per cent (Kolmer), while the flocculation tests varied from 65.7 per cent (Hinton) to 75.0 per cent (Mazzini), employing the respective standard antigens. With cardiolipin antigens the sensitivity rating of the Kolmer complement fixation test was 78.6 per cent, while varying from 69.4 (V.D.R.L.) to 76.6 per cent (Kline diagnostic) in four flocculation tests. It will be observed, therefore, that cardiolipin antigens gave 2.1 per cent more positive reactions in the Kolmer complement fixation test, 7.8 per cent more in the Hinton test, 4.0 per cent more in the Kahn standard and 10.2 per cent more in the Kline diagnostic tests. While these comparative results do not justify a final decision on the comparative sensitivity of standard and cardiolipin antigens in complement fixation and flocculation tests, they probably indicate that the latter are somewhat more sensitive, especially in flocculation tests.

As previously stated, one advantage of cardiolipin antigen is the fact that it is chemically reproducible. But while the cardiolipin antigens were supplied the various author serologists and state laboratories by a central laboratory, it will be observed in Table 114 that the variations in sensitivity ratings in state laboratories employing the Kahn, Kline and Kolmer tests were practically just as great with cardiolipin antigens as observed in tests employing standard antigens. In other words, it appears that the chemical reproducibility of cardiolipin antigen has not materially increased the uniformity of positive and doubtful reactions in various laboratories testing the same syphilitic serums.

Under the circumstances, it is quite apparent that there is no one satisfactory serologic test for syphilis as far as sensitivity is concerned although all of those shown in Table 114 are satisfactory from the standpoint of practical specificity in syphilis. In every stage of the disease, with or without treatment, a complement fixation test may give a true positive and a flocculation test a false negative reaction, or vice versa. In general terms, however, flocculation tests are likely to yield more positive and more persistently positive reactions in late cases of the disease under treatment than complement fixation tests. Similar results have been shown in previous serologic surveys so that it is quite definitely proved that the serum diagnosis of syphilis is best served by using two or three tests routinely whenever conditions permit, as shortly to be discussed in more detail. In my opinion at least one of them should be a complement fixation test of acceptable sensitivity.

TABLE 114

Tests	Antigens	Control Laboratories *		State Laboratories	
		Sensitivity †	Specificity ‡	Sensitivity †	Specificity ‡
Eagle complement fixation	Eagle	60.1	100.0	50.0–67.9	99.7–100.0
Eagle flocculation	Eagle	69.7	100.0	65.7–69.4	99.7–100.0
Hinton flocculation	Cardiolipin	73.5	100.0	—	—
Hinton flocculation	Hinton	65.7	100.0	42.9–78.4	95.7–100.0
Kahn standard	Cardiolipin	70.2	100.0	49.0–61.7	100.0
Kahn standard	Kahn	66.2	100.0	54.1–71.5	98.2–100.0
Kline diagnostic	Cardiolipin	76.6	100.0	71.2–85.3	90.0–100.0
Kline diagnostic	Kline	66.4	100.0	59.6–79.1	97.1–100.0
Kolmer simplified	Cardiolipin	78.6	100.0	73.3–85.6	98.0–100.0
Kolmer simplified	Kolmer	74.4	100.0	58.3–77.9	99.7–100.0
Kolmer quantitative	Kolmer	76.5	100.0		—
Mazzini	Mazzini	75.0	99.3	69.3–85.8	97.9–100.0
V.D.R.L.	Cardiolipin	69.4	100.0	68.8–81.4	96.4–100.0

* Tests conducted in the laboratories of the respective author serologists.
† Based on tests with the serums of 237 treated and untreated syphilitic donors.
‡ Based on tests with the serums of 141 presumably nonsyphilitic donors.

As shown in Table 114, the specificity of complement fixation and flocculation tests with the serums of normal presumably nonsyphilitic donors in the laboratories of the various serologists was practically 100 per cent, while varying from 95.7 to 100.0 per cent in state laboratories. Since the first National Serologic Evaluation Survey in 1935, however, specificity ratings have progressively improved. Thus, in the surveys from 1935 to 1943, including the Washington serologic conference of 1941, the specificity ratings of two complement fixation and eight flocculation tests in the laboratories of author serologists varied from 98.1 to 100 per cent, while in state laboratories the ratings of complement fixation tests varied from 90 to 100 per cent and of flocculation tests from 60 to 100 per cent.[26] Furthermore, cardiolipin antigens in the 1948 survey had specificity ratings of 100 per cent in the laboratories of author serologists but varied from 96.4 to 100 per cent in state laboratories.

Spirochetal Complement Fixation Tests. An analysis [16] of numerous reports on complement fixation tests conducted with the commercial antigen (palligen) prepared of cultures of the Reiter strain of alleged *T. pallidum* has shown 44.5 to 100 per cent positive reactions in 13,636 tests with the serums of syphilitic individuals. The incidence of positive Wassermann reactions varied from 30.4 to 100 per cent, although its sensitivity is difficult to express because of variations in the technic employed by different investigators. Specificity in tests with the serums of 36,255 presumably nonsyphilitic donors varied from 96.6 to 100 per cent in the spirochetal tests and from 97.3 to 100 per cent in the Wassermann tests.

Fewer reports have been made on complement fixation tests conducted with spinal fluids and "palligen" antigen. The incidence of positive reactions with those of syphilitic patients has been stated to vary from 84 to 100 per cent with 82 to 100 per cent positive Wassermann reactions.[16] Specificity, however, has been reported as being 100 per cent in the case of both the spirochetal and Wassermann tests conducted with the spinal fluids of nonsyphilitic donors.

In the Washington survey of October 1941 both Dr. Eagle and I also employed antigens prepared of the Reiter strain in our complement fixation tests. With the serums of all syphilitic patients sensitivity varied from 70.6 per cent (Kolmer) to 75.9 per cent (Eagle) while sensitivity in tests conducted with lipoidal antigens varied from 59.2 per cent (Eagle) to 74.1 per cent (Kolmer). In other words, the Eagle spirochetal complement fixation reaction was much more sensitive than the Eagle Wassermann reaction whereas the Kolmer spirochetal reactions were slightly less sensitive than the Kolmer Wassermann reaction.

With the serums of all normal individuals and nonsyphilitic patients, excluding those with leprosy and malaria, specificity varied from 93.7 per cent (Kolmer) to 98.1 per cent (Eagle). In other words, the Kolmer spirochetal complement fixation test yielded 6.3 per cent and the Eagle test 1.9 per cent falsely positive or nonspecific reactions. The percentage of Kolmer nonspecific reactions, however, was much lower than previously reported [24] due to the fact that the antigen was used in a smaller dose in order to reduce their incidence to a minimum.

Of further interest is an analysis of the results reported in the Oct. 1941 survey in relation to the stage of syphilis. In early syphilis (primary or secondary) with no treatment the Eagle spirochetal test showed 81.1 per cent and the Eagle Wassermann test 83 per cent sensitivity. The Kolmer spirochetal test showed 81.2 per cent and the Kolmer Wassermann test 91.1 per cent sensitivity. According to these results it would appear, therefore, that the spirochetal complement fixation test is less sensitive than the Wassermann test in early syphilis.

In syphilis of less than 4 years' duration, with varying amounts of treatment, the Eagle spirochetal test showed 59.2 per cent and the Eagle Wassermann test

46.3 per cent sensitivity. The Kolmer spirochetal test showed 55.0 per cent and the Kolmer Wassermann test 59.7 per cent sensitivity. In syphilis of over 4 years' duration, with or without treatment, the Eagle spirochetal test showed 84.1 per cent and the Eagle Wassermann test 61.6 per cent sensitivity. The Kolmer spirochetal test showed 77.2 per cent and the Kolmer Wassermann test 78.6 per cent sensitivity. According to these results it would appear, therefore, that the Eagle spirochetal test was more sensitive than the Eagle Wassermann test in both groups but that the Kolmer spirochetal test was slightly less sensitive than the Kolmer Wassermann test in both.

In tests conducted with the spinal fluids of patients with syphilis of the central nervous system, treated or untreated, the Eagle spirochetal test showed 82.7 per cent and the Eagle Wassermann test 85.6 per cent sensitivity. The Kolmer spirochetal test showed 83.1 per cent and the Kolmer Wassermann test 73.3 per cent sensitivity. No falsely positive or nonspecific reactions were observed with the spinal fluids of nonsyphilitic donors.

Under the conditions, no conclusions are as yet permissible on the comparative value of spirochetal complement fixation and Wassermann tests in the serum diagnosis of syphilis except the fact that the former are much more likely to yield falsely positive or nonspecific reactions with serums due, I believe, to the presence of natural spirochetal antibody in human serums, as previously discussed. In my opinion, spirochetal complement fixation tests as now conducted will probably not prove of material value in the serum diagnosis of syphilis unless it is shown that they possess special value in relation to treatment or some particular stage of the disease. They likewise show a high incidence of positive reactions in leprosy and malaria and consequently do not serve to distinguish between specific and biologically nonspecific reactions.

THE INTERPRETATION OF SEROLOGIC REACTIONS

The serologic tests for syphilis are no longer limited to individuals suspected of having the disease but are now being applied to increasing thousands and thousands of individuals in all walks of life. This is not only due to their more frequent use by physicians and the fact that many states legally require premarital tests and tests during pregnancy, but also to their wider and wider use in the industries as well as in the armed forces.

Under the circumstances, it has become increasingly important for physicians to have a good working knowledge of the clinical interpretation of serologic reactions. Unfortunately, many either depend too much upon serologic tests or do not depend upon them enough. As will be discussed later, negative reactions do not necessarily exclude syphilis nor do positive reactions necessarily prove its presence. Falsely negative or falsely positive reactions may not mislead expert syphilologists but the situation is apt to be quite otherwise in the case of those physicians possessing an inadequate knowledge of syphilis and of the interpretation of serologic reports. Even expert syphilologists have learned not to depend too much upon their clinical skill and judgment and especially since chronic latent or asymptomatic syphilis, so prevalent among both men and women in apparent good health and at a time of golden therapeutic opportunity, is detectable only by the serologic tests. Unfortunately, far too many physicians still place too much reliance upon a negative history for excluding the possible presence of syphilis, while others need to be reminded that the respectability of their patients is no bar to the penetrability of *T. pallidum* (Table 115).

Qualitative and Quantitative Serologic Tests. The amount of reagin in the serums and spinal fluids of syphilitic individuals varies greatly and especially in relation to the stage of the disease. It is usually influenced by the kind and amount of treatment given although apparently not influenced by race, sex, age, or season of the year.[27] Likewise, pregnancy does not appear to have an influence although it is well known that this state increases immunologic resistance to the disease. Furthermore, the reagin may fluctuate from day to day or within brief periods in untreated cases of syphilis although this is difficult to determine because of fluctuations in the sensitivity of the same test in the same laboratory at different times.

Tests conducted with single doses of serum or spinal fluid are only rough measures of the reagin content on the basis of ++++, +++, ++ or + reactions; for this reason I prefer to designate them as *qualitative tests*.[28] In general terms they suffice for diagnostic purposes although it is obvious that a ++++ reaction is more definite serologic evidence of syphilis than a + reaction.

Quantitative tests, however, may be conducted by either of two methods, namely, (1) using varying amounts of serum or spinal fluid in complement fixation or flocculation tests or (2) using a single dose with varying amounts of complement in complement fixation tests.[29,30,31]

The question arises as to whether or not quantitative tests possess any particular clinical value. Apparently the amount of reagin present in serum is in relation to the degree of infection but this is not necessarily in relation to the clinical status of the patient.[9] In other words, the signs and symptoms of syphilis are more in relation to the physiologic importance of the organs or tissues infected than to the numbers of *T. pallidum* present. For example, so few spirochetes may infect the posterior nerve roots and posterior columns of the spinal cord that a patient with advanced tabes dorsalis may give only doubtful or weakly positive or even falsely negative reactions, whereas another individual with a heavy infection of the skeletal system and giving strongly positive reactions may be in apparently good health.

In general terms, however, quantitative tests possess certain advantages. For example, qualitative tests may not reveal any serologic evidences of improvement due to treatment until the reagin-content is reduced below the ++++ level, whereas both physician and patient may be and usually are greatly encouraged by noting a progressive reduction in reagin in quantitative tests.[32] Furthermore, the latter usually afford better evidence than qualitative tests of seroresistance or "Wassermann-fastness" as well as impending or actual relapse under treatment. Quantitative tests are also of distinct value in the diagnosis of questionable cases lacking a history or clinical evidence of syphilis, since serums showing the presence of large amounts of reagin possess more diagnostic value than those showing small or doubtful amounts. Qualitative tests may also give falsely negative reactions due to the prezone phenomenon. However, one disadvantage of quantitative complement fixation tests is their expense although this has been so greatly reduced by my one-fifth method [33] that 5 cc. of complement is ordinarily sufficient for the conduct of 100 tests, 1 cc. of antigen for 1200 tests and 2 cc. of a 50 per cent dilution of antisheep hemolysin in glycerol for about 4000 tests.

As previously stated, however, qualitative tests are sufficient for diagnostic purposes as far as positive, doubtful or negative reactions are concerned since quantitative tests have but little advantage in this respect.[34]

TABLE 115. SUMMARY OF THE INTERPRETATION OF SEROLOGIC REACTIONS IN SYPHILIS

Subject	Interpretation
General Considerations	The constantly increasing use of serologic tests for the detection of syphilis renders necessary and advisable a proper understanding of the clinical interpretation of serologic reactions. Many physicians either depend too much on the serologic tests or do not depend on them enough. A negative history alone never reliably excludes the possibility of syphilis and respectability is no bar to the penetrability of *T. pallidum.*
Qualitative and Quantitative Tests	Apparently the amount of reagin present in serum is in relation to the degree of infection but the signs and symptoms of syphilis are more in relation to the physiologic importance of the organs or tissues infected. *Qualitative tests* are conducted with single amounts of serum or spinal fluid. They are usually sufficient for diagnostic purposes. *Quantitative tests* are more valuable for (1) the detection of serologic improvement in relation to treatment; (2) for the detection of sero-resistance or "Wassermann-fastness" and impending relapse and (3) in the diagnosis of questionable cases of syphilis.
Multiple Tests	Multiple serologic tests are always advisable when conditions permit since there is no one best test. One good test properly conducted, however, is better than two or more tests poorly conducted. A sensitive "screen test" may be employed but positive reactions should always be checked by one or more additional methods before the results are reported. Multiple tests, however, may yield discordant reactions and especially if serums or spinal fluids contain but small amounts of reagin. The same is true when portions of the same blood are sent to different laboratories or to the same laboratory under different names as "split specimens."
Serologic Reports	Serologic reactions may be reported as strongly positive, moderately positive, weakly positive, doubtful or negative. The U. S. Public Health Service recommends reporting them as positive, doubtful or negative. This is distinctly advantageous from the laboratory standpoint and especially in relation to overall reports when multiple tests are conducted with a separate report on each test. From the clinical standpoint, however, the author believes that reactions should be reported as strongly positive, weakly positive, doubtful or negative.

TABLE 115. SUMMARY OF THE INTERPRETATION OF SEROLOGIC REACTIONS IN SYPHILIS—(Continued)

Subject	Interpretation
Falsely Positive Reactions	Falsely positive reactions are divisible into two kinds: (1) *technical* and (2) *biologic* or those occurring with the serums of normal individuals and in diseases other than syphilis. The incidence of falsely positive reactions due to technic and with the serums of normal persons should not be over 1 per cent and preferably not over 0.1 per cent. They are less likely to occur in complement fixation than in flocculation tests. Biologic falsely positive reactions occur in yaws, pinta and less frequently in other spirochetal diseases and trypanosomiasis; also in leprosy, malaria, infectious mononucleosis, vaccinia, viral pneumonia and other conditions. Serologic tests conducted with cardiolipin antigen may give a lower incidence, especially in malaria. A final decision on whether or not a reaction is falsely positive should be made by the clinician; special examinations may be required.
True Positive Reactions	When the possibility of biologic falsely positive reactions can be excluded positive reactions by properly conducted tests of proved value are almost invariably indicative of syphilis. The incidence of true positive reactions in the different stages of syphilis varies according to the sensitivity of the tests employed. Positive reactions with the serums of *cord* bloods do not necessarily indicate syphilis of the infant. Unexpected positive reactions should always be rechecked before the patient is informed or treatment instituted in order to guard against falsely positive reactions. On the other hand, they should never be disregarded as they may be the only evidence of syphilis.
Doubtful Reactions	Doubtful reactions should not be ignored as they frequently indicate the presence of syphilis. The tests should be repeated along with clinical examinations for the purpose of reaching a final decision.
Negative Reactions	Since none of the serologic tests is sufficiently sensitive to detect all cases of syphilis, *falsely negative reactions* may occur in all stages of acquired syphilis with the possible exception of the secondary stage. The same is especially true in late or latent congenital syphilis. Consequently, negative reactions and even repeatedly negative reactions should not be permitted to over-ride clinical judgment.
Provocative Reactions	Sometimes of value in the diagnosis of untreated syphilis presenting suspicious lesions with negative serologic reactions; also in cases presenting no lesions with weakly positive serologic reactions. Of no value as a criterion of cure.
Serologic Tests in Relation to Treatment	The treatment of both seropositive and seronegative cases of primary syphilis should be thorough and adequate regardless of the results of serologic tests. After the cessation of treatment of early syphilis repeatedly negative serologic reactions over a period of two or more years are among the criteria of cure. At least one examination of the spinal fluid is also required. Persistently positive reactions in early and late syphilis in spite of treatment are indicative of persistent infection (seroresistance or "Wassermann-fastness").

TABLE 115. SUMMARY OF THE INTERPRETATION OF SEROLOGIC REACTIONS IN SYPHILIS—(Continued)

Subject	Interpretation
Serologic Relapse	Indicative of renewed activity of persistent foci of syphilitic infection. Usually indicates the need for treatment to prevent clinical relapse or the progression of the disease. It is possible that foci of infection may be activated by intercurrent diseases. It is also possible that positive reactions after a period of negativity may not be due to serologic relapse but to true reinfection or superinfection with *T. pallidum*.
Serologic Resistance or "Wassermann-fastness"	Refers to persistently positive reactions after six months of continuous treatment in early syphilis or after twelve months in late syphilis. In intimate relationship to the sensitivity of the tests employed. Cause unknown but apparently due to reagin produced in persistent foci of infection or to natural drug-resistance of *T. pallidum*. In early syphilis frequently due to inadequate treatment with infection of the central nervous system. Of less significance in late syphilis. Treatment required for the primary purpose of preserving health and longevity rather than merely for the purpose of securing serologic negativity. Advisable to explain matters carefully to the patient. Marriage safe for spouse and children after adequate treatment has been given.
Spinal Fluid Examinations	Complement fixation tests by quantitative methods are preferred. Positive reactions are usually indicative of invasion or infection of the central nervous system with *T. pallidum*. They do not, however, indicate the clinical type of neurosyphilis. Negative reactions do not exclude the possibility of neurosyphilis. Spinal fluid examinations are generally indicated in late syphilis. No case of syphilis can be regarded as cured without at least one or more examinations with negative complement fixation or flocculation and colloidal gold reactions along with normal total cell counts and protein determinations.

Multiple Serologic Tests. As previously stated, the results of all serologic surveys have shown that it is advisable for laboratories to conduct two or more different tests with each serum, whenever conditions permit, since there is no one best test. In other words, any one approved procedure may give a falsely negative reaction while another equally good method may give a true positive reaction; this is especially likely to occur in the case of serums containing small amounts of reagin. For over twenty years I have used routinely a complement fixation, a macroscopic and microscopic flocculation test in the examination of serums, since the marked differences in their technic and mechanism have appeared to give the best possible results in relation to sensitivity and specificity. In the examination of spinal fluids, however, only a complement fixation test has been employed. However, conducting complement fixation tests with two or three different antigens of varying sensitivity is now obsolete and should be abandoned as this does not constitute multiple tests in the meaning with which the term is here employed.

Needless to state, positive reactions with two or three tests of proved value constitute more conclusive serologic evidence of syphilis than a positive reaction with any single test and, of course, the same is true of negative reactions. However, even several tests may give falsely positive or falsely negative reactions so that safety does not necessarily lie in a multiplicity of tests because the possibility of technical errors is inherent in any and all methods. Certainly one good test, well conducted, is better than two or three tests poorly conducted. In this connection it is also to be stated that sometimes a test of low sensitivity will give a positive reaction when one of high sensitivity gives a falsely negative reaction.

In state or other laboratories required daily to conduct very large numbers of tests, this ideal serologic service may be and usually is impossible. Under these conditions, it is permissible and advisable to test all serums by a microscopic or macroscopic flocculation test of proved value as a "screen test." Negative reactions may be reported but positively reacting serums should *always be retested* by a complement fixation or another flocculation test before reporting the results. Undoubtedly a small percentage of cases of syphilis escape serologic detection in "screen tests" but the "tailor must make the suit according to the cloth." If, however, a positive "screen" reaction is not confirmed by additional tests it is usually inadvisable to report it to the physician. This is especially true if the "screen test" has been conducted by a supersensitive flocculation method.

The main advantage of using multiple tests, therefore, is the reduction to a minimum of the incidence of falsely positive and falsely negative reactions. But, unfortunately, only a relatively small percentage of physicians are qualified to interpret conflicting reports as evidence for or against the presence of syphilis. Indeed, as more tests are devised and older ones modified, the busy practitioner may find himself more and more confused. The same applies to conflicting reports received by him on "split specimens" in which parts of the same specimen of blood have been sent to two or three different laboratories or to the same laboratory under different names; likewise, when any laboratory reports a positive reaction on a patient at one time and a negative reaction a few days later on a second specimen of blood collected for the purpose of checking the results. Under these circumstances, there are three possibilities: (1) that the positive reactions were due to laboratory error; (2) that the patient is actually syphilitic but the serum contained such a small amount of reagin that it was detected only by a method of superior sensitivity, or (3) that the positive results were due to biologic nonspecific reactions which, in normal individuals, fortunately are but rarely observed.

Needless to state, it is necessary for the laboratory to report only what it has found, but if multiple tests have been used and conflicting reactions observed, it is advisable to combine the results in an overall report as positive, doubtful or negative in order to assist the physician as much as possible in interpretation and with the least chance of friction or tendency to undermine his confidence in the laboratory and the serologic tests for syphilis. Otherwise, however, interpretation is strictly up to the physician who alone must make a decision on the presence or absence of syphilis based upon a careful history and physical examination of the patient, supplemented by a repetition of the tests not once but several times, if necessary, in the same or another laboratory. When three tests are conducted the reactions may be interpreted as follows as an overall report:

1st Test	*2nd Test*	*3rd Test*	*Interpretation*
Positive	Positive	Positive	Positive
Positive	Doubtful	Positive	Positive
Positive	Negative	Positive	Positive
Negative	Negative	Positive	Doubtful
Doubtful	Doubtful	Doubtful	Doubtful
Negative	Doubtful	Doubtful	Doubtful
Negative	Negative	Negative	Negative
Negative	Doubtful	Negative	Negative

Serologic Reports. For over thirty-five years Wassermann reactions have been reported to physicians as strongly positive, moderately positive, weakly positive, doubtful or negative; later the same terminology was adopted in relation to the flocculation tests so that the terms have become widely known in the serology of syphilis. The Committee on the Evaluation of Serodiagnostic Tests for Syphilis, however, has advocated reporting reactions only as positive, doubtful or negative as being sufficient for all practical purposes. This simplifies matters for the laboratory and especially when rendering an overall report on the reactions observed with multiple tests although the reactions observed with each test should be reported to the physician at the same time.

For clinical reasons, however, I believe that it is advisable to divide positive reactions into "strongly positive" and "weakly positive."[35] For example, the great majority of serum tests for syphilis are conducted for physicians who have not had the opportunity to acquire special knowledge and skill in the detection of the disease. Under the conditions, they must depend on serologic diagnosis to a far greater extent than is true of expert syphilologists. Given a patient with chronic latent syphilis and especially one with a negative history and in good general health, a serologic report of "positive" may be discarded as being of no significance. But if the report is "strongly positive," as is frequently the case, such disposal of it is not likely to occur. It may be stated, however, that a report of "weakly positive" may likewise be discarded under these circumstances but hardly more so than one reported merely as "positive." Or, if syphilis is suspected clinically and the serum reactions are reported as "strongly positive" or even "weakly positive," it has been my experience that more practicing physicians will sit up and take notice than when receiving a report of only "positive." Furthermore, a patient starting off before treatment with a "strongly positive" reaction who later shows a "weakly positive" reaction, gives the physician at least some evidence of serologic improvement under treatment, whereas a mere "positive" report under such circumstances leaves him and the patient without this encouragement.

Falsely Positive Reactions Due to Technical Errors. Positive reactions do not necessarily constitute serologic evidences of syphilis because falsely positive reactions may occur. The latter are divisible into two kinds: (1) those due to *technical errors* and therefore avoidable, and (2) *biologic* falsely positive reactions which may be unavoidable. Because of their importance they may be discussed before true positive reactions are considered in relation to the serum diagnosis of syphilis.

Falsely positive reactions due to errors in technic may occur with any test and in any laboratory. Needless to state, however, their incidence is in relation to the skill and experience of those who conduct them. In general, they occur more frequently in flocculation tests, especially with supersensitive methods, than in complement fixation tests. It is significant, however, that in the various serologic surveys their incidence in the laboratories of author serologists has been less than 1 or 2 per cent and, indeed, usually have not occurred at all. For example, in the Washington serologic survey of Oct. 1941 four of the complement fixation tests commonly employed showed no falsely positive reactions with the serums of normal individuals without intercurrent illnesses or other pathologic conditions; the same was true of five of the flocculation tests although three of them showed 0.3 to 0.6 per cent nonspecific reactions. This shows what can be done with approved tests when they are properly conducted. However, since 1 per cent falsely positive reactions means one in a hundred, it is obvious that all serologists and technologists should aim to keep their tests as near as possible to 100 per cent specificity with the serums of normal persons. Indeed, the incidence of falsely positive reactions should not exceed 0.1 per cent since this alone means the possibility of one in every 1000 normal individuals.

Biologic Falsely Positive Reactions in Normal Individuals. A more important problem is in relation to those biologic nonspecific or falsely positive reactions that may occur with the serums of normal individuals in any laboratory regardless of the care and skill with which the tests are conducted.

As shown by several investigators [36-39] the serums of normal individuals may contain reagin or reagin-like substances capable of giving falsely positive flocculation reactions although the incidence among individuals in whom latent syphilis could be excluded with reasonable accuracy has been low, varying from about 1 in 4000 [40] to 1 in 1017.[37] But even though of exceptional occurrence they are, nevertheless, very disturbing. According to Barnard and his colleagues,[41] multiple bleedings (donation) tend slightly to increase the incidence of these biologic falsely positive reactions, while Boynton [42] has reported that the incidence tends to diminish under these circumstances. Since complement fixation tests are somewhat less sensitive and easier to read, they are much less likely to yield these reactions.[43]

Naturally, attempts have been made to eliminate these biologic falsely positive reactions in flocculation tests. For example, Kahn [44] has observed that they are especially likely to occur with normal human serums and those of the lower animals in tests conducted at 0° C. while true positive reactions with syphilitic serums were found to occur especially in tests conducted at 37° C. On this basis he has described a "verification test" based on the assumption that any serum showing a positive Kahn reaction at 0° C. as well as 37° C., may be suspected as giving biologic falsely positive reactions. Unfortunately, however, the serums of syphilitic individuals may also give positive Kahn reactions at 0° C. Under the circumstances, it does not appear that the "verification test" has solved the problem[45] while the status of the "differential method" of Rytz[46] requires further investigation.

In 1925 I called attention to the possibility of alterations in the serum globulins or other protein fractions being responsible for anticomplementary and nonspecific or falsely positive complement fixation reactions.[47] Cardon and his colleagues[48] subsequently reported that hyperproteinemia and hyperglobulinemia may be responsible for nonspecific or falsely positive complement fixation and flocculation reactions, with the suggestion that blood protein studies be instituted when they are suspected. Recently Neurath and his colleagues[49] have found that the *euglobulin* fraction of syphilitic serums, isolated by isoelectric precipitation, comprises about 13 per cent of the *gamma* globulins and contains 50 per cent or more of the total reagins or reactive antibodies. From another protein fraction of human serum a lipo-protein substance associated with the *alpha* globulins has been isolated which apparently inhibits or prevents falsely positive reactions in flocculation tests. The serologically active euglobulin fraction is subjected to quantitative flocculation titrations and another aliquot of this fraction then is titrated in the presence of specified amounts of the inhibitor. A true or syphilitic type of reaction is said to occur when the inhibitor fails to suppress the serologic activity of the euglobulin fraction; whereas, complete inhibition in the presence of the inhibitor is characteristic of a biologic falsely positive reaction. Since a final evaluation of the practical value of this "euglobulin-inhibition" test must await further investigation, the method has not yet been recommended for routine or practical use. Biologic falsely positive reactions, however, are much less likely to occur with the spinal fluids of normal individuals.

Biologic Falsely Positive Reactions in Disease and Other States. A review of the early literature on the Wassermann test shows that falsely positive reactions have been reported by some one at some time in practically all of the diseases of mankind which has created an erroneous impression still persisting in the minds of many physicians. This is very unfortunate because it is now certain that many of these reports were based on technical errors in the conduct of the tests.

It is now well known, however, that positive complement fixation and flocculation reactions occur in a high percentage of cases of *yaws, pinta* and *bejel.* This is to be expected since these diseases are caused by spirochetes so closely related to *T. pallidum* that Rein[50] has designated them "syphiloid reactions." Positive reactions may also occur in the *relapsing fevers, rat-bite fever* due to *S. minus* and the leptospiroses with special reference to *infectious jaundice,*[50] but the incidence is quite variable. They have also been reported in Vincent's angina[51] but the evidence is not conclusive.

In *leprosy* of all types the incidence of falsely positive complement fixation and flocculation reactions has varied from 16.3 to as high as 72 per cent,[43,52] including tests conducted with cardiolipin antigen. Likewise in natural and inoculation *malaria* the incidence has varied from 11.1 to 19.4 per cent[43] to as high as 80 per cent,[50] while Kitchen and his colleagues[53] have reported that serially repeated tests have shown as high as 90 to 100 per cent positive reactions at some time in the course of this disease. In both complement fixation and flocculation tests, however, cardiolipin antigen has reduced the incidence of these biologic falsely positive reactions. Thus, Kent and his associates [54] have reported that Kahn tests gave the highest and Kolmer tests the lowest incidence of positive reactions in sporozoite-induced vivax malaria, while cardiolipin antigen in a microflocculation test reduced the incidence of falsely positive reactions and practically eliminated them in the Kolmer complement fixation test.

A variable incidence of positive reactions has also been observed in *vaccinia* and *vaccinoid, infectious mononucleosis, viral pneumonia* including *viral pharyngitis* and *bronchitis* and other *upper respiratory tract* infections. In vaccinia the incidence has varied from 0.06 to 0.26 per cent to as high as 52.2 per cent and as high as 34.7 per cent in vaccinoid,[50] with the probability that the incidence is higher in flocculation than in complement fixation tests, but probably somewhat lower in tests conducted with cardiolipin antigen. In the majority of instances these reactions were observed during the second week following smallpox vaccination and have usually disappeared within three to four months. The incidence in infectious mononucleosis has varied from 0 to 18 per cent or higher,[55] depending on whether single or multiple tests were conducted during the course of the disease, being somewhat higher in flocculation than in complement fixation tests and probably somewhat lower in both tests conducted with cardiolipin antigen. The reactions, however, are weakly positive and usually disappear within two to twelve weeks following recovery. The incidence in viral and other infections of the upper respiratory tract and in viral pneumonia with both ordinary and cardiolipin antigens is so variable that it cannot be stated; in these the reactions are also weakly positive and usually disappear within three months following recovery.

Positive reactions are also stated to occur in *trypanosomiasis* and especially in Chagas' disease, but the incidence is unknown. The same is true of *lupus erythematosus*,[56] epidemic and murine *typhus fever* [50] and *subacute bacterial endocarditis*. It is now known, however, that normal *pregnancy* does not give falsely positive reactions when tests of proved value are properly conducted.[57] Nevertheless, an impression to the contrary still prevails and will not down. Positive reactions and especially repeatedly positive reactions are indicative of syphilis in this state. Likewise *menstruation* does not produce falsely positive reactions. The same is true of *jaundice* [57] although serums deeply tinged with bilirubin may become anticomplementary or otherwise unsatisfactory with the chances of yielding nonspecific complement fixation and flocculation reactions unless examined with proper technical care. Furthermore, it does not appear that *malignant disease* is a cause for falsely positive reactions. In serologic surveys the incidence of positive reactions with some of the flocculation tests has varied from 1.6 to 3.3 per cent [43] but the majority of the serologic tests employed have not given any falsely positive reactions at all.

It may be, however, that falsely positive reactions may occur when serums are tested during *febrile states*. In the first serologic survey in 1935 some tests yielded about 2 per cent positive reactions with serums of donors with natural or induced fever [57] although in the Washington survey of Oct. 1941 the majority of tests showed no nonspecific reactions at all with the serums of individuals with intercurrent febrile and afebrile illnesses while some gave an incidence of only 0.5 to 1.6 per cent. Apparently, however, *tuberculosis* is not as frequent a cause of falsely positive reactions as hitherto suspected. In the Washington survey the majority of tests showed none at all while others gave an incidence no higher than 0.8 per cent. Positive reactions have also been reported in the *hemolytic anemias* [58] and positive Kahn reactions in *viral hepatitis* [59]—likewise in rheumatic fever, brucellosis, leishmaniasis, filariasis, leukemia, pellagra, psoriasis, myocardial infarction, diabetes mellitus, lead poisoning, acute alcoholism, ether anesthesia, sulfonamide and serum therapy and after the prophylactic administration of typhoid and other vaccines,[60] but the evidence is not conclusive. Positive reactions in chancroid and lymphogranuloma venereum are always suspicious of coincident syphilis, especially if repeatedly positive reactions are observed. Apparently, penicillin therapy does not produce falsely positive reactions, since Turner [61] has reported negative Kahn and Kolmer reactions with normal serums to which this compound was added in vitro.

Management of Falsely Positive Reactions. Needless to state, the interpretation of falsely positive reactions with the serums of healthy and presumably nonsyphilitic individuals and those with diseases other than leprosy, malaria, infectious mononucleosis and vaccinia, is a problem of the first magnitude in practice. As previously mentioned, the Kahn verification test has not solved the situation, although it is worth conducting when falsely positive reactions are believed to have occurred. It is essential for the physician to recognize, however, that positive reports based upon supersensitive flocculation procedures as "exclusion" or "presumptive" tests, may not constitute a diagnostic problem in a patient who presents a negative history with no clinical evidences of syphilis; indeed, as previously mentioned, they should not be reported. With these excluded, however, there still remain individuals who consistently give conflicting serologic reactions and require very careful examinations before a decision may be reached on the presence or absence of syphilis.

According to Mohr, Moore and Eagle,[38] the physician is justified in suspecting positive reactions as being false or nonspecific when occurring in virgin females with no history of what may have been an extragenital chancre as well as, sometimes, in the case of males who deny sexual exposure; also in patients thought by the physician to be truthful, and who in the past have had negative reactions and deny sexual exposure; likewise in married individuals who, since marriage, have had one or more negative reactions preceding the positive one and whose marital partners are found seronegative. Otherwise and usually, however, the physician should base his conclusion on the results of an unusually thorough physical examination supplemented by blood tests for malaria and infectious mononucleosis, along with repeated serologic tests in different laboratories and by different procedures, including quantitative tests over a period of at least three to six months. If negative reactions occur syphilis is usually to be excluded. If persistently positive reactions are observed, however, I believe that it is advisable to regard chronic asymptomatic syphilis as present and to institute treatment for this disease. Spinal fluid examinations are also helpful in the sense that positive reactions would be indicative of the presence of syphilis; negative reactions, however, would not exclude this possibility. Finally, examinations of the family and contacts may be required.

True Positive Reactions. When diseases giving biologic nonspecific or falsely positive reactions are excluded, positive serologic reactions by properly conducted tests of proved value are almost invariably indicative of syphilis, since the incidence of falsely positive reactions due to technical errors or the presence of normal reagin is 1 per cent or less under these conditions.

Of course, the incidence of true positive reactions in the different stages of syphilis varies according to the sensitivity of the test or tests employed, as previously discussed. In the 1942 interstate serologic survey conducted by the Committee on the Evaluation of the Serodiagnostic Tests for Syphilis, the incidence of positive reactions in complement fixation and flocculation tests reported by author-serologists in primary syphilis varied from 70 to 74 per cent; in untreated secondary syphilis the incidence was 100 per cent; in early latent syphilis 84 to 90 per cent; in late latent syphilis 83 to 90 per cent; in cardiovascular syphilis 82 to 91 per cent; in neurosyphilis 92 to 95 per cent and in congenital syphilis 90 to 100 per cent. It is important to remember, however, that positive reactions with the serums of *cord bloods* do not necessarily indicate the presence of congenital syphilis, as these may be due to the passive placental transfer of reagin from a syphilitic mother. *It is much better to conduct the test two weeks after birth.* Even then, a positive reaction is not conclusive. Repeatedly positive reactions at subsequent intervals are required for establishing serologic diagnosis.

The greatest difficulty is experienced with the interpretation of *unexpectedly positive reactions* and especially those that are weakly positive or doubtful. Likewise and particularly when one test gives a positive and another a negative reaction, or when the reactions fluctuate from positive to negative, or the reverse, due to the presence of but small amounts of reagin in the blood and therefore highly influenced by the sensitivity of the test or tests employed. If the patient is known to have syphilis, the results are usually to be regarded as positive when avoidable errors can be excluded with reasonable certainty. In this connection it should be mentioned that when serums giving weakly positive flocculation reactions are re-tested within twenty-four hours they may give negative reactions presumably due to a loss of the reagin.[62]

However, if syphilis is not suspected clinically the physician should never jump to the conclusion that the disease is present until the tests have been repeated one or more times with similar results. The harm done by a mistaken diagnosis of syphilis based on a false positive reaction outweighs any number of false negative reactions. To inform the patient may do irreparable harm, as the "syphilitic scars of the spirit" are more difficult to cure than the disease itself.

But to discard a positive reaction simply because it was unexpected is likewise very reprehensible because of the chances of its being truly positive in cases of chronic latent syphilis where it may be the only possible evidence of syphilis at a time when the institution of proper treatment may prevent, or at least delay, the onset of obvious tertiary lesions. In other words, ***any physician who elects to discard an unexpectedly positive report without checking by one or more additional tests is assuming more responsibility than conditions permit or sanction.***

Doubtful Reactions. Doubtful reactions imply that the results of the test or tests, while suggestive, were not sufficiently clear-cut to justify a positive report. When occurring with properly conducted tests of proved value they should never be ignored, as they frequently indicate the presence of syphilis with small amounts of reagin in the blood when biologic nonspecific reactions can be excluded. This is especially true at the present time in relation to flocculation tests, since reactions formerly reported as "plus-minus," "one plus," or "two plus" are now being reported as doubtful. In my complement fixation test, however, I have always and purposefully reduced sensitivity to the point where "one-plus" and "two-plus" reactions may be safely reported as positive because I believe that such weakly positive reactions are almost invariably indicative of syphilis when diseases capable of yielding biologic nonspecific reactions can be excluded. Consequently, in my test only "plus-minus" reactions are reported as doubtful.

When doubtful reactions are observed, the patient should not be informed of the results until the physician has reached a final conclusion on the basis of a careful history, physical examination and a repetition of the tests in the same or another laboratory.

Negative Reactions. As previously stated, none of the present serologic tests is sufficiently sensitive to detect all cases of syphilis when conducted in such a manner as to avoid nonspecific reactions. This is not difficult to understand in the early stages of a chancre before sufficient time has elapsed for the production of reagin. But *falsely negative reactions* can occur in late syphilis in both the latent and active stages even when histologic evidences of syphilis are found after death.[63] Indeed, it is not reasonable to expect that any test can be rendered sufficiently sensitive to detect all cases of latent syphilis, since the infection may be so well held in check that the amount of reagin in the blood at any particular time may be insufficient for detection. Under the conditions, *a negative reaction and even repeatedly negative reactions should not be permitted to override clinical judgment.* No serologist can say that syphilis is absent merely on the basis of negative reactions. Final judgment and responsibility rests fairly and squarely with the physician.

Thus, in late untreated primary syphilis, falsely negative reactions may occur in about 25 per cent of cases, although they are almost invariably absent in untreated secondary syphilis. In early and late latent syphilis they may occur in 10 to 15 per cent of serums and in 10 to 20 per cent of cases of cardiovascular and hepatic syphilis. In tabes dorsalis serums may yield as high as 20 to 30 per cent falsely negative reactions although in juvenile and acquired paresis the incidence is not usually higher than 5 per cent. And while positive reactions are the rule in infants born with manifest lesions of syphilis, as high as 20 to 40 per cent falsely negative reactions may occur in older children. Indeed, it is to be regretted that the serologic tests so frequently fail to give positive reactions in late congenital syphilis even in cases presenting stigmata and other clinical manifestations. For this reason physicians need to be especially skilled in its clinical detection.

In both early and late syphilis the first injection or two of arsphenamine, neoarsphenamine, mapharsen or penicillin may produce an increase of reagin usually reaching its maximum between the fifth and tenth days, after which the titer usually subsides to or below its original level. This is called the "provocative reaction" as it is believed to be due to the temporary provocation of the infection with *T. pallidum*. If quantitative serum tests are employed, this provocative effect is almost uniformly observed at the start of treatment in early syphilis and less frequently in late syphilis.

This provocative reaction is sometimes of value in the diagnosis of a previously untreated patient and especially when negative serum reactions are observed in the presence of a lesion thought to be syphilitic, or if weakly positive reactions are observed in the absence of suspicious lesions. The tests should be conducted quantitatively before the injection of these compounds and then every other day for 14 days. The results are positive if reagin appears in a previously negative blood, or if it increases sharply in an individual who previously gave doubtful or weakly positive reactions.

While the test is of some value as a diagnostic procedure, it is of no value whatever as a criterion of cure. It is significant only when positive, as the absence of provocative effects does not signify freedom from syphilis; indeed, in late syphilis it is more often negative than positive.

In this connection it may be stated that some observers [64] have raised the question whether or not the arsphenamines may produce nonspecific positive reactions in normal nonsyphilitic human beings; but this does not appear to be the case.[65,66] Of further interest is the question whether or not the reagin may fluctuate in amount from day to day in both treated and untreated cases of syphilis. Not infrequently a serum will give a positive reaction and a few days later a negative reaction, or vice versa. Undoubtedly, fluctuations occur over long periods of time as would be expected in relation to fluctuations in the degree of infection, but whenever a serum reacts positively today and negatively two or three days later, it is practically certain that the reactions are not due to fluctuations in reagin but to technical factors. Indeed, in all tests it sometimes happens that the same serum tested on different days may give different results being an example of the frequent vagaries and paradoxical results of serologic tests due to obscure factors in their mechanism. As recently shown by Mohr and Smith,[67] daily variations in the course of repeated serologic reactions on the same individuals are not due to variations in the reagin-content of the serums, but primarily reflect daily fluctuations in the sensitivity of the tests employed.

SEROLOGIC TESTS IN RELATION TO TREATMENT

If the reagin in serum and cerebrospinal fluid is evidence of infection with *T. pallidum* rather than a direct expression of acquired immunity in syphilis, as I believe to be the case,[6] it is evident that as long as an individual continues to give positive reactions some infection remains in spite of the amount of treatment given and complete freedom from all detectable clinical symptoms and signs of the disease. This does not mean, however, that vigorous treatment should continue after thorough treatment has been given, merely to obtain persistently negative serologic reactions, as "clinical cure" may readily suffice for all reasonable purposes and objectives even though complete biologic cure is not obtainable. In early syphilis, however, one of the criteria of cure widely accepted is the finding of not one but repeatedly negative serologic reactions over at least two years following the cessation of treatment. Even under these circumstances one cannot be absolutely sure of biologic cure, since not sufficient time has elapsed for evaluating modern methods of treatment in terms of the ultimate fate of individuals. Indeed, upon the expiration of this period of observation, I commonly advise patients to have the tests repeated at least once a year for an additional period of five years, and even for the balance of life, since serologic relapse, with the return of positive reactions, is indicative of persistent relapsing infection where the prompt renewal of treatment will prevent or delay the onset of signs and symptoms of tertiary syphilis. This may be inadvisable in some cases, at least, as the patient and physician may well wish to regard the disease as definitely terminated, but for my own part, I prefer to be frank in stating that at the present time repeatedly negative reactions over one or two years following the cessation of the treatment of syphilis may not be as reliable an index of cure as commonly surmised.

But regardless of negative serologic reactions, the treatment of early syphilis with the arsenicals and bismuth or with penicillin should be thorough and adequate. This also applies to cases of primary syphilis proved by positive darkfield examinations for *T. pallidum* in which treatment is instituted before positive serologic reactions occur. However, if positive reactions occur after the first six months of continuous treatment with the arsenical compounds and bismuth, seroresistance or "Wassermann-fastness" is to be suspected. Furthermore, no case of treated early syphilis can be regarded as cured without at least one complete examination of the spinal fluid with negative results after the cessation of treatment.

Without doubt, however, patients completing thorough treatment with persistently positive reactions (seroresistance or "Wassermann-fastness") may become spontaneously negative with the further lapse of months or years of time without additional treatment. This may occur in both acquired and late congenital syphilis and is to be ascribed to the gradual eradication of infection or more likely to a high degree of latency due to the acquired immunity of the disease. But in the absence of biologic cure, relapsing infections may occur so I strongly believe in routine yearly tests over several years.

Serologic tests also play an important and decisive rôle in the evaluation of the penicillin treatment of early syphilis. Quantitative tests should be conducted with strongly positive serums or spinal fluids; qualitative tests are usually sufficient for detecting residual reactivity after the need for quantitative tests has passed. Both rapid and delayed serologic improvement may be observed. The majority of cases of early syphilis show negative reactions within four to six months. Weaker quantitative reactions during the first posttreatment month usually indicate a favorable serologic response, while negative reactions are observed in the majority of cases within the first six months of posttreatment observation, although most clinical and serologic relapses occur during this period. At all events, nearly all cases of early syphilis show negative serologic reactions by the end of eight months, while others respond more slowly. Some cases show weakly positive or doubtful reactions for as long as eighteen months following the completion of treatment.

Serologic Relapse. It may not be amiss to refer briefly to factors having a possible bearing upon the frequency with which, in human beings, positive serologic reactions may occur in well-treated syphilitic individuals after a period of negativity. As a general rule, these serologic relapses are interpreted in practice as indicative of renewed activity of persistent foci of infection and should result in the resumption of treatment in order to prevent clinical relapse or progression of the disease.

May an anamnestic effect, in the sense of an intercurrent infection, or the administration of vaccine, provoke a serologic relapse in serologic-negative cases of chronic syphilis? I have seen five cases of treated chronic syphilis with repeated negative reactions over two to five years, give temporarily positive Kolmer, Kahn and Kline reactions during attacks of epidemic influenza (three cases) and lobar pneumonia (two cases). For this reason, I always inquire carefully into the possibility of unsuspected latent or asymptomatic chronic syphilis in those occasional instances where temporarily positive serum reactions are observed in active tuberculosis, pneumonia, influenza, etc., diseases commonly thought to be alone responsible for falsely positive reactions.

Furthermore, as suggested by Chesney and Kemp,[68] it may be "that some of the relapsing Wassermann reactions occurring in patients with early syphilis who have been well treated may represent not a relapse of their first infection, as is generally held, but a true reinfection in which no lesion occurs at the portal of entry but in which systemic distribution of *T. pallidum* takes place and is accompanied by the reappearance of a positive Wassermann reaction." It is true that at present in clinical practice we have no means of differentiating such patients from those in whom the subsequent reappearance of a positive Wassermann reaction represents a true serologic relapse, but the possibility is to be admitted and borne in mind as it necessarily involves in an important manner our attitude toward the treatment of serologic relapse.

Serologic Resistance or "Wassermann-Fastness." Seroresistance refers to persistently positive serologic reactions in spite of treatment which is usually very disconcerting to both patient and physician. It also includes late cases of syphilis in which the serologic reactions fluctuate back and forth from negative to positive, due to the presence of small amounts of reagin.

Treatment-resistant syphilis may be of three kinds characterized by (1) the clinical persistence of lesions, (2) the persistence of spirochetes in lesions (most characteristic), or (3) the persistence of positive serologic reactions. All three need not be present in every case. A very extensive literature has accumulated on the subject which has been ably reviewed by Beerman[69] along with a consideration of methods for its therapeutic management which is admittedly difficult.

While there is no general agreement on what constitutes seroresistance or "Wassermann-fastness," the Clinical Cooperative Group regards early cases of syphilis as seroresistant when the serologic reactions remain positive after six months of continuous treatment with the arsphenamines and bismuth, and late cases as seroresistant if the reactions remain persistently positive after twelve months of continuous treatment. Needless to state, seroresistance is in intimate relationship to the sensitivity of the tests employed. Consequently, it is encountered more frequently at present than thirty-five years ago when the Wassermann test was very much less sensitive than the approved complement fixation and flocculation tests of today. On this basis the treatment of all types of syphilis is seemingly becoming less satisfactory and this is apt to become even more apparent as the tests are made more and more sensitive. In this connection it is to be stated, however, that flocculation reactions are particularly apt to remain persistently positive after thorough treatment and it remains to be determined whether or not they may be ignored if complement fixation tests of proved value are persistently negative. In other words, it is thought that the physician should worry more about the tests than about the patient.

The cause of seroresistance is unknown. By some it is regarded as the persistence of reagin in the blood after biologic cure similar to the persistence of agglutinins for *S. typhosa* after complete recovery from typhoid fever. As far as early syphilis of rabbits is concerned, however, I have found that the reagin disappears within fifteen weeks after biologic cure [6] and therefore this does not appear to be an acceptable explanation. Others believe that it is due to a natural or acquired resistance of *T. pallidum* to arsphenamine or other antisyphilitic compounds. So far, all experimental attempts to render *T. pallidum* drug-resistant have given inconclusive results but Beerman [69] has observed that a strain transmitted to rabbits from a case of treatment-resistant syphilis was unusually refractory to treatment. As far as seroresistance is concerned, the consensus is to the effect that it is due to the production of reagin in persistent foci of syphilitic infection. With this I agree and, consequently, believe that it is advisable to give follow-up periodic courses of treatment, not primarily for the purpose of merely securing negative serologic reactions, but as a safeguard against clinical relapse or the progression of infection.[6] As shown by the studies of the Clinical Cooperative Group, however, seroresistance is of much less significance in late than in early syphilis, as the ultimate fate of its victims cannot be measured or calculated on the basis of serologic tests, since a large percentage with or without follow-up treatment appear to do as well as seronegative cases. In fact, some become seronegative spontaneously within a few years with no additional treatment at all.

Furthermore, the Clinical Cooperative Group has shown quite conclusively that the incidence of seroresistance in early syphilis is in relation to the treatment given. For example, in primary and secondary syphilis treated by the continuous method, the incidence is usually 5 to 15 per cent, while it is 35 per cent in the case of intermittent treatment and 70 per cent in cases treated irregularly. Consequently, all physicians undertaking the treatment of early syphilis should impress upon patients the necessity of adequate treatment by the continuous plan not only because infectious relapses are at least four times more frequent among seroresistant than among seronegative individuals, but likewise because most of the former show infection of the central nervous system. For this reason all should have an examination of the spinal fluid with an appropriate therapeutic program if abnormal changes are found. In early congenital syphilis with adequate treatment seroresistance occurs in 5 to 20 per cent of cases.

In latent and chronic syphilis with adequate treatment seroresistance may vary from 10 to 25 per cent in tabes dorsalis to as high as 35 to 80 per cent in paresis, cardiovascular, hepatic and osseous syphilis. Consequently, all cases require an examination of the spinal fluid and a thorough clinical investigation of the cardiovascular (including roentgenologic examinations), central nervous and osseous systems for syphilitic lesions. In late congenital syphilis the incidence is from 60 to 80 per cent.

Furthermore, intelligent patients may become so discouraged and pessimistic as to abandon treatment. Consequently, the physician should always explain the situation and point out that the aim of treatment is not primarily to secure negative reactions but to maintain good physical health and longevity, since seroresistance is not incompatible with either; also that after appropriate treatment has been given, marriage may be safely contracted, since there is no more danger of transmitting syphilis to spouse and children than if a state of permanent seronegativity had been attained by treatment.

SPINAL FLUID EXAMINATIONS

It is important to remember that the cerebrospinal fluid may give positive reactions in both acquired and congenital syphilis when the serum reacts negatively. This is especially true in tabes dorsalis and other clinical types of syphilis of the central nervous system other than paresis, including asymptomatic neurosyphilis. Whenever late syphilis is suspected, it is generally advisable to conduct an examination of the cerebrospinal fluid in the diagnostic survey, including not only the complement fixation or flocculation tests but a total cell count, a test for increase of protein and a colloidal gold or mastic test, the former being preferred if properly conducted with a reliable reagent. Furthermore, no case of syphilis can be regarded as cured on the basis of negative serologic reactions without at least one or two complete examinations of the cerebrospinal fluid, since positive changes may be observed in spite of repeated and persistently negative serum reactions.

For various technical reasons a complement fixation test is preferred, and especially by a quantitative method, for diagnostic purposes and in relation to treatment as well. In view of their extremely high specificity, positive reactions are indicative of invasion or infection of the central nervous system. Syphilitic individuals, however, without infection of these parts may give temporarily positive complement fixation reactions during the course of acute pyogenic meningitis, presumably because the barrier of the choroid plexus to the passage of reagin from the blood to the spinal fluid is broken down.

Positive complement fixation or flocculation reactions, however, do not indicate the type of neurosyphilis, although a typical Zone I curve in the colloidal gold or mastic tests is usually indicative of incipient or manifest paresis, as discussed in

Chapter 14. In general terms, complement fixation tests of proved value give from 70 to 85 per cent positive reactions in all cases of neurosyphilis as a group, while the incidence with flocculation tests of proved value varies from 64 to 82 per cent. In tabes dorsalis the incidence may be 70 per cent and almost 100 per cent in paresis. As in the case of serum tests, however, a negative reaction does not exclude the possibility of neurosyphilis except, possibly, in cases of suspected paresis and especially if associated with a low or normal total cell count, no increase of protein, and a negative colloidal gold reaction. Spirochetal complement fixation tests give about 83 per cent positive reactions in neurosyphilis as a group along with 100 per cent specificity.

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19

THE CLINICAL INTERPRETATION OF IMMUNOLOGIC AND ALLERGIC SKIN TESTS

The only immunologic skin tests commonly employed are those of Schick and Dick for natural or acquired immunity to diphtheria and scarlet fever respectively, including the Schultz-Charlton blanching test sometimes of value in the diagnosis of scarlet fever. Possibly the intradermal injection of antitularemia serum (Foshay), employed as an aid in the diagnosis of tularemia, is to be included in this category although its mechanism is unknown at the present time.

Skin tests for natural or acquired allergy, however, are widely employed in the etiologic diagnosis of numerous allergic diseases with special reference to hay fever, asthma, vasomotor or allergic rhinitis, allergy to foods (gastro-intestinal allergy), the allergic dermatoses (urticaria, eczema), migraine, contact dermatitis, serum and drug allergy; also for allergy in various bacterial diseases (tuberculosis, brucellosis, tularemia, glanders, etc.) as well as in some of the viral and mycotic diseases and those due to animal parasites for diagnostic purposes.

While *hypersensitiveness* is the basic term for designating these diseases or states, the term *allergy*, coined by von Pirquet and meaning literally "changed or altered reactively," is now widely employed to include all the manifestations of hypersensitiveness in human beings, while the term *anaphylaxis*, coined by Richet and meaning "without protection," is reserved for those manifestations of hypersensitiveness produced in the lower animals in which an antibody in the blood has been demonstrated. The term *atopy*, suggested by Coca and meaning literally "a strange disease," is also employed for allergic diseases in human beings who are definitely subject to hereditary influences, with special reference to asthma and hay fever.

THE SCHICK TEST

This well-known test is now widely and universally employed for the detection of natural immunity to diphtheria or that acquired by active immunization with diphtheria toxoids or toxin-antitoxin mixtures (Table 116). When properly conducted, a *negative reaction* is indicative of immunity on the basis that the blood contains at least 1/500 to 1/250 of a unit of antitoxin per cc. of serum. It is indicated by the complete absence of erythema and edema at the site of injection of toxin, or, at the most, the appearance of a very small papule not more than 0.5 cm. in diameter. While, in the great majority of instances, this degree of antitoxic immunity is sufficient for protection against infection with *Corynebacterium diphtheriae,* nevertheless mild infections may sometimes occur, although usually without clinical evidences of toxemia.

TABLE 116. SUMMARY OF THE CLINICAL INTERPRETATION OF IMMUNOLOGIC SKIN TESTS

Test	Interpretation
Schick Test	A test of proved value for natural or acquired antitoxic immunity to diphtheria. *Negative reactions* are indicative of immunity due to the presence of at least 1/500 to 1/250 of a unit of antitoxin per cc. of serum. *Positive reactions* are indicative of susceptibility to diphtheria. *Pseudoreactions* are due to allergy to protein substances in the toxin; they are of the same significance as negative reactions. *Combined true and pseudoreactions* are indicative of susceptibility to diphtheria with allergic sensitization to protein substances in the toxin; they are of the same significance as positive reactions. Control injections advisable in older children and adults. Reactions best read on fifth to seventh days after injection. Positive reactions occur in 60 to 90 per cent of children between ten months and eight years of age; consequently, Schick tests preliminary to active immunization are not usually required in this age group. Positive reactions occur in 15 to 40 per cent of individuals ten to thirty years of age. Preliminary Schick tests are indicated as only positive reactors require immunization. Schick tests should be conducted about six months after active immunization; also at school age in the case of children immunized between one and three years of age. Positive reactors should be re-immunized.
Dick Test	A test of clinical value for natural or acquired immunity to scarlet fever. *Negative reactions* are indicative of antitoxic immunity usually sufficient for affording protection against scarlet fever although local infections with hemolytic streptococci may occur without the production of characteristic rashes. *Positive reactions* are indicative of susceptibility to scarlet fever. *Falsely positive reactions* due to allergy are so infrequent that control injections are not ordinarily required. *Pseudopositive reactions*, however, may occur following the injection of toxin contaminated with bacteria. Reactions should be read not earlier than eighteen hours or later than twenty-four hours after the injection of toxin. A common error is to regard weakly positive reactions as negative. Positive reactions occur in 50 to 75 per cent of children between one and five years of age; consequently, Dick tests preliminary to active immunization are not usually required in this age group. Positive reactions occur in 18 to 40 per cent of individuals between six and thirty years of age. Preliminary Dick tests are indicated as only positive reactors require immunization. Dick tests should be conducted about two weeks after active immunization; also at school age in the case of children immunized between one and three years of age. Positive reactors should be re-immunized. Also advisable during epidemics of scarlet fever in the case of children immunized three or more years previously; re-immunization of positive reactors both safe and advisable.

TABLE 116. SUMMARY OF THE CLINICAL INTERPRETATION OF IMMUNOLOGIC SKIN TESTS—(Continued)

Test	Interpretation
Schultz-Charlton Blanching Test	Of clinical value as an aid in the differential diagnosis of scarlet fever rashes from those occurring in rubella and drug allergies (quinine, salicylates, etc.). Consists of the *intracutaneous* injection of 0.5 cc. of convalescent scarlet fever serum or 0.1 cc. of standard scarlet fever antitoxin; latter preferred. Scarlet fever rashes usually show blanching eighteen to twenty-four hours later.
Foshay Anti-serum Test	Consists of the *intracutaneous* injection of antitularemia serum. An immediate reaction may occur in cases of tularemia giving positive allergic reactions to intracutaneous injections of heat-killed suspensions of *Past. tularensis.* The reaction to antitularemia serum is not allergic in character; mechanism unknown. Control serums should be injected at the same time.

On the other hand, *positive reactions* are indicative of susceptibility to diphtheria on the basis that the serum contains less than 1/500 to 1/250 of a unit of antitoxin per cc. or none at all. They sometimes develop within twenty-four hours but may not do so until the third or fourth day. Consequently, Schick reactions are best read and interpreted on the fifth to seventh days after the injection of toxin. Positive reactions (Fig. 33) may vary from a small area of erythema with slight edema about the size of a quarter (faintly positive) to that of a half dollar (definitely positive) or larger, with considerable edema (strongly positive). These slowly subside and often leave a circumscribed area of brownish pigmentation with scaling persisting for three to six weeks. In all instances the control site should show no reaction at all or at the most only a small area or papule not more than 0.3 cm. in diameter.

Unfortunately *pseudoreactions,* which have the same significance as negative reactions, may occur and prove disconcerting. They may develop within six to eighteen hours, reaching their height in thirty-six to forty-eight hours, but usually disappear by the fourth or fifth days. If, therefore, Schick reactions are read and interpreted on the fifth to seventh days, as previously advised, errors in interpretation are much less likely to occur. These pseudoreactions are characterized by a central area of dusky redness about the size of a dime with a characteristic secondary pale areola that shades off into the surrounding skin, with slight or no edema. They are allergic reactions to protein substances in the toxin-like bacterial products or the peptone used in the broth medium employed in culturing the diphtheria bacillus in the preparation of toxin. Pseudoreactions usually occur only in older children and adults; also in diphtheria convalescents and in persons previously immunized with toxoids or toxin-antitoxin mixtures. Since the control shows a similar reaction it should always be used under these conditions.

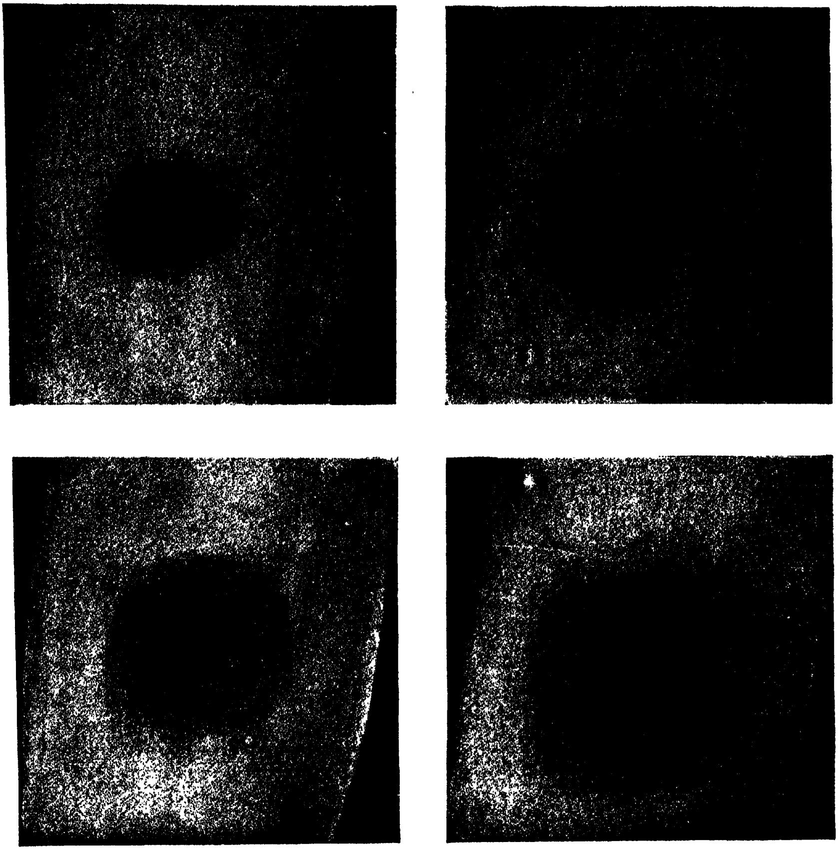

FIG. 33. POSITIVE SCHICK REACTIONS

A, positive reaction at end of 48 hours; *B*, positive reaction at end of 4 to 5 days; *C*, pseudopositive reaction; *D*, combined true and pseudopositive reaction.

Combined true and pseudoreactions may also occur in older children and adults due to the absence of antitoxin immunity along with the presence of allergic sensitization to some constituent in the toxin. Under the circumstances, they have the same significance as a true positive reaction. They are characterized by a

A B

FIG. 34. TECHNIC OF INTRACUTANEOUS INJECTIONS

A, by tuberculin syringe in upper arm (from Kolmer and Tuft, *Clinical Immunology, Biotherapy and Chemotherapy*, W. B. Saunders Co.); *B*, by Record syringe in upper arm (note wheal indicative of correct injection). (From Kolmer, *Infection, Immunity and Biologic Therapy*, W. B. Saunders Co.)

central zone of dusky erythema with considerable edema as observed in true positive reactions, along with a wide pale areola of erythema as occurs in pseudoreactions.

Since 60 to 90 per cent of children between the ages of ten months to eight years give positive reactions, it is the consensus that Schick tests are not required in this age group and that all should be routinely immunized with one of the diphtheria toxoids. In individuals of ten to thirty years of age, however, the incidence of positive reactions is generally reduced to between 15 and 40 per cent, so that preliminary Schick tests are always advisable in older children and adults since only positive reactors require immunization. In this connection the physician should remember that an attack of diphtheria may not necessarily render an individual immune to the disease.

It is always advisable, however, to conduct Schick tests about six months after active immunization. While one dose of alum-precipitated toxoid or two doses of plain toxoid generally suffice for producing antitoxic immunity with negative Schick reactions in 90 to 98 per cent of individuals, the incidence may be only 75 to 90 per cent in the case of immunization with toxin-antitoxin (T-A) mixtures. Positive reactors, therefore, are not immune and re-immunization is indicated. Furthermore, all children immunized below three or four years of age should be retested at six years of age and re-immunized if showing positive reactions.

Technic of the Schick Test. The usual method is to inject 0.1 cc. of the diluted toxin *intracutaneously,* using the cleansed skin of the inner surface of the forearm below the elbow or the upper arm near the site of insertion of the deltoid muscle (Fig. 34).

In the case of older children and adults, a similar injection of 0.1 cc. of the control fluid should be made either in the opposite arm, which is preferable, or several inches below the test site on the same arm. It is essential that the injections be given into the skin and not subcutaneously; otherwise, false negative reactions may occur. The presence of a *large white palpable wheal* (Fig. 34) indicates that the injection has been properly made.

Accuracy in the measurement of the amount of toxin injected may be insured by the use of a properly graduated tuberculin syringe, although a good Luer or Record syringe also is satisfactory. A 26-gauge ¼ to ½ inch hypodermic needle is best adapted for intracutaneous injections. If the test is being performed on a group of individuals, it is permissible to use the same needle for all injections, providing it is wiped off with alcohol after each injection.

THE DICK TEST

The Dick skin test is employed for determining the presence or absence of natural or acquired antitoxic immunity to scarlet fever. It does not have the same value and precision as the Schick test in diphtheria. This is due not only to greater difficulties in the preparation and standardization of the test toxin but also because a negative reaction, indicative of immunity to the erythrogenic toxin of hemolytic streptococci, may not indicate a sufficient degree of antibacterial immunity to the organisms themselves. Consequently, it is thought that individuals giving negative Dick reactions may still develop local infections with hemolytic streptococci but without showing the characteristic rash of scarlet fever. However, ample clinical experience has proved that the great majority of individuals giving *negative reactions* are immune to the disease (Table 116).

Reactions should not be read earlier than eighteen hours or later than twenty-four hours after the injection of toxin. *Positive reactions* are indicative of the absence of antitoxin in the blood and therefore of susceptibility to scarlet fever. They are characterized entirely by erythema without induration or edema and may be faintly or slightly positive (between 10 and 20 mm.), moderately positive (between 20 and 30 mm.), strongly positive (between 30 and 40 mm.) or very strongly positive (over 40 mm.) as shown in Figure 35. The slightest erythema, no matter how faint, constitutes a positive reaction if it measures as much as 10 mm. in any direction. A common mistake is to interpret weakly positive reactions as negative. On the other hand, *falsely positive reactions* are sometimes due to infection of the skin through bacterial contamination from careless handling of vials of the toxin. These usually do not suppurate, but last longer than a true

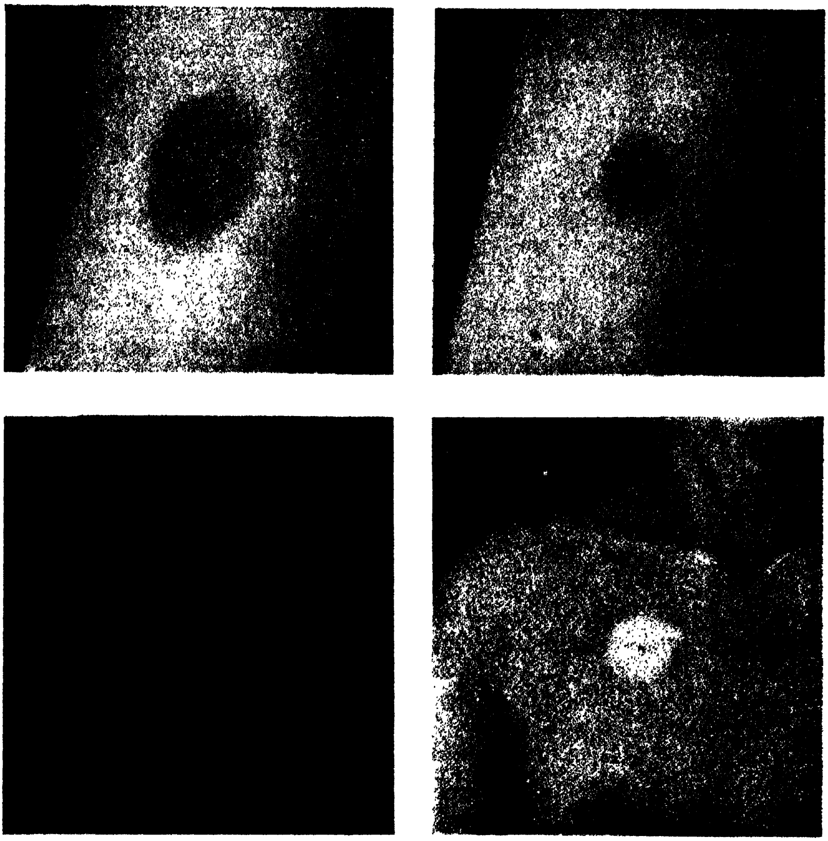

FIG 35. POSITIVE DICK AND SCHULTZ-CHARLTON BLANCHING REACTIONS

A, strongly positive Dick reaction at end of 24 hours; *B*, slightly positive Dick reaction at end of 24 hours; *C*, strongly positive Dick reaction in a Negro at end of 24 hours; *D*, Schultz-Charlton blanching reaction.

positive reaction and are often indurated. Pseudoreactions due to allergy, however, are so infrequent that control injections of the toxin, heated in boiling water for an hour, are not ordinarily required.

Since 50 to 75 per cent of children between one and five years of age give positive reactions, it is not generally necessary to conduct Dick tests in this age group in relation to active immunization against scarlet fever. But since only 18 to 40 per cent of individuals between six and thirty years react positively, a Dick test is advisable in this age group, as only positive reactors require immunization. But all individuals given the usual doses of toxin vaccine should be tested about two weeks after the last dose; if a positive reaction occurs it is well to repeat the last dose. Immunity is thought to last for about twelve years in about 90 per cent of individuals but in the case of children immunized between one and three years of age, it is advisable to retest at six years of age with the re-immunization of positive reactors. This is also advisable during epidemics of scarlet fever in the case of children immunized three or more years previously, as the re-immunization of positive reactors is both safe and advisable under these conditions.

Technic of the Dick Test. This is exactly like that of the Schick test (page 562) and consists of the *intracutaneous* injection of 0.1 cc. of Dick toxin carrying one skin test dose (one S.T.D.). The syringe and needle should not be sterilized with alcohol or other disinfectants, since retention of the latter may result in destruction of the toxin. Distilled water for boiling is preferable to tap water and as much of the water as possible should be expelled from the syringe and needle before use; this may be aided by ejecting a little toxin from the needle just before making the injection. As previously stated, rigid aseptic precautions are required to prevent any bacterial contamination that may result in falsely positive reactions. It will be noted, therefore, that the technic of this test is exacting.

The Schultz-Charlton Blanching Test in Scarlet Fever. This test has proved of clinical value as an aid in the differential diagnosis of scarlet fever rashes from clinically similar rashes occurring in rubella and the drug allergies (quinine, salicylates, etc.). It consists of the *intracutaneous* injection of 0.5 cc. of convalescent scarlet fever serum or 0.1 cc. of a potent scarlet fever antitoxin in the center of a large area where the rash is brightest, preferably on the abdomen or chest. The reaction is observed eighteen to twenty-four hours later. A *positive reaction* consists of blanching of the rash in a zone surrounding the central red spot where the injection was made (Fig. 35). The reading should be made while standing several feet from the patient. Rashes due to drug allergies are not blanched. The same is true in rubella (German measles) if the test is conducted with scarlet fever antitoxin. Convalescent scarlet fever serum may cause some blanching of nonscarlatinal rashes due to infectious diseases if it happens to contain specific antibodies for them. Consequently, the test is best conducted with standard scarlet fever antitoxin.

The Foshay Antiserum Test in Tularemia. Foshay has reported that patients with tularemia giving positive allergic reactions to intracutaneous injections of heat-killed suspensions of *Past. tularensis,* shortly to be described, also develop immediate reactions characterized by erythema and wheal formation lasting ten to fifteen minutes, when given *intracutaneous* injections of antitularemia serum.

The reaction is apparently specific but should be controlled by a second injection of normal serum from the same animal species, and preferably by a third injection of serum from the same species immunized against some other microorganism. The reaction should not be confused with any reaction indicating allergy to serum in preliminary tests before the administration of serum in treatment. Apparently this antitularemia serum reaction is not due to allergy and probably represents a new type of immunologic reaction of unknown mechanism at the present time.

THE TECHNIC OF SKIN TESTS

Allergens. It is now well known that human beings may possess natural or acquired hypersensitiveness to any of a remarkably large number of substances of animal or vegetable origin including chemical agents and drugs (Table 117). These exciting agents are known as allergens and may occur as *inhalants* (pollens, dandruffs and feathers of animals, dusts, etc.), *ingestants* (foods and beverages as well as various drugs), *contactants* (plants, drugs, cosmetics, dentifrices) and *injectants* (normal and immune animal serums, vaccines, drugs, insect venins). The detection of hypersensitiveness to these by skin or mucous membrane tests has proved of great value in relation to specific or etiologic diagnosis of allergic diseases as well as in relation to their treatment, which is based on preventing contact with exciting substances or attempting desensitization (hyposensitization) against them when this is not possible.

Furthermore, it is now generally believed that individuals may acquire hypersensitiveness to various pathogenic *bacteria* in chronic foci of infection (sinuses, tonsils, etc.) capable of producing some of the allergic diseases with special reference to asthma and vasomotor or allergic rhinitis, as well as, possibly, playing a rôle in the pathogenesis of rheumatic carditis, iritis, rheumatoid arthritis, and other diseases due to infection.

Human beings may also acquire hypersensitiveness to various pathogenic bacteria and *viruses* in the course of the acute or chronic infectious diseases, with special reference to tuberculosis, brucellosis, tularemia, glanders, mumps, lymphogranuloma venereum, etc.; also to various *yeasts, molds* and *fungi* producing the dermatomycoses and other mycotic infections, as well as to some of the *animal parasites* with special reference to *Echinococcus granulosus* and *Trichinella spiralis*. Allergic sensitizations to these living agents of disease may not always produce signs and symptoms, although these sometimes occur and especially the production of urticaria, but their detection by means of skin tests frequently yields information of diagnostic value.

The number of allergens required for skin tests in the specific or etiologic diagnosis of the allergic diseases is, therefore, extremely large and for this reason the diagnosis and treatment of allergy has properly become a specialty in medicine in view of the time and skill required. But all of the allergens required by the average practitioner for skin testing and treatment are commercially available. Otherwise, they may be prepared in any well-equipped laboratory and especially those of the dusts, dandruffs and foods.

TABLE 117. SUMMARY OF THE CLINICAL INTERPRETATION OF SKIN AND LABORATORY TESTS IN ALLERGIC DISEASES

Tests	Interpretation
Allergens	Natural or acquired allergy may be due to any of a remarkably large number of substances (allergens) of animal or vegetable origin, including chemical agents and drugs, classified as follows: (1) *Inhalants* (pollens, danders, dusts, etc.). (2) *Ingestants* (foods, beverages, drugs). (3) *Contactants* (plants, drugs, cosmetics, etc.). (4) *Injectants* (serums, vaccines, drugs, etc.). (5) *Bacteria* (tuberculosis, brucellosis, tularemia, focal infections) and *viruses* (lymphogranuloma inguinale, mumps, etc.). (6) *Yeasts, molds* and *fungi.* (7) *Animal parasites.*
Allergic Diseases	Positive skin reactions may occur to allergens without demonstrable clinical evidences of disease (*potential allergy*). The common allergic diseases and usual agents are: (1) *Asthma* (pollens, dusts, orris root, seeds, epidermals, foods, drugs, molds, bacteria). (2) *Allergic rhinitis* (same as asthma). (3) *Hay fever* (pollens of trees, grasses, weeds and flowers). (4) *Gastro-intestinal allergy* (foods; occasionally drugs). (5) *Migraine* (some cases; usually foods; rarely inhalants). (6) *Urticarial dermatoses* (foods, drugs, serums, pollens, physical agents and sometimes bacteria). (7) *Serum allergy* (immediate or delayed reactions and serum sickness). (8) *Allergic eczema* (chiefly ingestants and particularly foods; sometimes inhalants). (9) *Contact dermatitis* or "dermatitis venenata" (ivy, sumac, oak and other plants; chemical agents and drugs, dyes, paints, lacquers, metallic substances, dusts, etc.). (10) *Dermatophytoses and dermatophytids* (fungi, yeasts, molds).
Skin and Mucous Membrane Tests	(1) *Cutaneous or scratch tests* are simple, inexpensive, highly specific and safe; however, they are not as sensitive as intracutaneous tests. (2) *Intracutaneous tests* are highly sensitive and yield quicker reactions which are easier to interpret, but more likely to produce nonspecific and constitutional reactions; also more time-consuming and expensive. (3) *Ophthalmic or conjunctival tests* are of special value for determining hypersensitiveness to serum before administration, since positive reactions indicate the need for great care. (4) *Nasal tests* are of occasional value in hay fever and allergic rhinitis with positive histories but doubtful skin reactions. (5) *Patch tests* are used only for the etiologic diagnosis of tuberculosis and contact dermatitis or "dermatitis venenata." (6) *Passive transfer or indirect tests* of occasional value when skin tests are impossible or inadvisable. In suspected natural allergy to horse or other immune serums of the lower animals the cutaneous test is advisable; in suspected acquired allergy the intracutaneous test is advisable followed by the ophthalmic tests if positive reactions occur.

TABLE 117. SUMMARY OF THE CLINICAL INTERPRETATION OF SKIN AND LABORATORY TESTS IN ALLERGIC DISEASES—(Continued)

Tests	Interpretation
Reactions	A *positive reaction* usually signifies that the allergen is clinically important. On the other hand, it may be evidence of past or future clinical sensitivity. Great care is required in interpreting positive reactions in individuals with highly reactive or "dermographic" skins. Falsely *negative reactions* are more frequent and especially to foods; they may also occur in hay fever, allergic rhinitis and asthma; likewise in older individuals, those who have been allergic a long time, and in individuals with skins of the nonreactive type. Therapeutic or clinical trial is sometimes required for determining the importance of positive skin reactions or for determining specific etiologic factors in patients giving negative skin reactions, especially in the case of foods.
Laboratory Examinations	Differential leukocyte counts showing *eosinophilia* constitute additional evidence of allergy if there is no other apparent cause; its absence does not exclude allergy. Differential leukocyte counts of *nasal mucus* sometimes of value. An excess of eosinophils indicates allergic rhinitis. Its absence does not exclude allergy. A determination of the leukopenic *index* may be helpful in determining the clinical importance of foods giving doubtful or positive skin reactions.

Allergic Diseases. Manifestations of disease do not always occur in individuals who may show positive skin reactions to various allergens. But such reactions are designated *potential allergy,* since they indicate the possibility and probability of clinical manifestations later on in case the individual has contact with them.

Indeed, it is likely that symptom complexes and signs of disease of obscure or unknown etiology at the present time may be ultimately shown to be allergic in character. Otherwise, however, a large list of diseases are now known to be due to allergy, or hypersensitiveness plays some rôle in their production. The more important of these, along with the exciting agents commonly responsible, are shown in Table 117.

Kinds of Skin Tests; Advantages and Disadvantages. The two skin tests most commonly employed are the cutaneous or scratch and the intracutaneous tests. Each has its advantages and disadvantages. Consequently, it is not a matter of choosing one or the other, but of using either or both according to individual conditions.

Cutaneous or *scratch tests* have the advantages of simplicity and inexpensiveness since thirty or more may be conducted at one time. They are also highly specific with nonspecific reactions of rare occurrence. They are likewise safe since the absorption of amounts of allergens sufficient for the production of constitutional reactions does not occur. Their one important disadvantage is a lack of sensitivity. However, they are usually sufficiently sensitive for tests with the inhalant allergens in hay fever, asthma and allergic rhinitis; furthermore, they are frequently preferred in testing suspected cases of natural allergy to horse or other animal serums in which hypersensitiveness may be so exquisite as to render intracutaneous tests dangerous.

Intracutaneous tests have the great advantage of sensitivity and are, therefore, preferred in tests for the detection of hypersensitiveness to foods, bacteria, serum, fungi and animal parasites. The reactions are also quicker and generally easier to interpret. But they are more likely to yield local nonspecific and constitutional reactions, and are more time-consuming and expensive since not more than six to eighteen tests can be conducted at one time.

Ophthalmic or *conjunctival tests* are less sensitive than intracutaneous tests; hence they never give positive reactions when the latter are negative. On the other hand, however, positive ophthalmic and intracutaneous reactions indicate a higher degree of hypersensitiveness than a positive intracutaneous reaction alone. Consequently, they are particularly useful tests for allergic sensitization to serums preliminary to their administration in the prophylaxis and treatment of disease since a positive ophthalmic reaction indicates the need for great care. They are also sometimes used in testing for pollen allergy but only under special conditions. Needless to state, they cannot be employed in the presence of conjunctivitis nor in the case of crying children or adults.

Nasal tests have a very limited clinical application. They are of occasional value in allergic rhinitis in determining the clinical significance of certain inhalant substances giving doubtful skin reactions. They are also useful at times in detecting sensitivity in hay fever patients with positive clinical histories but negative skin tests or, like the conjunctival test, in differentiating the clinical significance of tests to pollens with overlapping pollination periods.

Patch tests are employed only in the etiologic diagnosis of tuberculosis and contact dermatitis or "dermatitis venenata" produced by contact with external irritants and characterized by a predominantly vesicular type of eruption. Substances for testing are carefully selected according to the history of the patient and applied in solutions, ointments or powders, according to their nature and to duplicate the conditions of exposure of the patient to them. A *positive* reaction indicates the presence of skin sensitivity of the contact type to the particular agent tested, providing it is not itself a primary irritant. Proof of the etiologic importance of this substance in relation to the patient's dermatitis must be established by clinical trial. It is in most instances of primary importance; however, it may sometimes be a secondary offender. A *negative* reaction usually, although not always, excludes the presence of a contact type of sensitivity; clinical sensitivity occasionally exists in spite of a negative patch test. This may be because the test has been applied at a time when the patient's skin is temporarily desensitized as, for example, after an acute attack. A negative reaction sometimes happens because the test is done in an area of skin which is not sensitive to the agent because the hypersensitiveness is local and not general—this occasionally takes place in patients with dermatitis of the face or neck in whom patch tests are negative when applied on the arm but positive when applied to the skin of the neck and back, even though the same agents are employed in both areas.

A negative reaction may also be obtained because the conditions under which the dermatitis resulted were not reproduced; for example, contact with substances responsible for certain occupational dermatoses is not constant but intermittent and is often aided by such predisposing factors as heat, friction, skin maceration or trauma. Since these conditions are not reproduced by the patch test, a negative result may follow.

The *passive transfer* or *indirect test* is based upon the demonstration of allergic antibody or reagin in the blood of the patient. It is of occasional value when cutaneous or intracutaneous tests are impossible or inadvisable, as in severe universal eczemas and especially in young children; in patients with severe intractable asthma who are constantly taking adrenalin; in patients too ill or too feeble for skin tests; in those in whom severe constitutional reactions are feared and, finally, as a check upon the specificity of positive skin reactions under special conditions.

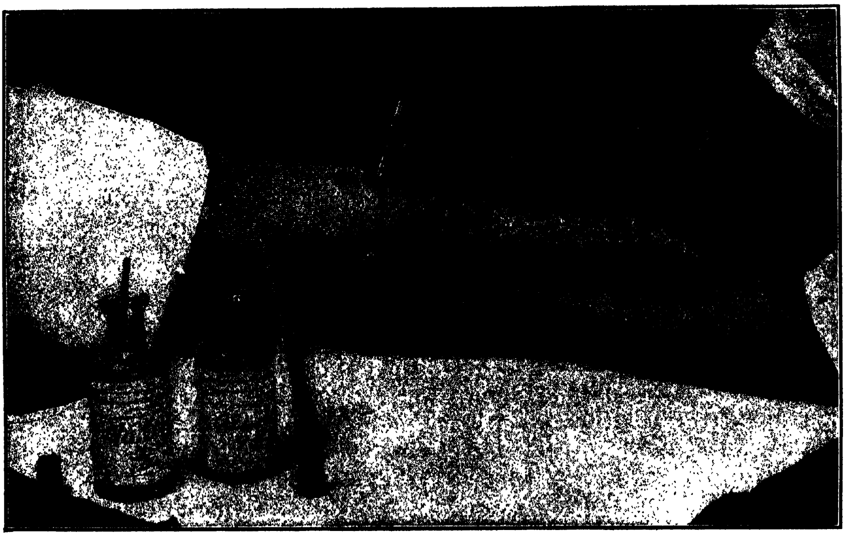

FIG. 36. METHOD OF MAKING SCARIFICATIONS FOR CUTANEOUS ALLERGIC TESTS WITH DALAND LANCET

(From Kolmer, *Infection, Immunity and Biologic Therapy,* W. B. Saunders Co.)

Technic of Cutaneous Tests. These are done preferably on the skin of the forearm or inner aspect of the arm in adults, and on the skin of the back in infants or young children. The skin is cleansed with alcohol and dried. A small superficial abrasion about ⅛ inch long is made through the epidermis by a small needle, a sharply pointed scalpel or a lancet (Fig. 36). Care should be taken not to draw blood. When more than one test is being done, the entire series of scratches should be made at one time in rows of four to eight. The scratches should be spaced at least ½ inch apart to insure clear definition of positive reactions. One of the scratches should be kept for a control—this usually is placed in the cubital fossa between the linear rows.

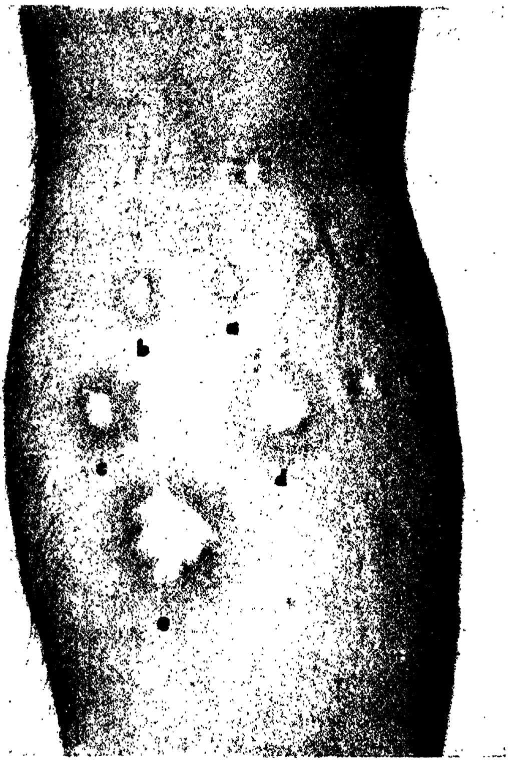

FIG. 37. SKIN REACTIONS AFTER SCRATCH TESTS WITH ALLERGENIC EXTRACTS

a, (negative or control); *b*, ± (doubtful); *c*, + (slight); *d*, ++ (moderate); *e*, +++ (marked). (From Kolmer and Tuft, *Clinical Immunology, Biotherapy and Chemotherapy*, W. B. Saunders Co.)

If the allergen is a liquid or a paste, it may be applied directly to the scratch. Very little rubbing is necessary following the application, since the scratch will usually absorb the material applied to it. If the allergenic extract is in powder form, a drop of a diluent which may be either tenth-normal sodium hydroxide or normal salt solution should be placed upon each scratch. The powdered extract is then added to the diluent from the blunt end of a toothpick, employing just enough of the powder to cover the end of the toothpick. This should then be mixed with the diluent by means of the toothpick and allowed to remain on the scratch. The control site should receive only the drop of diluent.

Reactions usually reach their maximum in from fifteen to thirty minutes, with an average of twenty. The material should therefore be left on the scratch until the reaction develops and then wiped off, so that the reaction may be read. Reactions may develop rather quickly in highly sensitive individuals and it is advisable to wipe off the material as soon as they are observed. This will prevent the remote possibility of a constitutional reaction.

Reactions may be recorded as follows (Fig. 37):

Negative (—)	No appreciable increase in size of wheal or areola as compared to control.
Doubtful (±)	Wheal less than 0.5 cm., slight to moderate areola or area of redness.
Slightly positive (+)	Wheal 0.5 cm.; moderate areola.
Moderately positive (++)	Wheal 0.5 to 1 cm. and without pseudopodia; moderate to marked areola.
Markedly positive (+++)	Wheal 1 cm. or more with pseudopodia and moderate to marked areola.

The reactions shown in Figure 37 were observed in pollen tests which are usually conducted by the cutaneous method. This method is likewise usually preferred in testing for food allergy if extreme hypersensitiveness is suspected (Fig. 38); otherwise intracutaneous tests are preferred. Cutaneous tests are likewise usually employed for the detection of drug allergies (Fig. 39).

Technic of Intracutaneous Tests. The skin of the outer aspect of the arm has been found more satisfactory and convenient for this type of testing than any other part of the body, although the skin of the forearm or back likewise may be employed when necessary. It is important that the arm or forearm be freed of any constricting bands, such as is often formed by rolling up a tightly fitting sleeve. The obstruction of the venous circulation produced by this "cuff" tends to inhibit or lessen the intensity of the skin reaction. The skin is cleansed with alcohol and dried. A very small amount (0.01 to 0.02 cc.) of the sterile liquid allergen (dilute extracts usually employed for routine testing) is injected by a tuberculin syringe into the outer layer of the skin (Fig. 34). Care should be taken that the needle is introduced into and not through the epidermis. This may be obviated by introducing the bevel of the needle far enough to pick the skin up with the point and then injecting the liquid extract. Following the injection of the material a small whitish pinpoint elevation or wheal should be visible (Fig. 34). As in the scratch technic, the tests should be arranged in rows of four to six, with at least ½ inch between tests. Six to twelve tests usually are performed at a sitting and although a large number may be done at one time without danger, it is not advisable, particularly with pollens or inhalants, because of the greater danger of constitutional reactions. The number of tests for young children at one time varies between four and eight.

Reactions are usually complete in from five to fifteen minutes, with an average of ten minutes, and should be read at the end of that period. An injection of the diluent constitutes the control site although in most instances a negative reaction may be used as the control. It is advisable, whenever possible, to recheck all positive reactions, employing the next higher concentration of the extract for those allergens giving only doubtful or slight reactions upon skin test.

The readings are made in the same manner as in the scratch or cutaneous tests. In some patients there may be no immediate reaction to the skin test, but within several hours (usually within twenty-four hours) there appears about the site of the test a zone of redness or erythema of varying size and with a tendency to persist for a longer period than the immediate skin reaction. This is known as a delayed reaction, and though it is recorded in the same way as immediate reactions, its exact significance is not known. Positive delayed skin reactions to food allergens are of occasion clinical importance.

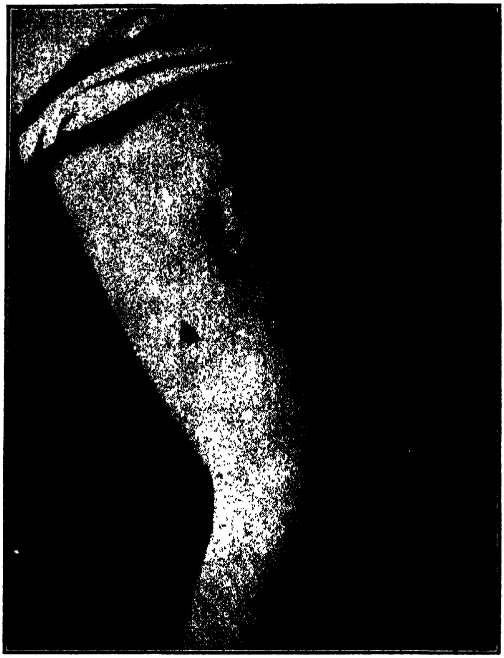

FIG. 38. POSITIVE CUTANEOUS REACTION TO BUCKWHEAT

Photograph one-half hour after applying the test.

Technic of the Ophthalmic or Conjunctival Test. One or two drops of the solution to be tested, or a minute amount of dried extract (usually pollen), is instilled into the conjunctival sac of one eye, the opposite eye being used as a control. Reactions, if positive, occur within five to ten minutes and are characterized by a definite redness due to injection of the conjunctival vessels and by considerable itching and lacrimation. Ordinarily, the reaction disappears within a few hours. When the reaction is marked, swelling of the conjunctiva or of the lower lid may occur and persist for twenty-four or forty-eight hours. Uncomfortable reactions are controlled readily by instillation of a drop or two of adrenalin (1:1000) into the eye. Reactions are read in the same manner as skin tests, although the degree of the reaction is more difficult to determine.

Technic of the Nasal Test. This test is performed either by spraying the liquid allergen into the nose or by holding the powdered allergen (*e.g.*, pollen) close to the nostrils upon the blunt end of a toothpick and having the patient inhale it, or by direct application of the allergen to the nasal mucosa. The reaction will manifest itself in sensitive patients by subjective symptoms such as sneezing and watery discharge or cough. Proper controls should be used to exclude a possible nonspecific reaction.

Technic of the Patch Test. The suspected agent is applied directly to the cleansed skin by means of a small square (¼ to ⅜ inch) of linen or blotting paper soaked in the solution or covered with the material in an ointment. If the agent is a powder, it should be moistened with normal saline or distilled water after being placed on the linen. After applying the suspected agent to the skin in this manner, the material is then covered with a large piece of rubber tissue, cellophane or oiled skin which in turn is held in place by a still larger piece of adhesive (1 to 2 inches in diameter). When many patch tests are being done it is convenient to have the adhesive already prepared with the cellophane, silk, or rubber covering adhering to its inner surface; this may then be applied directly over the piece of linen square containing the testing solution. It is important to make sure that the patch stays in place by employing fresh adhesive or applying thin strips to the edges. When the skin of the patient is sensitive or excessively irritated by the adhesive, plain cellophane (No. 600) may be employed instead of adhesive and the edges kept in place or sealed by flexible collodion or by a liquid nonirritant adhesive.

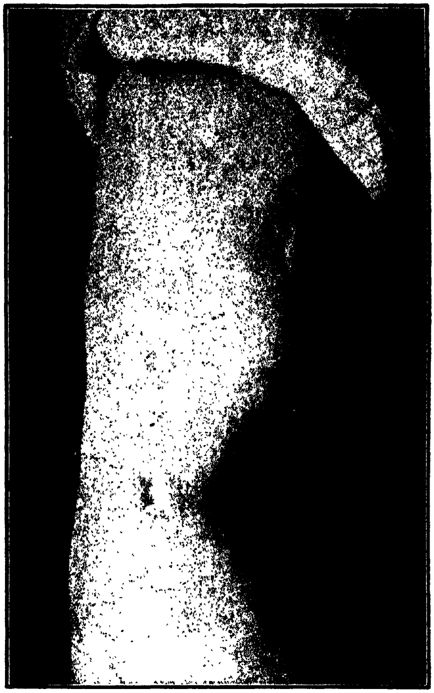

FIG. 39. POSITIVE CUTANEOUS REACTION TO QUININE

Photograph fifteen minutes after the application of quinine bisulfate. Upper, positive reaction; lower, control.

The patch may be applied to the skin of either the back, arm, or forearm. The back is very convenient for application of large numbers (10, 20, or more) of patch tests at a single sitting and is preferred for that purpose. When multiple patches are being used, the site of each patch should be marked on the patient's skin with indelible pencil or silver nitrate for future follow-up. If only a few patch tests are to be applied, the skin of the forearm or arm on the flexor surface is more convenient and also more sensitive.

The patch should be allowed to remain in place at least twenty-four hours unless it proves too irritating to the skin. If intense itching occurs before that time the patch should be removed. Occasionally it is necessary to leave it in place forty-eight or seventy-two hours before a reaction is obtained. On removing the patch, the reaction at the site of the test should be inspected. If no reaction is observed. daily observations should be made for the first few days and then at intervals over a period of two weeks before the reaction is called negative; delayed reactions sometimes occur with certain substances like trichophytin, and unless this precaution is observed, they will be missed.

A definitely positive reaction is indicated by the presence at the site of the patch of an erythematous area in which vesicles of varying size are present. The most typical reaction is one which reproduces the original lesion of the patient's dermatosis (Fig. 40). Negative reactions show no changes at the site of the patch. Occasionally a doubtful reaction may result. This consists of an area of redness without the appearance of any vesicles and should be watched for further change. If there is none, the test should be repeated with the patch left in place for a longer period of time.

It is important in reading the reactions to distinguish between a positive patch test to the agent being tested and that which may be due at times to the adhesive plaster. The latter reaction usually appears at the periphery of the tested area. It consists of an erythematous and papular eruption, develops more quickly than the specific reaction and disappears in a short time without leaving any evidence of its presence and without developing vesicles. It can be readily distinguished by the application to another area of the skin of a similar control patch without the use of the specific contactant. Most reactions to adhesive plaster are nonspecific in character. In a small proportion, however, there exists a specific sensitivity to the gum resin or rubber constituents of the plaster.

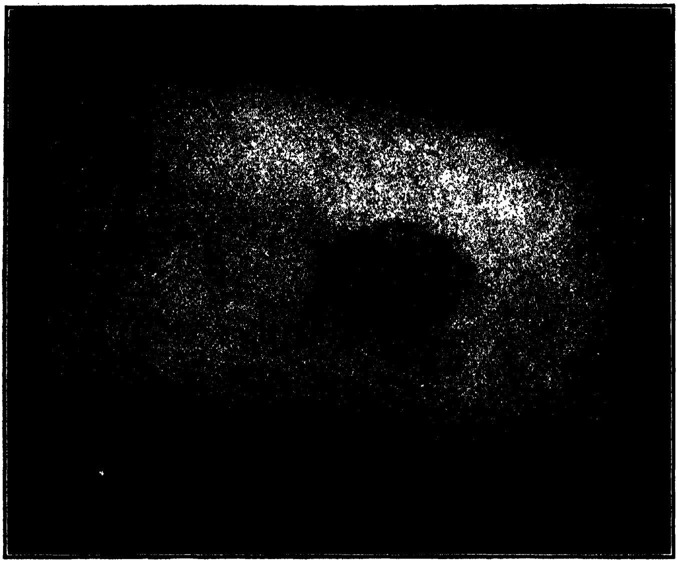

FIG. 40. POSITIVE PATCH REACTION TO FORMALIN

(From Kolmer and Tuft, *Clinical Immunology, Biotherapy and Chemotherapy*, W. B. Saunders Co.)

The Clinical Interpretation of Skin Tests; Therapeutic or Clinical Trial. While the technic and reading of skin tests are comparatively simple, their clinical interpretation requires skill, experience and sound judgment. A *positive reaction* simply means that the skin is specifically sensitive to the allergen employed; it does not necessarily imply that the patient is clinically sensitive to it with the production of signs and symptoms of allergic disease. In other words, positive reactions may occur in the absence of any definitely determinable clinical sensitivity to allergens. They may represent in some patients a sensitivity acquired in the past but bearing no relationship to their present clinical condition. However, positive skin reactions may likewise be the forerunners of future or potential clinical sensitivity. For example, positive reactions to ragweed pollen may be exhibited by children at varying intervals prior to the onset of the clinical manifestations of hay fever. Needless to state, great care is required in interpreting skin reactions in individuals with "dermographic skins," in whom mechanical injury from scratches or intracutaneous injections of various nonspecific agents may produce erythema and wheals. Therefore, controls are particularly important in this group.

Of equal importance is the interpretation of ***negative reactions.*** They may simply mean that the skin is without sensitivity and not necessarily imply that the patient is free of clinical sensitivity. This is particularly true in allergy to foods suggesting, although not proving, that clinical allergy may exist to digestion products rather than to a food itself. But such falsely negative skin reactions may also occur, although much less frequently, in those allergies with a hereditary factor as hay fever, allergic rhinitis and asthma due to inhalant allergens. Furthermore, in a small percentage of patients, especially in older individuals or those who have had their condition a long time, the skin may be of the nonreactive type and react poorly. Slight reactions under these conditions may, therefore, be just as important clinically as marked reactions in a more reactive type—hence, even the slightest increase in the size of the erythema and wheal should be regarded important until proved otherwise and checked by employing higher concentrations of the allergen.

Under the conditions, before conclusions are reached, it is sometimes necessary to resort to *therapeutic* or *clinical trial* in checking the results of both positive and negative skin tests. For example, in the case of positive reactions this may be accomplished either by noting the effect of elimination of the suspected agent or of exposure to it in relation to symptoms. Thus, if symptoms disappear when a patient is removed to a hospital and recur upon return home, it is a fair deduction that sensitivity to such an environmental factor as dust is important in the production of symptoms. Or, more importantly, when falsely negative reactions are suspected in individuals who are apparently allergic to foods, one may resort to trial or elimination diets, as, for example, those suggested by Rowe to discover foods responsible for symptoms.

LABORATORY EXAMINATIONS IN ALLERGIC DISEASES

When differential leukocyte counts show an ***eosinophilia,*** it is considered additional evidence of allergy if no other cause is apparent, although its absence does not necessarily exclude it.

Examinations of *nasal smears* for eosinophils are sometimes of aid in differentiating between rhinitis due to allergy and that due to infection. Proper collection of the secretions to obtain mucus is important. Frequent examinations may be required. An accurate differential count of smears, stained in the same manner as blood smears, may be difficult but usually the percentages of eosinophils and polymorphonuclear neutrophils may be determined. An excess of eosinophils suggests allergic rhinitis while an excess of neutrophils is indicative of infection. An excess of eosinophils with many neutrophils indicates an acute or chronic infection superimposed upon an allergic rhinitis. There is, however, no direct or proportional relationship between the eosinophils in the nasal secretions and those in the blood—the latter may or may not be increased in such patients without detracting from the value of the examination.

An additional laboratory aid in the diagnosis of allergy in patients with negative skin reactions, or with positive reactions that do not appear to be specific or in whom diet trial is difficult to conduct, is the *leukopenic index* described by Vaughan. It is similar to the hemoclastic test of Widal, Abrami and Iancovesco for liver function which, however, is not now generally employed. It is based on the occurrence of leukopenia instead of leukocytosis after the ingestion of food to which the individual is allergic. Because of the laboriousness of the technic, its chief usefulness is as an aid in the diagnosis of food allergy, particularly with skin-test positive foods in which there is doubt as to their etiologic importance and also with those which tend to give false negative reactions. The test may be conducted by the following method:

The patient reports to the office in the morning without breakfast. Two total leukocyte counts are made at ten-minute intervals. The patient then eats an average amount of the food to be tested. Following this, total leukocyte counts are made at fifteen-minute intervals for an hour. A final count is made at the end of an hour and a half. The same pipet and counting chamber are used for each examination. A range of fluctuation of 1000 leukocytes per c.mm. of blood, up or down, from the average of the premeal counts, is regarded as normal. A decrease of more than 1000 is considered positive. A decrease of less than 1000, if it occurs in all or nearly all of the counts, is considered suggestive or borderline.

SKIN TESTS IN THE DIAGNOSIS OF SERUM ALLERGY

Special mention should be made of skin tests for the detection of *serum allergy* in view of their importance in relation to the use of horse or other normal and immune serums for the prophylaxis and treatment of disease. *It is never safe or advisable to give serum of any kind other than human serum to an individual with a history of asthma until such tests are conducted*, since hypersensitiveness, and particularly natural allergy, may produce severe and even fatal reactions unless special precautions or methods of administration are employed.

If natural allergy is suspected, as in the case of asthma to horses, a cutaneous or scratch test is safe and usually sufficient. If acquired allergy is suspected resulting from a previous injection of an antitoxin, including T-A, or other immune serums, an intracutaneous test is preferred. This consists of the injection of 0.02 cc. of a 1:10 dilution of *normal horse serum;* it is inadvisable to use the immune serum to be administered. If a positive reaction results, the ophthalmic test should be conducted. This consists in the instillation of one drop of undiluted serum in the case of those giving slightly positive intracutaneous reactions. If stronger reactions are observed, it is better to use one drop of a 1:10 or 1:100 dilution of serum.

SKIN TESTS IN THE DIAGNOSIS OF BACTERIAL DISEASES

As previously stated, human beings may acquire allergic sensitization to some of the pathogenic bacteria and other living agents of disease during the course of acute, chronic and clinically unrecognized infections (Table 118). As an example of the latter, the allergic sensitization to products of the diphtheria bacillus, sometimes encountered among older children and adults, produces pseudoreactions in the Schick test. Whether or not natural allergy to some of the bacteria may exist cannot

TABLE 118. SUMMARY OF THE CLINICAL INTERPRETATION OF SKIN TESTS IN THE DIAGNOSIS OF INFECTIOUS DISEASES

Tests	Interpretation
General Considerations	Allergic sensitization may be acquired to many of the pathogenic bacteria during the course of acute and chronic diseases as well as from clinically unrecognized infections with special reference to tuberculosis, undulant fever and those due to focal infection. The allergy may produce symptoms (asthma, urticaria, arthritis, etc.) and its detection by skin tests is frequently of clinical value in diagnosis. Natural allergy may apparently exist to some of the pathogenic bacteria capable of producing excessive and dangerous reactions following the administration of vaccines for prophylactic or therapeutic purposes. Therefore, if administered to allergic individuals (especially asthmatics), due precautions should be taken. Allergic sensitization is also frequently acquired in the mycotic infections of the skin and mucous membranes in which skin tests sometimes possess diagnostic value. Allergic sensitization detectable by skin tests may occur in diseases due to the filtrable viruses as in lymphogranuloma venereum and mumps. Allergic sensitization may be acquired in many diseases due to animal parasites and especially in the helminthic infestments. Skin tests possess diagnostic value, especially in trichinosis and hydatid cyst disease. Allergic sensitization may also occur to the venins of bees, mosquitoes, flies and other insects yielding positive skin reactions sometimes of diagnostic value. Acquired allergic sensitization may be related to immunity, especially in smallpox, tuberculosis, undulant fever and tularemia.
Tuberculosis	Cutaneous tests (von Pirquet) highly specific but sometimes lacking in sufficient sensitivity. Intracutaneous tests (Mantoux) preferred; conducted with old tuberculin (O.T.) or better with the purified protein derivative (P.P.D.). The Vollmer patch test is reliable and useful. *Positive reactions* occur not only in clinically active tuberculosis but likewise in minor and healed tuberculosis; of greater diagnostic value in relation to active tuberculosis in infants and children under five years of age than in older children and adults. *Negative reactions* are indicative of the absence of tuberculin allergy and tuberculous infection except in the very early stages of tuberculosis. Falsely negative reactions may occur in fulminating tuberculosis and likewise in the poorly nourished and aged as well as during measles and other acute infectious diseases.
Brucellosis	*Positive reactions* following the intracutaneous injection of brucellergen are frequently of diagnostic value in association with positive agglutination and opsonocytophagic reactions but may not occur until late in the disease; may also occur in the absence of clinically detectable evidences of undulant fever and especially among those exposed to infection. *Negative reactions* do not exclude the possibility of undulant fever.

TABLE 118. SUMMARY OF THE CLINICAL INTERPRETATION OF SKIN TESTS IN THE DIAGNOSIS OF INFECTIOUS DISEASES (Continued)

Tests	Interpretation
Tularemia	Intracutaneous tests may be conducted with killed suspensions of *Past. tularensis* (Foshay). ***Positive reactions*** may occur as early as the third or fourth days of the disease. They may also occur years after recovery. *Negative reactions* do not exclude the disease.
Glanders	The ***mallein test*** is very useful for the detection of glanders in the lower animals but its value in human beings cannot be stated.
Allergic Diseases and Focal Infections	Intracutaneous tests preferred consisting of the injection of 0.1 cc. of heat-killed suspensions of bacteria carrying 100 million per cc. in a menstruum of saline solution. Autogenous suspensions preferred to stock suspensions. Cutaneous tests conducted with stock powders of dried organisms usually unsatisfactory because lacking in sensitivity. Positive reactions of more clinical significance than negative reactions. Tests advised before the administration of typhoid-paratyphoid or other vaccines for prophylactic purposes to allergic individuals (especially asthmatics).
Lympho-granuloma Venereum	Skin tests conducted by the intracutaneous injection of 0.1 cc. of 1:10 suspensions of sterilized pus (*Frei test*) or suspensions of brain of infected mice are valuable diagnostic aids although nonspecific reactions may occur from extraneous material. Suspensions of the cultivated virus are preferred in dose of 0.1 cc. because they are equally sensitive and more specific. Positive reactions, characterized by papules and erythema, occur in 90 to 95 per cent of cases although negative reactions may occur in the early stages of the disease. Positive reactions continue after recovery. A thermal reaction produced by the intravenous injection of mouse brain antigen has also been found of helpful diagnostic value.
Mumps	Positive reactions following intradermal injections of diluted heat-inactivated suspensions of the parotid glands of *rhesus* monkeys infected with the virus of mumps are indicative of immunity to the disease (Enders); the tests, however, are of no diagnostic value, since negative reactions occur during the acute stage of mumps.
Chancroid	An intracutaneous test consisting of the injection of 0.1 cc. of a heat-killed suspension of *H. ducreyi* possesses diagnostic value. Positive reactions occur in about 95 per cent of cases between eight and fifteen days after the development of lesions. Sensitivity lasts for many years and possibly for the balance of life. This may give positive reactions in individuals without chancroid at the time of the test because of previous infection. Negative reactions are of particular value in excluding active chancroidal disease.

TABLE 118. SUMMARY OF THE CLINICAL INTERPRETATION OF SKIN TESTS IN THE DIAGNOSIS OF INFECTIOUS DISEASES (Continued)

Tests	Interpretation
The Dermatophytoses	Intradermal tests employing *trichophytin* have proved of value in the diagnosis of the ringworms and other superficial dermatophytoses. The etiologic fungi, however, vary greatly in their capacity to produce allergic sensitization. Sensitization is more likely to be acquired in the deeper than in the superficial mycotic diseases of the skin. Sensitization may persist for long periods of time after apparent recovery. Nonspecific reactions may occur and especially in elderly adults. Negative reactions possess more diagnostic value, particularly in exudative inflammatory eruptions of the skin due to nonmycotic infections.
The Dermatophytids	A dermatophytid is a cutaneous eruption due, not to local infection with a fungus, but to allergic sensitization caused by infection in a focus somewhere else in the body. The intradermal trichophytin test almost invariably gives a positive reaction of great diagnostic value; it is essential for diagnosis.
Moniliasis	The *oidiomycin test* may give positive reactions in about 57 per cent of cases of cutaneous moniliasis but since 58 per cent of carriers without clinical types of moniliasis may give positive reactions as well as about 54 per cent of individuals who apparently had infection or were carriers in the past, the test has been largely abandoned as a diagnostic aid.
Coccidioidomycosis	Intradermal tests with *coccidioidin* are highly specific and of value in the diagnosis of coccidioidomycosis. Since sensitization may persist for long periods of time after recovery from primary infections of the lungs, positive reactions are not necessarily diagnostic of lesions of the skin suspected as being due to infection with *C. immitis*.
Sporotrichosis	Intradermal injections of *sporotrichin* are stated to be of aid in the diagnosis of sporotrichosis. Negative reactions are of value in excluding the disease. False positive reactions may occasionally occur.
Histoplasmosis	Intradermal tests with *histoplasmin* are of value in the diagnosis of histoplasmosis. Whether or not positive reactions in tuberculin-negative individuals with pulmonary calcinosis are indicative of previous infections with *H. capsulatum* cannot be stated at the present time. Cross skin reactions with histoplasmin, coccidioidin and haptosporangin have been reported in these individuals.
Echinococcus Disease	Intradermal tests with sterile hydatid fluid are of diagnostic value and particularly in preoperative cases of hydatid cyst disease. Pseudoreactions, however, may occur due to sensitization to sheep protein in the antigen, from trauma and in other cestode infestments. Negative reactions are valuable in excluding hydatid disease. Antigen may be obtained from the National Institute of Health.

TABLE 118. SUMMARY OF THE CLINICAL INTERPRETATION OF SKIN TESTS IN THE DIAGNOSIS OF INFECTIOUS DISEASES (Continued)

Tests	Interpretation
Trichinosis	Intradermal tests conducted with antigens prepared of *T. spiralis* have proved of great value in diagnosis. In marked cases of trichinosis positive reactions occur in from 92 to 98 per cent of cases. In mild and suspected cases the incidence of positive reactions is about 47 per cent. Positive reactions have been also observed in about 25 per cent of clinically well individuals probably due in most instances to a carrier state.
Filariasis	Intradermal tests conducted with antigens prepared of *D. immitis* give positive reactions in about 90 per cent of individuals infested with *W. bancrofti*. Similar results have been observed in loiasis and onchocerciasis.
Schisto-somiasis	Intradermal tests conducted with cercarial antigens give a high percentage of positive reactions in individuals infested with *S. haematobium*. Pseudo-positive reactions, however, may occur. Tests conducted with antigens of *S. japonicum* are particularly valuable for diagnostic purposes in endemic areas.
Ascariasis	Intradermal tests are of little or no clinical value in diagnosis.
Enterobiasis	Intradermal tests give positive reactions in a high percentage of individuals infested with *E. vermicularis*. Negative reactions, however, are of more value in excluding the disease than positive reactions in establishing its presence.
Uncinariasis	Intradermal tests have not proved of clinical value. Negative reactions are of more value in excluding the disease than positive reactions in establishing its presence.
Leish-maniasis	Intradermal tests conducted with suspensions of dead *L. tropica* almost invariably give positive reactions in oriental sore. Weakly positive reactions, however, may occur in about 10 per cent of noninfected individuals.
Trypano-somiasis	Intradermal tests conducted with suspensions of dead trypanosomes apparently possess no clinical value in the diagnosis of infestments with *T. gambiense* or other trypanosome diseases of human beings.
Drugs	Cutaneous, intracutaneous and patch tests are sometimes of value in the detection of natural and acquired allergies to drugs and other chemical agents including diodrast. They may be also employed for the detection of allergy to penicillin and streptomycin. These tests, however, may give falsely negative reactions in allergies to the sulfonamide compounds. The value of the "sulfonamide-containing serum method" of Leftwich has not been fully determined.

be stated, but the unusually severe reactions which sometimes follow the administration of typhoid-paratyphoid and other vaccines to individuals with some type of clinical allergy, suggest the possibility of its existence. For this reason, precautions are required in the administration of vaccines for prophylactic and therapeutic purposes to allergic individuals and especially asthmatics, to which further reference will be made.

While allergic sensitization acquired during the course of acute and chronic infections may not contribute to the production of signs and symptoms of disease. some believe that the joint and heart lesions of acute rheumatic fever may be allergic in character. Furthermore, in the opinion of some the rôle of chronic streptococcal, pneumococcal, or staphylococcal infections in the etiology of rheumatoid arthritis, recurrent iritis and other diseases of focal infection may be due, at least partly, to acquired allergic sensitization to the toxins or proteins of these or other micro-organisms. As previously stated, there is considerable evidence indicating that acquired allergic sensitization to hemolytic and nonhemolytic streptococci, pneumococci, staphylococci, *N. catarrhalis* and other micro-organisms occurring in chronic sinusitis or other foci of chronic infection may account for some cases of bronchial asthma as well as for urticaria and some allergic eczemas. Urticaria is especially likely to occur in acquired allergy to the proteins of animal parasites.

In many other bacterial and mycotic diseases and those due to animal parasites, however, acquired allergic sensitization is apparently clinically silent although its detection by skin tests may possess diagnostic value. This is especially true in such bacterial infections as tuberculosis, brucellosis, glanders, tularemia and chancroid. Allergic sensitization may also occur in gonococcal and *Esch. coli* infections and in typhoid fever, but so irregularly as to render skin tests of limited diagnostic value.

Whether or not acquired allergy bears a relationship to immunity in the bacterial diseases cannot be definitely stated at the present time, although there is considerable evidence indicating that this may be true. For example, the reaction occurring in one or two days after cowpox vaccination in the case of those who have had smallpox or who have been previously vaccinated, is regarded as indicative of immunity to smallpox, as originally observed by Jenner, and may be an allergic reaction to the virus. Likewise, in tuberculosis tuberculin allergy apparently enhances phagocytosis, aids in fixing the bacilli *in situ* and reduces the chances of spread of infection by leukocytes. Acquired allergic sensitization evidently plays a rôle in immunity to brucellosis since, according to Huddleson, the efficacy of brucellin in its treatment depends on the existence and continuation of a state of sensitization in the patient while under treatment.

Tuberculosis. No other living agent of disease compares with the tubercle bacillus in its capacity to produce allergic sensitization. Tuberculosis is also unique as the first bacterial disease in which hypersensitiveness was discovered by the subcutaneous injection of tuberculin by Koch. This acquired allergy to tuberculin is usually so exquisite that *positive reactions* may result from even minor and clinically unrecognized tuberculous infections as well as those apparently healed and inactive. Since the latter have been found in about 90 per cent of adults, the percentage of positive reactors increases from about 5 per cent in infants to 90 per cent in older individuals. Under the conditions, positive reactions in infants and children up to the age of five years are usually due to active tuberculous infection but, in older children and adults, they cannot be interpreted as necessarily indicative of active tuberculous infection although, of course, this may be the case. Needless to state, attempts have been made to utilize the test in a quantitative manner to determine the presence or absence of activity and in relation to healing, but these efforts have not proved successful.

Negative tuberculin reactions are of greater clinical value since they indicate the absence of tuberculous infection. Falsely negative reactions, however, may occur in the very early or incipient stage of tuberculosis before sufficient tuberculin hypersensitiveness has developed, as likewise in fulminating types of the disease due to desensitization or because the skin has become incapable of reacting (anergy). Falsely negative reactions also sometimes occur, and for unknown reasons, in the severely malnourished, in the aged, and during pregnancy, as well as during or immediately after several of the acute infectious diseases with special reference to measles, pertussis, typhoid fever and scarlet fever.

From the standpoint of *technic,* the cutaneous or scratch test of von Pirquet, employing old tuberculin, is highly specific but less sensitive than the intracutaneous test of Mantoux. Therefore, the latter is preferred and may be conducted with old tuberculin (O.T.) or the purified protein derivative (P.P.D.) of Seibert, which is preferred. If old tuberculin is employed, 0.1 cc. of a 1:10,000 dilution (0.01 mg.) is injected intracutaneously but if a negative reaction is observed a second injection of 0.1 cc. of a 1:100 dilution (1 mg.) should be made. If P.P.D. is employed, a tablet of the first test strength (0.00002 mg.) is dissolved in the vial of diluent supplied and the injection given intracutaneously, but if a negative reaction is observed, a second injection is given employing a solution of a tablet of the second strength dose (0.005 mg.).

Reactions should be read forty-eight hours later. Negative reactions show no edema with a very slight degree of erythema. Positive reactions (Fig. 41) are characterized by edema with erythema varying in degree from ± (erythema less than 5 mm. in diameter with only a trace of edema) to ++++ (marked erythema, edema and an area of necrosis).

The Vollmer *patch test* is also highly satisfactory, especially for "screening" purposes among infants and children.

Brucellosis. A skin test of aid in the diagnosis of this disease may be conducted by the intracutaneous injection of 0.1 cc. of *brucellergen* (Huddleson) composed of the protein nucleinate fraction of Brucella, after the lipid fraction has been removed. Reactions are best read forty-eight hours later and recorded in the same manner as tuberculin reactions.

Positive reactions are characterized by circumscribed areas of erythema, induration and edema (Fig. 42), sometimes accompanied by systemic reactions, which may persist for a week or longer. Frequently, they do not occur until the disease is well developed with positive agglutination reactions. But in the absence of the latter, they are usually indicative of the disease, provided there is a markedly positive opsonocytophagic reaction of the blood at the same time. Positive agglutination, brucellergen and opsonocytophagic reactions are practically

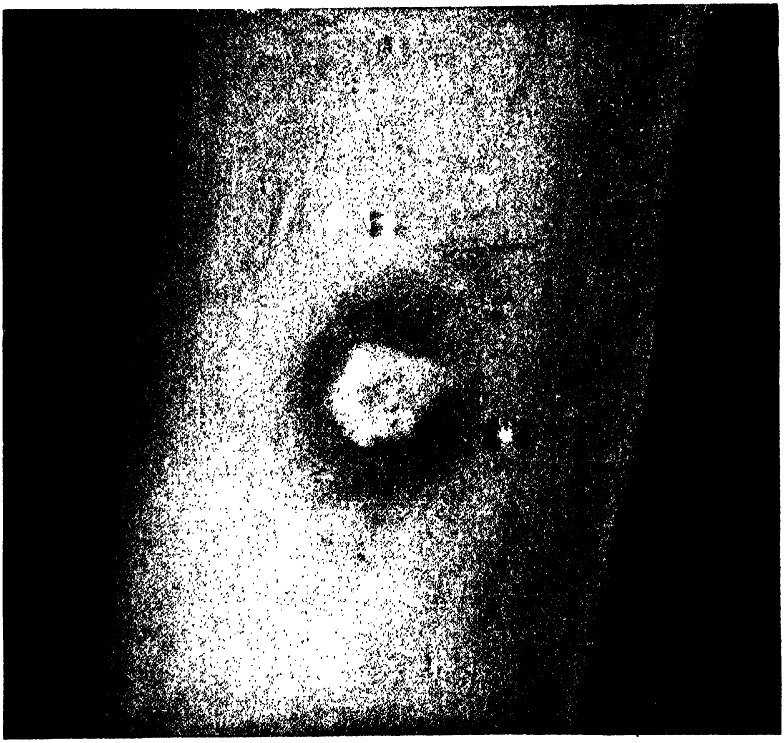

Fig. 41. Positive Tuberculin Reaction (Mantoux)

Twenty-four hours after the intracutaneous injection of 0.1 cc. of 1:1000 dilution of P.P.D. tuberculin.

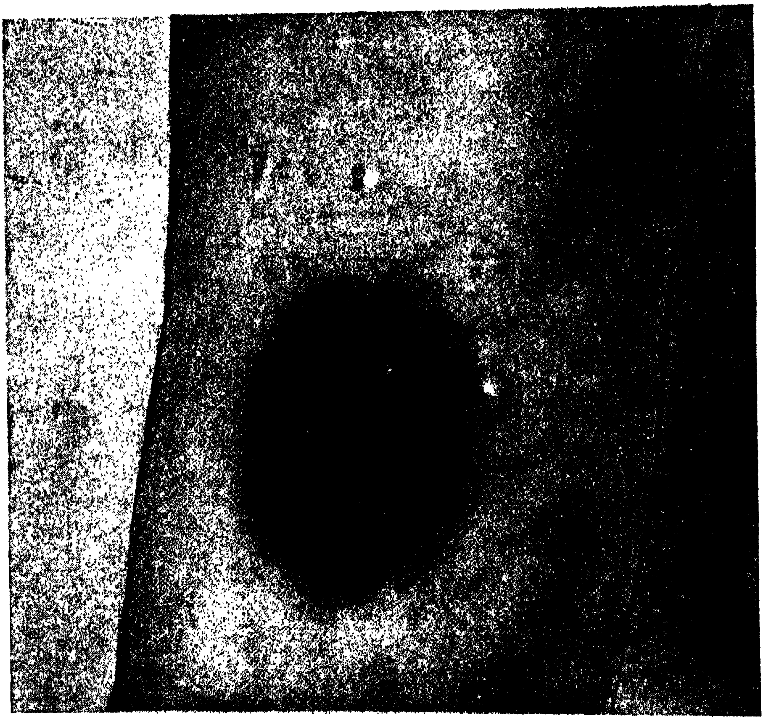

Fig. 42. Positive Brucellergen Skin Reaction

FIG. 43. POSITIVE SKIN REACTION IN TULAREMIA

Reaction 36 hours after the intracutaneous injection of killed suspension of *Past. tularensis*.

conclusive evidence of infection. As in tuberculosis, however, anywhere from 6 to 10 per cent positive reactions may occur in the absence of clinically recognizable undulant fever, apparently due to minor infections, and especially among those who, like veterinarians and laboratory workers, are particularly exposed to infection, and those accustomed to the use of raw milk. Allergic sensitization also tends to persist with positive reactions occurring during convalescence and for long periods of time after apparent recovery probably due to persistent infection. *Negative* reactions indicate the absence of allergic sensitization but not necessarily the absence of infection, since they may occur in individuals with brucellosis presenting such definite and conclusive evidences of infection as positive blood cultures.

Tularemia. Allergic sensitization to *Past. tularensis* is apparently acquired early in the course of tularemia. For this reason skin tests conducted by the intracutaneous injection of killed suspensions of the organism (Foshay) are sometimes of value in early diagnosis, as positive reactions are stated to occur on the third or fourth days of the disease (Fig. 43). As a general rule, however, agglutination tests are preferred. Negative reactions do not exclude the disease. Since the allergy usually persists for years after recovery, positive reactions do not necessarily mean active disease. When they are observed, however, in the presence of suspicious lesions, the latter is usually the proper interpretation. Apparently cross-reactions with suspensions of *Br. abortus* and *Br. melitensis* have not been observed, although they share a common antigenic constituent with *Past. tularensis.*

Glanders. In suspected *glanders* the mallein skin test may be employed, but owing to the rarity of the disease among human beings, it has not been used sufficiently to appraise its diagnostic value although it has been found by veterinarians to be a useful procedure in the detection of glanders among the lower animals.

Chancroid. It is now well established that allergic sensitization to *H. ducreyi* may be acquired in the course of chancroid. Consequently, an intradermal test has been proposed as an aid in the diagnosis of the disease.

The antigen formerly used was prepared by diluting pus aspirated from buboes with saline solution and sterilizing with heat.[1] At the present time it is prepared of cultures of *H. ducreyi* suspended in saline solution to carry about one billion per cubic centimeter and heated for thirty minutes at 60° C.[2]

The test is conducted by the intradermal injection of 0.1 cc. and the reaction read after seventy-two hours. It is stated that positive reactions occur between eight and fifteen days after the appearance of the local lesion.[3] About 95 per cent of cases of chancroid give positive reactions.[4] Allergic sensitization apparently endures for many years and possibly for the balance of life. Consequently, this may account for the positive reactions observed in some cases of lymphogranuloma venereum, lymphogranuloma inguinale and normal individuals in whom chancroidal infection was present at some time in the past.

Under the circumstances, bacteriologic examination of the pus for *H. ducreyi* appears to be more helpful than the skin test in the diagnosis of early or acute chancroidal lesions. In the case of venereal lesions, present for two weeks or longer, however, negative skin reactions are of value in excluding chancroidal infection although, as previously stated, positive reactions do not always indicate its presence as they may be due to previous infections with *H. ducreyi*. According to Dienst and Gilkerson,[5] the test is valuable in the differential diagnosis between chancroid and lymphogranuloma venereum although positive reactions in chancroid may not occur unless there is considerable infection of the regional lymph nodes.

Focal Infections. Skin tests for the detection of bacterial allergy in asthma, allergic rhinitis, urticaria and other allergic diseases and those due to *focal infection* are better conducted with autogenous suspensions of organisms recovered in cultures than with stock preparations because of the frequency of immunologic specific strains, with special reference to streptococci and pneumococci. Cutaneous tests conducted with stock preparations of the dried powdered organisms may be employed but are so greatly lacking in sensitivity that intracutaneous injections of heat-killed suspensions are greatly preferred and indeed necessary. Ordinarily these should carry approximately 100 million heat-killed organisms per cc. in a menstruum of saline solution. With the intracutaneous injection of 0.1 cc. as the test dose it is always advisable to give a control injection at the same time of 0.1 cc. of sterile saline solution carrying the same amount of phenol or other preservative. Positive reactions due to allergy usually occur within an hour but may be delayed for a day or two. Negative reactions do not necessarily exclude the possibility of allergic sensitization so that under the conditions positive reactions have greater clinical significance than negative reactions.

As previously stated, due care is required in the administration of stock vaccines for prophylactic purposes in allergic individuals and especially asthmatics, with particular reference to typhoid-paratyphoid vaccine. Under these circumstances, it is advisable first to conduct an intracutaneous test consisting of the injection of 0.1 cc. of a 1:10 or 1:100 dilution of the vaccine as dispensed. In case of positive reactions, immunization should be conducted by a series of injections with gradually increasing amounts of vaccine instead of the usual three injections.

SKIN TESTS IN THE DIAGNOSIS OF MYCOTIC DISEASES

Allergic sensitization may also occur in various mycotic diseases of the skin and mucous membranes due to infection with the pathogenic fungi with special reference to the dermatomycoses, dermatophytids, sporotrichosis, and coccidioidomycosis (Table 118). Allergenic extracts of some of these are available for diagnostic skin tests and treatment purposes with special reference to trichophytin.

The Dermatophytoses. Skin tests conducted by the intradermal injection of *trichophytin* have proved valuable along with clinical and laboratory examinations in the diagnosis and prognosis of many of the superficial mycotic diseases of the skin and scalp like the ringworms and other dermatophytoses such as tinea pedis ("athlete's foot"), tinea manuum, etc. They cannot take the place of mycologic examinations but may yield information which cannot otherwise be obtained.

A very large literature has accumulated on the subject which has been thoroughly reviewed by Lewis and Hopper.[6] Undoubtedly, many fungi infecting the skin produce allergic sensitization but vary in their capacity to do so. Thus *A. schoenleini, T. purpureum* and *T. cruris* are quite low in sensitizing capacity while *T. crateriform, M. lanosum* and *T. gypseum* are quite high.[6] Consequently, few patients having an infection due to *T. purpureum* react to trichophytin, while the majority infected by *T. gypseum* give positive reactions.

Furthermore, it is probable that the fungous infection must be severe enough to promote an inflammatory reaction for the production of allergic sensitization and for this reason positive skin reactions are more likely to be observed in the more severe and deeper dermatophytoses than in the superficial ones. It also appears that once sensitization has occurred it may persist for many years so that positive reactions may be observed in individuals without manifest infections although thorough search frequently reveals traces of infection, since spontaneous recovery from most fungous infections is rare.

Trichophytin, however, apparently may produce nonspecific reactions among individuals with inflammatory rashes which are not due to fungous infections. This is especially likely to occur in individuals 50 years or older, among whom the incidence may be as high as 35 per cent. In children and individuals under 20 years of age, however, the incidence is much lower (15 per cent) so that the diagnostic value of the test is highest in this age group.[6] Not infrequently positive reactions to trichophytin in individuals in whom microscopic and cultural examinations have proved negative for pathogenic fungi are due to previous treatment so that positive skin reactions under such circumstanc s indicate further laboratory examinations. But, undoubtedly, positive reactions may occur in some nonmycotic diseases of the skin although there is the possibility of their being due to previous mycotic infections.

Under the circumstances, the intradermal trichophytin test is to be regarded as highly specific. Certainly the majority of patients with primarily inflammatory mycotic infections proved by microscopic and cultural examinations give positive reactions. Negative reactions, however, possess even greater diagnostic value when the physician is trying to decide whether an inflammatory eruption is of mycotic origin. If the rash is of several weeks' duration (which would allow ample time for sensitization), if neither microscopic nor cultural examinations show fungi, and if the intradermal test gives a negative result, an exudative inflammatory eruption may be regarded as nonmycotic in origin.[6]

Trichophytin may be prepared of a single fungus like *T. gypseum* because all of the fungi producing the dermatophytoses appear to share a common allergen. However, apparently all likewise contain allergens more or less specific for the species. Consequently, it appears that trichophytin is best prepared of a mixture of three or four of the fungi like *T. gypseum, T. violaceum, M. lanosum* and *M. audouini*. As shown by Peck and Glick,[7] the skin-reacting factor is not necessarily identical with the desensitizing factor or substance.

Various preparations are commercially available. The test dose is usually 0.1 cc. of a dilution (usually 1:30 or 1:100) capable of producing reactions in sensitive individuals. The syringe and needle should be freshly sterilized by boiling. The trichophytin should be sterile. The readings should be made after 10 to 15 minutes (for an immediate wheal reaction which is especially apt to occur in infections with *T. purpureum*), after 48 hours (for the usual eczematous reaction) and again at the end of one week (Fig. 44). A control injection of sterile broth is advisable. Strongly positive reactions should lead to conservative methods of treatment since the prognosis is favorable; [5] when there is an exudative dermatitis the use of roentgen rays may be considered. Temporary falsely negative reactions may occur during some of the acute infectious diseases. Patch tests have also been employed but are not as satisfactory as intradermal tests.

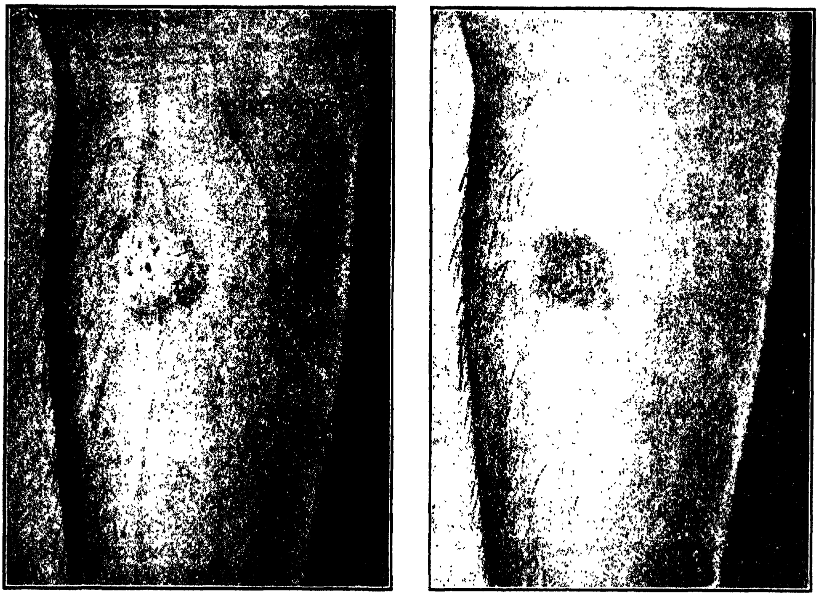

FIG. 44. POSITIVE SKIN REACTIONS TO TRICHOPHYTIN

A, immediate wheal type of reaction observed fifteen minutes after the injection of trichophytin; *B*, eczematous type of reaction after forty-eight hours.

Favin is a similar preparation of *A. schoenleini* but since this fungus is low in sensitizing capacity, the incidence of positive reactions is only about 20 per cent.

The Dermatophytids. A dermatophytid is a cutaneous lesion, frequently vesicular in character, due to allergic sensitization by a fungus producing infection in some other part of the body. The primary focus may be so small or inconspicuous as easily to escape clinical detection. The dermatophytids, therefore, are secondary lesions due to allergy. Consequently, fungi are not found in them by microscopic or cultural examinations.

The intradermal trichophytin test almost invariably gives a positive reaction and is, therefore, of great diagnostic value; indeed, it is required for establishing the diagnosis of dermatophytid. Irritation of the original focus sometimes causes an exacerbation of the cutaneous eruption and the same may occur after an injection of trichophytin. Lewis and Hopper [6] have reported 87 per cent positive reactions in a group of individuals with eruptions on the hands in which *T. gypseum* was isolated from the feet. In some of the patients with negative reactions the rashes on the hands were regarded as nonmycotic in origin.

Moniliasis. *Oidiomycin* is a similar preparation of *Candida albicans* injected intradermally as an aid in the diagnosis of cutaneous and other types of moniliasis. It apparently yields specific reactions in about 57 per cent of cases of cutaneous infections.[6] However, positive reactions have been observed also in as high as 58 per cent of carriers without lesions but with positive cultures for *C. albicans* in material from the tongue, skin or feces evidently responsible for sensitization.[4] Furthermore, about 54 per cent positive reactions have been observed among individuals without lesions and with negative cultures [6] which are also apparently due to sensitization sometime in the past. Consequently, the oidiomycin test has been largely abandoned as a diagnostic procedure and particularly since *C. albicans* is readily detected by microscopic and cultural examinations (Chap. 16).

Coccidioidomycosis. Dickson [8] has found intradermal injections of *coccidioidin* prepared of cultures of *C. immitis* of value in the diagnosis of coccidioidomycosis. The reaction is stated to be highly specific. But since sensitization may persist for long periods of time after recovery from primary infections of the lungs, positive reactions are not necessarily diagnostic of lesions of the skin suspected of being due to coccidioidomycoses. In conducting the test, the needles and syringes should not have been used for similar tests with trichophytin or other antigens. Haynes and Hess,[9] who have recently reviewed the literature, have reported 2.66 per cent positive reactions in a group of 413 individuals comprising nurses, medical students, and general hospital patients.

Sporotrichosis. Intradermal injections of *sporotrichin* prepared of cultures of *S. schenckii* are believed to be of aid in the diagnosis of sporotrichosis; indeed, negative reactions are stated to rule out the disease (Bloch). Falsely positive reactions, however, may occasionally occur and result in diagnostic errors.

Histoplasmosis. Van Pernis and his colleagues [10] have recently reported that intradermal injections of 0.1 cc. of a sterile broth culture filtrates of *Histoplasma capsulatum* undiluted, 1:10, 1:100 and 1:1000 produced specific immediate and delayed (18 to 24 hours) reactions in a case of systemic histoplasmosis.

Similar observations were made simultaneously and independently by Zarafonetis and Lindberg.[11] Positive reactions present both edema and erythema, the former having a diameter of at least 5 mm. Palmer,[12] after an extensive study of nontuberculous pulmonary calcification in tuberculin-negative student nurses, was of the opinion that positive reactions to histoplasmin should be regarded as evidence of previous infection with *H. capsulatum* or an immunologically related organism. Christie and Peterson [13] were of the same opinion and suggested that an antigen extract of the yeast form of the organism might logically be expected to give more satisfactory results than the broth filtrate with its extraneous antigens. According to Curtis and Grekin,[14] however, a positive histoplasmin reaction is of little or no significance although negative reactions are of aid in eliminating histoplasmosis in pulmonary disease. The value of the test has also been questioned by Olson and his associates [15] although Sontag and Allen,[16] as well as Waring and Gregg [17] believe that there is a relation between histoplasmin sensitivity and pulmonary calcinosis in tuberculin negative individuals. According to High and his associates,[18] disseminated bilateral pulmonary calcifications occur more frequently among individuals giving positive histoplasmin reactions than among tuberculin positive persons. In this connection, Groover and his colleagues [19] have reported 96 per cent positive tuberculin reactions, 58.6 per cent positive histoplasmin reactions and 31.1 per cent positive coccidioidin reactions in 1220 individuals with roentgen changes compatible with that of pulmonary tuberculosis. Under the circumstances, it appears that the histoplasmin skin test is of only limited value in the differential diagnosis of pulmonary disease. Calcinosis may be due to benign infections with *H. capsulatum* but cross skin reactions with histoplasmin, coccidioidin and haptosporangin do not permit this conclusion at the present time. Swerling and Palmer [20] have postulated that some disease other than tuberculosis and coccidioidomycosis accounts for a considerable number of cases in the United States of pulmonary calcinosis which apparently produces sensitivity to histoplasmin.

SKIN TESTS IN THE DIAGNOSIS OF VIRAL DISEASES

Whether or not allergic sensitization occurs to the pathogenic rickettsiae cannot be stated. Positive skin reactions have been reported as occurring in some cases of typhus fever but their diagnostic value has not been appraised.

Apparently, however, allergic sensitization may occur in some diseases due to the filtrable viruses. All efforts to demonstrate its presence by skin tests in anterior poliomyelitis and the encephalitides have met with failure although allergic sensitization occurs so characteristically in lymphogranuloma venereum and mumps as to render skin tests of practical diagnostic value (Table 118).

Lymphogranuloma Venereum. This disease, which is also known as lymphopathia venereum, tropical or climatic bubo and Nicolas-Favre disease, ranks third to syphilis and gonorrhea among the venereal diseases. It should not be confused with lymphogranuloma inguinale (due to infection with the Leishman-Donovan bodies), chancroid, or syphilis which present similar clinical manifestations. It is characterized by suppurative and nonsuppurative inguinal adenitis, acute and chronic proctitis (especially in women), fistulas, and acute and chronic ulcerations of the external genitalia with elephantiasis of the vulva. As originally shown by Frei, a skin test has proved highly specific and of great value in diagnosis, conducted by the intracutaneous injection of 0.1 cc. of a 1:10 dilution of sterilized pus removed by aspiration from an unruptured inguinal bubo. The reaction is read forty-eight to seventy-two hours later. A positive reaction consists of an inflammatory papule with erythema and is stated to occur in 95 per cent of cases with buboes, or 90 per cent with ulcerative lesions (Fig. 45). Negative reactions, however, may occur in the early stages of the disease. Unfortunately, nonspecific reactions occur and may be as high as 30 per cent. These reduce the practical value of the test, as it is difficult to use a control antigen.

Furthermore, since the antigen is of human origin it is frequently difficult to secure. Antigens prepared of the brains of mice infected with the virus have

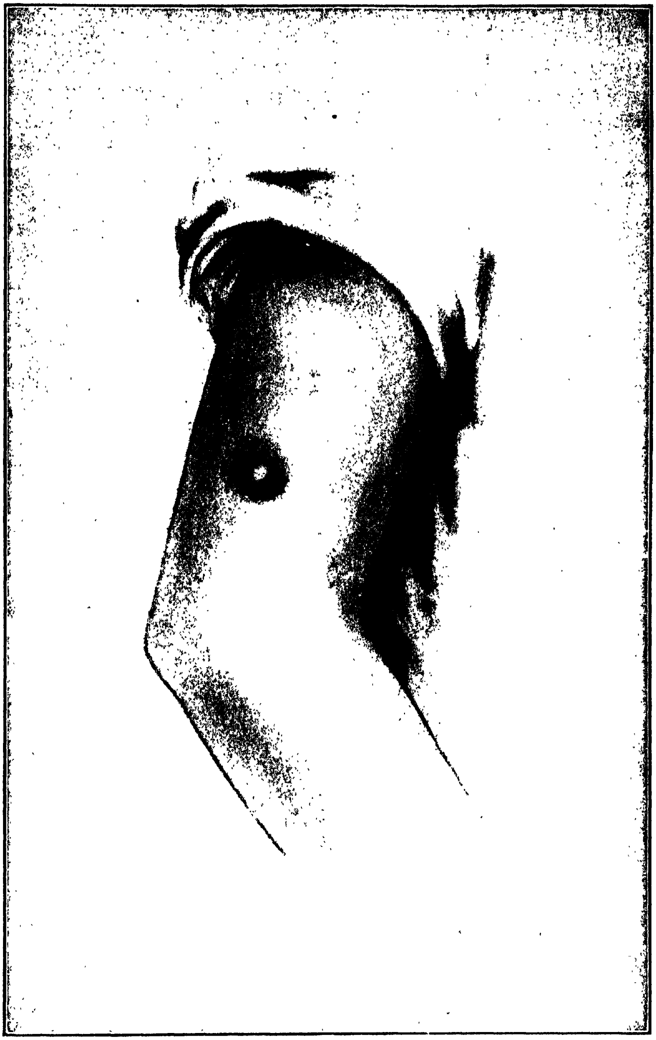

FIG. 45. POSITIVE FREI REACTION IN LYMPHOGRANULOMA VENEREUM
Pustular Type.

apparently proved more satisfactory and are commercially available. However, they may likewise produce nonspecific reactions so that it is always advisable to use a control antigen of normal mouse brain.

The virus has been successfully cultivated by the method of Rake, McKee and Shaffer in the egg yolk sac of the developing chick embryo, and by differential centrifugation may be obtained in a high state of purity. Suspensions in 0.1 per cent formalin are slightly hazy and almost colorless solutions with so little extraneous matter that intracutaneous injections of 0.1 cc. are not nearly as likely to produce nonspecific reactions as suspensions of sterilized pus or mouse brain. This type of antigen, therefore, is strongly recommended and is commercially available (lygranum). Positive reactions are characterized by reddish papules 6 mm. or more in diameter, surrounded by a fainter areola of erythema of varying size. The papule is the important part of the reaction and should be measured with a millimeter scale. Strongly positive reactions may show pustules or vesicles. Reactions gradually regress, leaving a faintly pigmented area with or without a slight scar.

The test may be also conducted by injecting 0.1 cc. of the Frei antigen of sterilized human pus intravenously. Positive reactions consist of a thermal reaction characterized by the production of a fever of 101° F., or higher, over a period of twelve hours or longer. Recently, mouse brain antigen in the same dose has been found more satisfactory and capable of yielding positive thermal reactions in about 95 per cent of cases of the disease.[21,22] Intravenous injections of similar amounts of antigen prepared of normal mouse brain are stated to produce similar reactions in about 17 per cent of cases of the disease; naturally one may surmise that these are nonspecific reactions produced by the proteins of the antigens, although it is stated that normal individuals do not show them. Intravenous injections of the virus cultivated in the chick embryo medium, however, have been found less satisfactory.[22]

Mumps. In 1943, Enders [23] reported the occurrence of skin reactions in children following the intradermal injection of a diluted heat-inactivated suspension of the parotid glands of monkeys infected with the virus of mumps. Reactions of erythema measuring 1–4 cm. in diameter and often slightly indurated developed in 24 to 48 hours. Negative reactions occurred during the acute phase of the disease but subsequent investigations have shown that persons exhibiting reactions exceeding 10 mm. in mean diameter may be, from the practical standpoint, regarded as resistant to mumps. In general, it is expected that the attack rate of the disease in exposed groups will be low when the incidence of positive reactions exceeds 50 per cent. The results have also shown that subclinical infections by the virus are frequent, accounting in young adults for about 35 per cent of past infections. In adults, the test has proved in most instances a more sensitive indicator of past infection, and hence of immunity, than the complement fixation test. Preliminary tests employing antigen prepared from the amniotic membranes of infected chick embryos have also given positive skin reactions in individuals hypersensitive to the virus or its products.

SKIN TESTS IN THE DIAGNOSIS OF DISEASES DUE TO ANIMAL PARASITES

Allergic sensitization also occurs in many diseases due to infestation with animal parasites (Table 118). This is especially true in hydatid cyst disease due to *Echinococcus granulosus* and trichinosis due to *Trichinella spiralis*, in both of

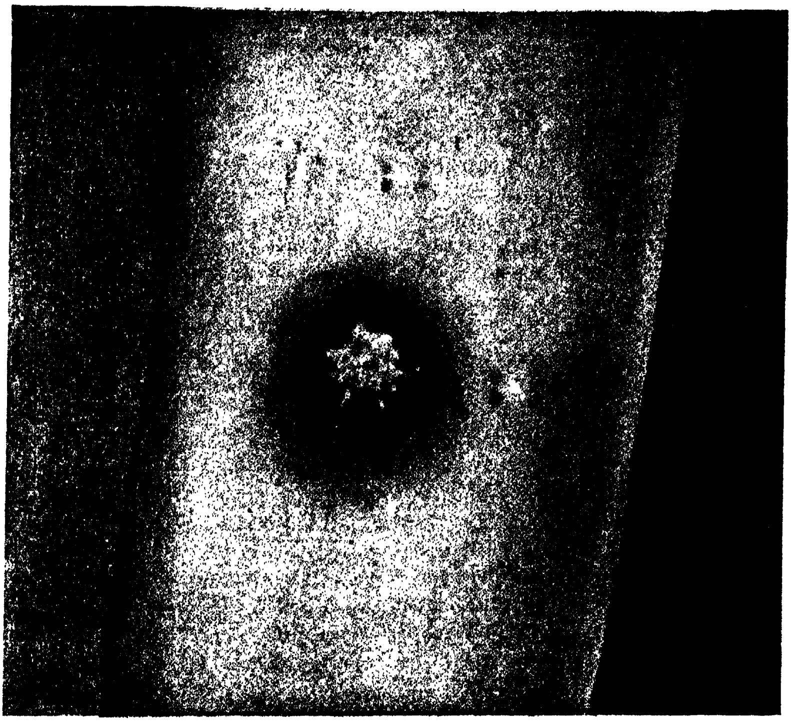

FIG. 46. POSITIVE INTRACUTANEOUS REACTION IN ECHINOCOCCUS DISEASE

which skin tests are frequently of diagnostic value. Likewise in some of the other helminthic diseases such as filariasis and schistosomiasis, although skin tests in these are not usually employed for diagnostic purposes in view of the irregularity of positive reactions, the difficulties of preparing or securing suitable extracts for intracutaneous injection and the greater ease of diagnosis by laboratory examinations.

Natural or acquired allergic sensitization may occur to the venins of bees, mosquitoes, house flies, sand flies, etc., which undoubtedly contribute very materially to the severity of local and constitutional reactions following bites. Extracts are commercially available for skin tests and treatment by desensitization.

Echinococcus Disease. An intracutaneous test has proved of value in the diagnosis of this disease,[21] although its clinical application may be hampered by difficulties experienced in obtaining a suitable antigen for its conduct. The latter is generally prepared of pooled sterile hydatid fluids obtained by puncture of unilocular hydatid cysts of sheep, pigs, oxen or human beings. After filtration and culture for sterility it is placed in ampules which, when kept in a refrigerator, are ready for use up to six months. With antigen supplied by the National Institute of Health, the technic and reactions are as follows:

The usual test dose is 0.01 cc. of 1:10,000 dilution. It is advisable to include a control consisting of the intracutaneous injection of 0.01 cc. of sterile saline solution. A positive reaction is of the immediate type, appearing usually within fifteen to twenty minutes after the injection of the antigen. In rare cases there may be a delayed reaction which does not reach its height before twenty-four hours. It is characterized by the formation of a wheal with a diameter larger by 3 mm. or more than that of the control, with or without pseudopodia. The wheal is usually surrounded by a zone of hyperemia, but this is not as important as the size of the wheal and the presence of pseudopodia (Fig. 46). The test is particularly useful preoperatively. In postoperative cases positive reactions may be observed over long periods of time. Positive reactions, however, are not absolutely indicative of the disease, since falsely positive or pseudoreactions may occur from hypersensitiveness to sheep protein in the antigen, from trauma, and in other cestode infestments. A negative reaction is valuable, but not conclusive, evidence for excluding hydatid disease.

Trichinosis. An intradermal test has proved of value in the diagnosis of infestment with *T. spiralis* and especially in mild cases with only vague symptoms. Indeed, the incidence of positive reactions with antigens prepared by different methods in clinically ill individuals has been as high as 92 to about 98 per cent,[25-29] so that the skin test is one of the most valuable aids in diagnosis. About 47.2 per cent of individuals with suspected trichinosis have been reported to react positively as well as about 24.8 per cent of normal controls,[29] suggesting that allergic sensitization of the skin may occur in mild infestments and even carriers of *T. spiralis*.

As previously stated, various methods for the preparation of the antigen have been described. That recently described by McNaught and his co-workers[29] is to be recommended. With this antigen the test dose is 0.1 cc. of a 1:10,000 dilution injected intradermally. A control injection of 0.1 cc. of sterile buffered saline solution is given at the same time.

During the second or third weeks of the disease an immediate reaction consisting of a wheal with a zone of hyperemia is commonly observed (Fig. 47). During the first few days of the disease, and especially in long-standing quiescent cases, a delayed reaction usually occurs, reaching a maximum size of 1 to 3 cm. in 20 to 24 hours and resembling a mild tuberculin reaction which then subsides.

According to Frisch and his colleagues,[30] the test is a valuable aid in diagnosis, provided typical wheals occur. The incidence of positive reactions, however, was found to vary a great deal according to the antigens employed. Best results were observed with an alkaline extract of the larvae from which the heat-precipitable material had been removed. Immediate reactions were observed in 28 per cent of 152 presumably infected but asymptomatic individuals; these reactions, however, were observed in only 0 to 6 per cent of 89 uninfected persons.

Filariasis. Intradermal tests conducted with antigens prepared of canine *Dirofilaria immitis* have proved of clinical value, since positive reactions occur in about 90 per cent of cases of filariasis due to infestment with *Wuchereria bancrofti*.[31] The reactions are usually of the immediate type but delayed reactions characterized by marked edema resembling Calabar swellings may occur. Similar results have been observed in *loiasis* and *onchocerciasis* with this antigen as well as with antigen prepared of *Onchocerca volvulus* so that the reactions are group-specific for filariae. Positive cutaneous reactions have been reported in *dracunculiasis* many years after recovery. In 215 individuals in British Guiana, Wharton [32] observed 89.8 per cent positive reactions, 5.1 per cent negative reactions and 5.1 per cent indeterminate reactions; 22 out of 23 children between 3 and 10 years of age reacted positively, indicating a general, early infection of the population. Twenty-six of 29 old elephantoid cases also gave positive reactions. These tests were conducted by the intracutaneous injection of 0.02 cc. of 1:100,000 of *D. immitis* antigen which was found highly reliable when freshly prepared. Intracutaneous injections of a corresponding dilution of dog serum were used as controls.

Schistosomiasis. Intradermal tests conducted with cercarial antigens prepared of snails infested with *Schistosoma spindale* or *S. bovis* give a high percentage of positive reactions in schistosomiasis of human beings due to *S. haematobium* and in a relatively small percentage of pseudopositive reactions in noninfested individuals due to hypersensitiveness to the proteins of the snail. Similar results have been reported with cercarial antigens prepared of *S. mansoni* [33] so that the reactions are of a group character. Intracutaneous tests, however, possess some diagnostic value and antigens prepared of *S. japonicum* have been particularly recommended for field work in endemic areas, since positive reactions occur but rarely in noninfested individuals. Undoubtedly, positive reactions continue to occur over long periods of time after apparent recovery.

Ascariasis. Positive intradermal reactions may also occur following the injection of extracts of Ascaris antigen. However, they are of little or no clinical value not only because diagnosis is made so readily by examinations of the feces for *A. lumbricoides* or its ova, but because individuals with no history or evidence of infestment may give positive reactions while those with the infestment may give negative reactions. This lack of correlation between hypersensitiveness and ascariasis is attributed to the toxicity of the antigen. True positive reactions are rare

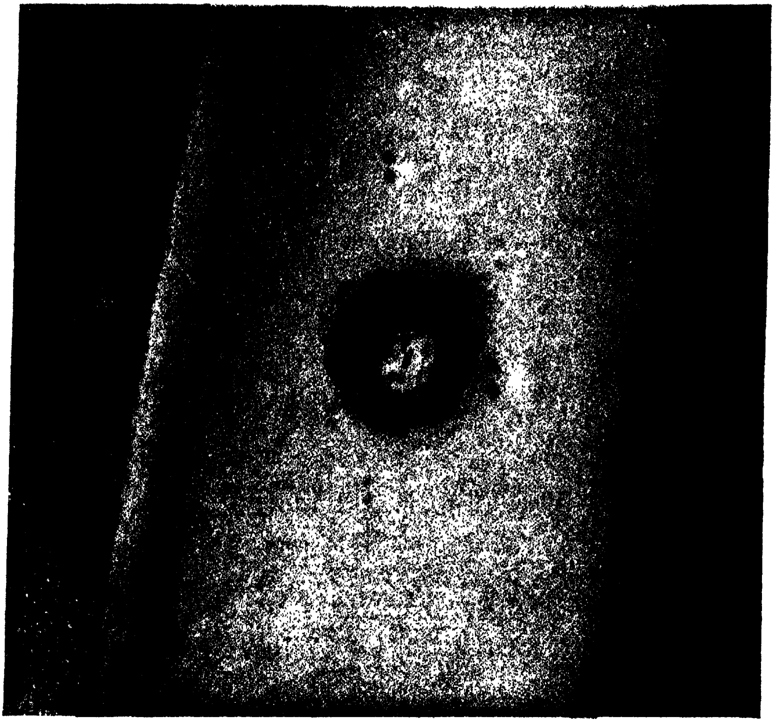

Fig. 47. Positive Skin Reaction in Trichinosis

in children under one year and in adults over 40 years of age. Continuous infestment over long periods of time, therefore, does not necessarily produce hypersensitiveness. Furthermore, the reactions to Ascaris antigen are of a group character as positive reactions may also occur in individuals infested with *Enterobius vermicularis* and *Trichuris trichiura.*

Enterobiasis. In this disease due to infestment with *E. vermicularis* immediate reactions, although absent in early infestments, occur in a high percentage of infested individuals. In low dilutions antigens prepared from adult worms give cross-reactions with other nematodes, but are fairly specific in high dilutions. Negative reactions are more valuable in excluding the disease than positive ones in establishing its presence.

Uncinariasis. In uncinariasis, or hook-worm disease, intradermal tests have not proved satisfactory for diagnostic purposes. Hypersensitiveness may occur in a variable percentage of infested individuals and persists indefinitely after the elimination of the parasite. Under the circumstances, negative reactions possess more clinical value than positive reactions but are seldom required for diagnostic purposes, since examinations of the feces for the ova, especially by concentration methods, are highly satisfactory.

Leishmaniasis. Intradermal tests conducted with phenolized saline suspensions of dead *L. tropica* almost invariably give positive reactions in oriental sore but may also give weakly positive reactions in about 10 per cent of noninfected individuals. Incidentally, Dwork [34] states that twenty-four cases of cutaneous leishmaniasis have been reported in the United States and Canada to which he has added four others. As stated by Dwork, one may expect that an influx into endemic areas of groups, such as expeditionary armed forces, which do not possess the immunity conferred by previous attacks, will be followed by the appearance of the disease in these groups.

Trypanosomiasis. Intradermal tests have also been conducted with antigen of trypanosomes recovered from the blood of infected rats, but apparently possess no diagnostic value in *T. gambiense* or other trypanosome diseases of human beings.

Pregnancy. Many attempts have been made to develop a diagnostic skin test for pregnancy. One of the most recent is that of Falls and his co-workers [35] consisting of the intradermal injection of colostrum obtained from primiparous pregnant women after at least twenty-eight weeks of pregnancy, diluted twice with sterile saline solution and preserved with merthiolate.

The test is conducted by injecting 0.02 cc. intradermally and reading the reactions at intervals of ten, thirty and sixty minutes. A papular elevation without a pinkish areola is considered a negative reaction and indicative of pregnancy, since the test is based on the assumption that sensitivity with positive reactions occurs during the nonpregnant state, with the absence of sensitivity during pregnancy.

The clinical value of the test, however, cannot be stated at the present time. Goldman and his colleagues [36] have recently reported 70 per cent correctly negative reactions in pregnancy and about 70 per cent correctly positive reactions in nonpregnancies. If these results are confirmed it would appear, therefore, that this intradermal colostrum test is of but limited value as a diagnostic procedure in pregnancy.

Drug Allergies. Skin tests are frequently indicated or required in cases of suspected natural or acquired allergies to various drugs and other chemical agents. Cutaneous or scratch tests may be employed by the local application of 0.1 to 1.0 per cent solutions in the case of soluble compounds. Otherwise, small amounts of powders may be applied. Intracutaneous tests are more sensitive and may be conducted by the injection of 0.1 cc. of 1:100 to 1:1000 sterile solutions; the reactions are usually of the immediate type. Patch tests may also be conducted employing solutions, powders or ointments of the compound and especially in cases of contact dermatitis.

In skin tests for suspected allergy to *penicillin*[37] the cutaneous method may be employed in which the powder or a solution in saline carrying 250 units per cc. may be applied. Intracutaneous tests, however, are preferred in which case 0.1 cc. of a sterile solution containing 1000 to 2000 units per cc. may be employed; the reactions are usually of the immediate type. Patch tests have been employed but may give falsely negative reactions, except in cases of contact dermatitis. Cutaneous and intracutaneous tests may also be employed in cases of suspected or acquired allergy to *streptomycin*.

Cutaneous tests, however, have usually proved unsuccessful in the detection of natural or acquired allergy to the *sulfonamide compounds* unless exquisite sensitization is present. Indeed, the same has been generally true of intracutaneous tests employing simple solutions of the compounds, although several investigators have reported positive reactions when the compounds were applied in patch tests. Leftwich,[38] however, has reported successful results with a method consisting of the intracutaneous injection of serum secured from individuals who had been receiving sulfonamide orally for at least 5 days in dosage to yield a concentration of the "free" compound in excess of 3 mg. per 100 cc. The reactions were of the immediate type, occurring within an hour after injection. The value of this "sulfonamide-containing serum method," however, cannot be stated at the present time and has been reported as ineffective in children.[39]

Since systemic reactions may follow the intravenous administration of *diodrast* for excretory pyelography, which are frequently disturbing and occasionally serious, Naterman and Robins[40] have stated that natural allergy to this substance may be one cause for their occurrence. Consequently, they advise that an intradermal test, consisting of the injection of 0.05 cc., should be done on all patients before diodrast is injected intravenously. A strongly positive reaction is characterized by the development of a wheal larger than 15 mm.; in such cases intravenous injections may give serious reactions and require that precautionary measures be taken.

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20

THE CLINICAL INTERPRETATION OF EXAMINATIONS FOR HORMONES AND VITAMINS

HORMONES

Hormones are chemical substances secreted by the ductless glands which, when carried to other glands and tissues of the body by the blood, stimulate or inhibit their functional activities. They are known as *hormones,* a name derived from the Greek word meaning to excite, because they are predominantly excitatory in nature. The ductless glands are nine in number, comprising the pituitary, pineal, thyroid, parathyroids, thymus, the islands of Langerhans of the pancreas, adrenals, testicles and ovaries (Table 119).

Apparently, however, other organs may likewise secrete hormones concerned in their own physiologic activities or those of other organs. Thus, the placenta apparently produces a hormone which, acting through the mediation of the ovaries, ensures its own physiologic integrity, although this hormone may be secreted elsewhere and merely stored in the placenta. Hormones are also secreted by the stomach and duodenum and possibly by the liver, spleen and other organs.

Hormones are not stored in the body except, possibly, in the glands producing them. Their secretion is never constant but only according to immediate needs. They cannot initiate any activity on the part of another gland but only stimulate its natural activities through a catalyzing-like effect. A hormone, however, does not stimulate the gland producing it and apparently it cannot inhibit the functional activities of another gland directly but may accomplish this indirectly by leaving the gland more or less to its own slow activities, by permitting the action of antihormones or through the medium of the autonomic and peripheral nervous systems. Some of the hormones have been isolated and definitely identified. Others are known to be produced on the basis of the results of investigations among the lower animals and by studies on human beings in whom one or other gland is known to be deficient or overactive.

The response of other glands, organs or tissues to a hormone depends on any one or a combination of factors as follows: (1) The quantity and, possibly, the quality of the hormones; (2) the capacity or degree of tissue response on the part of the organ upon which it acts (very important); (3) the state of the nervous system if the effects of the hormone are through this medium; (4) the condition of the blood as a vehicle of its transmission; (5) the influence of heredity; (6) the presence or absence of other hormones influencing its activity; (7) the presence or absence of antihormones, the existence of which has now been fairly well established; and (8) upon an adequate supply of certain vitamins concerned in the production of hormones.

TABLE 119. SUMMARY OF THE GENERAL ASPECTS OF THE HORMONES AND VITAMINS

Substances	Clinical and Laboratory
Hormones	Chemical substances secreted by the ductless glands which stimulate or inhibit the functional activities of other glands or tissues. They cannot initiate any activity on the part of another gland but only regulate its natural activities. Under normal conditions their secretions are never constant but only according to immediate needs. They are not stored in the body except, possibly, in the glands producing them. The response of other glands to their influence is conditioned by many factors of which tissue responsiveness is very important. An increased or decreased production of the hormones results in the production of a large and varied number of diseases or symptom-complexes. Remarkable success has attended the treatment of many diseases due to deficiencies. Practical laboratory examinations are available for the detection and estimation of but few of the hormones occurring in the blood or excreted in the urine. But various other laboratory methods are available as aids in the diagnosis of diseases or syndromes due to their increased or decreased excretion.
Vitamins	Accessory food factors essential to normal nutrition and growth. Although the body cannot make them it must have them through the ingestion of vitamin-containing foods. Many have been shown to be active fractions of enzymes essential for the breakdown of foodstuffs in intracellular metabolism by which the energy of foods is rendered available. A variety of metabolic disturbances in the avitaminoses are due to interference with oxidation-reduction processes. The first effects of vitamin deficiency may or may not produce symptoms with no signs due to anatomic changes and constituting the subclinical avitaminoses. Severe and prolonged deficiencies produce anatomic lesions with classic signs in the case of many of them. Practical laboratory examinations of the urine, blood, or both, are available as aids in the detection of some of the vitamin deficiencies with special reference to vitamins A, C, E and K and some of the factors of vitamin B complex (thiamine, riboflavin, nicotinic acid and pantothenic acid). The close relationship between the chemical and physiologic properties of the hormones and vitamins is becoming increasingly apparent.

An abnormal increase, decrease or complete absence of physiologically important hormones is capable of producing dysfunctions of many of the body systems and thus of a large number of diseases or symptom-complexes included in the subject of endocrinology. This is due to the fact that any one ductless gland is but rarely affected alone. Also, the less well-defined dysfunctions are particularly likely to produce many-sided clinical syndromes. Remarkable success has attended the treatment of diseases and syndromes due to deficiencies in the production of hormones and especially by the parenteral administration of the latter. With the exception of the hormone of the thyroid gland their oral administration, however, has been generally less effective or entirely ineffective. The treatment of diseases and syndromes due to the overproduction of hormones has been much less satisfactory.

Unfortunately, laboratory methods for the determination of the hormones occurring in the blood, or excreted in the urine, are only available in the case of the gonadotropic hormones produced by the anterior lobe of the pituitary gland, the hormones of the ovary and the hormones or androgens of the testicles. But various other laboratory methods are available for the detection of an increased or decreased production of some of the hormones as, for example, the basal metabolism test in relation to the thyrotropic hormone of the pituitary gland as well as of the thyroid gland hormone itself, blood glucose and glucose tolerance tests in relation to the ketogenic and adrenotropic hormones of the pituitary gland and of adrenalin of the adrenal gland, blood calcium and phosphorus determination in relation to parathormone, etc.

VITAMINS

Vitamins are accessory food factors which, in however small amounts, are essential or vital to life by reason of maintaining proper nutrition and growth (Table 119). Though the body cannot make them, it must have them through the ingestion of vitamin-containing foods. The amounts required are so ridiculously small that it is probable they act as catalysts. At least, various of the vitamins have been shown to be active fractions of enzymes which are essential for the breakdown of foodstuffs in intracellular metabolism. They have likewise been shown to have chemical structures which permit them to function in the transfer of hydrogen and phosphorus by which the energy of food is progressively liberated for the use of the individual cell. Consequently, a variety of metabolic and physiologic disturbances have now been associated with the avitaminoses directly traceable to interference with cellular oxidation-reduction processes.

Under the circumstances, the first effects of vitamin deficiency appear in an interference with the chemical processes of the cells. Symptoms may be produced with disturbances of normal body chemistry but physical signs due to anatomic lesions are absent. However, when the deficiency becomes more marked and prolonged, anatomic changes occur, with the production of the classic signs of the vitamin deficiency diseases. Consequently, it is likely that the avitaminoses are more prevalent than generally believed and certainly may be present in the absence of signs and symptoms of gross deficiencies. Fortunately, laboratory examinations of the urine, blood, or both, are available as clinical aids in the detection of some of the vitamin deficiencies. This is not only true of vitamins C and K but also of some of the factors belonging to vitamin B complex, especially thiamine chloride (B_1), riboflavin (B_2), nicotinic acid (P-P) and pantothenic acid.

Factors which may produce the avitaminoses are (1) inadequate intake as a result of vitamin loss due to improper cooking and processing, restricted diets, anorexia, nausea and vomiting due to gastro-intestinal disorders, congestive heart failure, pregnancy, or infectious diseases; (2) poor absorption in gastro-intestinal diseases, fistulas, obstructive jaundice or interference by mineral oil; (3) decreased utilization in hepatic disease or malignancy; (4) increased excretion during lactation or the result of administration of fluids by parenteral injection; (5) destruction by alkalis; (6) increased requirements in fever, infections, pregnancy, diabetes mellitus, hyperthyroidism or high carbohydrate diets and (7) inhibition of intestinal synthesis by the sulfonamide compounds or streptomycin by oral administration.

RELATION OF THE ACTIVITIES OF HORMONES AND VITAMINS

Since a close relationship between the physiologic activities of hormones and vitamins is becoming more and more evident, it is logical to discuss them together in the same chapter. At least, both appear to act as catalysts with a remarkably close relationship between their chemical and physiologic properties. The latter often run parallel either in the same direction as synergists or in opposite directions as antagonists.

Thus, the effects of vitamin A are not only similar to those of parathormone but a deficiency is frequently associated with atrophy of the adrenal glands, poor lactation, diabetes mellitus, dysfunctions of the testicles and ovaries. Deficiency also contributes to atrophy of the thyroid gland in males and its hypertrophy in females. Vitamin B complex deficiency may not only be a factor in hypothyroidism and gonadal hypofunction, but also in hypertrophy of the adrenal glands and atrophy of the thymus gland. A deficiency of vitamin C may not only exhaust the vitamin stored in the adrenal cortex and thereby reduce the production of adrenalin, but also exhaust that stored in the pituitary gland, thyroid gland, corpora lutea, pancreas and gonads and possibly exert an influence on their hormones. Vitamin D is directly related to calcium metabolism (fixation) and the parathyroid glands as well as to the sex functions. Vitamin E deficiency affects gonadal function, causing castration-like changes in the pituitary gland, atrophy of the testicles, and degenerative changes in the developing tissues if pregnancy occurs. Of course, constituents of food other than vitamins also have a relation to glandular function as, for example, the relation of calcium and phosphorus to the functions of the parathyroid glands and iodine to that of the thyroid gland.

THE PITUITARY HORMONES

While the total weight of the pituitary gland is only about 0.5 gm., it is nevertheless the master gland of the endocrine system because of the large number and great physiologic importance of the hormones it secretes, with special reference to those elaborated by its anterior lobe. However, none of the pituitary hormones have been completely analyzed chemically and consequently none have been prepared synthetically. Some co-operate and re-enforce while others antagonize or check the activities of hormones produced by other glands (Table 120).

Derangements may be due to an increased secretion of hormones usually caused by adenomas or to a decreased secretion resulting from atrophy occurring primarily or as the result of mechanical pressure by tumors. As a result of the confined position of the gland within the sella turcica it is likely to suffer from

TABLE 120. SUMMARY OF THE PITUITARY HORMONES

Hormones	Clinical and Laboratory Aspects
General Considerations	The pituitary gland is the most important of the endocrine system. It produces eight well-defined hormones and probably seven additional ones. None of its hormones have been completely analyzed chemically or prepared synthetically. There are no direct laboratory methods for the determination or assay of its hormones except in the case of the two gonadotropic hormones occurring in the blood and urine.
Growth Hormone	Secreted by the anterior lobe. Stimulates the growth of the bones and muscles. An *increase* may result in the production of gigantism or acromegaly; also partly responsible for the effects of Cushing's disease and hyperovarism. A *decrease* may result in dwarfism, acromicria or Simmond's disease; also plays a rôle in the etiology of Dercum's disease, the Fröhlich syndrome and hypo-ovarism.
Gonadotropic Hormones	Two hormones secreted by the anterior lobe: prolan A and prolan B. These constitute the sex-stimulating hormones. Both are increased during pregnancy. An *increase* may result in precocious sexual development of either sex. A *decrease* may result in sexual infantilism or under-development of the sexual organs and sexual characteristics.
Thyrotropic Hormone	Secreted by the anterior lobe. An *increase* may produce hyperthyroidism and a *decrease* hypothyroidism.
Parathyrotropic Hormone (?)	Secreted by the anterior lobe although its secretion has not been conclusively proved. An *increase* results in hypertrophy of the parathyroid glands with stimulation of calcium and phosphorus metabolism. This may be in relation to the etiology of osteitis fibrosa cystica and osteitis deformans. A *decrease* results in atrophy of the parathyroid glands and parathyroprival tetany.
Adrenotropic Hormone	Secreted by the anterior lobe. One fraction influences the cortex and a second fraction the medulla of the adrenal glands. An *increase* may produce hypertrophy of the medulla resulting in hyperadrenalism. A *decrease* may result in atrophy of the medulla leading to hypoadrenalism or a syndrome resembling Addison's disease.
Lactogenic Hormone	Secreted by the anterior lobe; also known as prolactin. Stimulates the secretion of milk after the ducts and acini have been prepared by estrin and progestin.
Hyperglycemic Hormone (?)	Secreted by the anterior lobe although its secretion has not been conclusively proved; also known as the diabetogenic hormone. Antagonistic to insulin. An *increase* may produce hyperglycemia and glycosuria. A *decrease* may produce hypoglycemia.

TABLE 120. SUMMARY OF THE PITUITARY HORMONES—(Continued)

Hormones	Clinical and Laboratory Aspects
Pancreatropic Hormone (?)	Secreted by the anterior lobe although its secretion has not been conclusively proved. Stimulates the growth and numbers of the islands of Langerhans. An *increase* produces an increase of insulin resulting in hypoglycemia.
Ketogenic Hormone (?)	Secreted by the anterior lobe or the pars intermedia although its secretion has not been conclusively proved. Regulates fat metabolism. A *decrease* retards or prevents the conversion of fats into glucose; may be in relation to the etiology of the Fröhlich syndrome.
Nitrogen Metabolism Hormone (?)	Secreted by the anterior lobe although its secretion has not been conclusively proved. Mechanism of action is unknown but is apparently involved in the conversion of proteins into glucose.
Erythropoietic Hormone (?)	Secreted by the anterior lobe although its secretion has not been conclusively proved; thought to be concerned in erythropoiesis.
Intermedin	Secreted by the pars intermedia: also known as the chromatophorotropic hormone. Concerned in pigmentation; it is also antidiuretic in diabetes insipidus.
Melanophoric Hormone (?)	Secreted by the pars intermedia although its secretion has not been conclusively proved. Influences pigmentation in Addison's disease and hyperthyroidism. It is also a powerful anti-diuretic. Has an influence on the adrenal glands. An *increase* may produce hyperadrenalism and a decrease hypoadrenalism.
Pitocin	Secreted by the posterior lobe; also known as oxytocin. Stimulates contraction of the uterus during parturition.
Pitressin	Secreted by the posterior lobe. On administration it raises blood pressure and relaxes the bronchial muscles with an increased respiratory rate; influences water metabolism; stimulates peristalsis and the gastro-intestinal secretions; probably antagonizes adrenalin; probably influences fat metabolism. A decrease may play a rôle in the etiology of obesity of the "pituitary type." Pituitrin is a combination of pitocin and pitressin.
Laboratory Examinations	Direct estimations of the pituitary hormones in the blood and urine are only possible in the case of the ovary-follicular stimulating hormone prolan A and of the luteinizing hormone prolan B. Blood chemistry and basal metabolic tests, however, are of clinical value in the detection of dysfunctions due to the thyrotropic, parathyrotropic, pancreatropic, adrenotropic, ketogenic, and hyperglycemic hormones.

pressure effects when one of its parts becomes enlarged. The site wherein a tumor arises and the nature of the cells of which it is composed often determine the predominant features of the resulting disease but because of pressure upon or irritation of the hypothalamus and optic chiasma any function—growth, sex, water elimination, the metabolism of carbohydrate, fat, protein, etc.—may become involved.

The hormones secreted by the anterior lobe are as follows:

1. The **growth hormone** is secreted by the acidophil cells and stimulates the growth and development of the bones and muscles. An *increase* before adolescence results in gigantism and after adolescence in acromegaly. An increase is also partly responsible for Cushing's disease or pituitary basophilism and hyperovarism.

A *decrease* in its secretion is responsible for dwarfism, acromicria and Simmonds' disease (pituitary cachexia). The Lorain-Levi type of dwarfism, however, is now thought also to involve dysfunction of the thyroid gland because there is no obesity although epiphyseal union of the bones is delayed. Decreased secretion probably also plays a rôle in the etiology of Dercum's disease (adiposa dolorosa) and hypo-ovarism. The Fröhlich syndrome (dystrophia adiposogenitalis), however, occurring as the infantile and adolescent types, is believed to be due not only to a deficiency of the growth hormone, but also to a lesion of the hypothalamus, while the Lawrence-Biedle-Moon syndrome is now thought to be due to involvement of the hypothalamus alone.

2. The **gonadotropic hormones** are two in number. One causes ripening of the follicles of the ovary, with the production of estrin in the female and the proliferation of the epithelium of the seminiferous tubules of the testes in the male; it is called "prolan A." The second stimulates the production of progestin by the corpora lutea and their luteinization in the female and the stimulation of the interstitial tissue of the testes of the male; it is called "prolan B." Vitamin E appears to be essential for their secretion and especially in women.

These hormones appear in the blood and urine at about the time of puberty. They then disappear from the urine of the male but occur in the urine of the female just before ovulation. The amount present in the blood of normal and non-pregnant females is too small for determination. They may be increased, however, in primary ovarian weakness or true hyperpituitarism with ovarian hyperfunction. In pregnancy the blood carries large amounts of both hormones and especially prolan B. The quantitative determination, therefore, of the gonadotropic hormones in the blood serum may be of value in differentiating primary and secondary gonadal disorders, as in amenorrhea and impotency.

Both hormones also occur in the urine during pregnancy comprising not only those produced by the anterior lobe of the pituitary gland but those produced by the placental (chorionic) tissue. Those produced by placental tissue, however, appear to differ from those produced by the pituitary gland, since they do not stimulate the follicles of the ovary. For this reason they have been called the "anterior-pituitary-like" hormones (A.P.L.) to which further reference will be made in the hormone diagnosis of pregnancy by the Aschheim-Zondek and Friedman tests employing urine. At present the tendency is to drop the designations "prolan A" and "prolan B" in relation to the gonadotropic hormones of the pituitary gland and to speak of "prolan" occurring in the urine of pregnant women produced by the chorionic tissue.

An *increase* in the secretion of the sex-stimulating or gonadotropic hormones of the pituitary gland results in precocious sexual development in both sexes. A *decrease* results in underdevelopment of the sex organs and secondary sexual characteristics in both sexes, designated sexual infantilism.

3. The **thyrotropic hormone.** An *increase* of this hormone may result in hyperthyroidism and a *decrease* in hypothyroidism, especially after removal of the pituitary gland.

4. The **parathyrotropic hormone.** There is little doubt that the anterior lobe of the pituitary gland influences the activities of the parathyroid glands. The existence of a parathyrotropic hormone has been disputed but it probably exists. An *increase* results in hypertrophy of the parathyroid glands, with an increase of calcium and phosphorus metabolism probably related to the etiology of von Recklinghausen's disease (osteitis fibrosa cystica) as well as to Paget's disease which may be only a phase or in relation to the former although blood calcium and phosphorus are usually normal. A *decrease* results in an atrophy of the parathyroid glands with the possible production of parathyroprivic tetany.

5. The **adrenotropic hormone** is probably to be divided into one factor influencing the cortex and a second influencing the medulla of the adrenal glands. An *increase* may produce hypertrophy of the medulla, resulting in hyperadrenalism, and a *decrease* atrophy of the medulla, with hypoadrenalism producing a syndrome resembling Addison's disease. Indeed, some cases of Addison's disease may be due primarily to a deficiency of the hormone instead of tuberculosis or some other destructive lesion of the adrenal glands.

6. The **lactogenic hormone**, or prolactin, stimulates the secretion of milk after development of the ducts and acini by estrin and progestin which are produced by the ovary.

7. The **hyperglycemic hormone,** or diabetogenic hormone, raises blood sugar by being antagonistic to insulin. Its existence has been disputed and these effects have been ascribed to the growth hormone, but a hyperglycemic hormone probably exists. Certainly the effects are not due to the thyrotropic or ketogenic hormones. An *increase* may result in hyperglycemia, glycosuria and lowered glucose tolerance. A *decrease* may result in hypoglycemia.

8. The **pancreatropic hormone** stimulates the growth and increases the numbers of the islands of Langerhans. An *increase,* therefore, produces an increase of insulin resulting in hypoglycemia. However, its secretion as a separate hormone has not been conclusively proved.

9. The **ketogenic hormone** regulates fat metabolism. Its secretion has been disputed, however, and the incomplete combustion of fats with the production of acetone and other ketone bodies ascribed to the influence of the thyrotropic hormone on the thyroid gland; however, a ketogenic hormone is apparently secreted. Some investigators believe it may be secreted by the pars intermedia instead of by the anterior lobe of the pituitary gland. A *decrease* retards or prevents the conversion of fats into glucose and may play a rôle in the production of the Fröhlich syndrome.

10. It is also thought that the anterior lobe may secrete a **nitrogen-metabolism regulating hormone.** Regulating effects, however, may be due to the thyrotropic hormone. If this hormone is secreted, the exact mechanism of its action is unknown although it appears to be involved in the conversion of proteins into glucose. An *increase* results in the excessive production of creatinine.

11. It is also thought that the anterior lobe produces an **erythropoietic hormone** concerned in erythropoiesis through stimulation of the erythropoietic tissues of the bone marrow. Its secretion, however, has not been proved.

The pars intermedia of the pituitary gland is supposed to produce two hormones as follows:

1. **Intermedin,** or the chromatophorotropic hormone, affects pigmentation and also the elimination of water in diabetes insipidus although without increasing the excretion of chloride.[1] It differs, therefore, from the antidiuretic effects of pitressin produced by the posterior lobe. It is also thought to be present in the colloid material of the posterior lobe which contains neither pitocin nor pitressin.

2. The **melanophoric hormone** also influences pigmentation but is believed to be different from intermedin. It apparently has an influence on the adrenal glands, so that an *increase,* as in basophilic adenoma, results in hypertrophy of both the cortex and medulla (producing hyperadrenalism) while a *decrease* results in atrophy or hypoadrenalism. Apparently it has an influence on pigmentation in both Addison's disease and hyperthyroidism. It also possesses a powerful antidiuretic effect. Some investigators regard this melanophore-expanding substance as a product of the posterior lobe and probably an effect of pitressin.

The hormones produced by the posterior lobe of the pituitary gland are two in number, namely, pitocin and pitressin. Pituitrin is a combination of the two.

1. **Pitocin,** or oxytocin, stimulates contraction of the uterus during parturition.

2. **Pitressin** (a) raises blood pressure on administration by contracting the smooth muscles of the arterioles although relaxing those of the bronchi and increasing the respiratory rates; (b) influences water metabolism by a diuretic and especially by an antidiuretic effect through its action on the tubular epithelium of the kidneys; (c) stimulates peristalsis by contracting the smooth muscle through its vagotonic effects as well as increasing the gastro-intestinal secretions and (d) antagonizes insulin so that its administration or increase reduces glucose tolerance probably by mobilizing glucose from the liver although this has not been proved. Apparently it also has an influence on fat metabolism by increasing the fat of the liver at the expense of that of the blood[2] although this also is a moot question. However, if pitressin possesses these properties a decrease in its production may play a rôle in the etiology of obesity of the "pituitary type."

Diabetes insipidus, therefore, may be due to a lesion of the posterior lobe resulting in a decreased production of pitressin. But since lesions involving the hypothalamus in the region of the tuber cinereum, including epidemic encephalitis, may result in polyuria it is probably more correct to regard the disease as due to a disorder affecting the integrity of the hypophyseal-hypothalamic mechanism rather than as being dependent specifically upon either the posterior lobe of the pituitary gland or the hypothalamus.

Laboratory Examinations in Pituitary Disorders. Unfortunately, the direct estimations of the pituitary hormones in the blood or urine as an aid in the clinical diagnosis of pituitary disorders are only possible in relation to the two gonadotropic hormones, prolan A and prolan B. Needless to state, they are only applicable in the case of males and nonpregnant females. In tests conducted with the blood serums of normal nonpregnant women as well as those with hypopituitarism, negative reactions are observed because of the presence of only very small amounts of the hormones.

In pregnancy an increase in the blood or urine is largely due either to the prolans or to the anterior-pituitary-like hormone (A.P.L.) produced by the placental (chorionic) tissues which is also known as the pregnancy urine factor (P.U.). The same is true in hydatidiform mole, chorionepithelioma and testicular tumors composed of malignant tissue (teratoma, epithelioma). In these conditions the presence of the hormones is usually determined by tests employing urine according to the Aschheim-Zondek or Friedman methods which are shortly to be discussed.

Prolan A, or the follicle-stimulating hormone, may be determined in the blood serum by the methods of Neustaedter or Fluhmann and in the urine by the methods of Levin and Tyndale, Katzman or Freed. Immature female white mice are employed. The presence of the follicle-stimulating hormone is indicated by the production of patency of the vaginal introitus with a change in the vaginal cells from mostly leukocytes with an occasional epithelial cell (weakly positive) to complete absence of leukocytes with mostly non-nucleated epithelial cells (strongly positive) as detected by examinations of vaginal smears. If the vaginal opening is patent but the cellular changes doubtful, the mouse may be killed and the uterus weighed, since a weight of over 15 mg. also constitutes a positive reaction.

Sections of the ovaries are also helpful, since the "ovulation" reaction shows the presence of normal ripening follicles which may rupture and then show beginning luteinization while the "lutein" reaction consists in marked luteinization of the performed "unripe" follicles with ova surrounded by luteinized cells.

Both hormones are likely to be increased along with diminished estrin in both the blood and urine of castrated women as well as during and after the menopause due to hypogonadism. An abnormal increase in nonpregnant women is evidence of hyperpituitary function as sometimes occurs in hyperthyroidism and hyperadrenalism.[3] A marked decrease or persistent absence of them, on the other hand, is indicative of hypopituitary function as seen in juveniles or as the consequence of severe infections. About 90 per cent of cases of amenorrhea and oligomenorrhea have shown abnormal hormone titers and about 50 per cent fall definitely into the class of hypogonadism with increased prolan excretion.[3] Hypopituitarism is also sometimes responsible for sterility while obesity in women may show an increased excretion of the gonadotropins indicative of a primary hypo-ovarism.

Examinations of male urine for the gonadotropic hormones are conducted only occasionally. Only increased values are regarded as significant as, for example, after castration, severe chronic orchitis, functional impotency and similar disorders. Methods for detecting them in the urine and blood have been described.[4,5,6]

Aid in the clinical detection of disturbances in the thyrotropic hormone, however, may be obtained by a determination of the basal metabolic rate; of the parathyrotropic hormone by determinations of blood calcium and phosphorus; of the hyperglycemic and pancreatropic hormones by blood glucose and glucose tolerance tests; of the ketogenic hormone by blood and urine examinations for the ketone bodies, and of the nitrogen metabolism hormone by the test for the specific dynamic action of protein.

THE OVARIAN AND PLACENTAL HORMONES

In addition to being the provider and custodian of ova, the ovaries are ductless glands producing two and possibly three hormones which alone, or in conjunction with other glands, bring about (1) the growth and development of the genital tract, mammary glands and the secondary female characteristics; (2) the initiation and maintenance of menstruation; (3) the bodily changes occurring during pregnancy and in preparation for parturition, and (4) an inhibition of the gonadotropic hormones of the anterior lobe of the pituitary gland mainly by preventing their entry into the blood. These ovarian hormones are summed up in Table 121.

1. **Estrin** or estrone, which is also known as the estrogenic hormone, is produced by the graafian follicles and corpora lutea, provided the ovaries have been stimulated by prolan A, one of the two gonadotropic hormones produced by the anterior lobe of the pituitary gland. Estrin not only supervises the first or proliferative half of the menstrual cycle, but governs the growth and development of the genital tract and the secondary sex characteristics. During pregnancy its secretion is greatly increased for the purpose of preparing the uterus for the effects of pitocin in parturition as well as promoting the development of the mammary ducts, the process being completed by the action of progestin in developing the acini and by prolactin (the pituitary lactogenic hormone) which actually stimulates the secretion of milk. Estrin is divisible into three variants, namely, (a) estradiol, (b) estrone (theelin) and (c) estriol (theelol).

The chemical structure of estrin has been well investigated and the hormone prepared synthetically. It is readily determined in the urine by assay methods employing immature female white mice or ovariectomized white rats weighing 140 gm. ± 20 gm. The mouse unit is the international unit; a rat unit is equal to 3 to 30 (average 5) international or mouse units.

In the urine, estrin variants are present in the form of free and combined estrones. They occur in the urine of children in amounts up to 50 international units per day, with larger amounts at puberty. Only very small amounts are present in the urine of adult males but women during menstrual life may excrete about 1500 units per month. However, estrin may be found in the urine of females sometimes after menstruation has stopped but is very low during old age. Its failure to appear in the urine of the mature female is indicative of ovarian failure. Increased excretion is less reliable because of the probable variation between the size of the various follicles and their luteinization. The estrogenic hormones excreted in the urine are greatly increased during pregnancy, reaching about 100,000 or more units at term. Their presence is indicative of the life of the fetus as they disappear upon its death.

An *increase* of estrin normally occurs between the time of ovulation and the onset of menstruation. It is also greatly increased during pregnancy, beginning about the eighth week. It is also frequently increased in follicular cysts of the ovary, adrenal cortical adenoma, Cushing's syndrome and granulosa cell tumors. It causes inhibition of ovulation, endometrial hyperplasia and menorrhagia. A *decrease* may result in delayed puberty, sexual infantilism, amenorrhea or hypomenorrhea.

TABLE 121. SUMMARY OF THE OVARIAN AND PLACENTAL HORMONES

Hormones	Clinical and Laboratory Aspects
Estrin	The estrogenic hormone; prepared synthetically. Secreted by the graafian follicles and corpora lutea under stimulation by prolan A of the pituitary gland. Governs the growth and development of the genital tract and the secondary sex characteristics; supervises the first or proliferative half of the menstrual cycle; prepares the uterus and promotes the development of the mammary ducts during pregnancy. Divisible into three variants: estradiol, estrone (theelin) and estriol (theelol). *Increased* normally between ovulation and the onset of menstruation; greatly increased during pregnancy. May be increased in follicular cysts of the ovary, in adrenal cortical adenoma, Cushing's syndrome and granulosa cell tumors. A decrease may result in delayed puberty, sexual infantilism and amenorrhea.
Progestin	Secreted by the corpora lutea under stimulation by prolan B of the pituitary gland. Also known as progesterone; is produced synthetically. Converted into sodium pregnandiol glucuronidate (pregnandiol). Governs the second or secretory half of the menstrual cycle; during pregnancy regulates the formation and maintenance of the decidua and placenta; renders the uterus resistant to pitocin; prepares the acini of the mammary glands; essential to nidation of the fetus. An *increase* may produce a delay in menstruation or amenorrhea; also uterine inertia during parturition. A *decrease* during pregnancy may result in abortion.
Relaxin (?)	Not definitely established. Source unknown. Supposed to produce relaxation of the ligaments of the symphysis pubis and sacro-iliac joints during latter part of pregnancy. These effects may be due to progestin.
Placental Hormone	The placenta produces or contains two hormones, estrone (theelin) and estriol (theelol). They probably constitute a single hormone differing from prolan A and prolan B of the pituitary gland and called the "anterior-pituitary-like" hormone (A.P.L.). Produced during pregnancy. Also produced in very large amounts in hydatidiform mole, chorionepithelioma and teratoma of the testes.

TABLE 121. SUMMARY OF THE OVARIAN AND PLACENTAL HORMONES—(Continued)

Hormones	Clinical and Laboratory Aspects
Laboratory Examinations	Estrin may be determined in the blood serum or urine. Pregnandiol may be determined in the urine. The "anterior-pituitary-like" hormone is best determined in the urine by the methods of Aschheim-Zondek or Friedman. Latter preferred in the diagnosis of pregnancy and especially in suspected hydatidiform mole, chorionepithelioma and malignant tumors of the testicle. *Positive reactions* occur in (1) 98 to 98.5 per cent cases of normal pregnancy; may be positive five to fourteen days after the first missed menstrual period; also (2) in about 50 per cent cases of ectopic pregnancy; (3) possibly in missed abortions if living placental tissue is present; (4) hydatidiform mole; (5) chorionepithelioma and (6) malignant tumors of the testes. Falsely positive reactions of phase I may occur in early menopause, endometrial hyperplasia, carcinoma of the uterus, primary ovarian failure and hyperthyroidism. *Negative reactions* occur in the absence of pregnancy, hydatidiform mole, chorionepithelioma and teratoma. *Falsely negative reactions* may occur (1) in normal pregnancy when the tests are conducted too early; (2) in normal and ectopic pregnancy upon death of the placenta and fetus and (3) in missed abortions upon death of the placenta.

2. **Progestin** governs the second or secretory half of the menstrual cycle. During pregnancy it regulates not only the formation and maintenance of the decidua and placenta but the proper preparation of the mammary glands for secreting milk, as well as rendering the uterus quiescent or refractory to the oxytocic effects of pitocin. It is essential to nidation of the fetus. The hormone which has been purified and crystallized is well known under the name *progesterone*.

Progestin is produced by the corpora lutea under stimulation of prolan B of the pituitary gland. It continues to be produced after pregnancy occurs, inhibits further ovulation, renders the uterus refractory to estrin and pituitrin, and allows the embryo to develop undisturbed.

An *increase* during the latter part of pregnancy may prolong the period of gestation or produce uterine inertia during labor. In nonpregnant women an increase may produce a delay in menstruation or amenorrhea. In this case a histologic (biopsy) examination of the endometrium may show unduly pronounced secretory changes. It is also likely that an increase may be responsible for prolonged lactation. A *decrease* during pregnancy may permit pitocin to cause uterine contractions with early abortion and death of the fetus due to loosening of the placental attachment.

Progestin has not been identified in either the blood or urine of human beings. However, through the action of the corpora lutea, as well as endometrial, hepatic and renal factors, it is converted into sodium pregnandiol glucuronidate (*pregnandiol*) which has been isolated from the urine in a pure and crystalline form. This differs from progestin in having a less quieting effect upon the contracting uterus. Since pregnandiol is produced only by the corpora lutea its presence in the urine indicates the presence of corpora lutea in the ovaries.

Pregnandiol, the excretion product of progestin, occurs in the urine as sodium pregnandiol glucuronidate which may be determined by the method of Venning and Browne.[7] Reinhart and his colleagues[8] observed an apparent agreement between endometrial biopsies and pregnandiol tests in 87 per cent of cases including cases of pregnancy, carcinoma of the cervix uteri, granulosum cell tumor of the ovary and pseudomucinous cystadenoma of the ovary.

A normal woman excretes from 5 to 10 mg. per liter of urine during the corpus luteum phase of the menstrual cycle and as high as 40 to 50 mg. per liter may be excreted during pregnancy, especially during the later months of this state.

3. A third hormone known as **relaxin** has also been described but its secretion has not been definitely established. It is regarded as responsible for relaxation of the ligaments of the symphysis pubis and possibly of the sacro-iliac joints in the latter part of pregnancy in preparation for labor. Its source is unknown. Nor has it been isolated. Some investigators believe that these effects are due to progestin.

Placental Hormones. As previously stated, the placental (chorionic) tissues apparently produce or contain two hormones, namely, (1) estrone or theelin and (2) estriol or theelol. Collip, employing acetone as an extracting agent, believes that estrone and estriol constitute a single hormone differing in its physiologic properties from the two gonadotropic hormones of the anterior lobe of the pituitary gland (prolan A and prolan B) and therefore designated the anterior-pituitary-like hormone (A.P.L.) or the pregnancy urine factor (P.U.). This opinion has been widely adopted. The anterior-pituitary-like hormone is also produced in hydatidiform mole, chorionepithelioma and malignant tumors of the testes with special reference to teratoma.

Laboratory Examinations for Estrin. Estrin in the blood serum may be determined by the methods of Neustaedter or Fluhmann previously mentioned for the detection of prolan A or the follicle-stimulating hormone of the anterior lobe of the pituitary gland. Estrin may also be determined in the urine by the methods of Levin and Tyndale, Katzman or Freed. However, these tests are only applicable to nonpregnant women, women without hydatidiform mole or chorionepithelioma and in men without malignant tumors of the testes. To the best of my knowledge, the tests do not differentiate between prolan A of the pituitary gland and estrin of the ovary; nor between prolan B of the pituitary gland and progestin of the ovary.

A marked decrease or persistent absence of estrin is indicative of ovarian failure and is more reliable evidence than the absence of the gonadotropins. However, the best way of determining the state of the ovarian function is by biopsy examination of the endometrium in which the changes must be interpreted in relation to the menstrual cycle and the quantitative degree of endometrial proliferation. A persistently marked increase of estrin with no gonadotropins is suggestive of hyperovarism due to hormone-producing tumors (granulosa cell) or follicular cysts of the ovary; an increase also occurs in about 50 per cent of cases of severe menorrhagia. Single determinations, however, are of but little value; it is better to conduct a series of tests at weekly intervals. An increase of estrin is also sometimes found in sterility, suggesting the possibility of habitual abortion as one of its causes. A decrease of estrin may be observed in about 70 per cent of cases of obesity, suggesting that primary or secondary hypo-ovarism may be important in the etiology of sterility in women and especially after the menopause.

The Aschheim-Zondek and Friedman Tests. The prolans, or rather the anterior-pituitary-like hormone (A.P.L.), produced by the placental (chorionic) tissue as well as by the tissues in hydatidiform mole, chorionepithelioma and teratoma of the testicles, may be determined in the blood serum by the method of Frank and Salmon, but is usually determined by urine tests according to the methods of Aschheim and Zondek (employing immature female white mice) or of Friedman (employing immature female rabbits). The latter is generally preferred. The rabbits must not weigh more than three pounds or be over four months old, although older animals may be employed, provided they have been isolated for at least two months before use, which allows sufficient time for the detection of pregnancy or for previously formed corpus haemorrhagica to disappear. Since much more of the anterior-pituitary-like hormone is excreted in the urine in hydatidiform mole, chorionepithelioma and malignancy of the testes than in pregnancy, the Friedman test is preferred as an aid in the detection of pregnancy because it can be conducted quantitatively.

Some observers, however, have stated that the concurrent use of the Aschheim-Zondek and Friedman tests results in the maximum percentage of correctly positive reactions in pregnancy.[9] Kelso[10] has described methods for conducting them in twenty-four hours and di Gioia[11] a method for conducting the Friedman test with blood serum instead of urine. Recently Weissman and his colleagues[12] have also described a method for conducting the tests for pregnancy, employing mature female South African clawed frogs (*Xenopus laevis*).

A rapid urine pregnancy test has also been developed by Zondek and his associates[13] employing infantile rats (3–5 weeks of age). This test depends on the production of hyperemia of the ovaries in six to twenty-four hours following two subcutaneous injections of 2 cc. of urine at an interval of one hour. In undisturbed pregnancies positive reactions are stated to occur in about 92.2 per cent of pregnancies at the end of six hours and in about 99 per cent at the end of twenty-four hours.

In a ***negative reaction*** by either the Aschheim-Zondek or Friedman tests the ovaries are pure white or light pink although large mature follicles may be observed. In young rabbits, not old enough to produce a positive reaction, the ovaries may be narrow and flat and have an opaque appearance which may give rise to falsely negative reactions. The uterine horns are pure white. Otherwise, negative reactions indicate (1) the absence of pregnancy and other conditions giving positive reactions; (2) in a known pregnancy, the death of the fetus except when living placental tissue is still present; (3) missed abortion and ectopic pregnancy following death of the placental tissue or (4) the performance of the test too soon after conception.

Positive reactions by either the Aschheim-Zondek or the Friedman test are indicated by the production of large hemorrhagic follicles or the presence of corpora lutea either enclosing the ovum or resulting from ruptured follicles. They occur (1) in pregnancy as early as five to fourteen days after the first missed menstrual period and especially toward the end of the first month with an accuracy of about 98 to 98.5 per cent.[14] Negative reactions, however, occuring in tests with urine voided less than ten days after the first missed period are not dependable and indicate a repetition of the test later on. The hormone slowly decreases and disappears from the urine during the first week after parturition. When the reaction is negative after having been positive, fetal death is indicated although positive reactions may occur for two to six weeks if functionally active chorionic tissue is present. (2) Positive reactions also occur in about 50 per cent of ectopic pregnancies, followed by negative reactions in about three weeks after the onset of vaginal bleeding; (3) possibly in missed and incomplete abortions as long as living placental tissue is present; (4) in hydatidiform moles; (5) in chorionepithelioma which should always be suspected when positive reactions occur longer than two weeks after the delivery of a hydatidiform mole; (6) in men with teratoma, embryonal carcinoma and choriocarcinoma of the testicles. When negative reactions after the surgical removal of hydatidiform mole, chorionepithelioma or malignant testicular tumors are followed in some weeks or months by positive reactions, recurrences or metastases are usually present. Twombly and his associates [15] have recently reported that a quantitative Aschheim-Zondek test in malignant tumors of the testicles reduces the percentage of errors. Eighty-two per cent of a group of fifty cases showing 2,500 or more mouse units per liter of urine ended fatally as likewise all who showed 10,000 units or more per liter. Under the conditions, therefore, the test is of prognostic value.

Possible errors in the interpretation of tests may result in *falsely positive* reactions due to early menopause, hyperthyroidism, ovarian cysts, endometrial hyperplasia, uterine carcinoma or primary ovarian failure when pituitary compensation has occurred. The reactions under these circumstances, however, are generally limited to the first phase characterized by ripening of the follicles and estrus in mice without hemorrhages into unruptured follicles or luteinization of the follicles.

THE TESTICULAR HORMONE

The testes are not only concerned in spermatogenesis but secrete a hormone called *testosterone*. It belongs to the class of the sterols, has been obtained in a crystalline form, and may be prepared synthetically. In the urine it occurs as two excretion products, androsterone or androkinin and dehydro-androsterone; both, especially the latter, are much less potent than testosterone. Together they constitute the *androgens* or male sex hormone (Table 122).

Testosterone is apparently secreted by the Leydig cells under stimulation by the gonadotropic hormones of the anterior lobe of the pituitary gland. Its functions are stimulation of (1) the development and maintenance of the accessory sexual characteristics; (2) the growth of the prostate gland; (3) the growth and distribution of hair; (4) the maturation of the skeleton, muscles and larynx; (5) the distribution of fat; (6) the control of the libido and potency and (7) the determination of the masculine psychology.

TABLE 122. SUMMARY OF THE TESTICULAR HORMONES

Hormones	Clinical and Laboratory Aspects
Testosterone	The male sex hormone secreted by the Leydig cells of the testes. Present in the urine as two excretion products: (1) androsterone and (2) dehydro-androsterone which, together, constitute the androgens. Stimulates the growth and development of the accessory sex characteristics of the male. Also present in the urine of females, especially during pregnancy. An *increase* occurs in hypergonadism; a *decrease* occurs in hypogonadism due primarily to a decrease of the gonadotropic hormones of the pituitary gland. A decrease may be caused by atrophy of the testes and cryptorchidism.
Inhibitin (?)	A second testicular hormone is supposed to be secreted by the germinal epithelium. Its production has not been proved. Supposed to inhibit the effects of the gonadotropic hormones of the pituitary gland and to play a rôle in the etiology of hypertrophy of the prostate gland.
Laboratory Examinations	Based on the effects of the male sex hormone in blood serum or urine on the growth of the combs of capons. Tests employing urine are of clinical value for diagnostic and assay purposes.

Just as estrin occurs in the urine of males, testosterone occurs in the urine of females, the source being unknown, and especially on the sixth to eighth days of the menstrual cycle, in pregnancy and in the presence of masculinizing tumors. Small amounts (1 to 32 i.u. per day) are passed in the urine of boys and girls before puberty, reaching 40 to 90 i.u. per day in the case of women.

Before puberty in males an *increase* of the hormone may result in hypergonadism characterized by precocious development of the secondary sexual characteristics and genitalia, precocious development of the muscles and premature closing of the epiphyseal lines. After puberty it may result in excessive libido, hypertrichosis, etc.

A *decrease* may result in hypogonadism usually due primarily to a pituitary disorder resulting in a decrease of the gonadotropic hormones. A decrease may also be due to eunuchoidism, destructive growths, atrophy of the testicles from mumps, typhoid fever, syphilis, prolonged and severe inanition and vitamin B_1 deficiency. Cryptorchidism is also accompanied by a decrease while, of course, the hormone is absent in eunuchism.

It has been claimed that a second hormone, called *inhibitin,* is secreted by the germinal epithelium of the testes [16,17] but this has not been proved. It is supposed to inhibit the effects of the gonadotropic hormones of the pituitary gland upon the interstitial cells of the testes. After middle age regressive changes in these organs, it is believed, result in a decreased production of this hypothetic hormone, with the result that the gonadotropic hormones overstimulate the testes to produce excessive amounts of testosterone which in turn produces hypertrophy of the prostate gland. It is improbable, however, that hypersecretion by the interstitial cells of the testes is responsible, since the secretion of the male sex hormone tends to decrease rather than to increase with advancing age. Other investigators [18] have postulated that prostatic hypertrophy may be due to a diminished production of the male sex hormone resulting in an excess production of the female sex hormones.

Laboratory Examinations for the Male Sex Hormone. The diagnosis of diseases due to the increased, decreased or nonsecretion of the male sex hormone is largely based on clinical manifestations. Laboratory methods are available, however, for its assay in the blood and urine based on its effect in promoting the growth of the combs of capons as worked out by Gallagher and Koch.[19] The method is sufficiently accurate with urine to be of clinical value and has been extensively employed. In cases of hypogonadism androgenic excretion is markedly decreased although small amounts occur in the urine of eunuchs. Women with adrenal tumors frequently excrete large amounts. The amount of androgens in the blood is very small, and due to the limitation in quantity available for analysis the methods for assay are usually crude; of these, that of McCullagh and McLin [20] is recommended as, likewise, the method employed in the Cleveland Clinic for the detection of androgens in the urine.

THE THYROID HORMONE

The hormone produced by the thyroid gland is known as *thyroxin,* of which iodine is an essential constituent, occurring as a phenyl ether of amino-acid tyrosine with four iodine atoms. It has been suggested that it occurs in combination with globulin to produce a physiologically active form of thyroglobulin. About 0.3 mg. of thyroxin is secreted daily, most of which is stored until needed in the colloid spaces as thyroglobulin. Thyroxin is prepared synthetically but apparently is not as physiologically active as thyroid extract. The vitamins of the B complex and vitamin E are favorable and vitamins A and C apparently opposed to thyroid activity (Table 123).

Thyroxin is believed to act as a catalyst increasing the oxidative processes of the tissues. It (1) regulates the speed of metabolism, producing about 40 calories of heat per hour per square meter of body surface; 1 mg. raises heat production about 1000 calories. It (2) stimulates the excretion of nitrogen (creatine), (3) mobilizes glycogen from the liver, (4) accelerates fat metabolism with reduction of blood cholesterol, (5) increases water elimination, (6) regulates heat production, (7) sensitizes the sympathetic nervous system to adrenalin and (8) stimulates the healing of wounds. The thyroid gland bears an intimate relationship to the pituitary, gonads, adrenals, thymus, parathyroids and islands of Langerhans of the pancreas.

While there are no direct *laboratory examinations* for detecting an increased or decreased secretion of thyroxin, a determination of the basal metabolic rate has been found extremely valuable in this connection. Determinations of blood cholesterol and iodine are also valuable, since cholesterol is generally reduced and iodine increased in states of hyperthyroidism while cholesterol is increased and iodine reduced in hypothyroidism.

TABLE 123. SUMMARY OF THE THYROID, PARATHYROID, ADRENAL AND OTHER HORMONES

Hormones	Clinical and Laboratory Aspects
Thyroxin	Secreted by the thyroid gland; iodine is an essential constituent. Produced synthetically. Acts as a catalyst increasing the oxidative processes of the tissues. Possesses important metabolic and other functions. Thyroxin production is estimated by the basal metabolic rate supplemented by blood cholesterol and iodine determinations. *Increased* in hyperthyroidism (exophthalmic goiter, toxic adenoma and masked hyperthyroidism). *Reduced* in hypothyroidism (cretinism, myxedema, hypothyroid and thyroid exhaustion states).
Parathormone	Secreted by the parathyroid glands. Its chief function is concerned with the regulation of calcium metabolism. Alterations in secretion may be detected by blood calcium, phosphorus and phosphatase determinations. An *increase* results in hyperparathyroidism which may cause generalized osteitis fibrosa cystica (von Recklinghausen's disease). Usually due to secreting adenoma of one or more of the parathyroid glands. A *decrease,* due to parathyroid deficiency, produces parathyroprival tetany.
Adrenalin and Cortin	*Adrenalin* or *epinephrine* is secreted by the medulla and *cortin* by the cortex of the adrenal glands. Both, along with the glands in general, bear an important relationship to the pituitary, thyroid, parathyroids, gonads and thymus. The medulla is not essential to life although adrenalin possesses many important physiologic properties. The cortex is essential to life because cortin controls sodium, potassium, water and other important metabolic functions. There are no practical laboratory examinations for determining adrenalin and cortin in the blood but various laboratory procedures are useful as aids in the detection of diseases of the adrenal glands involving the cortex, medulla or the glands as a whole and for 17-ketosteroids.
Insulin	Secreted by the islands of Langerhans of the pancreas; normally from 40 to 80 units per 24 hours. Its secretion bears an important relationship to the pituitary, thyroid, adrenal and gonadal glands. Insulin is extremely important in the metabolism of carbohydrates. There are no practical laboratory examinations for determining insulin in the blood but various laboratory procedures are useful in the diagnosis of ***hypoinsulinism*** (diabetes mellitus) and ***hyperinsulinism.*** ***Kallikrein,*** which occurs in normal urine and pancreatic extracts, has been regarded by some investigators as a pancreatic hormone but this has not been proved.
Pineal Hormone (?)	**Apparently the pineal gland belongs to the endocrine system but the secretion of a hormone has not been proved. The functions of the pineal gland are uncertain but it apparently bears a relationship to nutrition, growth and the sexual functions.**

TABLE 123. SUMMARY OF THE THYROID, PARATHYROID, ADRENAL AND OTHER HORMONES—(Continued)

Hormones	Clinical and Laboratory Aspects
Thymic Hormone (?)	Apparently the thymus gland belongs to the endocrine system although this has not been definitely proved. Extracts have a bearing on growth and maturity. The gland bears a relationship to the thyroid, pineal, pituitary and gonadal glands. If a hormone is secreted it is probably a product of Hassall's corpuscles.
Gastric Hormone (?)	A hormone may be secreted by the mucosa during digestion. Gastrin, however, is not a hormone but histamine. The "intrinsic factor" concerned in the production of the anti-anemic substance is apparently an enzyme.
Intestinal Hormones	*Secretin* is secreted by the duodenal mucosa and stimulates the production of pancreatic secretions. *Cholecystokinin* is secreted by the duodenal mucosa and stimulates contraction of the gallbladder. *Enterogastrone* is secreted by the intestinal mucosa and inhibits the motility and secretions of the stomach. *Incretin* is supposed to be secreted by the duodenal mucosa and to stimulate the secretion of insulin.

An *increase* of thyroxin, with an increased basal metabolic rate, occurs in exophthalmic goiter (Grave's disease), toxic adenoma of the thyroid gland and masked hyperthyroidism. A *decrease* of thyroxin, with a reduced basal metabolic rate, occurs in cretinism, myxedema (Gull's disease), hypothyroid states and thyroid exhaustion states. The basal metabolic rate is usually normal in nontoxic adenoma of the thyroid gland and normal or slightly reduced in colloid goiter.

THE PARATHYROID HORMONE

The parathyroid glands secrete a hormone known as *parathormone*. Its chief function is the regulation of calcium metabolism concerned in (1) the growth and maintenance of bone; (2) the maintenance of a normal calcium (including ionized calcium) content of the blood; (3) controlling nerve and muscle irritability and (4) indirectly influencing the permeability of cell membranes.

It is no longer believed that the parathyroid glands are necessary for the metabolism of methyl guanidine a failure in which at one time was regarded as the cause of parathyroprival tetany. Parathyroid deficiency, however, results in an increase of blood phosphorus and potassium, a decrease of blood calcium, a defective deposition of calcium in the bones and an increase of nerve excitability. Compensatory hypertrophy occurs in vitamin D deficiency and the glands probably protect against an excess of vitamin A. Their activity is also in relation to the pituitary, thyroid and adrenal glands with the possibility of menstrual disorders being related to hypocalcemia.

There are no direct *laboratory examinations* for estimating parathormone in the blood but the detection of alterations in its secretion is greatly aided by determinations of the diffusible and nondiffusible calcium of the blood as well as of blood phosphorus and phosphatase.

An *increase* of parathormone results in hyperparathyroidism which may be due to functioning adenoma of the glands or to hyperplasia without tumor. This may result in an excessive mobilization of calcium from the bones (causing rarefaction), a high degree of hypercalcemia, and frequently abnormal deposits of calcium in the way of renal or other calculi. When the bone lesions are extensive generalized, osteitis fibrosa cystica (von Recklinghausen's disease) results which is almost invariably due to secreting adenoma of one or more of the parathyroid glands. A *decrease* of parathormone due to parathyroid deficiency, surgical or otherwise, produces a type of tetany.

THE ADRENAL HORMONES

The medulla of the adrenal glands secretes a hormone known as *adrenalin* or *epinephrine,* while the cortex secretes a second hormone designated *cortin.* Both, and the adrenal glands in general, bear an important relationship to other endocrine glands with special reference to the pituitary, thyroid, parathyroids, gonads and thymus.

The medulla is not essential to life. The secretion of adrenalin is governed by the splanchnic nerves of the sympathetic system and possesses the following activities: (1) increases blood pressure and heart rate; (2) contracts the uterus; (3) inhibits peristalsis; (4) increases metabolism and kidney function; (5) dilates the pupils; (6) contracts the capillaries and (7) stimulates the arrectores pilorum and smooth muscle. Its secretion is stimulated by muscular activity or emotional stress, by the action of other hormones (especially thyroxin and insulin) and by extensive burns.

The cortex, however, is essential to life. Mainly through the secretion of cortin it (1) controls sodium, potassium and nitrogen excretion; (2) controls water and electrolyte metabolism; (3) contributes to carbohydrate metabolism; (4) maintains body strength—marked asthenia appears in its absence; (5) supports pregnancy and lactation and (6) exerts a masculinizing influence. The cortex also stores ascorbic acid or vitamin C.

While there are no practical *laboratory examinations* for the estimation of adrenalin and cortin in the blood, various other laboratory procedures are helpful in this connection. Thus, in general, in hypofunction of the adrenal glands (*hypoadrenia*) the basal metabolic rate is generally low, accompanied by hypoglycemia. In hypofunction of the cortex, especially in Addison's disease, the blood chloride is reduced, with an increase of urine chloride due to the increased excretion of chloride. In hyperfunction of the cortex (*hyper-adrenia*) resulting in the adrenogenital syndrome of the congenital or adult types and pubertas praecox, the urine may show an excessive excretion of the androgens while in tumors of the medulla there is usually hyperglycemia and glycosuria with decreased renal function on the affected side.

The excretion of non-phenolic *17-ketosteroids* in the urine may be taken as an index of the combined activity of the adrenal cortex and the male gonads. In the normal female their excretion is an index of the activity of the adrenal cortex alone. The method of Callow and his associates [21] has given satisfactory results in the determination of 17-ketosteroids although too little is known about the metabolism of adrenal cortical and gonadal hormones to give the method much clinical value. Excretion is low in hypo-adrenalism, Cushing syndrome, and adrenal tumors but in the group of questionable glandular dyscrasias the determination of the excretion of 17-ketosteroids has proved, so far, of but little diagnostic aid.

THE PANCREATIC HORMONE

In addition to an external secretion of great importance in the digestion of proteins, carbohydrates and fats, the pancreas, through the islands of Langerhans, secretes a hormone known as *insulin* which (1) maintains normal blood glucose; (2) aids in the conversion of glucose to glycogen in the liver and muscles; (3) accelerates the resynthesis and storage of muscle glycogen and (4) enhances the production and oxidation of glucose. About 40 to 80 units are secreted normally in 24 hours. Its secretion bears an important relationship to the pituitary, thyroid, adrenal and gonadal glands.

While there are no practical *laboratory examinations* for determining the amount of insulin in the blood, yet various laboratory procedures are extremely useful in this connection. Thus, an inadequate secretion, constituting *hypo-insulinism* and characteristic of diabetes mellitus, produces an excessive liberation of glucose from the liver along with an inadequate storage of glycogen resulting in hyperglycemia (with or without glycosuria and ketonuria), low glucose tolerance, high blood cholesterol, lowered carbon dioxide combining power of the plasma and high blood carotene. An excessive secretion, constituting *hyperinsulinism,* results in hypoglycemia, a flat glucose tolerance curve and an increase of the alkali reserve of the plasma (due to increased carbon dioxide absorption).

Kallikrein, discovered in normal urine by Frey and Kraut,[22] also occurs in pancreatic extracts although the site of its formation is unknown. It possesses vasodilating effects and has been used in the treatment of angina pectoris. Some investigators have regarded it as a hormone secreted by the pancreas but this has not been proved.

THE PINEAL HORMONE

The distribution of the blood supply to the pineal gland indicates that it belongs to the endocrine system but the secretion of a hormone has not been definitely proved. Indeed, the functions of the gland are uncertain but it apparently bears a relationship to nutrition, growth and the sexual functions. The gland is apparently antagonistic to the pituitary and thymus and contains large amounts of estrin.

THE THYMIC HORMONE

Whether or not the thymus gland belongs to the endocrine system is uncertain, as it may be a lymphoid organ with the same functions as other lymphatic organs.

However, while the lymphoid tissues involute after puberty its endocrine-like functions do not cease with advancing age. If a hormone is secreted it is probably a product of Hassall's corpuscles.

Undoubtedly, extracts of the gland have an important bearing upon growth, maturity and the development of bone centers and epiphyses. These effects may be due to products only stored in the gland and not produced by it. The endocrine nature of the gland, however, is indicated by its relationship to other endocrine glands. For example, it undergoes enlargement in hyperthyroidism, is probably antagonistic to the growth functions of the pineal gland and undergoes involution at puberty on maturation of the pituitary and gonadal glands.

In *thymic hypertrophy* (status thymicolymphaticus) it is usual to find a prolonged blood coagulation time and a low carbon dioxide tension of the blood with orthostatic albuminuria. In Timme's disease (thymic suprarenal pituitary "compensatory" syndrome) hypoglycemia is usual in the early stage followed by hyperglycemia in the later stage.

THE GASTRIC HORMONE

While *gastrin* was originally thought by Edkins [23] to be a hormone secreted by the pyloric portion of the gastric mucosa it is now known to be histamine. Available evidence indicates, however, that during digestion the gastric mucosa may produce a hormone which is apparently under the control of the terminals of the vagi in the wall of the stomach.

The "intrinsic factor" of Castle,[24] which is secreted by the gastric mucosa, is now regarded as an enzyme rather than a hormone. By acting on a precursory dietary substance, known as the "extrinsic factor" and present especially in beef muscle, liver, eggs and yeast, it produces the anti-anemic or hematinic substance which is largely stored in the liver until needed. The latter is actively concerned in erythropoiesis including the production of reticulocytes. The failure of the gastric mucosa to secrete the "intrinsic factor" is now commonly regarded as of primary importance in the etiology of pernicious anemia, while a similar anemia occurring in tropical sprue is ascribed to a diet deficient in the "extrinsic factor" or to failure in the absorption of the anti-anemic substance.

THE INTESTINAL HORMONES

The mucosa of the upper part of the small intestine and especially of the duodenum secretes a hormone known as *secretin*. Conveyed to the pancreas by the blood, it provides a powerful stimulus to the production of its secretions. It is also thought to stimulate indirectly the excretion of bile and probably the secretion of succus entericus. The hormone may exist as prosecretin. There is no evidence indicating that its production is under the control of the nervous system.

According to Ivy and Oldberg,[25] the duodenal mucosa also produces a second hormone known as *cholecystokinin*. It is secreted particularly through stimulation by acids and fats and especially by egg yolk and cream. It stimulates contraction of the gallbladder and, while related to secretin, is apparently a separate hormone since secretin does not have this effect nor does cholecystokinin stimulate the pancreatic secretions.

Ivy [26] has also prepared in purified form an extract of the intestinal mucosa which, when injected, produces a characteristic inhibitory effect on the secretions and motility of the stomach. The active principle appears to be a hormone or a hormone-like substance called *enterogastrone* which is free of vasodilator effects. Apparently it is secreted especially when fats are ingested and probably identical with the hormone (*chalone*) described by Lim.[27] The extract does not contain secretin or cholecystokinin.

Other hormones or hormone-like substances are regarded by some investigators as being secreted by the duodenal mucosa. These include *incretin* or *duodenin,* which is supposed to stimulate the secretion of insulin by the islands of Langerhans, and a hormone which is regarded as augmenting intestinal motility.

Other Possible Hormones. The liver has been regarded as producing a hormone (*hemopoietin*) capable of stimulating the bone marrow with the production of reticulocytosis. Also *eutonon* regarded as increasing the reserve strength of the heart, *anabolin* concerned in the detoxication of wastes and the conversion of toxins into urea, as well as factors promoting growth, accelerating hemoglobin production and affecting diuresis.

The spleen is also supposed to produce a hormone or hormones concerned in hemopoiesis, water storage by the tissues, chloride retention, the production of blood cholesterol and susceptibility to vagal stimuli. Their production, however, has not been definitely proved.

The kidney may also produce a hormone, since the injection of extracts may reduce blood pressure, increase urea clearance, reduce edema by diuresis, reduce blood cholesterol and produce peripheral vasodilation. Indeed, a hormone, *nephrohormone,* has been reported as isolated from the blood of the renal veins.

The heart may also produce a hormone or a hormone-like substance, since injections of extracts and extracts of the Keith-Fleck node are regarded as increasing the rate and strength of its beats.

It is also thought that *sympathin,* described by Cannon,[28] may be a hormone produced by the endings of the sympathetic nerves in the skin as well as by other sympathetic nerves.

VITAMIN A

Vitamin A is a fat-soluble vitamin formed in the liver from its precursors, the carotenes, and stored in this organ. It is a constituent of visual purple (rhodopsin), preserves the integrity of epithelium and influences calcium metabolism. It is synergistic to the sex hormones and cortin but antagonistic to the thyroid gland and insulin (Table 124).

An *excess* of the vitamin due to overdosage may produce toxic manifestations but this is quite rare. A *deficiency* may produce (1) keratinization of epithelium resulting in xerophthalmia, keratomylacia and follicular keratosis of the skin; (2) night blindness or nyctalopsia; (3) osseous metaplasia; (4) the formation of renal calculi, and it may possibly prolong the period of gestation. A marked deficiency may occur in alcoholic cirrhosis of the liver due to dietary deficiencies.[29]

Because of faulty absorption a deficiency may also occur in idiopathic steatorrhea (tropical and nontropical sprue and celiac disease). Although vitamin A is frequently referred to as "anti-infective" it is in no sense to be regarded as specific in the prevention or treatment of "colds," influenza, etc. Normal plasma levels have been observed in Darier's disease.[30]

The minimal daily requirements are 1500 I.U. units for infants, 3000 for children up to twelve years and 5000 for older children and adults. Deficiencies are best detected by biophotometric examinations of the eyes. *Laboratory methods* for the detection of deficiency, employing blood serum or urine, are less satisfactory and based essentially on the blue color reactions produced by the vitamin with antimony trichloride[31] as compared with a standard solution of copper sulfate in the Lovibund tintometer or in the Evelyn photoelectric colorimeter.[32,33] Normally, 100 cc. of serum contains between 10 and 40 Lovibund units. By the method of Pett and LePage[34] the normal varies between 35 and 40 international units. The method of Kaser and Stekol[35] is recommended; the normal varies from 50 to 300 international units per 100 cc. of plasma.

VITAMIN B COMPLEX

Vitamin B complex includes a number of well-defined substances, together with several factors not yet completely identified. All of them, however, are closely associated in nature, are widely distributed in natural foodstuffs and are water soluble.

Thiamine Hydrochloride (B_1). Vitamin B_1 has been synthesized and identified as thiamine hydrochloride. In foodstuffs and the body, B_1 combines with pyrophosphoric acid and functions as a co-carboxylase, an enzyme which has an important rôle in the metabolism (oxidation) of pyruvic acid.[36] It acts as a catalyst coenzyme in the metabolism of carbohydrate, probably facilitates the synthesis of fat from carbohydrate, promotes appetite and the growth of children and is necessary for proper nerve function. The daily maintenance requirement is not yet definitely known but is thought to be about 0.5 to 1.0 mg. in the case of children, 1.5 to 2.0 mg. in men and nonpregnant women, 2.5 mg. during pregnancy and 3.0 mg. during lactation.

To the best of my knowledge, no ill effects have been reported due to an *excess* of thiamine hydrochloride. A *deficiency* may result (1) in the production of neuritis and polyneuritis (infectious, alcoholic or due to pregnancy); (2) beriberi and the beriberi syndrome; (3) anorexia, gastro-intestinal disturbances and malnutrition, and (4) in functional disorders commonly classified clinically as neurasthenic but which are frequently masked pellagra. Deficiencies may occur in congestive heart failure, diabetes mellitus, hyperthyroidism, rheumatoid arthritis and prolonged diarrheas; also in about 50 per cent of cases of multiple sclerosis, dorsolateral sclerosis, about 80 per cent of cases of syphilis of the central nervous system and sometimes in tic douloureux.[37]

Various *laboratory examinations* have been proposed for the assay of thiamine in the blood and urine as aids in the diagnosis of deficiency states. Chemical tests are based upon the thiochrome reaction[36,38] which permits measurement of the fluorescence of partially oxidized thiamine. They are capable of detecting small amounts of thiamine in the urine and blood, although the percentage of error is admittedly high.

TABLE 124. SUMMARY OF THE VITAMINS

Vitamins	Clinical and Laboratory Aspects
Vitamin A	An *excess* due to overdosage may produce toxic manifestations but this is quite rare. A *deficiency* may produce (1) keratinization of the epithelium resulting in xerophthalmia, keratomylacia or follicular keratosis of the skin; (2) night blindness (nyctalopsia); (3) osseous metaplasia and (4) possibly the formation of renal calculi. It is not specifically "anti-infective." Deficiencies (dietary) may occur in alcoholic cirrhosis of the liver as well as in idiopathic steatorrhea due to faulty absorption. Deficiency is best detected by biophotometric examinations of the eyes; it may be detected also by chemical tests conducted with blood serum.
Vitamin B Complex	*Thiamine hydrochloride* (B_1). A *deficiency* may result in (1) neuritis or polyneuritis (infectious, alcoholic or due to pregnancy); (2) beri-beri; (3) anorexia, gastro-intestinal disturbances and malnutrition and (4) in functional disorders commonly classified as neurasthenia but which are frequently masked pellagra. Deficiencies may occur in congestive heart failure, diabetes mellitus, hyperthyroidism, rheumatoid arthritis, multiple sclerosis, syphilis of the central nervous system, etc. *Riboflavin* (*B_2 or G*). A deficiency constitutes ariboflavinosis which contributes to the etiology of pellagra, producing (1) ocular symptoms and lesions; (2) glossitis, cheilitis and fissuring of the commissures of the lips and (3) dermatitis of the seborrheic type involving the nasolabial folds, alae nasi, ears, etc. *Nicotinic acid.* Known as the pellagra-preventing or P-P vitamin. A *deficiency* plays the most important rôle in the etiology of pellagra. *Pyridoxine* (*B_6*). Apparently bears a relationship to nutrition by augmenting the activities of riboflavin and nicotinic acid in pellagra. *Pantothenic acid.* Probably also concerned in nutrition in association with riboflavin. *Biotin.* Rôle in human nutrition as yet unknown. *Para-aminobenzoic acid.* May be concerned along with pantothenic acid and biotin in regulating the pigmentation of hair. Promotes the growth of bacteria and inhibits the bacteriostatic effects of the sulfonamides upon them in cultures. *Inositol* which, along with pantothenic acid, may influence the growth and maintenance of hair. *Choline* which may play a rôle in sulfur and fat metabolism. In rats it prevents the excessive deposition of fat in the liver. *Laboratory examinations* employing urine are of value for the detection of thiamine, riboflavin and nicotinic acid deficiencies. Examinations employing blood are available for the detection of thiamine (in terms of pyruvic acid) and pantothenic acid deficiencies. Examinations of the urine for porphyrins may also aid in the laboratory diagnosis of pellagra.

TABLE 124. SUMMARY OF THE VITAMINS—(Continued)

Vitamins	Clinical and Laboratory Aspects
Vitamin C	Chemically identified as cevitamic or ascorbic acid and prepared synthetically. It possesses peculiar reducing properties which promote oxidative or respiratory mechanisms in the tissues including the maintenance of intracellular substances, normal capillary permeability, the functioning of odontoblasts as well as, possibly, influencing erythropoiesis and resistance to infection. The daily maintenance intake for infants is 10 mg.; children 15 to 20 mg. and adults 30 mg. A *deficiency* may cause (1) subclinical and clinical scurvy; (2) nonunion of fractures and (3) delayed healing of wounds. Deficiencies may occur in many diseases without primary involvement in their etiology. Deficiency does not appear to produce primary disturbances of protein, carbohydrate, fat or mineral metabolism. Under normal conditions the blood contains 0.7 to 1.4 mg. per 100 cc. of plasma with the excretion of 10 to 40 mg. per 24-hour output of urine. *Laboratory examinations* of the blood and urine fo- deficiencies are sufficiently accurate for clinical purposes and of value, especially in the detection of subclinical scurvy. "Saturation tests" for determining the degree of subsaturation of the tissues are of clinical value although complete saturation may not be required for the maintenance of good health. Deficiency may also be detected clinically by the capillary permeability test.
Vitamin D	The antirachitic vitamin. Not absorbed from the intestinal tract in the absence of bile. Chiefly concerned with the preservation of mineral balance and especially with the metabolism of calcium and phosphorus. D_2 is produced by the irradiation of ergosterol and D_3 by the irradiation of 7-dehydrocholesterol. *Viosterol* is commercial irradiated or activated ergosterol. A *deficiency* results in the production of rickets. Deficiencies may occur in pregnancy, lactation and various diseases. An *increase* of vitamin D from overdosage may produce hypercalcemia, hyperphosphatemia and diminished phosphatase activity. There are no *laboratory examinations* for the detection of vitamin D in the blood or urine. The detection of deficiency, however, may be aided by determinations of serum inorganic phosphorus, calcium and phosphatase activity.

Apparently thiamine is a nonthreshold substance when the kidneys are normal. Consequently, healthy individuals with no deficiency will show its presence in the urine. According to the method of Karrer [39] the blood contains approximately 6 micrograms and the urine about 8 micrograms per 100 cc. The total daily excretion in the urine varies from 70 to 150 micrograms, 1 microgram corresponding to 0.4 international units. According to the method of Melnick and Field [40]

TABLE 124. SUMMARY OF THE VITAMINS—(Continued)

Vitamins	Clinical and Laboratory Aspects
Vitamin E	Fat-soluble and contains three factors, namely, alpha, beta and gamma tocopherols. Alpha tocopherol is prepared synthetically for oral or parenteral administration. In female rats deficiency results in "resorption sterility" and in male rats degeneration of the germinal epithelium and spermatozoa. For these reasons the vitamin is frequently referred to as the "anti-sterility vitamin." Vitamin E is also antioxygenic, preventing the autoxidation of certain fats and oils. Deficiency in the lower animals may also result in muscular dystrophy. *Deficiency* in the woman may be a factor in the etiology of habitual abortion and abruptio placentae; in the man it may prevent maturation of spermatozoa with loss of libido. The daily maintenance dose is unknown. The administration of wheat germ oil, alpha-tocopherol or of all three tocopherols secured from natural sources, may be of benefit in the prophylaxis and treatment of sterility and habitual abortion as well as helpful in the treatment of neuromuscular disorders. There are no *laboratory examinations* for the detection of deficiency.
Vitamin K	Known as the "coagulation vitamin" because of its important function in the production of prothrombin by the liver. Occurs naturally and has been produced synthetically (K_1 and K_2). Cannot be absorbed from the intestinal tract unless bile is present. A *deficiency* results in hypoprothrombinemia and consequently in prolonged bleeding due (1) to a lack or (2) to the inadequate absorption of vitamin K because of lack of intestinal bile or intestinal disorders; (3) because of imperfect utilization of vitamin K by the liver in the production of prothrombin because of primary hepatic disease. There are no direct *laboratory examinations* for vitamin K in the blood or urine. Methods are available for the estimation of prothrombin in the blood.

from 55 to 160 micrograms are excreted per 24-hour output of urine while according to that of Perlzweig [41] the normal varies from 100 to 300 micrograms per 24 hours.

As previously stated, as long as any thiamine is found in the urine, deficiency may be clinically excluded, as only the surplus is excreted. On the other hand, its complete absence from the urine (zero excretion) is indicative of deficiency. On this basis Najjar and Holt [42] have recently described a method which apparently meets this simple purpose. The urine is collected under fasting conditions as follows: (1) The patient is allowed the usual evening meal; (2) next morning the urine is voided and discarded; (3) a glassful of water is taken and urine voided one hour later is used for the examination. This technic, however, does not estimate the degree of deficiency. If such is desired a thiamine "load test" is required.[43,44] The latter is based on the assumption that a deficient individual will retain in the tissues and therefore excrete less thiamine given in a meal than a normal nondeficient individual. The results, however, are affected according to the degree of intestinal absorption. Furthermore, when the test is conducted by the parenteral administration of thiamine, error may be introduced by conditions of the kidneys affecting its excretion.

Since a deficiency of thiamine results in an increase of pyruvic acid, blood tests are based upon quantitative determinations of the latter. According to the method of Platt and Lu,[45] the normal amount under fasting conditions is about 1 mg. per 100 cc. of plasma, and is increased to about 3 mg. in beriberi. According to the method of Bueding and Wortis,[46] the normal varies from 0.77 to 1.16 mg., averaging about 0.98 mg. However, since bisulfite may also combine with other compounds containing the carbonyl group and the total bisulfite-binding substances in the blood, the total calculated as pyruvic acid is stated to vary normally from 3.7 to 5.8 mg. per 100 cc. of blood with an average of 4.7 mg.[47]

Riboflavin (B_2). Riboflavin or vitamin B_2 is also known as vitamin G. It occurs as a fluorescent pigment widely distributed in plant and animal tissues. Yeast and liver are its richest sources although it also occurs in milk, eggs, spinach, etc. It is prepared synthetically and is closely related to nicotinic acid in its physiologic activities. The minimal requirement for adults has been estimated at 2.2 to 2.7 or about 3 mg. per day. It is of importance in nutrition and essential for growth in relation to carbohydrate metabolism and cell respiration.

Riboflavin is remarkably low in toxicity and an *excess* does not appear to produce toxic manifestations. A *deficiency* results in *ariboflavinosis* which apparently has a high incidence with indefinite manifestations, like lassitude and anorexia, before specific lesions develop.[48] A deficiency plays a rôle in the etiology of pellagra producing (1) ocular symptoms and lesions like itching, burning, dryness, mydriasis, granulation and extreme redness of the conjunctivas (particularly of the lower lids), superficial vascularizing keratitis and irregular pigmentation of the iris; (2) glossitis and cheilitis with fissuring of the commissures of the lips and (3) dermatitis of the seborrheic type involving the nasolabial folds, alae nasi, ears and occasionally the whole face and neck.

Slit lamp examinations of the eyes are particularly valuable for the detection of early ariboflavinosis.[49] Fortunately, *laboratory methods* are also available for the estimation of riboflavin in the urine.[50,51] As in the case of thiamine hydrochloride, its absence in the urine (zero excretion) is indicative of deficiency. The degree of deficiency, however, can only be determined by a "load test" following a meal containing an abundance of the vitamin in which the results are compared with those observed in a nondeficient individual. In the latter a marked excretion of riboflavin follows the meal, reaching a constant level in about eight hours. By determining excretion in the urine over a period of about fourteen hours, some idea is gained relative to the degree of tissue deficiency if such is present. According to the method of Strong,[41] about 480 to 800 micrograms are excreted per 24-hour output of urine.

Nicotinic Acid. Like thiamine and riboflavin, nicotinic acid is concerned with the continuous processes of cellular nutrition and respiration. While all three function in part as activators which are continually regenerated, they are also components of coenzymes which are used up and require constant replacement.

On the discovery that nicotinic acid is highly effective in the prevention of pellagra, it became known as the P-P vitamin. It is prepared synthetically, as is also nicotinic acid amide. Undoubtedly both riboflavin and especially nicotinic acid deficiencies are of most importance in the etiology of pellagra. Like riboflavin, nicotinic acid may be essential also to cell oxidation. The normal maintenance dose of nicotinic acid has been estimated as varying from 17.5 to 35 mg. per day although the former is probably too low.

A *deficiency* in nicotinic acid is responsible for many of the disorders seen in pellagra, including those of the alimentary tract like proctitis and diarrhea; dermatitis, pigmentation and thickening of the skin; glossitis; urethritis; vaginitis and nervous and mental disturbances. Indeed, a severe and acute deficiency may result in profound psychotic and stuporous conditions.[52] It is usually accompanied by a deficiency of other vitamins of the B complex.

Unfortunately, *laboratory methods* for the detection of nicotinic acid deficiency by examinations of the blood and urine do not correlate well with the nicotinic acid stored in the tissues. One of the simplest methods for the estimation of nicotinic acid and the nicotinamides is that of Rosenbloom and Jolliffe[53] which shows an excretion of 3.5 to 10.0 mg. per 24-hour specimen of urine in normal or nondeficient individuals. Najjar and Wood[54] have found that the urine contains an unknown fluorescent compound (known as F_2) which varies closely with the body store of nicotinic acid; they believe that it can be employed as a measure of nicotinic acid deficiency. By colorimetric methods[55,56] nicotinic acid appears to vary normally from 0.1 to 0.3 mg. per 100 cc. of blood plasma, with about five times as much in whole blood. The nicotinamides or coenzymes are included, since nicotinic acid is liberated by hydrolysis. According to the method of Klein and associates,[57] the normal for whole blood varies from 0.27 to 0.80 mg. per 100 cc., with the excretion of 20 to 50 mg. per 24-hour urine according to the method of Vilter.[58]

The relation of abnormal pigments in urine to pellagra is also of interest in connection with detection by laboratory methods. Porphyrins or "porphyrin-like substances" are stated to occur in pellagra and to disappear on the administration of nicotinic acid. The pigments have also been stated to be urorosein and another related substance which may occur in the urine of patients with diseases other than pellagra.

Pyridoxine. Pyridoxine or vitamin B_6 occurs in unrefined cereals, legumes. fish and meats. It is also prepared synthetically. Apparently it is of some importance in relation to nutrition[59] by augmenting the activities of riboflavin and nicotinic acid in correcting certain conditions associated with pellagra. It has also been reported as helpful in the treatment of pseudohypertrophic muscular dystrophy, multiple sclerosis and amyotrophic lateral sclerosis.[60]

There are no established daily requirements as far as human beings are concerned. According to the colorimetric method of Scudi and his colleagues,[61] it is absent or present only in traces in the urine under normal conditions. Investigations have shown, however, that *deficiency* produces acrodymia, hemorrhagic disease, "spectacle eyes" and paralysis in rats, as well as microcytic anemia in young dogs and malnutrition in chicks.

Pantothenic Acid. Apparently pantothenic acid is also an essential factor in the growth of living cells, probably being associated in function with riboflavin. It occurs naturally in milk and various vegetables and is prepared synthetically. It can be determined in the whole blood and is stated to vary normally from 0.019 to 0.32 micrograms per cc., corresponding to 19 to 32 micrograms per 100 cc. and averaging about 22.5 micrograms.[62] According to the method of Pelczar and Porter,[63] the normal for blood is 0.03 to 0.09 micrograms per 100 cc. For 24-hour urine the normal is 1.74 to 4.30 mg., according to the method of Pennington and his colleagues.[64]

The rôle of pantothenic acid in nutrition, however, has not been well defined nor has a definite group of signs and symptoms characteristic of its deficiency in human beings been described, except that it has been reported as reduced as much as 23 to 50 per cent of normal in individuals with pellagra, beriberi, and riboflavin deficiency.

Biotin; Para-aminobenzoic Acid; Inositol and Choline. Vitamin B complex also includes *biotin* or vitamin H. It is one of the "bios" factors essential for the growth of yeasts and the respiration of bacteria; it has been prepared synthetically. A *deficiency* causes dermatitis in rats on a diet of uncooked egg albumin but its rôle in human nutrition is as yet unknown. Oppel[65] has recently described methods for its detection and assay in the urine and feces.

Para-aminobenzoic acid, which is also a factor in vitamin B complex, acts as a preventive of nutritional achromotrichia of piebald rats. Animal investigations have indicated that along with pantothenic acid and biotin it may regulate the pigmentation of hair and affect the formation of melanin. There appears to be no justification at present, however, for its use in the treatment of premature graying of human beings. It also affects the activity of tyrosinase.

Para-aminobenzoic acid promotes the growth of practically all bacteria and can be used with advantage in all culture media in concentration of 5 mg. per 100 cc. This is particularly advisable in the case of blood and other cultures in individuals who have been receiving sulfanilamide, sulfapyridine, sulfathiazole and other sulfonamide compounds, since PAB is known to reverse the inhibitory or bacteriostatic effects of these compounds on streptococci, pneumococci, staphylococci and other micro-organisms *in vitro*.[66,67]

Inositol is known as the "mouse anti-alopecia factor" of vitamin B complex. Along with pantothenic acid it probably influences the growth and maintenance of hair although its value in this connection in human beings cannot be stated at the present time.

Choline is also included by many investigators in the vitamin B complex. Like muscarine it stimulates the parasympathetic nerve endings and, like nicotine, causes a preliminary stimulation and finally a paralysis of the autonomic ganglia, resulting in a marked drop in blood pressure, increased peristalsis and a general increase in the secretions. It also plays an important rôle in the *in vivo* methylation of homocystine to form methionine which may be important in relation to sulfur and fat metabolism and other methylations in the body. Occurring in the diet, choline is thought to prevent the excessive deposition of fat in the liver of rats in which a deficiency also produces extensive hemorrhages in the kidneys of about 90 per cent of these animals.

VITAMIN C

Vitamin C occurs naturally in the citrus fruits and various vegetables although the comparative ease with which it is destroyed by exposure to air, alkali and heat makes it impossible to retain fully the natural C content of foods when they are cooked for home use, canned, or dried, except in the case of tomato juice which, because of its high acidity, may be canned without much loss. Most fruit juices, however, may be concentrated *in vacuo* or by the spray process without appreciable loss of the vitamin.

Chemically, vitamin C has been identified with cevitamic or ascorbic acid and is prepared synthetically. Its unique action in the body is largely due to peculiar reducing properties promoting oxidative or respiratory mechanisms in the tissues. According to Dalldorf,[68] it is an essential nutrient required for the normal deposition and maintenance of intercellular substances, including collagen, alterations in the formation of which result in scurvy with increased capillary permeability and disturbances of bone and other cementum-forming cells. Consequently, it maintains capillary resistance, affects the functioning of odontoblasts in the formation of dentine and may have a specific effect on the formation of erythrocytes as well as an influence on antibody production and immunity to infection. It is synergistic to cortin and insulin. The daily maintenance requirements for infants are 10 mg.; about 15 to 20 mg. for children more than one but less than twelve years of age, and about 30 mg. for adults.

To the best of my knowledge an *excess* of vitamin C does no harm. A *deficiency* may cause (1) subclinical or clinical scurvy characterized by debility, anemia, malnutrition, swollen and bleeding gums, loosening of erupted teeth, increased capillary permeability with marked tendency to purpura, scorbutic carditis, and lowered resistance to infection; (2) non-union of fractures and (3) delayed healing of wounds.

Deficiency may also occur in tuberculosis, rheumatic fever, rheumatoid arthritis, gastro-intestinal disturbances, osteomyelitis, congestive heart failure, renal and hepatic disease, metabolic and endocrine disorders and malignancy.[69,70,71] A decrease of the vitamin in the cerebrospinal fluid has also been reported as occurring in herpes zoster, epilepsy and syphilis of the central nervous system.[72] An increase of elimination with a depletion of its tissue storage may also occur in children after the administration of acetylsalicylic acid as well as after ether anesthesia.

In spite of the importance of vitamin C in the maintenance of proper nutrition, its deficiency does not appear to produce primary disturbances of protein, carbohydrate, fat or mineral metabolism. For example, the well-recognized defect in skeletal calcification in scurvy is accompanied by no significant disturbances of calcium or phosphorus metabolism except in the very late stages, in which the disturbances are probably secondary manifestations of the general nutritional disorders.

In view of the frequency with which vitamin C deficiency is responsible for unsuspected or subclinical scurvy as well as, probably, playing a secondary rôle of importance in the causation of signs and symptoms of the diseases mentioned, *laboratory examinations* for its detection and estimation are of great importance. Normally it is stored in the pars intermedia of the pituitary gland, adrenal cortex, corpora lutea, thymus gland, pancreas, liver and other organs. In the blood it appears to be equally distributed between erythrocytes and plasma.

Most of the methods employed for determining the concentration of vitamin C in the blood, urine and other body fluids are based on titration with a standard solution of 2, 6-dichlorphenolindophenol in which the blue color is reduced to a colorless, or faintly pink compound. None possess a high degree of accuracy and different methods do not yield identical results. Mindlin and Butler [73] have described a method using the photoelectric colorimeter which eliminates the error due to other reducing substances.

/ Normally from 0.7 to 1.4 mg. of ascorbic acid or vitamin C occurs in the blood plasma under fasting conditions, according to the method of Magnusson and Osterberg,[74] the amount varying with its intake in foods. Thus, on an intake of 1.7 to 1.9 mg. of ascorbic acid per kilogram, the plasma will ordinarily show about 1 mg. per 100 cc. and its determination affords a useful method for determining vitamin intake in foods.[75] It is not only greatly reduced in scurvy but reduction is also quite common in the later stages of pregnancy and during lactation due to its utilization in fetal development and the production of milk.

Ascorbic acid excretion in the urine is also subject to wide fluctuations according to diet, the degree of tissue saturation and the procedure employed. According to the method of Harris and Ray,[76] it varies normally from 10 to 30 mg. per 24-hour output of urine but may be as high as 40 mg. At least, values below 10 mg. are apparently indicative of deficiency. By using a larger quantity of dye than will be reduced, with the addition of a crystal of ascorbic acid after the determination to complete its reduction, Bessey [77] has estimated that the total may be as high as 100 mg. per liter of urine.

When the blood plasma shows less than 0.7 mg. per 100 cc. and the urine less than 10 mg. per 24-hour output, it is evident that some depletion of vitamin C stored in the tissues ("subsaturation") has occurred. The required intake of vitamin C, however, to maintain a satisfactory degree of tissue saturation depends on the body weight or age. Furthermore, an individual may be well below standard and yet apparently in good health; in fact, a state of complete saturation may not be necessary for good health.

The degree of tissue depletion may be estimated by a "saturation test." After the administration of 600 mg., or 70 mg. per 14 pounds of body weight in children, there is normally a considerable rise in excretion during the following 24 hours; the total excretion of about 30 per cent of the dose is regarded as an approximately normal state; [78] of course less or none at all may be excreted in severe scurvy. Harris [79] has recently used the test in relation to the diet of children, pointing out the need for care in ensuring an adequate intake of potatoes and greens during the winter months, but stating that the test does not measure vitamin C exclusively, that after saturation excretion fluctuates appreciably from day to day, that individuals vary in their response and that other factors (fever, muscular work, kidney function) influence the results. Consequently, a determination of vitamin C in the blood appears to be preferable to the urinary saturation test

If laboratory examinations cannot be conducted, a good clinical test for vitamin C deficiency consists in a determination of capillary permeability. This is conducted with the cuff of a blood pressure apparatus inflated to the point of increasing the pressure within the capillaries which is followed in a few minutes by hemorrhages in the skin of the forearm below the constriction. Of course, increased capillary permeability occurs in conditions other than vitamin C deficiency.

VITAMIN D

Vitamin D is fat-soluble and occurs naturally in fresh liver oils as well as in egg yolk, liver (beef, pork, chicken), butter and salmon; whole milk is a poor source.

At least ten sterol compounds possess vitamin D activity but only two are of primary importance, namely, D_2 produced by the irradiation of ergosterol and D_3 formed by the irradiation of 7-dehydrocholesterol, a precursor of vitamin D present in the outer layers of the skin. In 1929 the Council on Pharmacy and Chemistry of the American Medical Association adopted the term *"viosterol"* for the commercial preparations of irradiated or activated ergosterol. It does not contain vitamin A.

Vitamin D is not absorbed from the intestinal tr..ct in the absence of bile. Its chief function appears to be concerned with the preservation of mineral balance and especially with the metabolism of calcium and phosphorus. The characteristic changes in deficiency are: (1) diminished serum inorganic phosphorus; (2) usually a diminished serum calcium; (3) increased serum phosphatase activity; (4) decreased corpuscular ester phosphorus and (5) a negative calcium and phosphorus balance with excessive elimination of these elements in the feces.

The minimal daily requirements are 400 U.S.P. units, irrespective of age. During the latter half of pregnancy, lactation, and for children under one year of age 400 to 800 units are advisable.

A *deficiency* in vitamin D may result in rickets; consequently, it is called the antirachitic vitamin. Deficiencies, however, may also occur in infantile tetany, osteomalacia, dental caries, celiac disease, hay fever, psoriasis, eczema, scleroderma and acne, as well as during pregnancy and lactation. It may also play a rôle in the slow healing of wounds and ulcers.

Ill effects due to an *increase* of vitamin D do not occur except from overdosage in which case hypercalcemia, hyperphosphatemia, diminished serum phosphatase activity and increased excretion of calcium and phosphorus in the urine may occur. Consequently, massive dosage should probably not be administered to elderly individuals with evidence of arteriosclerosis, particularly of the aorta, or to individuals with renal calculi. Furthermore, vitamin D should be administered with caution to individuals with nephritis, a sensitive colon and when there is a disproportionate intake of calcium and phosphorus.

Unfortunately, there are no *laboratory examinations* available for the detection or estimation of vitamin D in the blood or urine. All assay procedures depend on the calcifying effects of a stated dose given to severely rachitic rats. Determinations of serum inorganic phosphorus, calcium and phosphatase activity, however, are helpful aids in the detection of deficiency.

VITAMIN E

Vitamin E is fat-soluble and occurs principally in wheat germ oil, embryo cereals of oats and wheat, green lettuce, spinach, beef liver and egg yolk. It is stored moderately in muscles and fat and occurs as three factors, namely, alpha, beta and gamma tocopherols. Alpha tocopherol is prepared synthetically and appears to have all the properties of vitamin E isolated from natural products.

Vitamin E appears to be a factor concerned in rapid cellular division. Available evidence bearing upon its functions is almost solely in relation to experiments with the lower animals and especially rats and rabbits. Deficiency in the female rat does not interfere with conception but the entire litter of embryos may be absorbed, resulting in "resorption sterility." If she is again mated conception occurs and feeding the vitamin enables pregnancy to go to term with the birth of living young. In the male rat a deficiency is characterized by degeneration of the germinal epithelium and the spermatozoa, eventually resulting in complete loss of sexual power. For these reasons vitamin E is often referred to as the "anti-sterility vitamin." It is also sometimes designated as the "reproduction vitamin" but this is a misnomer because, as far as reproduction is concerned, it only aids or permits normal functions to occur.

A second important property of vitamin E is the prevention of autoxidation of certain fats and oils, referred to as its antioxygenic activity. Dietary deficiency in rats and rabbits is also stated to produce pathologic changes in the central nervous system resulting in muscular dystrophy; this also occurs in young dogs and guinea-pigs on vitamin deficient diets.

The maintenance dose in human beings is unknown. Furthermore, the effects of *deficiency* are not well understood. In the adult female it is thought, however, that deficiency may be a factor in the etiology of habitual abortion and abruptio placentae. In the adult male it may be a factor in preventing the maturation of spermatozoa and loss of libido. At least, these effects are suggested by clinical reports bearing upon the possible prophylactic and therapeutic value of large doses of wheat germ oil orally (4 to 20 cc. daily) as well as the administration of 50 to 100 mg. of alpha-tocopherol orally or 100 to 300 mg. parenterally per day. Clinical reports also indicate that the administration of alpha-tocopherol, or a mixture of the alpha, beta and gamma tocopherols concentrated from natural sources, may be helpful in the treatment of neuromuscular disorders like amyotrophic lateral sclerosis, progressive muscular dystrophy and atrophy, pseudohypertrophic muscular dystrophy, disseminated sclerosis and myasthenia gravis as well as primary fibrositis, tinnitus aurium, anorexia and malnutrition.

From the standpoint of *laboratory examinations* the methods of Mayer and Sabotka [80] and Minot [81] can be recommended for the determination of alpha tocopherol in the serum. Both are based on the isolation of the vitamin from all other impurities so that the sensitive iron bi-pyridil color reaction is not interfered with by other substances. According to both methods, the normal averages about 1 mg. per 100 cc. serum.

VITAMIN K

Vitamin K is known as the "coagulation vitamin" because of its important function in the formation of prothrombin by the liver. In the phenomenon of coagulation of the blood, the two principal substances are fibrinogen and thrombin which, together, produce fibrin. Thrombin is formed only from its precursor prothrombin. The latter, however, is produced in the liver, provided vitamin K is available. Intravascular coagulation is prevented by antiprothrombin (presumably heparin). In the event of a wound, thrombokinase is liberated by the platelets and tissues and mobilizes thromboplastin. The latter fixes antithrombin and the free prothrombin is then activated by the calcium salts of the blood with the production of thrombin.

Vitamin K occurs naturally in alfalfa, cabbage, spinach and cauliflower; also in fish meal, bran and other foods when stored in a moist condition permitting the production of the vitamin through the putrefactive activities of bacteria. It is produced synthetically in a form known as K_1 or 2-methyl-3-phytyl-1, 4-naphthoquinone and as K_2, the corresponding farnesyl derivative. In man, under normal conditions, the minimal requirements for vitamin K are believed to be met by that produced by bacteria in the lower intestinal tract. Since natural vitamin K is oil-soluble, its absorption, however, cannot occur unless normal amounts of bile are present. After absorption it is carried by the blood to the liver where it participates in the formation of prothrombin by a mechanism as yet unknown.

Under the circumstances, a *deficiency* of vitamin K results in hypoprothrombinemia and this in turn in prolonged bleeding. On the other hand, hypoprothrombinemia may be caused (1) by an actual lack of vitamin K due to temporary sterility of the intestinal tract which is also one factor in the etiology of jaundice of the newborn (icterus neonatorum); also (2) because of the inadequate absorption of vitamin K due to insufficient bile in the intestine as in biliary and gastrointestinal fistula, obstructive jaundice and intestinal obstruction, or because of impairment in its absorption as in ulcerative colitis and sprue; likewise (3) because of imperfect utilization of vitamin K by the liver in the formation of prothrombin due to primary hepatic disease as in cirrhosis, hepatitis, acute yellow atrophy, etc. Hemorrhage in hemophilia, and thrombocytopenic purpura, however, do not involve vitamin K, as hypoprothrombinemia is not responsible.

There are no direct *laboratory examinations* for the detection of vitamin K deficiency in the blood or urine. But several tests are available for determining the prothrombin level of the blood as those of Quick,[82] Cheney,[83] the "bedside" method of Smith and his colleagues [84] and the "serum volume test" of Boyce and McFetridge.[85] The average normal plasma coagulation time by the Cheney method is 5 to 6 minutes, varying from 2 to 8 minutes, when an optimum amount of calcium is used. Since vitamin K therapy may be ineffective in correcting hypothrombinemia due to extensive liver disease, Kark and Souter [86] have recently pointed out that a determination of the prothrombin level of the blood may be a reliable index of liver function, provided the intake of vitamin K is adequate; this has been referred to in Chapter 8.

FOLIC ACID AND VITAMIN B_{12}

Folic acid (pteroylglutamic acid) is the synthetic *Lactobacillus casei* factor. It occurs naturally in the liver, kidneys and other animal tissues; likewise in yeast, mushrooms, grass and green leaves. *Vitamin B_{12}*, of unknown chemical structure, was isolated from the liver by Rickes and his colleagues [87] in 1948.

Both compounds have been found effective in the treatment of pernicious anemia, nutritional macrocytic anemia and the macrocytic anemias of tropical sprue, pellagra and pregnancy, vitamin B_{12} being particularly effective in the treatment of subacute combined degeneration or sclerosis of the posterior and lateral columns of the spinal cord in pernicious anemia [88]. Both compounds, however, have been found ineffective in the treatment of primary aplastic anemia, iron deficiency anemias, secondary anemias, leukemia, idiopathic purpura and the leukopenias due to infections and drug allergies.

There are no *laboratory examinations* for the detection of deficiencies of either or both of these antianemic substances. Laboratory examinations are only of aid in the diagnosis of the particular macrocytic anemias in which the compounds have been found of therapeutic value. In this connection, however, glucose tolerance tests are of value in the diagnosis of tropical sprue since a "flat" curve is commonly observed, which is probably due to the poor or slow absorption of glucose from the intestinal tract as discussed on page 155.

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21

THE CLINICAL INTERPRETATION OF BIOPSY EXAMINATIONS

A *biopsy* refers to the removal of tissue from a living individual for a diagnostic examination. In addition to sternal bone marrow examinations in the diagnosis of diseases of the blood-making organs, biopsies are most frequently conducted and of greatest value in the early diagnosis of malignant tumors in which the prospects of successful treatment by surgical removal or irradiation therapy are maximum. In other words, a biopsy examination is unnecessary for the mere purpose of confirming the diagnosis of advanced malignant disease, although sometimes advisable in case of doubt and for the detection of diagnostic errors (Table 125).

GENERAL CONSIDERATIONS

Range. Biopsy examinations are of course readily possible as diagnostic aids in the case of those parts easily accessible for the removal of fragments of tissues, such as the skin, lips, tongue, oral cavity, nose, larynx, uterus (cervix and endometrium), testicles, mammary glands, superficial lymph nodes, etc. In recent years, however, the range of biopsy examinations has been progressively extended to include many of the deeper-lying tissues. It is now possible to examine tissue material secured by aspiration not only from the marrow of the sternum and other bones, but from the liver, spleen, and pleural cavities as well. A number of instruments are now available with which tissues may be obtained from other sites: from the esophagus by the esophagoscope, the stomach by the gastroscope, the trachea and bronchi by the broncoscope, the urinary bladder by the cystoscope, the rectum by the proctoscope, the sigmoid by the sigmoidoscope, and even to some extent from the peritoneal cavity by the peritoneoscope.

Biopsy examinations may also be conducted by frozen sections of tissues removed during surgical operations although they are likely to cause a delay of anywhere from 10 to 30 minutes. It is stated that the diagnoses agree with those arrived at by the examination of carefully prepared permanent sections in about 90 per cent of cases.[1] For many years, however, there has been an increasing consensus against such examinations for various reasons, including the very important one that they may give no more help in doubtful cases of malignancy, in which the surgeon needs most assistance, than reliance upon the gross appearance of the growth.[2,3,4] At least, this kind of biopsy examination should be preceded in every instance by a thorough clinical study, as close cooperation between the surgeon and pathologist is essential to accurate diagnosis.[5]

Accuracy. According to Reimann,[6] the percentage of error in the differential diagnosis between tumors (benign or malignant) and granulomas due to inflammation is no more than 3 per cent when the examinations are made by a skilful pathologist. In the naming or classification of malignant tumors or neoplasia, however, clinicians may obtain half a dozen different diagnoses from as many different pathologists examining the same slide. Fortunately, such discrepancies seldom involve the essentials of diagnosis as far as malignancy is concerned, although they indicate the serious need for a critical review of the nomenclature employed.[7]

TABLE 125. SUMMARY OF THE CLINICAL INTERPRETATION OF BIOPSY EXAMINATIONS

Subject	Clinical Applications
General Considerations	*Biopsy* refers to the removal of tissue from a living individual for a diagnostic examination. The *scope* of biopsy examinations is very large as fragments of tissue may be removed surgically or by aspiration from many parts of the body. Frozen section biopsies are of limited value. The percentage of *error* in the diagnosis of malignant tumors is not over 3 per cent when tissues are expertly examined. The *danger* of biopsy examinations in relation to the metastasis of carcinoma is not as great as formerly surmised. Precautions against infection, hemorrhage and the puncture of hollow organs must be observed in obtaining material. Healing of a biopsy incision after previous irradiation therapy may be delayed with the possibility of later ulceration.
Collection of Material	Methods employed for the collection of biopsy material are very important in relation to the value of examinations. Tissues should be promptly placed in a 4 per cent solution of formalin. Material secured by aspiration should be prepared in smears on glass slides or placed in 4 per cent formalin for the preparation of sections. Roentgenoscopic guidance is usually required in aspiration biopsies of tumors of the lungs, bones and other parts of the body.
Clinical Value	Of particular value in the early diagnosis of carcinoma; also for differential diagnosis between malignant and benign tumors and granulomas. Biopsy examinations of the endometrium are of value in determining its functional state in relation to sterility and irregular menstruation. Testicular biopsies are of value in determining the cause of infertility. Punch biopsies are of value in determining the cause of hepatomegaly and splenomegaly. Biopsy examinations are of value in the diagnosis of tumors of the skin, lips, tongue, palate, genitalia, etc. Sternal bone marrow biopsies are of value in relation to the diagnosis of diseases of the hemopoietic system, myeloma, metastatic carcinoma, infectious mononucleosis, aleukemic leukemia, etc.

Dangers. When fragments of malignant tissue are properly removed for examinations there appears to be much less danger of spreading the disease than was formerly surmised. In the case of tumors of the mammary glands, however, it is thought that biopsies are inadvisable and that the entire growths should be removed.[6] At least, the frequent palpation and squeezing of malignant tumors is apt to be more dangerous than biopsy examinations.

Indeed, it is stated that sufficient endometrium for examination in determining ovarian function can be obtained not only without the need of hospitalization but without anesthesia as well.[8] Obviously, due precautions against infection and hemorrhage are required in the conduct of aspiration biopsies. But the aspiration of lung tumors is regarded as a reasonably safe outpatient procedure which is often less arduous for the patient than a bronchoscopic examination [9] while aspirations of the liver are reported as being without complications or accidents other than transient weakness in the case of some individuals.[10,11] According to Hoffbauer,[12] however, there is the possible danger of hemorrhage and needle biopsy of the liver should not be attempted unless there are adequate facilities for coping with possible complications.

Furthermore, when a tumor has been irradiated the healing of a biopsy wound may be delayed and especially in the case of the skin. Ulceration may occur even some years after healing which usually results in a suspicion of recurrent malignancy and more irradiation therapy although the latter is contraindicated unless a second biopsy reveals malignancy and the need for it.[6]

COLLECTION OF MATERIALS

The diagnostic value of biopsy examinations depends a good deal on the methods employed in the collection of material. The fragment of tissue should include not only a portion of the growth but a bit of the surrounding tissue as well. Snipping off the top of papillomatous growths is worthless, as is likewise scraping the surfaces of ulcers. Indeed, tissue should not be removed from the central portions of an ulcerating lesion as it may include nothing more than necrotic tissue or exuberant granulations. Removal of tissue during operations with a fine electric cutting point is permissible, provided it is not destroyed for histologic examination by cooking. Testicular tissue for study in relation to the diagnosis of infertility is readily removed from the periphery of a testicle through a small incision in the scrotum.[9]

It is important that tissues should not be allowed to lie around for even an hour or two but should be promptly placed in a wide-mouthed bottle carrying 4 per cent formalin. Otherwise, alcohol may be used but it has the disadvantage of causing considerable shrinkage.

Endometrial tissue should be secured by curettage of the fundus unless there are special reasons for preferring the cervical canal. The aspiration of material from tumors or other lesions of the lungs requires the careful selection of the site of puncture by a combined roentgenologic and roentgenoscopic study unless peripherally located and capable of adequate localization by a roentgenographic examination.[10,13] Furthermore, aspiration should not be conducted under continuous roentgenoscopic observation. After the site of intended puncture has been selected, the needle should be advanced under direct vision of the red light, checking its position from time to time by roentgenoscopy.[13]

Roentgenoscopic guidance is not ordinarily required for the aspiration of large tumors in bones, but tumors in difficult regions such as the head, neck of the femur or the vertebrae may require this assistance. The same is true of tumors in obscure locations, such as those less than 5 cm. in diameter or situated at depths greater than 10 cm. as well as those in close proximity to vital structures like large blood vessels, the esophagus or heart.[13,14]

The technic of sternal puncture for the aspiration of material is described in Chapter 1. Cases of liver disease for needle-punch biopsy should be selected with care. It is particularly applicable to those patients in whom the organ is enlarged below the right costal margin with the surface located superficially just beneath the abdominal wall. Of course, the punch of a nodule is preferred if one is palpable. All cases should be tested first for a possible tendency to prolonged bleeding.[15,16] Before using the method on tumors in the epigastric or subcostal regions, a test puncture should be made with a gauge No. 20 needle in order to be certain that the apparent neoplasm is not a vascular tumor, a hollow organ or a simple abscess.[16]

In aspiration biopsies, blood is frequently sucked into the syringe. This material should be removed at once and inspected. Particles of suspected tumor tissue should be smeared between slides. The blood clot should be saved for sectioning. Otherwise, the material within the needle should be removed either by the stylet or by forcing the tissue on a slide by means of the syringe. The tissue should be flattened and smeared firmly between glass slides and sent to the laboratory for staining and examination. Otherwise the material should be carefully collected in one clump and placed in a 4 per cent solution of formalin for sectioning.

Naturally, many pathologists hesitate assuming the responsibility of making a diagnosis with punch or needle material because of disintegration of cells. At least, they should not be requested or expected to undertake the task when the question of malignancy is involved unless they have had experience and the material has been properly collected.

CLINICAL VALUE

Undoubtedly, biopsy examinations are of greatest clinical value in the early diagnosis of malignant and benign tumors and their differentiation from granulomas or other lesions. This is particularly true in the case of carcinoma in which the earliest possible institution of treatment is of the utmost importance. In early primary cancer of the lungs biopsy examinations not infrequently reveal the disease when bronchoscopy fails as a diagnostic procedure.[10] The same is true in the case of laryngoscopic, esophagoscopic, cystoscopic and sigmoidoscopic examinations.

Furthermore, biopsy of the endometrium provides the best way for determining ovarian function, as it provides exact knowledge of the functional state during any given menstrual cycle, and is particularly useful in cases of sterility and irregular menstrual bleeding. It also enables a check to be made on the results and dosage of hormone therapy.

Testicular biopsy examinations are likewise of value in determining the cause of infertility, particularly since the majority of cases have no endocrine disturbances but rather semen deficiencies resulting from degenerative lesions of the seminiferous tubules caused by either regional or constitutional inflammatory or toxic processes.[9]

Punch biopsies are also of value in determining the cause of hepatomegaly due to primary or metastatic carcinoma, Hodgkin's disease, syphilis, cirrhosis, hemochromatosis, etc., although they should never be used in suspected hemangioma or amebic abscesses.

Punch biopsies are likewise used in the diagnosis of epithelioma of the skin in which it has been stated that as high as 15 per cent may be diagnosed clinically as benign while 37 per cent diagnosed as keratoses have shown malignant changes.[17]

Sternal bone marrow biopsies are not only of value in the diagnosis of multiple myeloma [18] but likewise of Gaucher's disease, Banti's disease, malaria, leukemia, carcinoma with generalized bone metastases, infectious mononucleosis, agranulocytosis, pernicious anemia, aplastic anemia, polycythemia and any hematologic problem [19-24] although it has been pointed out that thorough clinical investigation deprives it of many of its uses [25] and that because of its diagnostic limitations it should not be used to supplant other recognized procedures but should be employed mainly as a means of gaining information regarding the morphology of the marrow cells as well as their respective number as far as diseases involving the hemopoietic system are concerned.

Needle or aspiration biopsies of the liver have been found most useful in the diagnosis of carcinoma and for establishing the nature of diffuse parenchymatous disease, including cirrhosis [11,12]—likewise in the differential diagnosis of chancroid and lymphogranuloma venereum,[27] eosinophilic granulomas of the skin,[28] histoplasmosis of the oral cavity,[29] etc.

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PART TWO

THE PRACTICAL APPLICATIONS OF LABORATORY EXAMINATIONS IN CLINICAL DIAGNOSIS

22

DISEASES OF THE BLOOD AND HEMOPOIETIC SYSTEM

With few exceptions, the phrase "diseases of the blood" is a misnomer in spite of its widespread use. Septicemia may be regarded as a true disease of the blood because it designates an actual infection of it. The same is probably true of those anemias due to hemorrhage, the destruction of erythrocytes by incompatible blood transfusions or hemolytic agents, as well as those resulting from the conversion of hemoglobin into methemoglobin by carbon monoxide or various other poisons and drugs, because such anemias are solely due to the effects of such agents on the blood.

Otherwise, however, the anemias due to blood loss, increased blood destruction or decreased blood production are not true diseases of the blood, since they are merely clinical and hematologic manifestations of changes in the hemoglobin, erythrocytes, leukocytes or thrombocytes due to deficiencies or diseases having their origin and location elsewhere in the body. Hyperglycemia, or hypoproteinemia, may be just as pronounced evidence of disease as an anemia but the phrase "diseases of the blood" has never been used to include abnormal changes in the plasma except in the case of hemolytic jaundice and hypoprothrombinemia in relation to delayed coagulation of the blood. The cerebrospinal fluid may also show pronounced changes in its cytology and chemical constitution, not only in certain diseases of the central nervous system but in some located elsewhere in the body, but no one has ever proposed grouping these under the designation of "diseases of the cerebrospinal fluid."

In other words, the great majority of so-called "diseases of the blood" are really diseases of the erythropoietic, leukopoietic or hemostatic systems of the body. This is true not only of many of the anemias but of the hemoglobinurias, the purpuras, hemophilia and other hemorrhagic disorders, erythemia, the leukemias, infectious mononucleosis, agranulocytosis, etc.

THE ANEMIAS AND HEMOLYTIC JAUNDICE

Classification. The most important single feature of all of the anemias is a reduction in the hemoglobin of the blood. The next important feature is a variation in the size and shape of the erythrocytes. The latter are generally reduced (*hypocythemic anemia*) but this is not always the case, since they may be present in normal numbers (*normocythemic anemia*) or, indeed, even above normal (*hypercythemic anemia*). In other words, anemia may be present regardless of a normal or increased number of erythrocytes per c.mm. of the blood due to a reduction in the total amount of hemoglobin when the erythrocytes contain less than a normal amount of it.

Thus, if the erythrocytes contain less than a normal amount of hemoglobin but are present in normal, above normal or below normal numbers, the anemia is designated *hypochromic*. If the erythrocytes contain more than the normal amount of hemoglobin, but are numerically below normal, the anemia is designated *hyperchromic*. And if the erythrocytes contain a normal amount of hemoglobin, but are also numerically decreased, the anemia is designated *normochromic*.

Variations in the amount of hemoglobin in red blood corpuscles are due to variations in their size as detected by the examination of stained smears of blood, a determination of the volume of packed cells, or by actual measurement of them. On this basis an anemia may be accompanied by erythrocytes of normal size (*normocytic*), below normal (*microcytic*) or above normal (*macrocytic*). As a matter of fact, at the present time the anemias are usually classified from the laboratory standpoint on this morphologic basis into *normocytic anemia*, *macrocytic anemia*, *simple microcytic anemia* and *hypochromic microcytic anemia*, as previously discussed in Chapter 1. Clinically, however, they are generally classified according to their causes because of the important bearing of the latter upon treatment and prognosis.

Etiology. Anemias may be due to any one or a combination of the following three basic etiologic factors: (1) blood loss; (2) increased blood destruction, or (3) decreased or defective blood formation, as follows:

I. *Anemia due to blood loss* as in:

1. Acute traumatic hemorrhage.
2. Acute or chronic hemorrhage from the gastro-intestinal, respiratory or genito-urinary tracts.
3. Hemorrhage due to failure of coagulation as in the purpuras, scurvy and hemophilia.

II. *Anemia due to increased blood destruction.*—This constitutes *hemolytic anemia* which may be acute, subacute or chronic. Needless to state, the degree of anemia depends upon the balance between the destruction or hemolysis of erythrocytes and their formation by the bone marrow. The exact mechanism of hemolysis varies with the hemolyzing agent. The stroma is completely broken up. When the globin-hematin combination in the hemoglobin molecule is split by the reticulo-endothelial cells, the iron in the hematin fraction is set free and bilirubin is formed. An excess of the latter produces bilirubinemia and usually jaundice (*hemolytic jaundice*). If the erythrocytes are destroyed in the circulating blood and not in the spleen hemoglobinemia results; if this exceeds the renal threshold for hemoglobin *hemoglobinuria* occurs.

1. Acute or subacute hemolytic anemia may be caused (a) by isohemolysins as in incompatible blood transfusions, erythroblastosis fetalis and paroxysmal cold hemoglobinuria; (b) by the toxic products of some of the bacteria and animal parasites as well as by the venins; (c) by endogenous toxic products as in severe burns; (d) by allergic reactions as in favis-bean poisoning and (e) by various chemical agents.
2. Chronic hemolytic anemia may be due (a) to chronic infections (bacteria, syphilis, malaria, etc.); (b) to poisoning by various metallic and organic compounds; (c) to

erythrocytes which are congenitally abnormal in shape, as in the spherocytic anemia of congenital hemolytic jaundice and sickle cell anemia; (d) to erythrocytes which are congenitally abnormal in the quality of the stroma as in Cooley's or Mediterranean anemia and sickle cell anemia; (e) to increased hemolysis from abnormal hemolytic activity of the spleen or other parts of the reticulo-endothelial system and (f) to increased hemolysis by changes in the *p*H of the plasma as in paroxysmal nocturnal hemoglobinuria.

III. *Anemia due to decreased or defective blood formation* as in:

1. A deficiency, failure of absorption, or failure of utilization of the antianemic factor responsible for pernicious anemia and concerned in the anemias of idiopathic steatorrhea, celiac disease, chronic diarrhea or other gastro-intestinal disorders, pregnancy, pellagra and other dietary deficiencies, hypothyroidism, chronic and extensive disease of the liver and "achrestic anemia."
2. A deficiency in the intake, absorption, storage or utilization of iron, as in the anemias due to iron-deficient diets, chlorosis and idiopathic hypochromic anemia of women as well as in the hypochromic anemias of pellagra, sprue, chronic diarrhea, pregnancy and in the anemia seen in infants due to a deficient antenatal storage or postnatal supply of iron.
3. Possibly, a deficiency in the intake or utilization of vitamin C and some of the factors of vitamin B complex, especially riboflavin.
4. Depression or injury of the bone marrow as in primary or idiopathic aplastic anemia and secondary aplastic anemia due to toxic agents (amidopyrine and related compounds, dinitrophenol, arsphenamine and other organic arsenicals, compounds of gold, hair dyes, mustard gas, etc.), chronic infections (syphilis, chronic sepsis, etc.), noninflammatory diseases (nephritis and malignancy) or irradiation.
5. Replacement or mechanical interference of the bone marrow resulting in myelophthisic anemia from metastatic neoplasms, multiple myeloma, Hodgkin's disease, leukemia, myelosclerosis, marble bone disease, etc.

Diagnostic Examinations. It is apparent, therefore, that the etiology of an anemia has a tremendous influence on its treatment and prognosis. Furthermore, diagnosis and especially etiologic diagnosis, may require any or all of the following procedures: (1) a careful history of the present illness relative to digestive disturbances, weakness, fatigue, dyspnea, jaundice, bleeding from any source, the occurrence of purpura, edema, etc.; (2) a careful social history relative to diet, occupation, and familial blood dyscrasias; (3) a careful physical examination not only for pallor, edema, purpura, gingivitis and glossitis, but for possible splenomegaly, lymphadenopathy, abnormal reflexes and sensory disturbances; (4) a complete examination of the blood not only for an accurate estimation of the hemoglobin and a careful count of the erythrocytes, but for the size and shape of the latter as well, including the volume of packed erythrocytes in some cases; (5) also, when necessary or indicated, a count of the reticulocytes, the number, size and staining reactions of the platelets, as well as total and differential leukocyte counts. Additional examinations may be required as follows: (6) an examination of the feces for occult blood, ova or parasites; (7) an examination of the stomach contents for hypochlorhydria, hypo-acidity or achylia; (8) a determination of the bleeding and coagulation times as well as tests for capillary fragility in the hemorrhagic diseases; (9) a determination of the tonicity of erythrocytes; (10) an estimation of bile pigments in the serum and urobilinogen in the urine in the hemolytic anemias and (11) examinations of the sternal bone marrow by aspiration or biopsy whenever necessary. Since this book is devoted to clinical diagnosis by laboratory examinations alone, the important or helpful changes detected by laboratory methods are summarized herewith.

Acute Posthemorrhagic Anemia. This anemia results from the more or less sudden and rapid loss of blood due to trauma, the rupture of ectopic gestations, peptic or typhoid ulcers, severe hemorrhages in hemophilia, acute leukemia, purpura haemorrhagica, aplastic anemia, etc.

1. The earliest effects are an increase of platelets, a shortening of the coagulation time and polymorphonuclear leukocytosis with a shift to the left. The erythrocytes, hemoglobin and volume of packed cells are misleadingly high due to vasoconstriction and the liberation of erythrocytes from storehouses such as the spleen. Blood volume is decreased due to loss of both cells and plasma. The plasma volume, however, is quickly restored, particularly if fluids are administered and, after the first twenty-four hours, may be actually greater than normal. Anoxemia may occur due to the reduction of hemoglobin.

2. Later there is an increase of reticulocytes with a decrease of erythrocytes and hemoglobin due to readjustment of the blood volume and dilution of the blood by tissue fluids. Hypercholesterolemia is not infrequent due either to decreased oxidation resulting from the diminution in erythrocytes or to excessive mobilization of lipids from the fat reserves in the tissues, as the neutral fats and fatty acids of the plasma are likely to be increased. Naturally blood iron is reduced.

The anemia is usually normocytic although macrocytosis may occur, as well as polychromatophilia, with normoblasts if the hemorrhage has been very severe. Blood regeneration is rapid leading to a normal leukocyte count in three or four days with no evidence of active red corpuscle regeneration after ten to fourteen days.

Chronic Posthemorrhagic Anemia. The anemia results from open or concealed bleeding over a long period of time, as from the uterus, peptic ulcers, hemorrhoids, malignant tumors, recurrent and severe epistaxes or hemoptyses, multiple hereditary telangiectasis, chronic thrombocytopenic purpura, hemophilia, etc.

1. It is of the hypochromic type characterized by the poverty of hemoglobin in the individual erythrocytes which, in extreme cases, may show large numbers occurring only as mere rings along with tiny microcytes and moderate numbers of poikilocytes.

2. The hemoglobin is reduced out of proportion to the red cell count and the volume of packed cells. Indeed, the erythrocyte count may be normal or even slightly above normal; consequently, the color index is low. The fragility of the erythrocytes may be normal but usually there is a slight increase of resistance to hypotonic solutions. The reticulocytes are normal in number or slightly reduced as are likewise the leukocytes, although a relative lymphocytosis is not uncommon. The platelets are usually normal in number and small in size.

3. The plasma is pale with no increase of bilirubin; hypoproteinemia is of frequent occurrence. Decreased glucose tolerance may occur as, likewise, increased

plasma fat and fatty acid, increased plasma phospholipid and decreased blood iron.

The Acute Hemolytic Anemias; Acquired Hemolytic Jaundice. Acute and subacute hemolytic anemia may be due to many causes as follows: (1) Incompatible blood transfusions; (2) severe bacterial infections and especially those due to hemolytic streptococci, *Str. viridans,* hemolytic staphylococci, *Cl. welchii, S. typhosa,* the Brucella, *Bartonella bacilliformis* (Carrión's disease or Oroya fever and verruga peruviana) and *T. pallidum;* (3) malaria; (4) chemical agents like phenylhydrazine, trinitrotoluene, dinitrobenzol, phenol, potassium chlorate, lead, acetanilid and the sulfonamide compounds (especially sulfanilamide) and (5) vegetable (favism) and animal poisons (venins) as well as those of endogenous origin (extensive burns). Severe hemolytic anemia resulting from any of these causes may produce *acquired hemolytic jaundice* due to the excessive production and defective excretion of bilirubin. Hemolytic anemia may also occur in malignant diseases including metastatic carcinomatosis, sarcoma, Hodgkin's disease, Boeck's sarcoid, the leukemias and reticulo-endotheliosis.[1]

The acute and subacute hemolytic anemias also include paroxysmal (cold) hemoglobinuria and erythroblastosis fetalis as well as Lederer's anemia. These will be considered separately as, likewise, the chronic hemolytic anemias of congenital hemolytic jaundice, sickle cell anemia, chronic hemolytic anemia with nocturnal hemoglobinuria (the Marchiafava-Micheli syndrome), and Cooley's or Mediterranean anemia. Chronic hemolytic anemia may also occur in association with pernicious anemia and, rarely, in leukemia, Hodgkin's disease and carcinomatosis.

The *laboratory findings* in the acute and subacute hemolytic anemias and acquired hemolytic jaundice are directly related to the rate and degree of blood destruction and may be summarized as follows:

1. Marked reduction in the erythrocytes, hemoglobin and volume of packed cells with a low color index. The anemia is usually normocytic but may be macrocytic. There is marked poikilocytosis with many normoblasts and macroblasts. Numerous erythrocytes show basophilic stippling and the reticulocytes are numerically greatly increased. Spherocytosis and increased fragility of the erythrocytes may also be observed in some cases.[1]

2. Due to stimulation of the bone marrow there is likewise leukocytosis with myelocytes and even, rarely, myeloblasts. The platelets are usually numerically increased and larger than normal; sometimes, however, leukopenia and slight thrombocytopenia occur.

3. The excessive destruction of erythrocytes may also result in the rapid and excessive production of bilirubin from the released hemoglobin. The resulting bilirubinemia produces *acquired hemolytic jaundice* due to the excessive production of the pigment and a reduced capacity of the liver to excrete it in the bile because of degenerative changes in the parenchymal cells resulting from anoxemia. As a result the blood plasma or serum shows a high icterus index and a positive indirect van den Bergh reaction.

4. The blood plasma also shows the presence of hemoglobinemia and a third

pigment, formerly regarded as methemoglobin but now identified as methemalbumin.[2] The neutral fats and fatty acids of the plasma are also likely to be increased along with hypocholesterolemia and not infrequently a reduction of blood iron.

5. Hemoglobinuria may occur and persist until the plasma level is as low as 30 to 50 mg. per 100 cc. The urine may also contain albumin but no bile (acholuric jaundice) although urobilinogen and urobilin are usually present; the latter also occur in the feces.

6. As might be expected, the sternal bone marrow is very hyperplastic with as many as 60 per cent nucleated cells belonging to the erythrocytic series instead of the normal of 20 per cent or less. Consequently, the number of leukocytes is relatively decreased.

Congenital Hemolytic Jaundice. This is a form of jaundice with splenomegaly and sometimes lymphadenopathy due to a variable degree of hemolytic anemia characterized by diminished resistance of the erythrocytes to hemolysis by hypotonic saline solutions. The disorder is chronic, congenital and often familial; it is also known as *chronic familial jaundice (icterus)*, *hemolytic splenomegaly*, *chronic acholuric jaundice*, and *spherocytic anemia*. The trait is a mendelian dominant and is transmitted by either parent. The manifestations usually appear during late childhood or early adult life but may be so mild that the disease is not recognized until late in adult life. The chief *laboratory findings* are as follows:

1. The erythrocytes may be numerically normal but are usually moderately reduced; during a hemolytic crisis they are greatly reduced. The hemoglobin and volume of packed cells may or may not be reduced proportionally. The mean corpuscular volume is usually below normal but may be normal or slightly increased.

2. Characteristically the erythrocytes are reduced in size, are of increased thickness, and are spherical rather than biconcave in shape (microspherocytosis). Haden [3] has recently described a new type of the disease characterized by the absence of spherocytosis and spontaneous hemolysis of erythrocytes probably due to an inherited defect of the stroma. On the other hand, macrocytes may be so numerous as to lead to confusion with pernicious anemia. Nucleated cells are not present unless the anemia is very severe. The reticulocytes are increased; the platelet count is usually normal.

3. Another characteristic change is unusual fragility of the erythrocytes. Hemolysis usually begins at about 0.51 to 0.72 per cent sodium chloride solution but may begin at 0.87 per cent. Complete hemolysis usually occurs at about 0.39 per cent.

4. The number of leukocytes is usually normal but leukocytosis occurs during a hemolytic crisis with a shift to the left; immature types may be so numerous as to give a "leukemoid" blood picture. Otherwise, lymphocytes, plasma cells and basophils may be increased.

5. Owing to excessive hemolysis and the overproduction of bilirubin, the icterus index is increased (15 to 30 or higher) accompanied by a positive indirect

van den Bergh reaction. Hemoglobinuria is very unusual but the urine contains increased amounts of urobilinogen and urobilin (no bile pigments or bile salts). The feces contain bile pigment as well as excessive amounts of urobilin. Blood cholesterol is usually reduced.

6. The sternal bone marrow shows erythropoietic hyperplasia of the normoblastic type; megaloblasts and abnormal leukocytes are absent.

Erythroblastosis Fetalis. This is an acute hemolytic anemia of the newborn including *congenital anemia of the newborn, icterus gravis* and *hydrops fetalis.* It is characterized by a severe macrocytic anemia, marked erythroblastosis, jaundice, edema, and enlargement of the liver and spleen. Petechial hemorrhages may occur. Permanent spastic paralysis and mental retardation may result. As stated in Chapter 17, the cause of the disease is the presence of the Rh factor in the erythrocytes of the fetus which results in the production of an antibody for Rh corpuscles by the mother which she passes back to the child through the placenta, resulting in the hemolysis of its erythrocytes.[4] Donahue and Fremes [5] have recently reported two cases of maternal Rh-Hr isoimmunization resulting in the birth of erythroblastotic infants, but in which normal infants were delivered in subsequent pregnancies. The usual *laboratory findings* are as follows:

1. Severe anemia of the macrocytic type present at birth or developing shortly thereafter in contradistinction to simple icterus neonatorum which develops about three days after birth. Erythrocytosis is a striking feature along with polychromatophilia, stippling and reticulocytosis. Fragility is usually normal.

2. Leukocytosis is marked, with myelocytes and sometimes myeloblasts. The platelets may be reduced, especially if purpura is present, in which case the bleeding time is prolonged.

3. The icterus index is increased, accompanied by a positive van den Bergh reaction usually of the biphasic type because of damage to the parenchymal cells of the liver, with partial obstruction of the bile ducts. Consequently, the urine contains bile although the feces are not usually acholic.

Lederer's Anemia. This is a rare type of acute hemolytic anemia of unknown etiology occurring at any age and in either sex. The onset is acute without premonitory symptoms. Fever is so common that the disease is sometimes known as "acute febrile anemia" and suggests an acute infection as the cause; it usually continues for one to three weeks or longer with vomiting, anorexia, subclinical or clinical jaundice, headaches and muscular pains, A comatose state with various neurologic disturbances may develop. The disease may also occur as a subacute type. Repeated blood transfusions are specific in treatment. The usual *laboratory findings* are as follows:

1. Rapidly developing severe anemia, usually of the macrocytic type, with marked reduction in erythrocytes and hemoglobin. Marked erythroblastosis is characteristic. The picture may resemble that of severe pernicious anemia. The reticulocytes are always markedly increased. The platelets are usually normal in number and size.

2. Marked leukocytosis occurs accompanied by myelocytes and even myeloblasts and may be so pronounced as to suggest myelogenous leukemia.

3. Excessive hemolysis and the overproduction of bilirubin lead to an increase in the icterus index with a positive indirect van den Bergh reaction. The urine does not contain bile pigment (acholuric) but shows the presence of excessive amounts of urobilinogen and urobilin, sometimes with albumin and casts.

Cooley's or "Mediterranean" Anemia. This hemolytic anemia is usually of the microcytic hypochromic type and of unknown etiology. First clearly separated by Cooley and Lee [6] from the symptom complex known as von Jaksch's anemia, it is also called *"Mediterranean" anemia, erythroblastic anemia* or *"target cell" anemia.* Fortunately rare, the classical form is characterized clinically by progressive anemia starting in childhood, a characteristic facies and splenomegaly, with a familial and racial tendency. For example, it is common for two or more siblings in a family to be affected and there is reason to believe that the parents of many affected children may show characteristics of the disorder. The usual *laboratory changes* may be summarized as follows:

1. Marked anemia with a particular reduction in hemoglobin and the volume of packed cells, of the microcytic hypochromic type.

2. One of the characteristic changes is a marked variation in the size of the erythrocytes. Most of them are microcytes containing so little hemoglobin that it appears only at the periphery of the corpuscles; indeed, few fully colored erythrocytes are present. The hemoglobin may also form circular areas in the centers of red blood corpuscles resulting in the so-called "target cells." Dameshek [7] regards these corpuscles as being congenitally defective and possibly the fundamental factor in Cooley's anemia; Bohrod,[8] however, regards them as newly formed cells produced by the bone marrow in response to blood loss from any cause. Sickling does not occur but large numbers of erythroblasts are characteristic along with polychromatophilia, stippling, Howell-Jolly bodies and reticulocytosis. The platelets are usually normal.

3. "Target cells" show increased resistance to hypotonic saline solutions [8] but the fragility of the erythrocytes as a whole is not increased.

4. Leukocytosis is usually accompanied by myelocytes and even myeloblasts. The monocytes may be increased as likewise the lymphocytes, the latter especially in infants.

5. Due to hemolysis and the excessive production of bilirubin, the icterus index is slightly increased (8 to 30) along with a positive indirect van den Bergh reaction. The urine usually shows an increase of urobilinogen or urobilin and sometimes the presence of iron-laden kidney epithelial cells.

Sickle Cell Anemia. Sickle cell anemia is also of the chronic hemolytic type. It is essentially peculiar of the Negro race or those of mixed blood and, being a dominant characteristic, the trait may appear in remote generations. However, sickle cell anemia may occur in white persons but is rare.[9] *Sicklemia,* without demonstrable sickle cell anemia, has been reported as occurring in 6 to 10 per cent of Negroes; [10,11] the transfusion of the latter with sicklemia blood may be dangerous although there is no correlation between sickling erythrocytes and the blood agglutinogens.[10] Singer and his colleagues,[12] however, have recently reported that the "trait" cells have a normal survival time of about 100 to 120 days so that it seems permissible to use them for therapeutic blood transfusions.

In the study of large groups of Negro women and infants, Watson and his colleagues[13] found 8 per cent of the former and 8.4 per cent of the latter with sicklemia. In the women, 84 to 100 per cent of the erythrocytes showed sickling, but only 0.5 to 29.5 per cent of the cells of infants showed this trait, which was ascribed to differences between adult and fetal hemoglobin. For this reason the survival of infants with sickle cell anemia during pregnancy may be due to the protective influence of fetal hemoglobin which is unable to sickle even at the low oxygen tension existing in the fetus.

The disease is characterized clinically by symptoms of anemia, rheumatoid pains with acute exacerbations, leg ulcers, jaundice during crises, and frequently splenomegaly. Hematologically it is characterized by peculiar sickle-shaped and oat-shaped erythrocytes with changes due to excessive blood destruction and active blood formation. The disease is also known as *meniscocytosis, Herrick's syndrome* and *drepanocytic anemia.* It occurs particularly in young individuals, probably because it is not compatible with long life. There is no predisposition according to sex. The usual *laboratory changes* are as follows:

1. Well-marked anemia due to a reduction of erythrocytes, hemoglobin and the volume of packed cells. In severe cases it is of the macrocytic type. As previously stated, a characteristic feature is sickling of the erythrocytes. Hypochromia is frequent, along with the presence of "target cells." Erythroblastosis, polychromatophilia, basophilic stippling and Howell-Jolly bodies are usual. The platelets are frequently increased and bleeding and coagulation times are normal. The resistance of the erythrocytes to hypotonic saline solutions is usually slightly increased. Sickle cells do not form rouleaux and sedimentation is slow.[14] An increase of cold agglutinins for group O corpuscles may occur.[15,16]
2. Leukocytosis occurs with a shift to the left; myelocytes may be found. Eosinophilia may occur, as likewise an increase of monocytes.
3. Owing to excessive hemolysis and the overproduction of bilirubin, the icterus index is increased (15 to 25) with a positive indirect van den Bergh reaction. The urine shows an increase of urobilinogen and urobilin, with a fixed low specific gravity and frequently albumin and casts. Gastric analysis shows no abnormalities.
4. The sternal bone marrow shows a marked increase of nucleated erythrocytes (50 to 70 per cent of all nucleated cells) largely composed of normoblasts, some of which may be unusual in shape.[17] There may be a moderate shift to the left of the leukocytes and an increase of megakaryocytes.

Pernicious Anemia. "Pernicious" anemia was so called before the discovery of liver therapy because the disease was inevitably fatal, but this term is now no longer appropriate. The same applies also to the phrase "primary anemia" which was also employed before the disease was discovered to be due to a deficiency in the production of the erythrocyte-maturing or antianemic substance which is stored in the liver and probably also produced to some extent by it.

The disease occurs as a chronic anemia of the macrocytic type, characterized clinically by insidious onset, achlorhydria or achylia and certain gastro-intestinal and neurologic disturbances. It is also known as "Addison's anemia" or *addisonian anemia* after Addison who first fully described it in 1855.

Pernicious anemia is a disease occurring chiefly in the temperate zone and among the white race although its rarity in full-blooded Negroes has probably been overemphasized and especially in those of mixed blood.[18] It is also rare in Orientals and uncommon in Jews. It is essentially a disease of late adult life and extremely unusual in children. In the United States and England males are more frequently affected than females but it is just the reverse in Germany and the Scandinavian countries. Heredity appears to be a factor in etiology. Pernicious anemia may occur in the males of a family and hypochromic anemia in the females, which is probably significant since both are fundamentally related to gastric secretory dysfunction. The usual *laboratory changes* are as follows:

1. The anemia varies tremendously depending on whether the disease is in remission or relapse. Without liver therapy and during relapse it is characterized by a severe reduction in the number of erythrocytes, hemoglobin and volume of packed cells. Macrocytes predominate with an increase in the mean corpuscular volume. Poikilocytosis is characteristic. Basophilic stippling occurs and both Howell-Jolly and Cabot's rings are commonly observed. Total blood volume is reduced, due to the decrease in the number of erythrocytes, but the plasma volume is variable. The resistance of the erythrocytes to hypotonic saline solutions is not significantly altered. The platelets are generally reduced and especially when the erythrocytes are below 2,000,000 per c.mm. of blood.[19]

2. While the total amount of hemoglobin is reduced, the erythrocytes are usually well filled with it. Consequently, the anemia is of the hyperchromic type with a high color index. Marked hypochromia occurs only when there is a coincident deficiency in iron. But while the erythrocytes are well filled with hemoglobin, they are not supersaturated with it; their dark appearance in unstained and stained preparations is due to a greater thickness of the cells.

3. Leukopenia is the rule, with an absolute neutropenia and a shift to the right. Many of the polymorphonuclear leukocytes are exceptionally large and multisegmented (*macropolycytes*). However, myelocytes and even myeloblasts may be observed and sometimes in such numbers as to suggest the presence of myelocytic leukemia. A relative lymphocytosis is usual. Eosinophilia is common and especially after the administration of liver or liver extract.[20]

4. The blood plasma during relapse is brownish-yellow. Because of excessive hemolysis and the overproduction of bilirubin, the icterus index is usually increased (20 to 25), with a positive indirect van den Bergh reaction; liver function (hippuric acid test) is usually impaired. Hypoprothrombinemia may occur which is not corrected by the administration of vitamin K.[21] Sugar tolerance may be reduced; hypoglycemia may be so pronounced during liver therapy as to produce hunger symptoms. Acid-base equilibrium, however, is normal. Anoxemia is usually due to decreased hemoglobin. Blood iron is reduced but serum iron is increased. The plasma fat and fatty acids are increased along with hypocholesterolemia.[22] Urea

clearance is likely to be slightly reduced but the nonprotein nitrogen of the plasma is not apt to be over 50 mg. per 100 cc. Serum uric acid is normal or slightly reduced during relapses but usually increased during remissions. There might be a slight decrease in the basal metabolic rate.

5. In untreated cases and those in relapse, the sternal bone marrow changes are characterized by hyperplasia of the cells of the erythrocytic series, with nucleated red corpuscles making up 30 to 50 per cent of all nucleated cells instead of 20 per cent or less as is normal. Most of these nucleated cells are megaloblasts. Megaloblastic hyperplasia is a sign that liver treatment is likely to be successful while in its absence this therapy is apt to be ineffective. However, since the intramuscular injection of liver extract may change a megaloblastic marrow to a mixed megaloblastic and normoblastic one in a few hours, it is important to delay its administration until a marrow study has been made.[23]

In addition, the marrow shows evidences of active leukopoiesis, with not only an increase of normal myeloid leukocytes but many which are abnormally large. "Reticulum" or "Ferrata" cells and lymphocytes are also more numerous than normal and there is a decrease of megakaryocytes many of which are abnormal. Complications may alter these typical changes.

6. Achlorhydria occurs in about 98 per cent of cases and is characteristic of the disease. Achylia may occur. The volume of gastric juice is also reduced.[24] Pernicious anemia, however, may occur with free hydrochloric acid present in the gastric contents. Trypsin of the pancreatic secretion may be decreased.

7. Owing to excessive hemolysis and the overproduction of bilirubin, the urine may show an increase of urobilinogen. The excretion of coproporphyrin I in the urine and feces is increased. The specific gravity of the urine is usually low and fixed with small amounts of albumin and casts sometimes present ascribed to injury of the kidney tubules by deposits of iron pigment. If edema is present these changes may lead to an erroneous diagnosis of chronic nephritis. Observations bearing on the metabolic behavior of tyrosine indicate that blood phenol levels are high and that the urinary excretion of total phenolic compounds is increased in untreated cases, while adequate therapy results in a reduction in excretion.[25]

8. The feces usually contain an excess of urobilin and sometimes so much mucus as to suggest the presence of mucous colitis.

Diphyllobothriasis and other Pernicious-like Anemias. A macrocytic anemia practically identical in its clinical and hematologic features with that of pernicious anemia may occur in persons infested with the fish tapeworm (*Diphyllobothrium latum*). But its incidence is so low [26] among infested individuals (0.1 to 0.2 per cent) as to suggest the coincident occurrence of pernicious anemia rather than a pernicious-like anemia due to hemolytic toxins produced by the parasite.

A macrocytic anemia resembling pernicious anemia may also occur in *tropical sprue* although it may be hypochromic microcytic in type while some cases do not show any anemia at all.

Macrocytic anemia may also occur in *idiopathic steatorrhea* and *celiac disease* although hypochromic microcytic anemia is more common, while an anemia of the "erythroblastic type" resembling Cooley's anemia may also occur in both diseases characterized by the presence of large numbers of normoblasts and microblasts. The bone marrow also shows a preponderance of normoblasts but megaloblasts are absent. The fragility of the erythrocytes is normal and while the icterus index may be increased in sprue, it is usually within normal in idiopathic steatorrhea.

Macrocytic anemia resembling pernicious anemia may also develop in individuals with *carcinoma of the stomach,* after *gastro-enterostomy* and *resection of the small intestine,* in *intestinal strictures* and *anastomoses, chronic dysentery, regional ileitis* and *chronic pancreatitis,* but is of rare occurrence. Not infrequently, however, a pernicious-like macrocytic anemia of mild degree is associated with severe *disease of the liver* of long duration,[27] especially in cirrhosis or cirrhosis combined with carcinoma, which may be due in some cases at least to a defective storage or synthesis of the antianemic substance. On the other hand, the anemia is frequently of the normocytic or microcytic type, the latter generally due to a chronic loss of blood.

A macrocytic anemia of mild or moderate degree associated with bone marrow hypoplasia may also occur in *hypothyroidism* which has been attributed to a physiologic adaptation to the diminished needs of the tissues for oxygen.[28] A rare type of anemia resembling pernicious anemia, which runs a chronic course responding only temporarily or not at all to liver therapy, and often fatal, has been termed *achrestic anemia.*[29] This anemia is apparently due to some interference with the proper action of the antianemic substance on the erythropoietic portion of the bone marrow. The bone marrow changes resemble those of pernicious anemia, being hyperplastic and largely megaloplastic.

"Tropical" macrocytic anemia, which affects the natives of tropical and subtropical countries and especially pregnant women in India, also presents clinical and hematologic changes closely resembling those of pernicious anemia;[30,31] it is generally relieved by the administration of liver or yeast.

Simple Chronic Anemia. This type of anemia is commonly referred to as "secondary anemia" because it is due to or associated with many chronic infections and other systemic diseases. A better designation, however, is "simple chronic anemia" because it is usually insidious in onset, runs a chronic course with slow recovery and shows no radical changes in the blood.

It is encountered in most cases of *subacute and chronic bacterial infections* like rheumatoid arthritis, rheumatic fever, subacute bacterial endocarditis, osteomyelitis, furunculosis, tuberculosis, bronchiectasis and pulmonary abscesses, pelvic inflammatory disease, urinary tract infections, bacillary dysentery, chronic brucellosis, tertiary syphilis, etc. In my opinion it also occurs in marked focal infections of dental, tonsillar or other origin. Needless to state, the correction or alleviation of the responsible infection is of fundamental importance in treatment.

This type of anemia is also frequently encountered in *parasitic infestments* causing impairment of nutrition, blood loss or secondary bacterial infections such as in uncinariasis, strongyloidiasis, taeniasis, leishmaniasis, filariasis, trichinosis, echinococcosis, clonorchiasis and schistosomiasis. In diphyllobothriasis, however, the anemia is macrocytic and of the "pernicious-type" while in malaria it is of the hemolytic type, as previously discussed.

Simple chronic anemia is also frequently encountered in diseases or states other than those due to infection or infestation, such as *subacute and chronic nephritis* of any type when marked nitrogen retention has occurred, *malignancy, chronic hepatic disease, biliary fistulas, chronic pancreatic disease, endocrine disorders,* especially hypothyroidism, Addison's disease and hypogonadism of the female, *pregnancy* and, possibly, some of the *avitaminoses* with special reference to vitamin C and vitamin B complex. It has also been stated that this anemia may be due to changes of *climate* but no adequate evidence has been presented in support of this view.[32]

As recently stated by Whipple and Robscheit-Robbins,[33] it would appear that the liver not only stores some of all the factors essential for the production of hemoglobin but that it is particularly concerned in the production or storage of globin and its precursors. According to these investigators, hypoproteinemia is almost always associated with a low reserve store of hemoglobin-producing factors in the liver, as in pregnancy, eclampsia and lactation, as well as depletion of the body protein reserve stores in general, which not only reduces the resistance of the liver to infection but renders it very susceptible to many toxic substances. The usual *laboratory changes* are as follows:

1. A mild or moderate degree of anemia due to a reduction in erythrocytes (2,000,000 to 4,500,000) and of hemoglobin (6 to 12 gm.) with reduction in the volume of packed cells. In chronic nephritis with marked nitrogen retention, however, the anemia is frequently more severe.

2. The type of anemia is usually normocytic but may be microcytic when severe. Anisocytosis is common but poikilocytosis is slight while polychromatophilia and erythroblastosis are conspicuous by their absence.

3. Slight leukocytosis is usually present due to an absolute increase of lymphocytes and especially when the anemia is caused by a chronic infection. Eosinophilia is commonly observed in parasitic infestments and especially in uncinariasis. In this connection it is to be stated, however, that eosinophilia may also be due to pernicious anemia, the administration of liver, or some of the dermatoses as well as occurring as a family trait [34] known as "familial eosinophilia."

4. The blood plasma is normal in color with no increase of bilirubin. Excessive amounts of iron and copper may be stored in the liver and spleen, especially in the malignancies,[35] with reduction in blood iron.[36,37] Hypoproteinemia and hypocholesterolemia may occur due to deficient diets and especially when marked edema is present as in uncinariasis.[38]

5. Urobilinogen and urobilin are not usually increased in the urine or feces.

Chlorosis. Chlorosis is not nearly as common at present as formerly, which has been attributed to better nutrition and hygiene. Classically it is an anemia of the hypochromic microcytic type occurring in girls before puberty for which the name *essential juvenile iron deficiency anemia* has been proposed by Alsted.[39] At the present time, however, it is being observed more frequently in older girls and women and while rare in males, cases have been reported as occurring among them.[40] Various causes for the disease have been proposed in the past but it appears to be due to a deficiency in iron or other factors in qualitatively and quantitatively insufficient diets with special reference to those deficient in meats and high in carbohydrates.

The *laboratory findings* are usually (1) an anemia characterized by a slight or moderate reduction in erythrocytes with a disproportionately marked decrease in hemoglobin. Consequently, the anemia is of the hypochromic type with a low color index; (2) the majority of the erythrocytes are microcytic with a reduction in the volume of packed cells. Anisocytosis is common and polychromatophilia may occur, but poikilocytosis and erythroblastosis are absent unless the anemia is unusually severe as is sometimes the case; [39,41] (3) basophilic stippling is usually absent with no increase of reticulocytes; (4) the platelets are normal; (5) the leukocytes are likewise normal or slightly reduced; (6) a characteristic feature is normal gastric function with no hypochlorhydria.

Other Iron Deficiency Anemias; Idiopathic Hypochromic Anemia. Hypochromic microcytic anemia due primarily to a loss, deficient intake or deficient utilization of iron, may not only occur in chlorosis but result from chronic hemorrhage, disorders of the alimentary tract with impaired digestion or iron-deficient diets. It is common in infants and young children as well as in pregnancy and is characteristic of uncinariasis. Like chlorosis, it is much more common in females than in males and is also known as *nutritional hypochromic anemia* or *chloranemia*.

When the cause cannot be discovered the anemia is usually designated as *idiopathic hypochromic anemia*. It is seen most frequently in well-nourished women at or near the menopause [42] and is apparently due to some unknown factor interfering with the absorption or utilization of iron. Only about 4 per cent of patients are men.[43] Recently Strauss and his colleagues [44] have described a familial type of the disease presenting a granular appearance of the skull in x-ray plates. Refractoriness to all forms of treatment is characteristic and a curious complaint sometimes presented is dysphagia, constituting the *Plummer-Vinson syndrome*.[45,46] The usual *laboratory changes* in these anemias are as follows:

1. Reduction in the number of erythrocytes per c.mm. of blood with the degree depending on the severity of the anemia although the erythrocyte count may be normal or even somewhat above normal. Extreme reductions, however, as in pernicious anemia, do not occur. The characteristic change is a marked poverty of hemoglobin in the individual erythrocytes most of which are microcytes. Polychromatophilia and poikilocytosis may occur but erythroblastosis is observed only in extreme cases. The reticulocytes are normal in number or slightly reduced, unless a recent hemorrhage has occurred. Basophilic stippling is uncommon. The fragility of the erythrocytes may be normal but a slight increased resistance to hemolysis by hypotonic saline solutions is of frequent occurrence.

2. The hemoglobin and volume of packed cells are markedly reduced and may reach extremely low levels. Consequently, the color index is low.

3. The platelets are usually normal in number and small.

4. The leukocytes may be normal in number or slightly reduced with a relative lymphocytosis in long-standing cases.

5. The sternal bone marrow is hyperplastic with an absolute increase of small polychromatophilic normoblasts roughly proportional to the degree of anemia and contrasting sharply with the poverty of the peripheral blood. Unlike pernicious anemia, megaloblasts are not found.

6. The blood plasma is very pale with no increase of bilirubin. Hypoproteinemia and hypocholesterolemia may occur along with increased plasma fat and fatty acids as well as a decrease in glucose tolerance. Both blood and serum iron are decreased. Blood and plasma volumes are variable.

7. There is no increase of urobilinogen in the urine or of urobilin in the feces.

8. Hypochlorhydria or achlorhydria are observed in the great majority of cases.

Aplastic Anemia. This term is applied to a severe, rapidly progressive anemia in which signs of excessive blood destruction are absent and which is characterized by a marked reduction not only in the erythrocytes but of the leukocytes (granulocytes) and the platelets as well (pancytopenia). In other words, it affects all three of the corpuscular elements of the blood produced by the bone marrow, namely, the erythrocytes, granulocytes and thrombocytes or platelets. Since these changes are usually due to an aplasia or hypoplasia of the bone marrow, the disease is called "aplastic anemia." But in some cases evidences of blood regeneration are found not only in the blood but in the bone marrow as well; indeed, the latter may be normally cellular or even hyperplastic.[47,48] In other words, it would appear that an arrest in the maturation of erythrocytes, granulocytes and platelets in the bone marrow is the underlying pathologic change. But in view of the fact that the disease is characterized by a great reduction of all three cells in the peripheral blood, Thompson and his associates have proposed calling it "progressive hypocythemia."[47] And since aplastic anemia (in which all of the hematopoietic elements of the bone marrow are depressed) is similar to agranulocytosis and thrombocytopenic purpura (in which the depression is selective) it has also been proposed to group all three together under the designation of "bone marrow insufficiency."

When the cause is unknown, the disease is designated idiopathic or *primary aplastic anemia*. It occurs in two forms. One, first described by Ehrlich in 1888, is very acute with death occurring in a few weeks; it affects individuals during adolescence or early adult life and is characterized by marked aplasia of the bone marrow. The other, which is characterized by partial aplasia or actual hyperplasia of the bone marrow and is sometimes called "pseudo-aplastic anemia," occurs mainly in adults twenty-five to seventy years of age. It is much less acute, and affected individuals may linger along for months and even one or more years. In both, however, the disease is progressive and fatal in spite of blood transfusions, the administration of liver, bone marrow, nucleotide or vitamins or any other kind of treatment.

When the cause is known, the disease is designated *secondary aplastic anemia*. It progresses more slowly with a much better prognosis since numerous cases have recovered. Among the causes are to be mentioned poisoning by benzol, mustard gas, hair dyes and volatile insecticides, the administration of the organic arsenicals, bismuth, mercury, colloidal silver, dinitrophenol and various analgesics and exposure to radioactive substances. Custer [49] has reported that the administration of atabrine for the suppression of malaria may also cause the disease. Parmer [50] observed that the parenteral administration of atabrine to rabbits results in a higher concentration in the bone marrow than in the plasma and erythrocytes. Two cases of aplastic anemia due to the administration of streptomycin have also been reported [51] as, likewise, aplastic anemia due to the administration of tridione.[52] Chronic bacterial infections have also been suspected as responsible for some cases but conclusive evidence has not been presented. The usual *laboratory findings* in both idiopathic and secondary aplastic anemia are as follows:

1. An erythrocyte count of 2,000,000 per c.mm. or less with a reduced volume of packed cells. The corpuscles are usually normal in size (normocytic) with polychromatophilia, basophilic stippling and few or no reticulocytes; nucleated erythrocytes are usually absent. In some instances, however, the anemia is macrocytic with moderate anisocytosis, poikilocytosis and erythroblastosis. A large number of nucleated cells, however, is always suggestive of an error in diagnosis even when the sternal bone marrow is found to be hyperplastic. The fragility of the erythrocytes is normal.
2. The hemoglobin is greatly reduced (3 to 6 gm. per 100 cc. of blood) and being commensurate with the reduction in erythrocytes, the color index is usually normal (1.0).
3. Marked leukopenia (1500 per c.mm. or less) due to the absence or a great reduction in the granulocytes with a relative lymphocytosis; an absolute lymphocytopenia, however, may be present as well. In cases showing hyperplastic bone marrow myelocytes may occur.[47]
4. Marked reduction in the platelets with a moderately prolonged bleeding time and poor clot retraction; the coagulation time is usually normal.
5. Blood iron is reduced but otherwise the plasma may show no changes. Since there is no evidence of increased blood destruction in the disease the bilirubin is not increased; consequently, there is likewise no increase of urobilinogen in the urine or of urobilin in the feces. Gastric secretion is not significantly affected.
6. Typically the sternal bone marrow consists chiefly of red corpuscles. The majority of the nucleated cells are lymphocytes. But, as previously stated, the marrow may be normally cellular or actually hyperplastic. In such cases there may be a moderate preponderance of lymphocytes. In other cases there may be a striking immaturity of the red and white series of cells but the megaloblasts of pernicious anemia and acute "aleukemic" myeloblastic leukemia are not found.

Myelophthisic Anemia. This anemia, which is due to space-occupying disorders of the bone marrow, is characterized by the presence in the circulating blood of immature leukocytes of the myeloid series as well as nucleated erythrocytes in numbers sometimes quite out of proportion to the degree of anemia. Consequently, it has also been called "leuko-erythroblastic" anemia, "myelopathic" anemia, and "osteosclerotic" anemia. Since it has many of the features of the anemia of leukemia and aleukemic myelosis, it is also sometimes called a "leukemoid reaction."

Metastatic carcinoma in bone marrow is perhaps the commonest cause but the anemia may also occur in multiple myeloma, myelosclerosis, osteopetrosis or "marble bone disease," neurofibromatosis, Hodgkin's disease and the primary xanthomatoses (Gaucher's disease, Niemann-Pick disease and Hand-Schüller-Christian disease). The usual *laboratory changes* are as follows:

1. A moderately severe anemia of the normocytic and normochromic type but sometimes of the macrocytic type. One of the characteristic features is the presence of unusually large numbers of erythroblasts largely composed or normoblasts. The reticulocytes are increased and polychromatophilia and stippling may occur. The resistance of the erythrocytes to hypotonic saline solutions is sometimes increased.
2. Leukopenia usually occurs but the total leukocyte count may be so high that leukemia is suspected. The granulocytes are present in normal proportions but the presence of myelocytes is another characteristic feature. Even an occasional myeloblast may be found.
3. The platelet count may be normal but is usually reduced.
4. Sternal bone marrow examinations are helpful in diagnosis and especially by biopsy instead of by the puncture method.[53] This is particularly true since the blood changes in myelophthisic anemia not only resemble those of myeloid leukemia but especially those of aleukemic myelosis. Differentiation from aplastic anemia is usually not difficult because of the marked erythroblastosis in myelophthisic anemia which is lacking in the former, although Rhoads and Miller [48] have stated that some cases of myelophthisic anemia due to sclerosis of the bone marrow may be classified as subgroups of aplastic anemia.
5. Unless the liver is involved, hyperbilirubinemia does not occur since there are no indications of increased blood destruction. Blood iron is generally reduced,[36] When the anemia is due to multiple myeloma it is usual to find hypercalcemia along with an increase of blood uric acid, urea nitrogen, fibrinogen and phosphatase with Bence-Jones proteinuria.

The Anemia of Pregnancy. According to Mull and Bill [54] the average number of erythrocytes per c.mm. of blood in women during the late stage of pregnancy is 3,780,000 (range 2,940,000 to 4,790,000) with an average of 11 gm. of hemoglobin per 100 cc. (range 8.68 to 14.08 gm.).

Simple *normocytic normochromic anemia* with a normal erythroid bone marrow along with myeloid and megakaryocytic hyperplasia is physiological, spontaneous recovery occurring after delivery.[55]

Simple *normocytic hypochromic anemia* is of common occurrence. It usually begins between the fourth and sixth months and is of moderate degree with erythrocytes about 3,500,000 per c.mm. and hemoglobin about 11 gm. per 100 cc. In the puerperium the erythrocytes and volume of packed cells increase rapidly but the hemoglobin more slowly. This type of anemia is usually regarded as "physiologic" and may be no anemia at all but rather hydremia resulting from an increase in plasma volume. But in many cases iron, protein, vitamin or other nutritional deficiencies, as well as infection and toxemia may play an important part in its etiology. It is generally relieved, either during or after pregnancy, by the administration of iron.

However, a severe *anemia of the microcytic hypochromic type* with less than 10 gm. of hemoglobin per 100 cc. may develop about midpregnancy and especially among women who have borne several children. Frequently, symptoms suggestive of anemia are present before pregnancy occurs. This anemia has been ascribed to iron-deficiency when its intake is insufficient to meet the demands of both mother and fetus. Vomiting is often a contributing factor. Normoblastic hyperplasia of the bone marrow occurs.[55] Iron is effective in treatment although recovery occurs after delivery.

Severe *anemia of the macrocytic hyperchromic type,* resembling pernicious anemia both clinically and hematologically, may also occur during pregnancy but is very much less common in temperate than in tropical countries. It is generally designated "pernicious anemia of pregnancy" but is, indeed, quite rare. It is apparently due to the failure of production by the stomach of the intrinsic factor of the antianemic substance [56] but in some instances antecedent anemia, hemorrhage, nephritis, eclampsia or puerperal infection have apparently played a rôle in its etiology, as well as dietary deficiencies with special reference to low protein intake due to severe nausea and vomiting. A megaloblastic type of bone marrow may occur. Of course true pernicious anemia may also occur during pregnancy.

Severe macrocytic hyperchromic anemia has usually been observed in women under thirty-five years of age. It generally becomes manifest during the latter half of pregnancy. Macrocytosis is not generally as severe as in true pernicious anemia but otherwise the blood and sternal bone marrow changes are quite similar to the latter. Of special significance is the fact that recovery takes place with the termination of pregnancy and on a normal diet, not specially supplemented with liver. Recurrence in succeeding pregnancies is stated to be rare. Before the advent of liver therapy the mortality was as high as 50 to 75 per cent. Iron as well as liver should be administered in treatment and supportive blood transfusions may be required. Fortunately none of the anemias of pregnancy cause any reduction of the hemoglobin or erythrocytes of infants at birth.[56]

The Anemias of Infancy and Childhood. The causes and manifestations of anemia in infancy and childhood may differ quite materially from those in adults. Growth is an important factor because of the greater need for hemopoietic substances and the highest incidence is during the second year of age. Diet also plays an important part as well as infections, the latter because they may have a more profound effect on hemopoiesis in children than in adults.

Furthermore, hemopoietic equilibrium is less well established in infants and young children, with the result that they react more readily to etiologic factors. Consequently, anemia and leukocytosis are apt to be more profound. Erythroblasts may appear where only polychromatophilia and reticulocytosis would show in an adult. Likewise, myelocytes and even myeloblasts may be found instead of a slight or moderate shift to the left as in adults. Lymphocytosis is much more likely with enlargement of the lymph nodes, spleen and liver. Perhaps bone marrow exhaustion may ensue more frequently.

Because normal values are not the same in infants and young children as in adults and since immature erythrocytes appear more readily, a classification of anemias on a morphologic basis is somewhat less useful. Anemia in the first two or three months after birth is predominantly macrocytic although rarely, if ever, due to a lack of the antianemic substance. Hypochromic microcytic anemia in an infant or young child, however, is usually due to iron-deficiency as is true in the case of adults.

Consequently, *von Jaksch's anemia of infants* or *anemia pseudoleukaemica infantum* is no longer regarded as a disease entity but a symptom-complex which may be due to malnutrition, gastro-intestinal disturbances, congenital syphilis, tuberculosis or other infections characterized by a deficiency in erythrocytes and hemoglobin along with marked anisocytosis, poikilocytosis, erythroblastosis, extreme leukocytosis with relative lymphocytosis, splenomegaly, hepatomegaly and lymphadenopathy usually ending in recovery.

Reference has already been made to *congenital hemolytic jaundice* occurring in children due to a variable degree of hemolytic anemia characterized by diminished resistance of the erythrocytes to hemolysis. Jaundice is present to a slight degree in almost all newborn infants (*icterus neonatorum*) which has been ascribed to temporary insufficiency of the liver to excrete bilirubin. Icterus of the newborn and infants may be also due to infections, syphilitic hepatitis, congenital cirrhosis of the liver and congenital obliteration of the bile ducts. Consequently, these types of icterus should not be confused with congenital or familial hemolytic jaundice.

Reference has also been made to *erythroblastosis fetalis,* a severe form of hemolytic anemia occurring in the newborn and apparently due to intravascular hemolysis, in which jaundice may be so severe as to be known as "icterus gravus"; likewise to *Cooley's anemia* starting early in childhood and likewise of the hemolytic type, as well as to *Lederer's hemolytic anemia* which may also occur in childhood. *Winckel's disease* is also an acute hemolytic anemia occurring in the newborn which is characterized by jaundice, hemoglobinuria, and cyanosis.[57] It has been attributed to sepsis although no micro-organisms have ever been demonstrated. With improved obstetrical technic, however, this disease has practically disappeared.

Hypochromic microcytic anemia is common among children from three months to three years of age. It occurs particularly in premature infants and twins and is more frequent in artificially than breast-fed infants. A similar type of anemia is observed among older children with abnormal eating habits. It may be due not so much to an actual lack of iron in the tissues as to its deficient utilization in the formation of hemoglobin. On the other hand, however, it may be due to an insufficient intake of iron, since milk is very low in iron content, and especially since the requirements of a growing child are greater than in the case of adults. A deficiency in vitamin C may also be responsible in some cases although anemia due to scurvy is less common in infants than in adults. It has also been suggested that some cases of anemia in the newborn may be due to a lack in the mother's diet of some substances present in yeast [58] while riboflavin deficiency may be concerned in the defective formation of hemoglobin. Infections of various types may also produce the anemia through derangements of diet and perhaps interference with iron metabolism. Chronic hemorrhage may also be a cause and especially when due to hookworm infestment, which is not uncommon in the first few months after birth and especially in young children.

A severe macrocytic anemia of the "pernicious type" has been observed in Germany and Italy among infants fed goat's milk exclusively and known as *goat's milk* *anemia.* Otherwise, macrocytic anemia is the result of nutritional deficiencies in infants and children in quite uncommon although it may be observed in celiac disease; true pernicious anemia, however, is rare if it ever occurs at all.

Aplastic anemia may occur in children but is very uncommon. *Chronic congenital anemia,* which occurs in the latter part of the neonatal period, resembles aplastic anemia but is less severe and acute.[59] It differs from erythroblastosis fetalis in that signs of active blood regeneration are lacking and the onset is relatively late. Repeated transfusions are the only means for sustaining life.

Banti's disease or *splenic anemia,* which may occur in childhood, is now known as *chronic congestive splenomegaly* because of the histologic changes presented by the spleen. At least, the histologic changes described by Banti are not specific for the disease as they may occur as the result of cirrhosis of the liver or of thrombosis of the portal or splenic veins. The etiology is unknown but the chronic congestion of the spleen with fibrosis has been ascribed to either portal hypertension [60,61] or to changes in the small splenic arteries permitting more blood to enter the spleen with resulting congestion which also produces portal hypertension and even degenerative changes in the liver cells.[62]

Splenomegaly may precede anemia in which case the latter, unless hemorrhages have occurred, is moderate in degree and usually normocytic in type. If repeated hemorrhages have occurred it is of the hypochromic microcytic type with reticulocytosis. When cirrhosis of the liver is well developed the anemia is usually of the macrocytic type and evidently due to a deficiency in the antianemic substance. The fragility of the erythrocytes is normal or slightly reduced. Leukopenia is a characteristic feature. The platelets are generally reduced although both bleeding and coagulation times are generally within normal. The sternal bone marrow does not usually show any changes unless frequent hemorrhages have occurred when it is likely to present normoblastic hyperplasia.

The *xanthomatoses* or diseases of lipid metabolism likewise usually occur in children or begin during childhood. Pick [63] has divided them into two groups, namely, generalized and localized. The former consists of the symptomatic or secondary type seen in diabetes mellitus, liver disease with jaundice and chronic nephritis, and the essential or primary type is seen in Gaucher's disease, Niemann-Pick disease and Hand-Schüller-Christian disease.

Gaucher's disease is a rare, chronic, familial disease of lipid metabolism of unknown etiology. Histologically it is characterized by the presence of large kerasin-containing cells ("Gaucher cells") occurring particularly in the spleen. The disease appears in childhood although it may not be discovered until adult life has been reached. The anemia is usually moderate in degree and normocytic in type. There is little or no evidence of active blood regeneration such as polychromatophilia or erythroblastosis. Leukopenia with a relative lymphocytosis is common; sometimes a monocytosis occurs. The platelets are usually reduced. There are no evidences of increased blood destruction. The total blood fats, cholesterol and lecithin are usually within normal although an increase of lipid nitrogen with a normal lipid phosphorus has been reported.[64]

Niemann-Pick disease, like Gaucher's disease, is also thought to be a familial disorder of lipid metabolism characterized by the "storage" of a phosphatide lipid, probably sphingomyelin. The disease occurs only in infancy, is more common in females, and has even a more marked predilection for the Jewish race than Gaucher's disease. An anemia of moderate degree is present along with leukopenia and a relative lymphocytosis and monocytosis. In some instances leukocytosis has been observed. The platelets are reduced. Blood cholesterol may be increased. Hyperbilirubinemia does not occur.

Hand-Schüller-Christian disease is also a disorder of lipid metabolism and likewise occurs most frequently in children. Unlike Gaucher's and Niemann-Pick diseases, however, it is not familial; furthermore, the spleen and liver are never conspicuously enlarged. The bone marrow is chiefly involved. Anemia is not always present and is rarely severe. It may be of the nonregenerative type, with leukopenia and slight thrombocytopenia. Otherwise it has the characteristics of pronounced myelophthisic anemia. Cholesterol, fatty acids, and phosphatides of the blood are increased.

THE HEMOGLOBINURIAS

Hemoglobinuria refers to the presence of free hemoglobin, oxyhemoglobin, methemoglobin or myohemoglobin in the urine. If large amounts are present the color is changed to a dark reddish-brown and may become almost black. A small amount of hemoglobin in the urine, however, may not produce a noticeable change in the color but is readily detected by spectroscopic or chemical methods with special reference to the benzidine, orthotolidin and guaiac tests. Microscopic examinations of the urinary sediments usually reveal the presence of dehemoglobinized or "shadow" red blood corpuscles. Hemoglobinuria, however, must be carefully differentiated from hematuria in which well-preserved erythrocytes are present. When it is suspected, freshly voided urine should be examined. Spontaneous hemolysis of red blood corpuscles may occur on standing and produce a "false hemoglobinuria."

It has been calculated that under normal conditions about 25 gm. of hemoglobin is released daily from about one billion worn-out erythrocytes and is converted into bilirubin by the reticulo-endothelial cells of the body and excreted by the liver. In those hemoglobinurias due to intravascular hemolysis, however, excessive amounts of free hemoglobin occur in the blood. A portion is converted into bilirubin which, being produced in excessive amounts, may exceed the excretory capacity of the liver and result in bilirubinemia, with or without jaundice, along with the elimination of excessive amounts of urobilinogen in the urine and urobilin in the feces.

If the amount of free hemoglobin is greater than the capacity of the reticulo-endothelial cells to convert all of it into bilirubin, the excess remains in the blood and constitutes *hemoglobinemia*. If this exceeds the threshold of the kidneys, free hemoglobin is eliminated in the urine and constitutes hemoglobinuria. It has been found experimentally that from 100 to 140 mg. of free hemoglobin per 100 cc. of plasma may be present before it is excreted in the urine[65,66] but since a portion is converted into bilirubin and other pigments it would appear that the normal renal threshold for hemoglobin may be about 60 mg. per 100 cc.

of plasma. In some of the hemoglobinurias, however, it is likely that the renal threshold is lowered. For example, the march type is ascribed to passive congestion with excessive intravascular hemolysis in the vessels of the kidneys but it does not appear possible for this to account for the hemoglobinuria unless the renal threshold were greatly reduced. Indeed, it would appear that this possible factor in the etiology of the hemoglobinurias is worthy of investigation by means of tolerance tests conducted by the intravenous administration of free human hemoglobin.

The Etiology of the Hemoglobinurias. At least six different clinical types of hemoglobinuria are recognized. The basic cause for most of them is increased intravascular hemolysis, with the possibility of a lowering of the renal threshold for hemoglobin in the case of at least some of them, as previously discussed. The causes of increased intravascular hemolysis, however, are not definitely known in some of the hemoglobinurias.

Under normal conditions, it is thought that free hemoglobin is released from erythrocytes as the result of their disintegration or fragmentation from being buffeted about in the circulating blood, the fragments being removed by phagocytosis by the cells of the reticulo-endothelial system.

It appears, however, that the reticulo-endothelial cells also produce an autohemolysin concerned in the destruction of effete erythrocytes under physiologic conditions. It probably sensitizes the erythrocytes to the lytic effects of the complement of the plasma although hemolysis may result after sensitization through the lytic effects of the lysolecithins or endocomplements of the corpuscles themselves.

An excessive production of this autohemolysin by the reticulo-endothelial cells of the spleen may be the important factor in the etiology of malarial and paroxysmal cold hemoglobinurias, especially since there is evidence indicating that the reticulo-endothelial cells may undergo hypertrophy. It is true that an actual increase of the hemolysin in the serum may not be demonstrable except in paroxysmal cold hemoglobinuria but it is likely that it may account for spherocytosis and the reduced resistance of erythrocytes to hemolysis by hypotonic saline solutions in some of the hemoglobinurias.

In paroxysmal nocturnal hemoglobinuria, however, the overproduction of this autohemolysin does not appear to be involved, since excessive intravascular hemolysis is apparently due to a change in the erythrocytes rendering them more susceptible to hemolysis by an increase in the *p*H of the plasma due to the accumulation of carbon dioxide during sleep. Nor does it appear to be involved in the etiology of march hemoglobinuria which is ascribed to increased intravascular hemolysis in the vessels of the kidneys probably associated with a lowered renal threshold for hemoglobin; or in the etiology of paralytic hemoglobinuria which is ascribed to the excessive production and elimination of myohemoglobin by the muscles which are unusually sensitive to the effects of lactic acid. But just why the erythrocytes are rendered unusually sensitive to hemolysis by a change in the *p*H of the plasma in paroxysmal nocturnal hemoglobinuria, or why the muscles are rendered unusually susceptible to the effects of lactic acid in paralytic hemoglobinuria, is unknown.

Symptomatic Hemoglobinuria. This refers to the hemoglobinuria sometimes seen in the severe types of acute hemolytic anemia. When occurring after incompatible blood transfusions and in erythroblastosis fetalis, it is reasonably ascribed to excessive intravascular hemolysis by the major or minor iso-agglutinins and hemolysins sensitizing erythrocytes to the lytic action of the complement of the plasma. Hemoglobinemia with hemoglobinuria after the intravenous injection of excessive amounts of hypotonic solutions, however, is apparently due to hemolysis resulting from a reduction in the plasma chloride and other electrolytes.

It is commonly thought that hemolytic anemia with hemoglobinuria due to chemical agents and drugs also results from direct hemolysis but the erythrocytes are so well protected against intravascular hemolysis by the proteins and other constituents of the plasma as to suggest that these agents may act primarily on the reticulo-endothelial cells (especially in the spleen) causing an excessive production of a hemolysin capable of hemolysing erythrocytes directly through action with their lysolecithins or endocomplement. The same may be true in the case of those hemolytic anemias with hemoglobinuria ascribed to excessive hemolysis by bacterial or protozoal toxins in some of the infectious diseases (notably malaria) as well as by endogenous tissue products produced by burns and in the course of lymphatic leukemia, Hodgkin's disease, lymphosarcoma and dermoid cysts acting principally on the reticulo-endothelium of the spleen.[66] It is true that in most cases an increase of hemolysin in the plasma cannot be demonstrated but, after all, this is not possible unless relatively large amounts are present, with the probability that spherocytosis is evidence of its presence and activity.[67] In this connection it is also to be remembered that in severe hemolytic anemias the etiologic agents may injure the reticulo-endothelial cells to such an extent as to interfere with their function of converting hemoglobin into bilirubin. Under these conditions, hemoglobinemia would result with hemoglobinuria when the excess in the blood was higher than the renal threshold.

Malarial Hemoglobinuria. This is also known as *blackwater fever*, *hemoglobinuric fever* or *hemorrhagic malarial fever*. It is now generally regarded as a form of acute hemolytic anemia with hemoglobinuria occurring in malaria and especially in infections with *Plasmodium falciparum*.

Since the administration of quinine appears to render erythrocytes more susceptible to lysis *in vitro* by bile and bile salts,[68,69] it is thought that the hemoglobinuria may be due to the erythrocytes in malaria being rendered more sensitive to lysis by quinine. If this is true, both atabrine and plasmochin have a similar effect.

It is now thought, however, that the hemoglobinuria is due primarily to malarial infection although it is true that parasites have not been observed in all cases; at least, it is treated by the cautious administration of quinine or neoarsphenamine along with general measures required for combating shock and prostration. Fairley [70] has postulated that chronic malaria may result in hypertrophy of the reticulo-endothelial cells with an abnormal increase in their phagocytic activity and the production of an increased amount of hemolysin which is liberated into the blood and is responsible for an increased destruction of erythrocytes, with resulting hemoglobinemia and hemoglobinuria. Others have attributed the hemoglobinuria to the effects of acquired sensitization to the protein of malarial parasites from earlier infections with the result that when a new infection occurs or quinine is administered the protein allergen is released with the production of an allergic reaction characterized by an acute hemolytic crisis.[71]

Paroxysmal Cold Hemoglobinuria. This condition is characterized by the sudden passage of hemoglobin in the urine at any time from a few minutes to seven or eight hours after exposure to cold, which may be slight and limited to only one part of the body. The attacks or paroxysms are characterized by muscular cramps, chills, fever and even nausea and vomiting. The patient may develop a slight jaundice with some enlargement of the spleen and liver. Vasomotor disturbances are common.

As a general rule, the first two or three specimens of urine are dark brown or almost black. Mild attacks, however, may show hemoglobinemia without discoloration of the urine, although hemoglobinuria is usually detected by chemical tests for occult hemoglobin. Spectroscopically the hemoglobinuria is found to be due to the presence of oxyhemoglobin and methemoglobin. The urine also contains some dehemoglobinized or "ghost" erythrocytes along with albumin. The blood findings are those characteristic of an acute hemolytic anemia.

With few exceptions the disease is caused by congenital syphilis although it may be due to acquired syphilis. Because vasomotor manifestations occur during attacks, it is also thought that disturbances of capillaries [72] or of the sympathetic nervous system [73] may play a rôle in its etiology.

Evidently the hemolysis of erythrocytes with resulting hemoglobinemia and hemoglobinuria is due to the presence in the plasma of an autohemolysin (discovered by Donath and Landsteiner), which is characterized by sensitizing only the patient's own corpuscles at a low temperature followed by hemolysis by the complement of the plasma after the blood has been warmed. Cases have been described, however, in which complement apparently played no part in the hemolysis; [73,74] these are apparently due to the fact that the autohemolysin may act in conjunction with the lysolecithins or endocomplement of the erythrocytes to produce hemolysis.

Just how infections with *T. pallidum* produces the disease is unknown. Apparently it is through some change in the reticulo-endothelial cells whereby they not only become more phagocytic but produce an excess of a hemolysin which is characterized by an affinity for only the patient's own corpuscles at reduced temperatures; in this respect it resembles the "cold" iso-agglutinins belonging to the blood subgroups.

Laboratory examinations should include not only the serologic tests for syphilis, examinations of the blood for hemolytic anemia and of the urine for hemoglobin, but likewise tests of the blood of the individual for the presence of the autohemolysin.[75]

Paroxysmal Nocturnal Hemoglobinuria. This is a rare type of paroxysmal hemoglobinuria characterized by marked hemolytic anemia, due to intravascular hemolysis occurring during sleep, either day or night, with the passage of hemoglobin in the urine on arising. It is also known as the *Marchiafava-Micheli syndrome.*

Most cases occur in men 20 to 40 years of age. The disease is characterized clinically by a protracted and fatal course with recurrent attacks of intermittent fever, slight jaundice, enlargement of the spleen, a predisposition to phlebitis with thrombosis and usually severe anemia. The latter is of the hemolytic type, with the production of hemoglobinemia which, upon exceeding the renal threshold, permits the passage of free hemoglobin in the urine. The hemoglobinuria, however, may be very slight and escape detection unless spectroscopic or chemical tests for hemoglobin are employed. The urine also contains an increased amount of urobilinogen along with a tobacco-yellow sediment composed of hemosiderin.

The anemia is of the macrocytic or normocytic type, characterized by polychromatophilia and erythroblastosis, the presence of some spherocytes, persistent reticulosis, marked leukopenia with a relative lymphocytosis, moderate thrombocytopenia and a normal resistance of the erythrocytes to hypotonic saline solutions. Bilirubinemia is usual, with positive icterus index and indirect van der Bergh reactions. The sternal bone marrow shows a characteristic normoblastic hyperplasia.

The cause of this paroxysmal hemoglobinuria is unknown. Since intravascular hemolysis occurs only during sleep, it is thought to be due to a change in the erythrocytes rendering them susceptible to hemolysis by carbon dioxide which accumulates during sleep with a consequent lowering of the *p*H of the blood. In this connection it has been shown that the patient's corpuscles show an unusual degree of hemolysis in the test tube when treated with carbon dioxide [76–78] and this test is employed for diagnostic purposes.[78,79] But just what renders the corpuscles more vulnerable to carbon dioxide is unknown. In this connection it has been suggested that in regions of the body where stasis and increased carbon dioxide tension occur, such as the spleen, intravascular hemolysis takes place. In addition to this fundamental change in the erythrocytes, however, a thermolabile autohemolysin may be produced,[80] presumably by the reticulo-endothelial cells.

March Hemoglobinuria. This is also a rare type of hemoglobinuria following strenuous exertion in the upright position, as by marching, or after standing a long time in the lordotic position.[81] Since lordosis is frequently found in affected individuals, the condition is thought to be due to hemolysis within the vessels of the kidneys due to stasis of the blood. It may be related to orthostatic albuminuria.

The symptoms are trivial and spontaneous recovery usually occurs after a few months. Anemia does not develop and jaundice is unusual. A slight leukocytosis is sometimes observed. The urine contains hemoglobin which, after a few hours, is followed by slight bilirubinemia and an increase of urobilinogen in the urine.

Paralytic Hemoglobinuria. This is likewise rare in human beings although not uncommon in horses. It is called "paralytic" because of extreme muscular weakness, cramps and sometimes paralysis with paroxysmal attacks of hemoglobinuria. Since the latter may escape detection, the disease may be mistaken for progressive muscular dystrophy. As a general rule, however, hemoglobinuria is detected by the dark color of the urine due to the presence of myohemoglobin.

The disorder is of unknown etiology but the paroxysms of hemoglobinuria have been attributed to the sudden release of lactic acid formed from excessive amounts of glycogen accumulated in the muscles during rest. The lactic acid is regarded as damaging the muscles with the excessive production of myohemoglobin in the blood which is eliminated in the urine. But just what renders the muscles unusually susceptible to damage by lactic acid is unknown.

A somewhat similar condition is known as "Haff disease," which occurs in Germany. It is likewise ascribed to the presence of myohemoglobin in the urine. This is thought to be due to damage of the muscles following the ingestion of poisonous resinous acids in fish and eels which have fed on the by-products of cellulose factories.[82]

THE PURPURAS, HEMOPHILIA AND OTHER HEMORRHAGIC DISEASES

Not infrequently the word "purpura" is employed as a diagnosis but like "jaundice," purpura is usually only a prominent clinical manifestation of a disease or some abnormal state. It merely designates hemorrhages in the skin, mucous membranes, internal organs or other tissues. But bleeding or an abnormal tendency to it, with or without purpura, is characteristic of hemophilia, hemorrhagic disease of the newborn, hereditary hemorrhagic telangiectasia and other hemorrhagic disorders which are commonly grouped together under the designation of the *hemorrhagic diatheses.*

Etiology. The important changes responsible for the abnormal bleeding characteristic of these disorders are (1) deficiencies in blood coagulation, (2) an increased fragility and permeability of the capillaries or (3) a combination of these factors.

Deficiency in blood coagulation is due to a numerical decrease in the platelets (*thrombocytopenia*) or a decrease in their functional activity resulting in a deficiency in the production of thromboplastin (*thrombasthenia*), as in hemophilia and hereditary hemorrhagic diathesis.

Thrombocytopenia may be due (1) to an interference with the formation of platelets, (2) an increased destruction of them in the circulating blood or, (3) to an increased demand upon them for plugging capillary defects. Decreased formation has been ascribed to the production of some substance by the spleen arresting the maturation of megakaryocytes in the bone marrow [83,84] which is supposed to explain the beneficial effects of splenectomy in the treatment of some cases of primary or idiopathic purpura haemorrhagica.[85] The rôle of the splenic factor in the etiology of this disease, however, has been denied.[86] But a decreased formation of the platelets may occur in congenital thrombocytopenia and in some of the anemias, myelogenous leukemia and other diseases characterized by extensive involvement of the bone marrow; likewise in some of the acute infectious diseases due, apparently, to the effects of toxic substances on the megakaryocytes.

Thrombocytopenia may be due also to a destruction of the platelets in the circulating blood. This may occur as a rare primary condition of unknown etiology, called purpura thrombolytica,[87] but occurs quite frequently as a secondary thrombocytolysis due to toxic chemical or physical agents as well as in some of the acute infectious diseases, splenic disorders and the xanthomatoses. Since a physiologic thrombocytopenia may occur premenstrually and since bruising or purpura haemorrhagica occurs preponderantly in females, it has been suggested that some factor concerned with the menstrual cycle may stimulate thrombocytolytic activity in the spleen [88] with the production of menstrual purpura. David [89] has reported a severe purpura occurring in women with thrombocytopenia in the late stages, which he ascribed to ovarian hormone deficiency.

According to Tidy, the platelets serve, normally, to protect the capillary walls and, by their deposition on the endothelium, act as a seal against the escape of blood into the tissues. On this basis a deficiency in platelets may play an important rôle in capillary bleeding [90–93] but, on the other hand, the thrombocytopenia may be due to the fact that an excessive number of platelets is being utilized for plugging the capillaries.[90, 93] But, either way, the rôle of the platelets in relation to capillary hemorrhage is of fundamental importance in the etiology of many of the purpuras. On the other hand capillary endothelium may be so defective in hereditary hemorrhagic telangiectasia, the anaphylactoid purpuras and purpura fulminans, or be so severely injured by the many factors responsible for symptomatic or secondary purpura, that bleeding may occur in the presence of platelets which are quantitatively and qualitatively normal. Indeed, MacFarlane [94] has recently found that bleeding in hereditary hemorrhagic diathesis may be due to a failure in the contraction of capillaries which are often distorted and bizarre in form.

However, defects in the capillary endothelium along with thrombocytopenia offer an explanation for the paradox of a prolonged bleeding time with a normal coagulation time of the blood. That is to say, in thrombocytopenia the platelets may be too few in number or size effectively to plug defects in the capillaries, which results in a prolongation of the bleeding time, but yet numerically sufficient to produce enough thromboplastin for normal coagulation although insufficient for a normal contraction of blood clots which requires additional amounts of it.

Furthermore, faults in blood coagulation and clot formation with retraction may be due to factors quite independent of the platelets as, for example, a deficiency in the production of prothrombin (hypoprothrombinemia) in hemorrhagic disease of the newborn, hereditary pseudohemophilia, idiopathic (familial) hypoprothrombinemia, severe disease of the liver and vitamin K deficiency. In idiopathic hypoprothrombinemia Stickney and Watson [95] have reported that the major deficiency was of the Quick B component, that affected by dicumarol, although the administration of vitamin K in large doses was ineffective in shortening the prothrombin time which may have been due to a primary disturbance of protein synthesis in the liver. Unfortunately, however, a determination of the prothrombin time is of little or no clinical value in the prediction or diagnosis of thromboembolic disease as this may occur when the prothrombin time is normal or even below normal.[96, 97]

Faulty coagulation and clot formation may also occur as a result of a deficiency in fibrinogen (fibrinogenopenia) as in constitutional fibrinogenopenia and severe disease of the liver as well as, possibly, in the presence of excessive amounts of heparin or other anticoagulants in the blood. The latter, however, has not been definitely proved although Copley and Robb [98] have recently shown that heparin reduces the platelets in the normal blood.

Classification. The purpuras and other hemorrhagic disorders may be classified, therefore, according to what is believed to be mainly responsible for bleeding. It is to be emphasized, however, that two or more factors may be involved in the etiology of any hemorrhagic disease. *Mechanical purpura* may be omitted from the classification because it is due to the rupture of normal capillaries from violent muscular contractions, as in whooping cough and convulsions, or from prolonged vascular constriction by a tight tourniquet.

1. *Due primarily to thrombocytopenia from a decreased production or failure of maturation of the platelets:*
   A. Primary forms: (1) Primary or idiopathic purpura haemorrhagica (Werlhof's disease); (2) congenital thrombocytopenia.
   B. Symptomatic or secondary forms: due to (1) aplastic anemia, myelophthisic anemia, pernicious anemia, hemolytic jaundice, chronic hypochromic anemia, myelogenous leukemia, malignant disease of the bone marrow and Hodgkin's disease (when the bone marrow is extensively involved); (2) in some of the acute infectious diseases.
2. *Due primarily to thrombocytopenia from an increased destruction of the platelets:*
   A. Primary form: (1) Purpura thrombolytica.
   B. Symptomatic or secondary forms due (1) to chemical or physical agents; (2) some of the acute infectious diseases; (3) splenic disorders (Banti's disease and Felty's syndrome); (4) the xanthomatoses (Gaucher's disease, Niemann-Pick disease and Hand-Schüller-Christian disease); (5) menstrual purpura and David's disease as well as purpura haemorrhagica in association with pregnancy.
3. *Due primarily to thrombasthenia or a functional deficiency of the platelets (thromboplastin deficiency):*
   A. Primary forms: (1) Hemophilia; (2) hereditary hemorrhagic diathesis.
4. *Due primarily to hypoprothrombinemia:*
   A. Primary forms: (1) Hemorrhagic disease of the newborn; (2) hereditary pseudohemophilia; (3) idiopathic hypoprothrombinemia.
   B. Symptomatic or secondary forms: (1) Diseases of the liver; (2) deficiency of vitamin K.
5. *Due primarily to fibrinogenopenia:*
   A. Primary form: Constitutional fibrinogenopenia.
   B. Symptomatic or secondary forms: due to severe injuries of the liver.
6. *Due primarily to increased capillary fragility or permeability:*
   A. Primary forms: Hereditary hemorrhagic telangiectasia; (2) anaphylactoid purpuras (Henoch's purpura, Schönlein's purpura, Osler's erythremia); (3) purpura fulminans.
   B. Symptomatic or secondary forms: (1) purpura simplex, purpura senilis, purpura cachetica, orthostatic purpura; (2) allergy (foods, drugs, etc.); (3) acute and subacute infections; (4) chronic cardiorenal disease and hemochromatosis; (5) chemical agents and venins; (6) menstruation and functional uterine bleeding; (7) deficiency of vitamin C and possibly vitamin P ("citrin"); (8) certain skin diseases (Ehlers-Danlos syndrome, Schamberg's disease, Majocchi's disease, etc.).

Diagnostic Examinations. The diagnosis of the hemorrhagic disorders on the etiologic basis is frequently difficult but always very important from the standpoint of treatment. Needless to state, a very careful history of the illness, particularly of the family history for bleeding, is extremely important as well as a thorough physical examination with special reference to the spleen and liver. Laboratory examinations are indispensable and especially platelet counts, determinations of the coagulation, bleeding and prothrombin times of the blood and the character of the clot and clot retraction. A determination of plasma fibrinogen concentration is also sometimes required as well as examinations for bilirubinemia and hypoproteinemia. Erythrocyte, reticulocyte and leukocyte counts with hemoglobin estimations and urine examinations (including urobilinogen) are routinely required.

A determination of *capillary resistance by the tourniquet test,* based on the production of petechial hemorrhages in the skin of the forearm (the Rumpel-Leede phenomenon), is always advisable and may be conducted as follows:

1. A blood pressure cuff is placed around the arm above the elbow.
2. Make careful note of any purpuric spots in the skin of the forearm.
3. Then inflate to between the systolic and diastolic pressures.
4. Maintain the venous stasis for about five minutes.
5. A more severe test of the ability of the capillaries to withstand trauma is conducted by vigorously flicking the skin three or four times with the thumb and forefinger.
6. The appearance of petechial hemorrhages constitutes a positive reaction, the number and size of the petechiae being a measure of capillary permeability and fragility (Fig. 48).

The *capillary fragility petechial index,* according to the method of Göthlin,[99] may be determined as follows: (1) mark off a circular area 6 cm. in diameter in each antecubital space, noting all blemishes which might be confused with petechiae; (2) inflate a standard blood pressure cuff about each upper arm to 35 mm. mercury for 15 minutes, when the pressure is reduced and the petechiae counted, using a good light and a magnifying lens; (3) repeat after an hour or longer, using a 50 mm. cuff pressure; (4) the petechiae which appear at 35 mm. are given a double value because those which appear at that pressure are more significant; (5) the second determination may be omitted if 2 or fewer petechiae appear with 35 mm. pressure; (6) if 6 or more petechiae appear at the lower pressure, the capillary fragility is considered abnormal; (7) the number of petechiae with 35 mm. pressure multiplied by 2, plus the petechiae with 50 mm. pressure, gives the petechial index; (2) an index of 8 or less indicates normal fragility, 9 to 12 borderline but probably increased fragility, and 13 or more increased fragility. Repetition of the complete test in less than 3 weeks is unreliable but it should be repeated every 6 weeks as long as previous tests reveal increased fragility and thereafter every 3 months.

When there is a family history of bleeding, the hemorrhagic disorder may be due to primary or idiopathic purpura haemorrhagica, hereditary hemorrhagic diathesis, hemophilia or constitutional fibrinogenopenia.

While the purpuras and other hemorrhagic diseases are conveniently classified according to what is known of the causes for bleeding or an abnormal tendency to it, many are best known according to clinical terms. On this basis the important laboratory findings in the purpuras and other hemorrhagic diseases are summarized in Tables 126 and 127.

The Primary or Idiopathic Purpuras. These include primary or idiopathic purpura haemorrhagica (Werlhof's disease), congenital thrombocytopenia, purpura thrombolytica, the anaphylactoid purpuras and purpura fulminans. The reasons for increased bleeding are fairly well understood but the causes for it are unknown; hence the designation of primary or idiopathic.

Primary or idiopathic purpura haemorrhagica is characterized clinically by petechiae or ecchymoses in the skin as well as by hemorrhages from mucous membranes and into various tissues. The onset and course of the disease are variable and may be marked by spontaneous remissions and relapses. It occurs most frequently in children (even in infants) and young adults, being uncommon late in life. Females are affected more frequently than males. A family history of ready bruising, frequent nose bleeds or other hemorrhages is not unusual. It is uncommon among Negroes. The platelets are reduced, the bleeding time is prolonged, the

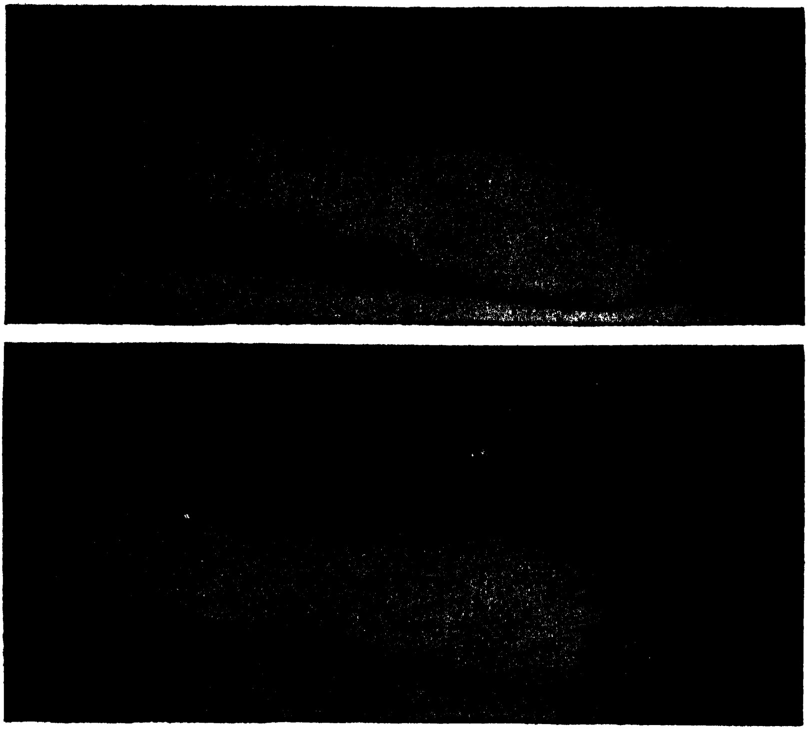

FIG. 48. THE TOURNIQUET CAPILLARY RESISTANCE TEST

A, before the application of the tourniquet in a case of purpura haemorrhagica; *B*, positive reaction after the application of the tourniquet.

coagulation time essentially normal, the tourniquet reaction positive and leukocyte changes insignificant with no anemia except that due to the loss of blood (Table 126).

Congenital thrombocytopenia is rare. Both mother and infant show marked thrombocytopenia. Purpura, however, may not occur if the remaining platelets show increased fragility with the release of sufficient thromboplastin to maintain normal bleeding and coagulation times of the blood.[100] *Purpura thrombolytica* is also rare and of unknown etiology.

The *anaphylactoid purpuras* are so-called because they are characterized by one or more of the common changes observed in allergy like edema, urticaria or

TABLE 126. SUMMARY OF THE CLINICAL INTERPRETATION OF THE LABORATORY FINDINGS IN THE PRIMARY AND SECONDARY PURPURAS

Purpuras	Laboratory Findings
Primary or Idiopathic Purpura Haemorrhagica	Etiology unknown. Thrombocytopenia; morphologic changes in the platelets in some cases. Prolonged bleeding time. Positive tourniquet reaction. Normal coagulation time. Failure of normal clot retraction. Anemia proportional to blood loss. Leukocytes usually normal but leukocytosis with a shift to the left may occur; also a relative lymphocytosis in long-standing cases. Increase of megakaryocytes in the bone marrow or changes due to blood loss. The urine may be normal, or blood or albumin may be present. The feces may contain blood.
Congenital Thrombocytopenia	Rare; etiology unknown. Thrombocytopenia of mother and infant; purpura may not occur if bleeding and coagulation times of the blood are normal. Usually a positive tourniquet reaction.
Anaphylactoid Purpuras	*Henoch's purpura* occurs particularly in children and adolescents and is characterized by gastro-intestinal symptoms. *Schönlein's purpura* occurs particularly in young adults and is characterized by arthritis or peri-arthritis. *Purpura fulminans* chiefly affects young children, is particularly severe and usually fatal in one to four days. The platelets are not significantly reduced. Coagulation and bleeding times and clot retraction are normal. The tourniquet test may give a positive or negative reaction. Anemia occurs only in case of excessive loss of blood.
Symptomatic or Secondary Purpuras	Usually only capillary weakness with positive tourniquet reactions but no well-defined abnormalities in the blood in purpura simplex, purpura senilis, purpura cachetica, orthostatic purpura, allergic purpura and vitamin deficiency purpura. In purpuras due to thrombocytopenia there is usually (1) a reduced platelet count; (2) prolonged bleeding time; (3) normal or slightly delayed coagulation time; (4) failure of clot retraction; (5) positive or negative tourniquet reactions; 6) variable changes in the leukocytes and (7) anemia proportional to blood loss. The urine may be normal, or blood or albumin may be found. The feces may show the presence of blood.

angioneurotic edema, diffuse erythema with or without edema, and necrotic areas of the skin which may be followed by the formation of bullae and ulcers.[101] They include Henoch's purpura, Schönlein's purpura, erythema simplex, nodosum, multiforme, bullosum and vesiculosum as well as urticaria pigmentosa and angioneurotic edema. These conditions are not due to thrombocytopenia or other hematopoietic disturbances but to increased capillary permeability allowing the passage of plasma or blood into the tissues. The etiology is unknown but is sometimes found to be due to allergic sensitization in which case the purpura and associated edema are more properly termed allergic purpura. *Henoch's purpura* is characterized by gastro-intestinal symptoms before the appearance of purpura in association with rheumatoid pains and moderate fever. It occurs most frequently in children and even in infants. Colic, tenesmus and abdominal tenderness without muscular rigidity are common; vomiting may occur as likewise obstinate constipation and sometimes intussusception. *Schönlein's purpura* usually affects young adults and is characterized by severe pain, tenderness and swelling of the joints, particularly of the lower extremities. The symptoms may resemble those of acute rheumatic fever but are not relieved by the salicylates. The laboratory findings are summarized in Table 126.

Purpura fulminans has also been included among the anaphylactoid purpuras.[102] It is rare, extremely severe and usually fatal in one to four days. This purpura chiefly affects children and is characterized by sudden onset, fever and ecchymoses. Henoch thought the centers of vascular innervations in the spinal cord were particularly involved.

The Symptomatic or Secondary Purpuras. These purpuras may be produced by so many factors that they are of common clinical occurrence. As previously stated, the detection or diagnosis of the cause in each case is of fundamental importance in relation to treatment.

They include the simple purpuras which are usually mild, confined to the skin and apparently due to capillary weakness without any well-defined abnormalities of the blood. *Purpura simplex, purpura senilis* and *purpura cachetica* (observed in elderly or ill-nourished individuals) and *orthostatic purpura* belong to this category. The latter develops in the lower extremities of some persons after prolonged standing or walking. A positive tourniquet reaction may be observed.

Symptomatic or secondary purpuras may also be caused (1) by *chemical agents,* with special reference to the organic arsenicals, gold salts, benzol, dinitrophenol, quinine, ergot, iodine, organic hair dyes and the barbiturates (since adults use these more commonly than children secondary purpuras are encountered more frequently among them); (2) by some of the venins of reptiles and insects; (3) by *physical agents* like roentgen rays and radium which produces thrombocytopenia, leukopenia and anemia; (4) by some of the *acute and subacute infectious diseases* with special reference to the septicemias, typhoid fever, the typhus fevers, Rocky Mountain spotted fever, miliary tuberculosis, vaccinia, smallpox, measles, subacute bacterial endocarditis, lupus erythematosus, etc.; (5) by *chronic cardiorenal disease* and *hemochromatosis;* (6) by *allergy* to foods, drugs, etc.; (7) by *deficiencies in vitamin C* and possibly vitamin P ("citrin"); (8) by *artificially induced fevers* due to capillary rupture; (9) by thrombocytopenia occurring in *splenic disorders* (Banti's disease and Felty's syndrome) and the *xanthomatoses* (especially Gaucher's disease); (10) by thrombocytopenia resulting from a decreased formation or maturation of the platelets by the bone marrow in some of the *anemias, myelogenous leukemia, malignant disease* and *Hodgkin's disease* (when the bone marrow is extensively involved); (11) by certain *skin diseases* (Ehlers-

Danlos syndrome, Schamberg's disease, etc.); (12) by *menstruation* and *David's disease* including the purpura haemorrhagica associated with *pregnancy*. The latter may be only a coincidence but is always serious and has a high maternal and fetal mortality.[103]

Symptomatic or secondary purpura may also be due to hypoprothrombinemia or fibrinogenopenia due to severe disease of the liver or vitamin K deficiency but these states usually produce prolonged bleeding after trauma or operations, as shortly to be discussed.

The laboratory findings vary according to the etiology of the purpura (Table 126).

Hemophilia. Hemophilia is a hemorrhagic disease transmitted by a sex-linked, recessive mendelian trait in affected males characterized by a life-long tendency to spontaneous hemorrhages and marked delay in the coagulation time of the blood.

The disease is limited to the male but is transmitted by him through an unaffected daughter to a grandson. The sons of an affected male are normal themselves and cannot transmit the disease to any of their descendants. The daughters of an affected male are normal but are capable of transmitting the trait to about half of their sons and as a recessive or hidden characteristic to about half of their daughters. Hemophilia of the female is theoretically possible, provided she is the daughter of a hemophiliac father and a conductor mother, but no authentic cases are known. Sporadic cases of hemophilia have been reported in which inheritance could not be demonstrated; long inheritance through females with the males, by chance, being unaffected may be a possible explanation for their occurrence. Boggs,[104] however, has recorded spontaneous hemophilia occurring in six brothers in which concealed inheritance on the part of the mother was thought to be excluded. The disease is rare in the Negro but may occur;[105] it is also rare among the Chinese.

As previously stated, the disease is characterized by a life-long tendency to spontaneous hemorrhages in the skin, muscles, urinary tract, joints and peritoneal cavity as well as from various mucous membranes (nasal, buccal, gastro-intestinal) along with excessive and prolonged bleeding after even trivial wounds or minor operations such as extractions of the teeth.

The fundamental abnormality is a greatly prolonged coagulation time of the blood (Table 127). This is not due to a numerical deficiency of the platelets but has been ascribed to a qualitative change in them resulting in a deficient production of thromboplastin (thrombasthenia). Certainly hemophilia is not due to a deficiency of calcium, an excess of antithrombin or an excess of antiprothrombin (heparin). The slow conversion of prothrombin into thrombin, however, may be a factor.[106] Recently Quick[107] has described a test for hemophilia based upon the clotting time of recalcified oxalated plasma which is also prolonged.

It has been suggested, however, that hemophilia is due to the lack of something else in the plasma of a protein nature and called the "coagulation globulin."[108,109,110] Even the intravenous or intramuscular injection of 30 to 40 cc. of blood may supply enough of this plasma factor to produce a marked shortening of the coagulation time with cessation of bleeding which may last several hours or even one or two days. A similar, if not the same, thromboplastic substance occurs

TABLE 127. SUMMARY OF THE CLINICAL INTERPRETATION OF THE LABORATORY FINDINGS IN HEMOPHILIA AND OTHER HEMORRHAGIC DISEASES

Diseases	Laboratory Findings
Hemophilia	Prolonged coagulation time of the blood and of recalcified oxalated plasma. Normal clot retraction when once formed. Normal bleeding time although sometimes delayed during episodes of active bleeding. Normal prothrombin concentration. Negative tourniquet reaction. Platelets normal or slightly increased. Variable anemia depending on the severity and frequency of bleeding. Polymorphonuclear leukocytosis may accompany posthemorrhagic anemia.
Pseudo-hemophilia	Occurs in and transmitted by both sexes. Normal coagulation but prolonged bleeding time of the blood. Familial epistaxis: normal coagulation and bleeding time of the blood.
Hereditary Hemorrhagic Diathesis	Occurs in and transmitted by both sexes. Normal coagulation time. Clot retraction may be delayed. Bleeding time prolonged. Positive tourniquet reaction. Normal platelet count.
Hemorrhagic Disease of the Newborn	Caused by hypoprothrombinemia; may be due to a deficiency of vitamin K of the mother at delivery. Coagulation and bleeding times prolonged. Abnormal clot formation and retraction. Platelets usually within normal.
Hemorrhagic Disorders due to Hypoprothrombinemia and Vitamin K Deficiency	Usually prolonged coagulation time of the blood. Bleeding time likely to be within normal. Platelets usually normal but may be reduced in constitutional fibrinogenopenia.
Hereditary Hemorrhagic Telangiectasia	Occurs in and transmitted by both sexes. Bleeding time, coagulation time and clot retraction normal. Platelets normal. Negative tourniquet reaction. Blood changes, if present, are those of acute or chronic posthemorrhagic anemia.

in the tissues, including those of hemophiliacs, but in the latter it may fail to stop bleeding. The bleeding time, however, is normal since the tissue thromboplastin is usually sufficient to produce coagulation although it may be slightly prolonged during episodes of hemorrhage. Venipuncture is without danger since the elasticity of the vessels is usually sufficient to close the wound. Sternal biopsies may be safely conducted if necessary or advisable in diagnosis. The megakaryocytes are morphologically normal but appear to have an accelerated rate of maturation and platelet formation.[111]

Pseudohemophilia. Pseudohemophilia is also a hereditary hemorrhagic disease closely resembling hemophilia clinically but differing from it in several particulars, namely, (1) by affecting both males and females; (2) transmission by either sex; (3) normal coagulation time but prolonged bleeding time.[112,113,114]

Familial epistaxis is also related to hemophilia as far as hereditary transmission is concerned but both the coagulation and bleeding times are normal.[115]

Hereditary Hemorrhagic Diathesis. This is a type of nonthrombocytic hemorrhagic disease which is familial but transmitted by either sex. Purpura is unusual but bleeding from the nose and other mucous membranes is common. The disease is also known as "hereditary hemorrhagic thrombasthenia;"[102] true hemophilia, multiple hereditary telangiectasia and primary or idiopathic purpura haemorrhagica must be excluded in diagnosis. The familial hemorrhagic disorder reported by Farber[116] affecting 25 individuals (14 males and 11 females) of a family of over 100 members covering 5 generation. apparently belongs to this category. The coagulation time is normal but the bleeding time is prolonged or intermittently prolonged (Table 127).

Hemorrhagic Disease of the Newborn. Spontaneous bleeding in newborn infants is not uncommon. The sites of bleeding are usually the intestinal tract or umbilicus (melena neonatorum). Bleeding, however, may occur in the skin, brain or other tissues. The hemorrhage may be present at birth, but usually begins shortly after or during the first week; bleeding after the second week is rare. The hemorrhages may be trivial or lead to rapid exsanguination.

The cause is now believed to be due to a deficiency in prothrombin (hypoprothrombinemia). The etiology is unknown unless the disease is due to a deficient vitamin K intake by the mother. At least, the administration of vitamin K to women before delivery increases the prothrombin content of the blood of the infant and reduces the incidence of the disease.

The prothrombin content of the blood of normal infants at birth is lower than that of adults. It progressively decreases after birth for 3 to 6 days, after which there is a progressive increase to the normal adult level. In some infants, however, the prothrombin may be decreased sufficiently to cause a prolongation of the bleeding or coagulation times but nevertheless severe bleeding may follow circumcision or other minor operations as well as trivial injuries. Intravenous or intramuscular injections of blood as well as the administration of vitamin K are effective in treatment. Survival of an acute attack results in a permanent cure.

Other Hemorrhagic Disorders Due to Hypoprothrombinemia, Fibrinogenopenia and Vitamin K Deficiency. Abnormal bleeding may also occur in severe degenerative diseases of the liver which produce hypoprothrombinemia and fibrinogenopenia. In obstructive jaundice there are no hemorrhagic manifestations as a rule, but in some individuals with complete obstruction of long standing there is a tendency to bleed and postoperative hemorrhages are common. Indeed, uncontrolled bleeding during or following operations on jaundiced patients accounts for about a third of the surgical deaths. Unfortunately, the hemorrhagic tendency is not necessarily related to the degree of jaundice; furthermore, the bleeding and coagulation times are not always reliable in estimating the danger. A determination of the prothrombin concentration of the blood is more reliable but even though approximately normal before operation, it may be reduced to the bleeding level by anesthesia, surgical shock, anoxemia, blood loss or other factors.

Hypoprothrombinemia may also be caused by a deficiency in the absorption of vitamin K from the intestinal tract due to the absence of sufficient bile as in complete obstructive jaundice, external biliary fistula, chronic ulcerative diseases of the intestines, intestinal fistula, short-circuiting operations, etc. If the vitamin is administered orally along with bile salts and is absorbed or, if it is given parenterally, the prothrombin is increased with a cessation of bleeding, provided there is sufficient liver parenchyma for the production of prothrombin.

As previously stated, abnormal bleeding may also occur in severe liver diseases due to a deficiency of fibrinogen. *Constitutional fibrinogenopenia,* however, may occur which may be a familial disorder with severe bleeding from the umbilicus or from trivial wounds.[87, 117, 118] Thrombocytopenia may be present at the same time. The coagulation time of the blood is delayed as well as the bleeding time. Delayed coagulation due to fibrinogenopenia, however, may be differentiated from that occurring in hemophilia by adding a few drops of the thromboplastin solution used in the measurement of the prothrombin time of the blood. In hemophilia the blood will coagulate promptly, whereas in fibrinogenopenia it fails to do so.

Hereditary Hemorrhagic Telangiectasia. This is a hemorrhagic disease occurring in and transmitted by both sexes as a simple dominant characterized by multiple dilatations of capillaries and venules in the skin and mucous membranes.

The etiology is unknown. The lesions may occur in childhood but increase with age. Bleeding may not occur until adult age is reached. Some individuals with the disease rarely or never suffer from hemorrhages. Epistaxis is common but bleeding may come from lesions in any location including the tongue, buccal mucosa, gastrointestinal, respiratory or genito-urinary tracts. It may occur spontaneously or follow slight trauma and vasomotor disturbances. The blood findings are only those which may result from the loss of blood (Table 127).

THE POLYCYTHEMIAS

Polycythemia is a general term signifying an increase above the normal in the number of erythrocytes in the circulating blood. This increase may be relative and temporary or absolute and even permanent. *Relative polycythemia* may be due to hemoconcentration through the reduction of blood plasma or to the shunting of erythrocytes into the circulation from some storehouse such as the spleen; it is usually temporary or transient in nature. *Absolute polycythemia* denotes an actual increase in the erythrocytes due to their excessive production by the bone marrow. If this is due to a known cause or stimulus, it is called *erythrocytosis* or pseudopolycythemia which is analogous to leukocytosis. If the cause is unknown, it is called *erythemia* or polycythemia vera which is analogous to leukemia.

Relative Polycythemia. Relative polycythemia may have many causes such as (1) a greatly reduced fluid intake; (2) marked loss of fluids from persistent vomiting, severe diarrhea or excessive sweating; (3) marked loss of sodium chloride with a shift of fluids to the tissues as in adrenal insufficiency; (4) shock with a peripheral shift of plasma into the tissues and (5) a possible shunting of erythrocytes from the spleen into the circulating blood. As a general rule, the total blood volume is actually reduced owing to a decrease in plasma volume. The laboratory findings are an increase in the number of erythrocytes per c.mm. of blood, an increase in the volume of packed cells and a corresponding increase of the hemoglobin.

Erythrocytosis. As previously stated, erythrocytosis means a polycythemia due to an absolute increase of the erythrocytes from known causes. It is transient in the physiologic erythrocytosis of the newly born and in short exposures to high altitudes but otherwise is likely to be permanent.

The cause of erythrocytosis is the effect of anoxemia on the bone marrow which may stimulate the excessive production of erythrocytes directly or indirectly by some by-product of asphyxia such as lactic acid. This anoxemia may be due to impaired pulmonary ventilation, decreased atmospheric pressure, defective pulmonary circulation or a reduction in the oxygen-carrying blood pigment (hemoglobin). Consequently, it may be encountered in (1) congenital heart disease; (2) acquired heart disease and especially mitral stenosis; (3) pulmonary disease and especially extensive emphysema; (4) Ayerza's syndrome due primarily to disease of the pulmonary artery or its branches; (5) high altitudes including *chronic mountain sickness* or Monge's disease and (6) as the result of the effects of various chemical agents and drugs in reducing the oxygen capacity of the blood with special reference to coal-tar derivatives, aniline and its derivatives, phosphorus and cobalt.

The usual laboratory findings are similar to those found in relative polycythemia. The total plasma volume may be reduced but the increase in the size of the red cell mass is so great that the total blood volume is increased.

Erythremia. This disease is also known as polycythemia vera, polycythemia rubra, Vaquez's disease or Osler's disease. It is insidious in onset, runs a chronic course (usually ten to fifteen years or longer) and is characterized by a peculiar reddish-purple color of the skin, a variety of vasomotor and neurologic manifestations, usually splenomegaly and a striking absolute increase of the erythrocytes with an increase of total blood volume.

The disease occurs in both sexes, probably somewhat more frequently in males, and especially in Hebrews; it is rare among Negroes. The age of onset is usually middle or late life but rare cases have been described in children with retardation of growth or infantilism. It is also claimed to occur sometimes as a family disease but this is often symptomless with no leukocytosis or "shift to the left" of the neutrophils as commonly occurs in sporadic cases.

Erythremia is undoubtedly due to a functional hyperactivity of the bone marrow resulting in the excessive production of mature erythrocytes but the cause is unknown. In other words, it is the antithesis of pernicious anemia but does not appear to be due to the excessive production of the antianemic substance by the stomach with overstimulation of the erythroblastic tissues of the bone marrow. It has been suggested that the disease may be due to a lack of balance in the hormonal control of erythropoiesis with special reference to pituitary basophilism and the suprarenal cortex [119] but the evidence is not convincing. Hyperplasia of the erythroblastic tissue of the bone marrow, however, may be associated with an increase of the leukoblastic tissue, with the result that some cases of erythremia suggest its possible relationship to chronic myelocytic leukemia. Furthermore, the disease does not appear to be due to anoxemia with compensatory hypertrophy of the bone marrow due to a plugging of the capillaries of the lungs with megakaryocytes or other causes. Because splenomegaly usually occurs and since tuberculosis of the spleen has been observed in some cases, it has been suggested that the spleen may play a rôle in the etiology of erythremia through (a) suppression of its function in the destruction of erythrocytes resulting in their longer "life," (b) a suppression of its regulatory action on the bone marrow or (c) a replacement of its lymphoid by myeloid tissue as a consequence of tuberculous infection or other causes. These suggestions, however, are purely conjectural and lacking in supporting evidence. The usual *laboratory findings* are:

1. A dark red color of the blood with a viscosity five to eight times greater than normal. Consequently, it may be difficult to draw blood up in a pipet and it spreads slowly between cover glasses. The total blood volume is characteristically increased. The specific gravity is increased from the normal of 1.055 to 1.065 to as high as 1.075 to 1.080.

2. A numerical increase of erythrocytes ranging from 6,000,000 to as high as 10,000,000 or more per c.mm. of blood. While the mean corpuscular volume may be below normal, the volume of packed cells is markedly increased.

3. The erythrocytes are usually normal in size, but slight anisocytosis, polychromatophilia and basophilic stippling may be observed. Poikilocytosis is unusual but an occasional normoblast may be found. The reticulocytes are not increased except sometimes after severe hemorrhages in which case the erythrocytes may be hypochromic and microcytic. The resistance of the erythrocytes to hemolysis by hypotonic saline solutions may be increased.

4. An increase of hemoglobin averaging 18 to 24 gm. per 100 cc. of blood.

5. Leukocytosis is common with a "shift to the left" in the myeloid series.

6. The platelets are frequently increased.

7. The sternal bone marrow is dark red in color and very cellular. The hyperplasia, however, usually involves all the marrow elements so that the ratio of the different types of cells to one another may not be strikingly different from the normal. But while polycythemia vera may be present with a normal bone marrow, Manning [120] states that the disease is to be suspected in cases in which secondary erythrocytosis may be excluded, when the sternal bone marrow shows more than

20 nucleated red cells per 100 white cells, along with more than 2 per cent reticulocytes with myeloid-erythroid ratios below 3:1.

8. Associated findings may include (a) bilirubinemia from increased blood destruction with positive icterus index and indirect van den Bergh reactions; (b) albuminuria with an increase of urobilinogen; (c) an increase of stercobilin in the feces; (d) sometimes hypoacidity or anacidity of the gastric juice and (e) a moderate increase in the basal metabolic rate in some cases. As a general rule, there are no significant changes in the albumin-globulin ratio or other chemical constituents of the blood except an increase of uric acid in some cases.

THE LEUKEMIAS

The leukemias are diseases of unknown etiology and fatal determination characterized by widespread proliferation of the leukocytes and their precursors in the tissues of the body, usually associated with quantitative and qualitative changes in the leukocytes of the circulating blood. The appearance of abnormal leukocytes in the blood, alone or in combination with alterations in total white and/or red cell counts, lymphadenopathy, splenomegaly and hepatomegaly, is not, however, an absolute indication of the presence of leukemia. Indeed, since the peripheral blood may represent an unreliable factor while the bone marrow so often indicates the fundamental lesion, the final diagnosis of leukemia should be made in some cases only when the bone marrow has been expertly examined for changes indicative of the presence of this disease.

Leukemia occurs in all human races as well as in mice and chickens. In human beings it is more common in males than in females. No racial or occupational predilection has been established although it is thought that the colored develop it less frequently than whites and that Hebrews are particularly susceptible to the chronic lymphocytic form of the disease. Apparently, heredity may have an influence and especially in relation to the lymphocytic form which has been described as a conditionally dominant autosomal type with great variation in the phenotype due to other genes or to external influences. No age is exempt. It has been observed at birth in some instances ("congenital leukemia") but has never been known to be transmitted from mother to child. It occurs in nurslings and, indeed, the incidence of the disease is higher in the first five years of life than any other age period, the great majority of cases up to twenty years being of acute forms of the disease. From this time until the age of fifty, chronic myelocytic leukemia predominates and chronic lymphocytic leukemia is more frequent after fifty, but acute leukemia may occur in the aged and chronic leukemia in children. Fortunately, the incidence of the disease is low but it has been estimated that more than 3000 to 4000 individuals die of it yearly in the United States. True leukemia is invariably fatal; alleged recoveries are due to errors in diagnosis. In acute leukemia death may result within two weeks after the onset of symptoms and the majority of patients succumb within two months. In chronic leukemia about 80 per cent survive for one year, 40 per cent for three years, 20 per cent for five years and 4 per cent for ten years or longer.[121]

Etiology. As previously stated, the cause of leukemia is unknown. Because acute leukemia resembles fulminating sepsis and since leukemia-like or leukemoid blood changes may be due to infection, many reports are to be found in the literature incriminating infections with staphylococci, streptococci, diphtheroid bacilli, tubercle bacilli and other organisms or their toxins as the etiologic agents or as predisposing causes. Indeed, some writers consider that a sharp distinction between bacterial infection and leukemia does not exist [122] but the tissue changes characteristic of leukemia do not occur in diseases of recognized bacterial origin.

However, the possibility of leukemia being due to a filtrable virus has commanded special attention and particularly since fowl leukemia can be transmitted by means of cell-free Berkefeld filtrates. Furthermore, lymphocytic leukemia, myelocytic leukemia, and possibly monocytic leukemia, exist spontaneously in mice and can also be transmitted. But fowl leukemia has never been shown to spread from bird to bird under natural conditions and it is yet to be proved that the leukemias of fowl and mice are identical with leukemia of human beings. Certainly all attempts to transmit leukemia from man to man, or the accidental administration of leukemic blood in transfusion, have not resulted in the transmission of the disease; [123] nor have attempts to transmit the human disease to the lower animals given unequivocal results.[124,125]

Leukemia has been observed in persons exposed to certain chemicals and especially benzol, pyridine and aniline dyes, as well as to roentgen rays, radium and other radioactive substances.[126,127,128] Furthermore, leukemia or leukemoid effects have been produced experimentally in mice by injections of benzol, indol, tar and other compounds. These results are doubtless due to the effect of these toxic agents on the leukoblastic tissues and naturally suggest that bacterial toxins may have similar effects although this has not been shown to occur to the best of my knowledge.

Trauma, however, has been held responsible for more than 70 cases of leukemia [129] and courts of law have awarded compensation in some cases. In the majority the injury has consisted in contusion of the abdomen or fractures of bones with the leukemia usually of the myelocytic form. There is no conclusive evidence, however, that trauma produces leukemia,[130] although the possibility of it aggravating a pre-existing and asymptomatic leukemia cannot be denied.

Since maturation of leukocytes in leukemia appears to be halted as is the maturation of erythrocytes in pernicious anemia, it has been suggested that a deficiency of a maturation factor may be involved in the etiology of leukemia but all attempts to prove this have failed. The same is true of attempts to prove that the disease may be due to a disturbance in the hormonal regulation of leukocytic production.

Finally, there appears to be a close relationship between leukemia and neoplasms.[131] At least, the resemblance of chloroma, which is a deposit of leukemic cells in the tissues, to new growths is striking. Furthermore, some of the peculiarities observed in leukemic cells are similar to those of neoplastic cells although, owing to technical difficulties, a relationship based on studies in cellular metabolism has not yet been definitely shown to exist. However, the possible relationship between leukemia and neoplastic diseases is indicated by the introduction of such terms as *leukosis* for leukemia in general, *myeloblastoma* for acute myeloblastic leukemia, *myelosis* for chronic myelocytic leukemia, *lymphoblastoma* for acute lymphoblastic leukemia, *lymphadenosis* for chronic lymphocytic leukemia, *reticulosis, reticulo-endotheliosis* or *reticulosarcoma* for monocytic leukemia, etc.

Classification. Leukemia is readily divided into (1) acute forms characterized by an acute course with a predominance of immature leukocytes in the blood and (2) chronic forms characterized by a chronic course with a predominance of mature leukocytes in the blood. Cases regarded as subacute usually resemble acute leukemia more than the chronic variety.

Both acute and chronic leukemia are best classified according to the predominating type of cell in the circulating blood as follows: (1) acute myeloblastic leukemia; (2) chronic myelocytic leukemia (neutrophilic, myelogenous or myeloid leukemia); (3) eosinophilic leukemia; (4) basophilic leukemia; (5) acute lymphoblastic leukemia; (6) chronic lymphocytic leukemia (lymphogenous or lymphoid leukemia); (7) acute monoblastic leukemia; (8) acute monocytic leukemia; (9) leukemic reticulo-endotheliosis or reticulosis; (10) megakaryocytic leukemia; (11) plasma cell leukemia (multiple myeloma with leukemia); (12) chloroleukemia (chloroma or chloroleukosarcoma); (13) lymphosarcoma cell leukemia; (14) erythroleukemia (leukemia associated with erythremia) and (15) aleukemic or subleukemic leukemia (leukopenic myelosis). To these may be added aleukemic megakaryocytic myelosis (chronic nonleukemic myelosis).

Not infrequently lymphocytic leukemia is confused with lymphosarcoma cell leukemia but the latter is now regarded as a distinct morphologic and clinical entity on the basis that the lymphocyte is a separate and distinct strain of cell.[132, 133] Monocytic leukemia has commanded particular attention within recent years with considerable confusion regarding its origin and terminology. According to Farrar and Cameron [134] and Osgood,[135] the monocyte is to be regarded as a definite leukocyte originating from monoblasts in the bone marrow which develop progressively to the premonocyte and mature monocyte in the blood. On this basis, therefore, it does not appear permissible to divide monocytic leukemia into the Naegeli and Schilling types as heretofore but to regard the Naegeli type as a variant of myelogenous leukemia and the Schilling type as a variant of leukemic reticulo-endotheliosis.[136] Unfortunately, even expert hematologists may experience difficulty in differentiating among the various types of acute leukemia and especially since some cases have been described in which the immature cells appeared to be so primitive that the name *stem cell leukemia* has been applied [137] as also the terms lymphoidocytic, embryonal or undifferentiated cell leukemia.

Acute Leukemia. Acute leukemia is of sudden onset with weakness, anorexia, pallor due to rapidly developing anemia, purpura, joint pains, fever, acute gingivitis and sore throat. Swelling and ulceration of the gingivae are particularly apt to be early manifestations of all types of acute leukemia and some writers have thought these lesions especially characteristic of monocytic leukemia. Not infrequently, necrotic and gangrenous lesions develop in the mucous membranes of the mouth and throat which may show an excess of spirochetes and fusiform bacilli in smears, leading to the erroneous diagnosis of Vincent's angina unless blood examinations are made.

Lymphadenopathy is not as marked as in chronic leukemia and may be insignificant or even absent in acute myeloblastic leukemia. Generalized adenopathy, however, is usual in acute lymphoblastic leukemia while great enlargement of the cervical glands is especially likely to occur in acute monocytic leukemia. Splenomegaly occurs in about 60 to 70 per cent of cases and especially in acute lymphoblastic leukemia. The liver is enlarged in about 50 per cent of cases and more frequently in acute lymphoblastic than in acute myeloblastic leukemia. Various types of leukemia cutis may occur but are relatively infrequent.

The diagnosis of acute leukemia and especially the differentiation and recognition of the different cell types cannot be made on the basis of the history and clinical manifestations alone; [138] blood examinations supplemented, if necessary, by sternal bone marrow studies are always required. The usual *laboratory findings* are as follows:

1. Characteristically the total leukocytes are greatly increased but not usually above 100,000 per c.mm. of blood. *But in the early stages the leukocyte count may be normal or even subnormal,* reaching as low as 400 per c.mm. of blood in some cases. Indeed, persistent leukopenia, together with anemia and thrombocytopenia, should arouse suspicion of leukemia.

2. Differential leukocyte counts are of primary importance in the recognition of the type of leukemia. A preponderance of myeloblasts and undifferentiated myelocytes with few polymorphonuclears and no eosinophils or basophils is characteristic of acute myeloblastic leukemia. Auer bodies occur in about 25 per cent of cells. In acute lymphoblastic leukemia, lymphoblasts constitute about 50 to 90 per cent of the cells. In acute and chronic monocytic leukemia, monoblasts, premonocytes and monocytes make up about 60 per cent or more of the cells, with the remainder composed of lymphocytes and polymorphonuclears along with a few myelocytes and myeloblasts as well as some plasma cells. A moderately large number of myelocytes indicates the Naegeli variant of myelogenous leukemia, while the presence of large cells with lacy chromatin, bizarre nuclei and irregular cell borders indicates the Schilling variant of leukemic reticulo-endotheliosis. Eosinophilic, basophilic and other types of leukemia are usually of the chronic variety shortly to be discussed.

3. The erythrocytes and hemoglobin are reduced and sometimes to the extent of constituting a severe anemia, which is usually of the normocytic type although moderate macrocytosis may occur. Polychromatophilia and normoblasts are frequently found.

4. A reduction in the platelets is characteristic, with counts below 100,000 per c.mm. of blood not unusual. Exceptionally large platelets may be observed.

5. Bleeding time is prolonged, with poor clot retraction. Coagulation time is also prolonged in some cases.

6. Positive tourniquet reactions are usual.

7. Sternal bone marrow studies usually show not only the evidences of severe anemia but a preponderance of myeloblasts, lymphoblasts, monoblasts or megakaryocytes, depending on the type of acute leukemia.

Chronic Leukemia. In contrast to acute leukemia, the onset of chronic leukemia is usually so gradual and insidious that it may be discovered only accidentally in the course of routine blood examinations or in individuals seeking medical assistance because of gingivitis, anemia, loss of weight, anorexia, flatulence, recurring attacks of diarrhea, unexplained fever, purpura or other lesions of the skin, enlarged lymphatic glands, or symptoms referable to enlargement of the spleen and liver as well as to the urogenital tract (hematuria, priapism, pain in the back, menorrhagia, metorrhagia or amenorrhea).

When the disease is well developed, however, there is usually marked pallor and loss of weight sometimes to the point of emaciation. The lymphatic glands are enlarged to some degree in all cases but especially in chronic lymphocytic leukemia, in which the cervical glands are particularly involved and sometimes associated with marked enlargement of the tonsils. In rare cases, the salivary and lacrimal glands may be involved (Mikulicz's syndrome). The spleen is also enlarged in all cases but especially in chronic myelocytic leukemia. The liver is likewise usually palpable and not infrequently decidedly enlarged, although jaundice is very uncommon and ascites rare. Anoxemia due to anemia may lead to enlargement of the heart with systolic murmurs and, along with the weight of an enlarged spleen, result in tachycardia, dyspnea and even cardiac failure with edema. Coughing due to pulmonary congestion, partial atelectasis or leukemic infiltrations of the larynx may occur. Gastro-intestinal symptoms may be so prominent as to lead to errors in diagnosis unless blood examinations happen to be made and, indeed, cases have been reported in which the symptoms have been due to extensive lymphocytic infiltrations of the tissues in the absence of typical blood changes constituting "pseudoleukemia gastro-intestinalis."

Bone pains are not unusual and especially in chronic myelocytic and monocytic leukemias with more or less characteristic tenderness of the sternum. These may be due to intramedullary tumors of leukemic cells (chloroma), which sometimes result in destruction or absorption of bone with fractures, to subperiosteal infiltrations or arthritis. Symptoms referable to the nervous system may develop which are likewise due to leukemic infiltrations. Purpura may occur in the late stages and especially in chronic lymphocytic leukemia—likewise herpes zoster and universal leukemic cutis due to infiltrations with leukemic cells, while in chronic myelocytic leukemia the skin lesions may be localized in the form of firm, sharply circumscribed and discolored masses. Leukemoid states may occur but are not characteristic of leukemia, since they are not due to leukemic infiltrations and may be formed in other conditions.

As in the acute leukemias, however, final diagnosis is based on laboratory examinations, especially of the blood. Furthermore, differentiation into the various forms of chronic leukemia is only possible by such examinations. The usual *laboratory findings* are as follows:

1. The blood is usually so thick and sticky that difficulty may be experienced in filling pipets and making smears. If mixed with an anticoagulant and allowed to stand in a hematocrit for an hour or two, three layers may be distinguished. The uppermost is cream-colored, 0.5 mm. or less in thickness and composed of

platelets. The middle is reddish-gray in color, composed almost entirely of leukocytes and of unusual thickness (especially in myelocytic leukemia). The lower is dark red in color and composed of erythrocytes but, because of anemia, thinner than normal. The total blood volume is usually increased due to an increase of both plasma and cells.

2. While there may be no reduction in the erythrocytes and hemoglobin in the early stage of chronic leukemia, especially the myelocytic form, yet anemia is usual and may be quite severe. It is generally of the normocytic type with anisocytosis while significant poikilocytosis is unusual. Polychromatophilia, basophilic stippling, reticulocytosis (especially in myelocytic leukemia) and erythroblastosis (normoblasts), however, are usual and particularly if severe anemia is present.

3. The platelet count is usually within normal during the early stages but frequently reduced in the later stages, especially if purpura is present. Persistently low counts are often of serious import. Fragments of megakaryocytes may be found.

4. Marked leukocytosis, however, is the most characteristic finding. In well-developed cases of *chronic myelocytic leukemia* the counts may range from 100,000 to as high as 800,000 or more per c.mm. of blood, although lower counts may be observed even in cases with pronounced clinical manifestations. Segmented neutrophils usually constitute about 30 to 70 per cent of the cells, with 30 to 50 per cent composed of metamyelocytes and myelocytes and 2 to 10 per cent of myeloblasts. The majority of the myelocytes are neutrophilic except in those comparatively rare cases of chronic eosinophilic or basophilic leukemias in which the majority are eosinophilic (about 90 per cent) or basophilic (25 to 60 per cent) respectively. A few cases of neutrophilic leukemia have been reported in which the leukocytes were polymorphonuclear neutrophils to the almost complete exclusion of myelocytes or other immature forms.[139]

In all cases of chronic leukemia, however, the eosinophilic leukocytes are likely to be present in normal or slightly increased numbers, while the basophilic leukocytes may be increased to 3 to 20 per cent. The lymphocytes may be slightly increased in absolute numbers but the percentage is low. The monocytes are at first increased in number but later reduced except in chronic monocytic leukemia.

In *chronic lymphocytic leukemia,* the total leukocytes are seldom over 250,000 per c.mm. of blood, with 90 to 99 per cent composed of well-preserved small lymphocytes and giving a "monotonous" type of blood picture. Large lymphocytes with indented nuclei are not infrequent but lymphoblasts are very unusual. Formerly, lymphatic leukemia was differentiated into the acute type based on a preponderance of large lymphocytes and the chronic type based on a preponderance of the small type, but this differentiation is no longer employed, since the acute type is based on a preponderance of lymphoblasts as previously described.

Chronic monocytic leukemia is stated to comprise about 11 per cent of all cases of monocytic leukemia; it is characterized by a preponderance of monocytes. *Mixed leukemia* probably occurs but at the present time this is regarded as doubtful, since the nongranular cells leading to its diagnosis are believed to be largely myeloblasts.

5. Sternal bone marrow examinations are of value but are only required when diagnosis is in question. Hyperplasia is characteristic of all types of chronic leukemia and may be diffuse or localized, depending largely on the duration of the disease. In chronic myelocytic leukemia, however, the changes are usually more pronounced than in chronic lymphocytic leukemia. In the former the differential count is remarkably similar to that of the blood while in the latter about 30 to 90 per cent of the cells are lymphocytes. Changes in the erythroblastic tissue are usually in relation to the degree of anemia.

6. Additional laboratory examinations of interest or of helpful diagnostic value are (a) an increase of the basal metabolic rate with a low blood iodine, especially in chronic myelocytic leukemia; (b) an increase of blood uric acid, total nitrogen, total lipids and fatty acids but a normal or slightly reduced cholesterol content; [140] (c) a decrease of total plasma proteins, sometimes associated with a reversal of the albumin-globulin ratio, especially in myelocytic leukemia; (d) sometimes albuminuria with hematuria and casts and (e) achlorhydria, especially in lymphocytic leukemia, in some cases.

Aleukemia or Subleukemic Leukemia. In this type of leukemia the total leukocyte count is normal or subnormal but repeated examinations usually reveal the presence of at least a few immature leukocytes in stained smears of blood. Consequently, diagnosis is difficult and usually requires one or more examinations of the bone marrow. It is likely that many cases are missed, especially in children in whom leukopenia is often a manifestation of leukemia. It has been estimated that 10 per cent or more of all cases of leukemia are of this type.[141] Acute and chronic forms occur and myelogenous, lymphogenous and monocytic types have been described.

Other Types of Leukemia. *Chloroleukemia* (*chloroma*) is characterized by the occurrence of localized tumors of the periosteum and ligamentous structures of the skull, paranasal sinuses, orbits, spine, ribs and sacrum as well as of the skin. The general clinical manifestations are similar to those of acute leukemia along with leukocytosis due to the presence of myeloblasts and myelocytes. Protrusion of the eyeballs with disturbances of vision and cranial nerve palsies are common manifestations. As in other leukemias, however, the disease may occur without leukocytosis. It is seen most often in children and young adults. The peculiar greenish color of the tumors has been ascribed to protoporphyrin.

Megakaryocytic leukemia is characterized by a flooding of the circulating blood with megakaryocytes, thrombocytopenia with atypical platelets, marked anemia, leukopenia and erythromyelomegakaryocytic hyperplasia of the bone marrow, along with the absence of splenomegaly and lymphadenopathy.[142] One form of the disease is known as *aleukemic megakaryocytic myelosis* and develops insidiously, occurs more frequently in women than men and runs a chronic course. Splenomegaly and hepatomegaly without lymphadenopathy but with moderate anemia are characteristic. The total leukocytes are normal or subnormal along with normoblastosis and polychromatophilia; atypical platelets and young megakaryocytes may be observed. Carpenter and Flory [143] have suggested that the disease is due to a chronic progressive hyperplasia of multipotential mesenchymal tissues and prefer designating it *chronic nonleukemic myelosis.*

Plasma cell leukemia is characterized by the signs and symptoms of leukemia, moderate leukocytosis (up to 60,000 per c.mm. of blood) largely due to young plasma cells (plasmoblasts), anemia and deposits of plasma cells in the liver, spleen, lymph nodes and bone marrow. Reiter and Freeman [144] have suggested that this leukemia differs from plasma cell myeloma with plasmocytes in the blood as much as lymphocytic leukemia differs from lymphosarcoma cell leukemia.

Lymphosarcoma cell leukemia is characterized by lymphosarcoma of the anterior mediastinum or other primary sites along with leukocytosis up to as high as 160,000 cells per c.mm. of blood. The latter is largely due to lymphosarcoma cells [145,146] which may comprise as high as 98 per cent of the total. The signs and symptoms are as varied as in other forms of leukemia but are due to glandular enlargements and tissue infiltrations. Splenomegaly sometimes occurs.

Erythroleukemia is characterized by the signs, symptoms and blood changes of erythremia along with the presence of myelocytes and metamyelocytes in the circulating blood. The disease, therefore, has much in common with myelocytic leukemia but is due to a hyperplasia not only of the erythropoietic but of the leukopoietic tissues of the bone marrow as well. A disease called *true chronic erythroblastosis of adults* has also been recently described by Heilmeyer and Schöner.[147] It resembles leukemia clinically but is characterized by erythroblastosis. Consequently, it resembles leukemia of chickens (erythroblastosis) which is attributed to infection with a virus.

LEUKEMOID REACTIONS

Leukemoid reactions are characterized by blood changes resembling those of leukemia. They were first clearly described by Krumbhaar [148] who divided them into two groups: (1) those presenting signs and symptoms suggestive of leukemia and (2) those that resembled leukemia only from the hematologic standpoint.

It is now known that leukemoid reactions are not uncommon and may have many causes. Needless to state, they are frequently difficult to differentiate from the true leukemias and doubtless all cases of alleged recovery from the latter were only leukemoid reactions. According to Hill and Duncan,[149] they may be due to causes producing (1) stimulation of the bone marrow with the overproduction of leukocytes; (2) increased liberation of leukocytes by the bone marrow or (3) extramedullary or ectopic hematopoiesis.

The leukemoid reactions may be as follows: (1) *High leukocytosis with small numbers of myelocytes* due to severe burns, eclampsia, osteomyelitis, fractures, Hodgkin's disease, chronic infectious granulomas of bones or reactions resulting from intravenous medication; (2) *lesser leukocytosis but many myelocytes and myeloblasts* due to septicemia, pneumonia, meningococcus meningitis, diphtheria, tuberculosis (especially miliary or involving the lymph nodes and spleen), mustard gas poisoning, severe hemorrhages, acute hemolysis (especially incompatible blood transfusions) or allergic reactions to the sulfonamide compounds; (3) *high leukocytosis with many lymphocytes* due to whooping cough, chickenpox, congenital syphilis or infectious mononucleosis or (4) normal leukocytes or even leukopenia but many myelocytes and myeloblasts due to ectopic hematopoiesis from destruction or overcrowding of the bone marrow as in myelosclerosis, multiple myeloma, lipoid histiocytosis, congenital hemolytic jaundice and prolonged untreated pernicious anemia.

Hill and Duncan have given the following table of laboratory differentiation between true leukemia and leukemoid reactions:

True Leukemia	*Leukemoid*
1. Leukocytes are atypical, particularly the immature ones.	1. Immature as well as mature leukocytes show normal morphology.
2. Myeloblasts may be numerous and as high as 99 per cent.	2. Myeloblasts usually less than 10 per cent.
3. Immature erythrocytes are rarely increased in proportion to the increase of immature leukocytes.	3. Erythroblasts often increased in proportion to immature leukocytes.
4. Platelets decreased, often severely; may be increased only in chronic myelocytic leukemia.	4. Platelets usually normal or increased but may be moderately decreased in some cases.
5. Progressive anemia which may become severe.	5. Anemia variable depending on the cause.

INFECTIOUS MONONUCLEOSIS

This is an acute, benign infectious disease of unknown etiology characterized clinically by irregular fever, sore throat, lymphadenopathy (especially cervical) and enlargement of the spleen. Hematologically it is characterized by leukocytosis chiefly due to highly differentiated mature lymphocytes,[150] an increase of agglutinin for sheep erythrocytes and a tendency to give temporary positive Wassermann and flocculation reactions. It is also known as *glandular fever, acute benign lymphoblastosis, acute lymphadenosis* and *lymphocytic angina.* The relatively large numbers of lymphocytes present in the blood are considered to be atypical "leukocytoid" lymphocytes which are regarded as benign cells originating in the reticulum of lymphatic tissue anywhere in the body.[151]

As previously stated, the etiology is unknown. Various bacteria and protozoa have been incriminated but none has been proved the cause. *Listerella monocytogenes* has commanded most attention but the supporting evidence is extremely meager.[152,153] At the present time the alleged experimental transmission of the disease to monkeys indicates that it may be caused by a virus.[154]

The epidemic form is chiefly confined to children but may occur among adults and especially physicians, medical students, nurses and laboratory technicians of the "hospital class." Sporadic cases may also occur in children but are more common among adults of 18 to 30 years, while very uncommon after 40 years of age. Males are somewhat more susceptible than females and, curiously enough, the disease is almost unknown in Negroes; at least but very few cases have been reported occurring among them.

The incubation period appears to be about eleven days. The prodromal symptoms are extremely variable but include usually headache of varying severity, malaise, sore throat and fever. The pharyngitis and tonsillitis, however, may be so severe with membranous exudates (anginose type) as to suggest diphtheria; indeed, if antitoxin is given without prompt results the disease should be suspected. Secondary infection with *T. vincentii* and *B. fusiformis* is a common complication. After one to four days the cervical lymph nodes enlarge (especially in children) and sometimes to the extent of resembling mumps. The axillary, mediastinal, inguinal and mesenteric glands may also become involved, the latter sometimes to a degree sufficient for the production of abdominal complaints sometimes simulating appendicitis.[155] Lymphadenopathy may precede other signs of illness by weeks; in some cases and especially in adults it may not develop at all. Splenomegaly occurs in about 50 per cent and slight hepatomegaly in about 12 per cent of cases. The latter is sometimes accompanied by obstructive jaundice. Cardiac and pulmonary symptoms are uncommon but nephritis may occur with albumin, blood and casts in the urine. Pain in the eyes is not uncommon followed by puffiness of the lids. Epistaxis is common, with various skin rashes occurring in about 18 to 20 per cent of cases. In a study of 34 cases Gall[150] observed an increase of blood alkaline phosphatase in almost all, along with a slight increase in the icterus index and positive cephalin and thymol turbidity tests indicative of dysfunction of the liver.

Mild cases are detectable only with the aid of laboratory examinations. The usual *laboratory findings* are as follows:

1. During the first week there may be a misleading leukopenia. Otherwise the total leukocytes may be normal but sooner or later a leukocytosis develops with 10,000 to 20,000 per c.mm. of blood and higher counts have been reported.

2. The polymorphonuclear neutrophils are increased early in the disease but in the fully developed stage 60 to 90 per cent or more of the leukocytes are of nongranular cells chiefly composed of what are now regarded as highly differentiated mature lymphocytes along with small lymphocytes and monocytes, both of which occur normally in the blood.

3. Anemia does not appear in uncomplicated cases. The platelet count is normal although thrombocytopenia may occur. The bleeding time may be prolonged but the coagulation time is normal.

4. Sternal bone marrow examinations may show an increase of lymphocytes or myeloid hyperplasia, with a moderate shift to the left of the myeloid leukocytes; these changes are chiefly of value from the negative standpoint in excluding leukemia.

5. Normal human serums contain heterophil agglutinin, which is not of the Forssman type, sufficient for the agglutination of sheep corpuscles in final dilutions up to 1:8, provided serum sickness or recent injections of horse serum are excluded. Under these conditions, agglutination at 1:16 to 1:32 or higher is suggestive of infectious mononucleosis (Paul-Bunnell test) while agglutination at 1:128 to 1:224 or higher is diagnostic. About 78 per cent of cases will show titers of 1:320 to 1:10,240 which may persist for many months. Repeated tests may be required before definitely positive reactions are observed. According to Warren,[156]

the titer of heterophil antibody is increased only when there is a considerable increase of the differentiated mature lymphocytes.

6. An average of about 20 per cent of cases have shown temporarily positive Wassermann and flocculation reactions; [157] repeated tests may be required before they are observed. Falsely positive Widal reactions have also been reported.

ACUTE INFECTIOUS LYMPHOCYTOSIS

In 1941 Smith described a benign infectious and contagious disease characterized by pronounced lymphocytosis which is distinct from infectious mononucleosis.[158] Some 58 cases have been reported, all but 4 occurring in children.[159–164] The incubation period is probably 12 to 21 days. In some cases there have been no constitutional symptoms whatever, whereas in others vomiting, irritability, fever and abdominal pain have occurred with signs of involvement of the central nervous system and morbilliform rashes in some cases. Infection of the upper respiratory tract may occur. There is no lymphadenopathy or splenomegaly. The disease, which is of unknown etiology, runs a benign course of 3 to 9 weeks with a favorable prognosis. It must be differentiated from infectious mononucleosis, lymphocytic leukemia, pertussis and other miscellaneous infections associated with lymphocytosis. The usual *laboratory findings* are as follows:

1. Total leukocyte counts of 40,000 to 150,000, with 60 to 90 per cent of small mature lymphocytes and usually no anemia.
2. No increase of heterophil agglutinins with negative Wassermann and cephalin flocculation reactions.
3. Negative serologic reactions for lymphocytic choriomeningitis and for types A and B influenza viruses.
4. Hyperplasia of the bone marrow, with mature lymphocytes composing up to 90 per cent of all nucleated cells.

LEUKOPENIA

Leukopenia refers to a reduction in the total leukocytes per c.mm. of blood in relation to age; for example, a count of 7000 in a young child constitutes a leukopenia although within normal for an adult. As a general rule, it is due to an absolute decrease of the granulocytes (neutrophils, eosinophils and basophils), this selective leukopenia being especially well marked in the syndrome known as "agranulocytosis." Leukopenia due to an absolute decrease of lymphocytes or monocytes is much less common but may occur in Hodgkin's disease or other affections of the lymph nodes. When leukopenia is marked, however, it is likely to be due to a reduction of all types of leukocytes but mainly of the granulocytes with special reference to the polymorphonuclear neutrophils.

The causes of leukopenia have already been briefly referred to on page 31. As recently discussed by Laurence,[165] it may be due to (1) a diminished formation or maturation of leukocytes by the leukoblastic tissues; (2) increased destruction of leukocytes in the peripheral blood; (3) increased elimination of leukocytes in the gastro-intestinal tract or peritoneal cavity due to infection; (4) a redistribution of leukocytes and particularly of the neutrophils both of which assume a marginal position in the venules of the liver, lungs, spleen and omentum due to intravenous injections of foreign proteins with special reference to the hydrophil colloids like gelatin, globulin or fibrinogen and (5) to a redistribution of the leukocytes in the body as a whole as sometimes occurs in the leukopenic phases of leukemia. Consequently, leukopenia may be due to various bacterial, viral, protozoal and metazoal diseases and especially overwhelming infections; various intoxications due to drugs, chemical and physical agents; cachectic and debilitated states; disorders of the hemopoietic system and especially those producing aplasia of the bone marrow or its replacement with foreign cells; reactions due to the intravenous administration of foreign proteins and in certain disorders of unknown cause as cirrhosis of the liver, Felty's syndrome, lupus erythematosus disseminata, etc.

Of course, leukopenia is just the antithesis of leukemia and leukemoid reactions as far as the hematologic changes are concerned. But the differences may be more apparent than real. Thus, Rothrock [166] has reported a case regarded clinically as acute agranulocytosis which showed all of the earmarks of a chronic leukemia on postmortem examination. Jackson [167] has also drawn attention to the fact that acute leukemia in an aleukemic or aplastic phase may resemble agranulocytosis both clinically and hematologically, while Strumia [168] has pointed out that the hematologic and anatomic lesions of the two diseases show many similarities, with cases of transition of one into the other. The deciding factor appears to be the mechanism involved in the release of immature and undifferentiated leukocytes into the circulating blood, release occurring in the acute leukemias but not in agranulocytosis.

AGRANULOCYTOSIS

Agranulocytosis is a syndrome, rather than a disease entity, characterized by marked leukopenia due to an absolute reduction in the granulocytes (neutrophils, eosinophils and basophils) in the circulating blood. In many instances, however, there is also an absolute decrease of the other leukocytes. First clearly described by Schultz [169] in 1922 as an acute syndrome of unknown etiology occurring in women of middle age, characterized by severe angina, marked prostration, agranulocytosis, sepsis and death, it has been found to occur more frequently than originally surmised with various names attached to it such as *agranulocytic angina, granulocytopenia, agranulosis, malignant neutropenia,* etc.

In my opinion, the syndrome may be divided into (1) the primary or idiopathic type of unknown etiology, and (2) the secondary type due to toxic depression or necrosis of the bone marrow by drugs or other chemical agents. Both types occur predominantly in adults, the incidence increasing after 25 years of age, while being extremely rare in children. Both types likewise occur much more frequently in women than in men, about 80 per cent of cases occurring among women, which suggests that the physiologic events of menstruation and the menopause may be in etiologic relationship to the syndrome. The primary type rarely occurs in Negroes but this race is susceptible to the secondary type, although the incidence is lower than in whites.

The etiology of the primary or idiopathic type is unknown. As previously stated, severe leukopenia may be caused by various septic infections but no one infectious agent can be regarded as the specific cause. At least all attempts to produce the syndrome experimentally in the lower animals by inoculation with organisms from patients have failed; the nearest to the human disease is the agranulocytosis produced by a virus affecting cats [170] but it is not at all likely to be responsible for agranulocytic angina of man.

Undoubtedly, however, the secondary type is due to the effects of drugs and other chemical agents on the bone marrow. This was first discovered by Kracke,[171] in 1931, in the case of oxidation products of the coal-tar derivatives of the "benzamine" group with special reference to aminopyrine and drugs containing this substance (allonal, amytal compound, amidophen, causalin, midol, cibalgine, etc.). Cases have also been ascribed, however, to the barbiturates as well as to dinitrophenol, the sulfonamide compounds with special reference to sulfanilamide, arsphenamine, neoarsphenamine, bismarsen, mapharsen, antipine, phenacetin, acetanilid, cinchophen and neocinchophen, gold salts, thiouracil, propylthiouracil, atabrine and pyribenzamine.

Apparently these, as well as other drugs, may produce the recurrent or chronic types of agranulocytosis through the destruction of leukocytes in the bone marrow as well as, possibly, through their destruction in the circulating blood. But a direct leukotoxic effect of this kind is not acceptable in explanation of acute agranulocytosis of the primary or Schultz type characterized by prodromal symptoms of malaise and fever followed suddenly by a chill, high fever, severe angina or necrotizing lesions of the gums, nose, vagina or elsewhere, along with jaundice and regional adenopathy in some cases and a fatal outcome in 70 to 90 per cent within a week or two. At least, this syndrome has not been produced experimentally in the lower animals by the administration of amidopyrine or other drugs. Under the conditions, it appears highly probable that acute agranulocytosis of human beings caused by drugs is due to a natural or acquired allergy to them, the latter generally the result of their prolonged or intermittent administration. This mechanism is indicated not only by the fact that other allergic manifestations may be present (like urticaria or asthma) but also because the attack may be precipitated by a very small dose and within less than twelve hours after its administration. It is true that allergic antibody cannot be demonstrated in the blood by the passive transfer method (as in the case of other drug allergies) and that positive skin reactions may not be elicited [172] but this, likewise, is not infrequent in acquired drug allergies. Furthermore, the sudden development of acute agranulocytosis due to a drug can hardly be ascribed to an interference with the maturation of the granulocytes in the bone marrow but their destruction or redistribution in the capillaries as the result of an allergic reaction is easily and readily possible.

Acute as well as subacute and chronic agranulocytosis, however, may occur in individuals in whom allergy to drugs may be definitely excluded. Such cases are generally characterized by fever, splenomegaly and splenic pain.[173] This type of the disease has been ascribed to excessive lysis or phagocytosis of granulocytes in the spleen which shows extreme clasmatocytosis with excessive phagocytosis of granulocytes. The cause of the phenomenon is unknown. Still another form of granulocytopenia has been described by Wiseman and Doan,[174] designated "primary splenic neutropenia." It is characterized by fever, splenomegaly with pain or discomfort as well as essentially normal, or somewhat hyperplastic, bone marrow obtained by sternal puncture. The manifestations may be acute, subacute or chronic with relief of all symptoms following splenectomy, the disorder being attributed to excessive lysis of neutrophils by the spleen.

In both the primary and secondary types of agranulocytosis, however, various infections of the throat and elsewhere commonly occur which is to be expected in view of the reduction in resistance due to depression or destruction of the bone marrow. Consequently, it is not unusual to find an increase of *Bor. vincentii* and *B. fusiformis* in smears of the gingivae and throat in cases of angina which may result in a mistaken diagnosis of Vincent's angina unless total and differential leukocyte counts are made. Furthermore, cultures may show the presence of diphtheroid bacilli with chances of an erroneous diagnosis of diphtheria; the same is true of staphylococci, hemolytic streptococci and pneumococci. As a matter of fact, all severe leukopenias, especially those due to agranulocytosis, are characterized by a marked reduction in the resistance of gingivae and throat to bacterial infections. Unfortunately, this reduction may be general with the result that sepsis or bronchopneumonia constitute great risks and are not infrequently the cause of death. In other words, the angina and necrosis of the gastro-intestinal tract in agranulocytosis are due to secondary infections: fortunately, the infectious agents are usually susceptible to penicillin which accounts for the remarkable success observed in the treatment of the disease with this compound.[175]

Needless to state, the diagnosis of agranulocytosis requires the exclusion of Vincent's angina, diphtheria and other severe infections of the throat, overwhelming sepsis, aplastic anemia and aleukemic leukemia. The characteristic *laboratory findings* in agranulocytosis may be summarized as follows:

1. Marked leukopenia with the total leukocytes less than 2000 per c.mm. of blood and frequently below 1000 in acute cases but rarely below 2000 in chronic or recurrent cases.

2. Complete absence of granulocytes (neutrophils, eosinophils and basophils) or their reduction to 2 per cent or less. Consequently, the majority of the cells are lymphocytes. A few monocytes may also be present and if they persist and increase, it is stated that the prognosis is more hopeful. Improvement is also heralded by the appearance of myeloblasts, metamyelocytes and myelocytes; mature neutrophils are the last of the series to reappear. Türk "irritation" cells may be found.

3. There may be no anemia or only an anemia of slight to moderate degree with no increase of reticulocytes. The sedimentation rate, however, is greatly accelerated. The icterus index may be moderately increased.

4. The platelets are usually within normal with no increase of the bleeding or coagulation times.

5. The bone marrow shows normal erythropoietic tissue with normal numbers of megakaryocytes but a striking deficiency of polymorphonuclears, metamyelocytes and myelocytes. Promyelocytes and myeloblasts are usually present while plasma cells, lymphocytes and reticulum cells may be increased.

The classification of tumors and tumor-like lesions of the lymphatic glands is a much disputed subject. The clinical types are numerous and sharp separations cannot be made, since they tend to fuse with one another. Probably Hodgkin's disease is clinically the best-defined disease of the group although it may occur in localized, generalized, mediastinal, osseous, splenic, typhoidal and acute forms. Needless to state, differential diagnosis is usually based on microscopic examinations of the glands removed by biopsy or after death. From this standpoint the lesions are usually divisible into (1) lymphosarcoma, (2) "reticulum cell" sarcoma, (3) lymphocytic lymphoma; (4) Hodgkin's disease; (5) Hodgkin's sarcoma; (6) follicular lymphoma and (7) "clasmatocytic lymphoma." Unfortunately, blood and bone marrow examinations are of limited diagnostic value although certain *laboratory examinations* are helpful, which in Hodgkin's disease may be summarized as follows:

1. Slight to moderate anemia of the normocytic type is present in 30 to 50 per cent of cases but sometimes is quite severe. If the spleen is particularly involved it may be of the hemolytic type with macrocytosis and spherocytosis associated with a decreased resistance of the cells to hypotonic saline solutions.
2. A slight to moderate leukocytosis (8000 to 16,000) is usual although higher counts may occur as well as normal counts. Lymphocytopenia and monocytosis are among the most consistent changes. Eosinophilia is likewise common with a tendency to neutropenia (especially if the bone marrow is involved).
3. The platelet count is usually within normal although sometimes increased in which case large bizarre forms may be found. Thrombocytopenia, however, may occur which is suggestive of bone marrow involvement.
4. Sternal bone marrow examinations may show no definite changes but usually reveal changes similar to those in the blood, namely, a slight or moderate shift to the left in the myeloid series, slight monocytosis or moderate eosinophilia, a reduction in lymphocytes, a relative reduction in erythroblasts and possibly an increase of megakaryocytes. The chief value of bone marrow examinations, however, is the ruling out of leukemia and especially the aleukemic form.
5. Other laboratory findings usually consist of (a) an increase in the basal metabolic rate, (b) an increase of blood uric acid and (c) an increase of alkaline serum phosphatase indicative of involvement of bones.

MULTIPLE MYELOMA

Multiple myeloma is a tumor characterized by multiple involvement of the bones with the production of pain, pathologic fractures, anemia, hyperproteinemia and the excretion in the urine of Bence-Jones protein. Fortunately, the disease is rare but occurs in all races, twice as frequently in males as in females and usually in later life with about 80 per cent of cases after the age of forty. The usual *laboratory findings* are as follows:

1. Normocytic anemia of moderate degree. On the other hand, anemia may be absent, even in fatal cases, or unusually severe (macrocytic). A few normoblasts may be found but no megaloblasts. Moderate polychromatophilia, slight baso-

philic stippling and reticulocytosis may be observed. Under these circumstances, the disease may be mistaken for pernicious anemia if the classical signs of myeloma are absent. Difficulty may be experienced in counting the erythrocytes due to increased blood viscosity from hyperproteinemia, rouleaux formation or autohemagglutination.

2. The leukocytes may be normal, slightly increased or slightly decreased. Differential counts may be normal or show an increase of lymphocytes or eosinophils along with a few myelocytes and even myeloblasts, plasma cells and Türk irritation cells.

3. The platelets are usually normal but sometimes reduced along with a prolongation of the bleeding time. Failure of clot retraction may be observed due to hypoproteinemia.

4. The sternal bone marrow findings are variable but the presence of "myeloma cells," which may make up 3 to 65 per cent of all the cells, is pathognomonic of the disease although their absence does not exclude it.[176]

5. Other changes consist of (a) hyperproteinemia; (b) an increase of serum calcium; (c) an increase of blood uric acid; (d) blood nitrogen retention in some cases and (e) a characteristic excretion in the urine of Bence-Jones protein which occurs in 65 per cent or more of cases.

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23

DISEASES OF THE KIDNEYS AND URINARY SYSTEM

Under this heading are included those diseases of the kidneys, ureters and bladder in which laboratory examinations are of diagnostic value. The latter include not only physical, chemical and microscopic examinations of voided urine and that collected from each kidney separately by ureteral catheterization, but also bacteriologic examinations when infections are suspected. Furthermore, since the functions of the kidneys embrace (1) the excretion of the end products of nitrogenous metabolism, useless foreign substances and certain crystaloids, (2) the retention of normal protein constituents, glucose and sodium chloride of the blood and (3) the synthesis of ammonia in the maintenance of acid-base equilibrium, various blood chemistry examinations are likewise of value, as discussed in Chapter 3, as well as renal function tests, discussed in Chapter 5.

PASSIVE CONGESTION OF THE KIDNEYS

In order to carry out their chief functions of excreting water, certain mineral substances and the waste products of nitrogenous metabolism, the kidneys possess an unusually abundant blood supply distributed in two systems of capillaries—the glomerular and the peritubular—of widely different hydrostatic pressures. Consequently, the kidneys are among the first organs of the body to show the effects of passive congestion due to cardiac failure from any cause. With a lowering of the hydrostatic pressure of the blood in the glomerular capillaries, filtration is not only reduced but permeability is likely to be increased, and plasma proteins and erythrocytes escape into the urine. Under the conditions, the chief *laboratory findings* are (1) oliguria or scanty urine which is high colored and apt to show a heavy deposit of "brick dust" urates upon cooling; (2) variable amounts of albumin but usually only traces and (3) a moderate increase of erythrocytes with possibly a few casts. Since the elimination of nitrogenous waste is good, except when passive congestion is due to cardiac failure from hypertension (cardiovascular renal disease), and (4) blood urea nitrogen and total nonprotein nitrogen are usually normal or but slightly increased and there is no increase of creatinine.

Long-continued passive congestion, however, may result in a diffuse fibrosis with some thickening of the glomerular tufts and more pronounced changes in the urine and blood chemistry determinations. This is particularly true in cardiac failure due to the advanced stages of essential hypertension in which the laboratory findings become part and parcel of that type of nephritis or Bright's disease known as ***nephrosclerosis*** (to be discussed later).

NEPHRITIS OR BRIGHT'S DISEASE

The term "nephritis" refers to inflammation of the kidneys from any cause and involving any part, but when used alone, custom has sanctioned its use for designating those diseases grouped together under the name of "Bright's disease." This, however, is to be regretted because Bright's disease includes not only true nephritis but various infiltrations, degenerations and fibroses of the kidneys which are not inflammatory in origin at all.

Classification. Consequently, many classifications have been proposed for nephritis or Bright's disease. Those based on etiology would be the most logical but, unfortunately, are impossible because of a lack of sufficient knowledge. Furthermore, classifications based on the nature of functional impairment (lack of concentration of the urine, nitrogen retention, chloride retention, etc.) are both theoretically and clinically impractical. Under the circumstances, the most logical classifications are those based on the nature (inflammatory or degenerative) and the site (glomeruli, tubules or vessels) of primary injury, as originally proposed by Volhard and Fahr, since these most nearly permit a correlation between the signs and symptoms on the one hand and the pathologic changes and alterations in function on the other. Unfortunately, however, even classifications on this basis are not entirely satisfactory because while the glomeruli, tubules or vessels (arterioles, arteries) may be the primary sites of injury, there is usually sooner or later secondary involvement, either anatomic or functional in nature, or a combination of both. In other words, mixed forms rather than pure types of nephritis or Bright's disease are the rule, and both anatomic and functional changes may be due entirely to extrarenal factors, especially passive congestion from cardiac failure as well as, possibly, to disturbances in lipid and protein metabolism.

Thus, nephritis or Bright's disease may be due to inflammation with the production of primary glomerulonephritis or tubular nephritis on the one hand, or to non-inflammatory infiltrations, degenerations or vascular scleroses on the other. Since infiltrations and degenerations of the tubular epithelium may interfere with the reabsorption of water and sodium chloride from the glomerular filtrates, edema may result. This is usually referred to at the present time as the "nephrotic syndrome," while the term "nephrosis" has largely replaced the older designation of "tubular nephritis." Furthermore, the term "nephrosclerosis" has largely replaced that of "vascular nephritis" for designating fibrosis of the kidneys originating in the arterioles or the larger branches of the renal arteries.

On this basis, therefore, nephritis or Bright's disease may be classified into three main varieties with subdivisions as follows:

1. *Glomerulonephritis:*
   - (1) Acute
     - (a) Diffuse
     - (b) Focal
       - Embolic
       - Nonembolic (toxic) or intercapillary
   - (2) Latent and subacute
   - (3) Chronic
     - (a) Without renal edema (chiefly azotemic)
     - (b) With renal edema (nephrotic syndrome)

2. *Nephrosis (tubular nephritis)*:

(1) Acute { (a) Exogenous (mercuric chloride poisoning, etc.)
(b) Endogenous (toxemia of pregnancy, etc.)

(2) Chronic { (a) Lipoid
(b) Amyloidosis

3. *Nephrosclerosis (vascular nephritis)*:

(1) Arterial or atherosclerotic (senile arteriosclerosis)

(2) Arteriolar (essential hypertension) { (a) Benign
(b) Malignant

(3) Combined arterial and arteriolar sclerosis

ACUTE DIFFUSE GLOMERULONEPHRITIS

Acute diffuse glomerulonephritis may begin so insidiously that, except for changes in the urine, there may be nothing to suggest its existence. Hematuria, however, is of such constant occurrence that the disease is frequently designated as "acute hemorrhagic nephritis," although the degree of bleeding may be so slight that it may be detected only by careful microscopic examinations of the urine, especially actual counts of the erythrocytes by the method of Addis.

While the etiology is still uncertain, there can be no doubt that acute infections are responsible for most cases of the disease. This is particularly true of streptococcal and pneumococcal infections of the respiratory tract, especially scarlet fever, tonsillitis, mastoiditis, pharyngitis, laryngitis and pneumonia as well as erysipelas, infected wounds, burns, etc., with exposure to cold and wet as important predisposing factors. But since streptococci or other micro-organisms are not to be found in the urine or in the kidneys, the mechanism of injury is obscure. Almost universally the nephritis develops or at least is first detected one or more weeks after the initial or primary infection. This suggests that the glomerulitis may be caused by exogenous or endogenous toxins similar to the production of glomerulonephritis by diphtheria toxin. Others have expressed the opinion that the nephritis may be due to acquired allergic sensitization to the proteins or toxins of micro-organisms.

Whatever may be the source and mode of action of the injurious agent, it may initiate processes that are capable of progressing in some cases without its continued presence although the permanent effects often appear to be the results of repair of the primary damage. Why all glomeruli are not involved is an unsolved riddle. One important result is an increase of their permeability, permitting not only the escape of plasma proteins but of blood itself. Consequently, as long as damaged glomeruli are capable of filtration the urine will contain albumin.

Mild hypertension is also frequently associated with acute glomerulonephritis. Apparently this is due to renal ischemia resulting not only from interference with circulation from proliferation of endothelium, thrombi in the capillaries and capsular exudates, but also to an increase of intrarenal pressure from gross swelling of the kidneys. These two factors also reduce the quantity of urine excreted, with variable degrees of retention of nitrogenous waste products.

Needless to state, the results of *laboratory examinations* are in relation to the duration and severity of the nephritis but in the average case may be summarized as follows:

1. A reduction in the volume of urine (*oliguria*) due to interference with glomerular filtration and prerenal deviation of water. Consequently, the urine is concentrated and of *increased specific gravity* although anuria is rare.

2. *Hematuria* is the most constant and characteristic change. Occasionally, bleeding is so acute that the urine is of a bright red color but usually a "smoky" or dark brownish-red color is observed. In mild cases, however, bleeding may be so slight that the number of erythrocytes barely exceeds the upper limit of normal. On recovery, hematuria is one of the last evidences of nephritis to disappear.

3. *Albuminuria* is a constant finding. The degree is variable and may range from as low as 0.5 to as high as 10 per cent with a general average of 2 to 4 per cent. Like hematuria, it is one of the last changes to disappear upon recovery. Fibrinuria is rare.

4. Numerous *casts* are present which are usually of the finely granular and cellular types along with an excess of leukocytes and epithelial cells.

5. In mild cases there may be no *nitrogen retention* but in most instances the blood urea nitrogen is increased from the normal of 8 to 18 mg. to 20 to 30 mg. per 100 cc. with an increase of the total nonprotein nitrogen. The creatinine is usually within the normal of 1 to 2 mg. per 100 cc. In severe cases, however, the urea nitrogen may reach as high as 40 to 160 mg., the total nonprotein nitrogen 200 mg., and creatinine 2 to 12 mg. or higher, due not only to impairment of excretion but especially to oliguria from the prerenal deviation of water and excessive protein catabolism. Consequently, high nitrogen retention with urine of low specific gravity is more significant than in the case of urine of high specific gravity.

6. There may also be some retention of plasma *chloride* above the normal of 570 to 620 mg. per 100 cc., especially in the presence of oliguria. In this case the urinary chloride is reduced from the normal of 10 to 16 gm. per 24-hour urine.

7. The daily loss of 4 to 5 gm. or more of albumin in the urine may produce *hypoproteinemia* with a reduction of the total plasma proteins from the normal of 6.4 to 8.0 gm. to as low as 5.5 gm. per 100 cc., with a change in the albumin-globulin ratio, which may be a factor in the production of edema.

8. Acidosis may develop due to a deficiency in the excretion of acid phosphate, sulfates and inefficiency on the part of the kidneys to produce ammonia.

9. Undoubtedly, the *urea clearance* test is the most reliable index of the degree of renal damage, since a reduction usually occurs in advance of a reduction in the excretion of phenolsulfonephthalein.

10. There is usually no *anemia* unless a marked degree of nitrogen retention (azotemia) occurs. A slight leukocytosis, however, is not unusual in the initial stage.

The *prognosis* is good as far as survival is concerned, as the mortality rate is usually no higher than about 4 per cent with death due to uremia. During the first few weeks, however, prognosis cannot be estimated on the basis of the degree of hematuria, albuminuria, nitrogen retention or hypertension. Recovery may occur even when the blood creatinine is 12 mg. or higher per 100 cc. of plasma. Blood urea nitrogen of less than 30 mg. and creatinine less than 2 mg. per 100 cc., however, are of good prognostic import. Urea clearance may fall to as low as 10 per cent of normal and yet be followed in time by apparent recovery. In other words, it is not so much the degree of reduction in urea clearance that counts as its duration. For this reason tests conducted during the first four months are of little value but when there is no tendency toward a return to normal in six months, the nephritis invariably progresses into chronic and terminal nephritis. However, if the plasma albumin is found to be quite low in the early stage, the probability of recovery is less favorable than if it is maintained near the normal.

Patients may show apparently complete recovery within a few months to a year or pass into a latent stage with variable degrees of persistent hematuria, albuminuria and impaired renal function. From this state gradual recovery may also occur or the disease may pass into subacute or chronic active glomerulonephritis. In some cases there is a direct progression of acute into chronic nephritis. As recently stated by Pittinos and his colleagues,[1] it is evident that minimum criteria should be established for normal kidneys before a patient is discharged as completely recovered. For this purpose they suggest a physical examination, blood pressure and urinalysis every three months over a period of two years, with special reference to Addis counts of erythrocytes in the urine.

ACUTE FOCAL GLOMERULONEPHRITIS

This is a special type of acute glomerulonephritis occurring in two forms. One is known as the *embolic* type occurring most frequently in the course of bacterial endocarditis, with special reference to subacute bacterial endocarditis due to *Str. viridans,* as well as during typhoid fever with bacteremia, malaria, etc. It is characterized by emboli of homogenous fibrin, coagulated plasma and bacteria in the glomerular tufts with some secondary leukocytic infiltrations. The second is designated the *nonembolic* or *toxic* type, characterized by the formation of hyaline fibrinous thrombi in the glomerular capillaries without secondary leukocytic infiltrations and is apparently due to the action of toxins from some distant focus of infection on the vascular endothelium. It is usually of little clinical significance when it occurs in conjunction with minor infections of the upper respiratory tract but may be serious when occurring during the nonbacteremic phases of endocarditis.

The *laboratory findings* are similar to those of acute diffuse glomerulonephritis but usually of milder degree. Hematuria and albuminuria along with granular and cellular casts are the chief changes. The hematuria may be detected only by careful microscopic examinations of the urine. Widespread or diffuse focal glomerulonephritis, however, may be just as severe as the diffuse type of the disease, with secondary tubular degenerations resulting in azotemia, edema and hypertension sometimes progressing to uremia with a fatal outcome.

LATENT AND SUBACUTE GLOMERULONEPHRITIS

As previously stated, acute glomerulonephritis usually ends in complete recovery although it may take several months to a year or more for all urinary changes and evidences of nitrogen retention to disappear, and it is always likely that some glomeruli and tubules may have been rendered functionally inactive for the balance of life.

On the other hand, however, a low-grade glomerulitis may persist without hypertension, edema or other pronounced clinical manifestations, which may be designated *latent* glomerulonephritis and is similar in these respects to chronic latent tuberculosis or syphilis. This latent form may follow a mild attack of acute glomerulonephritis characterized by little or no nitrogen retention but is always more likely to follow acute glomerulitis with marked retention of urea nitrogen and creatinine. Recovery may gradually occur but recurrent minor infections with exacerbations of the low-grade glomerulitis may result in the production of chronic active glomerulonephritis and eventually in a terminal nephritis.

Latent glomerulonephritis, therefore, is to be regarded as one form of subacute glomerulonephritis due to the persistence of low-grade inflammation. The latter, however, may also be due to the effects of repair of damaged glomeruli and tubules after inflammation and the causes for it have disappeared. The *laboratory findings* in latent glomerulonephritis and subacute glomerulonephritis due to repair are usually as follows:

1. Persistent hematuria usually detected by careful microscopic examinations of the urine, with special reference to counts of the erythrocytes by the method of Addis.
2. Persistent albuminuria which, however, may be slight and even disappear for short intervals. Casts and numerous leukocytes are usually observed. The sodium chloride of the urine may be decreased along with a coincident increase of plasma sodium chloride. Glucose sometimes occurs in the urine due to faulty reabsorption by damaged tubules.
3. The blood urea and total nonprotein nitrogens are approximately normal or but slightly increased with no increase of creatinine. Hypoproteinemia is usually absent and there is a normal albumin-globulin ratio of the plasma.
4. The urea clearance and concentration tests may be normal, or even above normal, but usually reveal renal damage. The concentration test is more sensitive than the urea clearance test for the detection of slight degrees of renal impairment; hence, if the former yields a normal result, the latter need not be conducted. But if the concentration test shows that the urine is of a fixed low specific gravity, the urea clearance test is indicated as it better determines the degree of impairment of renal function.
5. Slight anemia may be present due to a reduction in erythrocytes and hemoglobin.

CHRONIC GLOMERULONEPHRITIS

Chronic glomerulonephritis may be due to (1) the secondary effects of repair of glomeruli and tubules damaged by acute glomerulonephritis; (2) the persistence and progression of latent glomerulonephritis resulting from minor acute exacerbations or (3) it may occur as a primary disease in which the possibility of its being due to chronic streptococcal infections of focal origin (dental, tonsillar, sinuses, etc.) properly commands serious consideration.

In all instances most of the glomerular tufts and the membranes of Bowman undergo progressive fibrosis and hyaline degeneration, with the result that many become only functionally inactive masses of avascular scar tissue. Obliteration of the glomerular capillaries inevitably reduces the flow of blood in the peritubular capillaries, resulting in atrophy and degeneration of the tubules in addition to any damage sustained by previous or coincidental inflammation. In other words, chronic glomerulonephritis is never confined to the glomeruli alone as the tubules are invariably involved and especially the convoluted portions or loops of Henle. In the early stages of chronic glomerulonephritis, however, the clinical manifestations and laboratory findings usually permit the division of the disease into two kinds, namely, the (1) *azotemic type* characterized by marked nitrogen retention and hypertension without edema and (2) the *nephrotic type* characterized by much less nitrogen retention, little or no hypertension, but with edema largely due to hypoproteinemia from the excessive loss of albumin through the kidneys. Edema, however, may occur in the azotemic type due to cardiac failure and cardiac edema may also supervene in the nephrotic type.

The azotemic type is due primarily to severe proliferative and obstructive lesions in the glomeruli with resulting progressive hvpertension. Since the afferent glomerular arterioles are not involved the hypertension increases filtration by the intact glomeruli. The urine, however, not only flows more rapidly through the tubules but, owing to atrophic changes in them, is not properly concentrated by reabsorption. Polyuria results with urine of a fixed specific gravity (1.010 to 1.012) containing a slight or moderate amount of albumin. When progressive destruction of the glomeruli finally exhausts their functional reserve, nitrogen retention or azotemia becomes more marked. From this time on, uremia may occur suddenly and without previous warning. On the other hand, however, hypertension may proceed more rapidly than the azotemia in which case death may occur from congestive failure of a severely hypertrophied heart before renal insufficiency ends in uremia.

In the nephrotic type the peritubular capillaries, for some unknown reason, become hyperpermeable. As a result, large amounts of plasma albumin escape into the urine and there is marked albuminuria. Consequently, hypoproteinemia reaches the point at which the osmotic pressure of the plasma proteins is so reduced that more fluid passes from the systemic capillaries into the tissues than re-enters them with consequent edema. At this time, however, there is usually but little nitrogen retention and little or no hypertension. But, sooner or later, as the disease progresses, azotemia increases along with gradually increasing renal ischemia and hypertension. As a result, the albuminuria decreases and there is a rise in plasma proteins and spontaneous disappearance of edema. From this time on the progress of the disease is similar to that of the azotemic type, with ultimate death from uremia, although a fatal outcome may be due to cardiac failure and edema from the effects of hypertension. Consequently, the edema of chronic glomerulonephritis may be of two different types due to different causes and developed by different mechanisms.

Under the circumstances, the clinical manifestations and laboratory findings in chronic glomerulonephritis may be quite variable, depending not only on whether it is largely of the azotemic or nephrotic type but likewise on the degree of functional impairment. Function may be maintained so satisfactorily for variable periods of time, when it is known as glomerulonephritis with compensated renal functional impairment, that the disease may be discovered only accidentally as in the course of a life insurance examination. Sooner or later, however, as functional reserve is exhausted, the disease passes into glomerulonephritis with decompensated renal functional impairment. The results of various *laboratory examinations* may be summarized as follows:

1. The *volume of daily urine* varies according to the presence or absence of renal edema. In its absence, polyuria is the rule because of increased glomerular filtration from hypertension and the fact that the kidneys must dilute the solids for adequate elimination. Consequently, the urine is light in color and of a low *specific gravity*. As the concentrating power is lost, the latter tends to become fixed at or about the specific gravity of the protein-free blood plasma (1.007) but usually around 1.010 to 1.012 or lower. In the presence of renal edema or the nephrotic syndrome, however, the volume of urine may be decreased and of a normal or increased specific gravity. Polyuria may lead to dehydration and hemoconcentration, especially if there is a prerenal deviation of water. Indeed, it is not unusual for edematous patients to become dehydrated, especially if acidosis develops.

2. *Hematuria* is frequent and particularly in cases without edema. Usually, however, it is detected only by microscopic examinations, especially by counts of the erythrocytes according to the method of Addis.

3. *Albuminuria* is usually moderate or slight in cases without edema but very marked in those with edema. *Glycosuria* is not infrequent due to a decrease in the renal threshold and failure of reabsorption by the tubules. Indeed, fasting hyperglycemia with diminished glucose tolerance is not unusual.

4. *Casts* are invariably present. They are mainly of the hyaline variety but in cases of edema may be largely of the coarsely granular type and sometimes show the presence of fat droplets.

5. In compensated cases with polyuria and adequate elimination of solids there may be little *nitrogen retention,* but variable degrees of nitrogen retention are the rule in all cases. The blood urea nitrogen, total nonprotein nitrogen and creatinine gradually increase, especially in the azotemic type of the disease. Creatinine is usually the last to become elevated but high values are always of bad prognostic import, as they are indicative of severe renal impairment. Normally, the urea nitrogen is only about one-half that of the total nonprotein nitrogen but in the azotemia of chronic nephritis it may constitute as much as 80 to 90 per cent of the latter. Of course, marked retention of nitrogen may be largely due to extrarenal factors such as intercurrent infections, vomiting and diarrhea, excessive protein intake, water privation, myocardial failure, etc., with the result that too

much reliance must not be placed on nitrogen retention, but a urea nitrogen of 80 mg., total nonprotein nitrogen of 100 mg. and creatinine of 6 mg. or higher frequently indicate impending uremia. In nitrogen retention the urea of the saliva and cerebrospinal fluid is likewise increased. The blood *uric acid* is usually increased as, likewise, the amino acids and undetermined nitrogen and especially in terminal nephritis accompanied by edema.

6. *Urea clearance* is consistently reduced in all cases of active chronic glomerulonephritis. This may amount to less than 60 per cent of normal and usually less than 50 per cent; uremia can be expected when it is reduced to 5 or 10 per cent of normal. Diminished power of the kidneys to concentrate urine, however, is perhaps the first indication of renal functional impairment (*hyposthenuria*). As previously stated, this impairment usually reaches the point at which the molecular concentration of the urine approaches that of the protein-free blood plasma with a low fixed specific gravity of the urine (*isothenuria*). Since the power of concentration of the urine by the kidneys is not affected by extrarenal factors, a determination by the *concentration test* is of great clinical value for estimating renal function. Failure to eliminate water as determined by the *dilution test* is also of value but is not involved fundamentally in the mechanism of renal edema which is largely due to the prerenal deviation of water into the tissues, increased capillary permeability and decreased colloidal osmotic pressure of the blood due to hypoproteinemia. In most cases the retention of *phenolsulfonephthalein* parallels that of nitrogen retention but elimination of the dye may be normal in compensated cases of chronic nephritis in spite of relatively far advanced lesions. Furthermore, excretion may be decreased by factors favoring the extrarenal deviation of water in addition to the fact that in hepatic disease some of the dye may be removed from the blood. Clinically, the *creatinine clearance test* is no better than the urea clearance test. However, since creatinine is excreted by the glomeruli, the creatinine clearance test is an exact index of the degree of glomerular impairment, while the *phenol red test* is a fair measure of impairment of the excretory capacity of the tubules, since the dye is normally excreted by them.

7. Because of the loss of albumin through the kidneys, *hypoproteinemia* is usual in active chronic glomerulonephritis and especially in nephrotic cases where the plasma albumin may be below 2.5 gm. per 100 cc. In the terminal stages, however, the plasma proteins tend to return to normal levels with reduction or spontaneous disappearance of edema brought about by decreasing albuminuria and hemoconcentration.

8. Theoretically, renal insufficiency should reduce the urinary excretion of *sodium chloride* and increase the plasma chloride, but most patients with the azotemic type of chronic glomerulonephritis show a reduction in plasma chloride due to low chloride intake, vomiting or lowering of the renal threshold while, in acidosis of nephritis, there may be a shift of chloride from the plasma to the erythrocytes. In the nephrotic type of the disease, the plasma chloride is moderately increased and there is a reduction of the chloride in the urine but without any close parallelism between these changes and the occurrence of edema. An increase of the inorganic phosphorus of the serum may occur (*hyperphosphatemia*) as likewise retention of sulfates which, along with a deficiency in the production

of ammonia by the kidneys, predisposes to acidosis in advanced nephritis. As the latter approaches uremia there is also a concomitant decrease in serum calcium (*hypocalcemia*) with a reduction of total calcium below 9 mg. per 100 cc. due to a decrease of the nondiffusible fraction because of hypoproteinemia or hypophosphatemia. This hypocalcemia, when below 7 mg. per 100 cc., may produce neuromuscular excitation but the latter is very unusual. In rare cases, hypercalcemia may be observed which is attributed to defective urinary excretion and increased hydrogen-ion concentration of the blood rather than to hyperparathyroidism although the latter may occur as a compensatory mechanism. It may also be mentioned that the *basal metabolic rate* is sometimes increased, especially in chronic nephritis without renal edema.

9. In chronic glomerulonephritis, particularly in the nephrotic type, the excretion of *cholesterol* in the urine may be increased from the normal of about 0.5 mg. to as much as 90 mg. or more per day.[2] It appears to parallel the excretion of albumin. As a result, *hypocholesterolemia* is not unusual and is of serious prognostic import, especially in the azotemic type of the disease. In view of its high urinary excretion, the total blood cholesterol could be exhausted in a few months except for such compensatory factors as increased synthesis, decreased destruction or diminished excretion through the intestinal tract. The plasma *phospholipids* are not infrequently increased, especially in uremia.

10. *Anemia* is almost constantly found in chronic glomerulonephritis, especially the azotemic type, probably because of depressed activity of the hematopoietic tissues.[3]

THE NEPHROSES

The nephroses refer to those types of nephritis in which the tubules show the primary or principal lesions. They were formerly designated the *tubular nephritides*. But just as glomerulonephritis is invariably associated with secondary tubular changes of varying degree so, likewise, are the nephroses or tubular nephritides associated with glomerular changes. The only possible exception is that type of chronic nephrosis known as "genuine" or lipoid nephrosis. But even in this disease glomerular changes have occurred frequently enough for many observers to regard it as only a type of chronic glomerulonephritis with unusual degenerative changes in the tubular epithelium.

Acute Nephrosis. Acute nephrosis or acute tubular nephritis is ascribed to the effects of toxic agents on the tubules brought to the kidneys by the blood for elimination. Apparently they are excreted by the glomeruli along with glucose, urea, chlorides, and other physiologic substances with sufficient water to hold them in solution. But during reabsorption of the glomerular filtrates, toxic agents which are not selectively reabsorbed and therefore reach a high degree of concentration by the absorption of water, apparently produce cloudy swelling or more severe degenerative changes in the tubular epithelium with special reference to the proximal convoluted tubules. On the other hand, while the tubules do not participate in excretion under normal or physiologic conditions, it may be that foreign toxic agents are excreted by them; at least, it is known that the proximal convoluted tubules excrete such foreign substances as diodrast and phenolsulfonephthalein. If such occurs in the case of foreign toxic substances, an additional mechanism in the production of nephrosis would be operative and especially in relation to the acute toxic nephroses.

Be that as it may, acute types of nephrosis may be produced not only by such exogenous toxic agents as mercuric chloride, bismuth, lead, arsenic, barbiturates, phenol, methyl alcohol, etc., but also by the toxins of many of the acute infectious diseases (diphtheria, typhoid fever, cholera, malaria, etc.) which Fishberg has designated the "necrotizing nephroses." Acute nephroses may also be caused by apparently toxic agents of endogenous origin, as in the fevers (febrile nephrosis), obstructive jaundice, hyperthyroidism, intestinal obstruction, pernicious anemia and diabetes mellitus which, because of their usual mild and evanescent character, Fishberg has designated the "larval nephroses." The nephrosis of the toxemias of pregnancy, with special reference to eclampsia, is also to be ascribed to the effects of endogenous toxic agents although glomerular lesions characterized by swelling and proliferative changes in the endothelial cells of the basement membrane [4,5] may occur primarily and be of as much importance as hydropic, fatty or other degenerative lesions of the tubular epithelium resulting in blockade and renal failure.

Needless to state, the results of *laboratory examinations* vary greatly according to the severity of the nephrosis. In general terms, the urinary and blood chemistry changes are similar to those observed in acute glomerulonephritis except that (1) hematuria is less frequent; (2) albuminuria more marked (especially in the necrotizing nephroses and eclampsia); (3) hypoproteinemia with consequent edema more pronounced; (4) nitrogen retention less severe or entirely absent; (5) reduction in blood calcium more marked and (6) there is a greater tendency to hypocholesterolemia and lipemia. As a general rule, the volume of urine is reduced (oliguria) which in the case of severe mercuric chloride poisoning and eclampsia may amount to an anuria. This is due not only to reduced glomerular filtration but also to the fact that water is apparently absorbed by diffusion through the functionless tubular epithelium because of the high osmotic pressure of the plasma proteins in the blood of the peritubular capillaries. On recovery, however, a polyuria may be present for some time. Renal function tests may show impairment. In the case of the nephrosis of the late toxemia of pregnancy, the dilution or water-function test of Fishberg usually shows a decrease in the excretion of water by the glomeruli because of the characteristic lesion comprising swelling of the glomerular basement membranes.[6] This may also account for the fact that large amounts of albumin may be excreted in the urine in true eclampsia unassociated with nephrosis; coincident hepatic lesions may be a contributing factor.

Lipoid Nephrosis. This is a rare disease of unknown etiology characterized by severe albuminuria with hypoproteinemia and reversal of the albumin-globulin ratio, resulting in anasarca, lipemia and hypercholesterolemia, absence of nitrogen retention, normal blood pressure and the presence of doubly refractile lipoids in the urine identified by the use of the Nicol prism. In 1917 Epstein separated the syndrome from chronic glomerulonephritis and proposed regarding it as a distinct variety of chronic nephrosis.[7] Uremia does not occur and death usually results from some intercurrent infection, especially peritonitis.

The disease is characterized by degeneration of the tubular epithelium with the deposition of cholesterol esters in the cells and interstitial tissues. Cloudy swelling, necrosis and calcification of the tubular epithelium are less characteristic changes. The glomeruli are well filled with blood but otherwise show no alterations except that the walls of the capillaries, for some unknown reason, become hyperpermeable with the escape of large amounts of plasma protein and especially albumin.

By some investigators the tubular changes are regarded as due to an unknown toxin while others believe that they are due to a general metabolic disease in which the nephrosis is merely a part. Many are of the opinion that it is not a separate entity but merely a manifestation of chronic glomerulonephritis; at least, cases have been recorded in which apparent lipoid nephrosis in its terminal stages presented the clinical and laboratory manifestations of the former.[8] Epstein has proposed the hypothesis that the disease is due primarily to a disorder of protein metabolism, accompanied by a subnormal basal metabolic rate, with the excretion of an albumin different from the normal plasma albumin, although this has not been proved. Nevertheless, there appears to be little doubt but that lipoid nephrosis is associated with a marked disturbance of protein metabolism, with the probability that its catabolism is increased. One indication of depletion of body protein is the fact that large amounts of nitrogen are stored over long periods of time on diets containing large amounts of protein.

The characteristic *laboratory findings* are as follows:

1. Oliguria with urine of dark color and high specific gravity but increasing in volume during periods of reduced edema and anasarca. Frank hematuria does not occur, although a slight increase in erythrocytes and leukocytes has been reported in about 50 per cent of cases.

2. Severe albuminuria with the loss of as much as 20 to 30 gm. of protein per day; but because of the frequency of remissions, the degree of albuminuria varies considerably from time to time.

3. The presence in the urine of hyaline, granular and fatty casts along with large amounts of cholesterol and other lipoids. The latter are characterized by being doubly refractile and occur in the tubular epithelial cells. Some regard the presence of cholesterol and other lipoids as largely due to increased glomerular filtration while others believe that they are derived from the tubular epithelium as the result of lipoidal degeneration.

4. A marked reduction of chloride in the urine during periods of anasarca with the elimination of large amounts as it subsides. Large amounts of urea and other nonprotein nitrogenous substances are likewise present, largely due to the fact that they likewise are freely excreted by the hyperpermeable glomeruli with no impairment in the reabsorption of water by the damaged tubules. This hyperpermeability also accounts for glycosuria in about 50 per cent of cases due to a reduction in the renal threshold because the tubules cannot absorb the excess of sugar.

5. Owing to increased glomerular excretion the blood urea nitrogen, total nonprotein nitrogen and creatinine are within normal, although high values are

occasionally observed in patients with marked edema and extreme oliguria, associated perhaps with a high rate of protein catabolism. The plasma chloride is usually within normal but is occasionally subnormal or above normal.

6. Marked hypoproteinemia due almost entirely to a decrease in plasma albumin which may fall from its normal concentration of 4.5 to 5.5 gm. per 100 cc. to 2.0 gm. or less. This, however, is usually associated with an increase of plasma globulin as a compensatory change. As a result of these changes, the albumin:globulin ratio (normally 1.5 to 2.5:1) may be decreased, or, in some cases, actually reversed. The plasma fibrinogen may likewise increase, as a compensatory change from the normal of 200 to 400 mg., to as much as 1000 mg. per 100 cc.

7. Blood cholesterol, fatty acids and phosphatides are so greatly increased that in some cases the plasma is opalescent and at times almost milky in appearance. One striking feature of this lipemia is the increase of cholesterol esters, amounting, in many cases, to from 80 to 90 per cent of the total cholesterol.

8. The total serum calcium is usually reduced but since the reduction is largely due to the loss of the nondiffusible fraction "bound" to the plasma proteins, neuromuscular excitability does not usually occur.

9. There are usually no disturbances in acid-base equilibrium. Acidosis develops only with the onset of renal functional impairment dependent on progression into chronic glomerular nephritis.

10. Renal function is unimpaired over a period of years, especially in children, although the nephrosis is usually complicated e entually by a superimposed glomerulonephritis with renal functional damage. In this connection it is to be remembered, however, that changes in renal function tests may occur in the presence of severe oliguria due to prerenal deviation of water.

11. The basal metabolic rate is frequently subnormal (—20 to —35); this change appears to bear some relation to the degree of hypercholesterolemia.

Amyloid Nephrosis. Amyloid infiltration of the kidneys is usually only part and parcel of widespread amyloidosis in which the liver, spleen and other organs may be involved. Chronic active tuberculosis, especially of the lungs and bones, is responsible for at least 70 to 80 per cent of cases but the amyloidosis may be due to any chronic suppurative disease. As far as the kidneys are concerned, amyloid infiltration has been classified by Fahr as a nephrosis but as a matter of fact, it begins primarily in the glomeruli. Obstruction of the afferent arterioles gradually reduces the flow of blood in the peritubular capillaries, resulting in atrophy of the tubules. As the disease progresses, however, amyloid infiltration of the tubules also occurs with ultimate involvement of the whole organ.

Neither the clinical manifestations nor the laboratory changes are pathognomonic as far as the kidneys are concerned. The Congo red test has proved valuable in the detection of amyloidosis of the viscera in general but is not specific for involvement of the kidneys in particular. Frequently, mild cases are detected only at autopsy. During life the results of laboratory examinations are frequently interpreted as due to chronic glomerulonephritis but the possibility of amyloid infiltration of the kidneys should always be kept in mind in individuals with pulmonary or osseous tuberculosis as well as in those with other chronic suppurative infections.

It is true, however, that both the clinical manifestations and laboratory changes are usually more indicative of a nephrosis than of chronic glomerulonephritis as far as the early stages are concerned. For example, hypertension is infrequent but may occur if the disease is associated with an arteriolar nephrosclerosis. Edema is variable but always likely to be present; sometimes it is severe enough to produce anasarca. Hematuria is generally absent while albuminuria is fairly constant. As shown by Berg,[9] albuminuria is usually due to a high excretion of serum globulin but may be due to serum albumin without being influenced by the albumin-globulin ratio of the plasma; consequently, a determination of the urine protein fraction is of no differential diagnostic value. Fibrinogen may be also excreted.

As in lipoid nephrosis, nitrogen retention is commonly stated to be relatively infrequent but may occur in 10 to 20 per cent or more of cases.[10] Indeed, uremia is not infrequent and is apt to develop as a terminal event within three years.[11] This azotemia is due to a reduction in the glomerular excretion of nitrogenous waste products along with a plugging of the tubules by casts. The late stages of amyloid infiltration of the kidneys, therefore, may closely resemble chronic glomerulonephritis with renal edema.

The usual *laboratory changes* include (1) polyuria of variable degree; (2) albuminuria of varying severity; (3) the absence of hematuria, although this sometimes occurs to a very slight degree; (4) the presence of numerous hyaline, granular and waxy casts; (5) normal or but slightly increased retention of urea nitrogen, creatinine and total nonprotein nitrogen except in the late stages; (6) variable hypoproteinemia; (7) variable hypercholesterolemia and (8) rapid clearance of Congo red from the blood due in part to its absorption by amyloid material in the kidneys and other viscera and in part to excretion in the urine because of its adsorption by the albumin.

Congo Red Test for Amyloidosis. In studies on blood volume Griesbach[12] injected 10 cc. of a 1 per cent aqueous solution of Congo red intravenously without toxic reactions. The dye was found to be evenly distributed in the blood in four minutes. Ten minutes after injection it began to leave the blood but about 90 per cent still remained in circulation at the end of an hour with only traces remaining at the end of twenty-four hours due to excretion by the liver in the bile.

In 1923 Bennhold[13] discovered that when samples of blood were collected in paraffined tubes four and sixty minutes after injections of the dye in this dosage and the plasmas compared colorimetrically, in patients with amyloid disease of the liver the dye disappeared from the blood more rapidly than normally, the percentage remaining at the end of sixty minutes being expressed in terms of the four-minute specimen used as a standard. Using this technic, he found a disappearance of 11 to 29 per cent of the dye from the blood of normal persons in one hour, whereas the disappearance of 60 per cent or more indicated amyloidosis due to adsorption of the dye by amyloid material. This investigator also observed that the dye was excreted in the urine of individuals with albuminuria due to nephrosis and stated that the disappearance of 40 to 60 per cent from the blood in an hour indicated either nephrosis or amyloidosis.

Since then the test has been found extremely valuable in the diagnosis of amyloidosis when rapid disappearance of the dye from the circulating blood plasma occurs within four minutes, amounting to the disappearance of 90 per cent or more within an hour. This is especially true in differential diagnosis between enlargement of the liver and spleen due to amyloid infiltration and other states causing hepatomegaly and splenomegaly. However, the test may fail to detect slight to moderate degrees of amyloidosis so that a negative reaction does not prove the absence of the latter. According to Harmon and Kernwein,[14] the minimum time for the accumulation of sufficient amyloid to give a positive reaction is nine months, with an average of one and one-half to two years. Nor is the test of any value in indicating the distribution of amyloid infiltrations in the different organs of the body. Furthermore, the results of Congo red tests must be interpreted with great caution in the presence of albuminuria with hypoproteinemia. This is because the persistence of the dye in the blood is dependent to a certain extent on a normal concentration of plasma protein, particularly albumin, to which it is adsorbed. Consequently, in the nephrotic syndrome of chronic glomerulonephritis and in nephrosis, characterized by marked albuminuria and hypoproteinemia, the dye tends to disappear rapidly from the blood, as in amyloidosis, a large proportion of it appearing in the urine adsorbed to albumin. The test has been found without value in distinguishing between the stages of chronic glomerulonephritis or nephrosis or between these two states and orthostatic albuminuria in individuals free of amyloid disease.[14]

In conducting the test it is essential to avoid hemolysis, as the presence of hemoglobin in the serum results in erroneous findings. For this reason, Friedman and Auerbach [15] precipitate the serum proteins with ethyl alcohol while Taran and Eckstein [16] employ acetone, the precipitates being removed by centrifuging and colorimetric readings made with the supernatant fluids. The test may be conducted as follows:

1. The patient should be in the postabsorptive state and the test conducted in the morning before breakfast.
2. Inject intravenously 1 cc. of a 1 per cent aqueous solution of Congo red per 10 pounds of body weight.[16]
3. Exactly 4 and again 60 minutes after completing the injection remove 10 cc. amounts of blood into clean, dry test tubes.
4. Send the specimens immediately to the laboratory for the separation of the serums and the completion of the tests.

Harmon and Kernwein [14] have described a quantitative test for determining the rate of removal of the dye from the blood as follows:

1. Remove 10 cc. of blood into a tube carrying 2.5 cc. of a 1.4 per cent solution of sodium oxalate in distilled water. This is used as a source of plasma for the preparation of standards.
2. Inject intravenously 4 mg. of Congo red per kilogram of weight made up in a 1 per cent aqueous solution.
3. At intervals of 4, 9, 14 and 60 minutes thereafter remove 5 cc. samples of blood to tubes carrying 1 cc. of the 1.4 per cent solution of sodium oxalate.

4. Send the specimens immediately to the laboratory for the completion of the tests according to the technic described by these investigators. The results are expressed in terms of micrograms of Congo red per cubic centimeter of plasma.

As far as specimens of blood collected four minutes after the injection of the dye are concerned, the average amount of Congo red in the plasmas of normal individuals was found to be 85.7 micrograms and 29.6 micrograms in cases of amyloid disease and 71.5 micrograms in cases of chronic glomerulonephritis (Fig. 49).

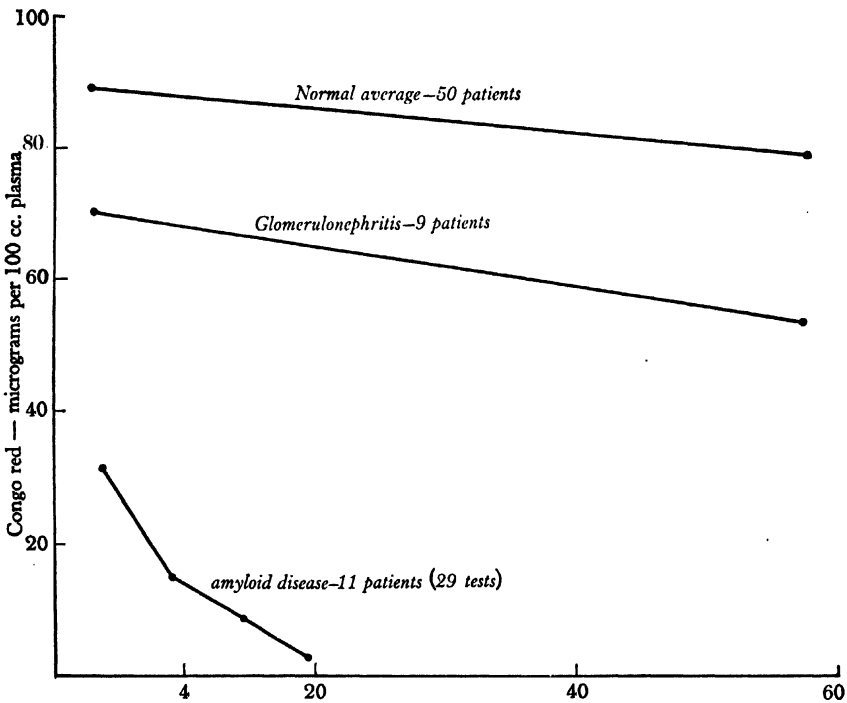

FIG. 49. AVERAGE RATE OF DISAPPEARANCE OF CONGO RED FROM THE BLOOD IN NORMAL PERSONS AND IN PATIENTS WITH GLOMERULONEPHRITIS AND WITH AMYLOID DISEASE

(From Harmon and Kernwein, Arch. Int. Med. *70*:416, 1942)

THE NEPHROSCLEROSES

Nephrosclerosis refers to that type of Bright's disease due primarily to sclerosis of the larger branches of the renal arteries, the arterioles or a combination of both. Consequently, hypertension with cardiac hypertrophy are prominent clinical manifestations. Ischemia of the kidneys due to vascular occlusion may result in an interference with glomerular functions along with degenerative changes in the tubules due to atrophic changes caused by defective circulation in the peritubular capillaries. Furthermore, fibrosis may extend from the sclerotic arteries or arterioles into the substance of the kidneys. For these reasons, the nephroscleroses of arterial or arteriolar origin are generally classified as degenerative types of Bright's disease and the resulting clinical syndrome commonly designated "hypertensive cardiorenal disease."

Arterial Nephrosclerosis. Sclerosis of the arcuate, interlobular or interlobar branches of the renal arteries produces that form of nephrosclerosis known as the *atherosclerotic type,* characterized by the infiltration and deposition of lipid material in the subintima as in the development of atheromata of the aorta or other large arteries. It is usually a part of generalized senile arteriosclerosis but may be particularly localized in the kidneys just as in other individuals it seems to be largely confined to the coronary, cerebral or peripheral arteries. As a result of complete closure of some of these branches the blood supply may be shut off from relatively large areas of the kidney, resulting in infarction.

As a general rule, however, these changes give rise to nothing more than a secondary hypertension and a mild degree of renal insufficiency. The latter seldom progresses to renal failure or uremia. Edema is generally due to failure or decompensation of a hypertrophied heart with death usually the result of a cerebral vascular accident, cardiac failure or an intercurrent infection with special reference to bronchopneumonia.

Under the circumstances, *laboratory changes* incident to renal impairment are usually of mild degree until cardiac failure supervenes in which case they become more pronounced due to passive congestion of the kidneys. In the average case they may be summarized as follows:

1. A gradual tendency to polyuria in which the specific gravity of the urine is reduced and ultimately fixed as 1.010 to 1.012 or lower (isothenuria). Nocturia may be an indication of weakness of the left ventricle, traceable to a delay in the diuretic response to fluids consumed in the latter part of the day.[17] With the onset of passive congestion due to cardiac failure the daily volume of urine is usually reduced.

2. A slight degree of albuminuria is usually present along with hyaline casts. The erythrocytes are not increased unless passive congestion due to cardiac weakness is present.

3. There may be a slight increase of blood urea nitrogen and of the total nonprotein nitrogen but azotemia is not likely to become pronounced until the urea clearance test has been reduced to below 50 per cent of normal or cardiac failure occurs. Albumin loss is so slight that hypoproteinemia does not develop, edema being usually due to cardiac failure.

4. Of the functional kidney tests, that of urea clearance is probably best. This test, as well as those for concentrating ability and phenolsulfonephthalein excretion, are positive in about 50 per cent of patients while positive dilution tests occur in about 25 per cent.[17]

Arteriolar Nephrosclerosis. Sclerosis of the arterioles and especially of those entering the glomeruli is far more serious and constitutes the *arteriolarsclerotic* *type* of the disease. High blood pressure is such a constant and characteristic feature that it has been designated "essential hypertension." As in arterial nephrosclerosis, however, it likewise appears to be secondary to arteriolar changes rather than primary in the production of them. At least, the first stage of the disease is apparently due to a functional vaso-constriction of the arterioles of unknown etiology and without demonstrable structural changes. This produces ischemia of the kidneys and, as shown by Goldblatt and his colleagues,[18] results in the production of "renin" which is activated in the blood to "angiotonin." The latter in turn appears to be capable of initiating a vicious circle since, as recently stated by Simonds,[19] the kidneys produce renin as long as they remain ischemic and may remain ischemic as long as they continue to produce renin.

Sooner or later sclerosis of the arterioles develops, the second stage being characterized by hypertrophy of the muscular coats with hyperplasia of the elastic lamina, while in the third stage the walls of the arterioles are transformed into hyaline tubes with marked occlusion. Consequently, there is a variable response to such depressor drugs as potassium thiocyanate and colloidal sulfur as well as to sympathectomy, in the treatment of the hypertension, with little or no chance of favorable results from either method of therapy in the third stage.

This arteriolarsclerosis is usually confined largely to the kidneys in which case the disease may pursue a prolonged course, constituting the "benign" type. On the other hand, especially in adolescents and young adults, arteriolarsclerosis may be widespread throughout the body, involving not only the kidneys, but the brain, retina and other organs as well. Under these circumstances, the disease may run a fatal course in the matter of a few months and is designated the "malignant" type of arteriolarsclerosis or "malignant hypertension."

As the result of long-continued constriction and sclerosis of the afferent glomerular arterioles, renal function is impaired not only by a reduction of blood supply to the glomeruli but by an early and progressive loss of tubular function as shown by the results of diodrast clearance tests. High blood pressure may maintain an adequate degree of glomerular filtration but as the disease progresses, especially in young individuals, so many glomeruli and tubules are rendered functionally incompetent by ischemia as to result in complete renal insufficiency with azotemia and death due to uremia. In older persons, however, and especially in the benign type of the disease, death more commonly follows cerebral hemorrhage or cardiac failure.

Under the circumstances, the *laboratory changes* are usually more pronounced than in the arterial type of nephrosclerosis. Needless to state, however, they vary in relation to the severity and progress of the disease, being more pronounced in the "malignant" than in the "benign" type of the disease. They may be summarized as follows:

1. Polyuria with the excretion of urine of low and ultimately of fixed specific gravity is the rule in the early stages, with oliguria developing in the later stages if marked azotemia or cardiac failure develops.

2. Moderate to marked albuminuria is usually present along with numerous hyaline and finely granular casts and an increase of leukocytes, especially in the

rapidly progressing "malignant" type of the disease. The erythrocytes are not usually increased unless passive congestion of the kidneys due to cardiac failure is present.

3. Hypoproteinemia, however, is not usually as marked as in chronic glomerulonephritis with renal edema, although it may be sufficiently pronounced to produce edema in the terminal stages.

4. Retention of variable amounts of blood urea nitrogen, total nonprotein nitrogen and creatinine is the rule, especially if urea clearance falls below 50 per cent of normal, and especially in the terminal stages with impending or actual uremia. Azotemia is also increased by passive congestion of the kidneys due to cardiac failure.

5. The urea clearance, concentration and dilution kidney function tests are of value in estimating the functional capacity of the kidneys, especially the urea clearance test, but in the "benign" type of the disease they may be approximately normal for several years.

6. Progressive secondary anemia of the hypochromic normocytic type is usual, especially in the "malignant" type of the disease.

UREMIA

True uremia is the terminal manifestation of renal failure associated with the retention in the blood of urinary waste products. *Pseudo-uremia* or *false uremia* is due to intracranial circulatory disturbances and has been so designated because some of the signs and symptoms resemble those of true uremia. It includes those states, like eclampsia and angioneurotic edema, characterized by epileptiform convulsions, which are due, apparently, to cerebral edema resulting from arteriolar constriction with consequent asphyxia and increased capillary permeability without renal insufficiency. It also includes "hypertensive encephalopathy" characterized by epileptiform convulsions, headache, vertigo, hemianopia, hallucinations, Cheyne-Stokes breathing, coma, etc., sometimes developing in hypertension and due to intracranial vascular disturbances alone without cerebral edema. Of course, "hypertensive encephalopathy" may occur in patients with true uremia but the symptoms are not due to the latter; indeed, according to Folhard, headaches and convulsions are absent in true uremia.

While the latter is associated with the retention in the blood of nitrogenous waste products, it is now well established that none of them (urea, creatinine, uric acid, etc.) are *directly* responsible for it. In fact, the cause is unknown. But, everything considered, it appears to be due to some unknown toxic substance although acidosis and reduced serum calcium (or high inorganic phosphorus) may also play a rôle in the production of some of the manifestations, *e.g.*, dyspnea, muscular twitchings, increased tendon reflexes, etc. In this connection it is to be stated, however, that while acidosis is generally present and often severe, it is not an essential part of uremia, as the latter may be present with a normal acid-base equilibrium and even in the presence of alkalosis.[20] Macquire [21] states, however, that since sodium depletion predisposes to uremia, the blood urea nitrogen and creatinine should be determined on all patients on a sodium-depletion diet, especially elderly individuals and those suspected of having renal disease.

In recent years attention has been directed to the possibility of an accumulation in the blood of putrefactive intestinal products, like the unconjugated or free phenolic compounds (phenol, paracresol, indole, etc.), being the toxic substances when renal insufficiency interferes with their elimination; [22,23] likewise, to the possibility of this toxic substance being guanidine (imido-urea) which is a compound of unknown chemical nature but presumably a product of abnormal protein metabolism due to hepatic insufficiency.[23] At any rate, the retention of nitrogenous waste products due to renal insufficiency appears to reduce the elimination of the toxic substances of intermediary metabolism, including the phenolic compounds and guanidine, with the result that their accumulation in the blood favors diffusion into the tissues of the central nervous system and cerebrospinal fluid with the production of true uremia, although Wallace and his associates [24] state that their retention is of little or no significance in producing the signs of depression of the nervous system. The usual *laboratory changes* may be summarized as follows:

1. Polyuria with urine of low and fixed specific gravity usually precedes uremia in chronic nephritis but during this state itself an oliguria, frequently amounting to an anuria, is usually present. Fluid restriction or the prerenal deviation of water, due to congestive heart failure or excessive vomiting and diarrhea from acidosis, may be contributing factors sometimes resulting in sufficient dehydration to produce a reduction in blood volume. Oliguria and anuria are likewise usually present in uremia due to acute glomerulonephritis or in states producing suppression of the excretion of urine, as in urinary tract obstructions from calculi, prostatic disease, or some other obstructive process.

2. The urine contains variable amounts of albumin and numerous casts along with an increase of erythrocytes and leukocytes.

3. There is a marked increase of the nonprotein nitrogenous constituents of the blood. The urea nitrogen is usually well over 70 mg., the total nonprotein nitrogen 100 to 300 mg. or higher and the creatinine anywhere from 5 to 30 mg. per cc.

4. Urea clearance is usually extremely low, being less than 5 to 10 per cent of normal. Phenolsulfonephthalein excretion may range from 0 to 10 per cent in two hours and is usually 0 in the five-minute specimen in the fractional method.

5. Hyperglycemia with reduced glucose tolerance is not infrequent. Some observers have attributed this to excessive hepatic glycogenolysis due to acidosis, others to poor carbohydrate utilization and still others to impaired glycogen formation.

6. If the plasma protein concentration has been previously low it may return to normal owing not only to dehydration and hemoconcentration but, apparently, in some cases to a reduction in the degree of albuminuria.

7. As a general rule, there is a reduction in plasma chloride largely because of deficient intake, vomiting, or a shift of chloride to the erythrocytes in acidosis. On the other hand, if dehydration is present the chloride may be within normal

and sometimes even above normal in cases of uremia with complete anuria and urinary tract obstruction.

8. Since acidosis is frequently present there is usually an actual base deficit (sodium) and depletion of the alkali reserve with a reduction of the carbon-dioxide capacity of the plasma to a degree seen in diabetic coma. Contributing to acidosis is a failure of the production of ammonia by the kidneys, excessive vomiting with loss of sodium chloride, excessive diarrhea with the loss of large amounts of base, and retention in the body of excessive quantities of anion, including phosphate, sulfate and undefined organic acids.

9. Consequently, there is usually an increase of inorganic phosphorus (phosphate) in the blood amounting to 7 to 15 mg. per 100 cc. but sometimes as high as 40 mg.,[25] with an increase in the cerebrospinal fluid. This increase in inorganic phosphate is regarded by many observers as of greater prognostic value than the blood urea nitrogen.

10. Largely as a result of hyperphospnatemia, some degree of hypocalcemia is also present due to a decrease in the diffusible fraction of serum calcium. Hypoproteinemia may also be a contributing factor owing to the loss of albumin in the urine to which the nondiffusible fraction is absorbed. Consequently, it would appear that neuromuscular irritability in uremia is not peripheral in origin but largely due to a "central" effect of the calcium ion deficit. An increase in serum magnesium has also been reported but normal values are observed in the majority of cases; the same is true in the case of serum potassium.

11. There is frequently a reduction in the plasma cholesterol, with values as low as 50 to 60 mg. per 100 cc. in which anemia, starvation and cachexia may be contributing factors. Hypocholesterolemia is usually accompanied by an increase of the total fat, fatty acid and phospholipid of the plasma.

12. Normally, the xanthoproteic reaction with serum is 0 to 50 but in uremia positive reactions may be observed due to the retention of phenolic compounds (Chapter 3). The normal serum guanidine varies from 0.032 to 0.048 (average 0.040) mg. per 100 cc. but may be likewise increased in true uremia.

RENAL RICKETS

Renal rickets, or renal dwarfism is so designated because it is a disease due to renal insufficiency resulting in a marked disturbance of calcium-phosphorus metabolism with so much demineralization of the bones as to render them soft with marked deformities and dwarfism. It is a rare disease which usually begins early in childhood, but may extend into the second decade of life and, in rare instances, into adult life. It is not improbable that in adults mild cases of the disease due to congenital defects in the kidneys may be responsible for the marked demineralization of the vertebrae and other bones seen in some cases and responsible for severe and chronic backache, pelvic pain, etc.

Although disease of the pituitary or parathyroid glands has been regarded by some observers as the primary cause it is now generally believed that congenital or acquired disease of the kidneys is responsible. Thus, it has been observed in association with agenesia (congenital absence of one kidney); hypogenesia (one kidney congenitally smaller than normal); congenital cystic kidney (polycystic kidney); hydronephrosis secondary to congenital or acquired obstructions of the ureters, neck of the bladder or urethra with or without retrograde infection; the acute and chronic types of nephritis; destruction of the kidney parenchyma by massive deposits of cystine, and other states resulting in renal impairment. Consequently, the disease has many of the features of chronic glomerulonephritis without renal edema although hypertension is rare until the late stages. The prognosis is very poor and especially in the case of children, with death ultimately due to uremia or intercurrent infection.

Renal impairment is usually severe enough to produce marked retention of inorganic phosphate (hyperphosphatemia). This results in an increased output of phosphate in the gastro-intestinal tract, partly in combination with calcium, as well as a deficiency in the absorption of calcium. Consequently, a hypocalcemia usually develops. This, however, may be offset to some extent by increased activity of the parathyroid glands as well as by a state of chronic acidosis (due partly to phosphate retention) which prevails in advanced cases and is regarded as promoting the mobilization of calcium from the bones (demineralization).

As might be expected, the *laboratory findings* are quite variable according to the stage of the disease and especially in early cases. In well-developed cases, however, they may be summarized as follows:

1. Polyuria with urine of a low and fixed specific gravity varying from 1.003 to 1.012. The urine also contains variable amounts of albumin, casts, and in some cases numerous erythrocytes.

2. Increased blood urea nitrogen, total nonprotein nitrogen and creatine over months or years, with rapidly progressing rises in the terminal stages.

3. Slight hypoproteinemia may be present due to albuminuria; this also tends to reduce the serum calcium.

4. Urea clearance, dilution, concentration and phenolsulfonephthalein function tests usually show renal impairment.

5. The inorganic phosphate of the serum, which in children varies normally from 4.0 to 7.0 (average 5.0) mg. per 100 cc., is usually increased and may reach as high as 9 to 16 mg. (hyperphosphatemia).

6. The total serum calcium, which varies normally from 9 to 11 mg. per 100 cc., is usually decreased (hypocalcemia) and may reach as low as 5 mg. when tetany is likely to develop. However, owing to the effects of chronic acidosis in promoting the mobilization of calcium from the bones, the total serum calcium may be within normal limits. The plasma cholesterol may be increased.

7. Serum alkaline phosphatase may be increased above the normal of 4 to 14 Bodansky units in the case of children or above the normal of 1.5 to 4 units in the case of adults.

8. Acidosis is frequent and usually chronic, with a reduction of the carbon dioxide capacity of the plasma from the normal of 55 to 65 volumes per cent to 30 to 40 volumes per cent or less.

Pyelitis and Pyelonephritis. In infections of the kidneys, pyelitis takes front rank from the standpoint of frequency, not only in infants and older children but in adults as well and especially during pregnancy. The term, however, is a misnomer and should be dropped in favor of a designation like "suppurative pyelonephritis," which is what it appears to be in the majority of instances, since the essential lesion is not usually confined to the pelvis except possibly in the earliest stage of infections due to *Esch. coli,* but in truth is likely to be an interstitial suppurative nephritis. The colon bacillus is by all odds the most frequent infecting organism with a special affinity or predilection for the pelvis, but the disease may be caused by *P. vulgaris, H. influenzae, Ps. aeruginosa* and by some of the pyogenic cocci such as the streptococci (especially *Str. faecalis*), staphylococci and pneumococci as well as in very rare instances by the gonococcus and *Actinomyces bovis.*

The mechanism of infection is apparently by way of the blood, as experimental data indicate that the organism first gains access to the lymphatics and finally the blood from the site of initial infection. This is now commonly thought to be the case even when the primary infection is in the bladder or other parts of the lower urinary tract, since ascending infection by way of the ureters is thought to be unlikely. Without doubt, however, obstruction to drainage by congenital or acquired strictures of the ureters, calculi, pressure by tumors, the pregnant uterus and the like are extremely important predisposing factors in the way of lowering local resistance and are always to be carefully looked for in all cases of pyelitis, especially in older children and adults. But apparently hematogenous infection with *Esch. coli, P. vulgaris, Ps. aeruginosa* and *Str. faecalis* may occur from the intestinal tract, especially in infants, and when infection is due to other streptococci, staphylococci, pneumococci, and *H. influenzae,* the primary focus is most likely to be found in the tonsils, teeth, or nasal accessory sinuses.

As previously stated, however, infection is seldom confined to the pelvis of the kidney even in those due to *Esch. coli* and *P. vulgaris.* These as well as staphylococci and streptococci, brought to the kidneys by the blood, may pass through the glomeruli without exciting inflammation only to reach the collecting tubules where they produce abscesses in the medulla as well as pyelitis and constitute pyelonephritis. From these locations the infection gradually spreads upward in a radiating fashion along the lymphatics until wedge-shaped areas are formed which have their apex in the pelvis and their broad base at the cortex, by reason of which they are frequently called "septic infarcts" from their shape, without being real infarcts at all, but with the danger of breaking through the capsule and producing perinephritic abscesses.

Pyonephrosis. In time, however, and especially in chronic infections, the pelvis and calyces may become extremely dilated with thick creamy pus associated with great destruction of the parenchyma, designated *pyonephrosis.* This may arise gradually in the pelvis from obstruction with super-added suppurative infection, or much more likely result from progressive destruction of the kidney in the course of advanced suppurative nephritis. The first part of the ureter shares in the dilatation, and when not occluded, the cystoscope is likely to reveal the very characteristic worm-like ropes of pus uncoiling from the ureteral opening. Finally, the renal tissue may be reduced to only a functionless shell and the kidney to only a bag of pus.

Tuberculosis. Tuberculous infection of the kidneys is always secondary to a primary focus elsewhere in the body, the bacilli being transported by the blood. Only minute lesions may develop which may, nevertheless, show bacilli in the urine. The risks of mistaking *Myco. tuberculosis* for *Myco. smegmatis* in the urine are greatly overemphasized. Apparently, minute lesions may heal by encapsulation with fibrous tissue just as they may elsewhere in the body if the patient is placed under the proper regimen, so that there is no justification for the surgical removal of a kidney simply because tubercle bacilli are found in the urine. But, on the other hand, when the lesions have become of the chronic ulcerative type so commonly called "renal phthisis," it is extremely doubtful if healing ever occurs and surgical removal of the kidney is required, provided the other one is able to carry on, although generally it is likewise infected. The initial focus is usually at the apex of a papilla or at the base of a pyramid, with progressive caseation and cavitation gradually destroying the medulla and finally the cortex, leaving the organ only a multilocular sac filled with thick tuberculous pus.

Syphilitic Nephrosis and Nephritis. Even *T. pallidum* may infect the kidneys, although syphilitic nephritis is comparatively rare. The acute form generally occurs during the secondary stage with such marked edema and large amounts of albumin with doubly refractile lipoid bodies in casts that the clinical picture is one of nephrosis rather than a nephritis. It characteristically responds so promptly to antisyphilitic treatment and especially with arsphenamine that the therapeutic test is of great value. By the same token it rarely comes to necropsy, although *T. pallidum* has been demonstrated in the tubules in a few instances.

Curiously enough, much different lesions are seen in late syphilitics where they are primarily those of glomerulonephritis with secondary changes in the tubules and interstitial tissues often associated with amyloid disease. These cases are likewise comparatively rare and seldom come to necropsy, but here again *T. pallidum* has been demonstrated. Rich has recently described lesions occurring principally in syphilitic Negroes consisting in masses of lymphocytes in the interstitial tissues compressing the tubules in a very characteristic manner with the presence of cholesterol crystals in their lumens which can be seen on the cut surface with the naked eye as glistening flecks. These, too, are regarded as possibly syphilitic in nature although *T. pallidum* has not been found in stained sections.

Laboratory Findings. 1. Bacteriologic examinations of the urine collected by catheterization with all precautions against possible contamination are of primary importance, as discussed in Chapter 15. This is also necessary for determining complete recovery, as residual infection with the presence of pus or other urinary changes may show positive cultures.

2. In acute and severe infections the volume of urine may be reduced, highly colored and of increased specific gravity. In all cases it is apt to be cloudy or frankly purulent due to the presence of pus, epithelial cells and bacteria.

3. Gross hematuria is usually absent but an increase of erythrocytes is usually

detected by microscopic examinations and especially by the counting method of Addis.

4. The urine is always likely to show the presence of variable amounts of albumin owing not only to renal irritation but to the presence of pus and blood. If the former is present in large amounts part of the protein may be due to the presence of nucleoprotein. In general terms, about 100,000 pus cells per 2 cc. of urine may yield as much as 0.1 gm. of protein per 100 cc. of urine. In mild cases of pyelitis, pyelonephritis and tuberculosis, a slight increase of pus is best detected by counting the leukocytes according to the method of Addis. If urine is not collected by catheter, proper precautions are required in interpretation from the standpoint of possible contamination with vaginal or urethral discharges.

5. There are usually variable degrees of increased blood urea and total nonprotein nitrogens. The blood uric acid and inorganic phosphate may be also increased above the normal.

6. Acidosis of variable degree is not infrequent, especially in children, with a reduction in the carbon dioxide combining power of the plasma.

7. The estimation of renal function is always of particular importance from the standpoint of the advisability of surgical intervention and the determination of the operative procedure to be employed. Since adequate elimination can be maintained by two-thirds of one kidney, the presence of marked nitrogen retention indicates extensive renal damage. The same is true in the case of low urea clearance. Whenever possible the functional efficiency of each kidney should be determined by the phenolsulfonephthalein, indigo carmine or other test by urethral catheterization or by direct inspection through the cystoscope. As far as determinations of blood urea nitrogen and total nonprotein nitrogen are concerned, only little information in relation to surgical risk is to be obtained by single examinations. In destructive renal lesions it is the persistence rather than the degree of nitrogen retention to which attention should be directed and by which the extent of renal damage should be estimated. In other words, the persistence of high nitrogen retention in spite of appropriate therapeutic measures is of definitely grave prognostic import. Because such individuals are poor operative risks, repeated preoperative estimations of the blood urea nitrogen, total nonprotein nitrogen, creatinine and urea clearance are of value in determining the time at which surgical operations may be attempted with a minimum risk to the patient.

POLYCYSTIC KIDNEY AND TUMORS

Polycystic or congenital cystic kidney is due to a wholesale failure of the metanephric and cloacal embryonic elements to fuse. In at least 95 to 98 per cent of cases both kidneys are affected. Apparently heredity may play a rôle in etiology. The kidneys may become sufficiently enlarged during intrauterine life to cause dystocia. Otherwise the disease may escape detection until early childhood and if a sufficient number of functional nephrons are present it may not be detected until adult age is reached. Some cases are latent and symptomless and discovered only at autopsy. Others develop the signs and symptoms of chronic glomerular nephritis, especially between 40 to 60 years of age. Still others are symptomless except for gross or microscopic hematuria with or without pain. Some are discovered only by finding enlarged kidneys in the course of physical examinations or on the development of such complications as acute or chronic pyelonephritis, perinephric abscess, urolithiasis, ptosis, etc. There are no pathognomic *laboratory changes* but any of the following may be observed:

1. A tendency to polyuria and especially nocturia. The urine is usually of low and fixed specific gravity.
2. Hematuria is present in most cases and tends to be intermittent. Gross bleeding occurs in about 10 to 20 per cent of cases and is sometimes fatal; minor bleeding detected only by microscopic examinations occurs in about 40 to 50 per cent. In cases complicated by the presence of acute or chronic pyelonephritis variable amounts of pus are found in the urine.
3. Variable degrees of albuminuria are common. In adult cases resembling chronic glomerulonephritis large amounts of albumin along with casts are found.
4. There is usually some retention of urea nitrogen and total nonprotein nitrogen. As renal impairment increases, retention of both nitrogen and creatinine becomes more marked. True uremia is a frequent terminal event.
5. Owing to renal impairment, chronic acidosis with a reduction of the carbon dioxide capacity of the plasma may occur.
6. Low urea clearance and reduction in the excretion of phenolsulfonephthalein as well as failure in the concentration of urine are commonly observed.

Tumors of the Kidney. Tumors of the kidney are rarely bilateral. Those occurring in the pelvis are commonly papillomas and squamous cell carcinomas. Cortical tumors or those involving the parenchyma may be nonmalignant (fibroma, adenoma, myoma, lipoma and hemanginoma) or malignant (malignant nephroma or hypernephroma, papillary adenocarcinoma, alveolar adenocarcinoma, malignant papillary cystadenoma and Wilms' tumor or adenosarcoma). The only characteristic *laboratory change* is hematuria which occurs sooner or later in about 60 per cent of all cases. The bleeding is usually intermittent in character and highly variable in both severity and duration. Severe bleeding is detected by dark-reddish or "smoky" urine sometimes showing the presence of coagula. Minor bleeding is detected only by microscopic examinations. Of course, hematuria is such a common manifestation of diseases of the kidneys that it is not diagnostic of tumors. Tumors of the pelvis are more apt to show hematuria than those of the parenchyma and malignant tumors much more likely than nonmalignant ones. The presence of blood may produce albuminuria. Blood casts may be found in cortical tumors with hematuria. Impairment of renal function largely depends upon the location and nature of the tumor. Cortical tumors, and especially those which are malignant, may destroy sufficient amounts of kidney to produce nitrogen retention and low urea clearance with defective excretion of phenolsulfonephthalein.

UROLITHIASIS

Urolithiasis refers to the presence of calculi in the kidneys (nephrolithiasis), ureters (ureterolithiasis), bladder (vesicolithiasis) or urethra (urethrolithiasis).

To these may be added calculi in the prostate gland (prostatolithiasis) derived from the urethra or formed in the substance of the gland by the deposition of calcareous material on the corpora amylacea. However, these are not usually included in the category of the urolithiases and for this reason are not discussed herewith. Urolithiasis also includes uroliths or deposits of crystals of the sulfonamide compounds in the uriniferous tubules sometimes occurring during sulfonamide therapy with special reference to sulfapyridine and sulfathiazole.

Incidence. Calculi in the kidney constitute about 50 per cent, in the bladder about 31 per cent, in the ureters about 16 per cent and in the urethra about 3 per cent of all cases of urolithiasis.[26] In nephrolithiasis, calculi occur in both kidneys simultaneously in about 20 per cent of cases. Nephrolithiasis occurs with about equal frequency in men and women; ureterolithiasis, however, occurs about twice as frequently in males as females while the incidence of vesicolithiasis is almost twelve times higher in men than in women because of the ease with which calculi formed in the kidneys are voided and the rarity of urethral obstruction in the latter. Urolithiasis may occur at any age but is uncommon in children. For example, as far as bladder calculi are concerned, the incidence is less than 1 per cent; among adults about 60 to 70 per cent of cases occur in individuals over 50 years of age. The incidence of urolithiasis is conspicuously low among Negroes; nephrolithiasis occurs nearly four times as frequently among whites. In nephrolithiasis most calculi occur free in the renal pelvis followed in frequency by calculi in a single or minor calyx, and coral or branching calculi in the pelvis and its calyces; calculi in the parenchyma are very rare. Coincident infection is not uncommon, especially in vesicolithiasis, and various other complications may arise as, for example, replacement lipomatosis in nephrolithiasis with infection, expulsion of calculi into the perirenal tissues or peritoneal cavity with peritonitis, perforation of a large renal artery or vein, nephrolithiasis with neoplasms, etc.

Chemical Composition and Radiopacity. Urinary tract calculi vary greatly in their chemical composition and radiopacity. From the chemical standpoint they may be comparatively pure, especially when formed in the kidney, but mixtures of various substances are quite common. The great majority are composed alone or of combinations of uric acid and urates (sodium, ammonium), calcium oxalate, calcium carbonate or the phosphates of calcium, ammonium or magnesium. Calculi composed of cystine or xanthine are rare. From the standpoint of radiopacity those largely composed of calcium carbonate are most opaque followed in order by those largely composed of calcium oxalate, calcium phosphate, ammonium and magnesium phosphate, cystine, urates, uric acid and xanthine.

The majority of calculi found in the kidney at operation are composed either of oxalates or phosphates, the latter being more frequent if infection is present. Since calculi composed of uric acid and urates are usually smooth and likely to be passed at intervals as small stones, or gravel, they are found much less frequently. Ureteral stones, which occur most frequently in the pelvic portion of the ureter, have the same chemical composition as renal calculi. Bladder stones are usually composed of uric acid and urates unless formed during cystitis due to infection when they are more likely to be composed of phosphates. Since calculi impacted in the urethra are derived from the bladder or kidney their chemical composition is in relation to their source.

The chemical analysis of calculi is always advisable whenever possible, as the results may yield data of value in relation to both etiology and treatment since dietary and metabolic factors may be of importance in at least some cases.[27,28] For example, calcium oxalate stones may reveal the presence of hyperoxaluria due to the excessive ingestion of foods rich in the oxalates (spinach, rhubarb, grapes, etc.); phosphatic stones may be due not only to infection but in part to the use of foods rich in calcium and phosphates in which an acid-ash diet along with the administration of ammonium chloride may be helpful in treatment; uric acid and urate calculi suggest the advisability of a diet low in the purines along with the administration of alkalies sufficient for rendering the urine alkaline for the conversion of uric acid into the more soluble urates; cystine stones may reveal the presence of cystinuria, due to an inborn error of protein metabolism characterized by failure to utilize cystine derived from the amino acid methionine, in which a low protein diet along with the administration of alkalies may be helpful, etc.

Etiology. However, in spite of a great deal of clinical and experimental investigation our knowledge of the morphogenesis of kidney and bladder stones is quite incomplete. Obviously they are due to the precipitation of crystalloids ordinarily held in solution in the urine with the assumption that they are present in the latter in excess of their solubility in water. But if this is true, the mechanisms by which they are kept in solution in the urine and especially the factors which promote their precipitation in the urinary tract are the questions at issue.

Undoubtedly, the reaction or *p*H of the urine may be an important factor although at the normal *p*H of 5.0 to 7.0 (mean 6.0) the concentration of most of the crystalloids is not greatly in excess of saturation; indeed, with respect to some of them (the phosphates of magnesium and ammonium and calcium carbonate) the urine is definitely undersaturated.[29] But within a *p*H range of 7.0 to 8.0 urine is very apt to become supersaturated with tertiary calcium phosphate with an enhanced tendency to precipitation; similar considerations are applicable to secondary calcium phosphate and calcium carbonate. Under these conditions, the crystalloids may precipitate out and form calculi in the absence of inflammation, provided there is stasis of the urine over a prolonged period of time.

One theory, widely held, is that the colloidal constituents of the urine increase the solubility of the crystalloids and that their dispersion results in a precipitation of the latter. According to Schade,[30] pure calculi composed of uric acid may be formed in the absence of inflammation when the protective or stabilizing action of the colloids is reduced by changes in the *p*H of the urine. Indeed, in his opinion, "stones" may be formed of pure albumin or fibrin through irreversible precipitation of some hydrophilic colloid of the urine, while a colloid concretion of this kind may become the matrix on which crystalloids are precipitated. Be this as it may, it would appear that most calculi are composed of successive deposits of crystals at right angles on a nucleus or matrix which may be a colloid concretion composed of albumin, fibrin, or amyloid material, or one composed of mucin, epithelial cells, pus cells or clumps of bacteria, with mucin or other organic substances acting as binding substances as well.

In the presence of normal amounts of crystalloids in the urine it would appear, therefore, that calculi may be formed in the kidneys or bladder if factors like obstruction or infection are present which may not only alter the *p*H of the urine with their precipitation but furnish a nucleus or matrix of mucin, cells or other foreign bodies of any sort on which crystals may be deposited. Undoubtedly, states producing a supersaturation of the urine with crystalloids are much more likely to result in their precipitation and the production of calculi not only by changes in the *p*H of the urine, but because excessive crystalluria may produce irritation with the excessive production of mucin, epithelial cells and leukocytes as matrices. Under these conditions, dietary errors and metabolic disturbances, as in gout, may readily become important etiologic factors along with the possibility that the incidence of urolithiasis may be increased by the habitual use of drinking waters containing large amounts of calcium carbonate or phosphate.

Furthermore, as far as diet is concerned, there is both clinical and experimental evidence [27] suggesting that a deficiency in vitamin A may be an etiologic factor by promoting the desquamation of keratinized epithelium from the mucosa of the urinary tract which may provide nuclei about which crystals are deposited. An excess of vitamin D may also be a factor. At least, kidney and bladder stones composed largely of calcium phosphate have been produced experimentally in rats by the administration of irradiated ergosterol in excess. It has also been suggested that the particular prevalence of nephrolithiasis in the tropics may be due to overirradiation with ultraviolet light.

Consequently, since so many calculi are composed of the salts of calcium and phosphorus, urologists have likewise become greatly interested in the possible relationship of hyperparathyroidism to calciuria and phosphaturia and to urolithiasis.[31,32] Griffin and his colleagues, however, have reported that hyperparathyroidism was found to be an etiologic factor in less than 0.2 per cent of 1,206 cases of urinary lithiasis.[33] However, parathyroid overactivity is not the only endocrine disturbance causing calciuria and phosphaturia. Atrophy of disuse, Cushing's syndrome and hyperthyroidism are thought by some observers to be among other endocrine disorders that may be etiologic factors. As previously stated, calculi composed of cystine are apparently due to a disturbance of the intermediate metabolism of proteins while those composed of xanthine are apparently due to disorders of the cholesterol metabolism of the reticulo-endothelial cells with or without changes in the cholesterol of the blood.

Laboratory Changes. There are no characteristic laboratory changes in urolithiasis. Urinary changes, however, are usually of helpful diagnostic value along with clinical manifestations and x-ray examinations, particularly if calculi are producing attacks of pain with ulceration of the urinary tract (urelcosis). Under these conditions any or all of the following may be observed:

1. Oliguria or anuria in case of obstruction followed by temporary polyuria on dislodgment of the calculus.

2. The presence of gross or microscopic hematuria along with albuminuria, blood casts and excessive numbers of leukocytes and epithelial cells. In case of gross bleeding blood clots may be found in the urine. The reaction of the urine

may be excessively acid or alkaline. Excessive amounts of crystals are usual, the varieties depending on the reaction of the urine. Pyuria is usual in cases complicated by pyelonephritis or cystitis. Under these circumstances, bacteriologic examinations of urine collected aseptically by catheterization are advisable, including examinations for the urea-splitting bacteria.

3. Blood chemistry determinations are of limited value as far as urolithiasis is concerned but always advisable in case renal impairment is suspected and especially for estimating preoperative risks in cases of chronic nephritis, long-standing obstruction or coincident infection including tuberculous nephritis. Blood calcium, phosphorus and phosphatase are stated to show no changes characteristic of urolithiasis.[33] Slight reductions in blood phosphorus are not unusual but the total serum calcium is apt to be constant and within normal. It is stated that patients with high serum calcium or phosphatase should undergo a thorough investigation for some other coincident disorder.[33]

4. Urea clearance, phenolsulfonephthalein excretion and other tests for renal impairment are apt to give approximately normal results unless the kidney or kidneys have been greatly damaged by coincident infection or hydronephrosis from obstruction.

5. As previously stated, a chemical analysis of calculi is always advisable for at least a determination of their chief constituents.

HYDRONEPHROSIS AND PYONEPHROSIS

If there is interference with the passage of urine, whether obstructive or nonobstructive, the resultant retention produces dilatation of the renal pelvis (pyelectasis) and its calyces (calyectasis) with possible destruction of the renal parenchyma; this constitutes *hydronephrosis*. The normal capacity of the pelvis is 7 to 10 cc. or even 15 cc. The urine which the kidney continues to excrete accumulates within the dilated pelvis with gradual flattening of the calyces; unless relieved, dilatation may proceed until nothing remains but a membranous sac in which kidney substance may not be found even microscopically. Hydronephrosis, therefore, may be slight, moderate or severe. It may be unilateral or bilateral depending on the nature and location of the obstruction. The retained urine may be sterile or show the presence of micro-organisms due to coincident infection. with the production of *pyelonephrosis*. The latter differs from hydronephrosis in the fact that there is necrosis or destruction of the parenchyma and the formation of pus pockets, while in hydronephrosis there is atrophy and degeneration of the renal tissue due to pressure and interference with nutrition with resultant fibrous replacement and scarring of the persistent stroma. Consequently, in a severe pyonephrosis there is but slight chance of a return of function because of destruction of the parenchyma, while a return of function is possible in hydronephrosis if the various obstructive and nonobstructive causes are removed before marked atrophy of the parenchyma has occurred. When obstruction is sudden the excretion of urine may stop with correspondingly less hydronephrosis than when obstruction comes on slowly and progressively. In addition to infection, hydronephrosis may be complicated by hemorrhage as well as by spontaneous or traumatic rupture.

Hydronephrosis may be due to congenital obstructive lesions in the penis and urethra or obstruction of the bladder due to enlargement of the prostate gland or other causes; in such instances dilatation of the bladder occurs with bilateral hydronephrosis. More frequently it is due to obstruction of a ureter (ureterectasis) or a renal pelvis (pyelectasis) in which case it is unilateral. Ureterectasis and pyelectasis may also develop, secondarily, as the result of retention of urine in the bladder due to dysfunction of the autonomic nervous system. It may also occur primarily as the result of similar disturbances, commonly of congenital origin, in the innervation of the ureter and renal pelvis. The ureterectasis and pyelectasis observed during pregnancy and the early postpartum period is now considered to be due to the action of toxic substances on the neuromuscular mechanism of the ureters.

Laboratory examinations are of value not only as aids in the diagnosis of the obstructive uropathies but especially for the estimation of renal function in relation to surgical procedures and postoperative management. In the majority of cases the problem usually consists in determining the extent to which existing renal functional impairment is the immediate result of obstruction and how much of it may have existed prior thereto. This is particularly true in cases of prostatic enlargement, since these fall within an age group where chronic nephritis is also to be expected. Blood chemistry determinations indicate that uncomplicated chronic hydronephrosis is often accompanied by no significant degree of nonprotein nitrogen retention. Certainly, single determinations are apt to be of but little clinical value, since it is not so much the degree of retention of urea nitrogen, creatinine, etc., that counts as its persistence. But because individuals with marked nonprotein nitrogen retention are poor operative risks, repeated preoperative estimations are of value in determining the time at which radical surgical measures may be attempted with a minimum of risk to the patient. As long as some urine is voided, the results of urea clearance and the excretion of phenolsulfonephthalein and indigo carmine or other renal function tests are apt to show good function in the absence of pre-existing renal impairment. However, cases occur in which back pressure from obstruction in a ureter or renal pelvis checks glomerular filtration almost completely, not only on the affected side but in the opposite kidney as well, which is known as "reflex anuria." Under such circumstances, remarkable grades of nonprotein nitrogen retention may be observed.

Some observers believe that in the obstructive uropathies the blood creatinine may remain within normal in the presence of extremely high urea nitrogen retention, in contradistinction to the findings in chronic nephritis.[34] But this is not generally the case. In fact, the reverse occurs more commonly, *i.e.*, with equal grades of urea nitrogen retention, blood creatinine is retained in higher degree in urinary obstruction than in nephritis which is exactly what would be expected in view of the purely mechanical nature of an obstruction.[35] As is well known, relief of obstruction is followed by a rapid decline of nonprotein nitrogen retention which may reach normal within a period of several days.

In hydronephrosis, therefore, the *laboratory changes* are quite variable but in general terms may be summarized as follows:

1. The volume of urine is reduced, especially in bilateral hydronephrosis. Relief of obstruction is followed by temporary polyuria. In "reflex" anuria the urine is very scanty, frequently amounting to a complete anuria. Hematuria occurs in about 10 per cent of cases. Casts and an increase of leukocytes are not unusual, along with traces of albumin.

2. In pyelonephrosis the urine is cloudy due to the presence of pus and bacteria. Albuminuria and hematuria are more pronounced.

3. In unilateral hydronephrosis and pyelonephrosis the blood urea nitrogen, total nonprotein nitrogen and creatinine may be within normal or but slightly increased unless complicated by a pre-existing chronic nephritis when all three are usually increased. Nitrogen retention is likewise more pronounced in bilateral hydronephrosis, especially in the presence of complete anuria.

4. There is usually an increase of blood uric acid and hypophosphatemia due to renal insufficiency is not unusual.

5. Serum sulfate may be increased also and reach 10 or even 15 mg. per 100 cc. due to renal insufficiency. The total blood cholesterol, cholesterol esters, fatty acids and phospholipids are frequently reduced below normal and especially in cases of prostatic hypertrophy.

6. The excretion of phenolsulfonephthalein and indigo carmine is reduced on the hydronephrotic side.

7. Urea clearance tests are probably best for estimating the degree of renal impairment.

CYSTITIS

Aside from rare or unusual cases of accidental or deliberate injection of irritating chemical agents into the bladder, and of thermal or electrical injuries, both acute and chronic cystitis are usually due to infection. The normal bladder, however, has a high degree of natural resistance to bacterial infections so that various predisposing factors are usually present which may embrace any of the following: (1) the presence of blood clots, calculi, foreign bodies, tumors or diverticula; (2) obstruction of the vesical neck, trigone or urethra; (3) pregnancy, constipation, exposure to cold, etc.; (4) residual urine in women following labor or after pelvic operations and (5) other conditions producing residual urine such as cystocele or atony of the bladder (due to neurogenic dysfunction, atherosclerotic changes or congenital defects). Under any of these conditions, infection may result from (1) the introduction of contaminated instruments (catheters, sounds, cystoscopes, foreign bodies); (2) by direct extension from infection of the posterior urethra; (3) by descending infection from the kidney and ureter; (4) by way of the blood (hematogenous route); (5) by way of the lymphatics although this is rare; (6) by way of fistulas between the bladder and adjacent viscera and (7) by reflex action due to disturbances of the detrusor muscle.

As previously discussed in Chapter 15, infection is commonly due to any of the following micro-organisms alone or in combination: colon bacilli, staphylococci, *M. catarrhalis*, hemolytic streptococci, *Str. faecalis*, *P. vulgaris*, *Ps. aeruginosa* or *Myco. tuberculosis*. *A. aerogenes*, *M. tetragenus* and urea-splitting organisms are usually secondary invaders. Infections with gonococci are rare except of the trigone in the case of women while those due to pneumococci are uncommon. Tuberculous cystitis is always secondary to tuberculosis of the kidney. Cystitis may be due also to irritation and ulceration by the ova of *Schistosoma haematobium* (vesical schistosomiasis) which is usually accompanied by secondary bacterial infection. Needless to state, exact bacteriologic examinations are always desirable and especially from the standpoint of the choice of a chemotherapeutic compound in treatment. These, however, are best conducted by examinations of urine collected by catheterization in order to avoid contamination. Otherwise examinations of *smears of freshly voided urine* may give information of value; voided urine may be used also in examinations for tubercle bacilli as well as for the ova of *S. haematobium.*

Cystitis may be acute or chronic. Special forms of the latter include (1) the so-called "elusive ulcer" of Hunner, which is now designated as panmural ulcerative cystitis (submucous or interstitial cystitis); (2) cystitis cystica (cystitis emphysematosa) which is of uncertain etiology and characterized by marked thickening of the mucosa with numerous small cystic cavities containing fluid and gas and (3) chronic trigonal cystitis which is particularly frequent in women and usually associated with a urethritis owing to the shortness of the urethra and its proximity to the vagina. The usual *laboratory changes* in cystitis may be summarized as follows:

1. Cloudy urine, including the second glass specimen, due to the presence of pus and bacteria. Cloudiness may be due to bacteria alone in the absence of frank pyuria. Bacteria may also be present in urine which is quite clear so that bacteriologic examinations of urine collected by catheterization are always required as final criteria of complete recovery. Needless to state, proper care is required for the exclusion of pus of extravesicular origin with special reference to vaginal or urethral discharges, prostatic secretions, etc.
2. Freshly voided urine is usually alkaline. A careful determination of the reaction with special reference to the *p*H or hydrogen-ion concentration is commonly of clinical importance in relation to chemotherapeutic treatment. Of course, such determinations should always be conducted with *fresh* urine.
3. Albuminuria of variable degree largely due to the presence of albumin and nucleoproteins derived from inflammatory exudates.
4. The urine also contains an excess of mucus; likewise variable amounts of blood. Hematuria may be so slight as to be detected only by microscopic examinations but some cases of acute cystitis are characterized by severe hematuria with changes in the gross appearance of the urine. Severe hematuria is characteristic of urinary schistosomiasis.

TUMORS OF THE BLADDER

Tumors of the bladder are similar to those which occur in the pelvis of the kidney. About 95 per cent are due to (1) benign papillomas, (2) malignant papillomas, or (3) transitional cell carcinomas. Less common types of malignant tumors comprise adenocarcinomas, colloid carcinomas and sarcomas; less common nonmalignant tumors include myxomas, fibromas, fibromyomas, angiomas, rhabdomyomas, chondromas, dermoids and teratomas.

From the laboratory standpoint *hematuria* is the most frequent finding. Not infrequently it is the first and only sign over long periods of time. It occurs in about 75 per cent of cases. It may be very profuse or very slight. The blood usually appears fresher and not so intimately mixed with the urine, as in bleeding from kidney tumors. In some cases it disappears spontaneously and may never recur, or only after a variable interval, the urine becoming clear and remaining so until the next attack of bleeding. This type of case frequently lulls the physician into a false sense of security with postponement of a thorough examination for the cause. Even in children the first symptoms are, likewise, always likely to be hematuria and frequency of urination. The degree of bleeding, however, is no criterion of the size or nature of the tumor, in both adults and children.

A second laboratory examination of great diagnostic value is a skilful microscopic examination of sections of the tumor tissue removed by biopsy through the cystoscope (Chapter 21).

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24

THE VENEREAL DISEASES

The diseases commonly transmitted by sexual contact comprise gonorrhea, syphilis, chancroid and lymphogranuloma venereum. For this reason they have been designated the venereal diseases. Granuloma inguinale is frequently included in this group as are, likewise, erosive and gangrenous balanitis (venereal fusospirochetosis) although they are not always transmitted by sexual exposure.

Importance of Laboratory Examinations. Undoubtedly, laboratory examinations are of great value in the diagnosis and differential diagnosis of these diseases of both the male and female genitalia. Indeed, it is to be regretted that so many physicians still rely too much upon the history and clinical manifestations which are admittedly unreliable in all but the most typical cases. Not infrequently this is not only disastrous for the patient, especially in the case of syphilis, but leads to the possible infection of others as well as to time-consuming and costly erroneous treatment.

For example, without the aid of bacteriologic examinations chancroid with phimosis, intra-urethral chancre, prostatorrhea and spermatorrhea, urethrorrhea and nongonococcal meatitis in the male as well as nongonococcal bartholinitis and abscess, vaginitis (including trichomonad vaginitis) and cervicitis in the female may be mistaken for gonorrheal infections. Likewise, chancroid may be mistaken for chancre, herpes genitalis, lymphogranuloma venereum, granuloma inguinale, simple ulcers or erosive and gangrenous balanitis due to fusospirochetal infection, not to mention the chances of mistaking any of these for syphilis. Furthermore, bacteriologic examinations, supplemented by complement fixation and skin tests, have proved of great value in the diagnosis and differential diagnosis of syphilis, chancroid, lymphogranuloma venereum and granuloma inguinale, as discussed in Chapters 15, 17 and 19.

GONORRHEA IN THE MALE

Acute gonorrhea of the male may be limited to the anterior urethra and chronic gonorrhea to the posterior urethra, prostate gland and seminal vesicles but the great majority of cases show anterior and posterior urethritis with some involvement of the prostate gland and seminal vesicles, while in some cases the entire seminal tract from the meatus to the epididymis is infected. In other words, unless acute anterior urethritis is promptly and adequately treated with penicillin or the sulfonamide compounds, of which sulfadiazine is the drug of choice, subacute or chronic urethritis is almost inevitable, with the production of some degree of prostatitis and seminal vesiculitis along with possible additional complications due to metastatic infection like epididymitis, periurethritis, periurethral abscess, septicemia, infections of the heart (acute endocarditis, myocarditis or pericarditis), acute or chronic arthritis, osteoperiostitis, myositis, bursitis, tenosynovitis, thrombophlebitis, peripheral neuritis, etc.

Mixed infections with micro-organisms like staphylococci, streptococci, *Esch. coli,* diphtheroid bacilli, etc., which are so likely to be present in the first two inches of the urethra and activated by the gonococcus, are not infrequent and, indeed, mixed infections are the rule in chronic urethritis with its associated prostatitis and seminal vesiculitis.

One attack of gonorrhea predisposes to subsequent attacks although they are usually less severe because the epithelium of the mucosa is more resistant plus the fact that a slight degree of acquired immunity may afford additional protection. Exacerbations of chronic gonorrhea (erroneously designated as recurrences), due to a flare-up of latent or residual infection in the seminal vesicles, are not uncommon; they usually occur within the first year after infection and are not infrequently mistaken for new attacks of gonorrhea.

As previously stated, gonorrhea of the male may be mistaken clinically for staphylococcal meatitis, chancroid with phimosis, intra-urethral or meatal chancres, urethrorrhea, prostatorrhea or spermatorrhea. For this reason, *laboratory examinations* are always advisable for diagnostic purposes and their clinical value may be summarized as follows:

1. Bacteriologic examinations of properly prepared and properly stained smears of the urethral discharge are of paramount importance; they may suffice for diagnostic purposes and especially in acute gonorrhea (page 396). In chronic gonorrhea it may be necessary to massage the prostate gland and seminal vesicles for the purpose of securing discharge from the penis or in the urine. In the case of the latter, the specimen should be centrifuged immediately and smears prepared of the sediment, including shreds. The Gram method of staining should always be included as, otherwise, staphylococci may be readily mistaken for gonococci. Cultures may be required and especially in chronic infections; they are essential as criteria of cure, as negative smears alone are not acceptable in this connection. In chronic infections, however, gonococci may not be found in either smears or cultures although micro-organisms of secondary infection are invariably present.

2. Intra-urethral and even meatal chancres with rather thin, watery discharges are frequently mistaken clinically for hyperacute gonorrhea. When suspected, darkfield examinations of the discharges for *T. pallidum* are required. Due care must be exercised against mistaking *T. refringens* for *T. pallidum.*

3. Macroscopic examinations of the urine by the two- or three-glass methods are usually of very helpful diagnostic value. The patient is required to retain his urine for at least two hours. In the two-glass test of Thompson he is then requested to pass at least six ounces of urine in the first glass and the balance in the second. In the presence of anterior urethritis, only the urine of the first glass will show cloudiness due to pus or shreds while the second will be clear. In posterior urethritis the urine in both glasses will be cloudy because pus in the posterior urethra gravitates toward the bladder as the result of the contraction of the external

sphincter overcoming that of the weaker internal sphincter. In mild posterior urethritis and chronic prostatovesiculitis, however, very little pus may accumulate in the prostatic and bulbous portions of the urethra, in which case the urine of the first glass may be cloudy but that of the second clear.

In the three-glass test of Posner the patient urinates in two glasses, as described, but retains some urine in the bladder. After massage of the prostate gland and seminal vesicles he passes the remaining urine in a third glass. This third specimen is usually somewhat opalescent or cloudy due to pus or characteristic shreds; the latter should be promptly examined bacteriologically before disintegration occurs. Cloudy urine, however, may be due to phosphaturia or oxaluria as well as to the presence of large numbers of spermatozoa. Normally some pale, small gelatinous crystals may be massaged out of the prostate gland and the secretions of the vesicles may appear as mucus and spermatozoa. Needless to state, variable degrees of albuminuria due to the inflammatory exudates are observed and the urine may show not only a great increase of leukocytes (pus cells) but an increase of erythrocytes as well.

4. The gonococcus complement fixation test is without value in the diagnosis of acute gonorrhea because positive reactions do not ordinarily occur until after the elapse of four to six weeks, but when the test is properly conducted, with special reference to the antigen, *positive* reactions possess diagnostic value. Negative reactions, however, do not exclude the possible presence of chronic gonorrhea.

5. The criteria of cure should include not only (a) complete absence of all symptoms and discharge but likewise (b) the complete absence of pus and shreds after repeated thorough massages of the prostate gland and seminal vesicles; (c) ten days to two weeks after apparent cure the sound test should be conducted not only for the purpose of detecting a stricture, if present, but for the expression of pus and gonococci from the urethral glands if complete recovery has not occurred; (d) sometimes it is advisable to inject an irritant intra-urethrally, like 0.5 to 1 per cent solutions of silver nitrate, to be followed by bacteriologic examinations of the discharge for gonococci. Likewise if the patient will indulge in beer or whisky, which are irritants to the genito-urinary tract, gonococci if present are likely to reappear in the discharge in the case of incomplete recovery; coitus by condom has the same effect. (e) Microscopic examinations of semen for gonococci are extremely important, as the organisms and pus in the prostate gland and seminal vesicles can very often be brought forth in no other way; (f) the complement fixation test is also of value because, if *positive* reactions continue after two or three months following apparent recovery, they are indicative of chronic persistent infection in the prostate gland and especially in the seminal vesicles. In this connection it is to be emphasized, however, that positive reactions do not have this significance in case gonococcus vaccine has been used in treatment, as the complement-fixing antibody produced in this manner may persist for six months to a year or longer; (g) finally, due emphasis should be placed on the value of negative cultures of pus and shreds for gonococci since, when properly conducted, they possess more value as criteria of cure than examinations of smears alone.

GONORRHEA IN THE FEMALE

Gonorrhea in women is usually far more serious than in men, because of the complexity and relationships of the female genito-urinary structures. It may be acute, subacute or chronic from the onset but is especially characterized by its chronic complications.

Acute and subacute gonorrheas of women are usually due to infections transmitted by males with acute gonorrheas while those which are chronic from the onset are usually due to transmission by males with chronic or so-called latent gonorrheas. Acute and subacute gonorrheas involve not only the urethra and endocervix but usually the glands of Bartholin and Skene and frequently the fallopian tubes as well, the latter prolonging the disease and usually accompanied by a pelvic peritonitis.

In chronic gonorrhea of the woman frank urethritis may be absent but the glands of Skene in the urethra are usually infected as well as the Bartholin glands (bartholinitis) and especially the endocervix (endocervicitis). When chronic from the onset, as is so likely to be the case when infection is due to sexual contact with a male with chronic gonorrhea, the woman may not suspect any trouble for about ten days or more after exposure, when she discovers a yellow vaginal discharge which may be the only manifestation of the disease at that time. Gonorrheal endometritis is apparently only a transient phase in the upward course of the infection but eventual salpingitis, with or without pelvic peritonitis, is so common as to be almost a phase of the disease. Verruca acuminata on the external genitalia commonly develop and harbor the infection.

Frequent complications are proctitis sometimes with perirectal abscesses and strictures, inguinal and femoral adenitis, trigonal cystitis, salpingitis and pyosalpingitis (which are usually bilateral) along with pelvic peritonitis and adhesions to the intestines and omentum, perioophoritis (which may progress into ovarian abscesses), periappendicitis, and arthritis (especially when the urethra and trigonal region of the bladder are infected). Septicemia and endocarditis are comparatively rare and not nearly as likely to develop as in gonorrhea of the male. But sterility and ectopic or tubal pregnancies are frequent sequelae of the salpingitis.

As in gonorrhea of the male, *laboratory examinations* are of great diagnostic value, especially in acute and subacute infections. They may be summarized as follows:

1. Bacteriologic examinations of smears and cultures of the inflammatory exudates are of paramount importance (page 394). All purulent discharges from the female genitalia should be looked upon with suspicion. Leukorrhea may be due to infestment with *Trichomonas vaginalis* (page 294), to infection with various bacteria other than the gonococcus or to other causes but it is always well to keep in mind that it may be due to gonorrhea. Proper care is required in the preparation of smears and cultures which are best prepared from the urethra after expression of Skene's glands and from around the cervix; likewise after expression of the Bartholin glands and ducts and especially if they are visibly

reddened. In virgins, vaginal smears or vaginal washings may be examined when the cervix cannot be exposed.

2. In spite of repeated and expert bacteriologic examinations of smears and cultures, gonococci may not be found, especially not in smears. Under these conditions, diagnosis depends on the clinical manifestations.

3. In chronic gonorrhea, however, and especially in the presence of salpingitis, pyosalpingitis and ovaritis, the complement fixation test is definitely of diagnostic value when properly conducted, particularly in reference to the preparation of antigen (page 506). That is to say, positive reactions almost invariably indicate the presence of gonococcal infection, provided gonococcal vaccine has not been given in treatment. Negative reactions, however, do not exclude the disease.

4. As in gonorrhea of the male, bacteriologic examinations, with special reference to cultures, are of great value in relation to criteria of cure. The gonococcus complement fixation test is also helpful in this connection, since the occurrence of persistently positive reactions several months after apparent recovery are acceptable as evidence of persistent infection. By the same token, negative reactions following previous positive ones are among the criteria of complete recovery.

SYPHILIS

For clinical purposes, syphilis is divided into the *acquired* and *congenital* forms of the disease. Strictly stated, however, all syphilis is acquired; congenital syphilis is merely acquired *in utero*. "Infantile syphilis" is not congenital syphilis; the term designates those comparatively rare instances of the disease acquired during or shortly after birth.

Whether or not specific strains of *T. pallidum* exist with a special predilection for certain organs, as for the central nervous system, cannot be stated. At the present time there is no experimental evidence indicative of their existence but the frequency with which the marital partners of paretics and tabetics develop similar types of neurosyphilis and the frequency with which syphilitic children born of neurosyphilitic patients develop congenital neurosyphilis, suggest that strains may exist with a special affinity for the central nervous system.

Transmission and Infectiousness of Syphilis. In from 90 to 95 per cent of cases, acquired syphilis is transmitted by sexual contact including intra-uterine infections (congenital syphilis). The remaining 5 to 10 per cent are transmitted by extragenital routes of infection, especially by kissing, resulting in the production of chancres of the lips and tongue, but including infections of the nipples, anorectal region and other parts, as well as those occasional cases of infections of the fingers occurring among physicians, dentists, nurses and midwives ("professional chancres").

Milk may account for the infection of some nursing infants; for this reason a healthy baby born of a recently infected mother should, for complete safety, be fed artificially from birth. Semen may be infectious when the male is in the early stages of syphilis but rarely, if ever, when the disease has been present more than five years, whether previously treated or not. Undoubtedly, syphilis may be transmitted also by blood transfusion, as many cases of "transfusion syphilis" have been reported. While it is clear that the blood of a syphilitic individual in the early stages of the disease is particularly dangerous, it is impossible to set any definite time limit on the infectiousness of the blood, since cases of transfusion syphilis have been reported from donors infected at least seven years. Consequently, it is safer to reject all syphilitic donors. Stored citrated blood and plasma are safer than fresh blood because *T. pallidum* dies off within 72 hours (page 482). In case of necessity, however, the fresh blood of a syphilitic donor may be rendered safe by the addition of neoarsphenamine or mapharsen before administration (page 482).

Because of the rapidity with which *T. pallidum* dies when deposited on eating and drinking utensils, clothing, toilet seats, etc., indirect transmission is rare and probably does not occur more frequently than once in every 8000 to 10,000 cases of the disease. Without doubt, breaks in the epithelial barriers of the skin and mucous membranes greatly favor infection, but it is also possible that *T. pallidum* may wriggle its way through apparently intact mucous membranes.

Infectiousness is always greatest during the first one or two years because of acute open lesions containing large numbers of *T. pallidum,* as during the primary and secondary stages, sometimes followed by relapsing lesions of the mucous membranes. Consequently, early diagnosis and the prompt institution of treatment of early syphilis is of paramount importance. It is assumed, however, that infectiousness is slight after five years even in untreated cases, although open lesions are always potentially dangerous. Reinfection or second attacks of the disease may occur after complete recovery; indeed, it appears that a second attack may occur before the first one is cured (superinfection) although without the production of a typical chancre.

Much less is known of the mechanism of infection in congenital syphilis. Paternal transmission, *i.e.*, infection of the fetus by the father without infection of the mother, apparently does not occur. In other words, it appears that syphilis is transmitted to a fetus only by a syphilitic mother. Under the circumstances, it is advisable to assume that all mothers of syphilitic children have the disease in spite of the absence of clinical manifestations and negative serologic reactions. The actual infection of the fetus is thought to be due to a spirochetemia (probably transient) of the mother. Certainly the more recent her disease the greater the chances of infection of the fetus.

Of course, the placenta may present an effective barrier to the passage of treponemes to the fetal circulation. In that case the child escapes infection; hence syphilitic women may give birth to nonsyphilitic children. But there is some evidence to indicate that treponemes may reach the fetal circulation from the maternal circulation by way of the placenta without causing detectable lesions in that organ although, in most instances, lesions are produced on its maternal side. At any rate, it is now known that the "law of Colles" to the effect that a mother of a syphilitic child may escape infection by reason of acquiring an immunity during pregnancy as, likewise, the "law of Profeta," based on the assumption that a fetus *in utero* may escape infection from a syphilitic mother by acquiring immunity are both erroneous because the mother, in the first instance, is probably always syphilitic, while the child in the second may or may not be syphilitic, depending on whether or not there has been a placental transfer of *T. pallidum.*

Incidence of Syphilis. The true incidence of syphilis is uncertain but on the basis of serologic surveys is known to vary greatly in different localities and social groups from as low as 0.2 to 0.5 to as high as 20 to 30 per cent, with an estimated general average of 8 to 10 per cent. If this is true, there are about 10 millions of infected individuals in the United States alone, the majority of whom either do not know that they have syphilis or regard themselves as having recovered from it. The incidence is higher in cities than in country districts.

About 91 per cent of persons are believed to contract the disease between 16 and 40 years of age, with an estimated incidence of 500,000 to 600,000 new cases per year. Every individual with acquired syphilis is thought to be responsible for the infection of one to three additional persons. During the incubation stage of the disease, an individual may transmit it to others but the greatest danger of transmission is during the first two years. It is likely that the danger of transmission is slight after five years whether treatment has been given or not.

It has been estimated that about one million of the potential mothers of this country have syphilis. Among syphilitic mothers only about 17 per cent of pregnancies result in the birth of living nonsyphilitic children. The remaining 83 per cent result in miscarriages, stillbirths, living children with syphilis, or infants who die during infancy. In other words, untreated syphilitic women have about one chance in six of bearing healthy children while nonsyphilitic women have at least three chances in four.

Under the circumstances, it has been estimated that every year about 25,000 fetuses are killed by the disease before birth with about 60,000 born alive with it, which is incomparably more serious than syphilis acquired during adult life. All of this could be largely prevented if *all* women would seek medical attention before the fifth month of pregnancy and if *every* physician would include serologic tests for syphilis routinely, repeating the tests at about the seventh month, promptly followed by adequate treatment of every case found to give checked positive serologic reactions. In private practice physicians frequently hesitate or refuse to have serologic tests performed during pregnancy because of fear of giving offense, but respectability is no bar to the penetrability of *T. pallidum.* Furthermore, blood examinations are so widely employed for various diagnostic purposes that it is always readily possible to include the serologic tests for syphilis without the knowledge of the patient. Undoubtedly, therefore, premarital blood tests and tests during pregnancy, as now legally required by many states, are highly desirable even though there may be objections to the former but certainly none that are logical to the latter.

Clinical Types of Syphilis. For clinical purposes syphilis is divided into two main groups, namely, (1) *acquired syphilis* or that contracted after birth and (2) *congenital syphilis* or that acquired during intra-uterine life. Acquired syphilis on the other hand is divided into four stages, namely, (1) the primary or chancre stage; (2) the secondary stage; (3) the stage of so-called latency and (4) the third or tertiary stage. From the standpoint of treatment, early syphilis embraces the primary and secondary stages while late syphilis applies to the ensuing period and is divided into chronic latent and chronic active syphilis.

Primary Syphilis. Following infection with *T. pallidum* the parasite rapidly invades the lymphatics and blood, but a sore does not appear at the site of inoculation until ten to ninety days later (average, twenty-one days), these days constituting the *incubation* period. The painless, indurated, raised and eroded papule or ulcer is the chancre and this, along with some painless, rubbery enlargement of the regional lymph nodes (satellite bubo), constitutes the primary stage of the disease. The chancre, however, may be so small or so situated (especially in women) as to escape attention. Indeed, about 30 per cent of men and 60 per cent of women with late syphilis are likely to deny all knowledge of infection. *A negative history therefore is of little or no value in excluding the possible presence of syphilis.* Furthermore, and most unfortunately, such cases of primary syphilis may unwittingly spread the disease to others and become a public health menace conceivably greater than patients with frank early lesions.

Every lesion on the genitalia should be suspected as syphilitic until proved otherwise and especially in the case of men. Clinical diagnosis is frequently difficult in differentiating chancres from chancroid, lymphogranuloma venereum, erosive balanitis, herpes genitalis, carcinoma, etc. Consequently, darkfield examinations for *T. pallidum* are of inestimable value and if routinely employed would prevent erroneous diagnosis in a large percentage of cases. Chancres are usually single lesions and if two or more are observed they develop simultaneously. Various clinical types are observed, including the indurated papule, eroded and ulcerative papules, "parchment" and "silver spot" lesions, multiple herpetiform chancres, etc.

In the meantime, *T. pallidum* has rapidly invaded the lymphatics and blood. Spinal fluid examinations indicate that the central nervous system is invaded in 20 to 30 per cent of cases although without the production of symptoms (asymptomatic neurosyphilis).

In untreated cases the chancre slowly heals in four to six weeks, frequently leaving no scar. This is followed by a period of four to eight weeks, sometimes called the "primary latent period," during which the patient may be without symptoms but may develop malaise, anemia, general lymphadenopathy, bodily aches and pains, headaches, etc.

Masking or Suppression of Syphilis. The administration of penicillin in the treatment of gonorrhea of adults may mask, suppress or delay the development of the lesions of primary or secondary syphilis without preventing asymptomatic, recurrent or relapsing syphilitic infection. The danger lies in the fact that the incubation period of syphilis is not only much longer than in gonorrhea but that individuals are frequently exposed to both diseases simultaneously, in addition to the fact that some may contract gonorrhea during the incubation period of syphilis. The incidence of the masking of early syphilis as the result of the treatment of gonorrhea with penicillin is unknown, but an increasing number of cases has been reported in recent years. In some cases it is possible that syphilis was contracted after the completion of treatment for gonorrhea but, on the other hand, there may be a considerable number of cases in which truly recurring syphilis remains masked or asymptomatic without adequate treatment of the disease. If the diagnosis of gonorrhea has been established and there is evidence or suspicion of coexisting syphilis, penicillin therapy should be withheld until a definite diagnosis of the latter disease is, if possible, established or excluded.

Secondary Syphilis. During the primary latent period the treponemes are occurring in the blood and disease is followed by the secondary stage characterized by macular, papular, nodular or pustular eruptions on the skin and as mucous patches on the lips, buccal mucosa, prepuce and vagina, or as broad, flat grayish condylomas in the axillas, perineum and female genitalia. As in primary syphilis, these lesions are highly infectious, but they may also be so light and evanescent as to go unnoticed. They also disappear spontaneously in untreated cases in from a few days to several months, depending on their type and severity. In some patients they never reappear while in others they recur, especially on the mucous membranes of the mouth and genitalia, during the ensuing three to five years. Since they, too, are highly infectious, they are also a menace from the standpoint of transmission of the disease. In rare instances during this period the chancre may recur at the same site, when it is known as "chancre redux." Spinal fluid examinations indicate that the central nervous system is invaded by *T. pallidum* in at least 50 to 75 per cent of cases although usually without clinical manifestations (*asymptomatic neurosyphilis*); as shortly to be discussed, however, this invasion results in the production of symptomatic neurosyphilis in a much lower percentage of cases during the tertiary stage.

Latent Syphilis. The patient then enters a period of undeterminate length, varying from a few months to a lifetime, but averaging about seven years, during which there are no clinical manifestations, and the disease is detectable, if at all, only by means of serologic tests of the blood and spinal fluid. This is the latent stage and involves a large number of individuals unknowingly infected with syphilis and those who believe themselves recovered from it.

Tertiary Syphilis. During the latent stage, however, especially in untreated or inadequately treated individuals, slowly progressive and chronic inflammatory changes take place in various infected tissues. These, sooner or later, give rise to the signs and symptoms of the tertiary stage which is characterized by the destructive effects of the gummas or by a breakdown of important tissues or organs resulting from the effects of chronic perivascular inflammation and fibrosis. The difference is largely one of degree, the essential pathologic changes being identical, but the lesions may affect many different organs or tissues of the body.

When these lesions involve the skin, mucous membranes, bones and joints they are frequently designated *benign chronic syphilis* but in about 36 per cent of cases lesions are also present elsewhere and especially in the cardiovascular and central nervous system. In other cases the lesions may affect organs of greater physiologic importance, constituting *visceral syphilis*. In about 10 per cent of cases the lesions are predominantly in the cardiovascular system (aortis, aneurysm, aortic insufficiency). Spinal fluid examinations show changes (positive Wassermann and colloidal gold reactions) in about 10 to 15 per cent of untreated cases. At least 1 to 3 per cent develop symptomatic neurosyphilis in spite of treatment and of these about 5 to 6 per cent are apt to be paresis and 3 to 5 per cent tabes dorsalis. All forms of neurosyphilis (excepting the purely vascular types) are especially likely to develop in white men while being less frequent in white women. The incidence is much less in Negroes, being lower in colored men than in white women while relatively uncommon among colored women.

Hepatic syphilis occurs in about 2 to 3 per cent of cases. Syphilis of the kidneys is not infrequent but syphilitic strictures of the rectum are probably very uncommon, most cases caused by venereal infection being due to lymphogranuloma venereum. While syphilis of the lungs, stomach, spleen, pancreas and bladder is rare and of the testicles uncommon, as far as clinical involvement is concerned, syphilis of the larynx is not infrequent and syphilis of the eyes (iritis, keratitis, uveitis, optic neuritis, etc.) is quite common.

Congenital Syphilis. Congenital syphilis is divided conveniently into two groups, *early* and *late,* depending on whether the child is younger or older than two years. Early congenital syphilis is similar in many of its manifestations to early acquired syphilis. Late congenital syphilis, however, differs in many important ways from late acquired syphilis.

Frank clinical manifestations of early congenital syphilis at birth (skin lesions, rhagades, snuffles, condylomas, etc.) are rare at present, since so many syphilitic women receive some treatment during pregnancy. As a result, those children who are born alive are apparently normal at birth. It is extremely important, however, to determine the presence or absence of syphilis in newborns as promptly as possible. For this purpose darkfield examinations of scrapings from the wall of the umbilical vein for *T. pallidum,* if positive, establish diagnosis within a few hours after birth, but negative results do not exclude the disease. Unfortunately, a single routine serologic test is of little value. A positive reaction does not necessarily mean that the child has syphilis, as it may be due to reagin passively transferred from the maternal blood through the placenta. A negative reaction does not exclude the disease which at the time may be in the seronegative phase. But if positive reactions are consistently observed at intervals up to six to eight weeks, syphilis is present, whereas if consistently negative over that period and at subsequent intervals of six months, one year and two years, it may be safely assumed that the child has escaped the disease.

Further aid in early diagnosis may be furnished by roentgenologic examinations of the long bones two weeks after birth, but only if *the mother received no bismuth* during pregnancy, as that compound may produce findings which are hard to differentiate from syphilitic osteochondritis. If diagnosis has not been established and treatment not instituted, clinical signs tend to appear within the first two or three months, if at all. These take the form of snuffles, skin lesions, osteitis and periostitis.

The clinical manifestations of late congenital syphilis are many and varied and frequently tax the diagnostic skill of the practitioner. They include the so-called *stigmata* ("saddle" nose, "dish-shaped" face, "sabre" skins, Hutchinson's teeth, rhagades, corneal scars, chorioretinitis and nerve deafness) or *progressive active lesions* (ocular, skeletal, juvenile paresis or taboparesis, and nerve deafness). Both types may, of course, occur simultaneously in any patient. But few infants and children with congenital syphilis will present such obvious manifestations that medical care is sought. Most of them are in a latent stage, but since syphilis is a family disease, unrecognized and unsuspected congenital syphilis is frequently discovered by the routine examination of the entire family of a parent who has the disease. The necessity for investigating the entire family becomes particularly important when the mother is found to have syphilis, or when another child is found to have congenital syphilis. Interstitial keratitis is by all odds the most common lesion of late congenital syphilis, being present in about 50 per cent of cases.

Laboratory Examinations. Needless to state, laboratory examinations are extremely important in relation to the diagnosis and treatment of syphilis. This is particularly true in relation to darkfield examinations for *T. pallidum* in the diagnosis of chancres and mucous patches (page 399); also in relation to serologic examinations of the blood (Chapter 18) and examinations of the cerebrospinal fluid (page 555). They may be briefly summarized as follows:

1. Darkfield examinations of chancres are positive in about 95 per cent of cases. They are applicable to the diagnosis of all extragenital chancres except those occurring in the oral cavity where the presence of *T. microdentium* may lead to diagnostic errors. Treponemes, however, may not be found in chancres well advanced in healing. As previously stated, darkfield examinations are advisable as a routine measure in the diagnosis of all sores about the genitalia. The same applies to chronic or slow healing sores or ulcers of the lips and fingers.

2. Serologic tests are of no value in the diagnosis of recent chancres. After ten days, however, positive reactions may be observed, with the result that as darkfield examinations for *T. pallidum* are increasingly negative serologic reactions are increasingly positive.

3. During the secondary stage of the disease positive serologic reactions are observed in almost 100 per cent of cases. Since the skin and mucous membrane lesions may not be as readily diagnosed as commonly believed, they are therefore of great diagnostic value.

4. During the latent stage of chronic syphilis, serologic tests possessing the maximum of sensitivity consistent with specificity are of inestimable diagnostic value and are usually the only means available for the detection of the disease, as discussed in Chapter 18. Needless to state, the physician should be thoroughly familiar with the causes for biologic and falsely positive reactions. Serologic tests are likewise of great value as aids in the detection of syphilis during pregnancy, of chronic active syphilis and congenital syphilis as, likewise, in relation to the treatment of the disease, as discussed in Chapter 18. Since falsely negative reactions may occur they should never be permitted to override clinical judgment.

5. Examinations of the cerebrospinal fluid for total cells, increase of protein, the Wassermann and a colloidal reaction (colloidal gold preferred) are always advisable in early syphilis after the first six months of treatment. If negative, it need not be examined again until after the first year of observation following the cessation of treatment. Cerebrospinal fluid examinations, however, are always advisable as part of a diagnostic survey in cases of latent or active chronic syphilis because, if abnormal changes are detected, it may properly lead to changes in the

therapeutic program. Certainly no case of syphilis can be regarded as cured without at least one, and preferably two, examinations with completely negative findings.

6. A macroscopic and microscopic examination of the placenta is always advisable when syphilis is suspected or known to be present, as the results are of value in relation to the probability of intra-uterine infection of the child.

7. Otherwise, additional laboratory examinations are not ordinarily required. Practically all cases of early syphilis, as well as a large percentage of cases of chronic active syphilis, will show the presence of a simple secondary anemia. As reported by Wile and his colleagues (Am. J. Syph., Gonor. & Ven. Dis. *25:* 133, 1941), cases of early syphilis frequently show monocytosis, eosinophilia, the presence of plasma cells and a type of cell, hitherto unreported, similar to that seen in infectious mononucleosis.

CHANCROID

The incubation period of chancroid may be less than three days but rarely longer than a week. It is purely a local infection with *H. ducreyi,* with extension to and involvement of the inguinal glands in over 30 per cent of cases. The latter is practically always a periadenitis with suppuration in from 30 to 50 per cent of cases. Frequently, mixed chancroidal and syphilitic infections occur. These begin as chancroids but after three or four weeks the lesions persist and become indurated. They are difficult to diagnose clinically and for this reason repeated darkfield examinations for *T. pallidum* and prolonged observation may be necessary. Because of the inguinal adenitis chancroid is included among the infectious inguinal granulomas and frequently confused clinically with lymphogranuloma venereum and granuloma inguinale (Table 128). Various *laboratory examinations* which are of helpful diagnostic and differential diagnostic value, may be summarized as follows:

1. Bacteriologic examinations of smears and cultures of the lesions and buboes for *H. ducreyi* with special reference to examinations of exudates aspirated from the glands (page 400).

2. In all but the most typical cases one or more darkfield examinations for *T. pallidum* should be made routinely for the exclusion of primary syphilis. These are especially indicated if the lesions heal slowly under sulfonamide therapy and show induration after two or more weeks which is always suggestive of mixed chancroidal and syphilitic infections.

3. Apparently, intradermal tests consisting of the injection of 0.1 cc. of heat-killed suspensions of *H. ducreyi* possess diagnostic value and are particularly helpful in differentiating chancroid from lymphogranuloma venereum and granuloma inguinale (page 581). Positive reactions do not occur, however, until chancroids have been present for at least eight to fifteen days. Allergic sensitivity may persist for many years after recovery from the disease and possibly for the balance of life. For this reason the test may give positive reactions in individuals without chancroid at the time it is conducted, as in lymphogranuloma venereum or granu-

loma inguinale, because of previous chancroidal infection. Negative reactions, however, are of particular value in excluding chancroid in causes of pudendal ulcerations and inguinal granulomas which have been present for two weeks or more.

4. Unfortunately, complement fixation and agglutination tests with antigens of *H. ducreyi* have not proved of practical diagnostic value in chancroid (page 508).

5. Biopsy examinations of excised tissues are very helpful in the differential diagnosis of chancroid, lymphogranuloma venereum and granuloma venereum.

TABLE 128. SUMMARY OF THE DIFFERENTIAL DIAGNOSIS OF THE INFECTIOUS INGUINAL GRANULOMAS BY LABORATORY EXAMINATIONS

Disease	Laboratory Examinations
Chancroid	Smears and cultures of the exudates show the presence of *H. ducreyi.* Darkfield examinations are negative for *T. pallidum.* Intradermal tests conducted with antigens of *H. ducreyi* usually give positive reactions 8 to 15 days or longer after the development of lesions. They may, however, be due to a previous chancroidal infection. Biopsy examinations are of diagnostic value. The intracerebral inoculation of mice with exudates or extracts of excised tissue are of helpful diagnostic value.
Lympho-granuloma Venereum	Darkfield examinations are negative for *T. pallidum.* Smears and cultures are negative for *H. ducreyi* and smears are negative for the "Donovan bodies" of granuloma inguinale. Positive complement fixation reactions with antigens of cultivated virus indicate (1) that the disease is clinically present; (2) that it is present but not clinically recognizable or (3) that unrecognized infection has occurred in the past. Intradermal tests are very helpful. Positive reactions, however, may be due to (1) existing disease; (2) previous unrecognized infection or (3) to mixed infections in gonorrhea, chancroid or syphilis. Biopsy examinations are very helpful in diagnosis.
Granuloma Inguinale	Properly prepared and properly stained smears of the lesions show the presence of "Donovan bodies" in at least 60 to 80 per cent of cases. Repeated examinations may be required. Smears and cultures are negative for *H. ducreyi.* Darkfield examinations are negative for *T. pallidum.* Intracerebral mouse inoculation tests are negative for the virus of lymphogranuloma venereum. Biopsy examinations are very helpful. Positive Wassermann reactions are due to co-existing early or chronic syphilis. Positive complement fixation and skin reactions with lygranum antigen are due to previous infection with the virus of lymphogranuloma venereum. Positive skin reactions with an antigen of *H. ducreyi* are due to previous chancroidal infection.

LYMPHOGRANULOMA VENEREUM

Lymphogranuloma venereum is essentially a venereal disease due to infection with a virus readily transmissible to mice by intracerebral inoculation. The virus is cultivatable and antigens prepared of it have proved of diagnostic value in skin and complement fixation tests.

While originally regarded as a tropical disease, lymphogranuloma venereum is now known to be a widely distributed disease with frequent occurrence in the United States. Originally called lymphogranulomatis inguinalis by Durand, Nicolas and Favre, much confusion has been created by the introduction of many additional names like venereal lymphogranuloma, lymphopathia venereum, climatic bubo, tropical bubo, pudendal ulcer, etc.

The disease occurs most commonly in males, preponderantly among the colored, but also occurs among whites. The onset is characterized by a small initial lesion on the genitalia which is transitory and heals rapidly. The patient is usually unaware of this initial lesion, with the result that the first symptoms are generally referable to buboes. The latter increase in size, become shiny, soft, begin to break down with fistulas and are accompanied by some constitutional symptoms. In men the infection may also involve the glands of the suprapubic and genitocrural regions as well as the skin of the buttocks. In women elephantiasis and trophic ulcerations of the vulva (esthiomene), due to chronic obliterative lymphangitis, are common along with extension to the intrapelvic glands leading to a genito-ano-rectal syndrome and the production of rectal strictures. In rare instances lesions may occur elsewhere as of the skin and iris. *Laboratory examinations* are of helpful diagnostic value and may be summarized as follows:

1. The detection of the virus in material aspirated from the buboes or excised bits of tissue of the latter by intracerebral inoculation in mice. Aspirated material may be diluted with an equal part of sterile saline solution and injected in dose of 0.1 cc. If excised tissue is employed, thick emulsions in saline solution should be centrifuged and 0.1 cc. amounts of supernatant fluid injected intracerebrally. Within a week mice usually develop symptoms consisting of muscular weakness and epileptiform convulsions and usually die within three to five days thereafter, sections of the cerebral meninges and brain showing extensive exudates of lymphocytes and large endothelial cells around the vessels of the meninges. Subcutaneous inoculation of such material into the inguinal region of guinea-pigs produces adenitis in four to eight days in about 50 per cent of animals which, upon histologic examination, show numerous small abscesses similar to those observed in human buboes. As previously stated, it is possible to cultivate the virus but cultural examinations of human lesions are not employed for diagnostic purposes.

2. Complement fixation tests employing antigens of cultivated virus have proved of considerable specificity and diagnostic value (page 515). Indeed, they may be more sensitive than skin tests in the detection of borderline and doubtful cases and their usefulness in epidemiologic surveys of lymphogranuloma venereum has been suggested. It is stated that the tests yield positive reactions in about 100 per cent of active cases of the disease. Positive reactions have been observed, how-

ever, in about 7 to 8 per cent of normal individuals apparently due to previous unrecognized attacks of the disease. They have also been observed in 50 to 75 per cent of cases of syphilis, gonorrhea and chancroid, indicating the possibility of mixed infections. Under the conditions, a positive reaction may indicate, therefore, (a) that the disease is clinically present; (b) that it is present but not clinically apparent, or (c) that unrecognized infection with the virus has occurred in the past.

3. The spinal fluid changes in acute syphilitic meningitis and lymphogranuloma meningoencephalitis may be strikingly similar except for negative Wassermann reactions in the latter disease.

4. The Frei intradermal test or modifications of it employing antigens of cultivated virus are likewise of helpful diagnostic value (page 586). This is particularly true of lygranum antigen prepared from the yolk sacs of developing chicken embryos inoculated with the virus. This antigen appears to be superior in sensitivity and specificity to both the Frei antigen of sterilized human pus and that prepared of mouse brain. It is stated that positive reactions occur in less than 1 per cent of normal individuals but the possibility of positive reactions due to previous unrecognized attacks of the disease as well as in cases of mixed infections in gonorrhea, chancroid and syphilis must be kept in mind, as stated above in relation to the complement fixation test. Paulson has described an antigen prepared from grossly fecal-free blood, mucus and pus or from tissue for use in cases of lymphogranuloma venereum with rectal lesions which is referred to in Chapter 25 in connection with the differentiation of the disease from nonspecific chronic ulcerative colitis.

5. Histologic examinations of tissues removed from buboes by biopsies are also of diagnostic and differential diagnostic value. Marked proliferation of the endothelial cells with the collection of large mononuclear cells in the form of small nodules are the earliest changes. Later, necrosis occurs in the centers of these nodules along with collections of polymorphonuclear leukocytes producing triangular or quadrangular shaped (stellate) abscesses.

6. During the course of the disease a large percentage of cases show a slight increase of the total serum proteins, especially serum globulin; these determinations, therefore, may be of additional diagnostic aid, as the changes are stated to occur in cases of buboes, chronic genital lesions, and in acute and chronic proctitis as well as in rectal strictures.

GRANULOMA INGUINALE

Granuloma inguinale is due to infection with a gram-negative bipolar bacillus successfully cultivated and identified by Goodpasture and designated *Donovania granulomatis*. This organism is to be found within monocytes in properly stained smears of lesions constituting the "Donovan bodies." Some investigators formerly regarded these organisms as forms of intracellular bacilli of the Friedländer group but since the disease usually responds to treatment with tartar emetic or other antimonyl compounds, as is true of the different clinical forms of leishmaniasis, others have regarded them as protozoa (leishmania) with the easily cultivatable bacillus as only a secondary invader.

Unfortunately, various other names have been applied to the disease, like lymphogranuloma inguinale and granuloma venereum, which have led to confusion with lymphogranuloma venereum. While included in the category of the venereal diseases, it appears that granuloma inguinale may be transmitted and contracted by nonvenereal routes of infection. It occurs almost exclusively among colored men and women, although whites of both sexes may contract it.

The disease usually begins as a nodular or elevated papule which is bright red in color, of soft consistency and without ulceration; it differs thereby from the lesions of chancroid, primary syphilis and balanitis. The lesion, however, develops into a serpiginous ulcer with easily bleeding granulation tissue. Characteristic button-like granulations may closely resemble hypertrophic syphilitic papules, although the latter are covered with epidermis and hence dry at the onset while whitish-grey in case of maceration and never bright red and velvety. The infection is predominantly one of the skin so that the absence of buboes is important. It spreads by continuity or contact auto-inoculation and in a few months usually involves the inguinal region over Poupart's ligament, the thighs and perineum almost to the anus. Its extension into the hairy parts of the genitalia is accompanied by the permanent loss of pubic hair, and in its progression it has the tendency to follow the moist folds of the skin in the genital and inguinal regions. Simultaneously with its progression scar tissue is formed which usually starts in the center of the ulcer. This scar tissue is rather atrophic with an extremely avascular and dry appearance and pigmentary changes. In contrast to this, however, the scars may be hypertrophic and persist for months or even years with flare-ups of the infection at various intervals. The clinical manifestations, therefore, may be sufficiently characteristic for diagnostic purposes but when small lesions occur on the prepuce or on the coronary sulcus they are frequently difficult to differentiate from chancres, chancroids, lymphogranuloma venereum or other chronic ulcers of the genitalia. Fortunately, *laboratory examinations* are of helpful diagnostic value and may be summarized as follows:

1. Examinations of properly prepared and properly stained smears of the exudates for *D. granulomatis* or "Donovan bodies" are of paramount importance (page 400). Repeated examinations are frequently required and a single negative smear never reliably excludes the disease. "Donovan bodies" are found in at least 60 to 80 per cent of cases. Care should be taken to prepare smears of the deeper portions of the granulations. The organisms occur both extra- and intracellularly but characteristically within large mononuclear cells which they may fill so completely as to obscure the outlines and structure of these cells. In very acute and fulminating lesions the organisms may not be encapsulated but in chronic cases encapsulated "bodies" usually predominate. Plastin bodies have also been described (Goldzieher and Peck) as occurring in endothelial cells as peculiar homogenously staining, round, short objects which may be regarded as indicative of granuloma inguinale even when "Donovan bodies" have not been found.

2. Needless to state, darkfield examinations for *T. pallidum* and serologic tests are frequently required for the exclusion or identification of syphilis since granuloma inguinale and syphilis (early or late) frequently occur concurrently.

3. Microscopic examinations of properly stained sections of tissue removed by biopsy are frequently required for diagnostic purposes, not only for "Donovan bodies," but for other rather characteristic histologic changes as well.

4. Of course, positive Wassermann reactions do not exclude granuloma inguinale as the latter may occur in syphilitic individuals. The same applies to positive intradermal and complement fixation tests with antigens of the virus of lymphogranuloma venereum, since granuloma inguinale may also occur in individuals who have had unrecognized attacks of the former. Furthermore, positive skin reactions with an antigen of *H. ducreyi* do not exclude granuloma inguinale, since the latter may also occur in individuals who have had chancroidal infection in the past.

5. Complement fixation tests conducted with antigens prepared of *D. granulomatis* possess diagnostic value as discussed in Chapter 17.

VENEREAL FUSOSPIROCHETOSIS

Mild types of erosive balanitis due to uncleanliness and infection with staphylococci or streptococci are not uncommon among men with phimosis. But it is now known that erosive and gangrenous balanitis, including phagedenic ulcers of the penis and scrotum, or of the vulva, may occur due to symbiotic infection with *B. fusiformis* and spirochetes constituting venereal fusospirochetosis. The disease is listed among the venereal infections as the primary form which is apparently due to moistening the sexual organs with saliva during coitus. It is particularly likely to occur in colored men. The infection usually begins at the corona or at the frenulum with gradual extension and destruction of tissue over many months when healing, with scarring, may occur spontaneously. In some cases so much of the shaft of the penis is destroyed as to require amputation while in the female the infection may involve the vagina with the production of a black gangrenous lesion (noma).

B. fusiformis and various spirochetes, including *T. refringens,* may occur in the smegma of the penis and clitoris of normal men and women as harmless saprophytes. They may, however, gain access to the lesions of chancroid or granuloma inguinale and produce secondary fusospirochetal infections but usually with no more than an increase in the odor and amount of discharge.

Primary venereal fusospirochetosis, however, is apparently due to fusiform bacilli and spirochetes of other origin in connection with which those from the mouth have been previously mentioned. Indeed, it may be that the infection of the genitalia is due to the same fusiform bacilli and *Bor. vincentii* producing Vincent's angina and fusospirochetal gingivitis. At least the spirochetes found in the lesions of the genitalia are of the large size closely resembling *T. macrodentium* and *Bor. vincentii* of the oral cavity. The fusiform bacilli of venereal fusospirochetosis are usually of the thin short type in acute rapidly progressive lesions while longer and thicker in chronic lesions; under penicillin therapy the spirochetes first disappear, the fusiform bacilli being more persistent. *Laboratory examinations* have proved of great value in diagnosis and may be summarized as follows:

1. Large numbers of fusiform bacilli and spirochetes are found in properly prepared smears stained with carbol-fuchsin. Darkfield examinations for spirochetes are not ordinarily required. Cultures are not employed because both the fusiform bacilli and spirochetes are strict anaerobes and difficult to cultivate. Animal inoculation tests are of no value.

2. If fusiform bacilli and spirochetes are not found in simple stained smears it may be advisable to make darkfield examinations for *T. pallidum* in case syphilis is suspected, although due care is required against mistaking *T. refringens* and other saprophytic spirochetes of the genitalia for *T. pallidum*.

3. Histologic examinations of sections of tissue removed by biopsy are of helpful value. In venereal fusospirochetosis a diffuse superficial necrosis with comparatively little tissue reaction at the edges of the necrotic areas constitute the usual findings. The smaller vessels in the deeper layers of the infected tissue are apt to be occluded by thrombi but granulation tissue is generally absent. Silver impregnation methods of staining show the presence of spirochetes which usually invade apparently healthy tissue in advance of fusiform bacilli.

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DISEASES OF THE STOMACH, INTESTINES AND PANCREAS

The clinical interpretation of laboratory examinations of the stomach and duodenal contents and of pancreatic function tests has been discussed in Chapter 10 and examinations of the feces in Chapter 11. They have proved of value in the diagnosis and differential diagnosis of certain organic diseases of the stomach, intestines and pancreas discussed herewith. Skilfully conducted fluoroscopic and roentgenologic examinations have proved of even more clinical value. Within recent years gastroscopic examinations have been employed in the diagnosis of diseases of the stomach but since there may be no correlation between symptoms and the results of gastroscopy, they require judicious and conservative interpretation in relation to diagnosis.

THE GASTRITIDES

Acute Gastritis. Acute gastritis is usually divided according to etiology into the (1) exogenous, (2) corrosive and (3) phlegmonous forms of the disease. *Laboratory examinations* are generally limited to the vomitus, which contains in addition to food residues, mucus, bile and at times blood. *When poisoning is suspected the detection of the agent by chemical analysis is very important.*

In acute gastritis due to exogenous causes (chemical, mechanical, thermal and bacterial) the secretory and motor functions of the stomach are increased. Consequently, gastric analysis is likely to show (1) an increased amount of gastric secretion; (2) the presence of an excess of rather thin mucus and (3) subacidity or anacidity in about 30 per cent of cases due to the neutralization of hydrochloric acid by regurgitated bile and duodenal contents. On the other hand, the acidity may be within normal in about an equal proportion of cases or even above normal in about 45 per cent. After an alcohol test meal a microscopic examination of the stomach contents frequently shows (4) a great excess of leukocytes which is of helpful diagnostic value.[1] In phlegmonous gastritis large amounts of pus, blood and shreds of gastric mucosa are commonly observed.

Chronic Gastritis. Opinions still vary as to whether chronic gastritis is a definite clinical entity. It is usually divided into (1) the catarrhal and superficial types occurring in about 30 to 40 per cent of cases; (2) the hypertrophic type which may approach polyposis occurring in about 50 per cent and (3) the atrophic type occurring in about 8 per cent. The latter, which Schindler considers is usually the end result of superficial gastritis, is particularly associated with pernicious anemia, chronic alcoholism with avitaminosis, carcinoma, pellagra and chronic conditions of the stomach and intestines. Undoubtedly, it is improper to designate all atrophic lesions of the stomach as "atrophic gastritis" in view of the fact that a large proportion of them are merely reflections of underlying deficiencies, similar to those occurring in the mouth due to avitaminoses, iron deficiency, etc. Scirrhous or sclerosing gastritis may also occur.

About 80 to 90 per cent of cases of chronic gastritis occur between the ages of 25 to 45 years and males are affected about eight times more frequently than females. Chronic gastritis is characterized by a tendency to decreased acidity of the gastric residuum and gastric secretions after the administration of a test meal. Recent investigations have indicated that the secretory activity of the gastric mucosa is not regulated *en masse* but that various nerves or chemical agents stimulate or inhibit each set of secretory elements separately.[2,3] Even "histamine proved" achlorhydria does not necessarily indicate true anacidity, as the state is frequently transitory. Consequently, the results of *laboratory examinations* are apt to be quite variable but in the average case may be summarized as follows:

1. The gastric residuum contains an excess of thick ropy mucus, especially in hypertrophic and polypoid gastritis.

2. Subacidity is present in about 30 to 40 per cent of cases and anacidity in about an equal percentage. Normal acidity, however, may be observed in about 20 per cent and even hyperacidity in 10 to 12 per cent. Free hydrochloric acid is particularly apt to be reduced or absent because of large amounts of mucin which combines with either acid or base.

3. The total chloride of the gastric residuum (normally about 500 mg. per 100 cc. in terms of Cl) as well as that during the height of digestion (normally about 550 to 600 mg.) is usually reduced to 200 to 300 mg. in the presence of true achylia while it is usually normal in false achylia.

4. Lactic and other organic acids may be present, especially in the presence of pylorospasm or chronic dilatation of the stomach. Bile is usually absent.

5. Large amounts of food remnants from previous meals occur in the gastric residuum. Erythrocytes, pus cells, sarcinae, yeasts and Oppler-Boas bacilli are commonly observed.

6. Pepsin and rennin are usually reduced and may be entirely absent in atrophic gastritis.

PEPTIC ULCERS

Peptic ulcers were so designated originally because peptic digestion of devitalized tissue was regarded as playing an important rôle in their production. They commonly occur in the stomach (*gastric ulcers*) or duodenum (*duodenal ulcers*); in either location they may be acute, subacute or chronic, single or multiple. Frequently, they occur concurrently in both organs. Gastric ulcers may develop anywhere from the cardia to the pylorus but usually on the lesser curvature. Peptic ulcers also occur in the lower end of the esophagus but are rare, as is likewise true of peptic ulcers of the jejunum. The true incidence is unknown but autopsy statistics indicate that approximately 10 per cent of individuals have had peptic ulcers sometime during their lives.

Duodenal ulcers are believed to be responsible for 10 to 12 per cent of cases of chronic recurrent dyspepsias. Among men, anywhere from about 8 to 12 per cent of gastric and duodenal ulcers are associated with chronic cholecystitis, while among women anywhere from 16 to 27 per cent of duodenal and about 14 per cent of gastric ulcers are associated with cholecystic disease. In other words, peptic ulcers appear to predispose to cholecystitis in both sexes and especially women; cholecystitis, however, predisposes to peptic ulcer to a much lesser degree (3 to 4 per cent). Indeed, the clinical manifestations of peptic ulcer are so diverse that many organic, functional, reflex and toxic conditions involving the stomach and duodenum may at some time or other give rise to symptoms closely resembling those of chronic duodenal ulcer. Furthermore, at operation about 45 per cent of duodenal and 35 per cent of gastric ulcers have been found to be associated with chronic appendicitis.

Peptic ulcers may occur at any age, including childhood, especially duodenal ulcers,[4] but most frequently from 20 to 40 years of age. Indeed, acute ulcers in the stomach or duodenum are not uncommon in newly born infants and may be the cause of melena neonatorum; they are frequently multiple and tend to heal rapidly unless death occurs due to hemorrhage.

Peptic ulcers are comparatively rare among the colored. In the United States duodenal ulcers are far more common than gastric ulcers, the proportion being approximately 9 to 1, with roentgenologic examinations revealing the presence of the former in about 17 per cent of patients whose histories are completely atypical of this common disease.[5] For some unknown reason gastric ulcers occur about three or four times more frequently among men than women. Furthermore, chronic gastric ulcers are far more likely to develop carcinoma than duodenal ulcers; indeed, malignant degeneration of the latter is distinctly uncommon.

Etiology. Peptic ulcers are due to the digestive action of acid gastric juice but in spite of a great deal of clinical and experimental investigation their exact etiology is still unknown. At all events, the mucosa fails to withstand the effects of gastric acids which may involve a lack of cellular resistance, the lack of sufficient mucin, the excessive production of hydrochloric acid, particularly during the night and fasting periods, and the possible effects of trauma in some cases. Heredity undoubtedly has an influence in view of the frequency with which peptic ulcers are encountered among members of the same families. Apparently, ischemia of localized areas of mucosa due to arterial spasm or embolism is of fundamental importance followed by the loss of tissue with ulceration or erosion, in which autolytic digestion probably plays a rôle, resulting in hematemesis and sometimes in perforation since the ulcers are always likely to involve the submucosa and muscular layers as well. Virchow originally laid great stress on embolism of branches of the gastric arteries, usually the right. This could be due to bacterial emboli produced during periods of bacteremia in chronic focal infections, or during the acute septicemias, with special reference to streptococci and other micro-organisms. On the other hand, there can be no reasonable doubt about the possible rôle of arterial spasm in the production of localized ischemia and especially since this, as well as functional alterations in the secretory activity of the gastric and duodenal glands, is known to be influenced by stimulation of the autonomic nervous system. At least, peptic ulcers are known to be in etiologic relationship to the high-strung nervous temperament, worry and other emotional states which, in turn, are so likely to produce the bolting of food, and dyspepsia of organic, reflex or functional origin. Indeed, the absence of such factors may well afford an explanation of the comparative rarity of peptic ulcers among the colored. Furthermore, as recently stated by Morrison and Feldman,[6] there appears to be a distinct relation between psychosomatic or constitutional changes of individuals and the etiology and activity of duodenal ulcers characterized by hypersensitivity, hyperirritability and hyperactivity which affect the duodenum, the digestive tract as a whole and the personality as well. The influence of the autonomic nervous system is also indicated by the frequency with which peptic ulcers develop or are activated by war conditions. Indeed, there is urgent need for the elimination of affected individuals from certain phases of war activity along with the rejection of suspects by draft boards.[7,8]

Laboratory Examinations. Undoubtedly, roentgenologic examinations are of more value in the diagnosis of gastric and duodenal ulcers than laboratory examinations although the latter are frequently helpful and may be summarized as follows:

1. The gastric residuum, which normally varies from 30 to 50 cc., is usually increased to about 100 cc. or more due to hypersecretion or pylorospasm.

2. Hyperacidity of the residuum and gastric contents after a test meal is far more likely to be observed in duodenal than in gastric ulcers. Indeed, in the latter the total acidity and free hydrochloric acid may be within normal or even reduced. After histamine stimulation, however, acidity is usually above the normal mean in both gastric and duodenal ulcers. Hypochlorhydria and hypo-acidity due to associated gastritis may occur therefore in about 5 per cent of gastric ulcers but persistent anacidity is usually incompatible with the diagnosis of both gastric and duodenal ulcers. Indeed, it is commonly thought that in peptic ulcers the pain is largely due to free hydrochloric acid which, however, may be only 40 units or less.

3. In the presence of hyperchlorhydria there is likely to be an increase in blood bicarbonate sometimes to the point of producing alkalosis. This is of importance and accounts, at least in part, for the intolerance to alkalies exhibited by some patients. Under the conditions, excessive alkali therapy may increase the carbon dioxide combining capacity of the plasma from the normal of 55 to 60 volumes per cent to 70 volumes or more. Patients with the most active ulcers and the highest acidity are most likely to show the least tolerance for alkalies and the greatest tendency to alkalosis.[9] Renal functional impairment may also be a factor in the retention of base and the development of alkalosis. Following the administration of calcium carbonate, alkalosis may develop with a marked reduction in the serum chlorides and consequent dehydration.[10]

4. Occult blood, due to bleeding, is not infrequently found in both the gastric residuum and the gastric contents after a test meal; under the circumstances it is also found in the feces.

5. Severe hemorrhages may produce a state of azotemia in patients with impaired renal function characterized by a marked increase of the urea nitrogen of the plasma due to the digestion of blood although there is no definite correlation

between the degree of azotemia and the prognosis for recovery.[11] Under the conditions of hypochloremia and azotemia the uriniferous tubules of the kidneys may show deposits of calcium.[12]

6. The majority of patients also show varying degrees of secondary anemia of the hypochromic type, especially those with chronic bleeding.

7. Serum lipase may be increased, especially in duodenal ulcers, due to involvement of the pancreas.

8. Not infrequently there is a high degree of correlation between the amount of pepsin and the severity and intractability of symptoms, although the enzyme may be also increased in tense and nervous individuals without peptic ulcers who present symptoms suggestive of their presence.[13]

CARCINOMA OF THE STOMACH

It has been estimated that in the United States about 27,000 persons die annually of carcinoma of the stomach; in other words, it kills more people than any other cancer of the body, since 18 per cent of the total deaths due to cancer result from this disease. About one-third of all carcinomas of men and about one-fifth of all carcinomas of women occur in this organ. Carcinoma of the stomach is about three times more frequent in men than in women. Unfortunately, the early symptoms may be so indefinite as to result in a long delay in diagnosis. Furthermore, from 25 to more than 50 per cent of ulcerating carcinomas may temporarily give symptoms similar to peptic ulcers. Under the circumstances, any doubtful gastric lesion should be considered malignant until proved otherwise, while those lesions which are considered benign should be kept under suspicion and closely followed in spite of normal acidity, size, or general x-ray and gastroscopic appearances.[14]

Gastric carcinoma is rare in childhood but after the age of 15 years the incidence rapidly increases for each five-year period. About 95 per cent of cases occur in individuals 40 to 70 years of age but the disease is by no means infrequent in subjects under 31 years of age.[15] The great majority of carcinomas of the stomach begin as such but at least 10 to 12 per cent of all gastric ulcers, including many that appear roentgenologically and macroscopically benign, prove to be malignant, including not only prepyloric ulcers, but those in other situations as well.[16] Indeed, a large proportion of lesions in the prepyloric region are ulcerous and malignant. Needless to state, great difficulty is frequently experienced in differentiating between malignant and nonmalignant ulcers by x-ray examinations and in many cases differentiation is impossible. From the pathologic standpoint about five different kinds of carcinomas of the stomach may occur with varying degrees of malignancy embracing (1) carcinoma simplex; (2) ulcerative adenocarcinoma; (3) papillary adenocarcinoma; (4) mucous or colloid carcinoma and (5) scirrhous carcinoma.

Aside from the relationship of gastric ulcers and irritation, the etiology of gastric carcinoma is unknown but it appears that chronic atrophic gastritis with low secretory activity (hypochlorhydria and achlorhydria) frequently antedates and predisposes to the disease.[17,18] Some investigators have contended that the disease never develops in a normal gastric mucosa but always results from atrophic gastritis with hyperplasia and papillomata (Konjetzny). Furthermore, the question of whether or not gastric ulcers develop into cancer is still controversial. This possibility, however, cannot be denied while there is no doubt that gastric cancers may undergo peptic digestion with the production of ulcers which may mimic benign ulcers in every respect.

Almost 90 per cent of cases exhibit less than 30 units of free hydrochloric acid on gastric analysis when the triple histamine test is employed. Under the circumstances, Wangensteen [19] has suggested that this type of gastric analysis is advisable in all individuals over 50 years of age, with follow-up x-ray examinations when hypoacidity is found, as a measure for the early diagnosis and treatment of the disease.[20]

Laboratory Examinations. Gastric carcinoma must always, if possible, be differentiated from such diseases as peptic ulcer, benign tumor, chronic gastritis, gastric syphilis, gastric tuberculosis and, especially when associated with a large crater, from phytobezoar. The various types of gastric sarcoma or lymphosarcoma, malignant polyp, and carcinomatous changes in other forms of benign tumor are regarded clinically as carcinoma. Unfortunately, there are no pathognomonic laboratory examinations for gastric carcinoma but, nevertheless, they are frequently of clinical aid in diagnosis and may be summarized as follows:

1. A low volume of secretion with reduced gastric residuum and in the fractional method of gastric analysis ten-minute volumes seldom above 10 cc., each largely composed of mucus.

2. Free hydrochloric acid is present in about 40 per cent of cases after the injection of histamine, which may be as high as 75 to 100 units, but hypochlorhydria and hypo-acidity are usual and likely to be constant changes. Anacidity even by the histamine method, however, may be present.[21] The majority of patients with peptic ulcer fall within the high-volume, high-acid range while the majority with carcinoma fall within the low-volume, low-acid range.

3. If stagnation is present and the stomach is not washed out 12 hours prior to a test meal gastric analysis, lactic and other organic acids along with Oppler-Boas bacilli are commonly found.

4. Occult blood in the gastric residuum and test meal gastric contents, as well as in the feces, is usually observed and especially in ulcerating lesions.

5. Varying degrees of anemia, usually of the hypochromic type, are usual and particularly in the late stages of the disease.

CARCINOMA OF THE DUODENUM

Duodenal cancers are comparatively rare, even in duodenal ulcers, probably because the duodenum is much less subject to irritation than the stomach. The incidence is apparently as low as 0.01 to 0.03 per cent [22] with even a much lower incidence of carcinoma of the ileum and jejunum. As expected, most cases occur in males. The lesions may be located in the first or supra-ampullary portion

(22 per cent), middle or ampullary portion (66 per cent) and lower, or infra-ampullary portion (12 per cent).

Clinical diagnosis is exceedingly difficult. The same may be true of roentgenologic diagnosis and especially in differentiating carcinomas of the first portion of the duodenum from obstructive ulcers or carcinoma of the pyloric region of the stomach. Laboratory examinations are unfortunately of but little assistance in diagnosis. Free hydrochloric acid is likely to be present in the gastric contents but this may also occur in obstructing pyloric cancer. Obstructive jaundice is particularly apt to be constant and persistent in carcinomas involving the middle of the duodenum and especially the ampulla of Vater. Under the conditions, the van den Bergh and icterus index tests may be of value in its early detection. In carcinoma of the first portion of the duodenum there is likely to be profuse vomiting, due to pyloric obstruction, with bile and pancreatic enzymes (amylase and lipase) present in the vomitus.

SYPHILIS OF THE STOMACH

Late acquired syphilis of the digestive system is very uncommon but when it occurs the stomach is much more frequently the site than the intestinal tract. Syphilis of the stomach may occur in any of the following four types: (1) single gummas, (2) multiple gummas as nodular ulcerative lesions, (3) diffuse nodular infiltrations and (4) as a chronic sclerosis. The last three may closely simulate carcinoma from a roentgenologic standpoint and especially since the lesions occur near the pylorus in nearly every instance.[23,24] There is no conclusive evidence that the lesions undergo malignant change and acute perforation is quite infrequent. About 70 per cent of cases occur in men and about 30 per cent in women in an age range of 30 to 50 years. About 27 per cent of cases present other clinical evidences of syphilis[25] and, as a matter of fact, "stomach trouble" in about 75 per cent of syphilitic patients is due to infection of the central nervous system.[26] Under these conditions it appears advisable to make a neurologic examination along with serologic and spinal fluid examinations before gastric analysis and roentgenologic examinations are employed. Since gastric syphilis may closely resemble carcinoma, both clinically and roentgenologically, it appears advisable to conduct an immediate exploratory laparotomy[27] because if the lesion is found to be syphilitic, treatment is just as effective after operation as before. Otherwise, treatment may be instituted as a therapeutic test.[25]

From the standpoint of *laboratory examinations,* the serologic tests give positive reactions in over 90 per cent of cases of gastric syphilis. In about 25 per cent there is retention with increased gastric residuum due to pyloric obstruction or an hour-glass stomach. Hypochlorhydria is present in about 85 per cent of cases and anacidity may occur even after histamine stimulation. Bleeding and the presence of occult blood in the stomach contents and feces do not occur in more than 5 to 10 per cent of cases. Secondary anemia, however, is not infrequent.

GASTRO-INTESTINAL TUBERCULOSIS

Stomach. Even though the esophagus is greatly exposed to infection by the swallowing of tubercle bacilli in sputum, *tuberculosis of the esophagus* is extremely rare. Furthermore, while tubercle bacilli swallowed in sputum commonly escape destruction by the hydrochloric acid of the stomach, gastric tuberculosis is very uncommon, the incidence in association with pulmonary tuberculosis being less than 1 per cent.[28] As in gastric syphilis, the granulomatous lesions are usually situated in the region of the pyloric antrum. There are no proved cases of primary tuberculosis of the stomach and all reported instances of the disease have been secondary to tuberculosis of the lungs, intestines or other parts of the body.

Clinically the disease may closely resemble gastric ulcer or carcinoma. Diagnosis is best accomplished by gastroscopic and roentgenologic examinations.[29,30] *Laboratory examinations* are of but limited value. Hypochlorhydria is generally observed but the results of gastric analysis may resemble peptic ulcer on the one hand or gastric carcinoma on the other. Blood is frequently found in the stomach contents. Tubercle bacilli may be found in smears, cultures, or by guinea-pig inoculation but such findings are of diagnostic value only in case repeated examinations of the sputum have proved negative.

Intestines. Curiously enough, tuberculosis of the *duodenum* is also very rare [31] although it may become involved by the extension of a gastric lesion. The same is true of the jejunum but tuberculosis of the *ileum* and *cecum* is not uncommon and especially in children (3 to 8 years of age) and young adults. In the case of the former it may be a primary disease resulting from the swallowing of bovine tubercle bacilli in contaminated milk but otherwise is almost invariably secondary to chronic ulcerative tuberculosis of the lungs resulting from the swallowing of human tubercle bacilli in sputum or by hematogenous infection. Sometimes the intestines are involved by extension of tuberculosis from the mesenteric lymph nodes, the peritoneum or tuberculous salpingitis. As a general rule, the lesions are ulcerative but hyperplastic tuberculosis is especially likely to involve the ileocecal valve and cecum, particularly in children, sometimes with involvement of the vermiform appendix and readily confused with regional ileitis.

Laboratory examinations are of but limited value as aids in diagnosis. Tubercle bacilli may be found in the feces and especially by concentration methods of examination including smears, cultures and guinea-pig inoculation tests but positive findings may be due to the swallowing of tubercle bacilli. Their presence in association with blood, mucus and pus, however, is always significant and especially if *S. dysenteriae, E. histolytica, Balantidium coli* and other causes of ulcerative enteritis are excluded.

Anus, Rectum and Sigmoid Colon. *Anorectal fistulas* constitute about 25 per cent of diseases originating in or about the anus and rectum. Various investigators have estimated that anywhere from 2 to 25 per cent of them are tuberculous. About 3.5 per cent of cases of pulmonary tuberculosis develop fistulas. They occur more frequently in men than women and particularly between 20 to 60 years of age. As far as laboratory examinations are concerned, tubercle bacilli may be found in scrapings but diagnosis is best made by the careful examination of tissue removed by biopsy.

The same applies to laboratory examinations in the diagnosis of *anal* and *perianal* tuberculosis in which the lesions may be ulcerative, verrucous, lupoid or miliary in character. It is necessary to exclude condyloma latum by darkfield examinations for *T. pallidum* and epithelioma by the examination of tissue removed by biopsy.

The lesions of tuberculous *proctitis* and *sigmoiditis* are usually ulcerative or hyperplastic in character. Ulcerative lesions constitute about 14 per cent of all cases of intestinal tuberculosis and are almost invariably associated with pulmonary tuberculosis. Nonspecific ulcerative, bacillary, amebic and balantidic enteritis must be excluded with the aid of laboratory methods. The examination of scrapings may reveal the presence of tubercle bacilli.

Hyperplastic lesions are always chronic. Some investigators have thought they may be primary infections. Certainly they are found less frequently associated with pulmonary tuberculosis than ulcerative lesions. Most cases occur in young adults, the disease being rare after 40 years of age. Strictures of the rectum may result. Examinations of tissue removed by biopsy are of most value from the standpoint of laboratory diagnosis, not only in relation to finding tuberculous infection, but for the exclusion of carcinoma and lymphogranuloma venereum. In connection with the latter, intradermal (page 586) and complement fixation (page 515) tests are also of value. Scrapings may reveal tubercle bacilli but negative results never reliably exclude the possibility of the disease.

INTESTINAL OBSTRUCTION

Intestinal obstruction may produce profound changes in the water and electrolytic equilibria of the blood and tissues. These are largely due to excessive vomiting with dehydration sometimes resulting in increased tissue destruction and decreased kidney function unless relieved by surgical measures and the parenteral administration of saline solution. Under the circumstances, high intestinal obstruction involving the pylorus, duodenum or jejunum is more serious in these respects than obstruction involving the lower ileum and colon. There are many causes which may be classified as follows:

- Pyloric
  - Pylorospasm
    - intrinsic or irritative
    - reflex
    - functional
  - Congenital or hypertrophic pyloric stenosis of infants
  - Hypertrophy of pyloric muscle of adults
- Duodenal
  - Carcinoma of duodenum, gallbladder or pancreas
  - Inflammatory adhesions and glands
  - Pancreatitis
  - Twisted mesentery and anatomic abnormalities
- Jejunal
  - Adhesions and ulcers
  - Tuberculosis
  - Congenital bands at the ligament of Treitz

Iliac

- Congenital and acquired strictures
- Obturation and compression
- Adhesions and bands
- Strangulated hernia
  - External
  - Internal
- Volvulus
- Intussusception
- Paralytic ileus
- Spastic or dynamic obstructions
- Masses of helminths
- Mesenteric thrombosis and embolism

Laboratory examinations may not be required for diagnostic purposes but are always helpful in high intestinal obstruction with excessive vomiting and dehydration persisting for twenty-four hours or longer and especially in atypical cases. The principal changes may be summarized as follows:

1. Hypochloremia or a reduction in the sodium chloride of the plasma below the normal of 570 to 620 mg. per 100 cc. due to the loss of the hydrochloric acid of the stomach from excessive vomiting.

2. This results in a corresponding increase in the bicarbonate of the blood. If protracted, the sodium of the serum is reduced below the normal of 315 to 340 mg. per 100 cc. because of excessive elimination in the urine in an attempt of the body to prevent the development of alkalosis. Serum potassium, however, is sometimes increased above the normal of 16 to 22 mg. per 100 cc. because of its absorption from the obstructed bowel.

3. As a result of the loss of plasma sodium chloride, there is present in the blood an excess of base, chiefly sodium and potassium, which is retained in the form of bicarbonate resulting in a disturbance of acid-base equilibrium with the production of alkalosis and the symptoms of tetany. Under these circumstances, the CO_2 combining power of the plasma is increased from the normal of 55 to 65 volumes per cent to 70 to 80 and even to 100 volumes per cent or higher and especially in pyloric and upper intestinal obstruction.

4. When complete obstruction first develops there is initially a fair degree of adjustment in the blood to the loss in chloride, acid ions, base and water, but with failure of the compensatory mechanism occasioned by the continued depletion of the extracellular fluid, the changes in the blood become rapidly progressive. These changes are not invariable. For example, in pyloric obstruction due to carcinoma with hypochlorhydria or anacidity severe vomiting will produce dehydration but the depletion of sodium chloride will be approximately equivalent to the loss of sodium. Under these conditions, alkalosis does not occur. On the contrary, in the presence of starvation ketosis with excessive ketonuria is apt to develop due to diminished carbohydrate utilization or hepatic glycogenesis. This reduces the oxidation of fats with the production of acetone and oxybutyric acid which tend to lower alkali reserve with the production of acidosis. Furthermore, if the intestinal and pancreatic secretions and bile are lost there is also a tendency to acidosis from a reduction in plasma base and bicarbonate while in obstruction of the ileum the loss of sodium chloride with bicarbonate excess are much less in evidence.

5. Dehydration also brings about increased tissue destruction and decreased kidney function largely responsible for azotemia in which the blood urea nitrogen may reach as high as 150 mg. and the total nonprotein nitrogen over 200 mg. per 100 cc. According to some investigators, impairment of renal function is due to the production of toxic substances in the obstructed intestine but this has not been proved. At any rate, azotemia tends to disappear rapidly with the relief of obstruction and the cause of death is still unknown. Unless promptly relieved, dehydration may also produce oliguria with albumin and casts in the urine. Hyperphosphatemia may also occur due to renal insufficiency in which the inorganic phosphates of serum are increased above the normal; likewise hypercholesterolemia which is of serious prognostic import.

6. Severe dehydration with the loss of plasma water may also produce hyperproteinemia, including fibrinogen, without alteration of the albumin: globulin ratio although this is of exceptional occurrence.

7. Severe dehydration may also produce a decrease in plasma volume, hemoconcentration, increased viscosity of the blood, decreased sedimentation of the erythrocytes and a reduction in the oxygen content of the venous blood. However, apparently normal values for erythrocytes and plasma volumes do not necessarily mean that hemoconcentration has not occurred, since these changes may be obscured by anemia or hypoproteinemia present before the occurrence of intestinal obstruction. Abdominal distention may produce leukopenia or "leukocyte exhaustion" in the absence of severe infection; [32] leukocyte exhaustion has also been reported as occurring postoperatively in cases of obstruction.[33]

8. In congenital or hypertrophic pyloric stenosis of newly born infants beginning before the third month of intra-uterine life, the meconium may fail to show the presence of keratinized epithelium in stained smears.[34]

9. Since recurrent pylorospasm may be due to food allergy, skin tests for its detection are frequently indicated. Gastric acidity is variable but hyperchlorhydria is commonly observed.

ACUTE DILATATION OF THE STOMACH

In this grave though comparatively uncommon condition, the stomach becomes greatly, sometimes enormously, distended; its cavity contains large quantities of fluid and gas which may stretch its walls to paper thinness. In the majority of instances it occurs as a sequence of surgical operations and especially those on the biliary tract, stomach or pelvic organs. It may also occur during the course of, or convalescence from, severe and wasting diseases, after childbirth, from errors in diet associated with sudden overloading of the stomach, in association with spinal deformities of various kinds and, apparently, spontaneously without any known direct cause. Age and sex give no exemption but most cases occur in individuals between twenty and thirty years.

The condition is apparently of reflex origin, the initial and essential factor being a paralytic ileus involving the gastric wall and the upper part of the small intestine and may also result from a depression of stomach tonus by ether or chloroform anesthesia.[33] As a result, the stomach becomes greatly distended by the accumulation of its own secretions which it is unable to expel, as well as by the regurgitation of fluids from the duodenum. Even the normal stomach, and also probably the upper part of the duodenum, cannot absorb the fluids which they secrete, so if the gastric secretions do not leave the stomach they simply collect and gradually increase in volume. Gastric hypersecretion, apparently, is not a factor in the early stage although it may become operative later on, since distention of the stomach probably acts as a stimulus to secretion.[33] Some investigators believe that the condition is secondary to obstruction in the terminal portion of the duodenum caused by its compression between the spinal column and aorta posteriorly and the mesenteric root anteriorly; apparently, however, this mechanical obstruction occurs when the dilatation reaches the degree where the dilated stomach, by its pressure, obstructs the third part of the duodenum.[35]

Although vomiting occurs in over 90 per cent of cases, it may be a late manifestation and, as pain disappears, is replaced by the effortless regurgitation of green, black, or brown fluid of offensive odor composed of bile, gastric and duodenal secretions. The stomach, however, does not empty itself and lavage may recover as much as 3 to 4 quarts of fluid. As a result, the sodium chloride of the plasma is reduced with changes in acid-base equilibrium producing alkalosis sometimes resulting in tetany. The results of *laboratory examinations*, therefore, are quite similar to those described in acute high intestinal obstruction. Consequently, continuous suction siphonage of the stomach by the method of Wangensteen, along with the parenteral administration of 1 per cent solution of sodium chloride and 10 per cent glucose are of paramount importance in treatment.

ALIMENTARY TOXICOSIS IN INFANTS AND CHILDREN

The outstanding symptoms of alimentary toxicosis in infants and children are severe vomiting and diarrhea, resulting in dehydration, acidosis and circulatory collapse. Infection accompanying or preceding the condition appears to be the cause. It is particularly apt to occur in the epidemic and other diarrheas of the newborn and infants[36] as well as in mastoiditis and other acute infections.[37] Due to vomiting, hydrochloric acid is lost and there is usually the additional loss of sodium chloride. The loss of acid ions, however, is not compensated by an increase in bicarbonate as in pyloric or upper intestinal obstruction, because considerable amounts of base are simultaneously lost in the diarrheal stools. These circumstances, combined with varying degrees of hemoconcentration, allow for considerable variability in the composition of the blood, but as a rule the total fixed base of the serum is diminished. The blood chloride is also reduced but proportionately less than the base, while the bicarbonate of the blood is reduced most conspicuously with the development of acidosis.

Consequently, *laboratory examinations* usually show the following changes:

1. Reduction in the CO_2 combining power of the plasma which may fall below 30 volumes per cent and, in severe cases, to 10 volumes per cent or even less. The *p*H of the serum is quite variable but frequently falls below the approximate normal of 7.35.

2. Oliguria is common because of dehydration. It may result in a marked de-

gree of azotemia with the total nonprotein nitrogen reaching as high as 200 mg. per 100 cc. of plasma in some cases.

3. Dehydration also tends to give an increase of inorganic phosphate of the serum which normally is present in 4 to 7 mg. (average 5 mg.) in infants and young children. The guanidine of the serum may be increased [38] above the normal of 0.032 to 0.48 mg. (average 0.4 mg.) per 100 cc.

APPENDICITIS

According to some investigators, total and differential leukocyte counts, with special reference to the Schilling method for the detection of a "shift to the left" of the polymorphonuclear neutrophils, are of distinct value in the diagnosis of acute, subacute and chronic appendicitis; [39–41] others have found them of but little or no value as diagnostic aids.[42–44] Much depends on normal standards. For example, the total leukocytes of children vary from 8300 to 10,800 per c.mm. while in adults about 7000 per c.mm. represents the normal. But when it comes to dividing the polymorphonuclear neutrophils into nonfilamented or immature and filamented or mature types, there is no unanimity of opinion on the relative percentages or absolute numbers of the two kinds. Thus, it is stated that the nonfilamented or immature cells may occur normally in 5 to 16 per cent [45] while others put the normal at about 5 per cent.[46] Most observers have adopted the latter. According to Yaguda,[39] a normal percentage of immature cells excludes appendicitis, over 5 but less than 14 per cent mild appendicitis, more than 14 up to 30 per cent diffuse suppurative appendicitis and over 35 per cent perforation with peritonitis. According to Smith,[47] the diagnosis of one out of five cases of acute appendicitis and about one-half the cases of subacute and chronic appendicitis will be missed if dependence is placed on total and differential leukocyte counts. It is to be pointed out, however, that he has based these statements on taking 10,000 leukocytes per c.mm. of blood and 20 per cent nonfilamented or immature and 50 per cent of filamented or mature polymorphonuclears as the upper limit of normal. Under these circumstances, total leukocyte counts about 10,000 per c.mm. with increased percentage of immature and mature polymorphonuclear neutrophils were observed in 78 per cent of cases of acute appendicitis, 34 per cent of cases of subacute appendicitis and 45 per cent of cases of chronic appendicitis. The percentages of immature or nonfilamented cells in the three groups were 84, 53 and 57 per cent respectively. In my experience, taking 20 per cent of immature or nonfilamented polymorphonuclears as the upper limit of normal is too high; I believe that 5 to 10 per cent is more correct and on this basis I have found that differential leukocyte counts based upon dividing these cells into the immature and mature types are of great value in the diagnosis of acute appendicitis at least and are frequently indicative of the disease when the total leukocyte counts are 10,000 or less per c.mm. of blood. Similar results have been observed in *acute diverticulitis*. Smith, Harper and Watson [48] state that the blood sedimentation time is shorter and the rate higher in acute salpingitis than in acute appendicitis but since sedimentation is increased in both. the test has not proved of definite value in differentiating these two diseases.

STEATORRHEA

Steatorrhea refers to an excess of fat in the feces which renders them pale and bulky. Like jaundice, it is not a disease entity but a characteristic manifestation of various diseases of known and unknown etiology. As previously stated (page 267), the total fat of the feces is composed of neutral fat, free fatty acids and fatty acids combined with calcium or magnesium to form soaps. When there is an excess of neutral fat and fatty acids, the feces are apt to be soft, greasy or oily and of a yellow color; an excess of soaps renders them white or gray, salve-like and rancid. Fat curds are soft and whitish while those composed of protein (like casein) are larger, firmer and yellowish. Steatorrhea may be accompanied by diarrhea (fatty diarrhea) in which case, as in tropical sprue, the stools are apt to be frothy. An excess of neutral fat is indicative of a deficiency in the splitting of fats, while an excess of fatty acids or soaps is indicative of deficiency in the absorption of fat.

In older children and adults on unrestricted diets the total fat in dried feces varies from 7.3 to 27.6 per cent per day (average 17.5 per cent) of which neutral fat averages about 7.3 per cent, free fatty acids about 5.6 per cent and combined fatty acids or soaps about 4.6 per cent. Under average conditions, a total fat amounting to more than 25 per cent, neutral fat, exceeding 11 per cent and soaps exceeding 15 per cent of the dried feces are indicative of steatorrhea.[49] Even on a fat-free diet there is a daily excretion of about 2 gm. of fat, the composition of which is very similar to the lipids of the blood. Indeed, it has been shown that under normal conditions the amount of fat in the feces and its composition are to a large extent independent of foods; under the circumstances, it is generally believed that at least a portion is derived from the blood by excretion into the small intestine.

Steatorrhea may be detected, therefore, by an inspection of the feces supplemented by simple laboratory tests. In many instances, however, accurate chemical analysis is required. Under these circumstances, it is advisable to maintain the patient on a known weighed diet and to determine the percentage of absorption, not only of fat, but of nitrogen as well.[50] Determinations of the total amounts of fat in dried stools frequently give the most valuable information. If the Schmidt diet is employed (page 256), the intake of protein is 118 gm., fat 111 gm. and carbohydrate 191 gm., amounting to 2234 total calories. Normally, over 94 per cent of the fat and over 92 per cent of the nitrogen are absorbed.

Classification. Steatorrhea may be (1) *primary* in which the cause is unknown, or (2) *secondary* in which the cause is known and summarized as follows:

Primary
1. Celiac disease
2. Nontropical sprue (idiopathic steatorrhea)
3. Tropical sprue

Secondary
1. Gastro-enteritis
2. Obstructive jaundice
3. Pancreatic disease
   - Pancreatitis
   - Tumors (especially carcinoma)
4. Obstruction of lacteals

In steatorrhea due to gastro-enteritis, obstructive jaundice and obstruction of the lacteals, the excess of fecal fats constitutes the main change from the laboratory standpoint. In steatorrhea due to pancreatic diseases as well as in all primary forms, more or less characteristic changes also occur in nitrogen and carbohydrate metabolism, summarized in Table 129 and shortly to be discussed. According to Thaysen,[51] celiac disease of infants and young children, nontropical sprue of adults and tropical sprue are not only very nearly related but probably identical and to be classified together under the designation of "idiopathic steatorrhea." It appears advisable, however, to consider them as separate entities although

TABLE 129. SUMMARY OF THE DIFFERENTIAL LABORATORY DIAGNOSIS OF THE STEATORRHEAS

Steatorrhea	Laboratory Examinations
Gastro-Enteritis	Largely due to increased intestinal motility with impaired digestion and absorption. Considerable increase of total fat. Slight increase of neutral fat, free fatty acids and soaps.
Obstructive Jaundice	Due to decrease or absence of bile salts. Feces pasty, greasy and light colored. Total fat greatly increased. Neutral fat normal or slightly reduced. Free fatty acids and soaps increased.
Celiac Disease	Etiology unknown. Large pale stools; frothy in diarrhea. Total fat greatly increased. *Free fatty acids and soaps increased.* *Fecal nitrogen usually normal.* Fasting hypoglycemia. Low flat glucose-tolerance curve. Slight to moderate or severe hypocalcemia. Serum inorganic phosphates sometimes slightly reduced. Normal serum phophatase. Hypochromic microcytic anemia.
Nontropical Sprue (Idiopathic Steatorrhea)	Etiology unknown; may be celiac disease continued into adolescence and adult age. Total fat greatly increased; 60 to 80 per cent due to *fatty acids* and about 30 per cent to *neutral fat.* Hypocalcemia usually present. Free cholesterol and cholesterol esters of the plasma normal or slightly reduced. *Fecal nitrogen normal* but may be slightly increased by a large protein intake. Blood urea and nonprotein nitrogen normal unless increased by dehydration due to diarrhea. Fasting hypoglycemia with a low flat glucose-tolerance curve due to faulty absorption of glucose. Hypochromic microcytic anemia common; moderate leukocytosis.

TABLE 129. SUMMARY OF THE DIFFERENTIAL LABORATORY DIAGNOSIS OF THE STEATORRHEAS—(Continued)

Steatorrhea	Laboratory Examinations
Tropical Sprue	Etiology unknown but probably a deficiency disease. Fatty diarrhea with light foamy stools. Total fat greatly increased, largely due to *fatty acids;* low neutral fat. Hypocalcemia but not as marked as in celiac disease and nontropical sprue. *Fecal nitrogen normal* but may be increased by a large protein intake. Blood urea and nonprotein nitrogens may be increased. Hypoproteinemia with reversal of the albumin-globulin ratio due to cachexia. Fasting hypoglycemia. Low flat glucose-tolerance curve. Hypochlorhydria and anacidity in severe cases. Hypo- or hyperchromic anemia, microcytic or macrocytic, changing from one to another spontaneously or under the influence of treatment.
Pancreatic Steatorrhea	Largely due to a deficiency in pancreatic lipase. Total fat greatly increased; largely due to *neutral fat;* free fatty acids and soaps approximately normal. *Fecal nitrogen greatly increased* (azotorrhea) *Large amounts of undigested muscle fibers* (creatorrhea) Hypocalcemia. Hyperglycemia frequent. Glucose tolerance decreased; diabetic type of curve. Glycosuria not infrequent. Blood diastase usually increased. Usually normal hydrochloric acid content of the stomach contents. Simple chronic anemia.

there is considerable evidence to indicate that nontropical sprue of adults may be a continuation of celiac disease of childhood.

Steatorrhea Due to Gastro-Enteritis. If conditions in the stomach and small intestine are normal, the food is digested and absorbed almost completely even though conditions of the colon produce diarrhea. On the other hand, if the food is rushed through the small intestine by reason of increased motility, as in gastro-enteritis, large amounts of undigested and unabsorbed foods, including fats, are apt to appear in the feces. Under these conditions, the total fat may be increased anywhere from 20 to 50 per cent above normal with a slight but definite increase of neutral fat, free fatty acids and soaps.

Steatorrhea Due to Obstructive Jaundice. In steatorrhea due to obstructive jaundice the changes are essentially due to failure in the absorption of fat. The feces are not only characterized by a greasy appearance but are light (clay) colored due to the absence of bile pigments and their derivatives. Under these circumstances, the total fat may reach 30 to 50 per cent of the dried feces. Neutral fat is usually normal or slightly reduced but the free fatty acids and soaps are increased.[49]

Celiac disease or intestinal infantilism (Gee-Herter disease) is a congenital disorder of unknown etiology, characterized by chronic intestinal indigestion (anorexia, abdominal distention, loss of weight, etc.), stunting of growth due to the faulty absorption of calcium as well as of vitamin A resulting in extensive demineralization of the bones (celiac rickets) which sometimes leads to tetany and convulsions and anemia. Most cases occur in children from 9 months to 2 or 3 years of age. According to some observers, the disease may be divided into those without and those with involvement of the pancreas with special reference to cystic fibrosis [52] although Harper [53] maintains that celiac disease may be distinguished from congenital pancreatic steatorrhea. The results of *laboratory examinations* are usually as follows:

1. The stools are usually large, pale, nearly white and offensive although at times of a brownish-greenish or gray color. In the presence of diarrhea, frothy stools are commonly observed. Excessive amounts of mucus may be present.
2. Owing to deficient absorption or an abnormal excretion of fat by the small intestine, the total fat of the feces is increased and especially during "bad" periods of the disease. Usually about 60 to 70 per cent occurs as fatty acids and soaps and only about 30 per cent as neutral fat. However, there may be exceptions in which the neutral fat is found to be the predominating fraction.
3. The nitrogen of the feces, which on an average diet varies normally from 0.5 to 1.0 gm. per day, is usually within normal. However, it may be slightly elevated (1.0 to 3.0 gm.) and especially if relatively large amounts of protein are being ingested apparently due to faulty tryptic digestion.
4. Fasting hypoglycemia (60 to 70 mg. glucose per 100 cc. of plasma) is not uncommon while the sugar tolerance curve is of the "flat blood sugar" type.[51] In this the maximum rise after the oral administration of a standard dose of glucose does not usually exceed 40 mg. and therefore less than normal. It has been abscribed to diminished or delayed absorption of glucose from the intestinal tract; liver glycogen storage is stated to be normal.
5. Slight to moderate or severe hypocalcemia is usually present due to the fact that the excess fatty acids combine with calcium to form soaps.
6. The inorganic phosphates of the serum may be slightly reduced. The serum phosphatase is usually normal.
7. The urine is usually normal although indicanuria is observed in some cases.
8. Anemia is almost always present and usually of the hypochromic microcytic type. The fragility of the erythrocytes is normal.
9. Hypochlorhydria is frequent and achlorhydria may occur in 30 to 40 per cent of cases.[54]

NONTROPICAL SPRUE (IDIOPATHIC STEATORRHEA)

The symptoms and laboratory changes in nontropical sprue or idiopathic steatorrhea (Gee-Thaysen disease) are similar to those occurring in tropical sprue but of lesser degree. The disease is so designated because it occurs in individuals who have never been in tropical countries. It occurs in adolescents and young adults but suggestive symptoms may develop so early in childhood that many observers have thought that it may be celiac disease continuing into adult age. Diarrhea and glossitis are not as pronounced as in tropical sprue and frequently there is but one pale bulky stool per day. Owing to calcium loss, skeletal changes are common and especially osteoporosis; osteomalacia may develop. Roentgenologic examinations frequently show the presence of megacolon. Characteristically, *laboratory examinations* show the following changes:

1. The fat content of the feces varies considerably but is always increased unless the fat content of the diet is greatly reduced. Fats, chiefly fatty acids, may make up as much as 60 to 80 per cent of the dried stool and neutral fat about 30 per cent. The steatorrhea is thought to be due to failure of absorption of fat; [51] at least, the administration of a fat meal is not followed by a normal increase in the blood fat curve. Diminished lipase activity has been noted in some instances but the deficiency is never as marked as in pancreatogenous steatorrhea.

2. Due to the loss of calcium in the feces, hypocalcemia is commonly present. The inorganic phosphates of the plasma are variable but sometimes reduced. Plasma phosphatase is usually normal.

3. The free cholesterol and cholesterol esters of the plasma are at the lower limits of normal or slightly reduced.

4. The nitrogen of the feces is normal although it may be slightly increased if the diet contains large amounts of protein. It is a matter of general experience, however, that a relatively high protein intake (110 gm. or more) is required for the maintenance of nitrogen balance. Normally and in idiopathic steatorrhea the fecal nitrogen usually does not exceed 10 per cent of the intake.

5. Nitrogen retention does not occur. In severe diarrhea with dehydration, however, the blood urea and total nonprotein nitrogens may be increased. Severe cachexia may produce a slight hypoproteinemia with reversal of the albumin: globulin ratio along with a slight increase of the basal metabolic rate due to starvation.

6. As in celiac disease, fasting hypoglycemia is almost invariably present with a low fat glucose-tolerance curve due to faulty absorption. On the ingestion of glucose the curve is of the low flat type. Following the intravenous injection of 20 gm. of glucose dissolved in 50 cc. of distilled water the curve is normal. The alkali reserve is normal or slightly reduced.

7. Except for a possible slight increase of urobilinogen and urobilin, the urine shows no abnormal changes.

8. Anemia may be absent but a hypochromic microcytic anemia is usually present along with moderate leukocytosis. The fragility of the erythrocytes is normal and the icterus index is normal or even slightly reduced. The bone marrow shows no changes.

TROPICAL SPRUE

While Ashford originally thought that tropical sprue was due to infection with a *Monilia* the disease is now generally regarded as a deficiency disease [55] with some points of resemblance to pernicious anemia, pellagra and Addison's disease. It occurs in both sexes and at all ages but about 90 per cent of cases occur in individuals from 20 to 40 years of age. It is quite rare, however, in full-blooded Negroes. There is a marked tendency to remissions and relapses.

Glossitis and stomatitis are usually more pronounced than in pernicious anemia but otherwise the mouth lesions may resemble those observed in pellagra. The abdomen shows dyspeptic distention and roentgenologic examinations usually reveal smooth segmentation of the intestinal loops with an enlarged and redundant colon. Patients are irritable, exacting, disagreeable, exasperating in behavior and stubbornly resistant to treatment. Petechial hemorrhages are common and photosensitive pellagrous rashes may occur. Dusky pigmentation similar to that observed in Addison's disease along with hypotension and emaciation are not infrequent. Muscular cramps, carpopedal spasm and other manifestations of tetany due to calcium deficiency are common. Some patients show signs of subacute combined sclerosis of the spinal cord while others present evidences of peripheral neuritis due to vitamin deficiency. The results of *laboratory examinations* are usually as follows:

1. Fatty diarrhea with numerous light foamy stools.

2. Great increase of total fat in the dried feces largely due to fatty acids but low in neutral fat. The steatorrhea is due to a fault in the absorption of digested fat as there is no deficiency in pancreatic lipase or bile salts.

3. Hypocalcemia is common although not as marked as in celiac disease and nontropical sprue; the plasma inorganic phosphates are normal or slightly reduced.

4. The free cholesterol and cholesterol esters of the plasma are at the lower limits of normal or slightly decreased.

5. The nitrogen of the feces is normal unless there is a large intake of protein.

6. The blood urea and nonprotein nitrogens are usually increased due to dehydration except in instances of impaired carbohydrate metabolism.

7. Hypoproteinemia with reversal of the albumin: globulin ratio may occur due to severe cachexia. A slight increase of the basal metabolic rate may be observed due to starvation.

8. Fasting hypoglycemia with a low flat glucose-tolerance curve due to faulty absorption are almost invariably present.

9. Hypochlorhydria and anacidity are common, particularly if the disease is severe, but free hydrochloric acid may be restored by effective treatment with liver extract. The "intrinsic factor" of the gastric secretion may be absent. The pepsin content parallels that of the free hydrochloric acid.

10. Anemia of the hypo- or hyperchromic type, microcytic or macrocytic, is present, changing from one form to another spontaneously or under the influence of treatment. The platelets and prothrombin are usually slightly decreased. The fragility of the erythrocytes is normal although the icterus index is sometimes slightly increased due to impaired liver function. The bone marrow shows erythroblastic changes and/or megaloblast arrest similar to the changes observed in pernicious anemia.[56]

THE DYSENTERIES

Acute and chronic dysentery may be caused by (1) different strains of *S. dysenteriae* (Shiga, Schmidt, Sonne, Flexner group), (2) *E. histolytica* or (3) *Balantidium coli.* The latter is rare or uncommon in temperate zones including the United States. Whether or not the so-called nonpathogenic amebas may produce symptoms, is still controversial although Rothman and Epstein [57] have recently suggested this possibility. These include not only *E. coli,* the cysts of which are found in the feces of 15 to 31 per cent of normal individuals, but also *Endolimax nana* (10 to 37 per cent), *Iodamoeba bütschlii* (2 to 4 per cent) and *Dientamoeba fragilis* (2 to 4 per cent).

Laboratory examinations are indispensable in the diagnosis and differential diagnosis of the dysenteries, since the clinical manifestations may be so similar that differentiation is not otherwise possible. This applies not only to the acute dysenteries but to the chronic forms as well which may be mistaken for nonspecific chronic ulcerative colitis, shortly to be discussed. The various diagnostic laboratory procedures have been considered in Chapters 12, 15, and 17 and are summarized in Table 130.

TABLE 130. SUMMARY OF THE LABORATORY DIAGNOSIS OF THE DYSENTERIES

Dysenteries	Laboratory Findings
Bacillary	*S. dysenteriae* present in cultures of freshly defecated feces or material obtained through the rectosigmoidoscope. Blood cultures may be positive in acute dysentery; negative in chronic dysentery. Feces contain excessive amounts of mucus and heavy cellular exudates composed of pus cells, epithelial cells, lymphocytes and particularly endothelial macrophages. Blood is usually present but may be so small in amount as to be discovered only by microscopic or chemical examinations. After ten days, agglutination of the Shiga bacillus 1:64 or higher, of the Flexner bacilli 1:128 or higher and of the Sonne bacillus 1:100 or higher is of diagnostic significance. Group agglutination commonly occurs. Antidysentery bacteriophage tests not sufficiently accurate or refined for routine diagnostic use.
Amebic	Commonly due to *E. histolytica; B. coli* dysentery rare in the United States. Vegetative parasites found microscopically in wet or stained preparations of *freshly* defecated stools or material obtained through the rectosigmoidoscope. Positive cultures of *E. histolytica* on special media but inferior in diagnostic value to direct microscopic examinations. Blood and excessive amounts of clear mucus in the stools with scanty cellular exudates and sometimes Charcot-Leyden crystals. Positive complement fixation reactions with antigens of *E. histolytica* of supplementary diagnostic value.

The etiology of nonspecific ulcerative colitis is unknown and it may not be a distinct entity. It is a colitis not due primarily to avitaminosis, the administration of metallic compounds such as mercury, food infections or those due to tubercle, dysentery or cholera bacilli, *E. histolytica, Balantidium coli* or the virus of lymphogranuloma venereum. Subjective and objective relief following the administration of amebacides in cases in which *E. histolytica* has not been found, does not necessarily incriminate the parasite as the etiologic agent in whole or in part. Nor is the disease that type of colitis seen at necropsy as one of the terminal manifestations in nephritis. As discussed in Chapter 15, various micro-organisms have been regarded as its cause but there is abundant and increasing evidence that there is no specific diplococcus, diplostreptococcus or streptococcus primarily responsible [58, 59] although they doubtless play a secondary rôle in etiology. *B. necrophorus* has been regarded as the etiologic agent or an important secondary invader [60] but its relationship to the disease has not been definitely proved. Food allergy may play some rôle in the exaggeration of symptoms but has not been definitely incriminated as the primary cause. In other words, no specific infection has been proved the cause of the disease although secondary bacterial infection may play a rôle in the production of the ulcerative lesions; for this reason the disease is designated *nonspecific ulcerative colitis.* A very attractive hypothesis, however, is that it is initiated by a specific dysentery and maintained by secondary infections. A psychogenic cause has also been suggested as playing a rôle in etiology and particularly since many patients show the presence of personality disorders and neurasthenic manifestations. On the other hand, however, the so-called functional disorders of the colon almost never develop into nonspecific ulcerative colitis. Under the conditions, the most important single diagnostic procedure is an accurate and detailed history supplemented by a physical examination, rectosigmoidoscopy and roentgenologic examinations. *Laboratory examinations* are of aid in diagnosis but chiefly for the exclusion of other causes of chronic colitis; they may be summarized as follows:

1. The presence of excessive mucus in the stools sometimes associated with blood and heavy cellular exudates.
2. Hypochlorhydria or anacidity may be present as an accompanying deficiency without being a gastrogenous cause of the disease.
3. Secondary anemia is not infrequent. The sedimentation rate of the erythrocytes is generally accelerated, except in cases with highly localized lesions, and repetitious determinations at varying intervals are of value as an index of advancement, no improvement in, or regression of, the disease. Blood chemical changes, when observed, are usually only secondary to debilitation.
4. Negative bacteriologic and agglutination tests for bacillary dysentery.
5. Negative examinations of the stools for *E. histolytica* along with negative complement fixation reactions; also negative examinations of the stools for *B. coli.*
6. Exclusion of lymphogranuloma venereum by intradermal tests conducted with the Frei antigen or antigens prepared of cultivated virus, as well as by complement fixation, serum neutralization, mouse inoculation and biopsy examinations. Paulson [61] states that a "bowel antigen" for intradermal tests, prepared from

grossly fecal-free blood, mucus and pus, or from involved tissue, offers a specific and more direct method for the determination of the presence of virus in intestinal tissue or discharge. The technic and interpretation of reactions are identical with those of the Frei test, which should be done simultaneously for comparative purposes. When there is a positive Frei reaction, "bowel antigen" is stated to offer a practical method for determining the connection between such a reaction and the etiology of the intestinal lesion. Even when the Frei reaction is negative, the virus may be present in the intestinal lesions. These are cases of anergy. A negative "bowel antigen" reaction, however, does not determine the absence of the virus as conclusively as a positive reaction indicates its presence.

7. When necessary or advisable, the exclusion of colitis due to lead, mercury, arsenic, aluminum and copper by chemical examinations of the stools.

INTESTINAL HELMINTHIASIS

The small and large intestines are frequently infested with any of a large number of animal parasites. The symptoms produced are not infrequently of a vague and general character and indeed may be absent, especially in adults. Under the circumstances, laboratory examinations are indispensable in diagnosis with special reference to examinations of the feces for parasites or their ova, discussed in Chapter 12. In a few instances serologic tests are of additional diagnostic value (Chapter 17) as well as intradermal tests for acquired allergic sensitization (Chapter 19). In addition to infestment with the protozoa *E. histolytica* and *B. coli* which produce the amebic dysenteries and various intestinal flagellates of lesser importance, a large number of helminths may produce infestment and disease. The more important of these, along with laboratory examinations of diagnostic value, are summarized in Table 131.

DISEASES OF THE PANCREAS

The most common disease of the pancreas is *diabetes mellitus* (discussed in Chapter 29) involving the islands of Langerhans although hyaline degeneration and fibrosis of them are found at necropsy in such a small percentage of cases as to raise the question of whether the lesions may not be the result of diabetes instead of the cause. Fortunately, *acute hemorrhagic pancreatitis* is uncommon and thought to be usually due to the backing up of bile into the pancreatic duct as a result of obstruction of the ampulla of Vater followed by infection with streptococci, *Esch. coli,* or other micro-organisms. *Acute and chronic interstitial pancreatitis* also occur characterized by periductile inflammation and fibrosis usually ascribed to ascending infection from the duodenal or excretory end of the duct. Benign adenomas arising from duct, acinar or island epithelium occur, but adenocarcinoma is by all odds the most common tumor, producing obstructive jaundice in about 80 per cent of cases since the head of the pancreas is most frequently involved. Males are affected more frequently than females and most cases occur between 40 to 60 years of age although the disease may occur in younger individuals. Hyperglycemia is observed in 5 to 20 per cent of cases and metastasis commonly occurs in the liver, regional lymph nodes and lungs, named in the order of frequency. Various *cysts* also occur due to obstruction of the main duct, the smaller ducts or alveoli, due to chronic interstitial pancreatitis as well as to trauma.

Aside from the production of obstructive jaundice, discussed in the succeeding chapter, the laboratory diagnosis of pancreatic disease is based mainly on the detection of changes due to dysfunction. As discussed in Chapter 10, the chief functions of the pancreas are (1) the production of insulin; (2) the production of trypsinogen, rennin and traces of erepsin; (3) the production of amylase (diastase) and small amounts of maltase; (4) the production of lipase and (5) the probable production of an unknown factor essential to life, since the complete loss of pancreatic juice results in death partly because of the loss of electrolytes

TABLE 131. SUMMARY OF LABORATORY EXAMINATIONS IN INTESTINAL HELMINTHIASIS

Parasites	Laboratory Findings
Schistosoma mansoni	Lateral spined ova in the feces. Eosinophilia, leukopenia and anemia. Positive complement fixation and precipitin reactions may be observed.
Schistosoma japonica	Ova in the feces. Eosinophilia; anemia. Positive complement fixation reactions.
Fasciola hepatica	Ova in the feces and bile (B and C). Positive complement fixation and precipitin reactions may be observed. Positive van den Bergh and icterus index reactions due to jaundice. Eosinophilia.
Fasciolopsis buski	Ova in the feces. Leukocytosis due to absolute eosinophilia; neutrophilic leukopenia.
Dicrocoelium dendriticum	Ova in the feces. Positive van den Bergh and icterus index reactions due to jaundice. Eosinophilia.
Heterophyes heterophyes	Ova in the feces. Eosinophilia; no anemia.
Opisthorchis felineus *Clonorchis sinensis*	Ova in the feces and bile. Positive van den Bergh and icterus index reactions due to jaundice. Eosinophilia and anemia in some cases.
Paragonimus westermani	Ova in the feces. Positive complement fixation reactions may be observed. Eosinophilia may occur.

TABLE 131. SUMMARY OF LABORATORY EXAMINATIONS IN INTESTINAL HELMINTHIASIS—(Continued)

Parasites	Laboratory Findings
Diphyllobothrium latum	Operculated ova with or without proglottides in the feces. Severe macrocytic anemia occurs in 0.2 to 0.1 per cent of cases. May be due to coincidental pernicious anemia. Eosinophilia may occur.
Dipylidium caninum	Ova with or without gravid proglottides in the feces.
Hymenolepis nana *Hymenolepis diminuta*	Ova with or without proglottides in the feces. Eosinophilia.
Taenia solium	Ova in the feces; cannot be differentiated from those of *T. saginata*. Differential diagnosis based upon finding gravid proglottides in the feces. Biopsy examinations for encysted larvae (*Cysticercus cellulosae*) sometimes possible. Eosinophilia and secondary anemia may occur.
Taenia saginata	Ova and gravid perglottides in the feces. Early leukocytosis followed by leukopenia; eosinophilia.
Trichinella spiralis	During first period larvae may be found in the dehemoglobinized blood and spinal fluid. During second and third periods encysted larvae in the muscles detected by biopsy examination. Leukocytosis with marked eosinophilia. Positive intradermal reactions after the seventeenth day. Positive precipitin reactions after the twenty-eighth day.
Trichuris trichiura	Ova in the feces. Eosinophilia.
Strongyloides stercoralis	Rhabitoid larvae in the feces; embryonate ova may be found in the feces. Early stage, leukocytosis and marked eosinophilia; chronic stage, moderate lymphocytosis and slight eosinophilia.
Necator americanus *Ancylostoma duodenale*	Characteristic hookworm ova in the feces. Hatched larvae may be found in the feces. Hypochromic microcytic anemia; eosinophilia.
Enterobius vermicularis	Detection of ova by the cellophane anal swab. Ova present in feces in only about 5 per cent of cases. Slight anemia with moderate eosinophilia in some cases. Positive intradermal reactions.
Ascaris lumbricoides	Detection of ova in the feces except in infestments where only male worms are present which may be the case in children. Ova may occur in vomitus. Anemia with eosinophilia not infrequent.

and water, resulting in severe dehydration. Under the conditions, the results of *laboratory examinations* in pancreatic disease may be summarized as follows:

1. Steatorrhea, characterized by such an increase of neutral fat in the feces that it may separate out as a yellowish oil (Table 129). This is largely due to a deficiency in lipase although in obstructive jaundice the total fats of the feces are also increased because of a deficiency of bile salts.

2. The lipase of the serum may be increased in about 37 per cent of cases of cancer of the pancreas and in practically all cases of acute hemorrhagic pancreatitis.[62] This is due to obstruction of the pancreatic ducts and the persistence of the capacity of the acini to produce this enzyme. Consequently, a high serum lipase is strongly suggestive of cancer although normal values do not exclude it.

3. Creatorrhea, characterized by an excess of undigested muscle fibers in the feces, and azotorrhea, characterized by 25 per cent or more of fecal nitrogen, are commonly observed, largely due to a deficiency in the production of trypsinogen.

4. Serum diastase (amylase) is also commonly increased in acute pancreatitis and may reach levels of 1000 or higher.[63] When the increase is moderate it is advisable to determine the urine diastase-blood diastase ratio. If this is lowered to unity or below, the primary cause is deficient renal excretion of diastase. The increase of the diastase level under such circumstances seldom exceeds 500, but in a few cases of exceptionally severe renal insufficiency levels as high as 1000 may be observed.[63]

5. Hyperglycemia with glycosuria and glucose tolerance curves of the diabetic type may be observed due to a deficiency in the production of insulin.

6. Due to fat necrosis in acute hemorrhagic pancreatitis, in which the lipase splits neutral fat into fatty acids and glycerin, with the deposition of the former as needle-like crystals which combine with calcium to form soaps, hypocalcemia is of frequent occurrence and especially between the third and eleventh days of illness.[64] Probably in no other disease is there such sudden demand for calcium, the amount needed depending on the extent of fat necrosis.

7. Of course, in jaundice there is hyperbilirubinemia with acholic stools which may contain blood, deeply bile-stained urine with the absence of urobilinogen, an increase of free cholesterol and cholesterol esters in the plasma, sometimes a decrease of serum phosphatase, a deficiency in blood prothrombin and, in late cases, impairment of liver function, with special reference to the excretion of hippuric acid. Duodenal drainage may show the absence of bile and the presence of bloody mucus.

8. In cancer of the pancreas with involvement of the stomach there may be an increase of gastric residuum with hypochlorhydria or anacidity in about 12 per cent of cases. Otherwise the gastric secretions are likely to be normal.

9. Leukocytosis occurs in acute hemorrhagic pancreatitis. In chronic pancreatitis, simple chronic anemia is usual and megalocytic hypochromic anemia has been reported.[65]

FOOD ALLERGY

Allergy to foods may produce manifestations limited to the alimentary tract; this may be properly designated *gastro-intestinal allergy*. As will be discussed shortly, however, food allergy may produce many other manifestations but, for the sake of convenience, all of these are being considered herewith.

Undoubtedly, food allergy is of very frequent occurrence although its exact incidence cannot be stated. It may certainly occur at any age but is far more frequent in infants and young children than in adults and much more frequent than allergies due to inhalants.

Allergenic Foods. *Allergy to practically any food may occur* but most commonly to wheat, eggs, milk, chocolate, the legumes, white potato, sea foods and meats (particularly pork). In the order of frequency and importance, the cereals include wheat, rice, barley, corn, oats, rye and buckwheat; the meats pork, beef, chicken and lamb; the vegetables beans, peas, white potato, tomato, celery, cabbage, lettuce, cauliflower and carrots; the fruits orange, apple, banana, strawberry and the melons; the nuts peanut, almond, cocoanut, brazil nut, chestnut, walnut, hickory, filbert, pecan, pistachio and hazel nut. Allergy may also occur to various spices and condiments as well as to tea, coffee, soft drinks (rarely) and to food substances employed in the preparation of alcoholic beverages (barley, rye, wheat, etc.). Allergy to honey is infrequent but the manifestations are usually of the gastro-intestinal type. Group reactions to closely related foods are frequently encountered both clinically and according to the results of skin tests.

Clinical Manifestations. The possible clinical manifestations of allergies to foods are many and diverse. For convenience they may be divided into the following groups:

1. *Respiratory,* including perennial allergic or vasomotor rhinitis, asthma and certain types of bronchitis. Although these manifestations occasionally may be due to the inhalation of dusts of various foods, *e.g.*, flour, they usually follow ingestion. Foods are more likely to produce asthma in children than in adults; in the latter food allergy is often responsible for coryzal symptoms.

2. *Gastro-intestinal,* including colic, diarrhea, edema of the lips and possibly of the tongue, canker sores, coated tongue and halitosis, dyspepsia, including epigastric pain and tenderness, sometimes mucous colitis, etc. As stated by Tuft, however, while there is little doubt that foods may produce any of these symptoms in a patient with other manifestations of allergy, the possibility that they are due to foods alone in individuals showing no other evidence of allergy is controversial.

3. *Cutaneous,* including dermatitis, eczema, urticaria, angioneurotic edema and pruritus. Instances of purpura, erythema multiforme and dermatitis herpetiformis due to food allergy also have been reported. Dermatitis occasionally may result from contact with a food allergen as in so-called "baker's eczema" involving the hands. Food allergy as a cause of eczema is most common in infancy and early childhood, decreasing in frequency with age. Allergy to eggs, milk and cereals is particularly frequent.

4. *Neurologic,* with special reference to migraine but possibly including in at least some instances Ménière's syndrome, vertigo, neuralgias, insomnia, lethargy, etc.

Laboratory Examinations. As with other allergies, diagnosis is dependent chiefly on thorough and detailed histories, skin tests and therapeutic trials with elimination diets. Skin tests for diagnostic purposes have been discussed and described in Chapter 19 as well as the leukopenic tests suggested by Vaughan. Because of the frequency with which negative skin reactions are observed, the intracutaneous method is preferred. The proper interpretation of skin reactions, either positive or negative, is very important. Negative skin reactions occur not infrequently even in the presence of definite clinical evidence elicited by the history or therapeutic trials with the elimination diets of Rowe. Even doubtful, slight or delayed reactions should be considered significant until proved otherwise. Corroboration of the specificity of positive reactions should be obtained whenever possible by adequate clinical trial. It is not always easy to prove that a particular food is responsible for a patient's symptoms, even in the presence of a suggestive history or positive skin reactions, because the latter are not always accompanied by specific reagins in the serum and because skin tests are usually conducted with allergens prepared of raw foods which are ingested after cooking.

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DISEASES OF THE LIVER AND BILIARY TRACT

The liver, gallbladder and biliary ducts are subject to many diseases in view of their physiologic importance, especially in reference to the rôle they play in digestion and metabolism. The clinical interpretation of the liver function tests and examinations of bile obtained preoperatively by duodenal drainage has been discussed in Chapter 8 while the practical diagnostic value of bacteriologic examinations of the gallbladder and bile has been discussed in Chapter 15. In this chapter are considered only those diseases of the liver and biliary tract in which laboratory examinations have proved of value as aids in diagnosis and differential diagnosis.

JAUNDICE

Clinical jaundice or icterus implies sufficient yellowish discoloration of the scleras, skin or mucous membranes to be detectable by simple observation alone. It is difficult to detect jaundice in Negroes and mild degrees may escape detection in whites when examinations are made under artificial light. Jaundice is due to an excess of bilirubin in the blood (bilirubinemia) but the latter may exist in the absence of clinical jaundice, being detected only by chemical examinations of the blood serum according to the van den Bergh and icterus index determinations (subclinical jaundice).

As previously discussed (page 115), bilirubin is produced extrahepatically through the hydrolysis of hemoglobin by the reticulo-endothelial and mesenchymal cells of the body. Under normal conditions this bilirubin varies from 0.1 to 0.25 mg. per 100 cc. of serum, as determined by the van den Bergh *indirect* test, which corresponds to 4 to 6 units in the icterus index test. Amounts over and above this are normally excreted into the cholangioles and bile ducts. This excreted bilirubin (sodium bilirubinate) gives a positive *direct* van den Bergh reaction but, not being absorbed under normal conditions, it is not normally present in the blood serum.

Excessive hemolysis, however, results in the excessive production of hemoglobin. If this exceeds the excretory capacity of the liver, hematogenous or hemolytic jaundice may result. In this type of jaundice, therefore, the indirect van den Bergh and icterus index tests show positive reactions with a negative direct van den Bergh reaction. In severe or prolonged hematogenous or hemolytic jaundice, however, the excreted bilirubin may be reabsorbed into the blood because of bile thrombi in the bile ducts (intrahepatic obstruction) or because of toxic permeability or rupture of cholangioles. Under these circumstances, both the direct and indirect van den Bergh tests show positive reactions along, of course, with positive icterus index reactions.

Negative direct with positive indirect van den Bergh reactions, therefore, are characteristic of hematogenous or hemolytic jaundice, especially congenital hemolytic jaundice and chronic hereditary jaundice,[1] provided intrahepatic obstruction does not occur. Negative direct with positive indirect van den Bergh reactions may be likewise observed in the very early stage of hepatogenous or hepatocellular jaundice. Otherwise, however, both tests give positive reactions due not only to failure of the liver cells to excrete bilirubin normally but because of the reabsorption of excreted bilirubin due to intrahepatic obstruction by bile thrombi or necrosis of the cholangioles and bile ducts. Both tests also give positive reactions in extrahepatic bile duct obstruction because the dammed-back bile not only injures the hepatic bile ducts and cholangioles with reabsorption of bile into the blood, but because the liver cells are likewise damaged with a reduction in the excretion of the bile normally brought to them by the blood.

Classification. Many classifications of jaundice have been proposed, based on pathogenesis, etiology, or both. Probably the most suitable for clinical use is that by McNee [2] in which it is divided into (1) *hematogenous* or *hemolytic jaundice* due to the excessive production of bilirubin with a negative direct van den Bergh reaction; (2) *hepatogenous* or *hepatocellular* (*toxic and infective*) *jaundice* due to injury of liver cells with decreased removal of bilirubin from the blood and, in severe damage, escape of bilirubin from canaliculi into blood, with a positive direct van den Bergh reaction after the very early stage; and (3) *obstructive jaundice* which is usually confined to extrahepatic duct obstruction but also to reflux of bilirubin from blocked canaliculi into lymphatics or directly into the blood, with a positive direct van den Bergh reaction. However, not all cases can be satisfactorily included under these three divisions.

Rich [3] has proposed a classification into (1) *retention jaundice* and (2) *regurgitation jaundice* based on pathogenesis and laboratory examinations. Retention jaundice is characterized by the overproduction of bilirubin with subnormal excretion by the liver, resulting in hyperbilirubinemia with negative direct and positive indirect van den Bergh reactions, increased urobilin in the feces and no urobilinogen or bile salts in the urine. Regurgitation jaundice is characterized by a reflux of bilirubin from the canaliculi of the liver into the blood because of obstruction to the outflow of bile or necrosis of liver cells (producing a disruption of continuity of the canaliculi) resulting in hyperbilirubinemia with a positive direct van den Bergh reaction, diminished output of urobilin in the feces and the presence of urobilinogen and bile salts in the urine. One drawback to this classification, however, is the fact that regurgitation jaundice includes those forms due to biliary obstruction and those caused by parenchymal liver damage, while in clinical medicine there is a need for distinction between these two forms of jaundice, since they represent entirely different diagnostic and therapeutic problems.

In 1943 Varela-Fuentes [4] classified jaundice into (1) that due to hyperhemolysis and (2) jaundice due to intercommunication of bile and blood divided into (a) subhepatic and (b) intrahepatic jaundice. Apparently, however, there are cases of hemolytic jaundice which are not caused by hyperdestruction of the blood.

The classification of McNee does not include the jaundice sometimes seen in infants due to a congenital patulous ductus venosus. Otherwise, McNee's classification may be summarized as follows:

Hematogenous or Hemolytic due to:

(1) Congenital hemolytic jaundice.
(2) Erythroblastosis fetalis; icterus neonatorum.
(3) Hemolytic anemias due to incompatible blood transfusions, various bacterial infections, malaria, hemolytic drugs and chemical agents, severe burns, favism, venins, etc.
(4) Pernicious, Lederer's, Cooley's, sickle cell, aplastic and Marchiafava-Micheli anemias.
(5) Internal hemorrhages (cerebral, ruptured ectopic pregnancy, hemophilia, etc.).
(6) Paroxysmal hemoglobinuria.

Hepatogenous or Hepatocellular due to:

(1) Infectious or viral hepatitis (sporadic and epidemic).
(2) Homologous serum jaundice.
(3) Virus pneumonia; yellow fever.
(4) Leptospiral jaundice (Weil's disease); early syphilis; relapsing fevers.
(5) Infectious mononucleosis; Carrion's disease (oroya fever); Rocky Mountain spotted fever.
(6) Ascending hepatitis secondary to cholangitis; abscesses (pyogenic and amebic); echinococcus cysts and other parasitic infestments.
(7) Hepatotoxic drugs and chemical agents.
(8) Eclampsia; acute yellow atrophy.
(9) Hepatic cirrhosis, (Laennec's, Hanot's, Charcot's, syphilitic, postinfectious hepatitis, hemachromatosis, etc.).
(10) Anoxia; passive congestion (congestive heart failure); hyperthyroidism; diabetes mellitus, etc.
(11) Severe or prolonged hematogenous or hemolytic jaundice.
(12) Malignancy; leukemia; Boeck's sarcoid; amyloidosis, etc.

Obstructive (extrahepatic) due to:

(1) Calculi (biliary or pancreatic).
(2) Carcinoma of the liver.
(3) Carcinoma of the gallbladder, common bile duct or duodenum.
(4) Carcinoma and cysts of pancreas.
(5) Strictures of the ducts from congenital atresia (usually of the common bile duct); adhesions; kinks (rare); parasites in the ducts.
(6) Aneurysm of the hepatic or renal arteries.

In an effort to obviate the disadvantages of these different classifications Ducci[5] has classified jaundice into (1) *prehepatic jaundice* divided into (a) hemolytic and (b) nonhemolytic types; (2) *hepatic jaundice* divided into (a) hepatocellular and (b) hepatocanalicular types, and (3) *posthepatic jaundice* divided into (a) completely obstructive and (b) incompletely obstructive types. This classification, therefore, is mainly based in the stage at which bilirubin accumulates in the blood during the process of excretion, whether within the liver or the extrahepatic biliary tract (posthepatic). In the former case it differentiates those instances in which bilirubin has been converted into the direct reacting type (hepatic) or the indirect reacting type (prehepatic). Prehepatic jaundice is characterized by hyperbilirubinemia with a negative or low direct van den Bergh reaction. When not due to overdestruction of blood it has received various designations like "familial cholemia," "familial nonhemolytic jaundice," "hereditary nonhemolytic bilirubinemia," etc.

Laboratory Aids in Differential Diagnosis. Needless to state, laboratory aids in the differential diagnosis of hematogenous, hepatogenous and obstructive jaundice are highly desirable from the standpoint of medical versus surgical treatment, depending on the kind of jaundice and its cause. Certainly laboratory examinations alone are no substitute for thorough histories and physical examinations in relation to diagnosis. Tests for urobilinogen and bilirubin in the urine are the only bedside laboratory examinations for dysfunction of the liver. Strongly positive reactions for urobilinogen (urobilinogenuria) are indicative of diffuse hepatitis with bile entering the intestine; this may occur in as high as 88 per cent of cases of infectious hepatitis.[6] A persistently negative reaction, however, indicates that little or no bile is entering the intestine which is indicative of extrahepatic obstruction so likely to be due to carcinoma. Positive reactions for bilirubin in the urine (bilirubinuria) may also occur in the preicteric and anicteric stages of hepatitis.

The characteristic laboratory findings are summarized in Table 132. The one constant change in all types of jaundice is hyperbilirubinemia. For purposes of routine examinations and diagnosis the following examinations are advisable: (1) direct and indirect van den Bergh tests; (2) total plasma cholesterol and cholesterol esters; (3) plasma albumin and globulin with the A-G ratio; (4) serum alkaline phosphatase; (5) two or more liver function tests, since no one alone is sufficient; (6) urine examinations for urobilinogen and bilirubin and (7) general blood examinations. The results of these at least serve the purpose of orientation; additional tests shown in Table 132 may be then employed if and when necessary or advisable. While impairment of hippuric acid synthesis is an early change in hepatogenous and obstructive jaundice, impairment may also occur in renal failure with the result that it is frequently advisable to determine urea clearance at the same time. Serum alkaline phosphatase is usually within the normal or but slightly increased in hepatogenous jaundice, showing no more than 10 Bodansky units or less,[7,8] although it may be more definitely increased.[9] Usually, however, this phosphatase in hepatogenous jaundice is no higher than 30 King-Armstrong units while in extrahepatic obstructive jaundice it may reach 90 or more units and is seldom less than 30 units. As shown in Table 132, total and differential leukocyte counts are also of value in differentiating between hepatogenous and extrahepatic obstructive jaundice.[10]

Biopsy examinations of the liver are not mentioned in Table 132 but are frequently very helpful in diagnosis and especially in differentiating between intrahepatic obstructive and extrahepatic obstructive jaundice; because of the usual longer duration of extrahepatic obstruction, the morphologic changes of secondary liver changes are more characteristic and pronounced than those observed in intrahepatic obstructive jaundice.

TABLE 132. SUMMARY OF LABORATORY AIDS IN THE DIFFERENTIAL DIAGNOSIS OF JAUNDICE

Type of Jaundice	Laboratory Findings
Hematogenous or Hemolytic	Negative direct van den Bergh reaction. Positive indirect van den Bergh reaction. Positive icterus index. Increase of fecal stercobilin; marked urobilinogenuria. Hypocholesterolemia; increase of plasma fat. Decrease of plasma phospholipid. Marked anemia; methemoglobinemia.
Hepatogenous or Hepatocellular	Positive direct van den Bergh reaction after very early stage. Positive indirect van den Bergh reaction. Positive icterus index. Urobilinogenuria and bilirubinuria; glycosuria in some cases. Decrease of cholesterol esters with increase of total cholesterol. Normal or slight decrease of plasma albumin with an increase of plasma globulin; reversal of A-G ratio. Normal or slightly increased serum alkaline phosphatase. Reduced plasma vitamin A. Positive galactose, levulose, hippuric acid, bromsulfalein, thymol turbidity and cephalin-cholesterol liver function tests. Neutropenia with lymphocytosis. Hypoprothrombinemia not materially influenced by vitamin K. Leucine and tyrosine in the urine in acute yellow atrophy.
Obstructive	Positive direct and indirect van den Bergh reactions. Positive icterus index. Hypercholesterolemia; increase of plasma fatty acid. Marked increase of serum alkaline phosphatase. Normal plasma proteins; hypocalcemia. Normal galactose, hippuric acid, thymol turbidity and cephalin-cholesterol liver function tests except in long-standing cases with hepatic injury. Leukocytosis; neutrophilia with decrease of lymphocytes. Low or negative fecal stercobilin with increased fecal fat. Low or negative urinary urobilinogen with indicanuria.

HEPATITIS

The liver is susceptible to infections with many of the living agents of disease resulting in the production of acute and chronic hepatitis with jaundice usually of the hepatogenous or hepatocellular type. These include not only various bacteria and spirochetes but rickettsiae, viruses, protozoa and helminths. Hepatitis may also occur in diseases of unknown etiology, like infectious mononucleosis. Various drugs and chemical agents may likewise produce "toxic hepatitis." Of course, chronic hepatitis also includes the cirrhoses of the liver which are separately discussed.

The bacteria include staphylococci, various streptococci, the Brucella, *Cl. perfringens*, *Esch. coli* and other gram-negative bacilli. These may produce nonsuppurative hepatitis, suppurative hepatitis or abscesses in the course of bacteremia or septicemia, or a primary cholangitis with secondary hepatitis. The viruses include those producing infectious hepatitis, homologous serum jaundice, yellow fever, primary atypical pneumonia, influenza, smallpox and vaccinia. The spirochetes include not only the leptospira of infectious jaundice (Weil's disease) but those producing the relapsing fevers and syphilis. The rickettsiae include those producing Rocky Mountain spotted fever and other diseases, while the protozoa and helminths producing hepatitis are listed in Table 133. Incidentally, since the terms "infectious hepatitis" and "infectious jaundice" may lead to confusion it appears advisable to designate them respectively as "viral hepatitis" and "leptospiral hepatitis."

Infectious Hepatitis. Infectious or viral hepatitis is a primary infection of the liver with a filtrable virus which has never been successfully cultivated or transmitted to the lower animals. This virus is not only resistant to 56° C. for at least 30 minutes but likewise to dessication, freezing and chemical agents. It occurs in the blood and feces during the disease and is largely transmitted by contaminated water, milk and other foods, although direct or droplet infection may occur. An attack is usually followed by a lasting immunity. Chronic hepatitis may ensue [11] but the so-called "posthepatic syndrome" of the disease has been regarded as a "hepatic neurosis," since the results of liver biopsies have usually shown no histologic evidences of permanent injury.[12,13]

Infectious hepatitis may occur epidemically (epidemic infectious hepatitis) or endemically. The endemic or sporadic disease has been known for generations under the name of "catarrhal jaundice" but its viral etiology was not recognized until World War II. Under the circumstances, the term "catarrhal jaundice" should be dropped as a clinical entity unless reserved for designating the type of jaundice produced by cholangitis due to bacterial infections.

The incubation period of infectious or viral hepatitis is thought to vary from 18 to 40 days. Epidemics usually occur during the fall and winter months and most commonly among children and young adults. There is some evidence that previous exposure to certain drugs, such as the organic arsenicals, may increase susceptibility to the disease or, at least, accentuate its clinical manifestations. The mortality rate is less than 0.5 per cent.

The *laboratory findings* are usually those of hepatogenous or hepatocellular jaundice listed in Table 132, although many cases never develop jaundice and thereby readily escape detection. There are no laboratory tests for the detection of the disease during the prodromal stage. The earliest changes are mild leukopenia with atypical lymphocytes resembling those seen in infectious mononucleosis.[14] The sedimentation rate of erythrocytes is normal or but moderately increased. According to Bohr,[15] the resistance of erythrocytes to hypotonic saline solutions is decreased but Zimmerman and his associates [16] have reported normal resistance in the early phase of the disease with increased resistance in the late stage. Eaton and his colleagues [17] have reported the presence of heterophil antibody in titer of 1:160 or over in 36 per cent of 68 cases, along with positive complement fixation reactions, employing an antigen prepared of human liver, in about 30 per cent of cases. Havens,[18] however, has reported that only 3 per cent of 508 cases showed the presence of heterophil antibody in titer of 1:56 which were reduced to 1:7 or negative by absorption of the agglutinin on boiled guinea-pig kidney; cold agglutinins were found in 0.6 per cent of cases.

Homologous Serum Jaundice. This disease was so named because it followed the administration of human or homologous plasma or serum containing a virus-producing acute hepatitis. Most cases have followed transfusions of plasma, especially pooled plasma, and whole blood. It has also occurred after the parenteral administration of convalescent human serums and vaccines containing human serum, like that formerly employed for active immunization against yellow fever; likewise after inoculations of malarial blood in the treatment of syphilis.[19,20] It may be transmitted by unsterilized syringes and needles contaminated with human blood, plasma or serum containing the virus.

Like that of infectious jaundice, the virus is also resistant to heat, freezing and desiccation and has never been successfully cultivated or transmitted to the lower animals. Under the circumstances, unsterilized syringes and needles employed in the administration of serum, plasma or blood are the only known vectors of the virus, although the oral administration of serum has been reported as transmitting it in one instance.[21] The virus occurs in the blood during the late stage of the period of incubation and during the active stage of the disease, but disappears during the convalescent stage. It also occurs in the feces during the active stage but apparently not in the nasopharynx so that transmission by direct contact or droplet infection probably does not occur.

The incidence following transfusions of plasma and blood has been quite variable. Thus, Scheinberg and his associates [22] have reported 11 cases following 2443 transfusions of plasma or blood (about 0.5 per cent) while Brightman and Korns [23] have reported an incidence of 4.5 per cent in follow-up studies of 649 cases receiving transfusions of pooled plasma. The incidence is usually higher in older than in younger adults and children and bears no relation to the amount of blood, plasma or serum administered.

The period of incubation is much longer than in infectious hepatitis, varying from 40 to 120 days or longer. The clinical manifestations are quite similar to those of infectious hepatitis although the mortality is much higher.[22,24]

Whether or not homologous serum jaundice is due to infection with the virus of infectious hepatitis has not been definitely determined. Available evidence, however, strongly indicates that this is the case, since the properties of the virus are practically identical with those of the virus of infectious hepatitis. The transmission of the virus by human blood, plasma or serum is apparently due to the fact that donors are accidentally and unfortunately selected with clinically inapparent infectious hepatitis without jaundice or other manifestations. The longer period of incubation in homologous serum jaundice may be due to the injection of relatively small amounts of virus, although there is no explanation at present for the higher mortality in this disease than in infectious jaundice. In the case of pooled plasma and stored blood it may be assumed that the virus undergoes some change by prolonged sojourn outside of the body but this does not apply to transfusions of fresh blood.

Needless to state, the occurrence of homologous serum jaundice adds an additional hazard to both plasma and blood transfusion therapy, as discussed in Chapter 27. Certainly individuals with a history of jaundice should not act as donors of blood or plasma for at least a year after the disappearance of jaundice, and pools of plasma should be furnished by no more than two donors. Furthermore, the need for the use of plasma or convalescent serum should be carefully considered because of the danger of transmitting the virus. Ultraviolet irradiation of plasma for destruction of the virus has been suggested as a solution of the problem; [22] in this connection Wolf and his associates [25] have reported that intravenous injections of irradiated human plasma were well-borne in 21 cases with the occurrence of urticaria in one. Prophylactic injections of 10 cc. of gamma globulin may be administered by intramuscular injection on two occasions one month apart, starting a month after a plasma transfusion had been given.

There are no pathognomonic *laboratory findings* in homologous serum jaundice; the usual changes are those observed in hepatogenous or hepatocellular jaundice shown in Table 132.

Toxic Hepatitis. Of course, hepatitis may be produced by the toxins of various organisms but the term is here employed in relation to hepatitis produced by various hepatotoxic drugs and other chemical agents. These include not only arsphenamine, neoarsphenamine and mapharsen, but the sulfonamide compounds, mercury, lead, chloroform, carbon tetrachloride, dinitrophenol, cinchophen, neocinchophen, thiouracil, propylthiouracil, tetrachlorethane, phosphorus, etc. Fortunately, penicillin and streptomycin do not produce hepatitis, although jaundice may occur in the treatment of hepatic syphilis with penicillin, which is ascribed to Jarisch-Herxheimer reactions with exacerbation of the infection.

The usual *laboratory findings* are those of hepatogenous or hepatocellular jaundice listed in Table 132.

CHOLANGITIS

Cholangitis refers to acute or chronic inflammation of the intrahepatic bile ducts while the term *choledochitis* refers to inflammation of the common bile duct. Both vary in severity from simple catarrhs to acute suppuration and are always associated with some degree of occlusion and, in cholangitis, with inflammatory changes in the parenchyma of the liver as well, which vary from simple leukocytic infiltrations of the periportal spaces to abscesses in and about the ducts.

Most authorities now agree that cholangitis affecting the intrahepatic and extrahepatic bile ducts is due to a widespread catarrhal inflammation with partial occlusion and a concomitant hepatitis producing jaundice. Under the circumstances, the latter is partly due to hepatitis and partly to intrahepatic and/or extrahepatic obstruction. Consequently, the disease may be called "catarrhal jaundice" if and when jaundice occurs. As previously stated, however, this term is not permissible for designating the sporadic or endemic type of infectious or viral hepatitis which for generations has been known as "catarrhal jaundice." Little is known of the source and nature of the infection but streptococci, staphylococci and *Esch. coli* are probably responsible for most cases of the disorder.

Suppurative cholangitis is almost always associated with cholecystitis. It is usually due to a lighting up of a chronic infectious cholangitis when there is obstruction to the flow of bile caused by stone, stricture, carcinoma or parasites. It rarely begins as an ascending infection in a nonobstructed duct system, except when there are fistulous connections between the gallbladder or ducts and the gastro-intestinal tract. It may, however, apparently occur through hematogenous infections, *i.e.*, during typhoid fever, influenza, pneumonia, etc. The usual infecting micro-organisms are streptococci, staphylococci, pneumococci, colon bacilli, typhoid bacilli and *Cl. welchii.* A special form has been described (*cholangitis lenta*) due to infection with *Str. viridans,* in which the liver, rather than the heart, bears the brunt of infection, but it is apparently quite rare.[26]

Chronic infective cholangitis may occur as a primary disease in connection with hepatitis or chronic cholecystitis of both calculous and noncalculous origin but in most instances it is associated with stone or a benign stricture of the common bile duct. One type of chronic infective choledochitis and cholangitis encountered at operation is seen most frequently in association with subacute pancreatitis and doubtless represents a more advanced and active stage of ascending biliary tract infection and hepatitis, with the possibility that it arises in the gallbladder, extends by continuity to the common duct and eventually involves the pancreas. A few cases are also on record of *obliterative cholangiolitis* involving the finer intrahepatic ducts with symptoms indistinguishable from those of chronic hepatitis with jaundice or Hanot's cirrhosis of the liver.[27]

Laboratory examinations are helpful in diagnosis. These include (1) examinations of bile obtained by duodenal drainage for physical changes, excess mucus, pus cells, desquamated epithelium and crystals, bacteriologic examinations of smears and cultures, examinations for parasites and (2) examinations of the blood for bilirubin, liver function and other examinations for hepatogenous or hepatocellular jaundice listed in Table 132.

ACUTE YELLOW ATROPHY

Acute yellow atrophy is characterized by severe necrosis of the liver, bearing no relationship to its anatomic arrangement, with severe jaundice and toxemia.

Since the liver is greatly reduced in size with a bright yellow color due to necrosis, fatty degeneration and bile pigmentation, the disease is called *acute yellow atrophy;* because of intense jaundice it is also known as *malignant jaundice* or *icterus gravis*. The necrosis, however, is nonspecific in origin and may have various causes; hence, acute yellow atrophy is a symptom complex rather than a disease entity. It is seen most frequently in association with the severe toxemias of pregnancy and for that reason occurs about ten times more frequently in women than in men. Otherwise, it is comparatively rare although children are not exempt. The disease is highly fatal but may occur in a milder or subacute form, especially affecting the right lobe, in which recovery may occur followed by a diffuse reparative fibrosis usually classified as toxic cirrhosis.

Acute or subacute yellow atrophy may be caused (1) by bacterial toxins during the course of syphilis, typhoid fever, diphtheria, septicemia, cholangitis, etc.; (2) by chemical agents or drugs as chloroform, phosphorus, cinchophen, arsphenamine and other organic arsenical compounds, the sulfonamides, etc., and (3) by endogenous toxic substances occurring in the toxemias of pregnancy and especially eclampsia. Symptoms usually develop acutely. *Laboratory examinations* are of value in diagnosis and may be briefly summarized as follows:

1. Strongly positive indirect and direct van den Bergh reactions. The latter may be biphasic (delayed or prompt) but has the same significance as the direct reaction due to the reabsorption of excreted bilirubin (sodium bilirubinate) because of intrahepatic obstruction of the bile ducts. Of course, the icterus index is strongly positive.

2. The blood urea nitrogen is greatly reduced because necrosis interferes with its formation from ammonia derived from the amino acids during the process of deamination. For this reason, the urea in the urine is also reduced.

3. The cholesterol esters of the blood plasma are reduced, usually associated with a diminution in the total cholesterol, and this is regarded as a measure of the degree or severity of the necrosis. It is apparently due to impaired esterification and storage of esters in the liver.[28]

4. The amino acid nitrogen of the whole blood is usually increased above the normal of 5 to 8 mg. per 100 cc., along with an increase of amino acids in the urine due to failure in deamination and extensive autolysis of the liver.

5. Blood uric acid and glucose are usually reduced below normal, along with reduced glucose tolerance. Hypochloremia commonly occurs.

6. Plasma fibrinogen is usually reduced below the normal of 0.2 to 0.4 gm. per 100 cc., accompanied by hypoprothrombinemia.

7. The bromsulfalein, galactose tolerance and other liver function tests show marked impairment.

8. The urine is bile stained (urobilinuria) and commonly contains casts and albumin. Crystals of tyrosine (due to autolysis) as well as crystals of leucine occur together in about 50 per cent of cases. Tyrosine tends to disappear on recovery and is of good prognostic import. Both tyrosine and leucine, however, may be absent in about 40 per cent of cases.

9. The feces show an excess of fats and an absence of bile pigment and are a pale grayish color. On the other hand, they may be quite dark due to the presence of blood.

THE CIRRHOSES OF THE LIVER

The term "cirrhosis," which was first applied by Laennec, refers to the tawny yellow color of the surface of the liver in portal cirrhosis, but it has since, by common usage, become synonymous with fibrosis of this organ.

Classification. Since the etiology of some types of fibroses or cirrhoses of the liver is uncertain or unknown, they are usually classified according to where fibrosis arises and becomes most marked, as (1) around the intralobular branches of the portal vein, (2) around the biliary ducts in the perilobular tissues or (3) diffusely and anywhere in the liver as the result of productive inflammation and repair. On this basis, a classification of the cirrhoses may be as follows:

Portal	1. Atrophic, alcoholic or Laennec's
	2. With progressive lenticular degeneration (Wilson's disease)
	3. In association with splenomegaly (Banti's disease)
	4. Due to chronic passive congestion
	5. Pigmentary (hemochromatosis)
Biliary	1. Hanot's or hypertrophic (rare)
	2. Obstructive (Charcot's)
	3. Infective largely due to cholangitis
	4. Cholangiolitic due to infectious or viral hepatitis
Diffuse	1. Syphilitic
	2. Repair of acute yellow atrophy
	3. Capsular (perihepatitis)

Etiology. Atrophic or Laennec's cirrhosis may occur in young children but most frequently in adults and about twice as often in men as in women. It is so commonly associated with chronic alcoholism as to be also known as "alcoholic cirrhosis." Just how or why alcohol so commonly produces the disease is unknown but recent investigations have led to the tentative conclusion that it is due to an insufficient protein intake, supplemented by an insufficient intake of some unidentified vitamin B complex factor.[29,30] In some cases cirrhosis is also thought to have been caused by intoxications with the heavy metals (lead, copper, phosphorus, arsenic) as well as by intestinal toxins, either bacterial or enzymic in nature, by way of the portal circulation. Pigmentary cirrhosis is most frequently seen in hemochromatosis due to a disturbance in iron metabolism in which the pancreas, heart and subcutaneous tissues are also involved, the curious bronzing of the skin producing so-called "bronzed diabetes."

Primary or Hanot's hypertrophic biliary cirrhosis, which occurs principally in young children, is very rare and of unknown etiology. Secondary types, which commonly occur in young adults, are due to prolonged obstruction of the large bile ducts by concretions, cancer or adhesions (Charcot's type) or by chronic infections by way of the hepatic artery with special reference to cholangitis. Recently, Watson and Hoffbauer [31] have described a cholangiolitic type of cirrhosis following infectious or viral hepatitis with normal or relatively normal hepatocellular function in the presence of marked regurgitant jaundice. It is characterized by prompt direct (1′) hyperbilirubinemia, hypercholesterolemia, increase of serum alkaline phosphatase and pruritus. This cirrhosis may progress into a type of cirrhosis indistinguishable from ordinary atrophic or portal cirrhosis.

Diffuse types of cirrhosis or fibrosis occur in congenital or acquired syphilis, on recovery from yellow atrophy or as a perihepatitis (capsular cirrhosis) associated with chronic capsulitis of the spleen, chronic proliferative peritonitis and sclerosis of the kidneys.

Laboratory Examinations. Most interest in laboratory examinations is in relation to the aid given in diagnosis between portal and biliary cirrhosis. In both types as, likewise, in diffuse cirrhosis, the liver function tests usually show varying degrees of hepatic impairment along with a decrease in blood diastase. Needless to state, biopsy examinations are of particular differential diagnostic value.

In *portal cirrhosis* of the Laennec type, jaundice occurs in about one-third of cases but is commonly slight and transient. The usual laboratory changes may be summarized as follows:

1. Positive van den Bergh and icterus index reactions of varying degree but usually slight.
2. Normal or but slightly increased serum alkaline phosphatase.
3. Normal plasma cholesterol.
4. Usually hypo-albuminemia probably due to impairment in the synthesis of serum albumin although with a positive nitrogen balance during periods of high protein intake.[32]
5. Usually an increase of serum globulin with a reversal of the albumin: globulin ratio.[33,34]
6. Frequently positive Takata-Ara serum reactions due to an increase of serum globulin.
7. Excessive urobilinuria even in the absence of hyperbilirubinemia.
8. Anemia, usually of the hypochromic type, with achlorhydria.

In portal cirrhosis due to hemochromatosis, glycosuria is present in about 75 per cent of cases along with decreased glucose tolerance. A deficiency in the adrenal cortical and androgenic hormones is usual. As a general rule, the plasma cholesterol is normal although an increase may occur.[35] Diagnosis is greatly facilitated by biopsy examinations of the liver.

In *biliary cirrhosis*, jaundice is always present and frequently very severe. The usual laboratory changes may be summarized as follows:

1. Strongly positive indirect and direct van den Bergh reactions with strongly positive icterus index reactions.
2. Hypercholesterolemia.
3. Increase of serum alkaline phosphatase.
4. Decrease of plasma prothrombin and fibrinogen.

5. Usually normal plasma proteins with no reversal of the albumin: globulin ratio.

6. Usually a negative Takata-Ara serum reaction.

7. Frequently leukocytosis and anemia characterized by macrocytosis [28, 36] sometimes associated with reticulocytosis.

8. A decrease of stercobilin (urobilin) in the feces along with an increase of fats.

9. Marked increase of urobilinogen in the urine.

THE CHOLECYSTITIDES

Cholecystitis and cholelithiasis are among the more common abdominal diseases. For reasons as yet unknown, they occur two to three times more frequently in women than in men although pregnancy may account for this to some extent at least.

Acute cholecystitis, which varies in severity from the acute catarrhal to the acute suppurative and gangrenous types of the disease, may be due to primary infection of the gallbladder. Most frequently, however, it occurs in association with cholangitis or as an acute exacerbation of an antecedent chronic cholecystitis which, in turn, is due to the presence of calculi (acute calculous cholecystitis). In the latter it is frequently associated with impaction of a stone in the neck of the gallbladder or the cystic duct, followed by an ulcerative mucositis with secondary infection rapidly spreading through the wall of the gallbladder.

Infection by extension from an adjacent viscus is rare. The same is probably true by ascension through the common and cystic ducts ("chologenous infection") in view of the normal activity of the sphincter of Oddi and the fact that the duodenum is not only normally sterile but the mucosa of the gallbladder unusually resistant, although infection may occur in the presence of duodenitis. Infection by way of the portal blood and hepatic lymphatics is probably much commoner as is also infection by way of the hepatic artery, while infection of the gallbladder by way of its other venous and lymphatic connections is apparently much less likely.

Chronic cholecystitis is the most common disease of the gallbladder. It may follow an acute attack but more often develops from infection secondary to cholesterosis (with or without papillomas), stones, or obstruction of the cystic or common ducts by adhesions, etc. If obstruction of the cystic duct is complete, the bile pigment is absorbed and the gallbladder distended with a clear fluid secreted by the lining epithelium (*hydrops*). Chronic cholecystitis may also result from a hematogenous infection of the outer wall of the gallbladder which gradually involves the mucosa and produces a chronic noncalculous cholecystitis.

In about 50 to 60 per cent of cases infection is due to bacilli of the colon-typhoid-paratyphoid group although it is frequently caused by staphylococci, streptococci (including *Str. viridans*), pneumococci or *Cl. welchii.* But since the infecting micro-organisms are always likely to be in the wall of the gallbladder, their presence is not always detected by bacteriologic examinations of the bile.

Laboratory examinations are frequently of value as aids in diagnosis. They embrace (1) examinations of the blood, urine and feces for bilirubinemia of hepatogenous or obstructive (extrahepatic) origin previously discussed. Jaundice is not uncommon and when present is more likely to be due to hepatitis or cholangitis (pyelophlebitis with multiple abscesses of the liver is rare) than to obstruction from distention of the gallbladder and Hartmann's pouch with pressure on the choledochus or stones in the biliary ducts; (2) examinations of the bile obtained by duodenal drainage for the absence of the "B" fraction, physical changes, excess mucus, flocculi, pus, excess gallbladder epithelium and crystals, as discussed in Chapter 8; (3) bacteriologic examinations of the bile obtained by duodenal drainage, although the value of these is limited by reason of the chances of contamination in collection as well as by the fact that the bile may be sterile because micro-organisms are always more likely to be in the tissues of the gallbladder; (4) examinations of the blood for leukocytosis, secondary anemia and especially the sedimentation rate of the erythrocytes.

CHOLELITHIASIS

Cholelithiasis is a general term referring to calculi occurring anywhere in the gall tract; *choledocholithiasis* refers to calculi in the common bile duct alone. Over 50 per cent of cases of cholelithiasis show stones in the gallbladder alone and about 6 per cent in the common duct alone; from 13 to 20 per cent show stones in both gallbladder and common duct and 10 to 15 per cent in both gallbladder and cystic duct. Calculi in the right and left hepatic ducts are uncommon and rare in the intrahepatic ducts. In the gallbladder they may vary in number from one to many hundreds. In the common bile duct single stones occur in about 60 to 70 per cent of cases with two to six present in the remainder; they are usually situated at the lower end but are confined to the ampulla in only about 10 per cent of cases.

It has been estimated that 2 to 25 per cent of the adult population have gallstones. They may occur at any age but the incidence in individuals below 25 years is not higher than about 0.4 per cent. Most cases occur after 40 years of age and women are affected two to five times more frequently than men.

Calculi may undoubtedly occur in the gallbladder without the production of ill health but the majority of cases have dyspepsia or other manifestations grouped under the designation of "masked or inaugural symptoms." Certainly there is no relation between the size, number or kind of stones in the gallbladder and the severity of symptoms that may be produced. In over 60 per cent of cases the symptoms are likely to suggest the presence of chronic catarrhal or chronic fibrous cholecystitis. Biliary colic is common—likewise symptoms referable to pressure and mechanical irritation by "fretting" sometimes resulting in ulcerations with hemorrhages. Recurrent infections with cholecystitis, cholangitis and hepatitis are common and especially if the cystic duct becomes occluded. Concomitant pancreatitis is not uncommon. Fistulas may be established with other organs, although the discharge of stones into the intestines rarely causes obstruction. Stones in the common duct can produce every known symptom referable to biliary tract and hepatic disease; diagnosis is difficult in at least 25 per cent of cases.

Composition and Formation of Calculi. From the chemical standpoint the calculi usually occurring in the gallbladder and bile ducts are divisible into four kinds as follows: (1) *mixed* or *mulberry stones* which are the most common—multiple, faceted and largely composed of cholesterol along with calcium carbonate and traces of iron, manganese, copper, lead and other substances; (2) solitary or multiple *cholesterol stones* containing about 99 per cent cholesterol but sometimes coated with bilirubin and traces of calcium carbonate; (3) *pigment stones* composed of bilirubin in combination with calcium and (4) *calcium stones* composed almost exclusively of calcium carbonate and phosphate. Concretions containing considerable amounts of calcium are more easily recognized in cholecystograms than those composed almost exclusively of cholesterol; the latter are usually visualized as transradiant areas if the bile surrounding the concretions is dense.

Apparently calculi are gradually formed over long periods of time but both clinical and experimental data indicate that they may be formed over periods as short as one to three months. Most are formed in the gallbladder but some are formed in the bile ducts as, for example, pure cholesterol stones in the intrahepatic ducts. Pure bilirubin concretions are likewise frequently formed in the intrahepatic ducts. Whether or not calculi can be formed in the common duct alone is still debatable but apparently very unlikely. It appears that they originate in the gallbladder as nuclei of cholesterol with outer layers of bilirubin and calcium with further additions of the latter substances in the common duct. Certainly many are too large to have traversed the cystic duct.

Etiology of Cholelithiasis. In spite of a great deal of investigation the etiology of cholelithiasis has not been completely elucidated. Needless to state, the fundamental mechanism involves the precipitation of cholesterol, calcium or bilirubin of the bile in the gallbladder or biliary ducts. It is practically certain, however, that this does not occur when these substances are present in the bile in normal amounts with a *normal galltract*. Supersaturation of the bile with these substances owing to metabolic disorders favors their precipitation but even under these circumstances it appears that the latter is due to stasis, infection, or other factors producing an initial injury of the structure or functions of the tract and especially of the gallbladder. Consequently, cholelithiasis is rarely a primary disease.

Supersaturation of the bile with bilirubin in hyperbilirubinemia and especially in the chronic hemolytic anemias, may alone result in the formation of bilirubin concretions in the biliary ducts, but even under these circumstances, it is always likely that stasis of the bile due to some obstruction is an important factor. Certainly the latter favors the precipitation of bilirubin as in strictures above the common duct, obstruction of the intrahepatic ducts by cancer or cholangitis, and in the common duct after cholecystectomy. Supersaturation of the bile with cholesterol esters due to hypercholesterolemia in pregnancy, chronic glomerulonephritis, hepatic disease, etc. may result in its absorption by the mucosa of the gallbladder with infiltration of its walls (*cholesterolosis*). But even in this common condition, which may or may not be associated with cholesterol stones, it does not appear that supersaturation alone is responsible but that stasis due to motor dysfunction from spasm of the sphincter of the common duct or of the circular muscular fibers of the neck of the gallbladder are important factors as well as, possibly, low-grade infection in at least some cases. Supersaturation of the bile with calcium in hypercalcemia is apparently very uncommon but even in its absence the precipitation of calcium is apparently largely due to its increased concentration in the bile, from stasis and the absorption of water. At any rate, calcium stones are comparatively rare and produced only when the cystic duct is completely obstructed. Under such conditions, it is thought, calcium carbonate or phosphate is precipitated out from the bile through the absorption of water with the production of soft white concretions or paste. In other words, stasis is predominantly involved in the precipitation of the calcium salts.

It appears, therefore, that supersaturation of the bile with cholesterol, bilirubin or calcium, due to disturbances in their metabolism, favors their precipitation in the galltract and especially in the gallbladder. But even under these circumstances it is evident that local factors in the galltract resulting in a reduction of their solubility, and consequently in their precipitation, are of fundamental importance in the etiology of cholelithiasis. As previously stated, it appears that these local factors alone may produce precipitation even in the absence of metabolic disturbances.

Among these local factors, it seems to me, stasis of the bile is of fundamental importance since it not only results in the absorption of water favoring the precipitation of calcium and bilirubin but of cholesterol as well. Stasis and infection may also be responsible for the reabsorption of bile acids and salts which reduces the solubility of cholesterol and thereby favors its precipitation through changes in the *p*H or other factors.[37,38] Stasis may be due to functional disturbances involving the motor activity of the gallbladder or common bile duct as well as to organic lesions, especially cholangitis and cholecystitis. Indeed, it is not unlikely that this largely explains the rôle of infection or the "lithogenous catarrh" of Naunyn in the etiology of cholelithiasis in addition to the fact that inflammation of the galltract due to infection, bacterial toxins or chemical irritants of exogenous or endogenous origin produce nuclei of desquamated epithelium, pus or mucus upon which cholesterol, calcium or bilirubin may become deposited.

Laboratory Examinations. Unfortunately, there are no pathognomonic laboratory changes in cholelithiasis comparable to cholecystography in the diagnosis of the disease. But the following are not infrequently of value as diagnostic aids:

1. Examinations of the bile obtained by duodenal drainage and especially for excessive amounts of crystals of cholesterol, calcium and combinations of these with bilirubin, as well as for excessive amounts of mucus, epithelium, leukocytes and erythrocytes as discussed in Chapter 8. Unfortunately, chemical examinations of bile obtained preoperatively by this method are not likely to be helpful.

2. Van den Bergh and icterus index determinations of the blood for bilirubinemia are indicated and especially when subclinical jaundice is suspected, since clinical jaundice may be absent as, for example, in about 26 per cent of cases with stones in the common duct. Complete obstruction, however, is absent in about 90

per cent of cases with some bile present in the duodenal contents and feces. Additional blood chemistry and hepatic function tests for differentiation between obstructive (extrahepatic) and hepatogenous jaundice may be helpful as well as examinations of the feces for a decrease of stercobilin and especially of the urine for an increase of urobilinogen. The latter is also likely to show the presence of albumin and casts after attacks of biliary colic.

3. Estimations of blood cholesterol are routinely advisable although cholelithiasis may occur in the absence of hypercholesterolemia.

4. Estimations of blood lipase and diastase are frequently indicated for the detection of pancreatitis, particularly if biliary colic is occurring from choledocholithiasis.

5. Blood sugar determinations are sometimes of value for the detection of temporary hyperglycemia after biliary colic.

6. Renal function tests are advisable, especially in elderly individuals, as renal impairment is not infrequent.

7. Secondary macrocytic anemia is not uncommon. Polymorphonuclear leukocytosis usually occurs in attacks of biliary colic along with an increase of the sedimentation rate of the erythrocytes.

AMYLOIDOSIS

Along with the kidneys and spleen, the liver is commonly a frequent site of secondary amyloid infiltration in diseases characterized by prolonged suppuration and cachexia with special reference to tuberculous and suppurative osteomyelitis, pulmonary tuberculosis, bronchiectasis, pulmonary abscess, chronic empyema, tuberculous enteritis, pyonephrosis, etc., as well as syphilis and cancer. Children and young adults are most frequently affected. The cause is unknown, as is likewise the exact chemical nature of the amyloid substance except that it appears to be protein. Clinical diagnosis is usually not difficult. Jaundice does not occur. The most valuable *laboratory examination* is the Congo red test which has been previously described in relation to amyloidosis of the kidneys (page 706). Additional laboratory changes of helpful diagnostic aid may be nitrogen retention with an increase of blood urea nitrogen probably due to associated amyloid infiltration of the kidneys and a tendency to hyperproteinemia due to an increase of plasma globulin.

PARASITIC DISEASES

The liver and biliary tract are subject to infestment by various animal parasites by way of the blood; these, along with the principal lesions produced and diagnostic laboratory examinations, are summarized in Table 133. The hepatic lesions may be caused by (1) mechanical effects due to blocking of the biliary ducts and pressure; (2) hepatitis and cholangitis due to irritation by ova or larvae; (3) hepatitis and cholangitis due to toxic substances produced by the parasites and (4) secondary bacterial infections. Cirrhosis of the liver is not uncommon and is usually designated "cirrhosis parasitica." Methods of laboratory diagnosis based on examination of the feces, of the bile removed by duodenal drainage and of

TABLE 133. SUMMARY OF LABORATORY DIAGNOSIS OF PARASITIC DISEASES OF THE LIVER AND BILIARY TRACT

Parasites	Lesions	Laboratory Diagnosis
E. histolytica	Abscesses of the liver.	Presence of parasites in aspirated pus of abscesses and feces. Positive complement fixation reactions.
L. donovani	Reticulo-endotheliosis and fatty infiltration of the liver.	Presence of leishmania in the polymorphonuclears and monocytes of the blood and in material removed from the liver by aspiration biopsy. Positive complement fixation reactions. Napier aldehyde and Sia precipitin reactions.
P. vivax *P. malariae* *P. falciparum*	Pigmentation and focal necrosis of the liver.	Presence of plasmodia in the blood and in material removed from the liver by aspiration biopsy. Positive complement fixation reactions.
Fasciola hepatica *Clonorchis sinensis* *Opisthorcis felineus* (liver flukes)	Cholangitis. Pericholangitis. Papilloma and adenoma of biliary ducts. "Cirrhosis parasitica."	Presence of ova in the feces or bile obtained by duodenal drainage.
S. haematobium *S. mansoni* *S. japonicum* (blood flukes)	Hepatitis; abscesses or pseudotubercles. Sclerosis of veins and of liver. Invasion of gallbladder.	*S. mansoni* most likely to produce hepatic lesions. Presence of ova in the feces or bile obtained by duodenal drainage. Positive intradermal reactions. Positive complement fixation reactions.
Echinococcus granulosus	Hydatid cysts in the liver. Daughter cysts in intrahepatic and common bile ducts.	Positive intradermal reactions. Positive complement fixation and precipitin reactions. Examination of cyst contents removed by aspiration biopsy of value but inadvisable because of danger of shock from peritoneal soiling and spreading of the infestment. Leukocytosis with eosinophilia.

material removed from the liver by aspiration biopsy have been discussed in Chapter 12 as well as serologic examinations in Chapter 17 and intradermal tests for acquired allergic sensitization in Chapter 19.

In addition to invasion of the liver by way of the blood, animal parasites may also occasionally invade the common duct, gallbladder and intrahepatic ducts by migration from the small intestine. Thus *Ascaris lumbricoides* sometimes invades the common bile duct and gallbladder,[28] often in association with gallstones, with the production of obstruction, suppurative cholangitis, abscess of the liver and pericholangitic fibrosis. Jaundice is not always produced and the administration of anthelmintics may increase pain and distress. *Taenia saginata* may also invade the common duct and gallbladder although this is of rare occurrence. Undoubtedly *Giardia lamblia* may invade the common bile duct and, possibly, even the gallbladder with the production of catarrhal choledochitis and cholecystitis.

It may be mentioned, however, that infection of the liver and biliary tract with pathogenic yeasts and molds is very rare except in the case of *Coccidioides immitis,* which may infect the liver and produce multiple caseous or cystic granulomatous masses resulting in biliary duct obstruction and periportal cirrhosis as part of the disease designated as *granuloma coccidioides.*

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DISEASES OF THE CARDIOVASCULAR SYSTEM

In general terms, the diseases of the cardiovascular system are divisible into (1) organic and (2) functional disorders. It is with the former that laboratory examinations are chiefly concerned, since there are no laboratory examinations of value in the diagnosis of functional disorders unless they have produced myocardial insufficiency (congestive heart failure). In several of the organic diseases of the heart, however, they possess direct diagnostic value as, for example, blood cultures in the detection of acute and subacute bacterial endocarditis, basal metabolism tests in the diagnosis of thyroid heart disease (thyrotoxicosis and myxedema), serologic tests in cardiovascular syphilis, blood sugar tests in diabetes, etc. Otherwise, laboratory examinations are frequently of helpful diagnostic and prognostic value, *i.e.*, those of the blood for anemia, infections, secondary polycythemia, changes in the sedimentation rate of erythrocytes, anoxia and anoxemia, changes in carbon dioxide tension of the plasma, nitrogen retention, the plasma proteins, hyperbilirubinemia, etc., as well as examinations of the urine including kidney function tests.

On account of the numerous causes of heart disease, however, its diagnosis may be very complicated and especially since two or more separate causes may occasion trouble not only simultaneously but in different and inconstant degrees. As far as possible, diagnosis in each case should include (1) the etiology, (2) structural defect and (3) the functional disorder. For example, instead of diagnosing simply rheumatic heart disease, or mitral stenosis, or auricular fibrillation in a given case, the complete diagnosis should be made such as, for instance, "rheumatic heart disease (etiologic) with mitral stenosis (structural defect) and auricular fibrillation (functional disorder)."

Classification of Organic Heart Disease. As far as organic heart disease is concerned, the causes of more than 95 per cent of cases may be classified as follows:

(1) Congenital anomalies

(2) Infections
- Rheumatic fever
- Pericarditis
  - Acute
  - Chronic constrictive
- Acute and subacute bacterial endocarditis
- Syphilis
- Diphtheria, scarlet fever, tuberculosis, etc.

(3) Endocrinopathies
- Thyrotoxicosis
- Myxedema
- Diabetes mellitus
- von Gierke's disease (glycogenic cardiomegaly)
- Addison's disease, etc.

(4) Arteriosclerosis
- General or systemic
- Localized
  - Coronary sclerosis and thrombosis
  - Cor pulmonale

(5) Hypertension
- Primary or essential
  - Benign
  - Malignant
- Secondary

(6) Miscellaneous
- Disorders of nutrition (beri-beri, etc.)
- Blood disorders
- Hemochromatosis of the heart
- Amyloidosis of the heart
- Trauma
- Thoracic and spinal deformities
- Toxic states other than infections
- Neoplasms

CONGESTIVE HEART FAILURE

Myocardial insufficiency giving rise to congestive heart failure develops eventually and often terminally in more than half of all cases of organic heart disease. It also occurs occasionally in individuals without organic heart disease but with sudden abnormal strain, as in the case of prolonged and extreme paroxysmal tachycardia. The most common causes are mitral and aortic valvulitis, chronic hypertension and myocardial infarction from coronary thrombosis. It is due to myocardial fatigue, as true chronic myocarditis is rare; at least, the anatomic changes in the myocardium itself are seldom sufficient in themselves to cause death. Congestive heart failure affects both sexes but as a rule occurs earlier and more severely in males than in females. About 75 per cent of cases occur in individuals over fifty years of age, although it occurs in younger persons and even in children with severe rheumatic pancarditis.

Passive congestion of the lungs, liver, kidneys, stomach, intestines and brain is the outstanding clinical and pathologic change with the production of edema, mostly interstitial in its site (even in the lungs). If long continued, the edema may result in fibrosis, especially in the liver and kidneys, due to stasis and compression. Venous congestion causes an elevation of capillary pressure which may become much higher than the colloid osmotic pressure of the plasma. As a result, the rate of filtration increases, while at the same time reabsorption of fluid from the tissues is decreased; these factors are of fundamental importance in the production of edema. In addition, it is reasonable to assume that the associated anoxemia may damage capillary endothelium sufficiently to render it more permeable to the passage of the plasma proteins into the tissues although definite proof of this is lacking. Furthermore, the development of edema is favored by the occurrence of hypoproteinemia due to malnutrition, dietary restrictions, nausea and vomiting which lowers the osmotic pressure of the plasma. Other contributory factors may be high sodium chloride and fluid intake, alkalosis and disturbed innervation of the capillaries.

There is a tendency to the retention of sodium chloride. As is well known, the administration of salt tends to increase the edema, while salt restriction aids in the withdrawal of fluids from the tissues and promotes diuresis. But in spite of the shifts of sodium chloride and water to and from the tissues, there may be little or no change in the blood; indeed, the blood chloride and bicarbonate are frequently unaffected although likely to show a wider range of variation than normal. No single factor underlies these variations. However, an increase of blood bicarbonate due to reduced elimination of CO_2 from passive congestion of the lungs may go hand in hand with a decrease of blood chloride while, on the contrary, hyperpnea produces a reduction in blood bicarbonate with an increase of chloride. Consequently, during recovery from congestive heart failure, blood bicarbonate increases and blood chloride decreases with a transient increase in the urinary excretion of sodium chloride.

The diagnosis of congestive heart failure is usually based on the signs and symptoms of the disease. Ordinarily *laboratory examinations* are not required for diagnostic purposes but changes due to chronic passive congestion, with or without edema, are frequently of helpful clinical value, including differentiation between cardiac edema and that due to malnutrition or nephritis; these may be summarized as follows:

1. Hypochromic normocytic anemia is not unusual. Secondary polycythemia, however, may occur, especially in mitral stenosis, due to anoxemia from impaired systemic circulation as well as pulmonary edema. In the presence of anemia, blood viscosity is reduced while in secondary polycythemia it is usually increased. A decrease in the oxygen saturation of the arterial blood owing to pulmonary congestion may be observed. Blood volume may be reduced due to widespread passive congestion, yet the volume of circulating blood may be too great for the strength of the heart, especially if a real hydremia is present.

2. Oliguria with an increase of the specific gravity of the urine, slight albuminuria with casts and hematuria frequently occur because of passive congestion of the kidneys. In patients with edema the sodium chloride of the urine is usually reduced regardless of its concentration in the blood.

3. Urea clearance and other kidney function tests frequently show renal impairment because of passive congestion. For example, the phenolsulfonephthalein test may show an excretion as low as 10 to 20 per cent in two hours as compared with the normal of 60 to 70 per cent. Of course, these changes may be due to an associated nephritis; consequently, it is better to wait until congestive failure subsides before drawing any deductions from renal function tests.

4. There may be some retention in the blood of urea nitrogen, creatinine and uric acid because of passive congestion of the kidneys although, in the absence of marked chronic nephritis, these changes are not pronounced.

5. The plasma chloride may be normal, increased or decreased, the presence of edema being usually associated with a tendency to hyperchloremia which, however, is not invariably present.

6. Hypercalcemia of mild degree (11.5 to 12.7 mg. per 100 cc.) is occasionally found when the CO_2 tension of the blood is increased, thereby enhancing its capacity for maintaining calcium in solution.

7. Acidosis may occur from deficient elimination of CO_2 through the lungs

due to pulmonary congestion and retarded circulation, resulting in a decrease of blood bicarbonate.

8. Hyperbilirubinemia with or without clinical jaundice is not infrequent. This is probably due to insufficiency on the part of the polygonal cells of the liver resulting from anoxemia caused by passive congestion.

9. While heart disease in itself has apparently no effect on the basal metabolic rate, in congestive heart failure values as high as +40 per cent may be observed. This is due partly to the increased activity of the respiratory muscles incident to dyspnea and cough associated with heart failure, partly to the increased oxygen consumption which results from slowing of the circulation in the tissues, and partly to the consumption of increased amounts of oxygen by the myocardium as the result of its hypertrophy and insufficiency.

CONGENITAL HEART DISEASE

Congenital heart disease comprises about 1.5 per cent of all organic diseases of the heart. It occurs in all races irrespective of social status. The cause is unknown except in occasional cases in which an intra-uterine endocarditis is almost certainly responsible. In most cases the anomalies have been ascribed to defects in the germ cells and to adverse influences of maternal origin during pregnancy. Heredity undoubtedly has an influence but the relationship of syphilis is of uncertain importance. Males are affected somewhat more frequently than females. Cases presenting cyanosis of varying degrees of severity are of particular importance, as for instance those with arteriovenous shunt with possible terminal or transient reversal of blood flow and cases of veno-arterial shunt (morbus caeruleus). Cyanosis is due to (1) the shunt of venous blood into the systemic circulation which must be about 30 per cent of the total blood to exceed the threshold for cyanosis; (2) the dilatation of the capillaries of the skin and mucous membranes with peripheral retardation of circulation, and (3) insufficient oxygenation of the blood in the lungs. The results of *laboratory examinations* may be as follows:

1. Leukocytosis due to an absolute increase of the polymorphonuclear neutrophils if infection is present.

2. Secondary polycythemia with an increase of erythrocytes and hemoglobin in cases with moderate to severe cyanosis due to a shunt of venous blood into the systemic circulation because additional erythrocytes and hemoglobin are required for the transmission of sufficient oxygen from the lungs to the tissues.

3. With secondary polycythemia an increase of the total volume and viscosity of the blood may occur as well as increased oxygen capacity which may be almost double the normal while oxygen saturation is nearly halved.

4. A reduction in the carbon dioxide content of the arterial and venous blood, probably owing to increased pulmonary ventilation whereby carbon dioxide, which is thirty times more diffusible than oxygen, is lost in the lungs, as well as to anoxia with tissue acidosis (due to defective circulation) with retention of bicarbonate in the tissues.

5. Oliguria with albuminuria and hematuria may be observed in the more severe types of congenital heart disease, partly due to engorgement of the capillaries because of secondary polycythemia and partly to the frequency of slight to moderate passive congestion from myocardial insufficiency.

6. In cases resulting in myocardial insufficiency (congestive failure), additional changes in blood chemistry determinations incident to edema, passive congestion of the liver and kidneys, etc., may be observed, as previously discussed.

RHEUMATIC HEART DISEASE

Rheumatic heart disease is one of the chief scourges of youth resulting each year in the crippling and killing of large numbers of children and young adults; indeed, it is one of the most serious of all types of organic heart disease. While rare in tropical and semitropical countries, in the colder and temperate climates it accounts for about 40 per cent of all heart disease, affecting all races and females somewhat more frequently than males in the proportion of 4 to 3 or 5 to 4. It is rare before 4 years and almost unknown before 2 years of age, practically all cases occurring between 4 to 50 years with over 90 per cent under 20 years of age. It occurs especially among the crowded poor where malnutrition and unsanitary living conditions prevail. In about 32 to 50 per cent of cases it appears to be a family disease affecting other members of the family or near relatives. This may be due to inherited susceptibility, close contact favoring the transmission of the infectious agent, or crowded unsanitary environment. The disease may apparently also occur in mild epidemics.

Rheumatic heart disease occurs in about 70 per cent of cases of rheumatic fever and 5 to 10 per cent of cases of chorea without rheumatism. The rheumatic fever may occur without involvement of the joints or muscles and without chorea, or be so light and evanescent as to produce so-called "growing pains." The disease may be acute, subacute or chronic. Pericarditis occurs in about 7 per cent of cases, when the disease is designated *pancarditis*. The mitral valve alone is involved in about 62 per cent of cases, the aortic valve alone in about 5 per cent and both valves in about 33 per cent. Involvement of the pulmonary and tricuspid valves is quite rare.

The cause of rheumatic fever and its associated rheumatic heart disease is still uncertain. Infection with streptococci, especially hemolytic streptococci, would appear to be responsible for the disease and certainly for recurrent attacks and recrudescences of the infection. The portal of entry appears to be the upper respiratory tract during attacks of tonsillitis, scarlet fever, etc. The possibility of the cause of the disease being a filtrable virus has been suggested but the evidence is inadequate at the present time. Whether or not the joint and cardiac manifestations are due to actual infection with streptococci, or the result of acquired allergic sensitization to them, is as yet unknown as discussed more fully in Chapter 15. Blood cultures sometimes show the presence of streptococci. When this occurs in recurrent attacks of rheumatic heart disease it may readily lead to a diagnosis of subacute bacterial endocarditis. Probably some cases of alleged recovery from the latter under sulfonamide or other types of therapy were really cases of recurrent rheumatic heart disease rather than subacute bacterial endocarditis.

The results of *laboratory examinations* may be summarized as follows:

1. Hypochromic normocytic anemia is of usual occurrence. In acute rheumatic heart disease a leukocytosis of 10,000 to 15,000 due to an absolute increase of the polymorphonuclear neutrophils commonly occurs.
2. The sedimentation rate of the erythrocytes is usually increased in proportion to the activity of the disease and may be the only evidence persisting in a chronic low-grade infection. It has, therefore, a relationship to the activity and prognosis of the disease.
3. Blood cultures by the massive method may show the presence of streptococci but are usually negative.
4. Oliguria with albuminuria and hematuria is common during acute rheumatic carditis.
5. Positive skin reactions have been reported as a frequent finding when the filtrates or nucleoproteins of hemolytic streptococci have been injected intradermally but these are now known to be nonspecific, as they may be due to hemolytic streptococcus infections without rheumatic fever.
6. In congestive heart failure additional laboratory changes may be observed, as previously discussed.
7. Cephalin-cholesterol flocculation tests for hepatic dysfunction have been reported as giving positive reactions in 72 per cent of 136 cases with no relation to the severity of the disease.[1]

ACUTE AND SUBACUTE BACTERIAL ENDOCARDITIS

Two types of heart disease have been called *acute* and *subacute bacterial endocarditis* respectively because of their clinical manifestations. They differ only according to the virulence of the infecting micro-organism and the duration of the disease. An arbitrary borderline of two months has been set between them. Both are highly fatal. They would be designated best according to the nature of the infection. For example, "*Staph. aureus* endocarditis" or "pneumococcus endocarditis" is preferable to "acute bacterial endocarditis" as a diagnosis and "*Str. viridans* endocarditis" is a better term than "subacute bacterial endocarditis."

Acute Bacterial Endocarditis. This disease consists of an acute nonrheumatic infection of the endocardium either uncomplicated or as part of some other acute illness. It is attended by the symptoms and signs of an acute infection ending in death or recovery in the course of two months. It is uncommon; cases in which it occurs are well under one per cent of all types of heart disease and of all types of endocarditis excluding the very fresh endocarditis of slight or moderate degree discovered at necropsies in individuals dying of a great variety of diseases.

Although acute bacterial endocarditis is more likely to occur in individuals with hearts previously damaged by rheumatic fever, congenital defects or arteriosclerotic valvulitis (about 60 per cent), it may involve the normal heart. The disease occurs at any age from intra-uterine life or infancy to old age, but most frequently after 40 years. Males are affected (about 73 per cent) more frequently than females (about 27 per cent). The infecting micro-organisms are usually hemolytic streptococci, *Staph. aureus,* pneumococci, gonococci, meningococci or *Esch. coli.* The rarer infections may be due to *S. typhosa, Str. viridans, M. tetragenous, H. influenzae, Past. pestis,* the Brucella or *Actinomyces bovis.*[2] These micro-organisms usually enter the blood and infect the endocardium during such acute illnesses as pneumonia, puerperal endometritis, gonococcal arthritis, abscesses, tonsillitis and quinsy, meningitis, etc. Instances of recovery from acute pneumococcal endocarditis have been reported.[3,4]

Laboratory examinations usually show the following changes:

1. Repeatedly positive blood cultures which are diagnostic when accompanied by the usual clinical signs and symptoms of the disease.
2. Secondary hypochromic normocytic anemia which develops rapidly but is not usually as severe as in subacute bacterial endocarditis.
3. Marked leukocytosis due to an absolute increase of the polymorphonuclear neutrophils.
4. Marked increase in the sedimentation time and rate of the erythrocytes.
5. Frequently hyperbilirubinemia of mild degree, with positive icterus index and van den Bergh reactions.
6. Sometimes oliguria with albuminuria and pronounced hematuria due to acute focal glomerulonephritis.

Subacute Bacterial Endocarditis. This disease occurs much more commonly than acute bacterial endocarditis, constituting from 1 to 2 per cent of all heart disease in the colder and temperate zones and affecting about one out of every 25 to 50 cases of rheumatic heart disease. When untreated it is almost invariably fatal after a long and lingering course. It occurs at any age but most commonly between 15 to 40 years; males are affected more frequently than females.

The chief predisposing cause is chronic heart disease, particularly old rheumatic valvulitis (in about 80 per cent of cases) and congenital cardiovascular disease (about 5 per cent), especially those with bicuspid aortic valves. It is stated to affect hearts previously undamaged, but personally I have not seen this occur in a series of 135 cases during the past twenty years. Rarely, aortic valves damaged by syphilis may be affected. In at least 90 to 95 per cent of cases the disease is due to infection with *Str. viridans* usually of focal origin with special reference to dental, tonsillar or sinus infections, as discussed in Chapter 15. In the remaining 5 to 10 per cent the disease may be caused by the gonococcus, *H. influenzae, H. parainfluenzae,* anaerobic streptococci or *S. typhosa* but in all of my 135 cases *Str. viridans* was identified as the cause by repeatedly positive blood cultures. Mycotic subacute bacterial endocarditis due to infection with *Candida parakrusei* and *C. guilliermondi* recovered in blood cultures are exceedingly rare but about five cases have been reported.[5,6] All have occurred in drug addicts taking heroin intravenously with the possibility and probability of the cause of infection being accidental skin contamination.

Laboratory examinations, with special reference to blood cultures, possess a high degree of diagnostic value. Indeed, I do not believe that a diagnosis of subacute bacterial endocarditis is warranted unless two or more positive blood cultures are observed. Laboratory examinations may be summarized as follows:

1. Repeatedly positive blood cultures. Several may be required before positive cultures are obtained and especially during the early stages of the disease.
2. Moderate to severe secondary anemia of the hypochromic normocytic type ultimately develops in practically all cases. Blood smears may show achromia but only rarely polychromatophilia. In a small percentage of cases (10 to 15 per cent) large endothelial phagocytic cells may be found of helpful diagnostic value, although they sometimes occur also in other diseases.
3. Polymorphonuclear leukocytosis of a slight to moderate degree is common but sometimes does not occur.
4. The sedimentation time and rate of the erythrocytes are increased.
5. The platelets are usually numerically within normal; purpura is usually due to increased capillary permeability or capillary embolism.
6. The serologic tests for syphilis sometimes yield temporarily nonspecific positive reactions, especially flocculation tests.
7. Hyperbilirubinemia, with or without clinical jaundice, is frequently observed. This is partly due to increased intravascular hemolysis with positive icterus index and van den Bergh reactions.
8. Owing to focal glomerulonephritis the urine frequently shows the presence of hematuria and albuminuria along with changes in blood chemistry (azotemia, etc.), as discussed in Chapter 23.

CARDIOVASCULAR SYPHILIS AND ANEURYSM

Cardiovascular syphilis ranks next to neurosyphilis or may equal and even exceed it as the most serious type of chronic acquired syphilis, both as a crippling and a killing disease. No wonder, therefore, that proper emphasis is placed on the thorough and adequate treatment of early syphilis, for it is thereby much easier to prevent than to cure after the development of advanced lesions. Indeed, of all the organs of the body *T. pallidum* in acquired syphilis most frequently invades and infects the tissues of the central nervous and cardiovascular systems, and commonly both; for example, it has been estimated that 10 to 35 per cent of cases of clinically recognized cardiovascular syphilis show asymptomatic or symptomatic evidences of neurosyphilis while from 20 to 25 per cent of cases of paresis and 15 to 50 per cent of cases of tabes dorsalis show clinical evidences of cardiovascular syphilis.

Cardiovascular syphilis may occur at any time from infancy to old age but is rare in congenital syphilis irrespective of race or sex; most cases are observed in individuals from 40 to 60 years. On account of greater physical activity the incidence is higher in men than in women, the proportions have been estimated as 2 to 1 and even as high as 5 to 1. The disease occurs at least twice as frequently among Negroes as whites, with aortic insufficiency as the most common lesion.[7]

T. pallidum shows a special predilection for the aorta and particularly its ascending portion. On the basis of necropsy studies, it has been estimated that aortitis may occur in over 90 per cent of cases of untreated or inadequately treated late syphilis. Clinically detectable cardiovascular syphilis, however, occurs in from only 5 to 10 per cent so that about 90 per cent of cases of acquired syphilis never show clinical manifestations of the disease. Uncomplicated aortitis is a diffuse chronic inflammation involving primarily the supravalvular portion, with or without dilatation, and occurs in 5 to 10 per cent of cases. According to Moore, Danglade and Reissinger,[8] it may be detected by (1) teleroentgenographic and fluoroscopic examinations for aortic dilatation; (2) increased retromanubrial dullness; (3) a history of circulatory embarrassment; (4) a tympanitic, bell-like, tambour accentuation of the aortic second sound; (5) progressive cardiac failure; (6) substernal pain and (7) paroxysmal dyspnea. Most physicians, including myself, however, question whether the diagnosis of uncomplicated syphilitic aortitis can be usually made unless the coronary ostia are involved.[9-12] Aortic insufficiency occurs in about 1 to 2 per cent of cases and syphilitic aortitis is the most frequent single cause of this valvular defect.

By reason of obliterative endarteritis of the vasa vasorum resulting in chronic inflammation of the median coat and destruction of its elastic tissue, *aneurysms* of the thoracic aorta occur in about 1 to 2 per cent of cases. Aneurysms, as well as thromboses, may also develop in other arteries but very infrequently. As far as the aorta is concerned, however, syphilis is always to be suspected as the cause of aneurysm unless proved otherwise. Chronic myocardial ischemia due to sclerosing lesions of the ostia of the coronary arteries without aortic valvulitis, and a very small group of cases of true syphilitic myocarditis, together constitute from 0.2 to 0.5 per cent of cases. Insufficiency of the pulmonic valve, heart block and coronary sclerosis may occur but are rare.

From the standpoint of *laboratory examinations* only the serologic tests are of clinical value in diagnosis. Since biologic true positive reactions may be due to syphilis of parts other than the cardiovascular system, they are, therefore, only serologic evidences of the presence of the disease. Under the circumstances, diagnosis must be based on clinical, roentgenologic and other examinations. Positive reactions occur, however, in over 90 per cent of cases of cardiovascular syphilis. Negative reactions should never be permitted to override clinical judgment. Even after adequate therapy has resulted in permanently negative serologic reactions, the signs and symptoms of uncomplicated aortitis, occlusion of the coronary ostia and aneurysms may continue but there is every hope and expectation that further progress may be prevented or delayed. Seroresistance or "Wassermann-fastness," however, is of frequent occurrence; indeed, in these respects cardiovascular syphilis ranks next to neurosyphilis.[13-16] Under the circumstances, the former should always be suspected in seroresistant cases if the latter can be excluded. In most cases seroresistance is due to a combination of both. In my opinion, cautious and moderate treatment comprising at least two courses per year should be continued in some cases for the balance of life.

Colloid goiter and *simple adenomas* of the thyroid gland cause no trouble with the heart or circulation unless the gland becomes so large that pressure on veins and arteries impedes the entrance of blood into or out of the heart. Thyrotoxicoses due to the overproduction of thyroxin, however, may materially affect the heart, as may myxedema and cretinism due to an absence or reduction in the secretion of thyroxin.

Thyrotoxic Heart Disease. Thyrotoxicosis or hyperthyroidism caused by the overproduction of thyroxin in exophthalmic goiter or toxic adenomas frequently results in the production of "thyroid heart disease," more properly designated *thyrotoxic heart disease*. The incidence varies in different parts of the world but in the United States has been estimated to cause from 3 to as high as 11 per cent of cases of organic heart disease. Thyrotoxicosis occurs more frequently in regions where there is a high incidence of simple goiters, due to a deficiency of iodine in foods and water, which in later life may change to toxic adenoma. Heredity may play a part in its etiology but race has little or no influence. Social and economic status, however, has an influence as, likewise, the education and intelligence of the laity in relation to its early diagnosis and adequate treatment—factors of great importance in the prevention of this type of heart disease. The age at which it occurs varies from 5 to 76 years but the most common age of onset is from 20 to 40 years. Females are affected about five times more frequently than males.

The mechanism by which thyrotoxicosis affects the heart may be dependent on three factors as follows: (1) a general increase of body metabolism producing overactivity of the heart or tachycardia; (2) the possibility that the heart itself is the seat of specific thyroxin stimulation with local increased metabolism favoring hypertrophy and congestive failure, and (3) a possible kind of arteriovenous shunt of blood through the widely dilated vessels of the thyroid gland favoring hypertrophy.[17]

As far as *laboratory examinations* are concerned, the characteristic finding is (1) an increase of the basal metabolic rate. This varies considerably in individual cases but + 50 to + 75 is not infrequent, while + 20 to + 30 demands close scrutiny for signs of thyrotoxicosis. In doubtful cases one or two determinations are inadequate for diagnostic purposes. In this connection it should be remembered that congestive heart failure alone may definitely raise the metabolic rate, as previously discussed. The possible relation of the advent of arteriosclerosis to the decreased activity of the thyroid gland so commonly associated with advancing age has been suggested.[18] Otherwise the laboratory changes are those observed in thyrotoxicosis in general, as discussed in Chapter 30, embracing (2) a tendency toward fasting hyperglycemia with glycosuria and decreased glucose tolerance; (3) hypocholesterolemia; (4) increased blood and urine iodine with increased iodine tolerance; (5) decreased plasma fat and fatty acids with increased alimentary lipemia and (6) hypochlorhydria or achlorhydria.

Myxedema Heart Disease. Hypothyroidism due to myxedema of adults or cretinism of children is only infrequently the cause of appreciable heart disease, although some abnormality in cardiac function is evident in almost every case.

Enlargement of the heart due to mucoid infiltration of the muscle is the usual finding in severe myxedema; generally it subsides rapidly under thyroid therapy. Arteriosclerosis with hypertension is also of frequent occurrence in myxedema; it sometimes produces chronic valvulitis and angina pectoris which may terminate in congestive heart failure. Myxedema is due to a deficiency or lack of secretion of thyroxin but, except in those cases caused by partial or total thyroidectomy, the etiology is unknown.

Myxedema heart disease may occur in young individuals but usually in middle age or later. It is likely that coronary sclerosis developing independently or favored by myxedema, may help to account for the greater frequency of cardiac dilatation in older persons. Apparently, sex has no relationship to the incidence of the disease.

From the standpoint of *laboratory examinations* the chief change is (1) a reduction in the basal metabolic rate to −30 or below. In borderline cases with −10 to −25, heart disease is frequent and true myxedema very rare. As discussed in Chapter 30, in relation to myxedema itself, additional changes may include (2) a tendency to fasting hypoglycemia with increased glucose tolerance; (3) hypercholesterolemia; (4) decreased blood iodine and (5) increased plasma fat and fatty acids.

Heart Disease Due to Other Endocrinopathies. Diabetes mellitus does not cause heart disease directly but frequently does so indirectly by producing arteriosclerosis with hypertension and therefore, secondarily, coronary sclerosis and thrombosis. At least 50 per cent of all diabetics die as a result of coronary occlusion or congestive failure. Marked atherosclerosis of the aorta with considerable dilatation is of common occurrence. Among men and women as a group occlusive peripheral arteriosclerosis develops much earlier and has been found approximately eleven times more frequent among diabetic than among nondiabetic individuals. It occurs about eighty times more frequently among diabetic than nondiabetic women.[19] Incidentally, it has been recently suggested that dermatomycosis may bear an etiologic relationship to thromboangiitis obliterans[20,21] while tobacco smoking is stated to produce a greater reduction in the oxygenation of the arterial blood in cases of arteriosclerosis than in normal individuals.[22]

An excess of insulin (*hyperinsulinism*) apparently does not seriously affect the heart unless heart disease is present, although it is stated that angina pectoris may occur due to a deficiency of carbohydrate in the myocardium. Insulin shock, however, may not only produce tachycardia and various arrhythmias but is dangerous in the presence of acute coronary thrombosis, severe angina pectoris, and congestive heart failure.

Hypoparathyroidism with hypocalcemia and tetany may gradually result in cardiac hypertrophy due to an increase in the duration of the systole. While *hyperparathyroidism* with hypercalcemia may increase the calcium content of the heart muscle, there is no proof that this is of any clinical significance.

Hypopituitarism is stated to cause microcardia and congenital heart block. *Hyperpituitarism,* however, is more important, as it commonly produces cardiac hypertrophy in acromegaly, especially of the left ventricle. Whether it is the result of a somewhat increased basal metabolic rate or due to some other factor, cannot be stated. Valvular sclerosis, coronary sclerosis and systemic arteriosclerosis may also occur. In gigantism, however, the heart is not affected; its size bears a normal relationship to body size.

Hypoadrenalism due to Addison's disease or destruction of the adrenal cortex produces hypotension and myocardial weakness but no organic heart disease. *Hyperadrenalism* due to adrenal cortical tumors may cause paroxysmal hypertension which, in turn, is likely to result in cardiac hypertrophy and arteriosclerosis.

Thymic hypertrophy is not usually attended by heart disease although microcardia and congenital heart block have been reported. Heart disease does not usually result from diseases of the *gonads,* but functional disorders with cardiovascular symptoms of the neurocirculatory asthenia type are commonly observed, especially in women during the menopause or following double oophorectomy. Hypertension, often temporary, frequently occurs and may affect the heart secondarily with the production of hypertrophy.

Laboratory examinations in the diagnosis of these endocrine disorders are discussed in Chapter 30.

HYPERTENSION AND HYPERTENSIVE HEART DISEASE

Primary and secondary hypertension are among the most common and most important causes of organic heart disease. Hypertension is responsible for about 30 per cent of all cases in the United States and is properly designated *hypertensive heart disease* although frequently referred to as "cardiorenal disease." In the United States it occurs about twice as frequently in Negroes as among white individuals although, since hypertension is less frequent in tropical and semitropical countries, it is said to be comparatively rare among Negroes in Africa. It has been estimated that nearly 100,000 persons die annually in the United States as the result of congestive heart failure due to hypertension and that 75,000 additional die annually from other consequences of the latter.

The cause of the heart disease is hypertension but the cause of the latter and especially of primary hypertension (benign and "malignant") is as yet unknown except that disorders of the kidneys are apparently involved, as discussed in Chapter 23. Sex has but little influence upon hypertensive heart disease although males may be affected somewhat more frequently than females. Heredity, however, ranks next to age in etiologic importance. Overeating and obesity are frequently associated with the disease but the relationship is very inconstant. High nervous tension and physical strain are undoubtedly important, at least in the aggravation of hypertension. Endocrine disturbances are frequently responsible, as previously discussed, with special reference to diabetes mellitus. The disease, like hypertension, is most common in middle age or thereafter. Signs and symptoms generally appear about ten years after the onset of sustained hypertension of considerable degree, except when there is valvular disease or coronary sclerosis to make its effects apparent more quickly.

There are no *laboratory examinations* directly applicable to the diagnosis of hypertensive heart disease. Those occurring in congestive failure (myocardial insufficiency) are described on page 793 while those due to associated nephrosclerosis have been discussed in Chapter 23. Otherwise, laboratory examinations are of interest only in relation to arteriosclerosis. An excellent review on the concept of the chemical changes in atheromatosis has been published recently by Page.[23] It is commonly stated that hypercholesterolemia does not occur in the absence of diabetes or nephrosis but Leary [24] regards arteriosclerosis as the "cholesterol disease" of human beings and states that it is associated with an intermittent excess of cholesteral esters in the plasma with the suggestion that diets limited or lacking in cholesterol (using vegetable oils instead of animal fats) may be of prophylactic value.

PULMONARY HYPERTENSION AND HEART DISEASE

The effect of pulmonary hypertension on the right ventricle is comparable to that of systemic hypertension on the left ventricle, except that in the case of the former there are many instances of sudden unexpected increases in blood pressure causing acute right ventricular strain. It may be acute or chronic.

Acute Pulmonary Heart Disease. Acute pulmonary hypertension and heart disease, or "acute cor pulmonale," is caused by sudden massive obstruction of the pulmonary circulation with dilatation of the right ventricle. Both sexes are about equally affected although the incidence is unknown. The disease usually occurs in the elderly, being rare under 35 years of age. In the majority of cases it is due to extensive pulmonary embolism originating from thrombosis of the abdominal, pelvic, or leg veins although the embolus may come from the right auricle in congestive heart failure. Pneumonia is not a cause. The sudden perforation of an aortic aneurysm into the pulmonary artery, however, can raise the pulmonary arterial pressure so suddenly that doubtless acute dilatation of the right ventricle antedates the inevitable death that ensues. Among those who survive the initial shock of acute embolism, hemoptysis and leukocytosis are apt to be the only laboratory examinations of interest or value.

Chronic Pulmonary Heart Disease. The cause of chronic pulmonary heart disease, or "chronic cor pulmonale," is prolonged increased resistance in the pulmonary circulation due to constriction of the capillary bed by extensive pulmonary fibrosis, severe emphysema, tracheal or bronchial stenosis, atelectasis, chest deformities and other intrathoracic conditions. In rare cases it may be due to primary endarteritis obliterans involving the pulmonary arteries; because of the deep cyanosis, afflicted individuals are sometimes referred to as "black cardiacs."

The disease occurs mostly among the elderly and is of about equal frequency in men and women, although the former are more exposed to pneumoconiosis. It is an important disease, although variable in incidence in different parts of the world. In the United States it probably constitutes about 0.9 per cent of cases of organic heart disease. Coronary disease is present in about half the cases, probably because of the advanced age of the majority of patients. There are no *laboratory examinations* of diagnostic value except, possibly, in relation to pneumoconiosis, discussed in Chapter 28.

Coronary sclerosis is a common disease, being found in about 38 per cent of necropsies but it does not always cause definite heart disease. In other words, it may limit cardiac action and reserve without actually producing organic lesions in the heart. The latter are particularly likely to occur in the atherosclerotic type of the disease due to a reduction in the blood supply to the myocardium. The cause is unknown, as is true of arteriosclerosis in general, although heredity and faulty cholesterol metabolism are probably important etiologic factors, as previously discussed. Overwork, mental strain, hypertension and infection are among the many additional causes suggested, but none has been proved or even consistently observed. Probably a combination of factors is responsible in most cases and the influence of diabetes mellitus and other endocrinopathies has been previously discussed. Syphilis is rarely responsible, while coronary disease due to rheumatic fever is relatively infrequent. Endarteritis obliterans, trauma, and congenital abnormalities of the heart are likewise rare etiologic factors. Alcohol, tobacco, tea and coffee are probably without direct influence, except that in some individuals angina pectoris appears to be precipitated or aggravated by excessive smoking and alcoholism, although alcohol may have a protective influence against the ill effects of coronary disease. About 94 per cent of cases occur after 40 years of age and men are affected somewhat more frequently than women.

Coronary thrombosis, which is responsible for acute myocardial infarction, is one of the most serious consequences of coronary sclerosis and especially in the atherosclerotic type of the disease. Sometimes thrombosis and even extensive fibrosis, if slow in development, may occur with a different clinical course from ordinary coronary thrombosis, and even clinically recognizable acute myocardial infarction may present a wide variety of clinical manifestations. But thrombosis of a large branch of either coronary artery is apt to be rapidly fatal although recovery from thrombosis of small branches is not uncommon. The age incidence of coronary thrombosis with cardiac infarction diagnosed clinically is younger than for coronary disease in general, but about 95 per cent of cases occur in individuals 40 years of age or older. *Coronary embolism,* although rare, may occur at any age, even in youth. The descending branch of the left coronary artery near its mouth is the place most commonly affected by both extensive sclerosis and by thrombosis.

Laboratory examinations are of but limited value in diagnosis except in relation to the detection of diabetes mellitus, thyrotoxicosis, syphilis, the nephroscleroses, etc., in relation to hypertension and arteriosclerosis. In sudden coronary thrombosis with cardiac infarction there is usually a polymorphonuclear leukocytosis of 12,000 to 15,000 per c.mm. of blood beginning a few hours after onset and lasting three or four days. Extensive infarction with survival, however, may show a leukocytosis of 20,000 to 30,000 and remain elevated for a week or two. The sedimentation rate of the erythrocytes is accelerated and usually remains so until healing is well established. A progressive decrease is of good prognostic import.

PERICARDIAL DISEASE

Pericarditis is of frequent occurrence, being sometimes serious and often fatal, or wholly unimportant. It frequently occurs as a complication of heart disease itself. It is found in about 5 per cent of necropsies and is present as an acute condition in one-half to two-thirds of them. The disease may be acute or chronic.

Acute Pericarditis. *Acute fibrinous pericarditis* frequently occurs in rheumatic carditis; also in other infectious diseases such as pneumonia, influenza, tonsillitis, septicemia, typhoid fever, tuberculosis, etc. In some cases it is but part of a polyserositis, being most commonly associated with pleuritis and infrequently with peritonitis. In all of these the pericarditis is due to infection but it may occur without infection in coronary thrombosis or embolism with cardiac infarction as well as a reaction to a foreign body and in uremia as a terminal event.

Serofibrinous pericarditis and *purulent pericarditis,* characterized by exudates of variable size, may develop under the same conditions except in the pericarditis of cardiac infarction and uremia in which the process is purely fibrinous with little or no effusion. *Hemorrhagic pericarditis,* in which the exudate contains large amounts of blood, is caused either by infection such as tuberculosis or by malignant disease. It should be distinguished from hemopericardium due to hemorrhage into the pericardium, and from the result of rupture of the aorta, heart wall or of a coronary vessel caused by infarction, aneurysm or trauma.

Acute pericarditis may occur at any age in accordance with the cause; in general it is most common between the ages of 10 and 40, with an average of about 25 years. For unknown reasons males are affected about three times more frequently than females.

The bacteriologic findings depend upon the primary sources of infection. Hemolytic streptococci, staphylococci and pneumococci are most frequent; *K. pneumoniae, Ps. aeruginosa, H. influenzae, Cl. welchii, Esch. coli, S. typhosa,* and *Myco. tuberculosis* are of less frequent occurrence.

Laboratory examinations are not usually required for diagnostic purposes but may be summarized as follows: (1) Leukocytosis due to an actual increase of the polymorphonuclear neutrophils is commonly observed and especially in serofibrinous and purulent pericarditis; (2) an increase of the sedimentation time and rate of the erythrocytes is usual and repeat examinations are of prognostic value; (3) bacteriologic examinations of exudates removed by paracentesis usually reveal the causative micro-organism in those cases due to infection, as discussed in Chapter 15; (4) physical, cytological and chemical examinations of the effusions, with special reference to the amount of protein present, are of value in differentiating between exudates and transudates, as discussed in Chapter 13.

Chronic Pericarditis. Chronic pericarditis is frequently very difficult to detect during life because of the absence of signs and symptoms. Fortunately it is for the most part of little or no importance.

The cause is acute pericarditis of rheumatic, tuberculous, septic or other, frequently unknown, infectious origin and cardiac infarction, neoplasms, and hemopericardium from trauma. The most common known causes are rheumatic carditis, pulmonary and pleural disease. Often the cause is unknown, the antecedent acute pericarditis having escaped detection. Infrequently tuberculosis, pneumonia, polyserositis, influenza, and rarely septic infections are known to be responsible as well as trauma which may produce a chronic adhesive pericarditis. *Chronic constrictive pericarditis* sometimes results and especially in the pericarditis of rheumatic carditis; it gradually results in severe hypertrophy of the heart and especially in children and adolescents.

Chronic pericarditis may occur at any age. In adults it is commonly observed in middle age, most cases averaging about 35 years. Males are affected two or three times more frequently than females.

Laboratory examinations are not usually employed for diagnostic purposes unless the pericarditis is accompanied by effusion, in which case it may be subjected to the same bacteriologic, cytologic and chemical examinations previously referred to in acute pericarditis. Hypochromic normocytic anemia is of frequent occurrence. Leukocytosis is generally absent although the lymphocytes are relatively increased and in the presence of leukocytosis may show an absolute increase. In cases due to infection, the sedimentation rate and time of the erythrocytes are generally increased but not usually to the degree observed in acute pericarditis.

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DISEASES OF THE RESPIRATORY TRACT

In acute and chronic rhinitis, nasal accessory sinusitis, otitis media, mastoiditis and tonsillitis, only bacteriologic and mycologic examinations are ordinarily employed for etiologic diagnosis; these have been discussed in Chapters 15 and 16 respectively. Other diseases of the upper and lower respiratory tracts in which various laboratory examinations are of aid, or essential in diagnosis and differential diagnosis, are considered herewith including a few, such as diphtheria, scarlet fever and pertussis, which are commonly classified under acute infectious diseases.

VASOMOTOR RHINITIS

Vasomotor rhinitis, or perennial allergic coryza, is one of the most troublesome diseases of the nose from both the standpoint of etiologic diagnosis and treatment. Characterized by frequent attacks of sneezing with profuse thin, watery discharge associated with nasal obstruction and sometimes with itching of the eyes and lacrimation, the attacks last only a few hours to two or three days and usually without fever, malaise or other constitutional symptoms. The disease may occur at any age, but most frequently between 20 and 40 years, and is undoubtedly more prevalent in children than generally surmised, being commonly regarded as an ordinary "cold."

Intranasal abnormalities such as septal deflections, spurs and polyps are not now regarded as the cause of the disease but they may be aggravating factors while polyps are commonly a result of it. By common consent the cause of vasomotor rhinitis is regarded as allergy with sensitization to inhalant allergens more frequent than sensitization to foods. *Dust*, either of the home or occupational variety,[1] is probably the most frequent exciting agent, with *orris root* a close second. *Feathers* are the most frequent offender of the animal dander group with that of the cat, horse and dog following closely in line. *Tobacco* is also an important offender while the insecticides, cotton seed, wool and other inhalant allergens are much less important. Allergy to *foods* is much less likely to be responsible unless they are inhaled as dusts, especially wheat, buckwheat and rye. Rice and corn flours in cosmetics are sometimes responsible. Allergy to ingested foods, however, may cause the disease, as indicated by positive skin reactions, and be benefited following the use of elimination diets. Among the most important of these are wheat, eggs, milk, chocolate, white potatoes, nuts and beans. Allergy to *drugs* is of less importance except in the case of pharmacists and others who inhale them. Allergic sensitization to *bacteria* and especially those associated with chronic rhinitis or sinusitis may be responsible in some cases but this conclusion is only justifiable in individual cases when all other possible allergies have been excluded. Allergy to *physical agents* like heat and cold may also be responsible and especially in cases where allergy to material substances has been excluded. These cases frequently belong to the group of nonallergic coryzas or so-called hyperesthetic rhinitides, which commonly present unusual difficulties in treatment.

Laboratory Examinations. 1. As in the etiologic diagnosis of hay fever and asthma, skin tests are of fundamental importance along with thorough histories and elimination diets when allergy to foods is suspected or known to be present. Positive skin reactions, no matter how slight, should be considered important until proved otherwise. Intracutaneous tests are preferred in the case of the foods.

2. Detailed cytologic examinations of the nasal secretions for eosinophilia are important and may also prove a valuable aid in diagnosis.[2] Thin smears prepared of mucoid secretions secured by blowing the nose on waxed paper, or collected by swabs, are preferred; they should be stained in the same manner as blood smears for the differential counting of leukocytes. Accurate differential counts are difficult. A few scattered eosinophils are without significance but large numbers are indicative of an allergic rhinitis while a preponderance of polymorphonuclear neutrophils is indicative of an infective rhinitis. An increase of both is generally indicative of allergic rhinitis with superimposed infection, as previously discussed in Chapter 19. Patients showing large numbers of eosinophils in their nasal secretions may or may not have a coincident eosinophilia of the blood.

HAY FEVER

Hay fever is a disease characterized by excessive sneezing, a watery nasal discharge, lacrimation and itching of the eyes, nose and palate, headache, malaise, etc., recurring *seasonally* in individuals allergic to pollens. "Pollen allergy" or "pollinosis" are more appropriate and accurate terms, since the disease may be due not only to allergic sensitization to the pollens of the grasses and weeds occurring in hay, but to those of various trees, flowers and others plants. The laity commonly call the disease "rose cold" and while hay fever may be due to allergic sensitization to the pollens of roses, it is obvious that the term is otherwise inappropriate. Seasonal hay fever, however, may be due also to various molds, spores of rust and smut in mill dusts and even to the dusts of certain flies.

The true incidence of hay fever is unknown but it has been estimated that it affects between 1 and 2 per cent of the population of the United States. It may occur in children but most frequently between 15 and 40 years of age. Sex has no influence on its incidence but Negroes and full-blooded American Indians are less subject to it than whites. Needless to state, climate has an important influence from the standpoint of pollination. Asthma may occur as an early manifestation of the pollen allergy, as an accompanying manifestation of hay fever in unusually sensitive individuals, or as a complication of hay fever due to allergic sensitization to other inhalants, ingestants, etc., shortly to be discussed.

The single important exciting cause is sensitization to one or more of the pollens. Theoretically, at least, human beings may become allergic to any pollen. Needless to state, however, the most important ones are those commonly borne by the winds. Heavy pollens which fall to the ground are not nearly as important as contact with them is much less likely to occur. Once the disease has been established, other factors may aggravate or prolong the symptoms as, for example, cold winds, the inhalation of irritating dusts, etc., as well as septal deviations, spurs, polyps, chronic sinusitis, etc.

Hay fever is strictly seasonal in character. When it occurs early in spring, it is usually due to sensitization to the pollens of certain trees; later on, to the pollens of various grasses and weeds, and still later to the pollens of various autumnal flowers. Since there is an important geographic distribution of pollens, physicians should acquaint themselves with those particularly prevalent in their individual communities. Allergy to molds [3,4] as well as to the spores of rust and grain smut in mill dusts [5,6] and to the dusts of caddisflies and mayflies (*Ephemerida*) are also known to be responsible for unusual cases.[7,8]

Laboratory Examinations. The diagnosis of hay fever is so easy that most affected individuals know when the disease is present. Etiologic diagnosis, however, with reference to the particular pollen or pollens responsible for the allergic sensitization or disease is of primary importance because of the chances of avoiding contact or securing relief and even complete recovery by thorough desensitization by way of injections of suitably prepared extracts of the offending pollens.

1. Skin tests are of inestimable value in this connection as discussed in Chapter 19. They should be as thorough as possible. In most instances individuals are hypersensitive to two or more pollens. Simple scratch or cutaneous tests generally suffice. Owing to botanical relationships, individuals frequently show positive reactions to pollens which do not occur in their locality. Intracutaneous tests are employed quite commonly by allergists. By either method, however, skin tests with the various pollens rarely fail in etiologic diagnosis. When they do, ophthalmic or nasal tests may be employed, as described in Chapter 19. Indirect tests for the detection of allergic antibodies or reagins in the blood by the method of passive transfer are seldom required or of clinical value.

2. The only other helpful laboratory examination is for the presence of large numbers of eosinophils in smears of the nasal secretions prepared of mucus obtained during an attack. However, they are not usually required. A slight eosinophilia may also be found in the differential leukocyte count of the blood.

ASTHMA

Asthma may be defined as a recurrent or paroxysmal type of dyspnea characterized by a wheezing or whistling type of respiration associated with a marked prolongation of expiration. In other words, it is easier to get air into than out of the lungs. In the great majority of cases it is due to allergy but "all is not allergy that wheezes." Allergic asthma may be regarded as a disease entity but otherwise "asthma" is a symptom-complex occurring in many pulmonary diseases. It is thought to affect at least 1 to 2 per cent of the population. In about 30 to 40 per cent it develops during childhood but at least 50 per cent of cases develop between 10 to 40 years of age after which the incidence rapidly declines. Up to puberty more boys than girls are affected but during adult life women are affected somewhat more frequently than men. For all ages, however, from 53 to 54 per cent of cases of asthma occur among males. It is much more common in the white race than in Negroes while rare among the Eskimos and American Indians. No difference in incidence exists according to social status as the rich and poor alike are equally affected. It is slightly more prevalent, however, among urban than country dwellers.

Etiology and Classification. Because of the many causes of asthma different classifications based on etiology have been proposed. For clinical purposes it appears sufficient to divide it into two main types as follows:

- *Allergic* due to sensitization to:
  - Inhalant allergens
  - Ingestant allergens
  - Injectant allergens
  - Bacterial allergens
  - Mycologic allergens
  - Parasitic allergens
- *Nonallergic* due to:
  - Reflex irritation
  - Bronchitis and bronchiectasis
  - Emphysema
  - Pneumoconiosis
  - Obstruction
    - Foreign bodies
    - Intrabronchial tumors
    - Extrabronchial tumors
  - Spasm of the pulmonary vessels
  - Cardiac failure including cor pulmonale

In allergic asthma *heredity* or the capacity for acquiring hypersensitiveness is the most important single predisposing factor. Among the *endocrinopathies,* however, only disturbances of the gonadotropic hormones of the female appear capable of exerting an influence at the time of puberty and the menopause or during menstruation and pregnancy. The possible relationship of the *avitaminoses* is uncertain but apparently a deficiency in vitamin C may be an aggravating factor probably due to undernutrition. At the present time it cannot be stated with certainty that disturbances in calcium and water metabolism, of acid-base equilibrium or other biochemical changes are important in relation to etiology. *Nervous and psychic states,* however, are important and especially in relation to provoking and aggravating the paroxysmal attacks. Physical exertion, fatigue, constipation, irritating gases and vapors as well as operations on the nose and throat, are likewise of importance as aggravating factors in some cases. Furthermore, there can be no doubt that *abnormalities of the nasal septum and turbinates,* including the presence of polyps, may be important predisposing factors. *Chronic sinusitis,* however, can be a primary cause of allergic asthma if it is true, as I believe it to be, that foci of infection in the sinuses, tonsils, adenoids, teeth, prostate gland or elsewhere may result in allergic sensitization to bacterial proteins even though conclusive evidence is absent because of a failure to demonstrate allergins in the serum for the bacterial allergens, since the former are strictly sessile or cellular as in tuberculin and other bacterial allergies. Furthermore, postnasal dripping due to chronic sinusitis is frequently responsible for chronic bronchitis which alone may produce a nonallergic type of asthma so commonly designated "asthmatic bronchitis." Not infrequently asthma is initiated by acute infectious diseases and particularly those involving the upper or lower respiratory tract such as influenza, pertussis, pneumonia, measles and scarlet fever.

There is also little doubt at present that asthmatic attacks can be produced by *reflex irritation* of the "trigger area of Hazeltine" located in the ethmoid region. Reflex irritation of this area may be due to septal abnormalities, polyps, chronic sinusitis, irritating gases or cold air, and may either produce a nonallergic type of asthma or aggravate one due to allergy. *Environmental factors* such as residence, occupation, seasons, climate, temperature changes and altitude may have an influence on both allergic and nonallergic asthma and especially the former.

As far as *allergic asthma* is concerned, the most important allergens are the inhalants—the dusts (home and occupational), orris root, pollens, animal danders and hairs (horse, cat, dog, goat, rabbit, cow, flies and other insects) sheep wool, feathers (chicken, duck, goose, turkey, canary, etc.), silk, glue, tobacco, seeds (cotton, kapok, flax), pyrethrum and other insecticides, drugs, molds and fungi. The ingestant allergens, embracing the foods and beverages, as well as some of the drugs, may also be responsible and particularly in children with special reference to wheat, eggs and milk. As previously stated, I believe that bacterial allergy may also produce the disease and acute asthmatic attacks may be induced by injections such as heterologous serums, bacterial vaccines and various other protein agents in the case of individuals having a natural or acquired allergic sensitization to these substances.

The dyspnea is due to obstruction or stenosis of the smaller bronchi resulting from spastic contraction, edema with the formation of fibrinous plugs, or to a combination of both. Because of the frequency and importance of bronchostenosis due to sticky exudates, bronchoscopic drainage is frequently of value in relieving attacks and in the treatment of the disease. These changes are ascribed primarily to the production of histamine or a histamine-like substance by the sensitized cells when allergen reaches them, but whether spasm of the bronchi with increased secretion is due entirely to this effect or is the result of irritation of the vagi, cannot be stated except to mention that considerable evidence is in favor of the latter view.

Laboratory Examinations. The clinical diagnosis of asthma is not difficult, but etiologic diagnosis, with differentiation between allergic and nonallergic asthma, which is so important in relation to treatment, including the detection of the exciting agent or agents responsible for the former, is frequently difficult and, indeed, sometimes impossible. Needless to state, thorough and detailed histories, physical examinations of the heart and lungs supplemented, if necessary, by roentgenologic and bronchoscopic examinations, as well as by examinations of the nose and throat, are of fundamental importance. Laboratory examinations are of great value and, indeed, skin tests are indispensable not only for the purpose of differentiating between allergic and nonallergic asthma but for the etiologic diagnosis of the latter, as discussed in Chapter 19. Laboratory examinations may be summarized as follows:

1. The skin tests should be thorough and exhaustive. The scratch or cutaneous method is usually sufficient, supplemented if necessary by intracutaneous tests which, however, are always preferred in the case of food and bacterial allergens as well as in the case of those prepared of molds, fungi and the animal parasites. Significant positive reactions are observed in about 60 to 75 per cent of cases of allergic asthma. Multiple positive reactions occur in more than 50 per cent but not all of them are necessarily of clinical significance. Unfortunately, falsely negative reactions may occur if the skin is nonreactive or if it does not share in the allergic sensitization; the latter is more likely to be true of allergens prepared of the foods, bacteria, molds, fungi, and animal parasites than of the inhalant allergens. The skin of certain individuals is extremely reactive and, in them, doubtful or slightly positive reactions may not be significant.

2. Bacteriologic and mycologic examinations of the sputum or secretions obtained by bronchoscopic aspiration are of value only in cases of asthma suspected of being due to allergic sensitization to bacteria, molds and fungi. In such cases I greatly doubt the value of skin tests conducted with stock bacterial allergens. In my experience it is far better to prepare separate vaccines of each microorganism carrying about 1000 million per cc. for intracutaneous tests. If positive reactions are observed to two or more of them, a mixture of equal parts may be used for therapeutic purposes.

3. Gross and microscopic examinations of the sputum for amount, consistency, color, "perles," Curschmann spirals, Charcot-Leyden crystals and an excess of eosinophils are of helpful diagnostic value, as discussed in Chapter 11, and especially the presence of the three changes last mentioned.

4. Differential leukocyte counts for the detection of eosinophilia are sometimes of value. Eosinophilia, however, may be observed in both allergic and nonallergic asthma, the average percentage in most asthmatics being between 4 and 7. More marked eosinophilia, however, usually occurs in allergic cases. The highest percentages are found during or following an attack. Eosinophilia may be absent; if present, its degree bears no relationship either to the severity of the disease or to the nature of the exciting factor. Certainly the absence of eosinophilia does not exclude asthma of either the allergic or nonallergic types.

5. The erythrocyte sedimentation time and rate may be increased in cases of asthma with infection but are otherwise normal.

6. Blood chemistry and basal metabolic determinations possess no diagnostic value; the same is true of urine examinations.

7. Gastric analyses frequently show the presence of hypochlorhydria and sometimes achylia. Liver function tests occasionally show some degree of dysfunction but none of these examinations is of particular diagnostic value.

DIPHTHERIA

Diphtheria occurs in all climates and countries but more commonly during the colder months of the year. It is most frequent in children from one to ten years of age, particularly from one to five years, while very uncommon under six months of age although newborn infants may contract it. Needless to state, the disease also occurs among adults. A slightly higher percentage of girls than boys contract it; Negroes are less susceptible and likewise show a higher incidence of negative Schick reactions, indicative of the presence of an antitoxic immunity. As discussed in Chapter 19, Schick-negative individuals, however, may sometimes contract diphtheria and especially with unusually virulent strains of the bacillus although the disease is apt to be mild and atypical and escape detection unless bacteriologic examinations are made. The bacillus may be transmitted indirectly by contaminated milk, toys, books and the like and presumably at times by some of the domestic animals but in the great majority of cases the disease is contracted through droplet infection by contact with individuals in its early stages, or with convalescent or healthy carriers.

The *incubation period* is usually one to four days. The disease may be very mild and catarrhal in type or very severe and especially in so-called *malignant diphtheria* (diphtheria gravis) due to infection with a strain of the bacillus of unusual virulence or toxicity known as *Corynebacterium diphtheriae gravis.*

The common clinical type is *faucial diphtheria.* Primary *nasal* diphtheria constitutes about 1 to 2 per cent of all cases and is most frequent in infants. It frequently escapes detection and is a common source of contagion with a tendency to run a chronic course. Primary nasopharyngeal diphtheria, however, is generally severe. Secondary nasal diphtheria is common, occurring in about 40 to 50 per cent of cases and adding to the severity of the disease. *Laryngeal diphtheria,* which is better designated laryngotracheal diphtheria, is the most dangerous type of all; laryngeal involvement occurs in about 15 to 25 per cent of all cases of diphtheria, is somewhat more common in boys than in girls and usually most frequent between two and five years of age. Laryngeal diphtheria is usually secondary to nasal or faucial diphtheria, but often appears to be primary when the former are slight or overlooked. It develops on or about the fourth or fifth day but not often after the first week. Diphtheria may also occur as a primary disease or secondary to diphtheria of the nose and throat in unusual locations such as wounds, or the conjunctivas, as well as by extension to the lips, oral cavity or scalp with occasional extension to the esophagus and stomach.

The morbidity and mortality rates have been steadily declining in recent years but the latter still averages about 5 per cent for all types of the disease, largely due to delayed diagnosis and the failure of prompt institution of adequate treatment with antitoxin. It is much more fatal in infants and young children than in adults and especially newborn infants. Involvement of the larynx usually increases the mortality up to 15 to 20 per cent while in laryngeal or tracheobronchial diphtheria it may be as high as 60 to 95 per cent.

Laboratory Examinations. 1. Since faucial diphtheria may be so mild as to escape clinical detection altogether, or be mistaken for staphylococcal tonsillitis, the streptococcus angina of scarlet fever, Plaut-Vincent angina, agranulocytic angina, leukemia, etc., bacteriologic examinations are of inestimable value in diagnosis. Indeed, they should be employed routinely especially in children. Properly prepared cultures are preferred to smears although the latter are required in case Plaut-Vincent angina is suspected. Since in diphtheria primary cultures may prove negative, the physician should always administer antitoxin if reasonably suspicious of its presence and especially since in diphtheria delay in its administration may prove disastrous. The same is true when nasal diphtheria is suspected and especially when there is a persistent irritating and blood-stained discharge although the latter may be due to a foreign body, syphilis, tuberculosis or the Plaut-Vincent infection. Bacteriologic examinations are more difficult from the standpoint of preparing cultures in laryngeal diphtheria but are always indicated for the aid so frequently given in diagnosis and differentiation from spasmodic croup and foreign bodies. Diphtheria is practically the only disease producing a pseudomembrane in the larynx. It is true that errors in bacteriologic diagnosis may be due to mistaking the diphtheria bacillus for *C. pseudodiphtheriticum* and diphtheroid bacilli, but the chances are greatly reduced by expert bacteriologic examinations. In case of doubt the sugar fermentation and guinea-pig virulence tests are of great differential value as, likewise, in relation to prolonged quarantine following recovery when only nonvirulent diphtheria or diphtheria-like bacilli are present in cultures. Blood cultures are of no value in diagnosis as they are usually sterile even in severe cases with the disease due almost entirely to the production of exotoxins in the local areas of infection.

2. Total and differential leukocyte counts should always be made whenever agranulocytic angina or leukemia are suspected. Indeed, this is particularly true in the majority of anginas occurring in adults unless the physician is quite certain of their absence. In diphtheria, leukocytosis of varying degree largely due to an absolute increase of the polymorphonuclear neutrophils is the rule. In very severe diphtheria a leukopenia may be present but never to the degree seen in agranulocytic angina and never with the sharp reduction of the granulocytes of the latter which may amount to their total absence, as discussed in Chapter 22.

3. Since diphtheria toxin is capable of producing a glomerular type of nephritis, the urine may show the presence of albumin, casts and erythrocytes.

4. Since mild or atypical diphtheria may occur in Schick-negative individuals, the Schick test possesses little or no diagnostic value except that positive reactions always indicate the possibility of diphtheria.

SCARLET FEVER

It is now generally believed that scarlet fever may be caused by any beta hemolytic streptococcus belonging to Group A of Lancefield although some investigators claim that a specific hemolytic streptococcus may be the cause. Since the portal of entry is usually by way of the nasopharynx, the disease is included herewith under diseases of the respiratory tract although scarlet fever may be due to infections of wounds and especially burns (*surgical scarlet fever*) or of the puerperal uterus (*puerperal scarlet fever*). The disease is due not only to the production of an erythrogenic toxin responsible for the rash and toxic manifestations but to invasiveness of the fixed tissues by the streptococcus itself sometimes associated with septicemia and showing positive blood cultures.

Almost invariably scarlet fever is characterized by an erythematous rash of varying severity which, however, may be hemorrhagic (*scarlatina haemorrhagica*). The angina with cervical adenitis may be unusually severe (*septic scarlet fever* or *scarlatina anginosa*) and occasionally the disease presents a degree of toxemia and prostration out of proportion to the severity of the angina (*malignant* or *fulminating scarlet fever*). Undoubtedly, scarlet fever may also rarely occur without a rash (*scarlatina sine eruptione*), due to invasion of the tissues by the streptococcus in those individuals carrying sufficient antitoxin in the blood for the neutralization of the erythrogenic toxin and thereby giving weakly positive or negative Dick skin reactions. Needless to state, such cases are particularly apt to occur in adults and escape detection; consequently, they are especially likely to be important sources of infection of susceptible children and adults. The *incubation period* is usually two to five days but varies from one to eleven days or longer; in severe cases and in surgical scarlet fever it may be as short as one day in some cases.

The disease is most prevalent in temperate zones and rare in the tropics. Negroes are said to be less susceptible but this may simply be due to the difficulty in recognizing the rash in them. It occurs most frequently between five and ten years of age and next between two and five years; the incidence under one year is less than 1 per cent of all cases. There is no proof that tonsillectomy lessens susceptibility to the disease. On the contrary, however, various infections of the throat, and especially diphtheria, may increase susceptibility. Contaminated milk and fomites may transmit the streptococcus but droplet infection through contact with early cases, unrecognized cases, convalescent and healthy carriers is usually responsible. The period of maximum infectivity is at the height of the eruption but transmission may occur during the latter part of the incubation period and during convalescence.

At present both morbidity and mortality rates are lower than formerly. The latter varies in epidemics, in different localities and especially according to age and treatment but at present has a general mortality rate of 2 to 2.5 per cent in the United States. The mortality, however, may be as high as 33 per cent among infants one year or younger when treated without antitoxin, penicillin or the sulfonamide compounds, about 25 per cent in children between two and three years of age, and two to five per cent after eight years of age. Acute glomerulonephritis and severe septic complications are of unfavorable import but death is always more likely to be due to complications than to the toxemia itself.

Laboratory Examinations. 1. Unfortunately, while bacteriologic examinations of cultures of the throat usually show large numbers or even pure cultures of beta hemolytic streptococci, it is not generally or easily possible to identify them as the cause of scarlet fever. Consequently, diagnosis depends largely upon the clinical manifestations of the disease. Similar results are commonly observed in epidemic sore throat which is likewise due to infection with hemolytic streptococci and sometimes accompanied by light erythematous rashes. Bacteriologic examinations, however, are of inestimable value in differentiating staphylococcal and pneumococcal anginas from that of scarlet fever. Also in the differential diagnosis between diphtheria and Plaut-Vincent angina, as well as for the detection of diphtheria and scarlet fever occurring coincidentally. Blood cultures may be positive in severe cases.

2. The Schultz-Charlton rash extinction test (Chapter 19), however, is fre-

quently of diagnostic value and especially in differentiating scarlet fever from the rashes of dermatitis exfoliativa, measles, rubella, secondary syphilis and allergic dermatitis as well as drug rashes due to other causes, especially those sometimes produced by quinine and the salicylates.

3. Blood examinations usually show the presence of an anemia along with a leukocytosis of 15,000 to 40,000 per c.mm. of blood largely due to an absolute increase of the polymorphonuclears which may be as high as 80 to 90 per cent. In mild and uncomplicated cases the leukocyte count generally returns to normal by the fifth to seventh day. An eosinophilia of 5 to 8 per cent or higher may occur on the fourth to the eighth days after onset and again during the third to fifth weeks.

4. The urine usually shows a small amount of albumin with casts but if acute glomerular nephritis develops, oliguria with heavy albuminuria and hematuria are observed as well as other changes described in Chapter 23.

PLAUT-VINCENT ANGINA

This disease affects both sexes and may occur at any age although it is observed most frequently in children and young adults. The onset usually is gradual with general malaise and fever and sometimes pain in swallowing. Usually there is only slight pain, but sometimes it is severe. The breath is characteristically offensive but the constitutional symptoms are seldom as pronounced as in diphtheria and acute tonsillitis. Heavy soft exudates occur on one or both tonsils which sometimes extend to the anterior pillars, uvula and hard palate. The exudates are commonly brownish in color owing to bleeding and are readily removed. The disease is characterized by the rapid ulceration or necrosis of the soft tissues. The infection may involve the nasopharynx with extension to the nasal mucosa and even the accessory sinuses.

The disease is ascribed to symbiotic infection with *Bor. vincentii* and *B. fusiformis*. Apparently it may occur as a primary infection but in adults is not infrequently secondary to a primary fusospirochetal gingivitis due to the same micro-organisms. Apparently vitamin C deficiency and blood disorders are sometimes important predisposing causes. At least, exudates characterized by the presence of large numbers of *Bor. vincentii* and *B. fusiformis* are not infrequently observed in clinical and subclinical scurvy, agranulocytosis, the leukemias and ulcerating syphilitic gummas.

Laboratory Examinations. These are extremely important in diagnosis and differential diagnosis and may be summarized as follows:

1. Stained smears show the presence of very large numbers of fusiform bacilli and spirochetes. Darkfield examinations are not necessary for their detection. Cultures are not employed as both micro-organisms are strict anaerobes and difficult to cultivate.

2. However, as the disease must be differentiated from diphtheria, cultures are always desirable. Cases of coincidental diphtheria and Plaut-Vincent angina may occur.

3. Total and differential leukocyte counts for the detection of agranulocytosis or leukemia are frequently required. In Plaut-Vincent angina a mild to moderate leukocytosis due to an absolute increase of the polymorphonuclear neutrophils commonly occurs.

4. Sometimes the disease must also be differentiated from chancre of the tonsil or an ulcerating gumma. In the former, *T. pallidum* is found upon darkfield examination but not in ordinary smears stained with carbolfuchsin; fusiform bacilli are absent or present in small numbers only. It is impossible, however, to distinguish between *T. pallidum* and *T. microdentium* by darkfield examinations. In ulcerating gummas the serologic tests for syphilis usually give positive reactions. It is stated that positive reactions may occur also in Plaut-Vincent angina but this has not been my experience in acute cases of the disease.

PERTUSSIS

Pertussis is an acute and highly infectious disease of the respiratory tract due to *H. pertussis*. It has been suggested that a filtrable virus may be the cause of the disease, but the evidence incriminating the bacillus is quite conclusive. While the disease is known best under the designation of "whooping cough," this is not a good term as it may occur in a light form without "whooping" accompanying the cough.

It is worldwide in distribution and is both endemic and epidemic. It may occur at any time during the year, but is most frequent during the winter and spring months. It is likewise more severe in the northern climates, possibly because of the higher incidence and severity of respiratory tract infections in general. Its exact incidence is unknown but it has been estimated that over 300,000 cases occur annually in the United States among children, with a general mortality rate of approximately 15 per cent. Mortality, however, is in relation to age. Thus, it may be as high as 25 to 40 per cent in infants under one year of age and 65 to 85 per cent of all deaths occur in this age period. Cases of congenital pertussis due to infection *in utero* have been reported, or the disease may be contracted within a few days after birth. Fully 80 per cent or more cases occur in infants and children up to the age of five years and about 97 per cent of deaths are in this age group. Indeed, it is realized increasingly that pertussis is among the most dangerous diseases of infants and children up to three years of age, largely due to the highly fatal bronchopneumonia, malnutrition resulting from vomiting, or other complications. In the United States more children die annually from pertussis than from diphtheria, scarlet fever, measles or tuberculosis; it exceeds the death rate from measles and scarlet fever combined. The incidence is slightly higher in girls than in boys. Its infrequent occurrence in adults depends largely on the fact that so many of them have had the disease early in life, plus the probability of acquiring immunity through clinically unrecognized attacks of the disease.

Pertussis is usually conveyed by droplet infection from contact with frank or clinically unrecognized cases of the disease and especially the latter occurring in adults. Clinically the disease is commonly divided into the incubational, catarrhal, paroxysmal and convalescent stages. The incubational and catarrhal stages last from eight to fourteen days. Unfortunately, the disease is most infectious at this time when it may be difficult or impossible to recognize it except by laboratory examinations, especially bacteriologic examinations for *H. pertussis*. Consequently, it is usually transmitted to others before its true nature is recognized. Paroxysmal coughing follows but, as previously stated, this may occur without the characteristic "whooping" which further adds to the difficulties of clinical diagnosis, especially in endemic cases.

Hemophilus pertussis is slightly invasive and in fatal cases may be found in the mucosa of the respiratory tract but it rarely invades the blood with positive blood cultures even in severe cases of the disease. Under the circumstances its pathogenicity appears to depend on the production of endotoxins and exotoxins which, however, appear to be identical although divisible into thermostabile and thermolabile fractions.[9] Consequently pertussis appears to be a local infection of the respiratory tract, the systemic effects being due to the absorption of the toxins. Recently toxoids prepared of these toxins have been proposed for active immunization against the disease as well as antitoxin for its prophylaxis and treatment.[10] When freshly isolated the bacillus is virulent, encapsulated, highly antigenic and grows in smooth (S) colonies, corresponding to Phase I of Leslie and Gardner.[11] But on cultivation, dissociation occurs into nonvirulent and nonantigenic or rough (R) colonies corresponding to Phase IV, with the possibility of two intermediate groups designated as Phases II and III.

Laboratory Examinations. Laboratory examinations are not required when clinical diagnosis is definite but they have proved valuable in the detection of the disease in the catarrhal stage and in atypical cases when clinical diagnosis is generally impossible or uncertain. Early diagnosis and prompt isolation are particularly important since the disease is most infectious at that time.

1. Fortunately, the "cough plate" method of bacteriologic diagnosis has proved very valuable in these respects. Indeed, it is stated that positive cultures may be observed in 88 to 98 per cent of cases [12–14] with decreasing positive cultures after the first two weeks of the disease. Brooks and her colleagues, however, have recently reported only about 50 per cent positive cultures by the "cough plate" method during the first two weeks of the disease, while the incidence was about 70 to 80 per cent by a nasopharyngeal swab method which they have described.[15]

2. Blood examinations are also sometimes of value, as a well-marked leukocytosis largely due to an absolute increase of lymphocytes frequently occurs during the catarrhal stage.[16] Needless to state, however, other diseases are capable of producing leukocytosis and lymphocytosis in both children and adults. Furthermore, the results must be interpreted in relation to age. Thus normal infants and children ranging from three months to three years of age usually show total leukocyte counts ranging from 10,000 to 12,000 per c.mm. of blood with 35 to 45 per cent lymphocytes. During the catarrhal stage of the disease, however, the total leukocytes may be increased to 14,000 to 28,000 per c.mm. of blood with 35 to 60 per cent lymphocytes.

3. Normally the serums of infants and children may agglutinate *H. pertussis* in final dilutions up to 1:20 [17] while most patients will show agglutination up to

1:160 at some time during the course of the disease. A rapid agglutination test has been described by Powell and Jamieson[18] but agglutinin production usually occurs too slowly to render the agglutination test of much practical value as a routine diagnostic test.

4. The same is true of the complement fixation test. While positive reactions occur somewhat earlier than positive agglutination reactions only about 50 per cent of serums show positive reactions during the paroxysmal stage.[19, 22]

5. The opsonocytophagic test has likewise failed as an aid in early diagnosis, although it is of some value for estimating the antibody response to active immunization.[20]

6. Positive skin reactions have been observed in some cases during the catarrhal stage following the intradermal injection of the vaccine or toxin.[21-24] It may be of some value in the detection of atypical or missed cases when bacteriologic examinations have proved negative but, otherwise, allergic sensitization is not acquired quickly enough to render skin tests of practical diagnostic value. It is likely, however, that they may prove of clinical value in the detection of susceptibility to pertussis in relation to active immunization against the disease. The same applies to intradermal tests conducted with the agglutinogen of *H. pertussis.*

THE LARYNGITIDES

As stated in Chapter 15, bacteriologic examinations are of value in the etiologic diagnosis of the various types of *acute laryngitis* and *abscess of the larynx* but are not ordinarily employed except in the diagnosis of laryngeal diphtheria and its differentiation from laryngismus stridulus as well as from laryngeal croup which is so likely to occur in children 2 to 8 years of age during influenza, pertussis, measles, scarlet fever and other of the acute infectious diseases as well as sometimes due to the presence of foreign bodies in the pharynx or larynx.

Bacteriologic examinations are likewise seldom employed in the diagnosis of *chronic nonspecific laryngitis* although of value as aids in the diagnosis of chronic laryngitis due to tuberculosis, syphilis, blastomycosis and other mycotic infections.

Tuberculous Laryngitis. Tuberculosis of the larynx is rarely primary. Characterized by infiltration, the formation of tubercles, and ulcers in the walls of the larynx, followed in many instances by edema, fibrosis, perichondritis, chondritis and even necrosis, it is usually secondary to pulmonary tuberculosis with 3 to 25 per cent of cases of the latter disease showing laryngeal complications. Indeed, it is the most frequent specific infection of the larynx. From 70 to 75 per cent of cases occur during chronic pulmonary tuberculosis. Tuberculous laryngitis occurs in both sexes and commonly between 20 and 40 years of age but more frequently in men than in women. It is uncommon in children and rare under one year of age. Differential diagnosis requires the exclusion of all other causes for hoarseness, including not only neoplasms but lupus, syphilis and other granulomatous infections like leprosy, glanders, and rhinoscleroma as well.

Laboratory examinations are of value in diagnosis and include not only (1) bacteriologic examinations of scrapings of lesions and sputum for *Myco. tuberculosis* with special reference to the method of fluorescent microscopy, but likewise in some cases (2) biopsy examinations of excised tissue and especially for differentiation from cancer, syphilis and other infectious granulomas.

Syphilitic Laryngitis. Primary syphilis of the larynx is rare. During the secondary stage, however, syphilitic laryngitis is not uncommon, especially 2 to 3 months following initial infection, although its incidence cannot be stated because the lesions are often so slight that even expert laryngologists cannot state with absolute certainty from the clinical manifestations that they are syphilitic. Condyloma are infrequent and usually situated on the edges of the vocal cords. Tertiary syphilis of the larynx, however, is comparatively common and may occur as circumscribed gummas, diffuse gummatous infiltrations or ulcerations, chondritis or perichondritis, chronic hypertrophies, stellate scars or vocal cord dysfunctions. Diagnosis is based mainly on the results of laryngoscopic examinations, a history of syphilis and the presence of lesions elsewhere in the body but is possible only after all other causes of chronic laryngitis have been excluded. Furthermore, it is always possible for various etiologic factors to coexist, which naturally increase diagnostic difficulties. However, the diagnosis of syphilitic laryngitis is always important because, if present, it is necessary to avoid Herxheimer reactions in treatment.

As far as *laboratory examinations* are concerned, (1) the serologic tests for syphilis are of great value although falsely negative reactions may occur in syphilitic laryngitis on the one hand while, on the other, biologic specific reactions do not necessarily indicate that a lesion in the larynx is syphilitic. In other words, repeatedly positive reactions simply indicate that the patient is syphilitic but the laryngeal disease may be due to tuberculosis, a neoplasm, etc. For this reason (2) biopsy examinations of excised tissue are frequently required and especially for the exclusion of tuberculosis, lupus, cancer, rhinoscleroma, mycotic granulomas, contact ulcers, etc.

TUMORS OF THE LARYNX

Tumors of the larynx may be intrinsic, or situated on or below the true vocal cords, or extrinsic when they are situated above this level. Next to chorditis nodosa (singers' nodes), *papillomas* of the larynx are without doubt the most common type of neoplasm found in relation to this organ. They are usually more or less pedunculated and occur in or near the anterior commissure of the vocal cords. While regarded as inflammatory in origin, papillomas may occur congenitally in children. In the latter they have a striking tendency to be self-limited although they may recur almost as rapidly as they are removed over a period of several years but usually not after adolescent age has been reached. Among adults there is an even greater tendency for lesions to recur after removal, probably because of mechanical strain and chronic irritation. In spite of multiple recurrences, however, only about 3 per cent of papillomas undergo malignant change.[25] Other benign tumors include fibroma, myxofibroma, angio-fibroma, polyps, cysts, etc.

Needless to state, malignant tumors of the larynx are much more serious. Fortunately, their incidence is about twenty times less than nonmalignant tumors. According to the Jacksons,[25] precancerous conditions are present in probably 75 or

80 per cent of cases embracing papillomas, pachydermia, leukoplakia, fibromas, aberrant thyroid, teratomas, branchial and other cysts.

Squamous cell carcinomas comprise about 92 per cent of all cases and other types of carcinoma about 2 per cent. In about 85 per cent carcinomas arise from the anterior third of the vocal cords in relation to the anterior commissure and in the same location as benign papillomas but may occur extrinsically in the epiglottis, aryepiglottic folds and hypopharynx. The remaining 6 per cent of malignant tumors include endothelioma, sarcoma, etc. Carcinoma of the larynx occurs about ten times more frequently in men than in women. About 90 per cent of cases occur after 40 years with the remaining 10 per cent from 18 to 40 years of age.

Diagnosis is based largely on the history, mirror examinations, palpation, roentgen-ray examinations and direct laryngoscopy. Differential diagnosis includes the possibility of syphilis, tuberculosis including lupus, rhinoscleroma, eversion of the ventricle or prolapse of the sacculus, blastomycosis and other mycotic granulomas, keratosis, pachydermia, perichondritis, benign tumors, hematoma, contact ulcer, recurrent paralysis, leukoplakia, cricoarytenoid arthritis and other inflammatory states.

Laboratory Examinations. Laboratory examinations are of inestimable value in diagnosis and differential diagnosis. They may be summarized as follows:

1. Routinely the serologic tests for syphilis. It is to be emphasized, however, that both benign and malignant tumors may occur just as frequently among syphilitic as among nonsyphilitic individuals. Consequently, positive reactions do not necessarily indicate that the lesion is syphilitic. As discussed in Chapter 18, malignant disease rarely yields biologic nonspecific or falsely positive reactions when tests of proven sensitivity and specificity are correctly conducted. Negative reactions exclude syphilis with high percentage of accuracy.

2. Bacteriologic examinations for tuberculous and mycotic infections are of value and are required when these are suspected clinically. Positive results are of value; negative results alone never reliably exclude their possible presence.

3. Under the circumstances, the biopsy examination of excised tissue is the most important single laboratory examination in the diagnosis of neoplasms of the larynx providing the proper tissue is removed. Indeed, it should be made in all cases of suspected malignant disease of the larynx regardless of opinion based upon clinical examinations. These include growths that on mirror examination seem benign, flat or slightly elevated growths with a suspicion of malignancy, and all large growths with both extrinsic and intrinsic involvement. As the life of a patient is dependent on early and conclusive diagnosis, it is a deplorable mistake to regard biopsy as a means of last resort.[25] Some physicians object to the procedure because of the danger of promoting metastases but these do not occur even when operation is delayed for considerable periods. The value of biopsy examinations depends, however, not only on the skill of the pathologist but on the removal of an ample amount of tissue, the complete removal of small growths, the removal of tissue from different parts of large growths including an edge of grossly normal tissue, serial sections and if need be, a repetition of the examination when the

results are inconclusive. Frozen sections are often disappointing and are not recommended. If tissue is removed by the electric cutting loop, the pathologist should be so informed because of the possibility of distortion of cells. A report on the grades of malignancy is frequently helpful although patients with histologic evidences of high grade malignancy (Grade IV) may run a mild course. Indeed, many clinicians now consider the grading or histopathologic indexes of cancers of the larynx as of little value. Certainly the degree of their aggressiveness may change and particularly Grade I into Grade IV; furthermore, one area of a large growth may show Grade I and another area Grade IV. Grade I comprises growths showing from 0 to 25 per cent undifferentiated and 100 to 75 per cent differentiated cells; Grade II, 25 to 30 per cent undifferentiated and 75 to 50 per cent differentiated cells; Grade III, 50 to 75 per cent undifferentiated and 30 to 25 per cent differentiated cells and Grade IV, 75 to 100 per cent undifferentiated and 0 to 25 per cent differentiated cells.

THE BRONCHITIDES

The bronchitides include *acute tracheobronchitis*, *chronic bronchitis* and *fibrinous bronchitis* (rare) due to bacterial infections; also acquired *bronchiectasis*, in which bacterial or mixed bacterial and spirochetal infections play an important rôle in etiology. Bacteriologic examinations of the sputum or bronchial secretions are required for etiologic diagnosis, as discussed in Chapter 15. For this purpose secretions secured during bronchoscopic examinations are always preferred because of the great reduced changes of contamination with saliva and especially if autogenous vaccines are to be prepared for the treatment of chronic bronchitis or bronchiectasis. Physical, chemical and microscopic examinations of the sputum are sometimes of additional diagnostic value, as discussed in Chapter 9. Bacteriologic examinations of the secretions of the nasopharynx or nasal accessory sinuses are always indicated because of the frequency with which chronic bronchitis and acquired bronchiectasis are due to secondary infections of the lower respiratory tract from these sources.

In this connection, *bronchopulmonary spirochetosis* or spirochetal bronchitis is worthy of special mention. It is surprisingly frequent and characterized by hemoptysis due to the superficial necrosis or ulceration of the tracheobronchial mucosa with the presence of large numbers of spirochetes in the secretions frequently associated with fusiform bacilli and various other micro-organisms. It may be acute in onset but is more frequently chronic accompanied by expectoration of large amounts of bloody and usually fetid sputum. Under the circumstances, it may simulate chronic pulmonary tuberculosis. Bronchopulmonary spirochetosis not infrequently leads to bronchiectasis and may result in the production of a diffuse pneumonitis and even lung abscess or gangrene. Pleural effusions due to the infection have been reported. Apparently the disease is due to the same spirochetes and fusiform bacilli responsible for fusospirochetal gingivitis with extension to the trachea and bronchi by the aspiration of secretions. The spirochetes and fusiform bacilli are usually discovered in ordinary stained smears of sputum or bronchial secretions but when the disease is suspected, darkfield examinations for spirochetes are always advisable. Since both the spirochetes and fusiform bacilli are strictly anaerobic and difficult to cultivate, it is important to remember that they are not detectable by ordinary aerobic cultures.

TRACHEOBRONCHIAL TUMORS

The diagnosis of benign and malignant tumors of the trachea and bronchi can only be reliably and conclusively made by bronchoscopic biopsy examinations. When the mucosa is involved the removal of a specimen of tissue is always possible and practical. In cases of primary endobronchial tumors, microscopic examinations of a small piece of the nodular or fungating lesion will suffice to show its nature. In peribronchial tumors, however, it appears inadvisable to bite through normal mucosa in an effect to obtain tissue no matter how characteristic may be the deflection and deformity of the bronchus.[26] For obvious reasons, aneurysm should always be excluded. Pulmonary tuberculosis and syphilis are usually also contraindications although their presence does not necessarily exclude either benign or malignant tumors. Indeed, biopsy examinations may be indicated in patients with syphilis or tuberculosis.

Extension of papillomas from the larynx into the cervical trachea, especially about tracheotomic wounds, is relatively common. Other benign tumors are not as rare as formerly believed; they include angioma, adenoma, teratoma, myoma, fibroma, lipoma, osteoma, chondrosteoma, retention cysts, aberrant thyroid, etc. Chondromas and osteochondromas may undergo malignant change. Primary carcinomas have been diagnosed with rapidly increasing frequency during the past decade. Edematous polypi and other tumor-like inflammatory growths are occasionally encountered and are always of the utmost importance because of the atelectasis, pulmonary edema or suppurative infections they may produce.

THE PNEUMONIAS

A remarkably large number of the living agents of disease may produce the pneumonias either as primary or secondary infections of the lungs. As a matter of fact, it is doubtful if pneumonia, excepting that due to *Past. pestis* and perhaps some of the viruses, is ever strictly primary in the sense that no previous infection of the upper or lower respiratory tracts or some acute infectious disease can be definitely excluded as a predisposing cause in the etiology of the disease.

These infectious agents include not only many of the pathogenic bacteria and possibly *T. pallidum* but several of the viruses and rickettsiae as well as several of the pathogenic fungi, protozoa and metazoa. Pneumonia may also result from mixed infections secondary to acute and chronic diseases, mechanical causes, shock, senility, etc., as well as from the aspiration of oils (lipid pneumonia) or other chemical agents, allergy or irradiation in which infection plays no part in etiology.

On broad clinical or pathologic grounds, the pneumonias are usually classified into the lobar or croupous, lobular or bronchial, interstitial and capillary bronchiolitic forms. Such classifications, however, are relatively unimportant except that the prognosis is better if the lesion is localized. Classification based on etiology is of far greater importance not only in relation to prognosis but especially in relation to chemotherapy. While for anatomic reasons the term "lobar pneumonia" is not always correct since all of a lobe may not be involved while several lobes or parts of them may be involved in the same patient, nevertheless, the term is as good as any other.

Pneumococcus Pneumonia. The incidence of lobar and atypical pneumonias due to pneumococcus infections varies greatly in different localities but it has been estimated that over 200,000 cases occur annually in the United States. In addition to being caused by Types I to XXXII pneumococci, seventeen new types have been described recently, nine of which are regarded as distinct from Types I to XXXII while eight are regarded as subtypes.[27] As is now well known, prognosis varies considerably according to the type of infection but is still most grave in those pneumonias due to Type III. Pneumococci are responsible for about 78 per cent of pneumonias of children with special reference to Types I, VI, XIV and XIX.[28] The disease in children, however, is never as grave as in adults and especially elderly individuals. Fortunately, prompt and adequate sulfonamide therapy alone or in combination with penicillin has greatly reduced the mortality due to all types in both children and adults. Pneumococcus pneumonia associated with bacteremia or septicemia with positive blood cultures is particularly dangerous as well as that occurring during the puerperium and when more than one lobe is involved. Possible complications include not only pleuritis, with or without empyema, but pulmonary abscess, gangrene, pericarditis, endocarditis, meningitis and arthritis as well.

Streptococcus, Staphylococcus and Other Coccal Pneumonias. About 25 per cent of atypical pneumonias are believed to be caused by *hemolytic streptococci* belonging to group A of Lancefield. These pneumonias are almost invariably secondary to streptococcus sore throat, scarlet fever, measles, pertussis or influenza. It is doubtful if pneumonia is caused by *Str. viridans* or nonhemolytic types of streptococci even though they are commonly present in the saliva and sputum. Pneumonia also occurs in from 0.6 to 1.4 per cent of cases of *acute rheumatic fever* but is seldom recognized clinically except in patients suffering from severe attacks of the disease. The etiology is still doubtful but apparently rheumatic fever is due to infection with beta or hemolytic streptococci.

About 10 per cent of atypical pneumonias may be caused by virulent strains of *Staph. aureus*. They are rarely primary and, like streptococcus pneumonia, are usually secondary to staphylococcus sore throat, influenza, measles, pertussis, etc. Staphylococci and streptococci may also produce superinfections in pneumonias due to pneumococci or other micro-organisms. *Micrococcus tetragenus,* the meningococcus, and *N. catarrhalis* may also produce atypical pneumonias which are likewise almost invariably secondary infections.

K. Pneumoniae and Other Bacillary Pneumonias. The incidence of pneumonia due to *K. pneumoniae* (*B. friedländeri*) varies greatly but occurs more commonly in the winter months. It is probably responsible for about 1 to 3 per cent of all pneumonias. The disease seldom occurs in infants and children, but chiefly among adults after middle age and more commonly among men than women. The mortality is very high although reduced by intensive treatment with sulfamerazine or sulfadiazine along with streptomycin in dangerously ill individuals. It may be primary and is usually lobar in type.

Atypical pneumonias may also be caused by *H. influenzae, H. pertussis, Ps. aeruginosa, Myco. tuberculosis, B. anthracis* (wool-sorter's disease), *Past. pestis* (pneumonic plague), the Brucella and especially *Br. melitensis, S. typhosa, Esch. coli, S. dysenteriae, C. diphtheriae, M. mallei* and *Past. tularensis.* Indeed, pulmonary tularemia is by no means uncommon [29,30] and may begin with pulmonary symptoms or develop during the course of infections of the fingers, hands or conjunctivas. Pleuritis with effusion is especially characteristic and the mortality is very high. The mortality of anthrax pneumonia is said to vary from 50 to 85 per cent while pneumonic plague is almost invariably and rapidly fatal.

Syphilitic Pneumonia. Of course massive or "white pneumonia" due to *T. pallidum* is well known in congenital syphilis. In acquired syphilis the pulmonary lesions usually consist of isolated gummas with arteritis or diffuse (chronic interstitial) pneumonitis.[31,32] Either may occur in about 5 per cent of cases, chiefly in men in the third decade of life, but are difficult to recognize clinically. Pulmonary tuberculosis and syphilis frequently occur coincidentally. It appears, however, that an acute or subacute atypical syphilitic pneumonia may be caused by *T. pallidum* in acquired syphilis although regarded as quite rare. It is believed that the pneumonia may be caused at times by an extension of the infection in the bronchi into the bronchioles and alveoli, but more likely is the result of the development of syphilitic granulomas in the perivascular tissues of the lungs. Many observers regard these pneumonias as due to secondary bacterial infections.

Primary Atypical and Other Viral Pneumonias. It is now fairly well established that a virus may produce a bronchopneumonia designated *primary atypical pneumonia.*[33-37] This is largely based on the exclusion of bacterial and rickettsial infections, more or less characteristic x-ray changes in the lungs, the failure of sulfonamide and antibiotic compounds in treatment, and the fact that, in 1946, the Commission on Acute Respiratory Disease succeeded in transmitting the disease by inoculating the upper respiratory tract of human volunteers with bacteria-free filtrates of sputums and throat-washings obtained from cases of the pneumonia. There is, however, no complete agreement among investigators as to the identity of the virus which has not yet been successfully cultivated. Furthermore, the results of attempts to transmit it to the lower animals have been confusing and conflicting while the results of serum neutralization and complement fixation tests for specific viral antibody have been doubtful and inconclusive.

In this connection it is also to be stated that Thomas and associates [38] have isolated from the lungs of fatal cases a nonhemolytic streptococcus MG for which a specific agglutinin develops during the second and third weeks of the disease in 25–75 per cent of cases; this agglutinin is distinct and different from the cold agglutinin for Group O human erythrocytes. Positive precipitin and skin reactions have also been observed to the capsular polysaccharides of this streptococcus. Under the circumstances, it has been suggested as the cause of primary atypical pneumonia but it appears that it may be a secondary infection or that the pneumonia may be due to symbiotic infection with a virus and this streptococcus.

It is now known that type A and type B viruses of *influenza* may produce pneumonia in man characterized by hemorrhagic and edematous lobular or peribronchial consolidation, with patchy areas of destruction of tissue and desquamation of the alveolar and bronchial epithelium. The gravest cases are those complicated by secondary infections with pneumococci, hemolytic streptococci, staphylococci, *H. influenzae,* or other micro-organisms; the mortality may be as high as 30 to 40 per cent.

The viruses of *psittacosis* and *ornithosis* may produce a similar pneumonia although the mortality is probably lower than 35 or 40 per cent and especially in young individuals. The virus of *measles* is also known to produce an interstitial type of pneumonia frequently complicated by secondary bacterial infections. Pneumonia has been ascribed also to the virus of *vaccinia* occurring after cowpox vaccination and as a complication of postvaccinal encephalitis. It is also common in *variola* and the most frequent complication in fatal cases of this disease. Pneumonia may be also caused by the virus of *lymphocytic choriomeningitis.*

The Rickettsial Pneumonias. In *typhus fever* due to infection with *Rickettsia prowazeki* tracheobronchitis is present so constantly that it is considered part of the disease. In a few cases actual pneumonia develops and is one of the main causes of death. It is characterized by accumulations of phagocytic endothelial cells in the capillaries of the alveolar walls. Frequently there is also a proliferation of the endothelium of the arteries and veins with perivascular accumulations of endothelial cells. Secondary bacterial infections occur commonly.

A similar pneumonia occurs in *Rocky Mountain spotted fever* due to infection with *Rickettsia dermacentroxenus.* It is somewhat less frequent than pneumonia in typhus fever but is largely responsible for the mortality of the disease. Rickettsiae, however, are not usually found in the pulmonary lesions.

The Mycotic Pneumonias. Various fungi and molds like penicillium, aspergillus, mucor, monilia, torula, blastomyces, coccidioides and actinomyces may gain entry to the blood and produce chronic forms of pulmonary disease. Some, however, may be inhaled and produce lesions which may be regarded as pneumonia.

Pneumonia due to *C. albicans* is uncommon and always occurs sporadically. The disease is usually chronic but frequently fatal with the lungs honey-combed by innumerable small abscesses along with large areas of consolidation. Pneumonia due to *Cryptococcus neoformans* also occurs. It is usually a chronic infection and often only part of systemic torulosis although a few cases of apparently primary infection of the lungs have been reported. As previously discussed in Chapter 16, coccidioidomycosis due to infection with *C. immitis* is usually due to the inhalation of the spores of the fungus with the production of bronchitis or atypical pneumonia. Primary infection may occur also through the skin. Systemic or generalized infection may occur by way of the blood in which almost any tissue or organ may become involved. The disease is practically confined to the San Joaquin Valley of California or its vicinity.

The Protozoal Pneumonias. Atypical pneumonia is a frequent cause of death in kala-azar due to infection with *L. donovani* involving the lymphoid and endothelial cells of the lungs along with secondary bacterial infections. The lungs are also the third most common site of localization of *E. histolytica,* largely the result of the rupture of amebic abscesses of the liver through the diaphragm with the production of abscesses rather than pneumonia. However, in rare instances the amebas themselves may be deposited in the lungs by the blood and produce pneumonia. Bronchitis and pneumonia are also rather common complications in malaria due to infection with any of the four *Plasmodia* of this disease but while the parasites are known to localize in the capillaries of the lungs, there is no evidence to indicate that they actually cause pneumonia which is thought to be due to secondary bacterial infections.

The Metazoal Pneumonias. During the life cycle of *A. lumbricoides* sufficient larvae brought to the lungs by the venous blood may occur in the alveoli to produce polymorphonuclear and eosinophilic exudates followed by epithelial desquamation, serous exudation or hemorrhage resulting in bronchiolitis and the consolidation of lobules with secondary bacterial infections; recovery is the rule. Similar lesions may be produced by the filariform larvae of *S. stercoralis.* As in the case of these two infestments, small numbers of the filariform larvae of the hookworms (*N. americanus* and *A. duodenale*) may pass through the lungs without the production of symptoms but when the infestment is sudden and massive, petechial hemorrhages and inflammatory exudates may produce diffuse atypical types of pneumonia along with secondary bacterial infections; recovery is the rule. Furthermore, the excysted metacercariae of *P. westermani* migrate through the wall of the duodenum, enter the abdominal cavity, penetrate the diaphragm, reach the pleural cavity, penetrate the lungs and finally lodge in the bronchioles where they develop into adult worms surrounded by fibrous capsules and produce lesions resembling tubercle-like abscesses with patches of pneumonia.

Laboratory Examinations. 1. *From the standpoint of specific therapy and prognosis, the etiologic diagnosis of a lobar or an atypical pneumonia, which is entirely dependent on bacteriologic or other laboratory examinations, is always advisable.* It is generally possible to obtain sputum for these purposes which should be collected in a clean, dry container and promptly delivered to the laboratory. Sputum can often be obtained by requesting the patient to co-operate by coughing or by a change of position in bed. Expectorant drugs, however, are seldom helpful in increasing the amount of sputum.

When sputum is not expectorated, it can often be obtained from the pharynx with a swab. Otherwise, secretions on the posterior pharyngeal wall may be collected on swabs and inoculated into tubes of broth or, preferably, sent to the laboratory for the preparation of smears and cultures. Material for examination may be obtained also directly from the involved area of the lung by *lung puncture.* The procedure is very reliable and fraught with practically no danger. The skin adjacent to the area of pneumonia, as located by physical or roentgenographic examination, is anesthetized with sterile novocaine solution. A long sterile 18- to 20-gauge needle attached to a small sterile glass syringe carrying a few drops of sterile broth, is inserted into the pneumonic area. Suction is made with the withdrawal of exudate and the needle quickly withdrawn.

2. It is also advisable to always make a *blood culture* whenever possible. For this purpose 10 to 15 cc. of blood is taken from a vein under rigid aseptic precautions and 1 cc. added to a tube of melted agar cooled to about 45° C. followed by rotation and pouring into a sterile petri dish for estimating the number of micro-organisms in case septicemia is present. The balance of the blood should be placed in a flask carrying 100 cc. of sterile hormone glucose broth to which 5 mg. of para-aminobenzoic acid has been added. Otherwise, the physician may collect 10 to 15 cc. of blood in a sterile test tube carrying about 2 cc. of a sterile 2.5 per cent solution of sodium citrate for the prevention of coagulation with prompt delivery of the specimen to the laboratory for the preparation of cultures.

3. In the laboratory it is always advisable to examine smears stained by the method of Gram prepared of sputum, material submitted on swabs, or secured by lung puncture. Almost invariably they show the predominant micro-organism which is very helpful in the interpretation of cultures which are so likely to show the presence of various bacteria in the case of cultures of sputum or pharyngeal swabs. Needless to state, the examination of fresh wet and stained smears is essential when a mycotic pneumonia or one due to an animal parasite is suspected. Special culture media are required in the case of those pneumonias suspected of being due to *Myco. tuberculosis, H. influenzae, H. pertussis, Past. tularensis, M. mallei,* the Brucella or pathogenic fungi. In pulmonary tularemia the agglutination test is of diagnostic value during or after the second week of the disease although it occasionally fails.[30]

4. Since penicillin and the sulfonamide compounds, especially sulfadiazine and sulfamerazine, are effective in all varieties of pneumococcal pneumonias, the *serologic typing of pneumococci* is not as essential as formerly but is still always advisable as a routine procedure whenever possible; it is absolutely essential in relation to type-specific serum therapy which is sometimes required in the pneumococcal pneumonias.

5. In *primary atypical pneumonia* two laboratory tests are of aid in diagnosis: (a) the cold-agglutination test employing group O human erythrocytes, and (b) the streptococcus MG agglutination test. Both are best conducted with serums obtained at weekly intervals during the course of the disease. If either or both give positive reactions, and especially if a significant increase in either agglutination titer is observed some weeks after onset, there is a high probability that the diagnosis is correct. If both tests give negative reactions it may be very difficult to establish a laboratory diagnosis. Morgan and Finland have reported 74 per cent positive cold agglutination reactions in 86 cases and 35 per cent positive MG agglutination reactions in 44 cases.[39]

In other viral pneumonias, however, laboratory diagnosis is difficult and frequently impossible. Only animal inoculation tests may be employed. Thus, in pneumonia suspected of being due to the viruses of influenza, ferrets may be inoculated intranasally with a few drops of filtered or unfiltered nasopharyngeal washings or material obtained by lung puncture. In the case of suspected pneumonia due to the virus of psittacosis, sputum or material secured by lung puncture may be injected intraperitoneally into white mice; the animals usually succumb within ten to fourteen days.

6. *Anemia* does not ordinarily occur during pneumonia of short duration although both the erythrocytes and hemoglobin may be reduced due to water retention and plasma dilution. Anemia is more likely to occur in patients with empyema or other complications. *Leukocytosis* of varying degree is the rule and due largely to an absolute increase of the polymorphonuclear neutrophils. While marked leukocytosis is of good prognostic import, yet patients with leukopenia due to severe infections may recover, although both extremely high or extremely low total leukocyte counts are of bad prognostic import. At the time of the crisis in pneumococcal pneumonia, the polymorphonuclear leukocytosis usually slowly disappears; its persistence or increase is always suggestive of a complication and especially empyema. Continued leukocytosis due to lymphocytosis, however, suggests delayed resolution. A transient monocytosis sometimes occurs at about the time resolution commences. In primary atypical or viral pneumonia, however, normal total and differential leukocyte counts may be observed. *Thrombopenia* of varying degree is of frequent occurrence and the *coagulation time* of the blood generally lengthened. The *sedimentation rate* of the erythrocytes is markedly increased. The *complement fixation* and *flocculation tests* for syphilis sometimes yield temporarily falsely positive reactions probably due to hyperproteinemia and especially in serum-treated cases of pneumococcus pneumonia.

7. *Arterial blood oxygen unsaturation* is of frequent occurrence and especially in the atypical pneumonias. Cyanosis is due primarily to insufficient oxygenation of the blood passing through aerated lung tissue and to nonoxygenation of that portion of the blood which passes through consolidated unaerated lung rather than to circulatory failure. Arterial blood oxygen unsaturation of 11.8 per cent may result in slight cyanosis which becomes severe at 26 per cent.[40] The *carbon dioxide combining power of the arterial blood* may increase early in lobar pneumonia soon followed by a decrease but this is not a constant finding.[41]

8. During the precritical stage of pneumococcal pneumonia, *hypochloremia* is of frequent occurrence although the plasma chloride varies considerably in different patients and in the same patient from day to day. After the crisis the plasma chloride quickly returns to normal. There is also a tendency to an increase of blood *uric acid* and a decrease of serum *sodium*. *Hypocholesterolemia* commonly occurs during convalescence. *Hypercalcemia* is frequent because of the changes in the CO_2 tension of the blood which keeps more calcium in solution.

9. *Nitrogen retention* of varying degree may occur often, followed by a marked increase of nitrogen excretion after the crisis. Slight *hyperglycemia* is of frequent occurrence while *acidosis* due to the retarded elimination of CO_2 is particularly apt to occur in the atypical pneumonias and especially in children. On the other hand, *alkalosis* may develop due to the increased respiratory rate resulting in hyperventilation. *Hyperbilirubinemia* with positive icterus index and van den Bergh reactions due to toxic hepatitis is not unusual while clinical jaundice is of poor prognostic import. *Hyperproteinemia* largely due to hyperglobulinemia

may occur, while a marked increase in plasma *fibrinogen* is characteristic of the pneumococcus pneumonias.

10. Febrile *albuminuria* due to nephrosis is of frequent occurrence. Acute glomerulonephritis with marked albuminuria and hematuria is sometimes observed and especially in streptococcus pneumonias. *Urobilinuria* may be observed, especially in the presence of hyperbilirubinemia.

ABSCESS OF THE LUNG

Abscess of the lung may be due to many sources of infection as follows: (1) As a sequence of lobar or atypical pneumonia and especially in delayed resolution with secondary staphylococcus or streptococcus infection. Thus numerous small abscesses are stated to occur in about 3 per cent of cases of pneumococcus pneumonia and especially in Type III infections, although they are not usually discovered during life. Lobar and atypical pneumonias due to hemolytic streptococci and *Staph. aureus*, however, are particularly likely to develop abscesses. (2) Foreign bodies, especially those composed of vegetable material, may be the cause either through the production of deglutition or aspiration pneumonias or the result of erosion and ulceration of the bronchi with secondary infection. (3) Undoubtedly, emboli or septic infarcts in the branches of the pulmonary artery are frequently responsible. These may gain access to the venous blood not only from the disintegration of thrombi in areas of phlebitis, but following wounds of the neck, tonsillectomy and other operations on the nose and throat and even the ears, as well as after the extraction of teeth. Apparently, however, the resulting pneumonitis and abscess under these circumstances may be due also to the aspiration of blood or other foreign matter and especially in individuals under general anesthesia. (4) Obstruction by bronchogenic carcinoma or other tumors with secondary infection may also be a cause as well as (5) perforating wounds of the lungs, (6) the rupture of subphrenic or liver abscesses into the lungs, (7) secondary infections of amebic and echinococcus cysts and (8) secondary infections of tuberculous or actinomycotic lesions, etc.

Laboratory Examinations. Diagnosis is usually based on the signs and symptoms aided by roentgenographic and bronchoscopic examinations. From the standpoint of infection, however, only laboratory examinations are capable of establishing etiologic diagnosis and may be summarized as follows: (1) Bacteriologic, mycologic or parasitologic examinations of material secured by bronchoscopic aspiration or drainage are always advisable in preference to examinations of the sputum. Indeed, owing to bronchial obstruction there may be but little or no expectoration until a large abscess has ruptured into a bronchus. (2) When this occurs the sputum is increased in amount, purulent and fetid, as discussed in Chapter 9. (3) The examination of smears and cultures is essential in diagnosis. *It is always important to include routinely anaerobic cultures.* Material secured by bronchoscopic aspiration frequently reveals pure or almost pure cultures of the infecting micro-organism. Smears and cultures of sputum, however, almost invariably show the presence of several bacteria adding greatly to the difficulties of clinical interpretation. Undoubtedly the most frequent micro-organisms responsible for lung abscesses are aerobic and anaerobic hemolytic streptococci, *Str. viridans*, nonhemolytic streptococci, hemolytic *Staph. aureus* and *albus*, pneumococci, *H. influenzae, K. pneumoniae, Bacteroides* and *Bact. melaninogenicum* along with *M. tetragenus, N. catarrhalis* and other diplococci of minor importance and frequently diphtheroid bacilli and *Bor. vincentii* or other spirochetes in association with various types of *B. fusiformis.* (4) Needless to state, blood examinations usually show a sharp leukocytosis due to an absolute increase of the polymorphonuclear neutrophils with a "shift to the left." (5) The sedimentation rate of the erythrocytes is increased. (6) Septicemia may supervene; for this reason blood cultures should be employed routinely.

GANGRENE OF THE LUNG

Fortunately, gangrene of the lung is comparatively uncommon but it may be due to any of the causes responsible for abscess of the lung. Its experimental production in the lower animals has shown the etiologic importance of complete bronchial obstruction.[42] Apparently severe vascular obstruction due to septic embolism with infarction is also frequently responsible. Infection with any of the micro-organisms producing abscess of the lung may be the cause but undoubtedly anaerobic bacteria are far more important in its etiology than aerobes as is likewise true in abscess of the lung.[43,44] I am convinced that primary, and especially secondary, infection with spirochetes and fusiform bacilli is of major importance, as claimed by Smith.[45] At least, these micro-organisms are present very frequently in the sputum and are known to be rapidly destructive of tissue with the production of fetid odor and hemorrhage as seen in Vincent's angina and severe erosive or gangrenous balantitis. But why some cases of abscess progress into gangrene is unknown. Undoubtedly, greatly lowered resistance is an important factor and especially debility during prolonged illness. The rapid destruction of tissue with no line of demarcation is evidently caused by severe proteolysis resulting from the excessive production of bacterial proteolytic enzymes or an imbalance between them and the anti-enzymes of leukocytes and other cells and of the plasma.

The clinical and roentgenographic manifestations are so pronounced that *laboratory examinations* are seldom indicated for diagnostic purposes except so far as they are required from the standpoint of etiologic diagnosis. The sputum is characteristically greatly increased, thin and watery, extremely offensive, "prune juice" in color due to bleeding resulting from necrosis, and separable into layers upon standing, as discussed in Chapter 9. The examination of stained smears supplemented by darkfield examinations for spirochetes is essential along with aerobic and especially anaerobic cultures for anaerobic hemolytic streptococci, Bacteroides, etc.

PULMONARY TUBERCULOSIS

Sufficient data have now accumulated to justify the view that most cases of pulmonary tuberculosis begin as a mild pneumonia or pneumonitis in the same manner as pneumonic infections caused by other micro-organisms. It is usually known as the *first infection type.* While heretofore this primary infection has been observed chiefly in infancy and childhood, it is now known to occur in young adults as well. Reinfection may occur in later life constituting the *reinfection type.* In its acute forms it may occur as an acute tuberculous lobar pneumonia but more frequently as an acute tuberculous bronchopneumonia also known as phthisis florida or "galloping consumption." It may also occur as an acute miliary tuberculous infection. Chronic tuberculosis of the lungs is generally designated as "chronic ulcerative tuberculosis" because of the frequency of progression with cavitation but also includes that type known as "fibroid tuberculosis" or "fibroid phthisis."

Laboratory Examinations. 1. *The presence of tubercle bacilli in the sputum and/or the gastric contents, in the absence of detectable tuberculosis of the upper respiratory tract, is the only absolute proof of pulmonary tuberculosis.* Failure to find them may be pardoned, but failure to search carefully for them admits of no excuse no matter how definite the diagnosis may appear. Examinations by the method of fluorescent microscopy may reveal their presence when smears stained by the method of Ziehl-Neelson are negative. The antiformin or other methods of concentration are frequently required as well as cultures on the Petragnini or other special media. Guinea-pig inoculation tests frequently reveal the presence of tubercle bacilli when they are too few for detection by direct microscopic or cultural examinations. Although one positive result outweighs many failures, it should be promptly confirmed. Tubercle bacilli may be found months before physical signs appear, and even before definite roentgenographic changes develop. Failure to find tubercle bacilli even on repeated examinations, however, does not exclude the possibility of tuberculosis, and postponement of diagnosis until they are found may be disastrous. Their presence in the sputum is an indication of ulceration. The number present in smears according to the scheme of Gaffky is significant but not necessarily of prognostic importance. Patients should be instructed to bring the early morning sputum and the little dense balls raised at infrequent intervals. No sputum should be regarded as negative until at least three to six, or more, specimens at intervals have been thoroughly examined, as the occurrence of closed pulmonary tuberculosis is now questioned. Tubercle bacilli can sometimes be recovered from swabs from the pharynx, or from the feces when they are not present in the sputum or stomach. Examinations of aspirated stomach contents for tubercle bacilli are particularly valuable.

2. Hemoptysis in chronic pulmonary disease is always suggestive of tuberculosis. In acute tuberculous pneumonia, the *sputum* at first resembles that of other types of pneumonia, but later becomes grayish. As chronic pulmonary tuberculosis progresses the sputum usually becomes more abundant, more purulent, and at times nummular and heavier than water. During improvement it becomes less and less purulent and the color may change from green to yellow to white. A sudden increase or decrease in the amount of sputum suggests the onset of complications or acute miliary tuberculosis. The rather uncommon chalky concretions or "lung stones" are usually seen in chronic tuberculosis and before ejection may cause hemoptysis. The presence of elastic tissue in the sputum without tubercle bacilli is evidence against tuberculous infection. The presence

of albumin in the sputum as well as other changes discussed in Chapter 9, are of helpful diagnostic value.

3. As discussed in Chapter 19, positive *tuberculin reactions* are an index of tuberculous infection but no tuberculin test accurately distinguishes active from inactive tuberculosis. In other words, positive reactions in infancy and childhood are generally indicative of active tuberculosis but in adults may be the result of clinically inactive or healed tuberculous infection, since tuberculin hypersensitiveness when once acquired is always likely to endure for many years and even the balance of life. The cutaneous (von Pirquet), intracutaneous (Mantoux) and patch (Vollmer) tests are commonly employed. The subcutaneous test (Koch) has the advantage of focal reactions being indicative of active tuberculous lesions.

4. The tuberculosis *complement fixation test* may be an additional diagnostic aid in some cases. Negative reactions are of little or no value in excluding tuberculosis of the lungs but positive reactions are significant as frequently indicative of active tuberculosis, as discussed in Chapter 17. The technic employed, with special reference to antigen, is very important. In this connection it is essential to remember that the antigen may contain sufficient lipids of tubercle bacilli to give positive complement fixation reactions in nontuberculous syphilitic individuals. In other words, the Wassermann test should always be included routinely and if a positive reaction due to syphilis is observed, a positive tuberculosis complement fixation reaction must be interpreted with great caution. Furthermore, biologic nonspecific Wassermann and flocculation tests may be observed in some cases of active pulmonary tuberculosis, as discussed in Chapter 18, although the incidence is very low. *Agglutination* and *opsonic index tests* possess no diagnostic value.

5. *Anemia* of the hypochromic normocytic type is common. The leukocytes may be normal or subnormal but a relative lymphocytosis is of frequent occurrence except in cases of acute secondary infection of tuberculous lesions when leukocytosis due to an absolute increase of the polymorphonuclear neutrophils may be observed.

6. The *sedimentation rate of the erythrocytes* is usually increased, frequently in relation to the degree or severity of infection. Repeat tests at intervals are of value in relation to progress and prognosis. Normal rates may be observed, however, in 20 to 40 per cent of cases, depending upon the criteria employed to determine activity of the disease.[46] The curves of sedimentation vary roughly, but not uniformly, accordingly to the degree of anatomic involvement.

7. The *gaseous composition of the blood* is essentially normal when the lesions are localized. However, if the latter are extensive, arterial oxygen saturation may fall below the lower limit of normal (95 per cent). Under these conditions, the carbon-dioxide content tends to rise progressively in most cases.

8. The *fibrinogen* of the blood is usually increased. Compared to an average normal of about 0.25 per cent, the concentration in active tuberculosis may rise above 1.0 per cent, although usually it is nearer 0.4 to 0.8 per cent. This may be responsible largely for the more rapid sedimentation rate of the erythrocytes. The total *plasma protein* is normal, or slightly elevated, due largely to an increase of globulin with an alteration of the albumin-globulin ratio. *Hypocholes-*

terolemia may be observed only in advanced cases. The *nonprotein nitrogenous constituents* of the blood are usually normal in the absence of renal impairment. However, *hypochloremia* may occur in some cases as likewise a lowering in the alkali reserve in the terminal stages with a marked tendency to acidosis.

9. Usually no significant changes are observed in the *urine* in the absence of renal impairment although febrile albuminuria is not uncommon. The diazo and urochromogen reactions, however, are often positive in the late stages.

THE PLEURITIDES

Pleuritis or pleurisy is an inflammation of the parietal, visceral, or diaphragmatic pleural membranes. It is commonly divided into four chief clinical types as follows: (1) *acute fibrinous pleuritis* (pleuritis sicca or dry pleurisy); (2) *serofibrinous pleuritis* (pleuritis serofibrinosa or wet pleurisy); (3) *suppurative pleuritis* (empyema) and (4) *chronic fibrous pleuritis* (pleuritis fibrosa). The pleuritis may be localized to the interlobar pleura and in empyema the pus may become encapsulated or encysted instead of free in the pleural cavity.

While pleuritis may follow aseptic irritation, it is my opinion that all acute and chronic types of the disease are due to infection. Pleuritis frequently develops over areas of cancer of the lungs or pleuras as well as over infarcts of the lungs, in which it is much more likely that local infection is primarily responsible. While it is conceivable that pleuritis may be due to primary infection, I prefer to regard all types as due to secondary infection. It is commonly stated that exposure to cold or dampness alone may cause acute pleuritis but while these may be important predisposing factors in the etiology of some cases, I believe that infection secondary to primary infection of the upper or lower respiratory tract is responsible.

The sources of the secondary infection in the acute and chronic pleuritides are quite numerous as follows: (1) by direct continuity or by way of the lymphatics in any of the lobar and atypical pneumonias, including pulmonary tuberculosis, as well as in abscess and gangrene of the lungs; (2) by either of these routes of infection in the bronchitides including bronchiectasis; (3) by involvement of the pleurae in pulmonary infarction and malignant disease; (4) by extension of inflammation from adjacent organs as in pericarditis, the pancarditis of acute rheumatic fever, mediastinitis or subphrenic abscess; (5) by the rupture into the pleural cavity of subdiaphragmatic or liver abscesses; (6) by metastatic infection in the septicemias; (7) by infection due to compound fractures of the ribs or other wounds of the thorax as well as thoracic paracentesis and (8) as a pleuritis with effusion as a complication in such diseases as chronic nephritis, rheumatoid arthritis, gout, leukemia, etc. The latter must be carefully differentiated by bacteriologic and other laboratory examinations from the transudates of hydrothorax, hemothorax and chylothorax.

Laboratory Examinations. The diagnosis of the four types of pleuritis is based on the signs and symptoms aided by roentgenographic and sometimes pleuroscopic examinations. But etiologic diagnosis is usually based on laboratory examinations of pleural effusions, when these are obtainable, as follows:

1. Careful bacteriologic examinations of smears (stained by the method of Gram) and cultures of the pleural fluids. These are preferably prepared of sediments obtained by the thorough centrifugation of 15 to 20 cc. amounts with rigid precautions against contamination. When tuberculous infection is suspected stained smears usually require prolonged examination for acid-fast bacilli; the fluorescent method of examination is helpful. Guinea-pig inoculation tests are also of great value providing the animals are inoculated with 10 to 15 cc. of fluid or large amounts of sediment. Both aerobic and anaerobic cultures should be made and especially if streptococcus pleuritis is suspected. Special culture media are required in pleuritis suspected of being due to *Past. tularensis, Myco. tuberculosis, H. influenzae,* etc., as likewise when it is suspected of being due to *A. bovis, C. albicans,* nocardiosis, sporotrichosis, *C. immitis* or other mycotic infections. Darkfield examinations for spirochetes should be included always when pleuritis is due to abscess or gangrene of the lung.

2. Total and differential cell counts (*cytodiagnosis*) of the fluid are likewise of helpful diagnostic value as well as physical examination referable to color, specific gravity and the presence or absence of coagula due to an excess of fibrinogen. As discussed in Chapter 13, these examinations along with quantitative determinations of total protein and chloride are particularly desirable in differentiating between transudates due to passive congestion or lymphatic blockade and exudates due to inflammation. Sometimes unusually large numbers of eosinophilic leukocytes are found (*eosinophilic pleurisy*) which is always suggestive of pneumonitis and pleuritis due to an animal parasite or allergy. In some cases, the cholesterol content of the effusion is unusually high with the presence of crystals (*cholesterol pleurisy*), and especially in pleuritis due to pulmonary tuberculosis.

3. Anemia is not unusually present in acute pleuritis but is very common in the chronic forms and especially empyema. Leukocytosis of varying degree due to an absolute increase of the polymorphonuclear neutrophils or monocytes is usually observed and especially in acute pleuritis with special reference to empyema. The sedimentation rate of the erythrocytes is almost invariably increased.

THE PNEUMOCONIOSES

Pneumoconiosis is a chronic disease of the lungs characterized by fibrosis caused by the prolonged inhalation of certain dusts. It constitutes one of the most frequent and important of the industrial hazards. According to the chemical constitution of the dust, the disease is divisible into (1) *silicosis* due to silica and seen in those working with quartz, granite, corundum, etc.; (2) *chalicosis* due to calcium dusts; (3) *siderosis* due to iron dusts among workers in iron, brass, bronze and especially mirror polishers; (4) *anthracosis* or *anthraco-silicosis* due to carbon and seen particularly in coal miners; (5) *asbestosis* due to the dusts of asbestos which is a hydrated silica of magnesium containing a small percentage of iron and nickel and (6) *baritosis* due to the dust of barium. Other silicates like talc, slate, etc., also may produce pneumoconiosis but are much less likely to do so than silica itself. Organic dusts like those of cotton, shoddy, flour, etc., rarely produce the disease although capable of producing irritation of the bronchi as seen in "shoddy fever," among bakers, etc.

All types of pneumoconiosis are productive of ill health but undoubtedly silicosis is the most dangerous of all because the pulmonary injury so greatly predisposes to tuberculosis as well as to influenza and pneumonia. The disease may occur acutely but is usually chronic due to the inhalation of silica over months or years of time. As far as granite dust carrying 35 per cent of quartz is concerned, it has been estimated that the safe limit of exposure is probably below 10 million particles per cubic foot of air while in the case of purer kinds of quartz the limit is probably less than 5 million per cubic foot. The examination of silicotic lungs has shown that practically all of the dust particles are less than 10 microns in longest dimension.

The injurious effects of these inorganic dusts is not necessarily due to the fact that they are sharp and cutting, since diamond cutters do not develop the disease. Apparently dusts are injurious in proportion as their chemical constitution is foreign to the body. For this reason silica and the silicates cause most injury, and carbon and the vegetable dusts least of all. After reaching and penetrating the bronchial mucosa, the dusts are usually taken up by phagocytic cells which tend to accumulate in the lymphatics with eventual blockade and the production of a low-grade chronic interstitial pneumonitis. All dusts, and especially silica, tend to "drift" toward the pleurae where dust-laden cells tend to accumulate in masses called "pseudotubercles." However, there is good reason to believe that only a part of the dust found so uniformly distributed throughout the lungs could have entered by way of the bronchi. Indeed, it appears from experimental investigations in anthracosis that the particles, on being swallowed, penetrate the intestinal mucosa and on reaching the portal circulation are conveyed to the lungs. The element of infection is not only important because dusts are carriers of bacteria, but especially because the resulting fibrosis greatly reduces resistance due to a lowering of blood supply. As previously stated, silica is especially dangerous not only from the standpoint of predisposing to tuberculous infection, but because it may activate latent lesions due not only to a reduction in local resistance but to the fact that silica in culture media is known to favor the growth of the tubercle bacillus.

Laboratory Examinations. Laboratory examinations, however, are of only limited value in the diagnosis of pneumoconiosis. They are confined to examinations of the sputum in relation to color, the finding of asbestos fibers and deposits of the dust and especially in phagocytic cells. Chemical examinations are also sometimes of diagnostic value in the identification of the dust. In silicosis there may be an increased urinary excretion of silicic acid but apparently with no increase in the blood above the average of 1 mg. of SiO_2 per 100 cc.[47]

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DISEASES OF METABOLISM

Many diseases produce or are accompanied by pronounced changes in metabolism, as discussed in Chapters 1, 3, 4, 5, 6, 8 and 20, but a comprehensive consideration of the metabolism of carbohydrates, proteins, lipids, minerals and water is beyond the scope of this book. Some symptom-complexes and diseases, however, are due to such pronounced disturbances in metabolism that they are classified as *Diseases of Metabolism*. The usual ones and especially those in which laboratory examinations are the only or helpful means in diagnosis, are considered herewith.

ACID-BASE EQUILIBRIUM

Normally the blood and all other fluids of the body are slightly alkaline, but the divergence from neutrality is so slight that the sum total of basic cations is practically equal to the sum of the acid anions. Sodium makes up the major part of the base, while potassium, calcium and magnesium are relatively unimportant. On the acid or anion side of the balance chloride predominates, bicarbonate being an important second. Because the *p*H of plasma is on the alkaline side of the proteins, the latter act chiefly as anions, with phosphate, sulfate and organic acids constituting the remainder. Within the cells it may be deduced that potassium is the most important base and organic phosphates and proteins the most important acids or anions. The *p*H of intracellular fluids appears to be slightly lower than that of the extracellular, but since the reactions of the two fluids are related, variations of extracellular *p*H will, therefore, be reflected in changes of the *p*H of interstitial fluids.

The mechanisms by which the acid-base equilibrium and the *p*H of the blood are preserved are inseparably connected with the maintenance of osmotic equilibrium and with water balance. In this connection dilution plays an important rôle, since it is quite evident that a change in the *p*H of the fluids in one part of the body is rapidly corrected by distributing it throughout the body by the blood aided by diffusion. The second mechanism resides in the buffers or salts of weak acids which mitigate changes of reaction. The chief buffers are proteins ably seconded by bicarbonates supported by phosphates. Since proteins occur in higher concentration in cells than in plasma, the buffer defense is more intracellular than extracellular in its mechanism.

Carbonic acid formed by the solution of carbon dioxide in water is among the most important of the buffers, since it is continually produced in the normal metabolism of the tissues and reacts with salts of the weaker buffer acids to form bicarbonates. It is, therefore, continually competing with other buffers for the possession of base aside from being volatile and consequently eliminated through the lungs. The quantity of bicarbonate in the blood and other body fluids depends on the base not combined with acids stronger than carbonic and constitutes the chief alkaline reserve of the body. If the total base or alkaline reserve becomes depleted by diarrhea or the accumulation of acids stronger than carbonic in the plasma, such as lactic or beta-hydroxybutyric acids, bicarbonate is reduced and acidosis results. On the other hand, if the total base or alkaline reserve is increased because of the administration of sodium bicarbonate or other alkaline salts, or if extra base is made available by loss of chloride in acid vomitus, plasma bicarbonate increases and alkalosis results. In acidosis a coincident change in the *p*H of the blood can be prevented by an increased respiratory output of carbon dioxide but such compensation is seldom quite complete. Consequently, a reduction in bicarbonate usually entails some reduction in *p*H while an increase in bicarbonate is usually associated with some increase of *p*H.

ACIDOSIS

Acidosis, therefore, is not a disease but a frequently occurring symptom-complex due to a disturbance of metabolism characterized by a reduction of the bicarbonates (alkaline reserve) in the blood below the normal level. This reduction may occur not only in conditions producing a removal of blood bicarbonate but likewise its loss by the intake or production of excessive amounts of organic acids, since the important function of bicarbonate is its buffering action in maintaining a normal hydrogen ion concentration of the blood. Under average normal conditions of activity and food intake, the acid waste products of metabolism are usually in excess of the basic or alkaline products but normal hydrogen ion concentration is maintained by: (1) the buffering properties of the blood principally by combining the acid radicals with the base of the bicarbonate, the liberated CO_2 being expelled through the lungs; (2) the excretion by the kidneys of urine much more acid than blood with the consequent return of base to form bicarbonate for transporting more acids to the kidneys and (3) the utilization of ammonia by the kidneys to combine with acid radicals for elimination as neutral salts.

As previously discussed (page 95), the most common cause of acidosis is the excessive production or retention of acid radicals and especially of beta-hydroxybutyric and aceto-acetic acids by faulty fat and protein metabolism. Less frequently other organic acids may be produced in amounts sufficient to produce it. Consequently, acidosis may develop not only in diseases associated with the production of large amounts of the ketone bodies resulting in a marked lowering of the alkaline or bicarbonate reserve of the blood, as in severe diabetes mellitus and cyclic vomiting of children, but likewise in some cases of acute and chronic nephritis associated with the retention of phosphoric and other acids along with a reduced capacity to form or utilize ammonia for their neutralization. Acidosis also occurs in some of the infectious diseases like pneumonia, rheumatic fever, typhoid fever, generalized tuberculosis and cholera—likewise in such chronic diseases as pernicious anemia, cirrhosis of the liver, cancer and starvation states as well as in some of the intoxications (methyl alcohol, salicylates, carbon monoxide), traumatic shock, severe burns, general anesthesia, pregnancy, etc. In normal pregnancy as well as in its toxemias, however, disturbances in the alkaline reserve of the blood itself appear to be of but minor importance.[1]

Laboratory Examinations. Marked acidosis may be detected clinically by headache, weakness, hyperpnea, fruity odor of the breath, drowsiness or coma. *Only laboratory examinations are capable of detecting mild cases before the production of symptoms.* Early recognition with appropriate treatment is always desirable for the possible prevention of serious acidosis.

1. For these purposes the most useful and reliable test is the determination of the CO_2 combining power of the plasma according to the method of Van Slyke. The normal range is 55 to 80 volumes per cent. Values between 50 and 40 volumes per cent are indicative of mild, 40 to 30 of moderate, and 25 to 10 of severe acidosis. A new and rapid colorimetric method requiring but 0.1 cc. of plasma has been described recently by Exton and his associates.[2]

2. Acidosis may be detected also by determining the CO_2 tension of the alveolar air of the lungs although this method is not now being generally employed. The normal varies from 5.2 to 5.7 volumes per cent. In mild acidosis 5 to 3.5 volumes per cent are observed, 3 to 2.5 in moderate and 2 or less in severe acidosis.

3. Determinations of the pH of the blood are not generally employed. The simplest and most satisfactory test is the colorimetric method of Cullen and Van Slyke.

4. A decrease in the diphosphoglycerate fraction of the organic acid-soluble phosphorus of the erythrocytes has been reported in severe acidosis of diabetes with adequate renal function, following the administration of ammonium chloride and in infants with severe diarrhea.[3]

5. Hypocalcemia may also occur.[4]

6. When acidosis is due to the excessive production of the ketone bodies, acetone and aceto-acetic (diacetic) acid are usually found in the urine along with beta-hydroxybutyric acid in severe cases and especially in diabetic coma.

7. Leukocytosis may occur, especially in the coma of diabetes mellitus, which has been ascribed to the hypertonicity of the plasma in this disease.[5]

ALKALOSIS

Alkalosis like acidosis is not a disease but a symptom-complex also due to a profound disturbance of the acid-base equilibrium toward the alkaline side with an excessive alkaline reaction of the body fluids. It may be due to an absolute or relative increase of alkali in the blood. An absolute increase is usually due to the administration of amounts of sodium bicarbonate, sodium citrate or other alkalies and their organic acids beyond the amount the body can neutralize and excrete. A relative increase is generally due to the rapid or excessive loss of acid radicals, especially carbon dioxide, as well as to a severe loss of hydrochloric acid in the stomach. The loss of carbon dioxide may be due to hyperventilation as in the acute fevers (especially pneumonia), exposure to high temperatures and high altitudes, hysteria, encephalitis and other disturbances of the respiratory center, etc. The loss of hydrochloric acid is generally due to severe vomiting from pyloric or other kinds of high intestinal obstruction, brain lesions, uremia, pernicious vomiting of pregnancy, the alimentary toxicoses of infants and children, x-ray, ultraviolet light or radium therapy, etc. In this connection it must be remembered that in nephritis or other diseases of the kidneys associated with marked dysfunction, the body is hindered in its acid-base regulatory mechanism by the partial loss of the ability of the kidneys to excrete an excess of base.

Laboratory Examinations. Marked alkalosis may be detected clinically by the signs and symptoms of tetany but, as in the case of acidosis, *only laboratory examinations are capable of revealing its presence in mild cases or before symptoms appear.* Early recognition is important because, as a complication in a diseased state, it may be a marked embarrassment to comfort, to treatment and even to life.

1. As in acidosis, alkalosis is detected best by the determination of the CO_2 combining power of the plasma according to the method of Van Slyke or the simplified method of Exton and his associates.[2] By the Van Slyke method 70 to 78 volumes per cent indicates mild, 85 to 90 moderate, and 100 or higher severe alkalosis.

2. It may be detected also by a determination of the tension of CO_2 in the alveolar air of the lungs although this method is not now generally employed. A tension of 6 to 6.5 volumes per cent is indicative of mild, 7 to 8 of moderate and 9 or higher of severe alkalosis.

3. In this connection it is important to remember that a patient may show acetone in the urine and still be in alkalosis.

DISTURBANCES OF WATER METABOLISM

Water constitutes about 70 per cent of the body weight, 50 per cent of the latter being due to intracellular fluids, 15 per cent to interstitial fluids and about 5 per cent to blood plasma. The sources of water are not only that which is preformed in liquids and solid foods absorbed from the gastro-intestinal tract, but that produced by the oxidation of foods to CO_2 and H_2O. Furthermore, it must be recognized that water continues to be formed by the latter means from endogenous sources even when neither food nor fluids of any kind are being consumed. The chief stimulus to its replenishment is thirst due to a deficiency of water or to a relative excess of sodium chloride in the body fluids or tissues. In the case of a full diet without excessive sweating this is usually sufficient. But when the diet is greatly reduced the situation may be altered if it entails a reduction in salt intake and salt depletion which, if carried too far, may also entail the elimination of carbohydrates and result in ketosis. Both ketosis and salt depletion promote dehydration and the former also abates thirst. Care should be taken, therefore, to always provide not only sufficient water but a minimum of carbohydrate (100 gm. of glucose) and sodium chloride (5 gm.) per twenty-four hours to prevent these disorders in the case of disease in which the instinct of thirst does not have a part. Large quantities of salt, as well as water, are lost by excessive sweating. Under these circumstances, when large amounts of water alone are taken muscular cramps may occur, especially in persons laboring at high temperatures, constituting *water intoxication.* If salt is taken as well as water, no cramps or other symptoms are likely to develop.

Water is excreted by the skin, lungs, kidneys and bowels. The excretory function of the skin and lungs is exercised chiefly in behalf of the regulation of the body temperature. Water lost in the feces may be regarded as waste incurred because of the necessity that unabsorbable residua of foods and intestinal secretions be diluted sufficiently to permit their ejection. As discussed in Chapter 2, on the kidneys, in mammals, falls the task of excreting excessive quantities of particular solutes which may accumulate in the body in excess, while retaining those which require conservation, in order to maintain a constant and optimal volume of fluids and their normal chemical constitution. By virtue of these powers, which seem to reside in the loops of Henle, the kidneys are peculiarly adapted to the conservation of water.

The object of water metabolism, therefore, is to maintain within the body an approximately constant state favorable to the operation of its vital activities. It is naturally divided into: (1) the exchanges of water between the body and its environment and (2) the movements of water within the body. As stated by Gamble,[6] the mechanics of the circulation of the blood make the maintenance of a normal blood volume much more important than a normal volume of interstitial fluid. Consequently, an increase in the volume of extracellular fluid, constituting a state of edema, is usually accomplished entirely by expansion of the interstitial spaces while, on the other hand, loss of plasma water as a result of dehydration is usually made up by the passage of interstitial fluids into the blood. Therefore, the volume of interstitial water exhibits a wide range of adjustability in defense of plasma water. From a clinical standpoint disturbances of water metabolism are chiefly of interest in relation to blood and plasma volume, edema and dehydration.

Blood and Plasma Volume. By the carbon monoxide method of determination, normal blood volume in adults has been found to vary from 63 to 76 cc. per kilogram of body weight or 2000 to 2900 cc. per square meter of body surface. By the dye method the normal limits are stated to vary from 72 to 100 cc. per kilogram of weight or 2500 to 4000 cc. per square meter of body surface. The normal limits for plasma volume are 49 to 59 cc. per kilogram or 1400 to 2500 cc. per square meter of body surface, the cells constituting 39 to 50 per cent of the total blood volume (average about 46 per cent). Both are about one-third lower in infancy but the age difference is less marked when the blood volume is calculated on the basis of body weight. A slight increase in blood and especially of plasma volume occurs in normal pregnancy.

An abnormal *increase* in total blood volume in relation to body weight or surface area may occur in polycythemia vera, sometimes in the leukemias before anemia develops and occasionally in diabetes insipidus as well as immediately after the administration of hypertonic saline solutions to individuals with edema. A slight increase may also occur in essential hypertension, Raynaud's disease, Buerger's disease, hyperthyroidism, the nephrotic syndrome and congestive heart failure although there is a tendency to a lower rather than a higher plasma volume. Increased hemoconcentration also follows extensive superficial burns which is regarded by Kapsinow [7] as an etiologic factor in the production of gastric and duodenal lesions (Curling's ulcer); this has received experimental confirmation in dogs.[8]

A *decrease* in blood volume may follow a decrease in the volume of corpuscles or of plasma, or both, as immediately after severe hemorrhage, pernicious anemia and congenital hemolytic jaundice. A marked decrease especially of plasma volume resulting in hemoconcentration is characteristic of shock. A decrease of total blood and plasma volumes with hemoconcentration may also be observed under the following conditions: (1) prolonged water restriction; (2) excessive water loss; (3) acidosis; (4) advanced diabetes mellitus; (5) excessive sweating and increased loss of water through the lungs; (6) occasionally in uremia; (7) sometimes after prolonged ether anesthesia and (8) in some cases of adrenal cortical insufficiency due to the excessive loss of sodium and chloride ions, with water, in the urine, or their passage from the plasma into the tissues.

Edema. Edema consists in the abnormal accumulation of interstitial fluid, either local or general. The clinical interpretation of examinations of edema fluids and transudates has been discussed in Chapter 13. The normal exchange of fluid between the blood and tissue spaces depends primarily on the following five factors: (1) the capillary blood pressure (13 to 35 mm. Hg.), which, being lower at the venous than at the arterial ends of capillaries, tends to drive fluid toward the tissue spaces; (2) the colloid osmotic pressure of the blood plasma (about 25 mm. Hg.) largely dependent on the concentration of plasma albumin counteracting the effect of the capillary blood pressure; (3) the permeability of the capillaries to protein; (4) the state of lymphatic circulation which aids in the removal of fluid from the tissue spaces and (5) perhaps tissue tension which varies in different parts of the body. The *primary* factors concerned in the pathogenesis of edema may be summarized as follows: [9]

1. Increased capillary pressure	(a) External pressure on veins. (b) Thrombophlebitis. (c) Congestive heart failure. (d) Dependency.
2. Reduced colloid osmotic pressure	(a) Loss of albumin. (b) Inadequate protein intake. (c) Impaired synthesis of protein. (d) Sudden plasma dilution.
3. Increased capillary permeability	(a) Inflammation. (b) Nephrosis. (c) Anemia. (d) Chronic anoxemia.
4. Lymphatic obstruction	(a) Lymphedema. (b) Increased venous pressure.

Contributory causes include low blood pressure, high salt intake, high fluid intake, heat and disturbed innervation.

Dehydration. Dehydration or a decrease in the interstitial fluids may result from the factors reducing blood or plasma volume previously mentioned. It should be emphasized, however, that with the possible exception of shock and adrenal cortical insufficiency, a marked decrease in plasma volume occurs only when the factors which operate to produce it act over long periods of time. In other words, any tendency toward the loss of fluid from the blood plasma is usually promptly counteracted by the passage of interstitial fluid into the blood, since the reserve supply in the interstitial tissues is about three times as great as the normal plasma volume. The results of dehydration are changes in the electrolytic composition and acid-base balance of the blood plasma and interstitial fluids.

Not infrequently it becomes necessary to repair the effects of dehydration by the administration of water and sodium chloride in amounts sufficient for restoring the electrolytic concentration as well as the volume of body fluids. Usually this can be accomplished by the subcutaneous or intravenous injection of physiologic saline solution. When salt has been more greatly depleted than water, however, hypertonic (2 per cent) saline solutions should be given. A decision on the proper amounts of salt and water to be administered may be facilitated by analyses of the serum for sodium chloride and bicarbonate. However, in this connection should be emphasized the fact that ketosis may occur in the presence of alkalosis as well as acidosis. For example, in intestinal obstruction, ketosis may develop as a result of carbohydrate deprivation, but the acidifying effect of the ketonic acids is usually insufficient to counterbalance the alkalinizing effect of the excessive accumulation of bicarbonate which results directly from the depletion of chloride ion. From the standpoint of treatment, therefore, it is of the utmost importance to recognize that in states of dehydration, changes in the chemical structure of the body fluids occur simultaneously with changes in their volume. Both defects are reparable by water, sodium and chloride, regardless of whether acid-base balance is altered in the direction of acidosis or alkalosis. The only defect of structure that cannot be repaired by these means is the presence of excessive amounts of ketone acids, which may be corrected by the administration of glucose. When adequate amounts of water and sodium chloride are administered, the correct degree of retention and excretion of the sodium and chloride ions necessary to re-establish the normal acid-base equilibrium depends on the efficiency of renal function.

DIABETES MELLITUS

Diabetes mellitus is a chronic inheritable disease of metabolism caused by a deficiency in insulin which is secreted by the islands of Langerhans in the pancreas. The result of this deficiency is the production of hyperglycemia, with or without glycosuria, due to a decrease in the combustion of glucose, a decrease in the normal deposition of glycogen in the liver and muscles, and an increased splitting up of glycogen by the liver. This disturbance in carbohydrate metabolism may be followed by disturbances in the intermediary metabolism of fats, leading to ketosis, acidosis and lipemia as well as by disturbances in protein metabolism.

The deficiency of insulin responsible for the disease, however, may not be due necessarily to its inadequate secretion by the islands of Langerhans. In some instances it appears that it may be secreted in normal amounts but that its effects are neutralized by some unknown antagonist. The islands constitute about 3 per cent of the weight of the pancreas and while a sufficient number may be destroyed by pancreatitis, tumors or calculi to produce the disease, yet detectable pathologic lesions at autopsy are apparently absent in about 25 per cent of cases. Otherwise, hyalinization and fibrosis of the islands constitute the chief pathologic changes. Hydropic degeneration is sometimes observed but none of these changes can be regarded as pathognomonic. They are, however, chronic in character and suggestive of the effects of some toxic agent of unknown nature and origin capable of gradually destroying the islands with the formation of new ones subject to the same destruction which eventually overcomes regenerative efforts. But in addition there is evidence in some cases that the reduction in insulin production may be secondary to disturbances in the anterior lobe of the pituitary gland, the adrenal glands and possibly the thyroid gland as well. Infection appears to play but a minor rôle in the etiology of the disease although the greatly lowered resistance of the diabetic is well known, especially predisposition to furunculosis, carbunculosis, septicemia and infections of the genito-urinary tract due to catheterizations, operations, etc.

In other words, while diabetes mellitus is known to be due to a deficiency of insulin the cause of the deficiency is still unknown in the great majority of cases. As is true of the influence of heredity in hay fever, asthma and other allergy diseases, the hereditary transmission of a tendency to acquire diabetes is undoubtedly of great importance. This is indicated by the higher incidence of the disease in blood relatives of diabetics, its almost simultaneous occurrence in twins, and the observation that mendelian ratios of the recessive type are to be found in many cases selected at random. In accordance with the view that diabetes is transmitted on mendelian lines as a recessive trait, Woodyatt and Spetz [10] have recently calculated that if a father and mother are both diabetic (or potentially so) 100 per cent of the children should develop the disease if they live long enough; or if one parent is diabetic and the other a carrier, 50 per cent; if both are carriers, 25 per cent; if one is a carrier and the other normal, 0 per cent, but with all children carriers capable of transmitting the disease.

However, while heredity greatly influences susceptibility to diabetes, obesity is by far the most important precipitating factor. As is well known, the incidence is particularly high among Jews but apparently this is not due so much to racial susceptibility as to the influence of heredity, inbreeding and obesity. At one time the disease was regarded as very uncommon among Negroes but it is now known that many are affected and the mortality in both sexes is relatively higher in early adult and middle age, but otherwise a little lower than that of the white race. It has been stated that the increasing incidence of diabetes in this and other countries is due to the increasing consumption of sugar but this is not substantiated by statistics. Indeed, it has been shown recently that a diet high in carbohydrate increases the production of insulin while one high in fats reduces it, with the suggestion that a high fat diet may be useful as a prophylactic measure in children whose family history suggests that they might ultimately become diabetic.[11] Worry and a strenuous life have been considered important in etiology but the evidence is not convincing. Trauma may activate latent diabetes but is not otherwise in etiologic relationship to the disease. Senility and arteriosclerosis are not to be regarded as causes although diabetes is an important factor in the production of the latter which, along with its complications, is among the most frequent causes of death in advanced diabetes.

The true incidence of diabetes in the United States cannot be stated but has been estimated as about 600,000 cases. In any community it is likely to be highest where the urine and blood sugars of the inhabitants are most frequently examined, where the inhabitants live the longest, and where they weigh the most. The disease is very uncommon in infancy but about 5 to 7 per cent of cases occur in children up to 10 years of age, about 17 per cent between 20 to 30 years, and about 75 per cent between 40 to 70 years. At the present time the incidence is about 15 per cent higher among women than men with an increasing incidence among the former between 40 and 50 years of age, largely ascribed to overweight, under-exercise and the readjustments in the endocrine balance associated with the menopause. It is also higher among married than single women. In pre-insulin days the total fetal mortality was as high as 44 per cent and while insulin treatment of pregnant diabetic women has not materially reduced it, yet it is now reported to be reduced to 6 per cent by estrin and progestin hormonal therapy.[12]

Formerly the average life expectancy after the development of diabetes was about five years but at the present time early detection and adequate therapy have prolonged this to over fifteen years. Indeed, uncomplicated diabetes itself is fatal in only about 2.5 per cent of cases; death usually ensues from other diseases or complications. Thus, about 3.6 per cent of deaths are due to coma which is an accident and usually inexcusable; about 60 per cent to cardiovascular-renal disease, especially the effects of arteriosclerosis; about 16 per cent to various infections and about 18 per cent to tuberculosis, cancer, inanition and other causes. At present nearly all diabetic children survive while formerly nearly all perished. The mortality among adults, however, is higher in cities than in country districts.

Laboratory Examinations. Laboratory examinations are indispensable and of the utmost value in the diagnosis and treatment of diabetes. Indeed, they are the only means available for the detection of the disease in its incipient or early stage. Even when signs and symptoms appear, they are indispensable since the early clinical manifestations are not pathognomonic. Diabetes develops so insidiously and is of such frequency that every physician and surgeon, regardless of his or her specialty, should include at least one examination of the urine of every patient for albumin and glucose. Neglect to do so may not only be a matter of embarrassment to the clinician, but sometimes one of disaster to the patient. The tests are so simple and so quickly made that the same applies to dentists who are in strategic position to detect glycosuria in both adults and children in apparent good health or who do not regard themselves as sick enough to consult physicians. Indeed, all physicians, surgeons and dentists would do well to advise and urge adults and members of their families to have an examination of the urine at least once a year. It is true that glycosuria is not always due to diabetes but it is likely in the great majority of instances.

Undoubtedly, however, a determination of the fasting blood glucose is even more valuable in the detection of diabetes and especially in its early stage than an examination of the urine, since glycosuria may not occur until hyperglycemia reaches or exceeds the renal threshold for glucose. For example, the fasting blood glucose may vary from 130 to 150 mg. or more per 100 cc. of plasma before glycosuria develops. Since early diagnosis is so important, physicians do well to include a fasting blood sugar determination as a matter of routine as frequently as possible and especially when there is the slightest suspicion of diabetes; this also applies to all members of the families of diabetes.

1. It is a common custom, especially in hospitals, to examine a specimen of morning urine. This is a mistake as far as the early diagnosis of diabetes by urine examinations is concerned. An evening specimen after the usual three meals of the day is greatly preferred. A sample of carefully measured urine collected over a period of twenty-four hours is best. The average normal *volume* is 30 to 50 ounces, or 1000 to 1600 cc. per day. In diabetic patients the volume is usually increased in relation to the quantity of glucose but this is not always true, since cases of "diabetes decipiens" are not uncommon. In other words, the 24-hour volume of urine may be normal and still contain as much as 5 per cent of sugar.

2. The normal average *specific gravity* of urine varies from 1.015 to 1.025. In diabetes accompanied by glycosuria, it is usually increased. But the glycosuria of diabetes may occur when the specific gravity is within normal or even below normal. For example, it has been stated that positive reactions for glucose may occur in 72.5 per cent of urine of diabetic and nondiabetic individuals with a specific gravity of 1.010 or less.[13]

3. The Benedict qualitative and quantitative tests for glucose are preferred, especially the latter. Positive reactions may be due to the presence of lactose (during lactation but not during pregnancy), pentose, levulose or galactose (in nursing infants). Saccharose is rarely present except when urine is collected in medicine bottles containing traces of syrup or after this sugar has been injected intravenously. Consequently, these sources of error should be kept in mind but it is rare for them to cause confusion. The urine of normal persons may also contain minute traces of glucose but not sufficient for giving positive Benedict reactions. Normal urine may also contain sufficient amounts of other substances capable of reducing copper and leading to confusion and error, but only rarely so; these include not only creatinine, uric acid, nucleoprotein, conjugate glycuronates and dihydroxyphenyl acetic acid of alkaptonuria but traces of chloroform as well.

Of course, glycosuria is not necessarily due to diabetes. If the renal threshold is very low it may occur in renal glycosuria and other types of nondiabetic glycosuria and especially after meals. But in the great majority of cases it is due to diabetes. Since in mild diabetes the renal threshold may be low, it may occur intermittently, chiefly following the ingestion of carbohydrate-rich meals. In more severe cases, however, the blood glucose being maintained at a relatively high level, glycosuria of varying degree occurs continuously. In mild cases of diabetes the 24-hour sample of urine may contain a trace to 1 per cent of glucose; moderately severe cases 1 to 3 per cent; severe cases 3 to 8 per cent. Concentrations higher than 8 per cent are rarely observed because of the difficulty of maintaining larger amounts of glucose in solution in the urine. In some cases of diabetes, however, glycosuria may be absent although the blood glucose concentration is above the normal renal threshold and, at times, may be extremely high. This occurrence of hyperglycemia without glycosuria is ascribed to an increase in the renal threshold for glucose and is observed most commonly in arteriosclerosis and chronic nephritis in elderly individuals with diabetes of long standing. However, sudden marked variations in the renal threshold for glucose may occur in such individuals, a phenomenon for which no satisfactory explanation can be made.

4. It is also advisable to examine the urine for the ketone bodies such as acetone, aceto-acetic acid (diacetic acid) and betahydroxybutyric acid since their presence is a satisfactory, though not an exact, indication of the existence of ketosis in diabetes. Because of the intimate relationship between ketosis and acidosis in the disease, the presence of ketonuria should always constitute a danger signal and an indication of the necessity for prompt and active therapy.

5. A determination of the nitrogen in the urine is also advisable because it furnishes an index to the quantity of protein which the patient is metabolizing. Tests for albumin at intervals are also advisable since the diagnosis of diabetes should not lead to neglect of the general treatment of the disease. Typical "showers" of casts are not infrequent in the urine of patients in diabetic coma even when only traces of albumin are present. They do not necessarily indicate a poor prognosis and are not necessarily due to an underlying nephritis. Tests of renal function are sometimes required.

6. As previously stated, blood glucose tests are not only of tremendous value in the diagnosis of diabetes but give a splendid index of the increasing or decreasing severity of the disease. Furthermore, in patients using regular or crystalline insulin exclusively, the level of the fasting blood glucose will indicate whether or not a bedtime dose of insulin is indicated. For patients taking protamine zinc insulin once daily before breakfast, the fasting blood glucose serves as an index of the amount to employ. Blood glucose tests made just before lunch or before supper serve as a valuable guide as to the amount of insulin which can be safely given before breakfast and before lunch, respectively. In this way insulin reactions may be avoided.

However, the degree of hyperglycemia is not necessarily an accurate index of the severity of diabetes because of the influence of such added factors as age, the state of nutrition, the available carbohydrate supply, the degree of acidosis, the presence of infection and the state of hepatic and renal function. Indeed, in mild cases the fasting blood glucose may be within normal limits and the disease detectable only by glucose tolerance tests or the respiratory quotient. Otherwise, the blood glucose may reach as high as 300 mg. per 100 cc. in moderately severe cases while in severe cases it may rise to 600 mg. or higher; values as high as 2000 mg. have been reported.

Needless to state, a determination of the blood glucose is imperative in the treatment of diabetic coma. There is, however, a remarkably wide range in variability in this condition. It may occur with blood glucose determinations as low as 130 mg. per 100 cc. and may be absent at values as high as 1500 mg. per 100 cc. Diabetic coma is stated to develop most likely in the presence of relatively low blood-glucose values in patients under twelve years of age and above the age of fifty years, and in those with acute infections.[14] Death may occur with values below 300 mg. per 100 cc. and recovery has been reported in patients showing as high as 1800 mg. per 100 cc.[15,16] The degree of hyperglycemia alone is not, therefore, a reliable criterion of the severity of diabetic coma in any individual case.

Blood glucose tests are usually conducted with specimens obtained by venipuncture but capillary blood is often desirable, especially in children, in patients from whom frequent samples are required, and in the case of those with veins difficult to puncture. In interpreting the results, however, it must be remembered that whereas in the fasting state the glucose content of capillary and venous blood is approximately the same, after a meal or after the administration of glucose the capillary blood glucose in normal individuals is from 20 to 50 mg. per 100 cc. higher than in the case of venous blood.

7. Glucose tolerance tests are of value in the diagnosis of latent or incipient diabetes and especially in the differentiation between true diabetes and renal glycosuria. They are also of value in the examination of individuals from diabetic families in relation to the detection of diabetes in a latent or potential form as well as for removing the suspicion of diabetes in cases of melituria due to the presence of levulose, lactose or other sugars in the urine. They are unnecessary and contraindicated, however, in known or proved cases of diabetes. The methods commonly employed, their sources of error and the interpretation of results are discussed in Chapter 4. There is an increasing consensus that the two-dose glucose tolerance test of Exton and Rose is the preferred method,[17] since it is relatively free from the influence of fairly marked changes in the antecedent diet or when it is not known, as is usually the case.

In normal individuals the ingestion of glucose is followed by a rise in blood pyruvate. In conditions associated with thiamine deficiency, the pyruvate curve following the ingestion of glucose is abnormally elevated and prolonged. In diabetes mellitus, however, there is little or no increase in blood pyruvate following the ingestion of glucose unless insulin is administered.[18]

8. When the diabetic is unable to utilize carbohydrates to the full measure of metabolic requirements, the deficiency is made up by the initiation and oxidation of fat in the muscles. However, a considerable fraction estimated at ⅓ to ½ of the total caloric needs from fat is obtained by a preliminary oxidation of fats in the liver to ketone bodies.[19] If this excessive fat catabolism continues unchecked, ketosis and coma follow. As previously stated, the ketosis is usually detected by examinations of the urine for acetone, aceto-acetic and betahydroxybutyric acids. It sometimes happens, however, that this state may be present in the absence of urinary changes. Under these circumstances, the blood test of Wishart for the detection of acetone and aceto-acetic acids may be employed, as may likewise Nanavutty's titrimetric modification of Van Slyke's gravimetric method for the determination of "total acetone bodies" in the blood and urine. The limit of accuracy is 5 mg. acetone per 100 cc. of blood. In normal individuals values approaching zero are found, but in diabetic coma as much as 190 mg. of acetone per 100 cc. have been observed.

9. In addition to ketosis, other disturbances of fat metabolism may occur with special reference to changes in the free and cholesterol esters of the plasma. In adequately controlled diabetes the total blood lipids are usually within the normal of 0.4 to 0.6 gm. per 100 cc. of plasma. In severe diabetes, and especially in coma, however, they may be increased to 2.5 gm. or higher per 100 cc., in which case the lipemia may give the blood a creamy appearance with lipuria.

From the clinical standpoint, determinations of the plasma cholesterol are of most interest and importance. Hypercholesterolemia is always reliable evidence that diabetes is uncontrolled; indeed, cholesterol determinations give a more reliable index of the severity of diabetes than blood sugar determinations. This is particularly true in diabetes of children, among whom the disease is frequently more severe and uncontrolled than in adults. High plasma cholesterol values, therefore, indicate that danger is present or imminent and that immediate steps to control the disease should be taken. However, hypercholesterolemia does not always occur in acidosis and coma; in about 50 per cent of cases it may be absent or of but slight degree. The degree of hypercholesterolemia does not appear to be dependent upon the severity of coma. Nor is there any close parallelism between the degree of hypocholesterolemia and hyperglycemia and glycosuria, especially in severe cases of diabetes. Furthermore, total cholesterol values of 400 mg. or more per 100 cc. of plasma do not present too grave a prognosis for life even in coma, but the prognosis for, and as the result of, complications, may be very grave with special reference to the danger of coma, nephritis, pneumonia, tuberculosis, severe arteriosclerosis, gangrene, cholelithiasis, retinitis, cataracts, abscesses, etc. Fortunately, both hyperlipemia and hypercholesterolemia can be rapidly reduced by the administration of adequate doses of insulin.

However, while in the past a bad prognosis has always been associated with abnormally high blood lipids, it would appear that hypocholesterolemia with values of 90 mg. or less of total cholesterol per 100 cc. of plasma have a much worse prognosis with the shortest duration of life and the highest mortality. It may occur with or without an increase in other blood lipids and especially in severe forms of the disease; the cause is unknown.

10. Laboratory examinations are of great value in the detection of acidosis in relation to coma in diabetes and, indeed, are indispensable for its early detection before signs and symptoms have become pronounced. The methods available have been discussed on page 98; of these a determination of the carbon dioxide capacity or combining power of the plasma is the method commonly employed.

11. While the elimination of nonprotein nitrogen by the kidneys of diabetic patients is usually strikingly good, moderate to striking retention may occur in cases of advanced diabetes and particularly in diabetic coma.[20] In some cases this is due to the presence of chronic nephritis. In other instances, however, no significant renal lesions have been found at autopsy. The cause of nitrogen retention under such circumstances is unknown but it may be due to any one or a combination of such factors as (a) dehydration due to acidosis, diuresis, vomiting or reduced fluid intake; (b) a state of shock; (c) excessive catabolism of protein or (d) hypochloremia.

12. Hypochloremia occurs chiefly in acidosis when chloride is lost through

vomiting, excessive excretion by the kidneys, or because of the transfer of plasma chloride to the erythrocytes. Despite the liberal administration of physiologic saline solution parenterally, it or hyponatremia (deficient sodium in the blood) may persist, and anuria result. In suitable patients hypochloremia and anuria may be strikingly relieved, however, by the intravenous injection of 60 to 130 cc. of 10 per cent solution of sodium chloride.[21] On the other hand, it has been shown that because of concomitant dehydration and hemoconcentration the plasma chloride may be actually increased following a period of reduction which has important therapeutic implications.[22]

13. Patients with diabetes are still occasionally encountered in whom injudicious dietary restrictions have resulted in extreme malnutrition with hypoproteinemia sometimes resulting in edema. Hypoproteinemia may be masked, however, by hemoconcentration due to vomiting in acidosis. On the other hand, active treatment of acidosis with intravenous injections of glucose and sodium chloride solutions may restore plasma volume to the normal but with such a reduction in the concentration of the total plasma protein as to produce edema.

14. An increase of glucose in the cerebrospinal fluid commonly occurs—likewise an increase of plasma phospholipid with a decrease of serum sodium and variable blood and plasma volume due to dehydration and hemoconcentration. As previously stated, leukocytosis may also occur in coma due to acidosis along with a deficiency in potassium.[23]

15. Finally, carotenemia may develop in diabetes because patients sometimes retain the yellow pigments of vegetables to a greater extent than normal individuals, apparently due to the inability of the liver in diabetics to convert carotin into vitamin A.[24,25]

RENAL GLYCOSURIA

Renal glycosuria may be defined as a state of intermittent or continuous glycosuria without hyperglycemia occurring in healthy individuals due to an abnormally low renal threshold for glucose. While not regarded as a disease of metabolism, it is discussed herewith because of the frequency with which it may be mistaken for diabetes mellitus.

In the great majority of cases renal glycosuria is of the *intermittent type* in which glycosuria occurs only when the low renal threshold is exceeded by the ingestion of a moderate amount of glucose or other carbohydrates. Marble and Joslin and their colleagues, however, regard only the *continuous type,* in which glucose occurs in the urine not only after a meal but in the fasting state as well, as being true renal glycosuria.[26] Because of confusion due to the occurrence of other types of nondiabetic melituria, the incidence of renal glycosuria cannot be given except to state that the continuous type appears to constitute about 0.15 to 0.35 per cent of all nondiabetic glycosurias.

The cause of renal glycosuria is unknown. As far as the continuous type is concerned, available evidence indicates that it may occur as a familial condition.[27] It seems certain that the low renal threshold and glycosuria are due to the incomplete reabsorption of glucose by the renal tubules. This, in turn, may be due to some deviation from the normal in connection with the phosphorylation mechanism, such as a deficiency in tissue phosphatase. It has been suggested that the low renal threshold may be due to some disturbance of hormonal control over the renal tubes by the posterior lobe of the pituitary gland.[28] Be that as it may, it would appear that recovery is possible in the intermittent type, as the renal threshold is restored to normal by diets low in carbohydrates and the avoidance of dietary excess in general. The continuous type, however, persists over years and years and even for the balance of life.

While it is stated that the continuous type never develops into diabetes mellitus,[26] it appears that this may occur in 8 to 10 per cent of cases of the intermittent type [29] and especially if the patient is careless of diet and becomes obese. In this connection it is to be remembered, however, that a low renal threshold may be present in some cases of early or incipient diabetes leading to the erroneous diagnosis of renal glycosuria.[30] Furthermore, there is always the chance of mistaking "potential diabetes" for intermittent renal glycosuria; in these cases glycosuria usually disappears on slight reduction of sugars and other carbohydrates in diet, while the fasting blood sugar is below 130 mg. per 100 cc. of plasma and never reaches 0.170 mg. after a meal. These constitute about 1.5 per cent of all cases of glycosuria.

Laboratory Examinations. *The diagnosis of renal glycosuria is based entirely on laboratory examinations.* It is asymptomatic although some patients may complain of easy fatigability and lassitude; under these circumstances incipient diabetes is always to be suspected. Insulin has little or no effect on the glycosuria. The results of laboratory examinations may be summarized as follows:

1. Intermittent glycosuria occurring only after meals, or continuous glycosuria occurring after fasting as well as postprandially.
2. Persistently normal fasting blood sugar values.
3. Glucose tolerance tests are of special value and importance in diagnosis. When conducted according to the technic of the standard test employing venous blood, as described in Chapter 4, the blood sugar curve in renal glycosuria is similar to that observed in normal individuals but glucose is present in all specimens of urine (except the preliminary one if the case of the intermittent type). The same is true if the test is conducted with capillary blood with the following results: a preliminary or fasting blood sugar of 120 mg. or less per 100 cc. of plasma; 200 mg. or less one-half hour after the administration of glucose and 120 mg. or less at the end of two hours.[29]
4. Needless to state, several fasting blood glucose tests should be made at intervals before a final diagnosis is made. Indeed, diagnosis on a single glucose tolerance test is always risky. It is important during the first year of observation that the patient have a fasting blood glucose and urine examination followed by tests after an ordinary meal every three months and thereafter at six- and twelve-month intervals for the balance of life.
5. The rate of utilization of carbohydrate as determined by the respiratory quotient is normal.
6. Ketosis and acidosis may develop during starvation but not after dietary excesses.

OTHER NONDIABETIC MELITURIAS

Melituria refers to the presence of any sugar in the urine. Consequently, it may be due to glycosuria, lactosuria, galactosuria, levulosuria or pentosuria. Sucrosuria, characterized by the presence of saccharose or cane sugar in the urine is very rare and usually due to the presence of syrups in bottles or other containers used in the collection of samples.

In the great majority of cases melituria is due to glycosuria. Needless to state, this is usually due to diabetes mellitus. However, glycosuria may occur in many nondiabetic states. The most frequent and important is renal glycosuria, previously discussed. If this condition is excluded, glucose and the other sugars mentioned are responsible for about 9 per cent of all nondiabetic meliturias which may occur under the following conditions, with some properly regarded as diseases of metabolism:

- *GLYCOSURIC*
  - (1) Nonhyperglycemic
    - Renal glycosuria
    - Alimentary glycosuria
    - Glycosuria of pregnancy
    - Phlorhizin glycosuria
    - Nephritis and nephrosis
  - (2) Hyperglycemic
    - Hyperthyroidism
    - Hyperpituitarism
    - Hyperadrenalism
    - Increased intracranial pressure
    - Arterial hypertension
    - Nephritis and nephrosis
    - Chronic hepatic disease
    - Ether anesthesia
    - Asphyxia
    - Acidosis
- *LACTOSURIC*
  - Late stages of pregnancy (some cases)
  - During lactation (physiologic)
- *GALACTOSURIC*
  - Alimentary galactosuria and especially in hepatic disease
  - Nursing infants with gastro-intestinal disturbances
  - Hyperthyroidism (some cases)
- *LEVULOSURIC*
  - Some cases of severe diabetes mellitus
  - Alimentary levulosuria and especially in hepatic disease
  - Essential or chronic levulosuria (rare)
- *PENTOSURIC*
  - Some cases of severe diabetes mellitus
  - Alimentary pentosuria
  - Essential or chronic pentosuria (rare)

The term *alimentary glycosuria* is employed to designate the urinary excretion of glucose by certain apparently normal individuals after the ingestion of excessive amounts of cane sugar, glucose or, at times, starch. In the absence of any abnormality of hepatic or tissue glycogenic function, it might be explained on the basis of increased capillary permeability of the intestinal mucosa for glucose resulting in its absorption at a rate more rapid than can be adequately handled by the liver. It therefore reaches the tissues, including the kidneys, in excessive amounts, with the result that a portion is eliminated in the urine. This occurs without hyperglycemia since tissue utilization of glucose is unimpaired.

Melituria during *pregnancy* is practically always due to glycosuria except during the terminal stages when a lactosuria may occur. The latter is usually responsible for melituria during lactation which it is to be regarded as a physiologic event. Of course, diabetes mellitus may have its onset during pregnancy or a known diabetic may become pregnant.

Laboratory Examinations. As in the case of renal glycosuria only laboratory examinations are capable of detecting the nondiabetic meliturias. Unfortunately, the copper of Benedict's and Fehling's reagents is reduced by all of the sugars, but special tests are available for differentiating glucose from lactose, galactose, levulose and pentose. Needless to state, every woman found to have glycosuria during pregnancy should be carefully studied for the possibility of diabetes. If thought to be a nondiabetic glycosuria she should be kept under close observation during the pregnancy and her blood and urine should be tested every three months during the first year following delivery and yearly or twice yearly thereafter for possible diabetes as advised in the management of renal glycosuria. Likewise in alimentary glycosuria it is always advisable to subject the individual to the same tests for the exclusion of incipient diabetes before a final diagnosis is reached although it may not be possible to differentiate alimentary glycosuria from renal glycosuria.

HYPERINSULINISM

In my opinion, hyperinsulinism is to be regarded as a symptom complex due to disease of the pancreas resulting in the over-production of insulin (*organic hyperinsulinism*) or to a disturbance in the mechanism regulating the release of insulin by that organ (*functional hyperinsulinism*).

Hyperinsulinism is characterized by hypoglycemia but is not to be regarded as synonymous with what is commonly designated *spontaneous hypoglycemia.* In other words, the latter is not a clinical entity but a sign of disturbances in carbohydrate metabolism due to (1) decreased hepatic glycogenolysis, (2) depletion of hepatic glycogen or (3) increased tissue utilization of glucose observed under physiologic conditions and in many diseases, as discussed on page 94. Consequently, "spontaneous hypoglycemia" is common while the clinical entity known as "hyperinsulinism" is comparatively rare.

Hyperinsulinism is usually of the organic type due to diseases of the pancreas resulting not only in the excessive production of insulin but apparently of insulin of excessive activity. It may occur in (1) adenomas of the pancreas involving the islands of Langerhans; (2) cancer of the pancreas involving the islands and (3) in states characterized by a general hyperplasia or hypertrophy of the insulinogenic cells of the islands. Treatment consists of subtotal pancreatectomy for the removal of excess insulin-producing tissue on the same principle as subtotal thyroidectomy in the treatment of hyperthyroidism. To these may be added (4) the hyperinsulinism occurring in infants born of mothers who have uncontrolled diabetes. In this serious type of hyperinsulinism, which may prove fatal unless anticipated and measures taken to prevent severe hypoglycemia during the first three days after birth, Bauer and Royster [31] have found the pancreas grossly enlarged in six of thirteen fatal cases. The enlargement appeared to be due to a numerical increase of the islands of Langerhans along with hypertrophy and hyperplasia of them. These changes are apparently due to the fact that unusual demands are made upon the pancreas of the fetus as the result of uncontrolled diabetes of the mother. The ensuing insular hypertrophy and hyperinsulinism of the fetus helps the mother but after delivery the infant is likely to possess an insulin-producing pancreas greatly in excess of its own needs.

Functional hyperinsulinism is still more uncommon and there is the probability that many cases so regarded are really instances of chronic spontaneous hypoglycemia due to extrapancreatic causes, notably disturbances of the sympathetic nervous system, and, less frequently, disturbances of the liver or of the endocrine glands. Otherwise, functional hyperinsulinism is due apparently to some disturbance in the mechanism regulating the release of insulin by the pancreas whereby while it is continually in excess of postdigestive needs, it is inadequate for the prevention of hyperglycemia and even glycosuria after meals. This may be the reason for diabetes mellitus and hyperinsulinism sometimes occurring simultaneously in the same individual. At any rate great caution is required in the diagnosis of functional hyperinsulinism because subtotal and even almost complete pancreatectomy may fail to yield a favorable result.[32]

Laboratory Examinations. Hyperinsulinism, like spontaneous hypoglycemia, may be recognized clinically by attacks similar to those produced by overdosage of insulin characterized by nervousness, trembling, sweating, weakness, unsteadiness of gait, emotional instability, inability to concentrate and increasing drowsiness sometimes resulting in unconsciousness with or without convulsions. But the diagnosis of *organic hyperinsulinism* is usually based on the results of laboratory examinations as follows:

1. A fasting blood glucose level of 60 mg. or less per 100 cc. of plasma.
2. A blood glucose level of 50 mg. or less per 100 cc. of plasma during an attack. An attack may be precipitated by having the patient omit breakfast and take moderate exercise although this valuable test need not be employed except in doubtful cases.
3. In *functional hyperinsulinism* there is little or no tendency for the disorder to progress. The attacks usually occur during the day, after the patient has had one meal and especially if the next meal is delayed. Attacks occur three to four hours after a meal. An increase in the carbohydrate intake increases the likelihood and severity of attacks. Spontaneous recovery from an attack is usual but rapid relief is afforded by giving food. Attacks are not likely to occur, however, after midnight when the patient is at rest. From the laboratory standpoint the blood

glucose during an attack is lower than the fasting level which is attributed to the insulinogenic effect of the carbohydrate of the preceding meal.

4. Unfortunately, glucose tolerance tests are not always of helpful diagnostic value in the diagnosis of either type of hyperinsulinism because of the variety of curves obtainable in any given patient. As suggested by Conn,[33] this may be due to the amount of carbohydrate in the diet on the preceding day or days before the test. Therefore, the test should be conducted under standard conditions (Chapter 4) in which case the results are usually as follows: (1) a subnormal preliminary or fasting blood glucose; (2) the highest peak seldom exceeds 120 mg. per 100 cc. and (3) a return of blood glucose to subnormal within two hours which is usually maintained throughout the third, fourth, fifth and sixth hours.

GLYCOGEN STORAGE DISEASE

Glycogen storage or von Gierke's disease[34] is a rare congenital disorder of carbohydrate metabolism occurring in infants and young children characterized by the storage of abnormally large amounts of glycogen not only in the liver but in the kidneys, heart, skeletal muscles and other organs.[35] Enlargements of the liver and heart, due to excessive deposits of glycogen in the cells along with fatty metamorphosis,[36] are characteristic features along with chronic hypoglycemia, acetonuria due to the incomplete oxidation of fats, malnutrition and retardation of growth.

The etiology is unknown but it appears that the disease may be inherited as a mendelian recessive characteristic.[37] It has been suggested (1) that the glycogen is in abnormal combination with proteins of liver and muscle cells, or (2) that the disease is due to a lack of liver glycogenase or (3) that it is due to dysfunction on the part of the anterior lobe of the pituitary gland. The stored glycogen does not appear to be altered chemically but possesses great stability, with the result that it is not readily converted into glucose. The result is a state of persistent hypoglycemia although symptoms and convulsions are very infrequent, even following exercise, because of its mild character. Curiously enough, patients may be unusually sensitive to the activity of insulin in promoting the withdrawal of glucose from the blood which may be another factor in the production of hypoglycemia;[38] they are also insensitive to injections of 0.5 cc. of 1:1000 solution of epinephrine which is further evidence of the inability of the liver to convert glycogen into glucose.

Laboratory Examinations. These are usually of great value in diagnosis and may be summarized as follows:[37]

1. The constant presence of acetone in the urine, generally in the morning, although not necessarily throughout the day.

2. A low fasting blood glucose which is not infrequently from 50 to 60 mg. per 100 cc. of plasma.

3. Failure of the blood glucose to rise normally (*i.e.*, over 30 mg. in 15 to 30 minutes) following the subcutaneous injection of epinephrine (see Chapter 4).

4. An increase of blood glycogen above the normal of less than 15 mg. per 100 cc.

5. A delayed fall, or other abnormality of the blood glucose curve, following a glucose tolerance test.

6. A rise, in some cases, of the blood sugar in the half hour following the administration of levulose (fructose).

7. An increase of the total lipids of the blood may be observed, especially of cholesterol.[39]

DIABETES INSIPIDUS

Diabetes insipidus is an uncommon and chronic disease of water metabolism characterized by excessive polyuria and polydipsia with tissue dehydration. Consequently, it may be mistaken clinically for diabetes mellitus, chronic glomerulonephritis or polycystic disease of the kidneys although on account of its chronicity it is not likely to be confused with the temporary polyurias or the intermittent polyuria of hysteria. At any rate, differential diagnosis is greatly aided by laboratory examinations. However, as far as diabetes mellitus is concerned it is to be remembered that transitory glycosuria may precede diabetes insipidus and that, very occasionally, both diseases may occur together in the same individual.[40-44]

The cause of diabetes insipidus is unknown but there is a growing consensus that the profound polyuria is due to an almost specific inhibition of the reabsorption of water by the renal tubules. This profound disturbance in water metabolism is thought to be due to the normal production of the diuretic hormone by the anterior lobe of the pituitary gland which, however, is uncontrolled because of a deficiency in the antidiuretic or vasopressor hormone secreted by the posterior lobe or by disturbances in the paraventricular and supra-optic nuclei in the hypothalamus and the supra-opticohypophyseal tract of nerve fibers which depress its secretion. Some investigators, however, believe that the disease is due to disturbances in the hypothalamus alone, but the antidiuretic action of pituitrin is an outstanding obstacle to this hypothesis.

At any rate, two types of diabetes insipidus may occur. In the *primary or idiopathic* type organic causes are not discoverable. Apparently it may be hereditary and transmitted by a simple mendelian dominant characteristic.[45] It usually appears at an early age and persists throughout life without affecting the patient's general health or longevity. In some cases the disease appears and disappears and in others it may subside entirely. In others it may be associated with other congenital physical or mental defects and especially with Laurence-Moon-Biedl syndrome. The *secondary or symptomatic* type is caused by changes in the hypothalamic area. These may be due to developmental abnormalities, skull injuries, acute or chronic infections, as well as to various tumors and infiltrations involving the base of the brain and especially the hypothalamic area. Xanthomatosis is a frequent cause in early childhood[46] and pellagra has also been stated to be an etiologic factor. In this form the disease polyuria and polydipsia may be also reduced by subcutaneous injections of pituitrin.

Laboratory Examinations. As previously stated, laboratory examinations are of great value in the diagnosis of diabetes insipidus and especially in its differentiation from diabetes mellitus; the characteristic findings may be summarized as follows:

1. A greatly increased volume of colorless or almost colorless urine of abnormally low specific gravity (1.001 to 1.005). In some cases traces of albumin and inosite may be present. Glucose is absent. The daily total excretion of solids is approximately normal.

2. A reduction in the total urinary excretion of sodium chloride below the normal of 10 to 16 gm. per 24 hours has been reported but according to Blotner,[47] while the excretion of chloride may vary from day to day, it is usually within the normal range even when large volumes of urine are excreted, under pituitrin therapy and on restriction of fluid-intake, provided a normal amount of salt is ingested.

3. The plasma sodium chloride is usually within the normal of 570 to 620 mg. per 100 cc. even when pituitrin has not been taken for several years.[47]

4. The plasma total nonprotein nitrogen, urea nitrogen, creatinine, uric acid, glucose and cholesterol are usually within normal limits.[48]

5. Appreciable changes in the basal metabolic rate are stated to be generally absent but an increase of the rate to as much as +30 to +45 has been reported. This is thought to be due to the enormous increased work of the kidneys incident to the elimination of excessive amounts of water. Diminution in renal work following the administration of pituitrin is stated to be followed by a return of the rate to normal levels.

HEMOCHROMATOSIS

Hemochromatosis is a rare chronic disease of metabolism characterized by a marked retention of iron in the body and the widespread deposition of hemosiderin and hemofuscin in the skin, liver, pancreas and other tissues with the exception of the brain, nervous system and parathyroid glands. In about 80 per cent of cases the skin, especially its exposed portions, gradually assumes a bronzed, bluish, leaden, or slate color. In some cases the pigmentation is principally or entirely due to melanin. The buccal mucosa is involved in about 16 per cent of cases. Apparently as the result of irritation by the deposited pigments, hypertrophic cirrhosis of the liver is gradually produced. For the same reason fibrosis of the pancreas gradually develops which, in 85 to 90 per cent of cases, produces diabetes mellitus. For this reason the disease is commonly known as "bronzed diabetes." Sexual hypoplasia is of frequent occurrence. Pigmentation produces extensive interstitial myocarditis only occasionally.

The etiology of hemochromatosis is unknown but the disease is generally believed to be due to an "inborn error of metabolism" characterized by an inherited abnormal avidity of the tissues for iron with an inability of the cells to rid themselves of the ferrous compounds.[49,50] Mallory[51] thought that the disease might be due to poisoning with copper but this hypothesis is no longer held although the liver has been reported to contain increased amounts of this element.[52]

It is quite likely that the disease begins early in life but in over 90 per cent of cases it becomes apparent clinically after 36 years of age. For some unknown reason, about 95 per cent of cases occur in men.[50] Hemochromatosis is distinct from hemosiderosis which is due to pigmentation of the tissues with hemosiderin alone resulting from the excessive destruction of erythrocytes. Furthermore, while the hemosiderin in hemosiderosis may be used again in the formation of hemoglobin, this does not occur in hemochromatosis.

Laboratory Examinations. Laboratory examinations are very helpful in diagnosis and may be summarized as follows:

1. Biopsy examinations of the skin usually show the presence of large amounts of iron containing pigment in the cells although it may be absent and excessive amounts of melanin found.[53] Care should be exercised against excising skin from the axillas or groin or from any part of the body where normally small amounts of iron pigment may be present with characteristically large amounts of melanin. Biopsy examinations of liver tissue removed during peritoneoscopic examinations are also of value in diagnosis.
2. Glycosuria and hyperglycemia are present in the majority of cases although the disease may occur without coincident diabetes. In some instances hemosiderin may be found in the cellular elements of the urine.
3. Hypercholesterolemia is frequently observed, sometimes accompanied by xanthomatous lesions of the skin. When liver function is impaired, the ratio of cholesterol ester to the total cholesterol may be reduced.
4. The plasma albumin may be below normal with a reversal of the albumin-globulin ratio.
5. Hyperbilirubinemia with positive icterus index and van den Bergh reactions due to cirrhosis of the liver is commonly observed even in the absence of clinical jaundice. Liver function tests may show impairment.
6. A moderate hypochromic anemia is usual.
7. The organic iron of the whole blood may be decreased within the normal of 50 to 52 mg. per 100 cc. although increased amounts have been reported in some cases.[54] The copper of the whole blood is within the normal limits of 0.14 to 0.15 mg. per 100 cc.
8. Moderate elevations of the basal metabolic rate have been reported.

ALKAPTONURIA, OCHRONOSIS AND PORPHYRIA

Alkaptonuria. Alkaptonuria is a rare and benign disease of protein metabolism characterized by the excretion of homogentisic acid in the urine. It sometimes produces a blackish discoloration of the cartilages and especially those of the ears. The alkapton bodies or aromatic oxyacids and especially homogentisic acid are products of the intermediary metabolism of phenylalanine and tyrosine. In alkaptonuria as much as several grams of homogentisic acid may be excreted in the urine daily. Such urine, on standing and becoming alkaline, gradually turns brown to black in color due to oxidation of the acid. The acid reduces the alkaline copper solutions of Benedict's and Fehling's reagents and may thus, by being mistaken for glucose, lead to an error in diagnosis. The acid does not, however, reduce the alkaline bismuth solution of Nylander's reagent nor is yeast fermented. Moreover, there is, as a rule, a fairly definite relation between the amount of homogentisic acid and total nitrogen excreted in the urine.

The etiology of alkaptonuria is unknown. But since it is usually a familial disease, it is thought to be an "inborn error of metabolism" inherited as a recessive mendelian characteristic with frequent occurrence in the children of consanguinous (first cousin) marriages. However, many cases have now been reported where no hereditary basis could be found.[55]

In infants and children the disorder is usually first suspected by noting dark stains on diapers or underwear. There are no subjective symptoms. *Laboratory examinations* of the urine for homogentisic acid are required for final diagnosis. It is stated that biologic nonspecific Wassermann and flocculation reactions may occur, with the possibility of mistaking alkaptonuria for congenital syphilis.

Ochronosis. Ochronosis is not a clinical entity but a rare and benign disorder due to metabolic disturbances accompanied by discoloration of the cartilages, tendons, fibrous tissues and sometimes of the skin by phenolic compounds, homogentisic acid, melanin or possibly other pigments of endogenous origin. Microscopically the pigment granules appear pale yellow or ochre in color, as originally described by Virchow, which is responsible for the name given the disorder.

Ochronosis is characterized clinically by (1) blue or black pigmentation of the cartilages of the ears, nose and tendons of the knuckles; (2) brownish discoloration of the scleras; (3) gray or bluish pigmentation of the skin; (4) a dark color of the urine and in about 50 per cent of cases by (5) degenerative changes in the involved cartilages and arthritis or arthropathies of the hypertrophic or deforming types (osteitis deformans ochronotica). In some instances, osteomalacia has been observed (osteomalacia alkaptonuria) and in the chronic course of the disorder premature arteriosclerosis may develop. Since the accumulation of the pigments is a very gradual process, it is readily understood that ochronosis does not usually become clinically apparent at an early age; most cases have been observed in individuals over 40 and rarely before 30 years of age.[56]

Laboratory examinations are frequently helpful in diagnosis and especially in determining the nature of the pigment responsible for discoloration. These usually consist of examinations of the urinary pigment supplemented in some cases by biopsy examinations of the skin with special reference to the detection of melanin.

Porphyria. Porphyria is a comparatively uncommon disorder of pigment metabolism characterized by the overproduction of porphyrins and their excretion in the urine along with various other clinical manifestations.

The disease may occur (1) as congenital (chronic) porphyria or (2) as acute porphyria. The latter is commonly subdivided into the acute toxic and the acute idiopathic forms. Günther,[57] who first described the porphyrias, expressed the opinion that the disease in all instances is due to a constitutional anomaly of pigment metabolism. Subsequent investigations have largely confirmed this hypothesis, with a growing consensus that it is inherited and transmitted as a dominant mendelian characteristic.[58,59] Under these circumstances, acute toxic porphyria is regarded as being precipitated by some toxic agent, usually a barbiturate, while the cause of acute exacerbations in idiopathic porphyria remains unknown.[60] Some investigators, however, believe that acute porphyria may be caused by toxic agents without involving a constitutional predisposition or liability to the disease.[61,62]

The porphyrins are pigments which occur naturally throughout the plant and animal kingdoms. They are related to two of the four isomers of etioporphyrin and are designated respectively Types I and III. Types II and IV have not been encountered in nature. Protoporphyrin Type III is combined with iron and globin as the basis of hemoglobin. Coproporphyrin Type I occurs normally in small amounts in the urine and feces; likewise smaller amounts of coproporphyrin Type III. Normal urine also contains traces of uroporphyrin Type I. The origin of Type III porphyrins may be explained on the basis of their relation to protoporphyrin of hemoglobin and other Type III compounds in the tissues but available evidence also indicates that they may be products of independent synthesis rather than of the destruction of hemoglobin. The origin of Type I porphyrins is still obscure but they, likewise, may be synthesized in the body quite independently of hemoglobin. It has been suggested that both series may have a common precursor. Both are produced physiologically at a relatively constant ratio, although the production of Type I porphyrins is much less than that of the Type III porphyrins. Under abnormal conditions, however, the relationships may be altered not only in their synthesis but in their distribution and excretion in the urine and feces as well.

Congenital or *chronic porphyria* may have its onset early in fetal life or infancy or first appear in adult life. The excretion of pink to almost black urine may first direct attention to it; or the urine may be of normal color and darken on exposure to light. As a general rule, these changes are due to the presence of excessive amounts of the Type I uro- and coproporphyrins, although Type II coproporphyrin may be responsible in some cases. The disease occurs predominantly in males. The patients usually show marked sensitivity to ultraviolet light and may develop skin lesions varying from simple erythemas to vesicles and large bullae filled with a colorless or blood-stained fluid (hydroa aestivale or hydroa vacciniforme) or epidermolysis bullosa.[63] Healing is followed by permanent scars and there is a tendency to brownish pigmentation of the skin and hirsutism. Brownish or pinkish pigmentation of the teeth may occur. In later life enlargement of the spleen and liver may occur.

Acute porphyria of the toxic or idiopathic forms is characterized by the excretion of red or dark reddish brown urine usually due to the presence of excessive amounts of Type III uro- and coproporphyrins and especially the former, which may be excreted as a zinc metal complex; [64] also by gastro-intestinal disturbances and especially colicky abdominal pains, psychotic manifestations and ascending paralysis (in the late stages), jaundice, renal damage, and, rarely, pigmentation of the skin or dermal photosensitivity. The majority of cases occur in women 30 to 50 years of age and the mortality is high. A family history of porphyria is frequent. In patients who recover, the porphyrins usually disappear from the urine in three to four weeks. Subsequent attacks, however, are always likely to occur and between them the porphyrins of the urine may be above normal. In addition to the porphyrins, the changed color of the urine may be due to other pigments like urofuscin, skatol red or urobilin.

Needless to state, *laboratory examinations* of the urine for the porphyrins are of fundamental importance in diagnosis. Spectroscopic methods are required. Owing to the dark red color of the urine, paroxysmal hemoglobinuria may be suspected and especially since the absorption bands due to the zinc metal complex of uroporphyrin may be mistaken for oxyhemoglobin. Nesbitt and Watkins, however, have shown how these may be differentiated spectroscopically.[60]

GOUT

Gout is a disease of purine metabolism characterized by the retention of uric acid in the body and the deposition of sodium urate in or about the joints and other tissues which results in the production of chronic arthritis with recurrent acute exacerbations and irregular constitutional symptoms.

The clinical manifestations may vary from asymptomatic hyperuricemia (high blood uric acid), usually with a diminished renal excretion of uric acid, to severe crippling arthritis. Apparently, sodium urate may be deposited in normal tissues although trauma, avascularity and other local factors may be predisposing causes. Acute exacerbations of the arthritis are apparently due to unsuccessful attempts on the part of the body to rid itself of uric acid and the deposited urates.

The articular cartilages of all joints are susceptible to the disease although those of the lower extremities, particularly the metatarsophalangeal joints of the great toes are most frequently and regularly involved. Why this occurs and why the urates are selectively deposited in articular cartilages is unknown. However, urates are also deposited in the periarticular tissues with the production of *tophi* although this term has come to denote deposits in tissues other than the joints, as in the helix and antihelix of the ears, the olecranon and prepatellar bursas, the tendons of fingers, hands, wrists, toes, feet, ankles, heels and less frequently in the skin, tarsal plates of the eyelids, nasal cartilages, scleras, kidneys, etc. The incidence of nephrosclerosis due to arteriosclerosis of the kidneys is particularly high and constitutes so-called "gouty nephritis." But while a defective excretion of uric acid by the kidneys plays an essential rôle in the etiology of gout, this nephrosclerosis is not to be regarded as the cause but as a result of the disease. The incidence of urinary calculi in gouty individuals is also relatively high and arthritis in connection with urinary tract lithiasis should always suggest the probability of gout, although there is no sound explanation for the frequent association of the two diseases. Generalized arteriosclerosis with secondary hypertension is also higher in gouty individuals than in control groups of comparable age but are usually late manifestations of the disease.

The incidence of gout is unknown but it undoubtedly occurs more frequently in England and Germany than in the United States where the disease, according to hospital admissions, does not appear to exceed 0.2 per cent. However, while gout occurs more frequently in private practice, it is by no means as frequently a "rich man's disease" as formerly thought to be the case. For some unknown reason slightly over 95 per cent of cases occur in men; women are also stated to be less susceptible to the development of tophi. Since heredity appears to play a rôle in the etiology of most cases of gout, one may expect to observe its manifestations early in life. As a matter of fact, the finding of hyperuricemia in apparently healthy young relatives of gouty patients and its presence with or without tophi in adults for years prior to the onset of arthritis, indicates that the disease antedates the development of symptoms which, however, only rarely occur before 30 years of age. Onset at an earlier age frequently portends severe seizures, often polyarticular in distribution, and may result in extensive crippling and severe nephrosclerosis before middle life. Allegedly a disease of cold climates, it is known to occur in the tropics and affects Negroes and other races as well as whites. It is not by any means confined to those addicted to over-indulgence in rich foods and alcoholic beverages. Previously the disease was assumed to be particularly prevalent among lead workers (*saturnine gout*) as secondary to nephrosclerosis of plumbism, but gout is more rarely a complication of lead poisoning than formerly supposed, especially in the United States.

Etiology. While gout is known to be a disease of purine metabolism, its etiology is still unknown in spite of a great deal of investigation devoted to the problem. As discussed in Chapter 3, uric acid is formed by the hydrolysis and oxidation of those nucleic acids (nucleotides and nucleosides) which are not only absorbed from the intestinal tract after the digestion of ingested nucleoproteins, but include those of endogenous origin as well. Most investigators have concluded that in normal as well as in gouty individuals, the purine ring remains intact and that uricolysis or the destruction of uric acid does not take place. In gout the excess of uric acid in the blood is not due to its overproduction since it appears that the intermediary metabolism of the purines is normal, but to the fact that it is not normally excreted by the kidneys. However, the cause of this specific impairment in the elimination of uric acid is unknown. It may occur in gouty individuals without demonstrable kidney disease. Many authorities are convinced that heredity is primarily responsible even though the disease shows a tendency to skip one generation. It is commonly stated that women, although infrequent victims of gout, are very likely to transmit it to their children. Just why the excess of uric acid in the blood of gouty individuals is deposited as urates in the articular cartilages and other tissues, while not occurring in other states of hyperuricemia like chronic leukemia and Bright's disease, is also unknown. The rôle of infection as well as the assumption of allergic sensitization of the tissues being responsible for a specific affinity for uric acid can be excluded. Under the circumstances, it appears that gout is due primarily to a diminution in the elimination of uric acid by the kidneys, with the possibility that trauma, avascularity and other local factors favor or induce the precipitation of sodium urate from a supersaturated solution in the blood even though urates do not precipitate readily from the latter because of the presence of protective colloids.

Laboratory Examinations. Gouty arthritis may be confused with traumatic arthritis, cellulitis, rheumatic fever, one of the specific infectious arthritides, rheumatoid arthritis, Heberden's nodes and acute bursitis, particularly when the latter is associated with a hallux valgus deformity. While clinical and roentgenologic examinations may suffice for differential diagnosis, laboratory examinations are almost indispensable. The characteristic changes in gout may be summarized as follows:

1. An increase of the uric acid of the whole blood above the normal of 2 to 4 mg. per 100 cc. is an almost invariable if not a constant feature of the disease and of great diagnostic value when other causes for hyperuricemia (page 106) can be excluded. In less than 2 per cent of cases the blood may show 4 to 6 mg. but otherwise almost invariably 6 mg. or more per 100 cc. In this connection it is to be remembered, however, that cyclic fluctuations in the degree of hyperuricemia commonly occur at irregular intervals. No characteristic or constant changes in the blood uric acid occur immediately before an acute exacerbation of gouty arthritis; in some instances it remains unchanged while in others it may be increased or even decreased. Needless to state, blood uric determinations are the only means available for the detection of asymptomatic gout or the so-called "gouty diathesis."

2. The fasting blood glucose is usually within normal limits. Abnormal glucose tolerance curves are occasionally observed but have no diagnostic significance. Blood total nonprotein nitrogen, urea nitrogen and creatinine are usually within normal unless a sufficient degree of nephrosclerosis is present to produce a low grade azotemia.

3. During acute exacerbations a mild to moderate leukocytosis may be observed sometimes due to an absolute increase of the monocytes. The sedimentation rate of the erythrocytes is frequently increased.

4. During quiescent periods the urine usually shows a decrease of urates which, however, are sometimes increased after an acute exacerbation. Otherwise, the urinary changes are those observed in nephrosclerosis if present in sufficient degree. Evidences of mild renal impairment revealed by the kidney function tests are commonly observed.

CALCINOSIS

Calcinosis is a general term designating rare diseases of calcium metabolism characterized by abnormal deposits of calcium carbonate and phosphate in the skin, subcutaneous tissues and other parts of the body. The deposits have essentially the same composition as normal bone, being composed not only of calcium salts but small amounts of magnesium carbonate and phosphate, cholesterol and fatty acids. The two principal diseases of calcinosis are (1) *calcinosis circumscripta* and (2) *calcinosis universalis* [65] although intermediate forms occur which are designated as "transitional." Calcinosis is also referred to in the literature by a variety of other names like "tendinitis calcarea," "calcinosis interstitialis," "petrificato cutis," "subcutaneous calcerous granulomata," etc.

Calcinosis circumscripta is confined almost exclusively to the regions of the terminal phalanges of the fingers and toes and to the extensor surfaces of the knees and elbows. When tophi are produced in the periarticular tissues, the disease is commonly known as "calcium gout" or "chalk gout." [66] Localized calcinosis develops insidiously, usually pursues a mild course, occurs at all ages including advanced, and somewhat more frequently in women than in men. According to

Steinitz,[66] scleroderma or sclerodactylia accompanies calcinosis circumscripta in more than 40 per cent of cases. Vasospastic attacks may occur similar to those of Raynaud's disease. Ulcerations of the skin may develop with the discharge of calcerous material.

In *calcinosis universalis* large areas of the skin may be converted into calcareous plaques, or large inflammatory masses may develop over which the skin may ulcerate with the discharge of gritty, chalk-like material. Deposits may also occur in muscles, tendons and nerve sheaths but the viscera are spared in both types of the disease. Most cases occur in children and may be detected early in infancy.[67-69] While gradual resorption of the calcium deposits may be observed in calcinosis circumscripta, this does not occur in calcinosis universalis, although intermissions are not infrequent.

The cause of calcinosis is unknown. It is not due to a deficiency in the excretion of calcium comparable to the etiology of gout characterized by a deficiency in the excretion of uric acid. Hypercalcemia does not occur. It has been suggested that local tissue changes may favor the deposition of calcium. This is largely based on the frequency of calcinosis universalis in scleroderma but the disease frequently occurs in individuals without this disease of the skin. It is probably a disorder of hyperparathyroidism. Otherwise, it is likely to be due to local tissue factors of an unknown nature.

Laboratory Examinations. The diagnosis of calcinosis is usually based on the clinical manifestations, supplemented by roentgenologic examinations. Laboratory examinations, however, are of value in differential diagnosis and in calcinosis may be summarized as follows:

1. The total calcium of the serum is within the normal of 9 to 11 mg. per 100 cc. The same is true of the phosphorus of the serum in terms of inorganic phosphate, which averages 5.0 mg. for children and 3.5 mg. per 100 cc. for adults. The serum alkaline phosphatase, which averages 4 to 14 Bodansky units in children and 1.5 to 4.0 units in adults, is likewise normal.

2. Balance studies have indicated a marked tendency to the retention of absorbed calcium and phosphorus and especially in the case of calcium.[67]

3. The blood serum uric acid, sodium, potassium and magnesium are within normal.

OBESITY

Obesity is a disorder of metabolism characterized by the excessive deposit of fat in the body. The deposition occurs principally in the abdominal wall, thighs, buttocks, shoulders and face while sparing the eyelids, nose, ears, hands, feet and genitalia probably because fat is to be regarded as a specialized tissue.

Adipose tissue constitutes about 18 per cent of the body weight of individuals under conditions of average nutrition. As stated by Wells [70] it is not to be regarded as merely ordinary connective tissue in which fat has been deposited in excess but a specialized tissue, probably part of the reticulo-endothelial system, and endowed not only with the property of playing a rôle in immunity [71,72] but possessing general metabolic functions of unknown nature, including the conversion of carbohydrates into fat.

Simple obesity is due to the ingestion of foods in excess of energy requirements or to the ingestion of faulty metabolic mixtures of foods as the result of habit and overindulgence or because of an uncontrolled inherited abnormal appetite. In some instances it is to be considered as a constitutional or endogenous disorder. Obesity may also be due to various endocrine dysfunctions and especially hypopituitarism, hypothyroidism, hypo-adrenalism and hypogonadism, to be discussed in the succeeding chapter.

As far as simple obesity is concerned, it is not possible to establish its true incidence in the general population or in relation to age groups, sex, race or geographic distribution. However, it occurs more frequently in women than in men; also more frequently among the married of both sexes. From the standpoint of etiology, heredity is a factor in many cases. But, if endocrine dysfunctions are excluded, the usual cause is overeating and especially of carbohydrates, since any energy intake in health in excess of the needs of the body is converted into and stored as fat. Needless to state, however, the energy needs vary greatly among individuals; consequently, the number of calories which may be ingested in foods and beverages without causing obesity is highly variable. It has been suggested, however, that obesity could be independent of food intake due to a difference in the specific dynamic action of protein, carbohydrate and fat in the obese.[73] A tendency of the obese for fat fixation (the lipophilia of Bergman) has also been suggested as the mechanism of a possible difference in control of hunger and satiety in the obese as compared to individuals who maintain normal weights.

Undoubtedly, obesity is a menace to health with an influence on longevity as well as being in important relationship to the etiology of diabetes mellitus, arteriosclerosis, hypertension, etc. The level of nitrogen exchange is the same as in normal individuals and is influenced by the same factors and in the same degree. In obesity there is no relation between creatinine excretion and the total volume of urine; it is independent of the total nitrogen excretion as well as of a positive or negative nitrogen balance.[74] There is a higher level of oxygen exchange because an obese individual actually consumes more energy than would otherwise be the case [75] but in simple obesity the basal metabolic rate is within normal in relation to surface area.[76] Daily variations in weight due to variable amounts of water in the adipose and other tissues occur more frequently than in normal individuals because of disturbances in the heat-regulating mechanism and water exchange. Furthermore, during reduction by diet, the obese may show periods of water storage with seemingly no fat loss in spite of a reduced caloric intake. Curiously enough, however, and for unknown reasons, obese individuals have a high tolerance for foods possessing a high ketogenic-antiketogenic ratio. Acetonuria may occur in some cases during the first few days of dieting but lasts only a few days. Consequently, ketosis and acidosis are not a menace to the obese or factors to be considered in planning treatment. A fasting hyperglycemia of moderate degree and even glycosuria may be observed in some cases but such changes are always suggestive of incipient or established diabetes. On the other hand, it has been observed that obese individuals are occasionally subject to attacks of hypoglycemia, suggestive of hyperinsulinism due to hypertrophy of the islands of Langerhans,[77] but in many cases glucose tolerance curves in the obese lie between those characteristics of diabetes and of normal individuals, especially the latter.[78]

It is apparent, therefore, that *laboratory examinations* have no value in the diagnosis and treatment of simple obesity, although they are of great value as aids in the diagnosis of obesity due to hypothyroidism (reduced basal metabolic rate) as well as to a lesser extent in the diagnosis of obesity due to other endocrine dysfunctions. In this connection it may be stated that Behnke and his colleagues [79] have recently proposed a classification of individuals in regard to overweight on the basis of the specific gravity of the body (hydrostatic weighing), using a tentative dividing line of 1.060 for the elimination of the obese in relation to the army, navy and allied services.

THE XANTHOMATOSES

The xanthomatoses are also diseases due to disturbances of lipid metabolism and are divided into two groups, namely, the primary and secondary.

The *primary xanthomatoses* embrace Gaucher's disease involving kerasin, Niemann-Pick disease involving phosphatide, and the Hand-Schüller-Christian disease, a form of generalized xanthomatosis, involving mainly cholesterol and cholesterol esters. All three are diseases of the reticulo-endothelial system and occur mainly in children. *Laboratory examinations* as aids in diagnosis have been previously discussed in Chapter 22.

The primary xanthomatoses also include a variety of lesions of the skin, lips, tongue, buccal surfaces, tonsils, larynx, trachea, bronchi, glans penis, vulva, anus, etc., as well as those which may arise from fascia, tendon sheaths and the periosteum. These lesions are variously designated according to their location and appearance as *xanthoma palpebrarum, xanthoma tuberosum, xanthoma planum, xanthoma desseminatum*, etc.

The *secondary xanthomas* occur in diabetes mellitus (*xanthoma diabeticorum*), jaundice (*xanthoma hepaticum*) and chronic nephritis.

In these xanthomatoses *laboratory examinations* consist of microscopic and chemical examinations of tissue removed by biopsy and determinations of the total lipids of the plasma with special reference to free cholesterol and cholesterol esters. Normal skin ordinarily contains less than 8 per cent total lipids, of which about one-fifth is cholesterol.[80] In xanthomatous tumors the cholesterol content is quite variable, but is very often the predominant lipid constituent.[80,81] In xanthoma desseminata the total lipids and cholesterol of the plasma have been reported as essentially normal but are sometimes below and occasionally slightly above normal.[81,82] Marked elevations of all lipids of the plasma and especially of cholesterol are stated to occur in xanthoma tuberosum; likewise in xanthoma palpebrarum although normal values are not uncommon. Hypercholesterolemia is also frequently observed in xanthomas of tendons and tendon sheaths as well as in xanthoma diabeticorum. The nature of these metabolic disturbances has not been completely elucidated but it does not seem that the lipemia is due to fats of exogenous origin.[83] There is, however, some evidence that the level of plasma lipids is influenced not so much by the elimination of animal fat from the diet as by a reduction of the total caloric intake irrespective of whether the reduction is at the expense of fat, protein, or carbohydrate.[80,83]

THE LIPOMATOSES

The lipomatoses refer to localized or diffuse collections of fat exclusive of obesity. Apparently, none are due to disturbances in lipid metabolism. However, some are due to disturbances of the pituitary gland while the etiology of many is unknown. They include a large number of diseases as follows: (1) *Adiposis dolorosa* (*Dercum's disease*) due to disturbances of the pituitary gland or structures lying over it; (2) *dystrophia adiposogenitalis* (*Frölich's syndrome*) due to hypopituitarism; (3) *adenolipomatosis or diffuse symmetrical lipomatosis of the neck* of unknown etiology; (4) *nodular circumscribed lipomatosis* of unknown etiology; (5) *lipogranulomatosis* due to the repair of local areas of fat necrosis; (6) *sclerema neonatorum* of unknown etiology and characterized by a deficiency of olein in the fat of infants; (7) *progressive lipodystrophy* of unknown etiology and (8) *pseudolipoma* occurring in hysterical women.

Laboratory examinations for diagnostic purposes are limited to microscopic examinations of tissue removed by biopsy. Taking the group as a whole there are no blood chemistry or other examinations of clinical value.

MALNUTRITION

Malnutrition in a broad sense includes both complete inanition as well as the dietary deficiencies. Obviously, it may be due to many causes including not only an actual lack of sufficient food, vitamin deficiencies with special reference to vitamin B complex, chronic alcoholism, nervous disorders, etc., but various chronic and wasting diseases as well. When well marked, with wasting and dehydration, its clinical diagnosis offers no difficulties. But the detection of marginal or developing malnutrition is important, especially in individuals of the nervous type, because malnutrition produces easy fatigability which, in turn, increases the severity of the subjective symptoms, such as nervous tension and irritability.[84] Anorexia due to various causes is frequently responsible for extreme malnutrition and especially anorexia nervosa and Simmonds' disease, due to hypopituitarism with atrophy of the adrenal cortex.

As far as *laboratory examinations* are concerned, the chief changes in well marked malnutrition are usually as follows: (1) Hypoproteinemia in which the plasma albumin is reduced below the normal of 4 to 5.5 gm. per 100 cc. of plasma with a consequent reversal of the albumin-globulin ratio and responsible for the edema of malnutrition because of a reduction in osmotic pressure, although a decrease of gamma globulin may occur.[85] Miranda [86] has recently pointed out that disturbances following insufficient nourishment may be due to (a) inadequate caloric intake, (b) vitamin deficiency, and (c) hypoproteinosis due to either a quantitative or qualitative deficiency of protein foods, with hypoproteinemia only one of the manifestations and not observed in all cases. (2) A low basal metabolic rate largely due to protein privation. Subnormal values, sometimes as low as —40, may be observed in patients with anorexia nervosa probably due largely to inanition. (3) Diminished glucose tolerance is commonly observed with curves resembling those seen in mild diabetes.[84,87,88] Indeed, the glucose tolerance test is advised as an aid in the diagnosis of developing or marginal malnutrition in which the curve has been designated as "pseudodiabetic."[84]

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DISEASES OF VITAMIN DEFICIENCY

Owing to the low toxicity of the vitamins and the elimination of amounts over and above actual needs, there are no symptom-complexes or diseases of human beings attributable to the ingestion of large amounts of them in foods. However, toxic effects may be produced by the oral or parenteral administration of excessively large doses of some of the vitamins. Such effects constitute the *hypervitaminoses.*

On the other hand, a large number of symptom-complexes or diseases are now known to be due to the ingestion, absorption, storage or utilization of inadequate amounts of the vitamins and the number is increasing with advancing knowledge. In my opinion, these are properly designated the *hypovitaminoses.* While they are commonly called the *avitaminoses,* I believe that this term should be reserved for those less frequent symptom-complexes and diseases due to a total lack of the intake, absorption, storage or utilization of vitamins. This would appear to be in better conformity with medical terminology. For example, the term "avirulent" applied to a micro-organism in relation to a given animal, usually implies a total lack of virulence instead of merely a reduction of it.

THE HYPERVITAMINOSES

The hypervitaminoses are very uncommon. They are usually readily recognized by the history of excessive dosage and the clinical manifestations. *Laboratory examinations* of the blood and urine for most of the vitamins concerned are available but are not usually employed as diagnostic aids.

Vitamin A. No ill effects are produced by the administration of ordinary therapeutic doses of the fish liver oils containing vitamin A (A_1 and A_2). Even the daily administration of from 50,000 to 300,000 or more U.S.P. units of vitamin A over periods as long as two to six months has been reported as being without harmful effects.[1] On the other hand, large doses have been stated to produce dermatitis and eczema of the face, scalp or other parts of the body although, in some cases, these dermatoses may have been due to allergic reactions to fish proteins in the oils.

However, if larger amounts of vegetables containing the yellow or red carotenoid pigments, the precursors of vitamin A, are ingested by normal individuals (especially children), those suffering from diabetes mellitus, myxedema or Simmonds' disease [2] as well as from diseases of the liver or kidneys, carotene may accumulate in the skin in amounts sufficient to produce a deep yellow color (xanthosis) known as *carotenemia.* This condition, however, produces no ill effects although it may be mistaken clinically for jaundice. Blood serum normally contains a small amount of carotene as well as the related pigment, xanthophyll, but together they do not usually exceed about 0.1 mg. per 100 cc. In diabetes, however, values ranging from 0.210 to 0.675 mg. or higher have been reported,[3] with the suggestion that the carotenemia in this disease, as well as in myxedema and hepatic disease, is due to impairment of the function of the liver to convert carotene into vitamin A or because of the depressing effects of lowered metabolism.[2,4,5] Since an excess of carotene in the serum may be mistaken for bilirubin in the icterus index test it is, therefore, always advisable to employ the van den Bergh tests for differential diagnosis between jaundice and carotenemia when such differentiation is required.

Vitamin B Complex. Although vitamin B complex may contain as many as thirteen factors, those of recognized importance are thiamine (B_1), riboflavin (B_2), nicotinic acid (P-P), pyridoxine (B_6), pantothenic acid, para-aminobenzoic acid, biotin and choline.

The toxicity of *thiamine chloride* by oral, subcutaneous, intramuscular or intravenous administration is very low so that ill effects are not to be expected even when large amounts are given although there may be a tendency to fatty infiltration of the liver in the presence of inadequate amounts of choline.[6] Allergic sensitization from parenteral administration, however, has been reported; likewise severe anaphylactic-like reactions with a few fatalities. Even large doses of *riboflavin* are regarded as harmless.[7] *Nicotinic acid* may produce flushing, pruritus, faintness, sebaceous gland activity and nausea but otherwise it is of low toxicity.[8] Nicotinamide, which is just as effective therapeutically, is stated not to produce these unpleasant effects except headache and nausea in some cases. *Pyridoxine* is likewise of very low toxicity[9] although excessively large doses (3.0 gm. per kilogram) may produce convulsions and death in the lower animals. While the human requirements and possible therapeutic dosage of *pantothenic acid* are unknown, it would appear to be nontoxic, since daily doses of as much as 100 mg. are stated to be well tolerated. *Para-aminobenzoic acid* is also of low toxicity; *biotin* is not used therapeutically, hence its toxicity for human beings cannot be stated. *Choline,* however, is regarded as relatively nontoxic since as much as 50 mg. may be given daily for a week without ill effects.

Vitamin C. Synthetic vitamin C (ascorbic or cevitamic acid) is of very low toxicity. As much as 6 gm. have been given orally and doses of 0.5 to 1.0 gm. of the crystalline compounds have been given intravenously to adults without ill effects although large doses in children may produce vagotonic reactions, such as bradycardia, increased peristalsis and erythema; these effects, however, have been ascribed to allergic sensitization rather than to hypervitaminosis.

Vitamin D. When amounts of vitamin D many times the therapeutic dose are administered to the lower animals, certain pathologic changes may be produced. In human beings, however, the vitamin is usually well tolerated. Rachitic children, who are known to have a high tolerance, have been given single massive doses as high as 1,000,000 units without ill effects.[10] But large doses in adults may produce lassitude, nausea, headache, anorexia and diarrhea with urinary frequency. Less common but more significant symptoms are vertigo, paresthesia, joint and muscle pains, tenderness of the teeth and gums, neuralgia of the mandibular branch of the trigeminal nerve, and impaired memory. If renal insufficiency is present with hypercalcemia greater care in dosage is required because the administration of the vitamin may increase the degree of the latter as well as hyperphosphatemia and diminished phosphatase activity. Under these conditions, the urine should be examined repeatedly for calcium as well as the blood for calcium and phosphorus.

Vitamin E. No toxic reactions have been reported in cases receiving alpha tocopherol and ordinary doses of 0.25 to 6 cc. of wheat germ oil per day. Larger doses, however, may produce minor symptoms. The danger of producing neoplasms by the continued ingestion of wheat germ oil, distilled tocopherols or alpha tocopherol, however, appears to be nonexistent.

Vitamin K. To date no serious untoward effects have been reported in human beings given reasonable therapeutic doses of natural concentrates of vitamin K or synthetic vitamin K_1. Concentrates of alfalfa in single oral or intramuscular doses as large as 2 gm. appear harmless. It has been observed, however, that 180 mg. doses of menadione administered orally to human beings may produce vomiting, porphyrinuria and albuminuria. It is also stated that some clinical consideration should be given to the possible undesirable effects which may be associated with conjugates resulting from the administration of this compound.[11] Such doses of menadione, however, are so much larger than those employed for therapeutic purposes that at present it appears safe to use these synthetic compounds.

THE HYPOVITAMINOSES AND AVITAMINOSES

Foods are the natural sources of vitamins but the margin of safety against deficiencies in average diets is quite narrow. Indeed, it is becoming increasingly apparent that nutritional and other disorders due to the hypovitaminoses are widespread and by no means limited to the lower economic groups. Furthermore, it is apparent that greater care is required in guarding against the loss of vitamins and minerals in the processing, preparation, marketing and storage of foods. In this connection it is also to be emphasized that the hypovitaminoses may develop gradually and insidiously from the use of special and restricted diets in the treatment of obesity, alimentary tract diseases, the food allergies, peptic ulcer, diabetes mellitus, hypertension, heart disease, chronic nephritis, etc. Under such circumstances, the possibilities of vitamin deficiencies should be kept in mind, since they are readily prevented by the supplemental administration of the vitamins.

Moreover, it is readily apparent that because of the narrow margin of safety in the amounts of vitamins ingested with foods, pregnancy and lactation, alcoholism and various acute and chronic diseases due to infection (especially of the alimentary tract) as well as congestive heart failure, hyperthyroidism, diabetes mellitus and other metabolic disorders may prevent the normal intake, absorption, storage or utilization of the vitamins. Increased physical activity also creates an increased demand for them, while rest reduces the need as seen, for example, in the remission of the manifestations of pellagra on rest in bed without a change in diet or the administration of nicotinic acid.

It is also obvious that individuals suffering from any particular disease of vitamin deficiency are likely to be victims of mixed deficiencies in which the clinical manifestations are readily masked and overlooked. As a matter of fact, it appears that diseases due entirely to deficiencies of but single vitamins are rare although some, like night blindness (nyctalopia), rickets, scurvy, beriberi and pellagra, are so largely due to deficiencies of particular vitamins that they are described as specific entities.

Unfortunately, the early clinical manifestations of the hypovitaminoses and avitaminoses are not well defined. Characteristic signs and symptoms may not appear for weeks, months or even several years. The prodromal symptoms, which are remarkably similar irrespective of the particular deficiency or deficiencies, usually embrace loss of weight and strength, lassitude, mental depression, irritability, insomnia, headaches, anorexia, gastro-intestinal disturbances, vague paresthesias, etc. But because the vitamin deficiency syndromes and diseases are progressive rather than self-limited, the importance of early diagnosis and specific therapy cannot be overemphasized.

Laboratory Examinations. The diagnosis of advanced or severe diseases due to the vitamin deficiencies is not usually difficult after some experience with them has been gained. But the detection of the early or subclinical hypovitaminoses, which occur most frequently, is much more difficult as, likewise, those uncommon cases in which the manifestations of deficiencies are so indefinite or so deceptive as to resemble other diseases.[12] A precise knowledge of previous diet, accurate histories and thorough physical examinations are of paramount importance supplemented, when necessary, by therapeutic tests.

Fortunately, examinations of the blood or urine, or both, are frequently of aid in the diagnosis of thiamine, riboflavin, nicotinic acid, vitamin C and vitamin D deficiencies, as discussed in Chapter 20. With the exception of vitamin C, however, the methods are quite complicated and not infrequently beyond the scope of many laboratories. Furthermore, many of the methods now available are lacking in sufficient sensitivity and specificity for the detection of those subclinical or atypical hypovitaminoses in which assistance in diagnosis is most urgently needed.

Additional laboratory examinations, however, are not infrequently of supplemental diagnostic value with special reference to blood chemistry determinations, hematologic examinations, blood prothrombin determinations, etc.

BERIBERI

Beriberi is a disease due to a deficiency in thiamine or vitamin B_1 characterized by multiple neuritis, often associated with congestive heart failure, anasarca and muscular atrophy. Because these manifestations vary greatly in type, severity and order of appearance, the disease in the adult has been classified into (1) *dry beriberi*, characterized by multiple neuritis; (2) *wet beriberi*, characterized by anasarca; (3) *fulminating beriberi*, characterized by acute cardiac failure and (4) *mixed beriberi* in which two or more types are present in combination. Except in the fulminating type, there is usually a long prodromal period of ill health characterized by emotional and intellectual changes.

The symptoms arising from involvement of the nervous system are predominantly those due to an ascending symmetrical, peripheral neuritis caused by degenerative changes along with congestion and edema of the brain and spinal cord. The cardiac changes, characterized by hypertrophy and dilatation ending in congestive failure, are essentially due to hydropic degeneration and edema of the muscle. The cause of the edema, which begins in the feet and legs with the ultimate production of pulmonary edema, hydrothorax, hydropericardium and ascites, is not definitely known but is probably largely due to circulatory failure and hypoproteinemia. Gradual loss of the subcutaneous, retroperitoneal and epicardial fat commonly occurs.

While beriberi is largely confined to the Orient, where the vitamin deficiency is ascribed to diets largely composed of polished rice, thiamine deficiency is now known to be an etiologic factor in the production of neuritis or polyneuritis (infectious, alcoholic or due to pregnancy), anorexia, malnutrition and gastrointestinal disturbances and anxiety states throughout the world. Deficiencies also occur in congestive heart failure, diabetes, hyperthyroidism, rheumatoid arthritis, multiple sclerosis, syphilis of the central nervous system, etc.

Laboratory Examinations. These are frequently of value in the diagnosis of beriberi and its differentiation from polyneuritis and anasarca due to other causes as well as for the detection of other thiamine deficiencies. The characteristic changes may be summarized as follows:

1. A reduction in the thiamine of the blood and urine as well as of pyruvic acid of the blood, as discussed on page 618.
2. A possible increase of the phospholipids and fatty acids of the plasma with a decrease of serum magnesium.
3. Hypoproteinemia with reversal of the albumin-globulin ratio in the wet type of the disease.
4. Hyperchromic anemia, which is sometimes of the microcytic type, occurs in practically all cases.

PELLAGRA

Pellagra is a common disease of slow and insidious onset with prodromal symptoms characterized eventually by dermatitis, diarrhea and dementia with periodic seasonal variations. It is now know to be due to a deficiency of nicotinic acid or the pellagra-preventing vitamin (P-P) in diet, although a deficiency in riboflavin (B_2) may be a contributing cause. Both vitamins belong to B complex.

The early or prodromal symptoms usually include anorexia, lassitude, easy fatigability, headache, vertigo, restlessness, nervousness, forgetfulness, irritability, apprehensiveness, insomnia and paresthesias. A symmetrical cutaneous lesion resembling sunburn (probably due to photosensitivity) develops on the back of the hands, wrists and forearms. These lesions may progress to ulceration and finally to atrophy, discoloration, desquamation and bleeding. Vaginitis may be present. Nausea, vomiting, colic and diarrhea with glossitis, gingivitis and stomatitis are common. In severe cases a profound dementia may develop. The symptomatology is similar in infants and young children, although the typical skin and oral lesions are sometimes absent. They are undernourished and underdeveloped for their age, do poorly in school and look like nothing so much as sad little old men and women.

Laboratory Examinations. Laboratory examinations are frequently helpful in the diagnosis of pellagra, especially of atypical cases, and may be summarized as follows:

1. The detection of the absence of nicotinic acid in the urine or its reduction in the blood as discussed on page 623. The results, however, do not correlate well with the nicotinic acid stored in the tissues.
2. The excretion of excessive amounts of porphyrin [13] or "porphyrin-like substances" and especially the chromogen of urorosein, which appears to be indirubin [14] in the urine, ascribed to impairment of the liver and thought to be responsible for photosensitization of the skin.
3. Normocytic or microcytic anemia of varying severity and especially in well-developed cases of the disease.
4. Sometimes hyperproteinemia due to dehydration from excessive diarrhea and vomiting which may also produce alterations in the acid-base equilibrium of the plasma.

SCURVY

Scurvy or scorbutus is a disease due to a deficiency in vitamin C characterized by general debility, irritability, pallor, general aches and pains, gingivitis, purpura, and subperiosteal hemorrhages.

Severe scurvy is not encountered among infants and adults as frequently as formerly but mild or borderline scurvy occurs quite commonly. Indeed, this type of the disease constitutes an important problem in diagnosis, since early detection and adequate vitamin C therapy are essential because the disease is not self-limited and may end fatally. It is far more likely to develop in artificially fed infants than in breast-fed infants because cow's milk contains four to five times less vitamin C. Most cases of infantile scurvy (Barlow's disease) occur between 8 to 13 months of age.

In infants, young children and adults the disease is always to be suspected upon the occurrence of apathy, disinclination to activity, weakness, anorexia, irritability, pallor, gastro-intestinal disturbances and soft, swollen, spongy gums (which bleed spontaneously or on the slightest provocation), frequently associated with fusospirochetal infection and halitosis. These manifestations are accompanied or soon followed by purpura due to fragility or increased permeability of the capillaries. Petechiae and ecchymoses of varying severity usually first occur in the skin of the lower extremities. Eventually bleeding may involve the mucous membranes, accompanied by intestinal hemorrhages and hematuria. Subperiosteal and periarticular hemorrhages with arthritic pains may suddenly supervene spontaneously or on the slightest trauma and especially in the legs. Under these circumstances an infant is likely to assume the "pithed frog" position in bed with flexion, wide abduction and external rotation of the thighs; it is apprehensive and cries frequently, especially when handled. In young children the so-called "epiphyseal separations" are common in severe cases and always to be suspected clinically by the persistence of localized tenderness after treatment has relieved the generalized tenderness. Loss of weight and nutritional edema, along with increased susceptibility to infection, are not infrequent in both children and adults. The growth and development of children are usually retarded.

Well-advanced scurvy does not usually present difficulties in clinical diagnosis. But the detection of early and subclinical cases may be difficult. In the case of infants and young children, roentgenologic examinations of the ends of bones and of the sternal junctions of the ribs frequently reveal cessation of the growth of bone and its replacement with connective tissue poor in collagen (in which fragments of densely calcified cartilage may be imbedded) even before the disease becomes manifest clinically. These changes, however, are quite similar to those observed in rickets and when the latter is suspected in association with scurvy, differential diagnosis may be difficult.

Laboratory Examinations. Fortunately, laboratory examinations for ascorbic acid in the plasma and urine are not technically difficult and are of value in diagnosis as discussed more fully on page 626. These examinations, especially of the blood plasma, are particularly valuable in the detection of ascorbic acid depletion of the tissues in the diagnosis of early scurvy, although it is stated that clinical manifestations may not appear until the tissues have been depleted for about three months. These and additional laboratory examinations may be summarized as follows:

1. A reduction of plasma ascorbic acid below 0.5 to 0.7 mg. per 100 cc.

2. A reduction of ascorbic acid in the total 24-hour urine to below 10 mg.

3. A positive "saturation reaction" which consists in an estimation of the amount of ascorbic acid excreted in the urine within a stated period following the administration of a test dose.

4. A positive tourniquet reaction indicative of increased capillary fragility and permeability. It may not be observed, however, and positive reactions may be due to causes other than scurvy.

5. Hypochromic normocytic or macrocytic anemia is common in adults but less common in infants. The platelets are not reduced. Coagulation and bleeding times are usually normal except in severe cases when either or both may be prolonged. In uncomplicated scurvy the total and differential leukocyte counts are usually within normal.

6. Changes in serum calcium and inorganic phosphorus may be observed but are not constant.

7. Hematuria occurs with great frequency; oliguria is common. Hyaline or granular casts, sometimes blood casts, and pyuria may be found.

RICKETS

Rickets or rachitis is a disease of calcium and phosphorus metabolism affecting infants and children due to a deficiency in vitamin D.

While cases of severe rickets are not encountered as frequently as at one time, it has been estimated that 50 to 90 per cent of all infants in temperate and especially northern climates may exhibit signs of it due to a deficiency in the quality and intensity of the ultraviolet rays in sunlight. Winter sunlight conveys much less of the rays than that of the warmer months and ordinary window glass filters out most of them.

While heredity apparently plays no rôle in the etiology of the disease, individual susceptibility due to a constitutional factor of unknown nature is important, since children are not predisposed to the disease in equal degree. Premature birth is a factor of great importance and rickets is of exceptional frequency and severity among premature infants. Rapid growth is also an important predisposing factor. Most cases develop between three to eighteen months after birth. Sex appears to have no influence although late rickets and infantile tetany occur more frequently in male infants while osteomalacia is of very rare occurrence among them.

The early manifestations of rickets are usually restlessness, irritability, head sweating and gastro-intestinal derangements. These are followed by the development of craniotabes, delayed dentition, enlargements of the wrists, knees, ankles and costochondral junctions of the ribs (due to changes in the epiphyseal-diaphyseal junctures), alterations in the bony thorax (Harrison's groove, "pigeon breast," "funnel breast") and later, after walking begins, by bowing of the legs (*genu valgum* or *genu varum*) with deformities of the spine in some cases.

Roentgenologic examinations are of diagnostic value and especially of rapidly growing long bones, since they usually reveal a flaring and widening of the diaphysis above the epiphyseal line; also increased thickness and fringy margins of the zone between the shaft and epiphysis along with coarse prominent trabeculae in spongy bone.

Laboratory Examinations. As discussed more fully on page 627, there are no available laboratory examinations for the detection or estimation of vitamin D in the blood or urine. However, other laboratory examinations of diagnostic value are available as well as helpful in differentiating rickets from infantile scurvy, congenital syphilis, chondodystrophy, osteogenesis imperfecta, cretinism, etc. They may be summarized as follows:

1. An increase of serum alkaline phosphatase above the normal of 4 to 14 Bodansky units per 100 cc. due to increased osteoblastic activity and frequently observed before roentgenologic changes or alterations in serum phosphate occur.
2. A decrease of inorganic phosphate of the serum below the normal of 4 to 7 mg. per 100 cc. along with a decrease of calcium in the urine and an increase in the feces.
3. A decrease of total serum calcium below the normal of 9 to 11 mg. per 100 cc. in some cases (frequently within normal) and especially in rickets associated with infantile tetany. An increase of calcium in the feces may be found along with a decrease in the urine.
4. Hypochromic normocytic or macrocytic anemia is of frequent occurrence.
5. Negative serologic reactions for syphilis.
6. Normal or slightly reduced basal metabolic rates.

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31

DISEASES OF THE ENDOCRINE GLANDS

The endocrine glands are commonly known as the "ductless glands." This is right and proper as far as their internal secretions of hormones are concerned, as these are absorbed directly into the blood. But some of the endocrine glands are not ductless but of a mixed character with both internal and external secretions, as in the case of the pancreas, testes and ovaries. Furthermore, organs other than those classified as endocrine glands, such as the duodenum, are apparently capable of producing internal secretions or hormones.

The endocrine glands are subject to diseases which may or may not materially affect their hormones. As far as laboratory examinations are concerned in relation to diagnosis, those diseases and syndromes due to disorders or perversions of the hormones are of particular interest; indeed, from both this and the clinical aspects, the latter could be properly designated "diseases due to dyscrinism."

GENERAL CONSIDERATIONS

Dyscrinism may be of several varieties as follows: (1) *hyperfunction* due to the excessive secretion of hormones; (2) *hypofunction* due to the insufficient secretion of hormones; (3) *dysfunction* due to qualitative rather than quantitative changes in the hormones or to disorders characterized by alternate hyperfunction and hypofunction in the same individual and (4) *afunction* due to the complete loss of hormones. If dyscrinism involves only one gland, a pair (ovaries, testicles, suprarenals) or four (parathyroids) the disorder is referred to as a *monoglandular* disease. If the functions of other glands are involved which, because of the intimate interrelationships of the endocrine glands, is usually the case, the disorder is designated a *pluriglandular* disease. Some of the diseases and syndromes due to hypofunction and afunction are amenable to treatment through the administration of the deficient hormones but diseases and syndromes due to hyperfunction are more difficult from the standpoint of treatment except in the case of those glands in which the surgical removal of an excess of tissue, or its depression by x-ray treatment, is possible.

Primary dyscrinism may be due (1) to congenital deficiencies or the complete absence of a gland as in cretinism; (2) its surgical removal in whole or in part, or the result of its injury; (3) its destruction by inflammation or (4) by tumors. *Secondary dyscrinism* is due to effects of a primary disorder of one endocrine gland on the functions of one or more other glands. It is of such frequent occurrence and complexity that endocrinology is one of the most difficult subjects in medicine.

As discussed more fully in Chapter 20, there are as yet no direct laboratory methods available for the determination of the hormones in the blood, or excreted in the urine, in relation to the diagnosis of endocrine diseases, except in the case of the gonadotropic hormones secreted by the anterior lobe of the pituitary gland, ovaries and testicles. But various other laboratory examinations are available for the detection of dyscrinism, especially in reference to the basal metabolic rate and blood chemistry determinations. Otherwise, diagnosis is based on objective signs (physical or skeletal abnormalities, mental or nervous disturbances, menstrual disorders, etc.) and subjective symptoms (nervous, circulatory or general), the latter being usually due to an imbalance of the autonomic nervous system (embracing the parasympathetic and sympathetic divisions) as a result of hyperfunction or hypofunction on the part of different endocrine glands which normally exercise a regulatory influence over it. In this connection it must be remembered, however, that some nonendocrine disorders may produce signs and symptoms which may be erroneously attributed to dyscrinism. Furthermore, heredity alone may be an important factor in relation to both physical and mental development.

HYPERPITUITARISM

Hyperpituitarism is caused by the excessive secretion of one or more hormones by the pituitary gland. Almost invariably it is due to disorders of the anterior lobe. These disorders, in turn, are most frequently due to the overproduction of the growth hormone resulting in such disorders as gigantism, acromegaly or Cushing's disease. Not infrequently, however, there is a coincident increase of the gonadotropic, thyrotropic, adrenotropic or other hormones which are also secreted by the anterior lobe and responsible for secondary signs and symptoms of disease. Anterior hyperpituitarism is most frequently due to functional changes or adenomas involving the eosinophilic cells (gigantism or acromegaly) and less frequently of the basophilic cells (Cushing's disease). Adenomas also affect the chromaphobe cells of the anterior lobe but since they do not secrete hormones the signs and symptoms are those due to the space-occupying lesions rather than to anterior hyperpituitarism. As the result of secondary atrophy of eosinophilic and basophilic cells, however, the signs and symptoms of anterior hyperpituitarism are likely to be replaced sooner or later by those of anterior hypopituitarism. It is readily understood, therefore, that both the clinical and laboratory manifestations depend a good deal upon whether hyper- or hypo-anterior pituitarism predominates at the time examinations are made.

GIGANTISM

Gigantism is a rare disease due to the overproduction during infancy, early childhood or adolescence of the growth hormone of the anterior lobe before the closing of the epiphyses of the long bones which normally occurs at 18 to 20 years of age. Males are affected more frequently than females. The disease is apt to run its course within thirty or forty years, or less, and death is frequently due to pituitary cachexia or infections resulting from reduced resistance.

Three types of the disease are recognized as follows: (1) *Normal gigantism,* which develops during pre-adult life, is usually due to simple functional changes in the eosinophilic cells and but rarely to adenomas. It is characterized by overgrowth of the long bones without adiposity and with average sexual and mental development. (2) *Eunuchoid gigantism,* which is due to the same causes but in which the body measurements are different along with sexual infantilism because of a coincident deficient secretion of the gonadotropic hormone. (3) *Acromegalic gigantism,* in which overgrowth of the flat bones continues into adult life because it is generally due to adenomas of the eosinophilic cells, is commonly regarded as a combination of gigantism and acromegaly.

During the early *hyperpituitary period,* rapid growth is accompanied by great muscular strength, early sexual maturity, virilism and hirsutism; during the later *hypopituitary period,* however, physical and mental vigor declines, hirsutism is replaced by a scanty growth of hair and virilism by impotency in the male or amenorrhea or other menstrual disturbances in the female due to atrophy of the gonads.

Laboratory Examinations. The diagnosis of gigantism is usually readily made on the basis of the clinical manifestations supplemented if need be by roentgenologic examinations of the sella turcica. Consequently, laboratory examinations are not generally required but are sometimes of helpful diagnostic aid. They may be summarized as follows:

1. During the *early* stage of anterior hyperpituitarism (a) the basal metabolic rate is frequently increased due to stimulation of the thyroid gland by the excessive production of the thyrotropic hormone; (b) this increase of metabolism may also accelerate the specific dynamic action of protein, with an increase of blood creatinine above the normal of 1 to 2 mg. per 100 cc. and an increase of urinary creatinine above the normal of about 1.25 gm. per 24-hour urine; (c) decreased sugar tolerance with fasting hyperglycemia and glycosuria may be observed due to the overproduction of the hyperglycemic hormone which reduces the secretion of insulin.

2. During the *late* state of anterior hypopituitarism, however, the changes are usually reversed with (a) a reduction of the basal metabolic rate below normal; (b) increased sugar tolerance; (c) normal or approximately normal blood sugar and (d) absence of glycosuria.

ACROMEGALY

Acromegaly is also due to an overproduction of the growth hormone caused by eosinophilic adenomas of the anterior lobe of the pituitary gland which develops after closure of the epiphyses of the long bones. The disease occurs more frequently than gigantism, affects both sexes and usually develops in early middle life between 20 to 40 years of age. It may pursue a rapid course, ending fatally in three to four years, but in most cases is a slowly progressive disease over a period of ten to twenty years.

The short and flat bones show marked growth with exaggeration of all bony prominences of the head and face. The supra-orbital ridges become enlarged along with the development of a massive and protruding lower jaw (prognathism) producing malocclusion. The anterior teeth are usually widely spaced. The ears, lips and tongue become thick and heavy along with a general enlargement of practically all of the viscera (splanchomegaly). The hands become large, broad, thick or "spadelike" along with enlargement of the feet. Kyphosis develops in many cases and is sometimes accompanied by neuralgias and osseous pains due to pressure on the spinal nerve trunks.

During the *early* stage, other anterior pituitary hormones are likely to be produced in excess, resulting in hypergonadism, abnormal hyperplasia of the mammary glands in both sexes, hyperthyroidism, etc., along with a voracious appetite. Bradycardia with cardiac arrhythmias are of common occurrence. During the *late* stage, signs and symptoms of anterior hypopituitarism may develop due to the effects of atrophy of the adenomas from pressure and characterized by hypogonadism, hypothyroidism, hypo-adrenalism, etc.

Laboratory Examinations. As in the case of gigantism, diagnosis is usually readily made on the basis of the clinical manifestations. Laboratory examinations, however, are sometimes of helpful diagnostic value and especially in atypical cases. Before hypopituitarism supervenes they may be summarized as follows:

1. The basal metabolic rate is increased in about 70 per cent of cases.[1]

2. An increase of blood iodine, increased iodine tolerance and increased urinary excretion of iodine may be observed.

3. Sugar tolerance is likely to be low with a tendency to fasting hyperglycemia and glycosuria, although both hyperglycemia and glycosuria may be absent.[2] Increased insulin resistance may occur.

4. The specific dynamic action of protein is likely to be increased along with increased blood and urine creatinine.[3]

5. Hypocalcemia with a marked increase in urinary calcium may be observed which is apparently responsible for the development of osteoporosis in some cases.[4]

6. Hypocholesterolemia with hypolipemia as well as an increase of blood uric acid and nonprotein nitrogen sometimes occurs.

7. Serum sodium and serum chloride may be increased, with salt retention.

8. Polyuria is not infrequent and the urine may show the presence of albumin and casts.

9. Hypochromic normocytic anemia may develop in severe cases along with hypochlorhydria or achlorhydria.

PITUITARY BASOPHILISM

Pituitary basophilism is usually due to adenomas of the basophilic cells of the anterior lobe of the pituitary gland although they may be exceedingly small and escape discovery unless a careful microscopic examination of the pituitary tissue is made. Many of the clinical manifestations are due to hyperpituitarism although other glands (especially the adrenal cortex, gonads and parathyroids) are so commonly involved that the symptomatology is quite variable. Indeed, clinical differentiation between basophilic adenomas and adrenal cortex neoplasms may not be possible. When the disorder is due to basophilic adenomas alone it is frequently designated *Cushing's disease* but when this is associated with adrenal cortical tumors, it is commonly known as *Cushing's syndrome.*

Pituitary basophilism is quite uncommon and usually occurs in females. The onset is generally between five and twenty-five years of age. Very few cases have been observed in older patients or in males. The outstanding clinical manifestations are extreme fatigability and weakness, abdominal and especially lumbosacral pains, severe intermittent headaches, obesity sparing the arms and legs, stunted growth, purplish abdominal striae, hirsutism, osteoporosis, sexual dystrophy and hypertension. Edema of the hands and legs may occur.

Laboratory Examinations. Diagnosis is usually based on the clinical manifestations. Roentgenologic examinations reveal no changes in the sella turcica but do reveal evidences of skeletal decalcification. Laboratory examinations are sometimes helpful in diagnosis and may be summarized as follows:

1. An excess of the pituitary gonadotropic hormones (prolan A and prolan B) may be found in the urine. The estrogenic hormone (estrin) is usually absent. An excess of the latter in the female or an excess of the androgens in the male is usually indicative of adrenal cortex hyperplasia or neoplasm.
2. Transient glycosuria may occur in about 50 to 60 per cent of cases.
3. Reduced glucose tolerance with moderate hyperglycemia are commonly observed ascribable probably to the excessive secretion of the pancreatropic hormone.
4. Increase of serum calcium and increased urinary calcium due to the excessive secretion of the parathyrotropic hormone. Serum phosphorus is normal.
5. Hypercholesterolemia following secondary hyperplasia of the adrenal cortex may occur, as may likewise an increase of the total nonprotein nitrogen of the blood.
6. The basal metabolic rate is usually increased owing to the excessive secretion of the thyrotropic hormone or to hyperplasia of the adrenal cortex. In the late stage of the disease the rate may be decreased.
7. Polycythemia may be observed.

HYPOPITUITARISM

As in the case of hyperpituitarism, the diseases and symptom-complexes characterized by hypopituitarism are likewise principally attributable to involvement of the anterior lobe of the pituitary gland. In the latter, as in hyperpituitarism, however, not all of the hormones are necessarily involved, so that the clinical manifestations of hypopituitarism vary greatly according to which of the hormones are being secreted insufficiently.

As previously stated, the clinical manifestations and laboratory findings characteristic of hyperpituitarism are frequently followed by those of hypopituitarism as the result of atrophy or other changes in the eosinophilic or basophilic cells of the anterior lobe. Otherwise, however, hypopituitarism may be primary and due to many causes among which may be mentioned the influence of heredity, congenital abnormalities, infectious diseases during childhood, congenital or acquired syphilis, trauma and hemorrhage, neoplasms, embolism, etc. Among the principal diseases are dwarfism of the Lorain-Levi type, infantilism, mongolism.

adiposogenital dystrophy (Fröhlich's syndrome) and Simmonds' disease (pituitary cachexia).

DWARFISM AND INFANTILISM

Dwarfism is a failure of somatic and sexual development in which the individual past the age of early childhood presents characteristics normally found in one much younger. The Lorain-Levi type is characterized by shortness of stature combined with sexual retardation, small head, hands and feet, delay in the closure of epiphyseal lines, and frequently a premature senile appearance (geroderma). It may be due not only to a deficiency in the secretion of the growth hormone of the anterior lobe of the pituitary gland (pituitary dwarfism) but to a deficiency in the secretion of the thyrotropic or gonadotropic hormones resulting in the thyroid and gonadal types of the disease. Furthermore, dwarfism may follow deficiencies in the hormones concerned in growth and development thought to be secreted by other glands. As a group they constitute the "endocrine dwarfisms." When the hypopituitarism occurs in later life, however, after normal stature and development have been attained, the clinical manifestations are likely to be obesity of the girdle-mammary-mons type with menstrual disorders and frigidity in the female and impotence in the male with loss of libido.

Infantilism may be due also to dyscrinism resulting from the inheritance of what may be called "primary endocrine weakness." However, many cases are thought to be due to intra-uterine damage of those organs of the fetus which are acted upon by the endocrine secretions by reason of syphilis, alcoholism, tuberculosis or other diseases of the parents; also by such factors after birth as severe malnutrition or traumatic brain diseases of the child.

Laboratory Examinations. As a general rule, the diagnosis of dwarfism and infantilism is readily made on the basis of clinical manifestations. As far as hypopituitarism is concerned, the results of laboratory examinations may be as follows:

1. The basal metabolic rate is usually reduced and the specific dynamic action of protein may be low.
2. There is a tendency to increased glucose tolerance and fasting hypoglycemia with increased insulin sensitivity.
3. A tendency to hypochloremia with an increase of urinary chloride is of frequent occurrence.
4. Plasma cholesterol is usually normal or slightly decreased.

ADIPOSOGENITAL DYSTROPHY

Adiposogenital dystrophy or Fröhlich's syndrome is primarily a disease of anterior hypopituitarism usually associated with dysfunction on the part of the pars intermedia and the neighboring structures of the diencephalon. Heredity probably plays a part in etiology. Injury of the pituitary gland by infection, trauma or hemorrhage may be responsible as likewise atrophy of the anterior lobe resulting from pressure from without by hemorrhages, cysts, tumors or gummas. The most common lesion is a benign chromophobe adenoma producing pressure and atrophy and especially of the basophilic cells with a reduction of their secretions.

The clinical manifestations vary considerably according to the age at which the disease develops. Obesity, due to disturbances in fat, carbohydrate and water metabolism and sparing the forearms and lower legs, is the most characteristic change, leading to the development of the feminine contour in males. The individual may remain short in stature but the majority of patients are of normal or even considerable height. Underdevelopment of the internal and external genital organs and secondary sexual characteristics is the second salient feature of the disease and especially when it begins in infancy or childhood. The onset of menstruation is delayed and among adults of both sexes libido is diminished or absent. Children are usually mentally alert but during adolescence and adult life both sexes are likely to develop drowsiness, mental and physical sluggishness, inferiority complexes and seclusiveness.

Laboratory Examinations. Laboratory examinations usually show (1) a reduced metabolic rate due to a deficiency in the thyrotropic hormone but in some cases it is within normal. If the rate is materially decreased (2) the plasma cholesterol is usually below normal along with a reduction of the specific dynamic action of protein. (3) A rather characteristic change is a considerable increase of glucose tolerance responsible for craving for sweets and rich foods. (4) Fasting hypoglycemia is of frequent occurrence and an increase of blood uric acid may be observed. (5) Secondary hypochromic normocytic anemia with slight to moderate lymphocytosis or eosinophilia may be observed.

SIMMONDS' DISEASE

Simmonds' disease or pituitary cachexia is characterized by extreme general debility and weakness, marked loss of weight often progressing to cachectic emaciation, loss of sexual function and sterility and a low basal metabolic rate. The great majority of cases occur among women, with the highest incidence between 40 to 60 years of age. Not infrequently these manifestations are preceded by pituitary obesity and premature senility.

The disease is due to progressive atrophy of the anterior lobe of the pituitary gland. This may be the result of embolism, thrombosis or infarction of the lobe following childbirth, chronic inflammation due to syphilis or tuberculosis or to trauma, neoplasms, cysts, etc.

The most difficult problem is differentiation from *anorexia nervosa.* The occurrence of severe weight loss, a low basal metabolic rate and pronounced psychopathic disturbances in a young woman who has never been pregnant favors the diagnosis of the latter; indeed, a claim of cure in any case of true Simmonds' disease is open to question unless a pituitary tumor has been successfully removed.[5] On the other hand, the occurrence of severe weight loss, amenorrhea and a low basal metabolic rate in a woman over 30 years of age whose symptoms date from a postpartum hemorrhage and collapse, or whose sella turcica is deformed or shows calcification, and who in the course of the disease loses axillary and pubic hair, warrants a diagnosis of Simmonds' disease.[5]

Laboratory Examinations. It is usually possible readily to differentiate Simmonds' disease clinically not only from Addison's disease but also from the cachexias due to tuberculosis, malignant disease and thyrotoxicosis. Laboratory examinations, however, are of helpful value and in Simmonds' disease the characteristic changes may be summarized as follows:

1. *A marked reduction in the basal metabolic rate.*
2. The specific dynamic action of protein, however, is usually normal but may be slightly reduced.
3. Fasting hypoglycemia with increased glucose tolerance is usual. Glycosuria may occur because of a low renal threshold for glucose.
4. Hypochlorhydria or actual achylia may be observed.
5. Moderate to severe anemia is usually present and may lead to the erroneous diagnosis of pernicious anemia. The total leukocytes are generally within normal limits but eosinophilia and lymphocytosis frequently occur.
6. Marked retention of sodium chloride and of water following oral administration has been reported.[6]

HYPEROVARISM

Hyperovarism is a disorder ascribable to the excessive secretion of estrin by the graafian follicles and corpora lutea of the ovaries. When occurring in childhood, it is characterized by precocious development of the primary and secondary sex characteristics with the early onset of menstruation. Among adults it is usually characterized by increased libido and fecundity and sometimes nymphomania. Excessive menstruation with menorrhagia or metrorrhagia may also occur, in which case changes in the uterine mucosa are commonly observed characterized by the presence of dilated and irregularly shaped glands with proliferative changes. In both children and adults an excess of estrin is commonly found in the blood with its consequent overexcretion in the urine and feces. In older girls and adults, however, premenstrual tension with or without pelvic pain and pronounced psychologic disturbances may occur instead of excessive menstruation; under these circumstances, an excess of estrin is found in the blood with but little or no excretion of it in the urine.

Since estrin is secreted by the ovaries under stimulation by prolan A of the anterior lobe of the pituitary gland, hyperovarism is commonly due to anterior hyperpituitarism resulting in the excessive secretion of this gonadotropic hormone. Heredity may also play a rôle in the etiology of hyperovarism. Inflammations, follicular cysts, and granulosa cells tumors on the ovaries may be contributing factors, but apparently hyperovarism does not occur as the result of changes in the ovaries alone. It is also thought that destructive lesions of the pineal gland may be responsible in some cases due to the loss of a hormone believed to have an inhibitory effect on the secretion of estrin. Adenomas of the adrenal cortex causing the excessive excretion of cortical hormones are also believed to produce hyperovarism.

Laboratory Examinations. Hyperovarism may be recognized in children from the clinical manifestations alone but in the adult the diagnosis is not usually justified without the corroborative evidence of laboratory examinations. The early development of secondary sex characteristics is usual in disturbances of the adrenal cortex. In the latter, however, the patient usually shows evidences of masculinization, due to the excessive secretion of the male sex hormones, along with an overgrowth of hair involving the entire body instead of being mainly confined to the pubic and axillary regions as in hyperovarism. The main laboratory changes may be summarized as follows:

1. The estrin content of the urine is increased and may reach more than 150 mouse units per 1000 cc. along with an increase of estrin above normal in the blood. In cases characterized by premenstrual tension, however, little or no estrin is found in the urine but blood estrin is likely to be increased 10 to 100 times. Normally, an adult should excrete 1200 to 1500 mouse units of estrin in the urine during the month but in hyperovarism it may reach a total of 4000 to 10,000 units, with especially marked excretion during the menstrual cycle when the excretion should be almost nil, except in cases characterized by premenstrual tension.
2. Endometrial biopsy examinations are of great value in differentiating menorrhagia and metrorrhagia due to hyperovarism from other causes for these conditions.

Placental Hyperhormonism. As stated in Chapter 20, the placenta apparently produces or contains two hormones, estrone (theelin) and estriol (theelol). They probably constitute a single hormone differing from prolan A and prolan B of the pituitary gland called the "anterior-pituitary-like" hormone (A.P.L.). According to Joslin and his colleagues,[7] an increase of this hormone in the blood after the fifth month of pregnancy accurately forecasts the accidents of pregnancy, namely, premature delivery, toxemia, stillbirth and neonatal mortality. In their experience the administration of estrin and progestin has controlled the rise in serum placental hormone in diabetic women, with the result that fetal mortality has been reduced from 32 to 6 per cent which is near the level of fetal mortality in nondiabetic pregnant women (3.4 per cent).

HYPO-OVARISM

Hypo-ovarism is a disorder due to greatly reduced or absent secretion of estrin by the graafian follicles and corpora lutea of the ovaries. It may occur at any age and be primary before puberty or it may be secondary during sexual life and after the menopause. It is one of the most common disorders of the endocrine glands; most cases come under observation during the childbearing age and at the menopause.

Primary hypo-ovarism, the type occurring before puberty, may be due to disorders of the anterior lobe of the pituitary gland producing a deficiency in the secretion of prolan A and consequently resulting in a deficiency in the secretion of estrin. Heredity may be a factor, as a mother with any endocrinopathy may transmit an ovarian deficiency. Ovaritis due to mumps during childhood may be responsible. Vitamin deficiencies, with special reference to vitamins A and E, are also thought to be causes in some cases. As the girl approaches adolescence a retardation or failure in sexual development is observed. The individual is usually thin and slender with eunuchoid measurements, undernourished and anemic with immature breasts and immature internal and external genital organs along with scanty pubic and axillary hair. There may be, however, a tendency to pituitary obesity with unduly large breasts. Menstruation is delayed and both irregular and scanty after it begins. Libido fails to develop or is only rudimentary.

Secondary hypo-ovarism, the type acquired after normal puberty, is of course caused by complete bilateral ovariectomy producing artificial menopause in which case it is permanent unless relieved by the administration of the ovarian hormones. It may be due also to destructive lesions of the anterior lobe of the pituitary gland resulting in a deficiency in the secretion of prolan A and prolan B; also to hyperfunction of the adrenal cortex or hypothyroidism. Secondary hypo-ovarism may be also the result of local conditions in the pelvis, such as ovarian cysts, cellulitis or uterine neoplasms. However, temporary hypo-ovarism may be caused by emotional disturbances, the fear of pregnancy or an intense desire for it. The individual shows no abnormalities in sexual development. The clinical manifestations are those of the menopause. Menstruation is irregular and scanty or absent. In the presence of complete bilateral ovariectomy pregnancy, of course, cannot occur; otherwise, however, pregnancy may occur but is much less likely. Furthermore, in secondary hypo-ovarism there is an increased tendency to abortions due to a deficiency in the secretion of progestin (progesterone) secreted by the corpora lutea. These manifestations may be only temporary or extend over many years until the menopause occurs as a physiologic change. Indeed, hypo-ovarism may occur without clinical manifestations, in which case its presence is detected only by laboratory examinations revealing a decrease of estrin in the blood or urine.

Laboratory Examinations. The diagnosis of hypo-ovarism is usually readily made according to the clinical manifestations. In view of its numerous causes, however, laboratory examinations are of value in relation to establishing etiology and may be summarized as follows:

1. In all cases there is usually a deficiency of estrin in the blood and urine. If menstruation occurs the normal increase during the preceding week does not occur.
2. When due to anterior hypopituitarism a deficiency in prolan A and prolan B in the blood and urine is also generally observed in addition to a deficiency of estrin in the blood and urine and of progestin in the urine, the latter being estimated in terms of pregnandiol (sodium pregnandiol glucuronidate).
3. If not due to anterior pituitary deficiency but to disease of the ovaries themselves, or their removal, estrin in the blood and urine is likewise decreased or absent but prolan A is not only present in normal amounts in the blood and urine, but frequently increased. Under these conditions, a marked deficiency in the secretion of estrin is also indicated when vaginal smears show most of the cells to be leukocytes with only a few epithelial cells, none of which are cornified. A moderate number of leukocytes and of nucleated oval epithelial cells, with a moderate number of irregularly shaped large epithelial cells containing very small faintly staining nuclei with considerable amounts of mucus, constitutes a moderately positive result indicative of a decrease of estrin secretion. If the smear

shows almost exclusively large irregularly shaped nonnucleated epithelial cells with very little mucus, the secretion of estrin is usually regarded as being adequate.

4. Biopsy examinations of the uterine mucosa are also helpful since in prolonged and pronounced hypo-ovarism the endometrium is usually fibrotic with few and inefficient glands.

5. A reduction in the basal metabolic rate may be observed due to hypothyroidism. The associated hypo-ovarism may indicate failure of direct stimulation of the ovaries because of a deficiency in thyroxin or indirectly due to a lack of secretion of the thyrotropic hormone by the anterior lobe of the pituitary gland.

HYPERORCHIDISM

Hyperorchidism is due to the excessive secretion of the male sex hormone or testosterone by the Leydig cells of the testes. In children heredity may be a factor in etiology as, likewise, basophilic adenoma of the anterior lobe of the pituitary gland, resulting in the excessive secretion of the gonadotropic hormone. Destructive tumors or other lesions of the pineal gland may also be a cause, probably because of a deficient secretion of a hormone normally inhibiting testosterone. For the same reason premature involution of the thymus gland may be a factor in etiology. Among adults a neoplasm, especially carcinoma, of the adrenal cortex is the most frequent cause. Tumors of the testes involving the Leydig cells and resulting in the excessive secretion of testosterone may also produce the disorder.

In children hyperorchidism is characterized by precocious puberty with excessive as well as premature development of the testicles, penis, prostate gland and secondary male characteristics. Skeletal growth is rapid until checked by early closure of the epiphyses. Premature dentition may occur with marked malocclusion. The face is sometimes of the mongolian type but mentality is not necessarily below normal.

In adults the disorder is usually characterized by hypertrophy of the testes and scrotum, exceptional muscularity, deep voice, heavy beard and excessive libido.

Laboratory Examinations. Laboratory examinations are not ordinarily required as aids in diagnosis. The urine, however, may show the presence of excessive amounts of the male sex hormone occurring as androsterone and dehydroandrosterone which, together, constitute the androgens. In case of testicular tumors, the urine may also show an excess of the pituitary gonadotropic hormone, reaching from 50 to 50,000 international units per 1000 cc. depending upon their nature and size. Differential diagnosis of adrenal cortex tumors may be difficult because of hirsutism and precocious sexual development with virilism, while the urine is apt to contain large amounts of estrin.

HYPO-ORCHIDISM

Hypo-orchidism is due to a deficiency in the secretion of the male sex hormone (testosterone) by the Leydig cells of the testes. The clinical manifestations are most marked in eunuchism, eunuchoidism and cryptorchidism occurring before puberty but signs and symptoms of hypo-orchidism also occur when eunuchism and eunuchoidism are acquired after puberty, and in many adults over fifty years of age undergoing the male climacteric.

Eunuchism is a condition characterized by the complete absence of testicular activity in the male. It may be due to congenital absence of the testicles, a condition which is of doubtful occurrence, or complete atrophy of both testicles, which is probably extremely rare.

When occurring before puberty, it is manifested by failure in the development of the secondary sex characteristics, changes in anthropometric measurements, hypotrichosis and impotence. When occurring in the adult male it is manifested by some regression of the sex characteristics, reduction of energy and endurance, and marked nervous and psychic symptoms.

Eunuchoidism is far more common and is due to a deficiency in testicular activity of males. It may occur at any age and the earlier it begins the more marked are the clinical manifestations. Heredity may be an important predisposing factor. Direct causes may be severe bilateral orchitis following mumps or other infections, excessive exposure to roentgen rays, partial castrations and destructive tumors like sarcomas, carcinomas or teratomas. Among the most frequent causes are deficiency in the secretion of the gonadotropic hormone by the anterior lobe of the pituitary gland, dysfunctions of the thyroid gland and adrenal cortex, a persistent thymus gland and hypo-insulinism.

The clinical manifestations are characterized by changes in anthropometric measurements and lack of definition of the secondary sex characteristics, with a tendency to simulate the female in body contour as well as psychic characteristics.

Cryptorchidism or undescended testicles is more common than generally surmised. In about 80 per cent of cases it is unilateral and in about 20 per cent bilateral. The two types of cryptorchidism are: (1) those in which mechanical obstruction prevents the passage of the testicles into the scrotum; and (2) those in which the endocrine secretions fail to provide a sufficient impetus for the mechanical factors to bring about normal descent.

Of course, the principal clinical finding is an absence of one or both testicles in the scrotum. The latter is usually undersized and thickened, the penis is small, and in the case of adults the prostate gland and seminal vesicles are likely to be underdeveloped. Generally there are also signs and symptoms of hypo-orchidism, especially in bilateral cryptorchidism.

It is now generally believed that many, if not all, men pass through a *male climacteric period,* somewhat similar to that of women, usually in a less severe but more prolonged form, characterized by a progressive deficiency in testicular activity. The age of onset is much less definite than in the case of women, but early manifestations are usually apparent at about fifty years of age. It is usually characterized by a decrease in potency and libido, but may be accompanied by marked changes in the nervous system due to an imbalance between the two divisions of the autonomic nervous system, psychic disturbances which may amount to involutional melancholia, and even circulatory and general disturbances similar to those observed in women passing through the climacteric following the cessation of menstruation.

Laboratory Examinations. Laboratory examinations are not generally required as aids in the diagnosis of these disorders of hypo-orchidism, but assays of the androgens and estrin excreted in the urine are frequently helpful. In this connection it is to be remembered, however, that their excretion in the urine varies considerably from day to day under normal conditions.[8] Consequently, only assays conducted over considerable periods of time possess diagnostic value. The androgens are excreted normally in the urine of both boys and girls[9] but very little estrin is excreted by children before 10 to 11 years of age.[10, 11] The average excretion of the androgens in the total daily urine of a normal adult male *averages* about 66 international units per day; the *average* excretion of estrin is about 100 international units equivalent to about 10 gammas of theelin.

1. In *eunuchism* the urine is free of both the androgens and estrin.
2. In *eunuchoidism* the excretion of the androgens in the urine is about one-third and of estrin about one-fifth of normal.[8] If failure of excretion of the gonadotropic hormone of the anterior lobe of the pituitary gland is involved in the etiology of eunuchoidism the urine also shows a deficiency in prolan.
3. In pronounced cases of the male climacteric and in bilateral cryptorchidism the androgens in the urine are likewise usually reduced below normal. In about 50 per cent of cases of bilateral cryptorchidism the prolan of the urine is increased, indicative of insufficiency in the secretion of testosterone by the testicles with compensatory overactivity in the secretion of the gonadotropic hormone by the anterior lobe of the pituitary gland. In the remaining 50 per cent of cases, however, an excess of prolan in the urine is not observed because of a deficiency on the part of the pituitary gland, or because the testicles are able to respond to stimulation by it in spite of their position.
4. In eunuchism and eunuchoidism the basal metabolic rate may be reduced to −20 or lower, and glucose tolerance may be lowered.
5. In eunuchoidism and the male climacteric an examination of the seminal fluid immediately after ejaculation frequently fails to show live and motile spermatozoa, indicating deficiency of spermatogenesis by the testicles.

HYPERADRENALINISM

Hyperadrenalinism is usually attributable to excessive secretion of cortin by the cortex of the adrenal glands resulting in cortical hyperadrenalism. Changes in sexual development are so constant that the resulting disorders (pseudohermaphrodism, pubertas praecox and virilism) are classified under the designation of the *adrenogenital syndromes*. Hyperadrenalinism due to the excessive secretion of adrenalin by the medulla occurs less frequently.

Pseudohermaphrodism is a congenital type of cortical hyperadrenalinism characterized by the presence of partially developed testicles, a feminine external appearance, a precocious development of the genital organs, and a tendency to gonadal tumors.

Pubertas praecox, prepubertal and adolescent cortical hyperadrenalinism, is usually due to hyperplasia or tumors of the cortex. Clinically, it is characterized by rapid growth of the genitalia with emphasis on male characteristics, hirsutism and obesity. This disorder affects girls about five times more frequently than boys.

Virilism or cortical hyperadrenalinism of adults may accompany benign tumors, which later become carcinomas, but is frequently functional, with no structural changes, as evidenced by the fuchsinophil reaction of the cells. It affects both sexes in about equal degree. In women it is characterized by the masculinization of the secondary sex characteristics with hirsutism and obesity. Sexual desire is usually lost, and sometimes there is even a transfer to homosexuality. In the Achard-Thiers type of the disorder, diabetes is frequently associated with the syndrome, while in other cases there may be complete loss of subcutaneous fat, without hirsutism or sexual change. Males also show pronounced hypertrichosis and occasionally develop the secondary sex characteristics of the female.

Medullary hyperadrenalinism is usually due to highly malignant neuroblastomas (sarcomas) affecting children. It is less frequently associated with benign ganglioneuromas affecting children and adults and chromaffinomas of adults, which involve the medulla and result in the excessive secretion of adrenalin. It is usually characterized by the signs and symptoms of atypical hyperthyroidism, but may also produce primary or essential hypertension as an important manifestation.

Laboratory Examinations. Laboratory examinations are frequently of aid in the diagnosis of pseudohermaphrodism, pubertas praecox, and virilism; the usual findings may be summarized as follows:

1. An increase of the excretion products of testosterone (the androgens) along with increased urinary 17-ketosteroids and an increase of estrin in the urine in both sexes and variable pregnandiol excretion.[8,12]
2. Blood chemistry changes may be just the opposite of those observed in hypo-adrenalinism (Addison's disease), characterized by an increase of serum sodium and bicarbonate, with a decrease of serum potassium and sodium chloride along with salt and water retention.[13]
3. The total plasma cholesterol may be increased.
4. In medullary hyperadrenalinism there is a tendency toward an increase of the basal metabolic rate, fasting hyperglycemia, decreased sugar tolerance and glycosuria.

HYPO-ADRENALINISM AND ADDISON'S DISEASE

Hypo-adrenalinism or hypo-adremia occurs in two clinical types: (1) the functional and (2) the organic or Addison's disease.

Functional hypo-adrenalinism is characterized by the absence of detectable lesions but apparently involves chiefly the medulla with a temporary or prolonged decrease in the secretion of adrenalin. It is of very frequent occurrence and is commonly observed not only in fright, grief, shock, and allergic diseases but in many of the infectious diseases, especially influenza, pneumonia, diphtheria, scarlet fever, chronic focal infections, tuberculosis, and rheumatoid arthritis, as well as in intoxications of nonbacterial origin due to burns, dehydration, etc. Weakness and exhaustion, easy fatigability, anorexia, loss of weight, and tachycardia are among the usual manifestations, including the syndrome known as "neurocirculatory asthenia," the "exhaustion syndrome" or "soldier's heart."

Addison's disease, or organic hypo-adrenalinism, is usually due to tuberculosis, syphilis, or neoplasms as well as fibroid atrophy, amyloidosis, hemorrhage and infarctions mainly involving the cortex of the adrenal glands. A deficiency in the secretion of both cortin and adrenalin occurs. Males are affected about three times more frequently than females; most cases develop around 40 years of age.

The disease is characterized by a peculiar and characteristic pigmentation of the skin and mucous membranes, extreme asthenia, hypotension, subnormal temperature, anorexia, nausea, vomiting, vertigo and attacks of syncope, insomnia, amenorrhea in women and testicular atrophy in men, with a tendency to impotency and loss of libido.

Laboratory Examinations. Laboratory examinations are of great value in the diagnosis of Addison's disease, and the usual changes may be summarized as follows:

1. A characteristic reduction of plasma chloride below the normal of 570 to 620 mg. per 100 cc. with an increase of chloride in the urine. In suspected cases the following test by Cutler, Power and Wilder [14] is of diagnostic value, although it should always be conducted in a hospital because critical crises may occur: (a) The diet should provide no more than 0.95 gm. chloride and 4.1 gm. potassium per day; if adrenal cortex is being administered it should be stopped for a day prior to the test; (b) on the afternoon of the first day and the morning of the second, the patient is given orally 42 mg. of potassium citrate per pound of body weight with generous amounts of water; (c) on the second day the fluid intake is limited to 40 cc. per kilogram; (d) on the third day it is 20 cc. per kilogram before 11 A.M.; (e) urine is collected from 8 P.M. of the second day to 8 A.M. of the third day and from 8 A.M. to 12 noon of the third day. All specimens are sent to the laboratory for quantitative chloride determinations. Normally the total chloride in the various specimens varies from 17 to 141 mg. per 100 cc. with an average of 54 mg. In Addison's disease the total chloride is likely to be as high as 229 to 356 mg. per 100 cc. or more with an average of about 290 mg.

2. There is a tendency to an increase of serum potassium above the normal of 16 to 22 mg. per 100 cc. with a decrease of potassium in the urine.

3. The serum sodium is usually decreased below the normal of 315 to 340 mg. per 100 cc.

4. The total nonprotein nitrogen of the blood is frequently increased above the normal of 25 to 40 mg. per 100 cc. due to renal failure; albumin and casts may occur in the urine.

5. Fasting hypoglycemia occurs in about 60 to 70 per cent of cases along with increased glucose tolerance and increased insulin sensitivity.

6. A decrease in the blood eosinophils of 50 per cent or more of the initial level following the intramuscular injection of 25 mg. of purified adreno-cortico-

tropic hormone is stated to indicate a satisfactory adrenal response, but if this does not occur a complete absence of adrenal cortical reserve may be present.[15]

7. Increased urinary sodium and chloride with decreased urinary potassium.
8. Decreased 17-ketosteroid excretion.
9. The basal metabolic rate is frequently reduced below normal.
10. The total plasma proteins are sometimes increased above the normal of 6.4 to 8.0 gm. per 100 cc. due to dehydration and hemoconcentration.
11. Slight to moderate normocytic anemia is the rule; simple microcytic anemia may occur, but macrocytic anemia is rare.

HYPERTHYROIDISM; THE TOXIC GOITERS

Hyperthyroidism is due to the excessive secretion of thyroxin, resulting from hyperplasia of the acinar cells of the thyroid gland. Thyroxin contains about 65 per cent of iodine and an amino group. It is believed to act as a catalyst to increase the oxidative processes of the tissues. Under normal conditions about 0.33 mg. are secreted per day, but about 0.5 mg. must be administered daily to maintain an approximately normal metabolism in the thyroidless adult.

The excessive secretion of thyroxin increases tissue oxidation or metabolism with the production of "toxic" signs and symptoms of disease. Clinically these diseases are grouped together under the designation of the "toxic goiters" embracing (1) Graves' disease or diffuse exophthalmic goiter; (2) nodular exophthalmic goiter in which nodules develop in the thyroid gland as the result of previous cycles of hyperplasia and involution; (3) simple diffuse toxic goiter in which a colloid goiter develops into the toxic type; (4) toxic adenoma of the thyroid gland and (5) various hyperthyroid states in which goiter is not detectable.

As far as general signs and symptoms are concerned, hyperthyroidism is generally characterized by loss of weight in spite of a good appetite and the high intake of food, loss of strength, hypersensitiveness to heat, flushing, sweating, persistent tachycardia, tremulousness, irritability, restlessness and emotionalism. On the other hand, the patient may be apathetic, with relatively low pulse and basal metabolic rates giving a particularly grave prognosis.[16] Furthermore, since the basal metabolic rate may be increased in many diseases as discussed in Chapter 6, hyperthyroidism may be masked by functional or organic heart disease (auricullar fibrillation, paroxysmal tachycardia, transient auricullar flutter, angina pectoris, congestive heart disease) as well as by abdominal conditions such as appendicitis, cholecystitis and even spastic colitis.[17] Imbalance of the autonomic nervous system, including neurocirculatory asthenia, characterized clinically by sweating, tremor, flushing, brilliance of the eyes, palpitation, dyspnea, precordial discomfort, faintness, vertigo, insomnia, etc., presents one of the greatest difficulties in differentiation from hyperthyroidism although there is usually no increase of the basal metabolic rate and persistent tachycardia does not occur. Indeed, it may be impossible at times to decide between early hyperthyroidism and essential hypertension, since the basal metabolic rate may vary from +17 to as high as +40 in the latter disease.[18] Even in advanced tuberculosis the basal metabolic rate may be as high as +30 although it is normal in early cases.[19] Furthermore, while hyperthyroidism may develop during the menopause it is frequently difficult to differentiate it from vasomotor symptoms of the menopausal state without hyperthyroidism.[20]

Laboratory Examinations. 1. From the standpoint of laboratory examinations the most characteristic and constant change in hyperthyroidism is an increase of the basal metabolic rate. In this connection it is important to remember, however, that hyperthyroidism may exist without the rate being higher than the upper limit of normal (+15). Thus an individual with a normal rate of −5 or −10 may show only +5 to +10 upon the development of hyperthyroidism; these values are still within the normal range but are increased for the individual concerned. Consequently, physicians should not necessarily exclude hyperthyroidism simply because even repeated basal metabolic tests under acceptable conditions give rates falling within the normal range.

2. A second fairly constant change is an increase in blood iodine.[21–23] Normally, the total blood iodine (organic and inorganic) will average about 8 to 16 micrograms (average 12) per 100 cc. but in hyperthyroidism, even when no iodine has been administered, the values are usually from 16 to 21 micrograms or higher, although lower values are usually observed in chronic cases.

3. Under the conditions, there is also an increased excretion of iodine in the urine (normally 36 to 78 micrograms per day) as w ll as in the feces [24] indicating that in hyperthyroidism thyroxin is drained out of the thyroid gland despite its increased production. The urinary excretion of radioactive iodine, however, is reduced.[25]

4. The iodine tolerance test (see Chapter 6) usually gives a "flat" type of curve, indicating that in hyperthyroidism there is an undersaturation of iodine in the thyroid gland and tissues.[26]

5. Furthermore, while the plasma cholesterol and total lipoids are usually increased above normal in hypothyroidism, they are generally decreased in hyperthyroidism.[27]

6. The liver is frequently damaged, the changes varying from simple acute fatty degeneration and necrotic lesions to a chronic form of atrophy and cirrhosis. As a result, glucose and especially galactose tolerance tests, as well as the cinchophen oxidation and hippuric acid synthesis tests, are stated to show functional impairment (usually of slight to moderate degree) in 45 to 95 per cent of cases.[28–30] It has been suggested that liver injury may be due to a depletion of glycogen in hyperthyroidism which renders the liver more susceptible to damage by bacterial toxins, endogenous metabolic products and thyroxin.[29] There is a tendency toward fasting hyperglycemia, and glycosuria may occur with decreased glucose tolerance.

7. Creatinuria is commonly observed [31] although it disappears on the administration of iodine.[32]

8. However, while the creatinine tolerance test has been stated to show decreased retention in about 90 per cent of cases of hyperthyroidism,[33] similar results have been found to occur so frequently in autonomic imbalance, menopause,

hypertension and nontoxic goiters that the test has failed to provide an additional aid in the laboratory diagnosis of Graves' disease.[34]

9. For unknown reasons the excretion of calcium and phosphorus in the urine is higher in hyperthyroidism than in any other disease;[35] this may be accompanied by an increase of serum alkaline phosphatase.[36]

10. Hypochlorhydria or achlorhydria may be observed.

HYPOTHYROIDISM; CRETINISM AND MYXEDEMA

Hypothyroidism is due to a deficiency in the secretion of thyroxin by the thyroid gland. As a result, tissue oxidation or metabolism is reduced, with the production of signs, symptoms and laboratory findings just the opposite to those observed in hyperthyroidism.

Cretinism is the result of thyroid deficiency in infants and young children. It is due to a congenital defect in the functional development of the gland, as a result of which it is hypoplastic and atrophic. However, in most cases the gland is of normal size, or even enlarged, with histologic evidences of compensatory hyperplasia or the acinar cells. This is probably the result of an effort to maintain growth during intra-uterine life in the presence of an iodine deficiency on the part of the mother. But after birth the deficient gland is unable to secrete an adequate amount of physiologically active thyroxin. The typical clinical manifestations are: (1) retarded and abnormal skeletal growth; (2) arrested sexual development; (3) mental deficiency often amounting to idiocy; (4) sometimes deaf mutism; (5) coarse "pig-like" facies; (6) failure in the normal ossification of the bones, and (7) a basal metabolic rate reduced from 20 to 40 per cent or more below the normal. The disease occurs most commonly in districts where colloid goiter is particularly prevalent, but some of the worst cases occur in children born of nongoitrous parents, this being due to prenatal or early postnatal atrophy of the thyroid gland, its congenital absence, or its destruction by thyroiditis.

Myxedema, or Gull's disease, is caused by acquired thyroid deficiency in older children or adults resulting from atrophy or destruction of the gland, or resulting from an operation in which too much of the gland has been removed (operative myxedema or cachexia strumipriva). The chief characteristics are (1) a low basal metabolic rate (—20 to —40 or more); (2) a thick, puffy appearance of the skin with dry sparse hair; (3) a mongoloid facies; and (4) apathy, lethargy and slow cerebration, although general intelligence is usually within normal. While the edemalike appearance of the skin was formerly thought to be due to the accumulation of mucin in the subcutaneous tissues it is now known to be due to the deposition of a semi-fluid albuminous substance containing over 13 per cent of protein and representing an increase in the normal quantity of stored or deposited protein.[37]

Acquired hypothyroidism, however, may occur without myxedema in the form of *hypothyroid states* caused by an inadequate intake or utilization of iodine, subtotal thyroidectomy, thyroiditis, or deficiency in secretion of the thyrotropic hormone by the anterior lobe of the pituitary gland. It may also occur as a *thyroid exhaustion state* since it has been estimated that more than 50 per cent of all cases of hyperthyroidism, unless adequately treated, change into hypothyroidism because of degenerative changes in the gland. The clinical manifestations, in addition to a reduction in the basal metabolic rate, are quite diverse but likely to include easy fatigability, increased sensitivity to cold, various skin disorders, a neurasthenic state, constipation, "rheumatic" or vague abdominal pains, especially in the lower right quadrant, changes in weight, retardation of osseous development in the case of children, enuresis, menorrhagia or other menstrual disturbances, sterility in the case of men, etc. As a general rule, however, hypothyroidism does not occur in diffuse or nodular colloid goiters or nontoxic adenomas, although the basal metabolic rate is sometimes slightly reduced below −15.

Laboratory Examinations. Cretinism and well-marked myxedema are generally recognized by the clinical manifestations, but laboratory examinations possess great value in confirming diagnosis and are particularly valuable in the detection of other hypothyroid states and thyroid exhaustion. Of course, the changes vary according to the severity of the hypothyroidism, but may be summarized as follows:

1. A basal metabolic rate ranging from −5 to −45, the latter being generally indicative of athyreosis or the complete absence of thyroxin secretion.
2. A decrease of blood iodine.
3. An increased excretion of iodine in the urine following its administration, due to decreased iodine tolerance which indicates that neither the atrophic thyroid gland nor other tissues can take up iodine as completely as in the normal state.[38]
4. Sometimes a slight increase in glucose tolerance with a tendency toward fasting hypoglycemia.[39]
5. Hypercholesterolemia occurs so constantly that plasma cholesterol determinations are not only of great value in diagnosis but of value in relation to treatment.[40,41]
6. In some cases there is also a tendency toward an increase in plasma neutral fat, total fatty acids, and phospholipid.[27]
7. Reduced urinary excretion of creatinine may be observed in children (not in adults) along with increased retention as demonstrated in the creatinine tolerance test.[42,43]
8. While the calcium and inorganic phosphorus of the serum are usually within the normal range in myxedema, yet a reduction in the urinary excretion of calcium and inorganic phosphorus has been reported.[44]
9. Decreased urinary 17-ketosteroids and carotinemia may occur.

HYPERPARATHYROIDISM

Hyperparathyroidism follows an excessive secretion of parathormone by one or more of the parathyroid glands, resulting in a more or less profound disturbance of calcium and phosphorus metabolism. It may be primary or secondary.

Primary hyperparathyroidism is almost invariably due to adenomas involving one or more of the parathyroid glands. The result is such an excessive secretion of parathormone as to lead to demineralization of the bones with the production of generalized osteitis fibrosa cystica or von Recklinghausen's disease.

Secondary hyperparathyroidism follows hyperplasia of the parathyroid glands with the possible overproduction of parathormone occurring in some cases of such diseases as severe rickets, osteomalacia, osteitis deformans, fragilitas ossium, multiple myeloma, metastatic carcinomas of bones and nephrolithiasis. Taken as a group, it appears that about 60 per cent of all cases of parathyroid hyperplasia have bone lesions.[45] Indeed, the hyperplastic changes in the parathyroid glands are occasionally so pronounced that histologic differentiation from adenomas may be difficult or impossible. The cause of hyperparathyroidism under these conditions is unknown. It has been suggested that it may be compensatory in nature due to deficiencies in vitamin D or calcium as far as rickets and osteomalacia are concerned.[46,47] On the other hand, it may follow the excessive secretion of the parathyrotropic hormone, which is supposed (but not proved) to be secreted by the anterior lobe of the pituitary gland, resulting in hyperplasia of the parathyroid glands with the excessive secretion of parathormone.

In most cases the diagnosis of primary hyperparathyroidism may be confirmed or excluded by consideration of the history and clinical manifestations, roentgenologic examinations of the bones, determinations of the calcium, phosphorus and phosphatase of the blood serum and estimations of the urinary excretion of calcium and phosphorus. Differentiation of generalized osteitis fibrosa cystica from those diseases of bones producing secondary hyperparathyroidism is very important because surgical removal of parathyroid adenomas is usually followed by extraordinary improvement if carried out before advanced skeletal lesions or renal impairment prevents good recovery. Differential diagnosis, however, may be difficult and particularly in the presence of marked renal insufficiency, or when the disease is in its early stages, is only mildly progressive, or is in remission.[48]

Osteitis Fibrosa Cystica. As previously stated, the generalized type of this disease of hyperparathyroidism is due usually to adenomas involving one or more of the parathyroid glands. It affects women more frequently than men but may occur in children. Localized osteitis fibrosa cystica is much more common, is particularly apt to affect individuals under 20 years of age, and is characterized by solitary cysts occurring in the femur, humerus or tibia. Curiously, however, adenoma of the parathyroid glands is but rarely observed in this type of the disease.

The generalized form is characterized clinically by slow and insidious onset with (1) pain in the bones of the extremities or back, (2) hypertonicity and weakness of the muscles with disturbances in gait, (3) various gastro-intestinal disturbances, (4) deformities of the bones of the limbs or spine, (5) spontaneous fractures of the femur or humerus, (6) renal symptoms of polyuria and colic due to calculi composed of calcium, and (7) sometimes small palpable tumors in the neck (parathyroid glands). Tumors of the epulis type, and cysts, occur most frequently in the long bones but may also occur in the jaws and cranium.

Laboratory examinations in addition to the clinical and roentgenographic manifestations are extremely valuable in diagnosis and may be summarized as follows: (1) Hypercalcemia with an increase of total serum calcium above the normal of 9 to 11 mg. per 100 cc. in at least 80 per cent of cases; (2) an increase in serum alkaline phosphatase in practically all cases; [49] (3) a reduction in the inorganic phosphate of the serum below the normal of 2.0 to 5.0 mg. per 100 cc. in adults and (4) a marked increase in the excretion of calcium and phosphate in the urine. When renal impairment is marked there is likely to be an increase of the total nonprotein nitrogen of the blood along with hypochloremia and varying degrees of dehydration and hemoconcentration.

Osteitis Deformans. The etiology of osteitis deformans, or Paget's disease, is unknown although some authors have expressed the opinion that it may be due to hyperparathyroidism.[50] It is an extraordinary fact, however, that in spite of the extensive changes which may occur in the bones in this disease, the serum calcium and inorganic phosphorus remain within normal limits. Under the conditions, if changes occur in the parathyroid glands it would appear that they are a secondary rather than a primary cause of the disorder.

The disease usually affects males and begins, as a rule, after 40 years of age, sometimes with indefinite pains in the arms and legs which are worse at night, but more frequently with gradual enlargement of the head. The process is a rarefying osteitis involving the center of bones with new bone formation, especially in the subperiosteal bones. Enlargement and deformity may not only affect the skull but the tibiae, femora, pelvis, vertebrae and other bones. As a result there is progressive reduction in stature with progressive dorsocervical kyphosis and bowing of the legs. Marked arteriosclerosis sometimes occurs.

Diagnosis is made best by roentgenologic examinations of the bones. From the standpoint of *laboratory examinations* the only constant change is an increase of the phosphatase of the serum. This appears to be in relation to the severity and prognosis of the disease. Extensive involvement of the skull is liable to give the highest values.[49] In early cases, however, the rise in serum phosphatase may be insignificant but a definite rise, in conjunction with suggestive roentgenographic changes, is of great diagnostic value. According to Williams and Watson,[51] the hyperphosphatemia is due to an actual increase of the concentration of the phosphatase in the serum rather than in relation to the presence of the activator of the enzyme in the blood.

Multiple Myeloma. Cases of multiple myeloma with diffuse involvement of the bones, along with hypercalcemia, roentgenographic findings and slight enlargement of the parathyroid glands (rare), have led some investigators to postulate an associated state of secondary hyperparathyroidism in this disease. From the standpoint of *laboratory examinations* the most characteristic changes are (1) the occurrence of the Bence-Jones protein in the urine, and (2) hypercalcemia. The inorganic phosphorus of the serum is within normal limits except when renal insufficiency causes retention of phosphates. The serum alkaline phosphatase is usually within normal limits although increased values have been reported in a few cases.[52]

HYPOPARATHYROIDISM AND TETANY

Hypoparathyroidism results from a deficiency in parathormone. It is characterized by a reduction in the calcium of the serum and especially of the diffusible fraction. Consequently, the outstanding clinical manifestation is *tetany*.

The tetany of hypoparathyroidism may have various causes involving the parathyroid glands, but is usually due to the removal of one or more of the glands in subtotal parathyroidectomy, or thyroidectomy, or injury of them by hemorrhage, postoperative fibrosis, etc.; consequently, this type of tetany is usually designated *parathyroprival tetany* or tetania parathyropriva.

Tetany, however, may occur as an idiopathic disorder in infants, as the result of hyperventilation of the lungs, alkalosis or loss of gastric hydrochloric acid, as well as in sprue, celiac disease, pregnancy and lactation, osteomalacia and various infections and intoxications. It is generally accepted that the determining factor in all etiologic types of tetany is a deficiency of calcium in the plasma and extracellular fluids of the body. The deficiency is not always referable to a reduction of the total calcium of the serum but especially of the diffusible or ionic fraction. In nephritis, for example, the total calcium of the serum may be reduced to 3 or 4 mg. per 100 cc., yet tetany does not occur, presumably because the concentration of ionic calcium has not been reduced to the critical level. As a general rule, however, a reduction of total serum calcium from the normal of 9 to 11 mg. to 7 or 8 mg. per cent, results in *latent tetany* in which increased neuromuscular excitability can be elicited by galvanic or mechanical stimulation. When the total serum calcium is reduced to 5 or 6 mg. or less per cent, *manifest tetany* usually develops. The calcium concentration of the tissues themselves (muscles or brain) is not altered, the increased neuromuscular excitability being apparently due to an imbalance between the concentration of ionic calcium in the extracellular fluids and within the tissue cells.

The tetany of alkalosis, however, is difficult to explain on the basis of calcium deficiency, since both the diffusible and nondiffusible fractions are usually within normal limits. It has been suggested, however, that the shift of the acid-base equilibrium of the blood toward the alkaline side causes a reduction in the diffusible or ionic calcium without altering the total calcium of the serum. Moreover, it should be remembered that tetany is simply the clinical manifestation of neuromuscular excitability. It is conceivable that abnormal conditions other than calcium deficiency may alter the excitability of cells. In other words, it need not be assumed that all forms of tetany have a common cause.

Emotion, some undue strain, *e.g.*, pregnancy, lactation or a failure in general health, may precipitate an attack of manifest tetany in an individual with latent tetany. Certain clinical tests are employed to detect this incipient or latent form of the disorder: (1) *Chvostek's sign*—tapping over the facial nerve in front of the ear causes twitching or spasm of the facial muscles. (2) *Trousseau's sign*—obstruction of the circulation in the arm causes the hand to assume a typical position ("accoucheur's hand"). The effect is probably due to the anoxemia induced in the muscles of the hand and forearm. (3) *Erb's sign*—increased excitability of the muscles to the galvanic current already referred to.

Manifest tetany is characterized clinically by neuromuscular excitability or spasmophilia. Children are especially apt to show jerking and generalized convulsions. Spasms of the muscles of the hands and feet (carpo-pedal spasm) are usual. Spasms of the eye muscles may occur, while spasm of the larynx (laryngysmus stridulus) is particularly common in infants. Spasmodic retention of the urine is not uncommon. Rapid respiration and fever may be observed.

Laboratory Examinations. Laboratory examinations are of great value not only in the detection of latent tetany, but likewise in the etiologic diagnosis of the disorder. The usual changes may be summarized as follows:

1. Hypocalcemia, referable to the total serum calcium or its diffusible fraction according to the cause, as shown in Table 134.

TABLE 134. SUMMARY OF LABORATORY CHANGES ACCORDING TO THE CAUSES OF TETANY

Causes	Serum Calcium			Serum Phosphate	Bicarb. of blood	Plasma Chloride
	Total	Diffusible	Nondiffusible			
Hypoparathyroidism	R *	R	N or R	I	N	—
Infantile (idiopathic)	R	R	N	N or R	N	N
Hyperpneic	N	N	N	N	I	—
Alkalosis	N	N	N	N	I	R
Gastric	N	N	N	I	I	R
Sprue and celiac	R	R	N	N	N	—
Pregnancy	R	I	R	N	—	—
Osteomalacia	R	R	N	N or R	—	—

* N = normal; R = reduced; I = increased

2. Hyperphosphatemia may occur in tetany due to hypoparathyroidism and in gastric tetany. Hypophosphatemia may be observed in the idiopathic or infantile type of the disorder.

3. The bicarbonate of the plasma is increased in tetany because of hyperventilation, alkalosis and gastric tetany. This change is commonly associated with hypochloremia.

4. There is usually a decrease of calcium in the urine and feces.[53] The urinary excretion of phosphorus (inorganic phosphate) is also decreased in tetany and following subtotal thyroidectomy. It seems that thyrotoxicosis, followed by subtotal thyroidectomy, or subtotal thyroidectomy alone, brings about this fall, and that it does not depend on the parathyroid glands.[53]

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MISCELLANEOUS INFECTIOUS DISEASES

It has been estimated that from 40 to 50 per cent of all the diseases of man are definitely or presumptively due to infection. The infectious agents include about 56 different bacteria, 8 spirochetes, 12 rickettsiae, 38 filtrable viruses, 63 molds, yeasts, actinomyces and pathogenic fungi, 16 protozoa and 31 helminths. To these living agents of disease may be added about 51 arthropods, not only because they transmit various pathogenic organisms to man, but also because many play a direct rôle in the production of disease. Infection is determined, not merely by the fact that an infectious agent finds lodgment in or upon the body of a susceptible individual, but also by its ability to survive, multiply and produce injurious effects in the living and actively resisting host. Infection involves, therefore, two living entities: the *infectious agent* and the *host.*

Unfortunately, laboratory examinations are not available as aids in the diagnosis of all of the infectious diseases, but in many due to bacteria and spirochetes they are extremely helpful or pathognomonic, as discussed in Chapter 15; the same applies to many of the mycotic diseases, as discussed in Chapter 16, as well as to many diseases due to the protozoa and helminths, discussed in Chapter 12. Infectious mononucleosis has been considered in Chapter 22, the venereal diseases (gonorrhea, syphilis, chancroid, lymphogranuloma venereum, granuloma inguinale and venereal fusospirochetosis) in Chapter 24, and diphtheria, scarlet fever, Plaut-Vincent angina, pertussis, the pneumonias and pulmonary tuberculosis in Chapter 28. The purpose of this chapter is to summarize briefly the laboratory examinations of diagnostic value in various other infectious diseases although many of them have been discussed previously as, for example, serologic examinations, in Chapter 17, and skin tests in Chapter 19.

THE MENINGITIDES

Meningitis refers to an inflammation of the meninges which may be acute (including the fulminant or so-called malignant type), subacute, chronic or relapsing. It is sometimes limited to the cerebral meninges (*cerebral meningitis*), or to the spinal meninges (*spinal meningitis*), but in most instances of acute meningitis both are involved (*cerebrospinal meningitis*). In cerebral meningitis the inflammation may be confined to the meninges covering the base of the brain (*basilar meningitis*).

Infection with various bacteria and viruses is the usual cause, but on the other hand, the meninges are susceptible to irritation or inflammation caused by bacterial toxins in the blood, by lead, alcohol and other chemical agents, as well as by trauma. This constitutes *meningismus* or *serous meningitis* characterized by congestion of the meninges with edema and sterile cerebrospinal fluid. The meninges are also highly susceptible to irritation and inflammation by sterile fluids injected intrathecally, especially penicillin, streptomycin and serum, producing *aseptic meningitis.*

As a general rule, meningitis involves all three of the membranes, but in acute meningitis the pia and arachnoid are chiefly involved (*leptomeningitis*), while in chronic infections the dura mater may be principally involved (*pachymeningitis*). Not infrequently the brain itself shares the inflammatory processes (*meningoencephalitis*).

Anatomically and immunologically the meninges are peculiarly exposed and susceptible to infection. It may occur through open fractures or through those communicating with the paranasal sinuses or ears; also by direct extension of infection from the sinuses or from the ears and mastoid cells (*otitic meningitis*), as well as from other neighboring tissues (*meningitis sympathica*). Otherwise, infection is usually by way of the blood. It may occur in the course of bacteremia, as in tuberculous and syphilitic meningitis, or in the course of septicemia due to the meningococcus, streptococcus, pneumococcus, staphylococcus, influenza bacillus, etc. For many years it was thought that the meningococcus reached the meninges by direct extension along the lymphatics accompanying the olfactory nerve, but this idea is no longer held. If it were true, one would expect a higher incidence of meningitis from organisms so commonly found in the nose and especially in chronic sinusitis. It appears rather that the meningococcus reaches the lymphatics, draining the upper respiratory tract and finally the blood, causing an initial septicemia. This is followed by localization in the meninges through an inherent selective affinity of the organism for these tissues.

Owing to the low resistance of the meninges to infection it is likely that any virulent bacterium, virus or other pathogen is capable of exciting a meningitis, but aside from those instances of direct infection, meningitis apparently is induced only by those living agents of disease capable of originating bacteremia, septicemia or virusemia. In the order of frequency, these are *T. pallidum,* meningococcus, tubercle bacillus, pneumococcus, hemolytic streptococcus and the influenza bacillus, with much less frequent infections due to staphylococcus, the Friedländer bacillus, colon bacillus, typhoid bacillus, *Ps. aeruginosa, Cryptococcus neoformans, C. albicans,* etc. Mixed infections may occur but are uncommon.

As will be discussed later in more detail, *Listerella monocytogenes* may also produce a suppurative type of meningitis in human beings. The mortality in 19 reported cases has been about 70 per cent. Monocytes are present in the spinal fluid with intracellular and extracellular gram-positive bacilli. The latter have been mistaken in some instances for diphtheroid bacilli.

So-called *primary meningitis* is usually due to the meningococcus, pneumococcus, streptococcus and the influenza bacillus, since meningitis may arise without an apparent or pronounced preceding focus of infection, while *secondary meningitis* is usually due to infection by *T. pallidum,* the tubercle bacillus, pneumococcus, streptococcus, staphylococcus, the Friedländer bacillus, etc., from a preceding focus of infection. But, as a matter of fact, such a classification is of limited value since, in the so-called *primary meningitides,* clinically undetected foci of infection may be present in the nasopharynx or elsewhere. Indeed, the writer does not believe in so-called primary meningitis at all, because even in the case of meningococcal and influenzal meningitis, it is always likely that some infection of the upper respiratory tract antedated the onset of meningitis.

Laboratory Examinations. The signs and symptoms of acute meningitis may be so pronounced and characteristic that an examination of the cerebrospinal fluid is not required for diagnosis; however, this is essential for etiologic purposes except, possibly, in epidemics of meningitis which are invariably caused by the meningococcus.

Normal spinal fluid is perfectly clear. Any departure from a clear state indicates meningitis even when only slightly opalescent, provided blood is not present from the accidental puncture of a vein. On the other hand, a perfectly clear fluid may still be pathologic, as in serous, tuberculous and syphilitic meningitis and lymphocytic choriomeningitis. Indeed, it may be clear in the earliest stages of meningococcal, pneumococcal and other infective meningitides if the total cell count is below 200 cells per c.mm. of fluid.

Etiologic diagnosis requires, therefore, a prompt bacteriologic examination of the cerebrospinal fluid. If this is cloudy, the examination of direct smears or of the sediment, stained by the method of Gram with *thorough decolorization* before counterstaining, will frequently indicate the nature of the infection. The presence of gram-negative diplococci indicates meningococcal meningitis while gram-negative bacilli, or threads, suggest influenzal meningitis and gram-positive cocci indicate pneumococcal, streptococcal or staphylococcal meningitis (influenza bacilli are frequently overlooked on account of their small size and faint staining). A good laboratory should be able to submit such a helpful preliminary report within an hour after receipt of the fluid. It is true, however, that smears may not show sufficient organisms in fluid taken in the earliest stages, in which case etiologic diagnosis must await the outcome of cultures. In this connection it is important to prepare cultures as promptly as possible, and especially in the case of suspected meningococcal meningitis because the organisms die rapidly. Furthermore, it is important to culture 0.5 to 1 cc. of the fluid, as a loopful or two is likely to be insufficient. Needless to state, an enriched medium is required. Great emphasis is properly placed on the need for as *early* etiologic diagnosis as possible in cases of the suppurative meningitides because a delay of hours and certainly of days in instituting proper specific therapy has a tremendous influence upon prognosis. There is need for a greater realization of this fact by both physicians and laboratory technologists.

In addition to bacteriologic examinations, the gross appearance of the fluid, cytologic examinations (total and differential cell counts) and chemical examinations (especially for total protein, sodium chloride and glucose) are of value, as discussed in Chapter 14; also serologic examinations, when syphilis is suspected or known to be present, as discussed in Chapter 18. The changes are variable and especially in relation to the duration and severity of the meningitis but those summarized in Table 135 may be regarded as fairly typical.

Antibiotic and Sulfonamide Therapy. Additional examinations are frequently indicated or required in relation to specific therapy. Thus in *meningococcal menin-*

TABLE 135. SUMMARY OF CHANGES IN THE

Examinations	Normal	Serous	Purulent
Pressure	100–200 mm. H_2O 0–8 mm. Hg.	Increase	Increase
Appearance	Clear	Clear	Cloudy
Color	Colorless	Colorless	Grayish or yellow-green
Coagula; Sediments	Absent	Absent	Present
Totals cells *	0–8 10–12 borderline	Normal or sl. inc.	Mod. to marked inc.
Kinds of cells	Lymph.	Lymph. and endothel.	Polymorpho.
Protein (Pandy)	—	. .	++ to ++++
Protein (quant.)	15–40 mg.†	Normal or sl. inc.	Marked inc.
Glucose	50–90 mg.†	Normal	Dec. or absent
Sod. chloride	720–750 mg.†	Normal	Normal or sl. dec.
Colloidal gold	Neg.	Neg.	Men. curve
Wassermann	Neg.	Neg.	Neg.
Smears and cultures	Sterile	Sterile	Meningococcus *H. influenzae* Streptococcus Pneumococcus Staphylococcus *K. pneumoniae* *Esch. coli*, etc. *C. immitis* *C. neoformans*

* Per c.mm. of fluid. Slight increase (15–100); moderate increase (100–500); marked increase (1000–5000 or higher).

† Per 100 cc. of fluid.

CEREBROSPINAL FLUID IN THE MENINGITIDES

Tuberculous	Lymphocytic Choriomen-ingitis	Syphilitic			
		Asympto-matic	Meningo-vascular	Tabes	Paresis
Increase	Increase	Normal or sl. increase	Normal or sl. inc.	Increase	Increase
Clear or opaque	Clear or opaque	Clear	Clear	Clear	Clear
Colorless	Colorless	Colorless	Colorless	Colorless	Colorless
Present	Absent	Absent	Absent	Absent	Sometimes present
Mod. inc.	Mod. inc.	Normal or sl. inc.	Mod. inc.	Sl. inc.	Sl. to mod. inc.
Lymph.	Lymph.	Lymph.	Lymph.	Lymph.	Lymph.
++++	± to +	± to +	+ to ++	+ to ++	+ to +++
Marked inc.	Sl. inc.	Sl. inc.	Sl. inc.	Sl. inc.	Mod. inc.
Sl. dec.	Normal	Normal	Normal or sl. dec.	Normal or sl. dec.	Normal or sl. dec.
Marked dec.	Usually nor-mal	Normal	Normal	Normal	Normal
Men. curve	Men. curve	Zone II curve	Zone II curve	Zone II curve	Zone I curve
Neg.	Neg.	Frequently pos.	Usually pos.	Usually pos.	Pos.
Tubercle ba-cilli	Sterile	Sterile *	Sterile *	Sterile *	Sterile *

* Sterile cultures although *T. pallidum* may be discovered by intratesticular inoculation of rabbits with cerebrospinal fluid.

gitis sulfadiazine appears to be the compound of choice, but if penicillin is employed alone or in combination with this compound it is frequently advisable to determine the susceptibility of the infecting meningococcus to penicillin in vitro as a guide in the dosage to employ in treatment. Furthermore, it is sometimes advisable to test cerebrospinal fluid for meningococcus polysaccharide by overlaying 0.2 cc. of perfectly clear polyvalent or type-specific antimeningococcal serum in a small test tube with 1 cc. of perfectly clear centrifuged spinal fluid. A positive reaction is indicated by the formation of a white ring of precipitate at the line of contact. If occurring within 10 minutes at room temperature, it is usually indicative of a severe infection requiring intensive treatment.

Likewise in *pneumococcal* and *streptococcal meningitis* it is always advisable to determine the susceptibility of the organisms to penicillin in vitro as a guide to dosage in treatment. In pneumococcal meningitis a test for polysaccharide in the spinal fluid may be also indicated, since a positive reaction is indicative of a severe infection requiring intensive treatment. The test is conducted in the same manner as described for meningococcal polysaccharide except that rabbit type-specific antipneumococcal serum is employed.

In *influenzal meningitis* streptomycin is usually the antibiotic of choice in treatment. Some strains of *H. influenzae*, however, are more susceptible to penicillin in vitro than to streptomycin. Susceptibility tests, therefore, are always advisable for choosing the antibiotic to employ in treatment. In mild cases sulfadiazine alone in adequate dosage may suffice, providing its administration is continued for at least two weeks to allow adequate time for antibody production. The writer, however, always prefers combination therapy with sulfadiazine and streptomycin or penicillin. In addition to these compounds it is also advisable to administer Alexander's immune rabbit serum in the treatment of severe or moderately severe cases of the disease. The dose to employ may be estimated upon the basis of the amount of sugar in the cerebrospinal fluid as described by Alexander, Ellis, and Leidy,[1] the test being conducted as follows:

Tube	Spinal Fluid cc.	Reduction of 1 cc. of Benedict's Qualitative Reagent *					
1	0.05	+	o	o	o	o	o
2	0.1	+	+	o	o	o	o
3	0.15	+	+	+	o	o	o
4	0.2	+	+	+	+	o	o
5	0.25	+	+	+	+	+	o
Mg. % sugar		over 50	40 to 50	30 to 40	20 to 30	10 to 20	<10

* + = Reduction of reagent; o = no reduction of reagent.

Alexander prefers to treat with sulfadiazine for 24 hours before administering the immune serum. A preliminary skin test for hypersensitivity is first conducted with normal rabbit serum diluted 1:10. The immune serum is injected intravenously. Intrathecal injections are usually unnecessary but if deemed advisable should be delayed, if possible, until 24 hours after the first intravenous dose. A reaction in the spinal fluid may result from the combination of the soluble polysaccharide or *H. influenzae* and the antibodies of the serum, which can cause increased congestion of the meninges. If a partial block is suspected, MacPherson and his colleagues [2] suggest that 10 mg. of heparin in 1 cc. volume be injected before giving the serum intrathecally.

The dosage of serum to employ is based upon the spinal fluid sugar as follows:

Spinal Fluid Sugar (Mg. per cent)	Mg. Antibody Nitrogen Indicated
<15	100
15 to 25	75
25 to 40	50
over 40	25

THE ENCEPHALITIDES

Encephalitis refers to inflammation of the brain. When the cerebral meninges are also involved the disease is known as *meningoencephalitis.* Not infrequently the spinal cord is likewise involved, constituting *encephalomyelitis.* Encephalitis usually involves the upper motor neuron with hyperactive deep tendon reflexes, the loss of superficial abdominal reflexes, and the development of abnormal reflexes (Babinski, Oppenheim, Chaddock, Stone) along with spasticity, in co-ordination of muscles without loss of function, etc. When combined with lower motor neuron lesions, diminished reflexes, flaccidity and loss of motor function are usually observed, or a meningeal type of reaction may be produced with positive Kernig, Brudzinski and other hyperactive reflexes, stiff neck, opisthotonos, etc.

Classification. The causes of encephalitis, meningoencephalitis and encephalomyelitis are numerous and diverse. These include not only infections with some of the filtrable viruses, various bacteria and other living agents of disease, but intoxications of exogenous or endogenous origin, mechanical injuries of the brain and unknown factors as well. Indeed, every meningitis is accompanied by some degree of encephalitis or myelitis, or both, due to extension of inflammation from the meninges to the brain or spinal cord. By common consent, however, the term encephalitis is usually reserved for inflammatory lesions occurring primarily in the substance of the brain.

Under the circumstances, the encephalitides may be briefly classified according to etiology as follows:

(1) *Viral*
- Infectious:
  - Type A (chiefly sporadic): von Economo's
  - Type B (chiefly epidemic):
    - St. Louis
    - Japanese B
    - Equine encephalomyelitis
    - Australian X
    - Russian Far East
- Postinfectious: Influenza, measles, rubella, mumps, vaccinia, variola, etc.

(2) *Bacterial or Other Infections:* These include infectious and postinfectious as well as hemorrhagic types of encephalitis occurring in pertussis, scarlet fever, tonsillitis, erysipelas, streptococcus septicemia or bacteremia (following extraction of teeth), abscesses of the

brain, chorea, pertussis, pneumonia, typhoid fever, bacillary dysentery, syphilis, relapsing fever, typhus fever, malaria, etc.

(3) *Exogenous Intoxications:* As in poisoning by carbon monoxide, lead, arsenic, thallium, bismuth, alcohol, benzene, etc.

(4) *Endogenous Intoxications:* As in uremia, the toxemias of pregnancy, fat embolism of the brain from fractures of long bones, etc.

(5) *Mechanical Injuries:* As in tumors of the brain, fractures of the skull, cerebral arteriosclerosis, embolism, thrombosis, etc.

(6) *Unknown Causes:* As in Schilder's disease (*encephalitis periaxialis diffusa*), encephalitis neonatorum, acute disseminated encephalomyelitis, multiple sclerosis, etc.

To this classification may be added acute anterior poliomyelitis because of an associated mild encephalitis in some cases; for this reason the disease is frequently designated *polioencephalomyelitis.*

Laboratory Examinations. Unfortunately, the only two ways for definitely establishing the etiologic diagnosis of a viral encephalitis is by finding the virus in the brain or spinal cord after death by animal inoculation tests, or by demonstrating the presence of acquired specific virus-neutralizing and complement fixing antibodies in the serum after recovery. Even these examinations are applicable only to the St. Louis, Japanese B, Australian X, Russian Far East and equine types of encephalitis. Paired specimens of serum should be examined simultaneously, the first being collected as early as possible in the disease and the second during convalescence. If both yield negative results, the disease is excluded; if the first gives a negative and the second a positive result, the disease has been present. Cultures and animal inoculation tests of the cerebrospinal fluid during life for the viruses are not available for diagnostic purposes at the present time although highly desirable.

Other examinations of the spinal fluid, however, may be of helpful diagnostic value, especially in reference to gross appearance, total cells and protein determinations. At least, they are extremely valuable in differentiating between an encephalitis and a bacterial or mycotic meningitis. Differentiation between encephalitis and lymphocytic choriomeningitis is more difficult as far as spinal fluid examinations are concerned. The same is likewise true in some cases of tuberculous meningitis during its early stages. It is hoped, however, that the usual changes observed in the different forms of meningitis (Table 135) and the more important forms of encephalitis, summarized in Table 136, will be helpful in connection with such clinical data as the appearance, age, and sex of the patient, as well as the history, temperature, pulse and respiratory rates, neurologic and mental changes, etc.

Acute disseminated encephalomyelitis is particularly difficult to differentiate from an encephalitis. The latter may also be mistaken for multiple sclerosis. If, however, the patient develops spotty signs of motor and sensory involvement, especially a dissociated appreciation of pain and temperature changes, along with paralyses and bizarre reflexes, disseminated encephalomyelitis should be suspected. Such a syndrome cannot be differentiated from acute multiple sclerosis but if the patient recovers and has recurrences, multiple sclerosis is likely. It is particularly important for physicians to remember that the cerebrospinal fluid in multiple sclerosis may give positive colloidal gold, benzoin or mastic reactions in about 80 per cent of cases, although the Wassermann test gives a negative reaction.

THE ARTHRITIDES

As is well known, the acute and chronic arthritides are very common diseases, especially in the colder climates. Various classifications and many different names for the arthritic diseases have resulted in much confusion. Needless to state, general and specific therapeutic measures should be based largely on the causes. In spite of a great deal of clinical and laboratory investigation, however, the etiology of two of the most common varieties of chronic arthritis, namely, rheumatoid or atrophic arthritis and osteo- or hypertrophic arthritis, remains uncertain and obscure. Furthermore, while much progress has been made in the treatment of these, yet it is likely that many physicians still agree with what Osler is reputed to have stated, that "when a patient with arthritis walks into my office I feel like jumping out of the window."

It is generally agreed that some acute and chronic types of arthritis are the result of infections with various specific micro-organisms although there is much difference of opinion on whether the inflammatory changes in and about the joints are due to the actual presence of the micro-organisms themselves, their toxins, or local allergic reactions to them. There is also consensus that acute and chronic types of arthritis may be the result of many noninfectious causes. This group includes osteo- or hypertrophic arthritis although there is much uncertainty regarding the nature of these noninfectious factors in the etiology of this common and chronic disease. Incidentally, it may be mentioned that differentiation between atrophic or rheumatoid arthritis and hypertrophic or osteo-arthritis is not always easily made by roentgenologic examinations, with the result that mixed types of chronic arthritis are frequently encountered.

Undoubtedly, most uncertainty and difference of opinion is in relation to the etiology of rheumatoid arthritis. Some, including myself, believe that this common disease is always due, at least in part, to infection, especially with various streptococci of focal origin. As previously discussed in Chapter 15, this does not necessarily involve the question of whether or not streptococci, staphylococci, pneumococci or other micro-organisms in foci of chronic infection of dental, tonsillar or other origin acquire a selective localizing affinity for the joints. In other words, vascular or other local conditions in the latter may favor their localization during periods of bacteremia, aside from the possibility of the arthritic changes being due to bacterial toxins alone or to acquired allergic sensitization to bacterial proteins. Others are of the opinion that the only relation chronic focal infection has to the disease is through a lowering of general resistance favoring the operation of other etiologic factors.

Classification. Aside from rheumatoid arthritis, however, there is general agreement in regard to the etiology of other forms of the disease. Many are known to be due to specific infections on the basis of the finding of micro-organisms in the synovial fluids, or because of their occurrence in the course of infectious diseases of known etiology. These are classified under the heading of the *specific*

TABLE 136. SUMMARY OF THE CEREBROSPINAL FLUID CHANGES IN THE ENCEPHALITIDES AND ENCEPHALOMYELITIDES

Examinations	von Economo	St. Louis	Equine	Post Infectious	Japanese B	Australian X	Schilder's Disease	Acute Multiple Sclerosis
Pressure	Normal or sl. increase	Normal or sl. inc.	Normal or sl. increase	Increase	Normal or sl. increase	Normal or sl. increase	Normal or sl. increase	Normal
Appearance	Clear	Clear or opaque	Clear or opaque	Clear	Clear	Clear	Clear	Clear
Color	Colorless or sl. yellow	Colorless	Colorless	Colorless	Colorless	Colorless	Colorless	Colorless
Coagula	Rare	Rare	Rare	Rare	None	None	None	None
Total cells	10–200	50–1000	200–2000	10–100	10–100	10–100	Normal	Normal or sl. increase
Kind of cells	Lymph.	Lymph.	Lymph.	Lymph. and mononuc.	Lymph.	Lymph.	Lymph.	Lymph.
Protein (Pandy)	± to +	− or +	++ to ++++	± to +	± to +	+ to ++	Neg.	− to ++

Protein (quant.)	Sl. inc.	Inc. (70%)	Increase	Sl. increase	Sl. increase	Sl. increase	Normal	Inc. (50%)
Glucose	Normal	Usually inc.	Normal	Normal or sl. dec.	Normal	Normal	Normal	Normal
Sod. chloride	Normal	Normal or sl. increase	Normal	Normal	Normal	Normal	Normal	Normal
Colloidal gold	Usually pos.	Positive (80–90%)	Usually pos.	Usually pos.	Usually pos.	Usually pos.	Usually neg.	Zone I (50%) Zone II (30%); Neg. (20%)
Wassermann	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.
Smears and cultures	Sterile	Sterile	Sterile	Sterile	Sterile	Sterile	Sterile	Sterile
Virus neutralization test *	None	+	+	None	+	+	o	o
Complement fixation	None	+	+	None	+	+	o	o

* + = neutralization of virus by convalescent serum; none = tests are not available; o = etiology unknown.

TABLE 137. SUMMARY OF LABORATORY EXAMINATIONS IN GONOCOCCAL, TUBERCULOUS AND OTHER FORMS OF SPECIFIC INFECTIOUS ARTHRITIS

Examinations	Gonococcal	Tuberculous	Other Forms
Hematologic	Sedimentation rate increased. Leukocytosis with shift to left in acute stage; shift to left alone in chronic stage. Hypochromic normocytic anemia. Normal sugar tolerance. No increase of blood uric acid.	Sedimentation rate increased. Slight leukocytosis with a shift to left may occur. Hypochromic normocytic anemia. Normal sugar tolerance. No increase of blood uric acid or cholesterol.	Sedimentation rate increased. Leukocytosis with shift to the left. Hypochromic normocytic anemia.
Serologic	Antistreptolysin titer normal. No increase of streptococcal agglutinins. Positive gonococcal complement fixation reactions in 70 per cent acute and 80 to 90 per cent chronic cases. Positive reactions with synovial fluid in about 80 per cent of cases.	Antistreptolysin titer normal. Usually no increase of streptococcal agglutinins. Positive tuberculosis complement fixation reactions may occur with serum and synovial fluid.	Antistreptolysin titer and increase of streptococcal agglutinins frequent in hemolytic streptococcus arthritis. Positive agglutination reactions in typhoidal and brucellosis arthritis.
Bacteriologic	Positive blood cultures in about 4 per cent of fulminating cases. Smears and cultures of synovial fluid positive in 15 to 70 per cent of cases. Synovial fluid usually cloudy due to increase of cells (mostly polymorphonuclears).	Sterile blood cultures. Tubercle bacilli may be found in synovial fluids by smears, cultures, or guinea-pig inoculation tests. Positive biopsy examination of excised tissue may be observed.	Blood cultures frequently positive. Synovial fluid usually cloudy due to increase of cells and especially polymorphonuclears. Positive cultures of synovial fluid frequently observed.

infectious arthritides. Others are definitely known to be due to causes other than infections and consequently are classified as the *noninfectious arthritides*. This leaves a third group in which infection with streptococci or other micro-organisms of focal infection may or may not play an important primary or secondary rôle in etiology. Most students of arthritis classify them under the heading of the *nonspecific infectious arthritides*. To this group belongs rheumatoid or atrophic arthritis. Since the clinical manifestations of Still's disease of children corresponds to severe rheumatoid arthritis of adults in association with splenomegaly and lymphadenopathy it would appear permissible to include it in this category. The same is probably true of Felty's syndrome of adults, characterized by splenomegaly, lymphadenopathy, yellow-brown pigmentation of the skin, secondary anemia with leukopenia in association with a chronic atrophic type of arthritis. Under the conditions the arthritides may be classified on the basis of etiology as follows:

- (1) Specific Infectious
  - Rheumatic fever
  - Gonococcal
  - Tuberculous (including tuberculous rheumatism or Poncet's disease)
  - Suppurative or nonsuppurative
    - Streptococcal
    - Staphylococcal
    - Pneumococcal
    - Meningococcal
    - Typhoidal
    - Influenzal
    - Malarial
    - Lepral
    - Mycotic, etc.
  - Brucellosis
  - Haverhill fever (erythema arthriticum epidemicum)
  - Syphilitic
    - Arthralgia of early syphilis
    - Gummatous of late syphilis
    - Hydro-arthrosis of congenital syphilis
    - Charcot's joints of tabes, etc.
- (2) Nonspecific Infectious
  - Rheumatoid or atrophic arthritis of adults
  - Marie-Strümpell spondylitis
  - Still's disease of children
  - Felty's syndrome of adults
- (3) Noninfectious
  - Osteo-arthritis or hypertrophic arthritis
  - Osteo-arthritic spondylitis.
  - Senile arthritis
  - Malum (Morbus) coxae senilis
  - Traumatic arthritis
  - Ochronodal arthritis
  - Acromegalic and other endocrinal arthritides
  - Metabolic arthritis (gout, rickets, etc.)
  - Psoriatic arthritis
  - Hemophiliac arthritis
  - Menopausal arthritis
  - Allergic arthritis (serum sickness, etc.)
  - Hypertrophic pulmonary osteoarthropathy
  - Neoplastic arthritis
  - Trophic arthritis (nerve injury, syringomyelia, etc.)

TABLE 138. SUMMARY OF LABORATORY EXAMINATIONS IN RHEUMATIC FEVER, RHEUMATOID (ATROPHIC) ARTHRITIS AND OSTEO-ARTHRITIS (HYPERTROPHIC)

Examinations	Rheumatic fever	Rheumatoid Arthritis	Osteo-arthritis (Hypertrophic)
Hematologic	Sedimentation rate increased. Leukocytosis during febrile stage. Variable shift to left even in absence of leukocytosis Hypochromic normocytic anemia in late stages.	Sedimentation rate increased in 90 per cent during active stage. Leukocytosis with shift to left in active stage. Hypochromic normocytic anemia usual. Low sugar tolerance (60 per cent). Blood uric acid usually normal. Increase of plasma globulin with reversal of A-G ratio frequent.	Sedimentation rate rarely slightly increased. Leukocytosis with shift to left rare. Usually no anemia. A-G ratio normal. Blood uric acid normal or slightly increased. Normal glucose tolerance. Slight increase in plasma cholesterol in some cases.
Serologic	Antistreptolysin titer usually increased in febrile stage. No increase of streptococcal agglutinins. Negative gonococcal complement fixation reaction.	Antistreptolysin titer may be increased in early stage. Streptococcal agglutinins increased in 40 to 80 per cent. Negative gonococcal complement fixation reaction.	Antistreptolysin titer not increased. No increase of streptococcal agglutinins. Negative gonococcal complement fixation reaction.
Bacteriologic	Blood cultures sterile. Usually sterile synovial fluid. Synovial fluid may show increase of cells.	Blood cultures by massive method sometimes positive. Usually sterile synovial fluid. Synovial fluid may be cloudy due to leukocytes and especially polymorphonuclears.	Blood cultures sterile. Usually no increase of synovial fluid. If obtained, usually sterile with no increase of cells.

Laboratory Examinations. Laboratory examinations are always of pathognomonic diagnostic value when the micro-organisms producing the specific infectious types of arthritis are found in synovial fluids removed by aspiration with rigid precautions against contamination. This is particularly true in the suppurative forms usually due to infections with hemolytic streptococci or *Staph. aureus*. Laboratory examinations are likewise of diagnostic value in gonococcal, pneumococcal, meningococcal and tuberculous arthritis, especially if the infecting micro-organisms are recovered in smears and culture of the synovial fluids. In the nonsuppurative forms of specific infectious arthritis, however, diagnosis is usually largely based upon the knowledge that the arthritis has developed as a complication in the course of the infectious disease. The usual changes seen in these specific infectious forms of acute and chronic arthritis are summarized in Table 137.

Otherwise, laboratory examinations are only of helpful diagnostic value and are not usually employed except as aids in differentiating among rheumatic fever, rheumatoid (atrophic) arthritis and osteo-arthritis (hypertrophic); the usual or typical changes in these are summarized in Table 138.

BRUCELLOSIS (UNDULANT FEVER)

The prediction of Simpson [3] in 1930 that brucellosis would soon become an important public health problem has been amply verified. Surveys conducted during recent years in different parts of the United States have not only shown its widespread occurrence but also that it is a frequently unrecognized cause of disabling illness. Indeed, its true incidence is as yet unknown. In 1940, 3358 cases were officially reported to the U. S. Public Health Service but it is practically certain that the number of cases actually occurring each year is much larger. Recent surveys [4,5,6] conducted by skin and serologic tests in widely separated endemic areas indicate that approximately 10 per cent of these population groups may have sustained clinically unrecognizable infections with Brucella, and particularly with *Br. abortus*, through the use of raw milk and contact with infected cattle.

Etiology. Brucellosis may be caused by any one of the following three kinds of Brucella:

1. The *caprine* or goat variety, caused by *Br. melitensis*, the species originally described by Bruce as the cause of Malta fever. This strain is rather widely distributed among goats. It is found in the udder, spleen and lymph nodes, giving rise to interstitial mastitis and splenic lymphadenitis. It results in human infection, either through the drinking of the contaminated milk or the handling of the infected goats, and is the most pathogenic for man of all three strains. It has also been found in the milk of infected cows, both in the United States and Europe, and in cultures from aborted fetuses of sheep and goats in Italy, France and Argentina.

2. The *bovine strain* or *Br. abortus*, described originally in cattle by Bang, and responsible for infectious abortion disease in these animals. It is the least virulent of all three, at least in man, as it has been found in raw milk without causing symptoms from its ingestion. It has been recovered from various naturally infected animals—like horses, dogs, fowl, sheep, deer, etc.—in all parts of the world, but not from the hog.

3. The *porcine type* or *Br. suis*, first isolated by Traum [7] in 1914 from aborted fetuses of hogs both in United States and abroad. While the hog is the natural host for this strain, it has also been isolated from the horse, cow, dog and fowl. It may also be responsible for the disease in human beings, being midway between the other two strains in its pathogenicity for man.

While these various species of the Brucella group are closely related in their characteristics, they each possess certain immunologic and cultural differences.

Evans[8] was the first to recognize this in her original study in 1918 when she separated the Brucella into a *melitensis* and an *abortus* group. It was considered at that time and for many years thereafter that *Br. suis* belonged to the *abortus* group as a porcine variety, since it differed very little from the *abortus* strain. Subsequent studies have indicated that the two are clearly separable and that the differentiation as *Br. suis* is warranted. Differentiation into serologic types has been greatly facilitated by the agglutinin-absorption test and numerous studies have been made with it. Probably the most comprehensive was that of Evans[9] who studied 68 strains of Brucella obtained from all parts of the world—she was able in these studies to establish two main serologic types; one, a smooth *melitensis* strain, and the other composed of smooth strains of both the *abortus* and *suis* types, combined. In addition, she described six subtypes, distributed under each of the main types, which comprise the antigenic variants or "para" strains. It is to be remembered in classifying these types that it is not possible to differentiate the *Br. abortus* and *Br. suis* strains by the agglutinin-absorption method alone. This simply serves to differentiate *Br. melitensis* from the other two. To differentiate *Br. abortus* and *Br. suis* one must employ methods based on the relative ability to utilize glucose, the rate of production of hydrogen sulfide (using lead acetate paper for the determination) and the differences in the bacteriostatic action of dyes when the three strains are grown on media prepared according to the method of Huddleson so as to contain thionin, methyl violet and basic fuchsin.

Clinical Types. Undulant fever is a subacute or chronic generalized infection with a gradual or insidious onset. It is characterized by an irregular remitting fever of uncertain, but often prolonged duration, by profuse sweats, chills (or chilliness), occipital or frontal headache, pains in the joints and muscles, nervous disturbances, constipation, secondary anemia and progressive weakness. While these symptoms are present to a greater or lesser degree in most patients, the clinical characteristics and course of the disease are extremely variable. Hughes, as far back as 1897, appreciated this fact and as a consequence, in his original classical description of undulant fever, he differentiated the following three clinical types based on the temperature curve. This classification applies today, not only to *melitensis,* but to *abortus* infections as well, and is as follows:

1. An *intermittent type* comprising the majority of cases, with insidious onset, symptoms corresponding to those mentioned above, and irregular temperatures varying between 100° F. in the morning and 101° F. to 104° F. in the evening. This form persists for from six weeks to four months, the patient spending about one-third of the time in bed. A fair proportion (possibly 25 per cent) of these are ambulatory cases with mild, almost negligible, evidences of illness, so that some continue employment throughout. The temperature is only slightly elevated, rarely above 101° C. in the evening, and other symptoms are much milder. The duration in these ambulatory patients varies between two weeks and several months, being more frequently longer than one month and less than four.

2. The *undulant type* is characterized by frequent relapses, following febrile periods during which the usual symptoms of the intermittent form of the disease

appear. Because these symptoms resemble influenza and occur in the summer, this form is often diagnosed as "intestinal influenza" or "summer flu." Sometimes symptoms recur almost before the patient has recovered from the first febrile period. This may be repeated several times but usually with decreasing intensity and duration. As a rule, this form of the disease does not last longer than the usual type although this cannot be stated with any degree of certainty.

3. The *malignant type* is rare. When it does occur, the onset is sudden, the temperature is high and maintained, prostration is marked, severe chills and muscular pains are present, and fatal termination takes place usually in less than three weeks.

In some respects it is unfortunate, however, that the disease has become known as undulant fever, since this term is mainly applicable to acute brucellosis only. It is true that an undulatory, remittent, or intermittent fever frequently occurs in both the acute and chronic types of the disease but this is not always the case. Indeed, the disease is frequently mistaken for typhoid fever, malaria, tuberculosis, influenza, rheumatic fever and other diseases as well. Many so-called "neurasthenics" whose chief complaints are exhaustion, insomnia and irritability with a variety of aches and pains have been found to be victims of brucellosis. The signs and symptoms of acute brucellosis may be sufficiently characteristic for clinical diagnosis but, in dealing with chronic brucellosis, the diagnostic acumen of the physician is usually severely taxed. As stated by Simpson,[10] no disease, not even syphilis and tuberculosis, is more protean in its various and diverse clinical manifestations.

Laboratory Examinations. Under the circumstances, physicians naturally turn to the laboratory for diagnostic aid. The finding of Brucella in *blood cultures or by the inoculation of guinea-pigs with blood* according to the technic described by Poston,[11] are the only methods by which the diagnosis of brucellosis may be completely established. Unfortunately, however, the incidence of positive blood cultures is still so low, aside from the fact that they usually require at least two weeks' time, that bacteriologic methods are not usually helpful in early diagnosis.

Furthermore, as discussed in Chapter 19, while a negative *skin reaction* is generally acceptable as excluding present or past infection with Brucella, a positive reaction does not necessarily mean that the symptoms presented by the patient at the time of the test are due to brucellosis. In other words, since allergic sensitization tends to persist for years after recovery from even minor infections with Brucella, positive skin reactions occurring in individuals with such diseases as active tuberculosis, Hodgkin's disease, typhoid fever, malaria, or subacute bacterial endocarditis may readily result in a mistaken diagnosis of brucellosis.

From the standpoint of serologic examinations, two tests are available: (1) the agglutination test and (2) the opsonocytophagic test of Huddleson. The complement fixation test is not employed. But since the injection of heat-killed Brucella or brucellergen in the conduct of skin tests may produce both agglutinins and opsonins,[12,13] it is important for physicians to take blood for the agglutination and opsonic tests beforehand.

Next to the recovery of Brucella by bacteriologic methods, it appears that strongly positive *agglutination reactions* possess most diagnostic value and particularly in acute brucellosis. Agglutinins may be produced as early as the fifth day after the onset of symptoms but not ordinarily until the second or third weeks. Sometimes they occur only during the initial stage and then disappear or persist in very low titers. In other cases they occur intermittently and at unpredictable intervals. As a general rule, the tests should be repeated at intervals of three to five days, as a progressive increase of agglutinin is of more diagnostic value than single positive reactions. Once acquired, however, agglutinins tend to persist in the serum for months or years.

High titers are seldom observed in chronic brucellosis. Unfortunately, agglutinins may be acquired by some individuals as the result of exposure to infection without clinically detectable illness in just the same way as allergic sensitization with positive skin reactions are acquired. Therefore, positive agglutination reactions, like positive skin reactions, occurring in individuals with typhoid fever and other diseases resembling brucellosis, may readily result in diagnostic errors.

On the other hand, negative agglutination reactions do not exclude either acute or chronic brucellosis. Indeed, it is stated that as many as 6 to 10 per cent of cases showing positive blood cultures fail to develop agglutinins for reasons as yet unknown. In chronic brucellosis negative agglutination reactions have been reported in as high as 46 per cent of cases.

In other words, a negative agglutination reaction does not exclude either acute or chronic brucellosis; nor does a positive reaction necessarily establish the presence of the disease, as a positive reaction may occur in individuals with other diseases as the result of a previous clinically unrecognizable Brucella infection.

Furthermore, agglutination reactions vary to some extent according to the strains of Brucella employed in the preparation of antigens. Unfortunately, the test has not yet been standardized although this would be highly desirable. Cross agglutination may occur with *Past. tularensis* but usually this presents no difficulties. However, fever from any illness may sometimes produce an increase of Brucella agglutinins resulting in falsely positive or anamnestic reactions.

It is commonly stated that agglutination at 1:80 constitutes a positive reaction. As a matter of fact, agglutination at 1:40 may be regarded as positive, as the great majority of normal individuals giving negative skin reactions do not agglutinate even at 1:10 and only occasionally at 1:20. In skin-positive normal individuals, however, while about 70 per cent react negatively at 1:10, about 30 per cent may give positive reactions in final dilutions as high as 1:100 as the result of clinically unrecognized infections. Needless to state, this adds greatly to the difficulties experienced in the interpretation of positive agglutination reactions.

The inadequacies of the agglutination and skin tests, particularly in distinguishing between present and past *Brucella* infection, led Huddleson, Johnson and Hamann [14] to reintroduce the *opsonocytophagic reaction,* a modification of the Leishman-Veitch technic for determining the phagocytic activity of the blood in the presence of serum opsonins and homologous leukocytes. The opsonocytophagic test is sometimes employed in conjunction with the skin test or the agglutination test, or both, to determine the immunity status of an individual giving positive reactions by either or both methods.

According to Huddleson's interpretation, the opsonocytophagic power of the blood is low during the active infective phase of the disease and becomes marked after recovery. On this basis, it is considered that individuals have developed immunity to *Brucella* if 60 per cent or more of the polymorphonuclear leukocytes show marked phagocytosis. If as many as 40 per cent of the leukocytes show moderate to marked phagocytosis the patient may be infected and has not yet developed any immunity, or he may be uninfected.

While theoretical considerations lend support to the contentions of Huddleson and his associates as to the value of the opsonocytophagic test, it still lacks confirmation. Evans and her colleagues [15] regard the opsonocytophagic test as the least reliable of the diagnostic tests in cases of chronic brucellosis since they found strongly positive (immune) reactions in four cases from which *Brucella* were cultivated, and weak or moderate reactions in recovered cases.

Foshay [16] has found that a significant proportion of patients yield aberrant and unexpected results in relation to their immunity status when the opsonocytophagic test is employed in conjunction with cultural methods, agglutination tests and skin tests. High phagocytic titers (immune reactions) occurred in some patients with severe and uninterrupted brucellosis, proved by cultures. Certain recovered patients, asymptomatic for months or years, exhibited marked fluctuations from month to month, running the entire gamut from high to low phagocytosis, or sometimes none at all. Until more extensive studies have been made on culturally proved cases of brucellosis the results of the opsonocytophagic test, therefore, should be interpreted with caution and with reservations.

Hematologic examinations indicate that leukopenia occurs in the majority of patients with acute brucellosis. In chronic brucellosis, either leukopenia, moderate leukocytosis, or normal leukocyte levels may be found. Calder and his colleagues [17] have directed particular attention to the occurrence of active lymphocytogenesis as the most striking and constant feature of the blood changes in all of the manifestations of brucellosis. The lymphocytosis is evidenced by an increase in both percentage values and in absolute numbers of lymphocytes, and by an unusually high proportion of immature lymphocytes (lymphocytic shift-to-the left). Mild anemia of the macrocytic, hyperchromic type is the rule. The erythrocyte sedimentation rate is usually not high, except when regional complications are present.

TYPHOID FEVER

Typhoid fever is an acute infectious disease caused by *S. typhosa* (*B. typhosus*) which has a selective affinity for the reticulo-endothelial tissues of the body. Infection occurs only when the bacilli in contaminated water or foods are swallowed, escape destruction in the stomach, and reach the small intestine. There the alkaline contents and bile are a favorable culture medium and the bacilli apparently proliferate during the stage of incubation, penetrating the inflamed mucosa through the lymphatics to infect the Peyer's patches and solitary lymph follicles. The organism, however, is highly invasive and during the succeeding ten or twelve days reaches the blood by way of the lymphatics, producing a bacteremia. At that time the initial symptoms of fever, headache, malaise, diarrhea or constipation with vague abdominal discomfort become more pronounced. It is for this reason that blood cultures are of valuable diagnostic aid in the early stage of typhoid fever whenever the disease is suspected. During this initial bacteremia or septicemia the bacilli are widely distributed throughout the body, but show a marked selective affinity for the reticulo-endothelium of the spleen, bone marrow, the liver and, less regularly, the lungs, periosteum and other tissues.

In the meantime the preliminary inflammatory hyperplasia of the reticulo-endothelium of the Peyer's patches and solitary lymph follicles has usually progressed to ulceration with the ultimate danger of hemorrhage and perforation. Gradual enlargement of the spleen occurs from the same hyperplasia of the reticulo-endothelium, resulting in occlusion of the vascular channels with congestion, giving the typical acute red type of splenic tumor. Similar changes affect the mesenteric and retroperitoneal lymph nodes and the bone marrow with focal necrosis of the liver. Meningitis and typhoid periostitis, especially of the tibia and spine, may follow, as well as other sequelae.

During this time the fever mounts to a fastigium of 102° to 103° F. with a disproportionately slow pulse (rarely exceeding 100) accompanied by tenderness of the abdomen and demonstrable peristalsis with enlargement and tenderness of the spleen. There is also a development of "rose spots" on the abdominal wall which are the result of minute emboli of typhoid bacilli in the superficial capillaries, and a leukopenia which arises from diffuse infiltration of the bone marrow by large mononuclear cells resulting from reticulo-endothelial hyperplasia, crowding the normal leukoblastic tissue. In severe infections the patient is likely to be highly toxic, show disorientation, carphologia and involuntary twitching or "subsultus tendinum." This stage lasts for approximately an additional two weeks, during which the patient may die of the toxemia, or from hemorrhages which are due to erosion of a blood vessel at the base of an ulcer in the ileum. Such may take place concomitantly with perforation, causing intra-abdominal hemorrhage and secondary peritonitis. Otherwise convalescence follows, with persistent infection in the gallbladder or kidneys in about 2 to 4 per cent of cases. The latter are potential carriers of the bacillus. The mortality rate averages about 10 per cent; relapses are not infrequent.

Laboratory Examinations. Laboratory examinations possess great value in the diagnosis of typhoid fever, especially during the early stages of the disease. They are also of value as aids in the detection of atypical cases and of carriers; likewise in the differentiation of typhoid fever from brucellosis, paratyphoid fever, miliary tuberculosis and other diseases which may bear a clinical resemblance to it. Naturally the results of laboratory examinations vary a good deal according to the severity and duration of the disease but in general terms may be summarized as follows:

1. Undoubtedly *blood cultures* are of most value in early diagnosis. With proper technic, positive cultures may be expected in about 90 per cent of cases during the first week of the disease. As the infection progresses and localizes,

however, the percentage is likely to drop to about 75 per cent by the second week and to about 30 per cent by the third week of the disease.

2. On the other hand, only 10 to 15 per cent of cases are likely to show typhoid bacilli in the *feces* during the first ten days of the disease. Thereafter the percentage rapidly increases, reaching about 50 per cent by the third week and from 80 to 90 per cent during convalescence. Under the conditions, bacteriologic examinations of the feces possess diagnostic value and especially in suspected or atypical cases of typhoid fever with negative blood cultures and doubtful agglutination reactions. Needless to state, however, typhoid bacilli may be found in the feces of carriers suffering from diseases other than typhoid fever.

Of course, bacteriologic examinations of the feces are extremely valuable for the detection of typhoid carriers. About 11 per cent of cases are positive for eight to ten weeks after recovery (convalescent carriers) and from 2 to 4 per cent for a year or longer (chronic carriers). Bacteriologic examinations of bile secured by duodenal drainage are also of value in the detection of carriers.

3. Typhoid bacilli do not ordinarily occur in the *urine* until after the second week of the disease, when only about 25 to 30 per cent of cases show their presence. Bacteriologic examinations of the urine, therefore, are not ordinarily required or indicated for diagnostic purposes except in atypical or doubtful cases of the disease. Needless to state, specimens of urine secured by catheterization are greatly to be preferred.

During the early period of convalescence about 12 per cent of cases show the presence of typhoid bacilli in the urine. Consequently, repeated bacteriologic examinations of both feces and urine are indicated before quarantine is lifted to guard against the possible discharge of carriers. In a small percentage of cases chronic carriers continue to discharge typhoid bacilli in the urine over months or years of time, but the incidence is not as high as in the feces.

4. Since time is required for the production of antibodies, *agglutination tests* are not likely to give positive reactions until after the first seven to ten days following the onset of symptoms. During the early stage, therefore, negative reactions should not be permitted to override clinical judgment. Indeed, if positive blood cultures have been observed, agglutination tests are not required for diagnostic purposes. On the other hand, positive reactions may be due to a previous attack of typhoid fever or to vaccination against the disease. Furthermore, weakly positive reactions may be due to influenza or some other infection responsible for anamnestic reactions. In typhoid fever, however, the reactions become progressively stronger when repeated at intervals of three to five days. The technic employed has considerable influence upon the results.

As discussed in Chapter 17, it is the consensus that the serum diagnosis of typhoid fever is best served by using H (flagellar) and O (somatic) antigens routinely. The serums of normal individuals who have never had typhoid fever or been vaccinated against the disease may agglutinate H antigen in final dilutions up to 1:20 and O antigen up to 1:80 or 1:100. In an individual who has never been vaccinated and who presents symptoms suggestive of typhoid fever, agglutination of H antigen at 1:40 and of O antigen at 1:160 is suspicious; agglutination of H at 1:80 or higher and of O in dilutions higher than 1:160 is usually indicative of typhoid fever in the majority of instances.

Typhoid fever occurring in a vaccinated individual is apt to show a particularly high titer of O agglutinin. On the other hand, a high titer of H agglutinin with a low titer of O agglutinin suggests an anamnestic reaction. However, a very high titer of H agglutinin as, for example, 1:1280 or 1:2560, is also indicative of typhoid fever under these circumstances, since it is unusual for H agglutinin to persist in titers higher than 1:640 for longer than six months after immunization. Consequently, when the disease is suspected in a previously vaccinated individual the tests should be repeated every three to five days with the same antigens. If the titers progressively increase, especially for O antigen, typhoid fever is most likely present.

An increase of H agglutinin, and especially of O agglutinin, is also of aid in the detection of typhoid carriers among nonvaccinated individuals. To be significant, the titer of H agglutinin must be above 1:20 and O agglutinin above 1:100.

5. As discussed in Chapter 17, the *complement fixation test* is also valuable in the serum diagnosis of typhoid fever including cases of the disease occurring in previously vaccinated individuals. Indeed, available data indicate that it may be superior to the agglutination test in both specificity and sensitivity.

6. *Hematologic examinations* are likewise of diagnostic value. Hypochromic normocytic anemia with leukopenia commonly occurs. Neutropenia with a relative lymphocytosis and a normal total leukocyte count is not infrequent. Leukocytosis is usually indicative of a complication, especially perforation with peritonitis. Even in cases showing leukopenia there is likely to be a "shift to the left" with an increase of immature or nonfilament polymorphonuclear neutrophil leukocytes probably due to an inhibition in the maturation of neutrophils in the bone marrow.[18]

7. In general, it is advisable to examine the feces at intervals for *occult blood,* as its presence in large amounts is indicative of bleeding and may be a warning of progressive ulceration of Peyer's patches with impending perforation.

8. Examinations of the urine for the *diazo reaction* are not employed for diagnostic purposes as frequently as was formerly the case. Positive reactions usually occur about the middle of the first week or ten days after the onset of symptoms. The reaction usually disappears during the third week and its early disappearance is thought to be of favorable prognostic import. Its reappearance is usually indicative of a relapse.

PARATYPHOID FEVER

Paratyphoid fever is practically indistinguishable from typhoid fever except by bacteriologic and serologic methods. As a general rule, the signs and symptoms are similar except that paratyphoid fever is generally milder and of shorter duration, in which case it may resemble mild typhoid fever. At times, however, the disease is quite severe, with rose spots, enlargement of the spleen, and positive blood cultures, especially in *S. paratyphi* B (*S. schottmülleri*) infections, which are responsible for most cases of paratyphoid fever in the United States.

In contradistinction to typhoid fever, the temperature in paratyphoid fever (especially in *S. paratyphi* B infections) may rise more abruptly and remain more irregular throughout the disease. Gastric symptoms, vomiting, and nausea are often more prominent than in typhoid fever, and enlargement of the spleen is present less frequently. Peyer's patches and the solitary lymph follicles of the small intestine, and likewise the mesenteric lymph nodes and the bone marrow, are involved less severely. Hemorrhage and perforation are, consequently, less likely to occur. The mortality is lower, usually about 4 per cent, with none at all in many small epidemics.

The paratyphoid fevers are usually due to infections with bacilli of the Salmonella group and especially *S. paratyphi* A and *S. paratyphi* B (*S. schottmülleri*). To these must be added, however, *S. paratyphi* C (*S herschfeldii*), as well as *S. kentucky, S. cholerae-suis, S. enteritidis* and others. These may occasionally produce typhoid-like fevers but are more likely to produce acute gastroenteritis or dysentery-like symptoms due to enteric infections, not infrequently complicated by lesions of a septic or suppurative type. Many other bacilli of the Salmonella group are also responsible for food infections with dysentery-like lesions and symptoms, to which further reference will be made.

Laboratory Examinations. The diagnosis of the paratyphoid fevers is purely a laboratory problem based on bacteriologic and serologic examinations. In view of their close clinical similarity to typhoid fever, all bacteriologic examinations of the blood, feces and urine for the typhoid bacillus, as well as all serologic examinations for typhoid fever, should include, routinely, examinations for paratyphoid and Brucella infections. As in typhoid fever, repeated negative bacteriologic examinations of the feces are required before quarantine is lifted. In the paratyphoid fevers the results of laboratory examinations may be summarized as follows:

1. *Blood cultures* are frequently positive during the first week or two after the onset of symptoms, but the incidence is not as high as in typhoid fever. Consequently, even repeatedly sterile blood cultures do not exclude the disease.

2. Bacteriologic examinations of the *feces* are particularly valuable and especially during or after the second week of the disease. Repeated examinations may be required before the bacilli are found.

3. Bacteriologic examinations of *urine* collected by catheterization may show the presence of paratyphoid bacilli, but the incidence of positive findings is lower than in typhoid fever.

4. *Agglutination tests* possess diagnostic value but are inferior to bacteriologic examinations of the blood, feces and urine. H and O antigens of *S. paratyphi* A and *S. paratyphi* B should be used routinely. Since paratyphoid fever may be caused by other Salmonella it is advisable, however, to include tests with antigens of *S. paratyphi* C and other species if mild typhoid-like symptoms are present. In general terms, the serums of normal individuals who have not had paratyphoid fever or who have not been vaccinated against the disease may agglutinate the H antigens in final dilutions up to 1:20; agglutination at 1:80 or higher is indicative of the disease. Normal serums may agglutinate O antigens in final dilutions

up to 1:80; agglutination at 1:160 or higher is also indicative of paratyphoid fever.

In typhoid fever, agglutinins for paratyphoid bacilli may increase but not nearly to the degree that H and O agglutinins do for the typhoid bacillus. In paratyphoid fever some increase of agglutinins for the antigens of the typhoid bacilli may occur but not as much as do agglutinins for the paratyphoid bacillus responsible for the infection. Consequently, serum diagnosis usually requires quantitative agglutination tests with the H and O antigens of the paratyphoid bacilli as well as of the typhoid bacillus. Usually two or three tests at intervals of three to five days are required before diagnosis is established on the basis of increasing agglutinin for the particular paratyphoid bacillus producing infection. The agglutinin absorption test of Castellani may be required.

As in typhoid fever, the diagnosis of paratyphoid fever in an individual previously immunized with typhoid-paratyphoid vaccine largely depends upon observing a progressive increase of agglutinin for the O antigen of the particular paratyphoid bacillus producing the disease.

FOOD INFECTIONS

Food infections are due to the ingestion of living pathogenic micro-organisms in raw or partly cooked foods. They should be distinguished from the *food intoxications* due to the ingestion of bacterial toxins which may occur in uncooked foods or those in which toxins escape destruction during the process of cooking. The most noteworthy example of food intoxication is that due to the ingestion of the exotoxin produced by *Cl. botulinum* contaminating vegetables and escaping destruction during the process of canning.

As a general rule, food infections are due to *Staph. aureus* or bacilli of the Salmonella (paratyphoid) group. The latter include not only *S. enteritidis* and *S. aertrycke* (*S. typhi-murium*) and several subtypes of each, but the Newcastle bacillus, the Newport bacillus and various subtypes, the Thompson bacillus, *S. panama, S. derby, S. aberdeen,* etc., comprising a group of over twenty-five different bacilli.[19]

The food infections are characterized clinically by the signs and symptoms of acute gastro-enteritis. Indeed, some of these may so closely resemble those of bacillary dysentery, especially that produced by the Newcastle bacillus, that several investigators have recently reported outbreaks of "bacillary dysentery" due to infection with the Newcastle bacillus.[20, 21]

Laboratory examinations are indispensable in diagnosis. These embrace bacteriologic examinations of the stools supplemented by bacteriologic examinations of the suspected food or foods. Since staphylococci occur normally in the feces only their presence in foods serves to establish definitely the etiology of food infection due to *Staph. aureus*. As far as the Salmonella or bacilli of the paratyphoid group are concerned, final identification of the infecting bacillus is largely based on biochemical reactions and agglutination tests. The latter are conducted with various immune serum from which group agglutinins have been removed by absorption. Unfortunately, the Salmonella group of bacilli is exceedingly complex serologically. Each species possesses one to three distinct antigenic components in the body of the bacillus (O or somatic antigens) and other distinct components in the flagella (H or flagellar antigens). The latter occur in many species in two alternate phases, the specific phase and the group phase, each possessing different antigens.

THE DYSENTERIES

Dysentery is a general term given to a number of disorders characterized by acute or chronic inflammation of the intestines, especially of the colon, and attended by pain or discomfort in the abdomen, tenesmus, and frequent stools containing blood and mucus.

TABLE 139. SUMMARY OF LABORATORY EXAMINATIONS IN THE DIFFERENTIAL DIAGNOSIS OF THE DYSENTERIES

Examinations	Bacillary	Amebic	Balantidic
Feces	Positive cultures of a bacillus of the dysentery or Shigella group. Heavy cellular exudates of pus cells, epithelial cells, lymphocytes and especially of endothelial macrophages.	Motile trophozoites of *E. histolytica* present. Discharge largely composed of clear mucus streaked with blood; scanty cellular exudate of erythrocytes, degenerated leukocytes, epithelial and tissue cells. Many Charcot-Leyden crystals.	Motile trophozoites of *B. coli* present
Blood	Blood cultures usually sterile but may be positive in severe acute infections. Agglutination reactions by the patient's serum may occur, especially in subacute and chronic infections. Agglutination of the Shiga bacillus 1:64 or higher is positive; agglutination of the Flexner, Newcastle or Sonne strains at 1:128 or higher is positive.	Blood cultures sterile. Negative agglutination reactions with antigens of the dysentery bacilli. Positive complement fixation reactions with antigen of *E. histolytica* may be observed.	Blood cultures sterile. Negative agglutination reactions with antigens of the dysentery bacilli.

Etiologically they may be divided into those due to (1) the dysentery bacilli and allied species belonging to the genus Shigella and producing bacillary dysentery (2) *Endamoeba histolytica,* producing amebic dysentery; and (3) *Balantidium coli,* producing balantidic dysentery. About eighteen different dysentery and paradysentery bacilli belonging to the genus Shigella have been described, but it is evident that some have not been adequately investigated and little is known in regard to their cultural characteristics, biochemical activities, and antigenic structure.[22] As previously discussed, the Newcastle bacillus of the Salmonella group is particularly likely to produce outbreaks of acute gastro-enteritis resembling bacillary dysentery and, indeed, is included among the dysentery bacilli by some investigators. At the present time, however, with the exception of knowledge of the existence of the exotoxin produced by the Shiga strain of *S. dysenteriae,* very little is known in regard to the factors in dysentery bacilli responsible for dysentery in man, and it has not been explained why one species may cause epidemic outbreaks of the disease while a very closely related species does not.

Laboratory Examinations. While clinical manifestations may aid in differentiating among bacillary, amebic and balantidic dysenteries, yet in the final analysis definite diagnosis is based upon laboratory examinations, especially of the stools. Needless to state, etiologic diagnosis is extremely important in relation to specific treatment with chemotherapeutic compounds. The usual or characteristic laboratory findings are briefly summarized in Table 139.

ASIATIC CHOLERA

Cholera is an acute, specific, communicable disease, epidemic in character (especially prevalent in India), caused by a spiral organism or vibrio, the *Spirillum cholerae asiaticae* or *Vibrio comma.* It is characterized clinically by a profuse, painless diarrhea with stools of a so-called "rice-water" character and by vomiting, muscular cramps, and a rapid tendency toward collapse. The diarrhea is often the first symptom and is so severe that the stools quickly lose their form and consistency. Because of the presence of whitish epithelial flakes, they take on the appearance of water in which rice has been cooked—hence the name, "rice-water" stools. One of the striking effects of the disease is the extreme dehydration of the tissues caused by the marked purgation and by the vomiting; in fact, this probably contributes more toward a fatal outcome than the toxemia engendered by the organisms themselves. This fluid loss may vary from one-third of the total body fluid, in mild cases, to more than two-thirds in severe ones. It leads to marked concentration of the blood, with tendency to hemolysis, acidosis, marked reduction in chlorides, suppression of the urine, and, in many instances, to uremia. Recognition of this extreme disturbance of the body fluids is important in treatment, since failure to correct it in the early stages has been responsible for the high mortality rate. The mortality rate has varied in past epidemics from 30 to 80 per cent. The course of the disease usually is comparatively short, recovery ensuing in the nonfatal cases within one to two weeks. Except for the acidosis and uremia already referred to, complications and sequelae are rather uncommon.

The mode of transmission of cholera infection is similar to that of typhoid fever and bacillary dysentery. Infection results from ingestion of food or drink contaminated directly or indirectly by organisms originating either in another infected human patient or in a carrier. Patients with cholera infection discharge an enormous number of vibrios from the intestinal tract, especially because of the numerous bowel movements. If due care is not taken to dispose properly of these infected excreta, they subsequently may contaminate the food or water supply. Small endemic outbreaks of the disease may follow the contamination of vegetables or other uncooked foods (seldom milk because of the tendency to inhibition of the organisms by acid formation) or follow fly transmission; however, the most important and probably the most frequent mode of transmission is through the water supply, a mode of transmission which has been responsible for severe epidemics.

Carrier infection likewise is an important means of conveying the disease. Although large numbers of organisms are produced during the early stages of the disease, they usually disappear after the second week and seldom persist for more than one or two months. Such convalescent carriers are potential sources of contagion. Chronic active carriers are rare, but normal, healthy, passive carriers exist. They constitute an important means of transmission because they are not recognized and may cause contagion in insidious fashion. Some of them may develop the disease themselves, often after a preceding gastro-intestinal upset. They are responsible, not infrequently, for localized outbreaks of endemic character. Carriers are found more often in regions where the disease is prevalent and are rare in other localities.

The significance of direct contact with infected cases, in the spread of the disease, is evidenced by its frequency in overcrowded areas among people living under poor sanitary conditions; also by the spread of past epidemics along the direct routes of commercial travel, or in Asiatic countries along routes taken by pilgrims. The rapidity of its spread is in direct relationship to the speed of communication in these areas.

Laboratory Examinations. In view of the characteristic signs and symptoms of the disease, laboratory examinations may not be indicated or required, except as aids in the diagnosis of mild or atypical cases. Bacteriologic examinations of the stools, however, furnish the most definite and conclusive evidence of the disease when the cholera vibrios are found. Needless to state, such examinations are the only means available for the detection of carriers. Final identification of the micro-organism is based on agglutination tests and the Pfeiffer bacteriolysis test employing a known anti-cholera serum of good titer.

Blood cultures are usually sterile and therefore of little or no diagnostic value. Unfortunately, agglutination tests conducted with patient's serum are likewise of little or no value because of insufficient time for the production of agglutinin in acute cases of the disease. Normal human serums may agglutinate the vibrios in final dilution of 1:10. Higher titers may be observed in individuals who have been vaccinated against the disease within one or two years previously.

PLAGUE

Plague is an acute infectious disease, primarily of rodents, communicable to man and from man to man. It is caused by *Pasteurella pestis* (*B. pestis*). Sweeping, time and again, over large areas of the world with frightful mortality, it has written one of the most terrifying chapters in the history of epidemic diseases. Introduced into Europe from China during the fourteenth century, it produced a devastating epidemic resulting in a loss of more than 25,000,000 people, constituting nearly one-fourth of the entire population of central Europe. Numerous violent outbreaks occurred during the succeeding four centuries. One of the most notable was the Great Plague of London in 1664 and 1665, during which there were nearly 70,000 deaths in a total population of approximately 500,000. Gradual subsidence of the disease took place so that by the middle of the nineteenth century it had almost completely disappeared from Europe. However, in 1894 it reappeared suddenly and without known reason in China (Yunnan province), reached Hong Kong, and spread from there by way of the trade and travel routes in all directions, eventually involving almost every country in the world, including the United States and its possessions. This pandemic was as severe as that of the fourteenth century, especially in India where as many as 5,000,000 deaths resulted in a single decade. It still is endemic in India and other parts of Asia, in Central Africa, and in certain parts of South America (Argentina, Peru and mountains of Ecuador). The first endemic focus in the United States was in California, where sporadic outbreaks occurred between 1900 and 1928 and appeared to be transmitted by ground squirrels. The Gulf States—Louisiana, Texas and Florida—had a few outbreaks between 1914 and 1920 which were quickly controlled. Since 1934 it has not been reported in the United States outside of California and Nevada.

As stated, plague is primarily a disease of rodents, especially of rats, the most important species being *Epimys morvegicus* and *Epimys rattus*. Since the latter lives in closest relationship to man, it is perhaps the most dangerous. Other rats, however, can also be infected, and the danger of plague exists wherever rats are found. Creel has estimated that in the United States alone the rat population is probably as great as the human population with an economic loss of $167,000,000 annually due to their depredations.

Past. pestis, however, is also pathogenic for mice, ground squirrels, various species of ground moles, and flying squirrels. Indeed, as reported by Meyer [23] in 1938 for the Committee on Sylvatic Plague, investigation of the extent of infection of fleas and other insects on the American Continent, by means of pooled samples of thousands of fleas infesting wild rodents, showed the prevalence of plague bacilli in these insects; also their presence in the tissues of flying squirrels and field mice, often without inflammatory lesions. These organisms were virulent for guinea-pigs.

The bacillus may be transmitted among rodents by ingestion because of their cannibalistic habits. Transmission takes place much more often, however, through fleas, particularly the *Xenopsylla cheopis*. *Nosopsyllus fasciatus* and *Pulex irritans* may also be transmitters. Since fleas habitually infesting dogs and cats may also infest rats, the problem of flea extermination must be general. The climatic and geographic distribution of both fleas and rats must also be taken into account in dealing with the disease.

The first development of plague in rats is a heavy blood infection. The bacilli are taken into the intestines of fleas where they can live for a long time and multiply tremendously between feedings. The duration of life of the infected flea will depend largely on the ratio between temperature and moisture. Climatologic conditions have a definite effect on determining the length of time a flea may live and transmit the bacillus from rodent to rodent, from rodent to man, and from man to man.

The exact manner in which fleas infect rodents and man has been a little in doubt. The bacilli undoubtedly occur in the feces and may be deposited on the skin and gain entrance to the tissues through scratching and superficial abrasions. Infection may also occur in the same manner through regurgitation of infected blood by the flea as well as through bites. It is certain, therefore, that while contact infection and other means of direct and indirect transmission may take place, the usual manner of spread of plague is from rat to rat, rat to man, or man to man, by the agency of fleas. The ordinary rat fleas leave the body of the dead rat within about three days and can remain alive about three or four weeks. In the California outbreak infection from ground squirrels to man was definitely shown. According to McCoy, nearly all cases have the peculiarity of showing the primary buboes in the axillae, because the fleas in the course of infection attack the upper extremities; on the other hand, when the disease is contracted from rats, the fleas are more apt to bite the legs and thus produce inguinal buboes.

Such transmission does not hold good, however, for the pneumonic type of plague. This is believed to be due to direct inhalation of the bacilli coughed up by human beings with that form of the disease. The organisms are then sprayed into the atmosphere in droplets of mucus and may remain alive for considerable periods if the weather is cold and charged with moisture. In other words, pneumonic plague is largely direct infection from man to man and rarely from rats. Pneumonic plague is very uncommon in rats although it does affect ground squirrels. The latter animals have been responsible for pneumonic plague in California.

Human carriers have not been commonly regarded as a source of possible transmission of plague. However, patients recovering from the bubonic form of the disease may retain the organisms in their glands for some time, or in the sputum in the recovered pneumonic patient, and thus act as a potential source of contagion. Plague organisms have been found even in individuals with mild glandular swelling, representing apparently unrecognized instances of pestis minor. These sources may account for the occasional endemic outbreaks appearing in localities or under circumstances in which rodent transmission seems unlikely or unimportant.

Clinical Types of Plague. Largely according to the source and manner of infection, plague may, therefore, occur in man in several clinical types as follows: (1) the *bubonic* form which is most frequent. It is characterized by lymphadenitis or buboes and, because of subcutaneous hemorrhages from secondary septicemia, was formerly designated the "Black Death." This type is always caused by flea infection. When due to rat fleas, the inguinal glands are usually involved because the infection commonly arises through the skin of the legs, whereas in infection from squirrel fleas, the axillary and cervical glands are frequently first involved through infection of the skin of the arms or neck. The buboes may reach considerable size and not infrequently break down and discharge infectious pus. The incubation period is three to seven days and occasionally up to fourteen days. The mortality is 60 to 95 per cent.

2. *Ambulatory plague,* or pestis minor, which is a much milder variety of the bubonic type with swelling and tenderness of the glands but with milder constitutional symptoms, giving little or no inconvenience. These cases are dangerous from the standpoint of contagion because so easily overlooked. They are analogous to the latent plague of flying squirrels and mice.

3. *Pneumonic plague,* which is fortunately less common. It is even more fatal than the bubonic type, death occurring almost invariably on the third or fourth day. As previously stated, it is thought to be due to the direct inhalation of the bacillus by droplet infection rather than secondarily to an initial septicemia. It is characterized by peribronchial inflammation and later hemorrhagic pneumonia of the lobar or lobular type.

4. *Septicemic plague,* which may result initially from an overwhelming invasion of the blood stream by the infecting organisms. It is usually fatal, before the lymph glands have a chance to form buboes. It also may be secondary to the bubonic or pneumonic forms and occur as a terminal infection. Constitutional symptoms are quite marked, hemorrhages from the gastro-intestinal tract and of the skin are frequent and severe, and death may take place within twenty-four hours after the onset.

Laboratory Examinations. The laboratory diagnosis of plague in man is largely dependent upon bacteriologic examinations as follows:

1. Smears and cultures of the small vesicles, sometimes occurring on the legs in the early stages, from flea bites, may show the presence of plague bacilli.

2. The bubo, which is generally in the inguinal region, should be aspirated, and the material examined by smears, cultures, and guinea-pig inoculation. In mild or chronic cases, where the diagnosis is in doubt, and the bubo is small, hard, and difficult to aspirate, it should be excised for the preparation of cultures and the inoculation of guinea-pigs with tissue. In frozen cadavers it is stated that the bacillus can be cultivated from buboes up to 102 days.

3. In pneumonic plague the bacilli are readily discovered in the sputums or lung exudates secured by aspiration, by means of smears, cultures and guinea-pig inoculation tests.

4. In severe, and sometimes even in ambulant, cases plague bacilli may be found in blood cultures. Septicemia is not always present. It may appear early or late in the course of the disease, and is subject to marked fluctuations.

5. The serums of normal individuals who have never had plague and who have not been vaccinated against the disease may agglutinate the plague bacillus in final dilutions up to 1:10. On or about the ninth day of bubonic plague the titer may increase and ultimately reach about 1:40. Under the circumstances, the agglutination test is not of any value in early diagnosis, and negative reactions are of no value in excluding the disease. Positive reactions, however, may be of value in the diagnosis of mild or atypical infections. They may also assist in the decision as to whether or not a recovered individual actually had the disease.

6. After death histologic, cultural, and guinea-pig inoculation tests may be relied upon for diagnosis, the best material being the bubo or spleen. In putrid

cadavers the thermoprecipitin test, conducted with extracts of the spleen or bubo, may likewise be used.

TULAREMIA

Tularemia is an infectious disease caused by the *Past. tularensis* (*B. tularense*). It occurs primarily in wild rodents, especially rabbits and hares, and is transmitted secondarily and accidentally to man.

The disease was first described by McCoy [24] in 1911 as a fatal "plague-like disease of rodents" occurring in Tulare County, California, and transmissible to rodents and to monkeys. The following year McCoy and Chapin [25] described the bacillus and named it *Bacterium tularense.*

It remained for Francis,[26] however, between the years of 1917 and 1920, to establish definitely the relationship between an outbreak of this infection in jack rabbits, and that occurring during the deer-fly season in more than two dozen human patients in Utah suffering with a febrile disease previously termed "deer-fly fever." By means of guinea-pig inoculation, Francis isolated the organism either from the blood stream (two cases) or from the pus from the glands (five cases). He proved its identity with the causative organism of the "plaguelike disease of rodents" described by McCoy and gave the disease the name of *tularemia.* Since that time tularemia has been found to be more widely prevalent in human beings than heretofore surmised and has been extensively studied in all parts of the world. It has been reported from practically every state in the Union, from Japan in 1925, from Russia in 1928 where extensive epidemics have since been reported, from Norway in 1929, Canada in 1930, Sweden in 1931, and Austria in 1935. It is probably more common than suspected and appears to be on the increase, although this may be due in part to a greater facility of recognition. Fifteen cases were reported prior to 1924, and 6159 between 1924 and 1935, with a mortality of 4.8 per cent.[27]

Transmission. Tularemia, like plague, is primarily a disease of wild rodents. It is accidentally transmitted to man either by the bites of infected blood-sucking flies or ticks, or by contamination of the hands or the conjunctival mucosa with the tissues or body fluids of infected animals, ticks or flies. The disease exists widespread in nature among wild animals, but rabbits and hares (cottontails, jacks and snowshoes) are most important since they are responsible for more than 90 per cent of the cases of tularemia occurring in the United States, especially in the Western States. It is believed that about 1 per cent of these animals are naturally infected. Since the disease occurs as a septicemia, it is spread among rabbits chiefly by the rabbit tick but also by other blood-sucking ticks, flies and lice. The frequency with which wild rabbits are involved explains its greater prevalence among market men, housewives, and hunters who dress these animals with their bare hands, as well as among laboratory workers who examine them without gloves. Domestic rabbits, though susceptible, seldom are infected naturally, due no doubt to their freedom from ticks.

Other animals in which natural infection has been found include ground squirrels in California and Utah, wild rats in Los Angeles, wild mice in California, quail, grouse and gray foxes in Minnesota, sage-hens and their ticks in Montana, sheep in Idaho, as well as coyotes, deer, opossums, skunks and other animals. However, these animals are very uncommon sources of human infection.

Occasional instances of tularemia have followed the bites of certain animals, for example the coyote, ground squirrel or hog, but these cases were due probably to the fact that the mouth parts of the animals were contaminated by the ingestion of infected dead rabbits, rather than to actual infection of the animal. Likewise, one instance of transference of the infection from human to human occurred in a woman who contracted the disease from the prick of her thumb, sustained while she dressed the tularemia ulcer of her fly-infected son. This transference was purely accidental, since *tularemia is not directly communicable from man to man.*

The most common method of transmission of the disease to man, however, is by contamination or self-inoculation. The specific acts in which this inoculation is brought about are listed by Francis as follows: (1) Skinning and dressing of rabbits by the market man for his patrons. (2) Dressing of rabbits for the table by the housewife, servant, or cook, or by the hunter. (3) Touching of the eye by the farmer while he is pulling ticks from his horse or cow. (4) Skinning and cutting up of jackrabbits for fish bait, coyote bait, animal food, or for the table or market. (5) Performance of or assistance by laboratory workers at autopsies on infected guinea-pigs, rabbits, or white mice, or the handling of infected living rabbits or guinea-pigs or infected living ticks. In most of these instances a small wound, either in the form of a scratch, cut, or puncture is produced during the process and acts as the portal of entry. In the insect-borne cases, the bite constitutes the wound. Infection may occur also, however, in the absence of a definite wound and apparently through the unbroken skin.

Finally, infection may be transmitted by the ingestion of incompletely cooked rabbit meat from infected animals. This is a rather uncommon source, however, since the organisms are readily destroyed by heat.

Clinical Types. The incubation period may be as short as a few hours or as long as twenty-one days, with an average of two to five days. Extensive clinical investigations have shown that the disease manifests itself in four principal clinical varieties:

1. The *ulceroglandular type* occurs in about 90 per cent of cases. The onset is frequently abrupt with chills, fever, drenching sweats, headache, malaise, and various gastro-intestinal symptoms. Great prostration is the rule. A primary sore develops at the site of inoculation (fingers, hand or elsewhere) at the onset, or within twenty-four to forty-eight hours. It begins as a small papule which rapidly enlarges and ulcerates, leaving a punched-out ulcer with a necrotic floor. It is accompanied by a regional adenopathy. Superficial lymphangitis does not occur unless there is a secondary streptococcal or staphylococcal infection. There is usually a primary bacteremia which persists for seven to ten days and sometimes results in focal necroses in the lungs, liver, spleen and lymph nodes. Capillary bronchitis, bronchopneumonia or lobar pneumonia develop in approximately 18 per cent of cases. Pleural effusion may occur at the onset, but develops more

frequently during the course of the infection. It may or may not be associated with pulmonary lesions.

If resistance is low the initial bacteremia may progress into a septicemia or the latter may develop secondarily. This occurs in the majority of fatal cases (5 to 6 per cent). Under these conditions the patient is desperately ill with drenching sweats, chills, high septic fever, severe prostration, tympanites, diarrhea, slight jaundice, splenomegaly, hepatomegaly, acute glomerulonephritis, cyanosis, pneumonia and delirium progressing into coma and death.

2. The *oculoglandular type*, which is similar to the preceding except that the primary lesion is a severe conjunctivitis, usually unilateral and accompanied by regional lymphadenitis. As the disease progresses, small discrete ulcers of the conjunctivas appear. This form is due to contamination of the eye with infected material during the handling of diseased animals. It is much less common than the preceding type. Septicemia with a fatal termination may occur.

3. The *glandular type*, which is very uncommon and corresponds to the first form except that there is no evident primary lesion or ulceration. Septicemia with a fatal termination may occur.

4. The *typhoid type*, which is most frequent in laboratory workers, caused by accidental infections. Protracted fever is its most characteristic symptom, there being no primary or regional lymphadenitis. The site of infection is obscure, and suggests that it is possibly transmitted either through the unbroken skin or by droplet infection *via* the respiratory route.

The disease is generally rather benign, the mortality being about 5 to 6 per cent. Some patients remain ambulatory throughout the course of the disease. In most cases convalescence is slow and protracted, with a persistent weakness and malaise that lasts several months or even as long as a year or more. The development of pulmonary complications is of bad prognostic import. Death in most instances is due to septicemia, pneumonia, or to severe meningitis.

Laboratory Examinations. Laboratory examinations are usually indispensable in diagnosis. The local lesions occurring on the fingers, hands or elsewhere accompanied by lymphadenopathy are readily mistaken for pyogenic infections or syphilis. The typhoid type may be mistaken for typhoid fever, paratyphoid fever or brucellosis. The results of laboratory examinations may be summarized as follows:

1. Cultures of the lesions on blood dextrose cystine agar, or on a coagulated egg-yolk medium, may be tried but are likely to be unsatisfactory because of partial healing, difficulties in cultivating the bacterium, or the presence of secondary infections obscuring *Past. tularensis*. The examination of direct smears is hardly worth while. Darkfield examinations for *T. pallidum*, however, are frequently indicated for excluding the possibility of a chancre. The presence of streptococci or staphylococci in cultures should not be permitted to override a clinical suspicion of tularemia.

2. Blood cultures should be made routinely, not only in the early stage, but at subsequent intervals. Hormone glucose cystine broth is preferred. The bacterium, however, grows slowly, frequently requiring four days or longer. This

factor adds to the difficulty of bacteriologic diagnosis. In severe septicemia the patient may succumb before a positive blood culture is observed.

3. The bacterial skin test of Foshay [28] is stated to be an aid in early diagnosis, since positive reactions may occur on the third or fourth day of the disease. Since the allergy usually persists for years after recovery, positive reactions do not necessarily mean active disease. When they are observed in the presence of suspicious lesions, however, the latter is usually the proper interpretation. Apparently cross reactions with suspensions of *Br. abortus* and *Br. melitensis* have not occurred in the few patients tested, although they share a common antigenic constituent with *Past. tularensis*.

The test is conducted by injecting about 0.05 cc. intradermally. The positive reaction begins to appear in thirty-six hours with edema and erythema, which progresses until, at forty-eight hours, there is a tender elevated area of erythema with a pale hard center. The reaction lasts for about five days and then slowly recedes, leaving a small area of brownish pigmentation. Positive reactions have been obtained by Foshay as early as the second day of the disease and were highly specific. This makes the test of special value, since the agglutination reaction is usually negative during the first week of the disease and often during the first twelve days. The allergy persists for long periods of time and as long as fourteen months in the cases studied by Foshay.

4. Foshay has also described an intradermal test employing goat or horse anti-tularemia serum. Positive reactions are characterized by erythema and wheal formation lasting ten to fifteen minutes. An intradermal injection of normal control serum from the same animal species is required. However, the occurrence of nonspecific reactions greatly reduces its diagnostic value.[29]

5. After the first week of the disease, agglutination tests conducted with the patient's serum are the most constant and reliable diagnostic procedures. The serums of normal individuals do not usually agglutinate antigens of *Past. tularensis* in final dilutions higher than 1:10 to 1:20. Therefore, if a titer of 1:10 or 1:20 is followed a few days later by one of 1:40 or higher, the presence of tularemia should be strongly suspected. Agglutination at 1:80 or higher is regarded as diagnostic for tularemia, provided brucellosis can be excluded. Cross-agglutination reactions between Brucella and tularensis antigens occur and may cause confusion. In a series examined by Francis,[28] 129 out of 570 human tularemia serums tested gave positive cross-agglutination reactions, whereas the remainder failed to show any agglutination of Brucella in spite of high titers (1280 to 2560) for *Past. tularensis*. This tendency to cross agglutination makes it essential that *every serum submitted from patients suspected of having either tularemia or brucellosis should be examined for agglutinins to both organisms.*

In tularemia, agglutinins do not usually appear in the blood until some time during the second week, reaching their maximum during the fourth to the seventh week. Consequently, the test should be repeated at weekly or more frequent intervals until either tularemia is excluded or its presence indicated by the occurrence of agglutinins in increasing titer. A rising titer is perhaps the most definite corroborating diagnostic procedure next to isolation of *Past. tularensis* by culture. It is important to remember, however, that agglutination tests conducted a week or two after the bacterial skin test may raise the titer to 1:20 or 1:40 in the absence of tularemia.[29] Agglutinins may persist in recovered cases for many years, even up to twenty-four years, after recovery. In the experience of Francis, no recovered case has ever become negative.[30]

6. The opsonocytophagic reaction tends to parallel agglutination in time of occurrence and is stated to be of value in differentiating brucellosis from tularemia, when cross agglutination occurs.[29]

GLANDERS

Glanders is an infectious disease of equine animals (horses, mules and asses) caused by *Malleomyces mallei* (*B. mallei*). It is sometimes transmitted to human beings, attacking chiefly those who come into close contact with horses and mules. Infection results most frequently from contamination of a scratch or wound with a glanderous discharge, but primary infection of the nasal mucosa may occur. Many cases have been reported in laboratory workers; indeed, probably no organism, with the possible exception of *Past. tularensis,* is so dangerous to work with as the glanders bacillus. But when one considers the high infectivity of the organism in cultures, it is surprising that the disease is not more common among those who come into contact with glandered animals. It is possible, however, that occult glanders may occur among human beings more frequently than surmised with the possibility of being readily overlooked.

In man, glanders may be acute or chronic, and it may be localized chiefly in the respiratory organs, or in the skin and subcutaneous tissues. In *acute glanders* there is generally fever, a mucopurulent nasal discharge, and a degree of prostration out of all proportion to the clinical signs. A generalized pustular eruption is frequent. Almost invariably, death occurs within ten days. In *chronic* glanders there may be coryza and multiple subcutaneous and intramuscular abscesses, often associated with lymphadenitis. Nodules may form in the mucosa of the respiratory tracts and have a tendency to break down and ulcerate. Necrotic foci may also appear in the bones and other viscera. The disease may remain active for weeks, months, or even years. The mortality is about 60 per cent. Sometimes after apparent recovery the disease may break out again; latent periods up to ten years have been observed.

Laboratory Examinations. Laboratory examinations have proved of great value in the diagnosis of human glanders, as they have in the detection of the disease in horses and mules. They may be summarized as follows:

1. Cultures of discharges on appropriate media may reveal the presence of the bacillus but are frequently negative. Smears are of little or no value. Blood cultures may be positive in severe acute glanders.

2. From the bacteriologic standpoint the intraperitoneal inoculation of male guinea-pigs with fragments of diseased tissue, scrapings from ulcers, or some of the nasal discharge is usually preferred. A positive reaction is indicated by the development of an acute orchitis, usually on the second or third day—constituting the *Straus reaction.* Together with the orchitis, there are severe general symptoms with the development of grayish nodules in the spleen and other internal organs,

proving fatal in twelve to fifteen days. While the test is not absolutely specific, nevertheless it is frequently of diagnostic value.

3. Unfortunately, the agglutination test is not usually helpful in diagnosis. The serums of normal persons may have a titer of 1:100 but in acute glanders may reach as high as 1:1000 to 1:2000. On the other hand, the titer is usually very low in chronic glanders and several cases have been reported in which no increase of agglutinins could be found.

4. The complement fixation test, which has proved very valuable in the detection of chronic and latent glanders in horses and mules, is sometimes helpful in the detection of the disease in human beings.[31]

5. The mallein skin test may be helpful in the detection of chronic and latent glanders, as it has in horses and mules, but it has been used so seldom in man that it is difficult to express any opinion of its value.

ANTHRAX

Anthrax is primarily a disease of sheep, cattle and goats and, to a lesser extent, horses and hogs, transmissible to man. It is due to the *Bacillus anthracis,* a large spore-forming bacillus which has the distinction of being the first micro-organism proved definitely to bear a specific etiologic relationship to an infectious disease. In man the disease occurs more frequently in Europe, China and other foreign countries than in the United States, because of the greater prevalence of the disease among the lower animals in those countries.

Transmission. Since the spores are highly resistant to destruction they may survive for long periods of time in the wools, hairs and hides of infected animals and in the soil. Man is usually infected indirectly by handling such materials in the leather, wool and hair industries, especially those imported from abroad. Domestic products seldom cause the disease. Direct infection may result from contact with infected animals or from the process of skinning them. Infection from person to person is rare and with ordinary care physicians, nurses and attendants incur no unusual risks. Workers in hairs and hides may be carriers of the spores on the skin, as there are recorded instances of other members of the family being infected by contact with them.

The spores survive not only in wools, hairs and hides, but likewise in dusts. Methods for the disinfection of these products have greatly reduced the incidence of infection but the problem has been the destruction of spores by processes which do not injure the materials. As an example of their high resistance is the fact that men have been infected by the use of shaving brushes, in spite of precautions taken in their manufacture to destroy the spores. There are also cases on record where human beings have become infected through wounds of the feet contaminated with soil.

Clinical Types. The disease in men usually occurs as an infection of the skin, called external or *cutaneous anthrax.* It is characterized by a local lesion frequently mistaken for a furuncle or carbuncle and known as the "malignant pustule." Severe local lesions are diffuse in character and designated as the "malignant edema" type. As expected, the lesions occur generally on the exposed parts of the body—the hands, arms, the face, neck and chest. Frequently, infection is initiated by razor cuts or the picking of pimples, or by scratches and abrasions. Bronchopneumonia may result from the inhalation of dust contaminated with spores. This is *pulmonary anthrax* and is known as "wool sorters' disease." *Intestinal anthrax* may result from the swallowing of spores in dust, or through the contamination of uncooked foods by the hands, or by the ingestion of the partially cooked meats of infected animals. The mortality in external or cutaneous anthrax is usually 10 to 20 per cent while it is about 90 per cent in pulmonary and intestinal anthrax. Septicemia may occur in all types and always adds greatly to the mortality of the disease.

Laboratory Examinations. Bacteriologic examinations of the cutaneous lesions are of great value in diagnosis. Indeed, they should be made routinely in all cases of furunculosis occurring among those especially exposed to the risks of anthrax, like workers in the hide, hair and leather industries. Bacteriologic examinations of sputum are also of diagnostic value in pulmonary anthrax. The examination of smears of cutaneous lesions is valuable in relation to early diagnosis but the latter usually requires confirmation by cultural examinations. Blood cultures should be made routinely in all cases, including those presenting only small cutaneous lesions.

Since only small and variable amounts of agglutinins, opsonins and complement-fixing antibodies appear during the course of anthrax, serologic examinations have not proved of value in diagnosis. Extracts of the organs of animals succumbing to the disease contain a thermostable antigen (apparently the polysaccharide haptens of the capsules) which give a precipitate with potent antianthrax serum. This is the Ascoli precipitin reaction.

LEPROSY

Leprosy is a chronic infectious disease due to *Mycobacterium leprae* (*B. leprae*). The etiologic relationship of the bacillus to the disease, however, is based almost solely on the regularity with which it is found in the lesions by direct smears and tissue examinations. It has never been cultivated with certainty and leprosy has never been indubitably transmitted to the lower animals; in other words, the postulates of Koch for establishing the etiologic relationship of an organism to a disease have never been completely fulfilled.

Leprosy is practically worldwide in distribution with a calculated incidence of approximately four million victims in 1933. In Europe it occurs chiefly in Norway, Russia and Iceland. It is more common in China, the Philippine Islands, Hawaii and Central Africa. In the United States the chief endemic foci appear in California, Texas, Louisiana, Mississippi, Florida, Minnesota, Wisconsin and New York, but sporadic cases appear in every state of the Union.

Transmission. It is now generally agreed that leprosy is transmitted through prolonged and intimate contact. But the exact mode for transmission is unknown. Many physicians and nuns working among lepers for years have escaped infection, while a few others, notably Father Damien, have contracted the disease. Instances of familial transmission have been clearly traced. Sometimes the child has developed leprosy before its parents. In other instances one or both parents have developed it before the child. The disease is obviously not highly contagious and certainly not nearly as much so at present as in Biblical times. Other factors in transmission are obscure. It is possible that the bacillus may be carried from an infected person to others by flies, bedbugs, mosquitoes and other blood-sucking insects but, if so, the transference is apparently mechanical as there is no evidence that the organism has a stage of its life cycle in an insect. Leprosy of rats, a disease occurring naturally among these rodents, presents many analogies to human leprosy, but has not hitherto yielded data of significance in relation to the latter, owing in large part to difficulty in cultivating the specific organism involved as is true in the case of leprosy of human beings.

The portals of entry of the bacillus into the human body are thought to be the mucous membranes of the nose and pharynx, the respiratory and gastrointestinal tracts, and the skin. Early localization of lepra bacilli in the nose, along the septum, makes the nasal portal of entry highly probable and stained smears of that region are of considerable aid in bacteriologic diagnosis. Indeed, it is likely that the upper respiratory tract and the skin are the chief, if not the only, portals of infection.

Clinical Types. The period of incubation is unknown, but has been estimated by various observers to be from three to six or more years. Clinically the disease has been divided into the *cutaneous* (nodular) and *neural* (anesthetic) types, but as a matter of fact most cases are a mixture of the two. Purely cutaneous leprosy is seldom seen. According to the system agreed upon in the International Congress on Leprosy, held in Manila in 1931, cases are recorded as C for cutaneous and N for neural lesions, with *1* indicating slight, *2* moderate, and *3* severe involvement. Death is rarely due to leprosy itself. Tuberculosis, nephritis and amyloid disease are the most frequent causes.

Laboratory Examinations. Laboratory examinations for lepra bacilli are of considerable diagnostic value and especially in the cutaneous or nodular type of the disease, as follows:

1. Smears of the nasal mucosa stained by the acid-fast method may be positive in 80 per cent or more of cases. In obtaining material the most satisfactory method is to scrape the mucosa gently with a blunt scalpel. Sometimes the bacilli are present in the nasal mucosa before skin nodules have developed; thus they are said to be demonstrable occasionally in apparently healthy contacts.

2. In the nodular form a piece of skin removed with a safety razor blade or sharp pair of scissors curved on the flat, may be pressed downwards on a slide for the preparation of a film to be stained for acid-fast bacilli. Or sections of the tissue may be prepared and stained by the acid-fast method. As a general rule, large numbers of acid-fast bacilli are found. In early cases of generalized leprosy, the bacilli can often be found in clippings from the lobule of the ear. If there is any doubt as to whether the organisms found in the nose or skin are true leprosy bacilli, it is advisable to inject a suspension of ground-up material into guinea-pigs; if they are leprosy bacilli, there will be no result; if, on the other hand, they are tubercle bacilli, the animals will develop tuberculosis. When the lungs are involved,

the sputum may contain lepra bacilli, which are easily distinguished from tubercle bacilli by cultures and guinea-pig inoculation tests.

3. The complement fixation test has been employed for the diagnosis of leprosy, but it appears to possess no practical value since positive reactions may be observed with antigens of tubercle bacilli.[32] In this connection it may be stated that the serums of a large percentage of cases of leprosy yield biologic nonspecific Wassermann and flocculation reactions, as discussed in Chapter 18.

4. Attempts to diagnose leprosy by means of allergic skin tests (lepromin) have been made [33,34] but apparently they have not proved of practical value.

YAWS

Yaws, or frambesia tropica, is a chronic infectious disease due to *T. pertenue.* It affects the dark-skinned races of tropical countries and is characterized principally by large papillary cutaneous eruptions. The disease occurs particularly in childhood. The most common site for the primary lesions is on the lower extremities. The secondary eruption usually appears two to four weeks later. Yaws is more vulnerable than syphilis to treatment with penicillin and the organic trivalent arsenicals (arsphenamine, neoarsphenamine, mapharsen).

The organism is indistinguishable from *T. pallidum* of syphilis. Indeed, opinion is divided as to whether yaws is a tropical form of syphilis or a separate disease; available evidence favors the latter view. In experimental infections of rabbits, yaws apparently does not protect against infection with syphilis. Similar, though inconclusive, observations have been made in human beings. The determination of the precise relationship of yaws to syphilis, however, must await further investigations on the immunology of both diseases.

Transmission is chiefly or entirely by contact and is favored by nakedness and unsanitary conditions. The disease is not as a rule of venereal origin. Flies can transmit the disease experimentally but whether they and other insects transmit it to human beings is uncertain. Some observers believe that infection occurs through the skin and is transmitted by gnats. In contradistinction to syphilis, the disease is apparently not transmitted *in utero.*

Apparently there is no natural immunity to yaws. Acquired immunity is similar to that in syphilis except that it is apparently developed in higher degree and is more persistent following recovery. *Treponema pertenue,* therefore, does not usually infect the cardiovascular or central nervous systems and other viscera as is the case in untreated syphilis. Indeed, it is believed that recovery may occur spontaneously.

Laboratory Examinations. Laboratory examinations are of great value in diagnosis. In contradistinction to syphilis, large numbers of the organisms are present in the cutaneous lesions so that they are readily found by darkfield examinations, although morphologically indistinguishable from *T. pallidum,* as previously stated. The organism also produces a reagin exactly similar to that produced in syphilis. Consequently, the Wassermann and flocculation tests with serums yield from 90 to 100 per cent positive reactions in well-defined cases of the disease. As in the serologic tests for syphilis, however, biologic falsely positive reactions may occur in malaria, leprosy and other conditions, as discussed in Chapter 18. As a general rule, there are no spinal fluid changes with normal total cell counts, no increase of protein, negative colloidal gold and negative Wassermann reactions.

RELAPSING FEVER

Relapsing fever is an acute infectious disease due to *Borrelia recurrentis* (*Spirochaeta obermeieri*). There is, however, a group of spirochetes producing this disease which may be sharply differentiated from each other by immunologic procedures. For example, the relapsing fever of Europe is ascribed to *Bor. recurrentis,* that of Africa to *Bor. duttonii,* that of India to *Bor. carteri,* and that of the United States to *Bor. novyi.*

The disease is common in Eastern Europe, India, Africa, and most of the warmer countries. A few epidemics have taken place in the United States, especially in the southern portion. It comes on abruptly with a chill, fever, generalized pains, great prostration and occasionally delirium. The spleen enlarges and jaundice may develop. After three to ten days the fever and other symptoms subside. After a free interval of one to three weeks a relapse may occur but it is usually less severe and of shorter duration because of acquired immunity. Three, and even four, attacks may develop although the disease is not often fatal.

Relapsing fever is *transmitted* by lice and ticks. The organisms are highly invasive. They rapidly multiply in the blood during the onset of each attack and diminish as it declines. They are never numerous but may be found in wet or stained smears of blood and by darkfield examination. Mice, rats and guinea-pigs are susceptible. Intraperitoneal inoculation with human blood may therefore be infective even when the spirochetes can no longer be found by direct examinations.

Laboratory Examinations. Examinations of the blood for the spirochetes are of great value in diagnosis. They are usually readily detected by darkfield examinations or in stained smears. The numbers present vary from case to case. At the height of the first pyrexial attack they are often numerous—several organisms to a field—but they may be relatively few and hard to find. During the decline of the fever their numbers diminish, the spirochetes become less motile, and frequently assume irregular shapes or accumulate in rosettes. These changes are regarded as indicative of lysis or agglutination due to the production of antibodies. After the subsidence of the fever they can no longer be found microscopically in the blood. But the intraperitoneal inoculation of mice with blood may give rise to infection. At the onset of a relapse they again become demonstrable microscopically in the blood, although not always in such large numbers as in the first attack. During the interval between the pyrexial attacks the spirochetes remain latent in the tissues.

THE LEPTOSPIROSES

The leptospiroses embrace a group of diseases of worldwide distribution due to infection with various species of pathogenic leptospira. The latter commonly occur among wild rats, field mice, dogs and other animals. Man is usually infected through contact with water contaminated by the excreta of these animals and especially by the urine of wild rats in which the leptospira commonly occur. In this connection the diseases of man due to leptospiral infections have been recently reviewed very thoroughly by Walch-Sorgdrager.[35]

Probably the best known of the leptospiroses is infectious jaundice. It was first described by Weil [36] in 1886, although the cause was unknown until 1916 when Inada and his colleagues [37] proved that Weil's disease was due to an organism which they named *Spirochaeta icterohaemorrhagica*. Shortly thereafter Noguchi [38] found that it was not a true spirochete but a leptospira and renamed it *Leptospira icterohaemorrhagiae*. In this connection, however, it should be mentioned that in 1907, Stimson [39] described an organism called *Spirochaeta interrogans*, which he discovered in silver-stained sections of the kidneys of an individual succumbing to what was thought to have been yellow fever. Since this organism is now known to be identical with *Lept. icterohaemorrhagiae,* Sellards [40] has recently suggested that the latter be named *Lept. interrogans*. Various German investigators have called it *Spirochaeta icterogenes*.

Infectious Jaundice (Weil's Disease). Jaundice of variable degree is likely to develop in all of the leptospiral diseases of man. In infectious jaundice, or Weil's disease, it is particularly characteristic. However, as pointed out by Havens and his colleagues,[41] it appears that Weil's disease may occur in subclinical or mild ambulatory forms without jaundice; also in moderately severe forms with or without jaundice. Under the circumstances this type of leptospirosis of man may be far more prevalent than commonly surmised. If this is true, it is apparent that the disease may be readily overlooked unless the physician keeps it in mind in the illnesses of those especially exposed to leptospiral infection through exposure to rats, and particularly stagnant water likely to be contaminated by *Lept. icterohaemorrhagiae* excreted in the urine of these animals, as well as, possibly, by gophers and foxes.

Furthermore, it is now known that *Lept. icterohaemorrhagiae* produces a disease in dogs with acute jaundice ("yellows") similar to acute Weil's disease in human beings. As in man, dogs are infected by wild rats. In other words, dogs constitute another source of infection of man with this organism. Dogs are also subject to infection with *Lept. canicola* producing a nonjaundice type of leptospirosis known as "canine typhus." Unfortunately, this organism may also infect man and is also sometimes responsible for infectious jaundice.[42,43] In other words, in the United States and other countries, leptospirosis with severe jaundice (Weil's disease), or without marked jaundice, may be due to infection with *Lept. icterohaemorrhagiae* transmitted by rats or dogs, or to infection with *Lept. canicola,* transmitted by dogs only. In Denmark infections have also been reported due to *Lept. sejroe*.

The great majority of infections of human beings with *Lept. icterohaemorrhagiae* and *Lept. canicola* are due to contact with water contaminated by the excreta of wild rats or dogs. Under the circumstances most cases have occurred among sewer workers, divers, seamen, reed cutters, swimmers and those working in abattoirs, butter factories, barns and stables. Indeed, Stiles and Sawyer [44] have properly called attention to leptospiral infection, including Weil's disease, as an occupational hazard. Infection is considered possible through the intact skin and mucous membranes, as well as through these tissues after injury. Usually it follows contact of the abraded or sodden skin with stagnant water, slime, or mud contaminated with the leptospira. Infection may also follow bites of wild rats, dogs and ferrets; [45] also bites by white rats.[46] It is also possible for infection to be transmitted by coitus [47] and the urine of infected human beings has been regarded as hazardous.[48] Several infections have been contracted during the conduct of postmortem examinations as well as by laboratory workers in the conduct of bacteriologic and serologic examinations.

As far as leptospirosis with severe jaundice and bleeding is concerned (infectious jaundice or Weil's disease), the incubation period is usually six to twelve days. The disease is of sudden onset with chills, fever, headache, cramping muscular pains, conjunctival congestion, intestinal disturbances and albuminuria. On or about the seventh day the fever falls by lysis while jaundice and purpura develop in 40 to 60 per cent of cases along with anemia, emaciation, marked prostration, nervous disorders and cardiac failure. Death occurs mainly during this period, the mortality varying from 5 to 50 per cent with an average of about 33 per cent. Otherwise, convalescence sets in after the thirteenth or fourteenth day.

Leptospira are highly invasive and may be found in the blood during the initial febrile stage by guinea-pig inoculation. On or about the seventh day they lodge particularly in the kidneys and are abundantly excreted in the urine until the twenty-fifth day when their excretion begins to decline, generally ceasing after forty to sixty days. An intermittent, symptomless, "after" fever may develop during convalescence, probably due to the absorption of lysed leptospira or their products.

Whether or not natural immunity exists cannot be definitely stated, but acquired immunity is quickly developed. Unlike other spirochetal infections, recovery from infectious jaundice confers a definite and lasting immunity which is apparently humoral in character. Agglutinins and lysins may appear in the blood as early as the fifth day after onset, following which the leptospira begin to disappear. They usually reach the maximum concentration on or about the fifteenth day after onset of the illness and remain in the blood for many years after recovery. It is stated that the Wassermann and flocculation reactions may become temporarily positive.

Other Leptospiral Diseases. Stagnant water may also contain various nonpathogenic leptospira grouped under the designation of *Lept. biflexa* [49] or *Spirochaeta pseudoicterogenes.* Uhlenbuth and Zuelzer [50] have reported observations which suggest that these water leptospira are avirulent forms of *Lept. icterohaemorrhagiae* whose virulence may be restored by animal passage, but these results have not been confirmed by other investigators.

Lept. grippotyphosa,[51] however, is regarded as the cause of a disease prevalent during the summer and early autumn months in southern Bavaria and certain parts of Russia, variously known as *"swamp fever,"* "water fever," "field fever," "harvest fever," "mud fever" or "slime fever." Clinically it closely resembles Weil's disease in a mild form—the case mortality is less than 1 per cent—but there is no jaundice except occasionally in the scleras. The organism is immunologically distinct from *Lept. icterohaemorrhagiae* but the animal reservoir of the infection is unknown.

Japanese seven-day fever (*nanukayami*) is a relatively mild febrile disease, with practically no jaundice, due to infection with *Lept. hebdomadis* [51] which is carried by the field mouse. There are few, if any, fatalities but a common sequel is opacification of the vitreous humor.

Hasami fever, occurring in Japan, is also a mild disease due to infection with *Lept. autumnalis*, conveyed by the field mouse and wild rats. Jaundice occurs only in the scleras and the case fatality rate is low (1.9 per cent); opacification of the vitreous humor, however, occurs in about 90 per cent of cases. A similar disease, known as *Pomona fever*, occurs in Australia. Little is known of the leptospiral diseases characterized by jaundice that are endemic in Sumatra, Batavia, Macassar, Borneo and the Andaman Islands and designated as *Rachmal disease, Salinem disease, Andaman A fever*, and other names.

Laboratory Examinations. As previously stated, it is possible that leptospirosis of human beings due to infection with *Lept. icterohaemorrhagiae* or *Lept. canicola* occurs more frequently in the United States and other countries than is generally surmised. It is also likely that some cases regarded as catarrhal jaundice, or jaundice due to other causes, are leptospiral infections. For these reasons it is important for physicians to keep in mind, more frequently than they generally do, the possibility of leptospirosis. Unfortunately, laboratory examinations are not always capable of detecting the disease, especially subclinical or mild forms of it, but when leptospirosis is severe enough to produce jaundice (as in Weil's disease), they are always likely to be very helpful in diagnosis. They may be briefly summarized as follows:

1. During the initial fever the leptospira may be found in the blood by darkfield examinations. Undoubtedly, however, the presence of artefacts due to particles of fibrin are important sources of error. Under the conditions, the results of darkfield examinations should always be checked by stained smears of the blood, as the leptospira are readily stained.

2. It is always advisable to inoculate guinea-pigs intraperitoneally with blood during the first week of the disease, although these animals do not always develop infection and rarely develop jaundice or succumb to infection sooner than a week to ten days after inoculation. On the development of illness, the animals should be killed with the preparation of sections of the kidneys and liver stained by a silver impregnation method for leptospira.

3. Cultures of the blood are also advisable, as the leptospira are more readily cultivated than other spirochetes.

4. After the first week guinea-pigs should be inoculated with the urine of the patient, as leptospira are commonly excreted during the seven to twenty-five days after the onset of symptoms. They may continue to be excreted in diminishing numbers up to forty to sixty days.

5. Normal human serums agglutinate *Lept. icterohaemorrhagiae* and *Lept. canicola* in final dilutions up to 1:40. On or about the fifth day after the onset of illness the titer may reach about 1:100. By the fifteenth day it may reach as high

as 1:100,000. Strongly positive reactions continue to be observed for about seven weeks after which the agglutinins gradually decline to titers of 1:300 to 1:900. However, mild cases may show no increase of agglutinins or but weak increases which return to normal within a few weeks.

6. As stated in Chapter 17, the agglutination-lysis test conducted with patient's serum on or after the tenth day of the disease is also of diagnostic value.

7. A mouse protection test has also been proposed, but its value cannot be stated at the present time. It consists of inoculating mice intraperitoneally with lethal doses of *Lept. icterohaemorrhagiae* or *Lept. canicola* along with patient's serum. Mice between four and six weeks of age must be used.

8. Needless to state, the icterus index or van den Bergh tests for hyperbilirubinemia are indicated when jaundice is suspected.

9. The urine is likely to show the presence of albumin, casts, and increased numbers of erythrocytes due to nephritis by leptospira in the kidneys.

10. In fatal cases diagnosis is largely based on finding leptospira in sections of the liver, kidneys or other organs, stained by a silver impregnation method.

LISTERELLOSIS

Listerella monocytogenes (*Bact. monocytogenes*) is a small gram-positive motile bacillus discovered by Murray and his colleagues [52] in 1926 as the cause of a spontaneous disease occurring in stock rabbits and guinea-pigs, characterized chiefly by acute mononucleosis with occasional involvement of the mesenteric glands and focal necrosis of the liver, terminating fatally. The bacilli produce hemolysis on blood agar and, since they may be beaded, are sometimes mistaken for streptococci or diphtheroid bacilli in bacteriologic examinations.

Since 1926 the organism has been reported by various investigators as causing a similar disease in gerbilles of South Africa, meningo-encephalitis in sheep, cattle and goats, a respiratory tract disease in silver foxes, and massive myocarditis with necrosis in chickens.

Julianelle [53] has recently warned that listerellosis may eventually become an important disease among human beings. As a matter of fact, it is now known that *L. monocytogenes* may produce an acute meningo-encephalitis in man.[54, 55, 56] It has been observed in all ages from infancy to maturity, although the majority of nineteen cases thus far reported have occurred in young individuals with a mortality of about 70 per cent. The spinal fluid changes are those characteristic of a suppurative meningitis. The cells are largely of the mononuclear type. The bacilli are readily found in smears and are often ingested by the mononuclear cells; in some instances they have been mistaken for diphtheroid bacilli. Some cases have shown leukocytosis with mononucleosis. Septicemia may occur [57] with metastatic pneumonia and focal necrosis of the liver.

In view of the fact that *L. monocytogenes* infections of rabbits and human beings may produce leukocytosis with mononucleosis, it has been suggested that the bacillus may be the cause of infectious mononucleosis of human beings. As stated in Chapter 26, however, the evidence is not conclusive and there is a suspicion that the disease may be due to infection with a filtrable virus.[58,59] Normal human serums may agglutinate *L. monocytogenes* in final dilutions of 1:5 to 1:20. In infectious mononucleosis titers ranging from 1:20 to 1:160 have been observed.[53] While agglutinin for sheep corpuscles (heterophil antibody) is characteristically produced in infectious mononucleosis, various investigators have observed that this antibody is not produced in rabbits by *L. monocytogenes*.[53,60] Furthermore, while temporarily positive Wassermann and flocculation tests may occur in infectious mononucleosis, as discussed in Chapter 18, the reagin is not produced in rabbits inoculated with living or heat-killed *L. monocytogenes*.[60]

TYPHUS FEVER

Epidemic typhus fever is a specific acute infectious disease due to *Rickettsia prowazekii,* appearing chiefly in epidemic form and transmitted from person to person through the bite of an infected body louse or rat flea. After an incubation period of five to twenty days (average twelve) it is manifested clinically by abrupt onset with fever, chills, nausea, vomiting and prostration, with marked cerebral symptoms like headache, vertigo and even delirium or convulsions; also by the occurrence of a continued fever of about two weeks' duration, terminating by crisis or rapid lysis and by the appearance on the fourth day of a macular eruption which tends to become hemorrhagic. Bronchitis is such a frequent complication that it is really a part of the disease. Bronchopneumonia is the chief cause of death. The mortality rate of the disease varies in different epidemics—in severe ones it may be more than 50 per cent; on the other hand, in milder outbreaks, the rate may be only 5 to 10 per cent. The average mortality rate during epidemics probably is between 10 and 20 per cent. Murine typhus fever is caused by *R. typhi* (*R. mooseri*).

Clinical Types. In 1910 Brill[61] described a disease subsequently known as *Brill's disease,* which he had observed in endemic form in New York since 1896. This is now regarded as a mild type of typhus fever. The general clinical characteristics are the same as those of the epidemic type of the disease except that they are milder and fatalities very uncommon (less than 1 per cent). Furthermore, the disease does not seem to spread to other patients in the ward or home, but occurs in isolated sporadic cases, in contrast to the epidemic form of Old World typhus. Cross-immunity experiments have shown that Brill's disease is identical with Mexican typhus fever—monkeys inoculated with the latter were immune against the former, and vice versa. Subsequent investigations likewise have not only confirmed the identity of Brill's disease with the epidemic or ordinary form of typhus fever, but have also established the fact that the endemic type differed only in being transmitted by the rat flea instead of by the body louse.

As the result of these investigations it is now recognized that typhus fever exists in two clinical types:

1. *Epidemic typhus,* also referred to as Old World or European typhus, prevalent mostly in European and Asiatic countries as well as in Mexico. It is transmitted from man to man by the body louse, is highly contagious and likely to be

rather malignant in some epidemics. It is most common in the winter or spring, especially in cities and in crowded places like camps, ships, jails, hospitals—hence the names "jail fever," "ship fever," "camp fever" and "hospital fever."

2. *Endemic typhus,* formerly designated as *Brill's disease,* transmitted to man only secondarily from rats through infected fleas, and resembling plague in this respect. It occurs only sporadically or in small localized outbreaks, especially among food-handlers or those who are likely to come in contact with rats, and rarely or never in epidemics. It has no predilection for the lower classes of society nor has it any relationship to a condition of lousiness, as does the epidemic type. It is now more common in rural than in urban communities and especially in the late summer and fall in contrast to the winter and spring prevalence of the epidemic type. It has been reported in this country from nearly every state in the union, and while formerly more prevalent mainly in the South Atlantic states and along the Gulf coast, seems to be spreading inward to other states. At present over two thousand cases occur in the United States each year with a mortality of about 0.6 per cent.

Laboratory Examinations. Laboratory examinations are of great value and especially as aids in the diagnosis of mild and atypical cases of the disease. They may be summarized as follows:

1. Normal human serums do not ordinarily agglutinate antigen OX_{19} of *Proteus vulgaris* in final dilutions higher than 1:25 or 1:50. By the sixth day of typhus fever, however, the Weil-Felix reaction is usually positive with a titer of about 1:80, on the tenth day about 1:1280, and at the end of two weeks about 1:5120 or higher It is advisable to conduct duplicate tests with antigen OXK of *P. vulgaris* because in tsutsugamushi disease the titer is high, while low or negative with OX_{19}, as discussed more fully in Chapter 17.

2. Agglutination tests can also be conducted with suspensions of *R. prowazekii* of both murine and human varieties. Cross agglutination tests carried out by Zinsser and Castaneda [62] have shown much higher agglutination titers with the homologous rickettsiae than with rickettsiae of the other strain.

3. Complement fixation tests conducted with antigen prepared from yolk sacs infected with the wild rat strain of endemic typhus fever have also proved highly specific and of diagnostic value in the diagnosis of present or past typhus fever and its differentiation from other rickettsial diseases, like Rocky Mountain spotted fever and "Q" fever.[63]

4. In the majority of cases the intraperitoneal injection of 5 cc. of the patient's blood into each of two guinea-pigs during the first few days of illness will result in a febrile disease with involvement of the scrotum or scrotal sac.[64] This phenomenon, which is the result of rickettsial infection, is almost diagnostic of typhus and Rocky Mountain spotted fevers. By killing a guinea-pig and making smears from the scrotal sac, a presumptive diagnosis can usually be made, since in typhus fever the cells are packed with rickettsiae. In Rocky Mountain spotted fever, however, the organisms are fewer and larger, and tend to show the characteristic lanceolate forms in smears and sections of tissue.

5. In order to establish diagnosis during convalescence, cross-protection tests with strains of typhus and Rocky Mountain spotted fever rickettsiae and patient's serum have proved of value.[65]

ROCKY MOUNTAIN SPOTTED FEVER

Rocky Mountain spotted fever, also known as Rocky Mountain fever or spotted fever, is an acute infectious disease due to the *Rickettsia rickettsii*, transmitted to man by various ticks. It closely resembles endemic typhus fever in its clinical manifestations, being characterized similarly by sudden onset with chills, headache, neuromuscular pains affecting the back and extremities, moderately high continued fever terminating by lysis, and by the appearance of a macular eruption which subsequently becomes petechial, affecting at first the ankles, wrists, forehead and lumbar regions, and finally the entire body. The incubation period is two to twelve days and in most instances between three to seven days.

The disease was first described by Maxcy[66] in 1899. Outbreaks occurred in Bitter Root Valley in Montana and Snake River Valley of Idaho and were originally believed to be limited to those regions. Subsequent observations showed that the disease occurred in other states of the Rocky Mountain region (hence the name) up to the western coast. In the past few years cases have been reported in other states as well—in fact, at present a total of twenty-six states have shown sporadic outbreaks. The more recent of these have shown a characteristic geographic distribution in that they affect particularly those states along the Atlantic seaboard which are east of the Appalachian Mountains, extending from New York to Georgia. This variety has been called the *eastern type* of spotted fever, as distinguished from the *western type* affecting the Rocky Mountain area. Cross-immunity tests have shown that such separation is purely arbitrary, since both types appear to be caused by the same organism, although transmitted by slightly different vectors. It is difficult to estimate accurately the frequency of each type of spotted fever—probably not less than 750 cases occur annually in the western area and about 200 in the eastern area.

The disease varies in severity in different areas. In Idaho the mortality is less than 5 per cent; on the other hand, in Montana some outbreaks have had a mortality rate reaching as high as 80 per cent. The mortality of the eastern type averages about 25 per cent. As with typhus fever, this difference in mortality rate may be due to a reduced virulence of the rickettsiae because of different arthropod vectors. The highest mortality occurs among older individuals.

The transmission of spotted fever to man usually results from the bites of various infected ticks. The rickettsiae have been found to invade all of the tissues of these vectors, including even the testicles and spermatozoa, which accounts for their transmission by succeeding generations.

Although these insects are widely distributed, only certain species seem capable of being vectors and of transmitting the organisms to man. The western type of the disease is transmitted by the wood tick, *Dermacentor andersoni,* found especially on such smaller wild animals as squirrels, rabbits, chipmunks, badgers, and prairie dogs. The eastern type is transmitted by the dog tick, *Dermacentor vari-*

abilis. These ticks become infected either by feeding on infected animals or through transovarial infection. That man acts merely as an accidental host and plays no part in the perpetuation of the rickettsiae can be appreciated when one considers the life cycle of the tick, which usually requires two years for its completion.

Laboratory Examinations. Laboratory examinations are usually very helpful in the diagnosis of Rocky Mountain spotted fever and especially in differentiating the disease from severe measles, typhoid fever, smallpox, severe meningococcal meningitis, streptococcal septicemia, and Colorado tick fever. Unfortunately, differentiation from typhus fever is not readily accomplished by laboratory examinations, but this is usually possible according to geographical distribution and clinical manifestations, as discussed in Chapter 17. The results of laboratory examinations may be summarized as follows:

1. While the Weil-Felix reaction may be negative before or at the time of appearance of the skin eruption, positive reactions with serum titers of about 1:160 or higher are commonly observed shortly thereafter. If negative, a second test should be conducted between the tenth and fifteenth days of the disease. At that time positive reactions are observed in the majority of cases and almost invariably by the time convalescence is established.[67] There is no agreement on the best strain of *P. vulgaris* to employ as antigen; antigens OX_{19} and OX_2 give about the same results, as discussed in Chapter 17.
2. The intraperitoneal injection of male guinea-pigs with 5 cc. amounts of patient's blood is of helpful diagnostic value. Extremely severe scrotal lesions usually result. Smears of the scrotal lesions usually show lanceolate forms of rickettsiae within the endothelial cells. Both typhus and spotted fever rickettsiae produce febrile reactions in guinea-pigs, as well as characteristic lesions in the brain, which distinguish them, not only from other febrile diseases, but even from one another.
3. Differentiation between the two diseases may also be aided by cross-immunity tests, as discussed in Chapter 17.
4. Complement fixation tests conducted with antigens of *R. dermacentroxenus* are also stated to be of diagnostic value and of aid in differentiating Rocky Mountain spotted fever from typhus fever and "Q" fever,[68] although its exact value cannot be expressed at the present time.
5. Leukocytosis of varying degree occurs along with secondary anemia. In elderly patients the urine may show the presence of albumin and casts.

Other Rickettsial Diseases. The only other rickettsial diseases in which laboratory examinations are definitely of diagnostic value are São Paulo typhus fever, tsutsugamushi disease of Japan (due to infection with *R. tsutsugamushi*) and South African tick fever due to *R. sanguineus*. In São Paulo typhus fever, Weil-Felix reactions conducted with antigen OX_{19} are usually positive; the disease is not typhus but caused by rickettsiae immunologically identical with those of spotted fever, with a mortality of about 70 per cent. In tsutsugamushi disease, positive agglutination reactions with antigen OXK are commonly observed; negative reactions occur with antigens OX_{19} and OX_2. In South African tick fever, positive Weil-Felix reactions are stated to occur with antigens OX_{19}, OX_2 and OXK. To the best of my knowledge there are no laboratory examinations of value in the diagnosis of trench fever (probably due to infection with *R. quintana*), fièvre boutonneuse, Kenya fever, mite fever of Sumatra, "rural typhus" of Malay, or "Q" fever (due to *R. burneti*) except, possibly, Weil-Felix agglutination tests with various antigens of *P. vulgaris.*

RICKETTSIALPOX

Rickettsialpox is a new rickettsial disease caused by *Rickettsia akari,* transmitted by bites of infected mites (*Allodermanyssus sanguineus*), an ectoparasite of house mice (*Mus musculus*).[69-72] The rickettsiae are transmissible to mice, guinea-pigs and chick embryo, but not to monkeys.

Following the period of incubation, which is unknown (probably about 10 days), the disease is ushered in by the development of a firm, red papule at the site of the mite bite. This papule develops into a deep-seated vesicle which ultimately shrinks and dries to form a black eschar. About a week after the development of this local lesion there is an abrupt onset of chills, fever, sweats, headache, backache and general muscular pains followed in three or four days by a rash. The latter is maculopapular in character and develops into vesicles frequently resembling those of certain stages of chickenpox. Regional, but not generalized, lymphadenopathy develops with splenomegaly in some cases. Recovery occurs with no sequelae.

Laboratory examinations usually show no anemia but moderate leukopenia. Negative agglutination reactions have been observed with different strains of *P. vulgaris* OX_{19}, OX_2 and OXK although positive complement fixation reactions usually occur with antigens of *R. akari,* as discussed in Chapter 17.

YELLOW FEVER

Yellow fever is an acute infectious disease characterized by fever, jaundice, albuminuria, and hemorrhages, including the vomiting of blood. The fever is not remarkably high, and the jaundice is not intense, but there is rapidly developing necrosis of the liver. Under epidemic conditions the majority of deaths occur toward the end of the first week of illness, with a mortality of about 60 per cent among adults. The disease is rarely fatal, however, in young children. Sometimes the symptoms are of only moderate severity, then suddenly become severe during convalescence. In contrast to rapidly fatal infections, there are patients without jaundice who develop merely a mild fever of short duration.

The Commission of the United States Army in Cuba in 1902 established that yellow fever is due to a filtrable virus. This was confirmed by Stokes, Bauer and Hudson[73] in 1928. They found that the disease was transmissible to rhesus monkeys, and this opened the way for intensive investigations. The virus in both man and monkey has a special affinity for the liver where it causes necrosis. It was discovered subsequently that the virus was also transmissible to white mice and guinea-pigs where, because of a great increase in its neurotropism, it provoked encephalitis with corresponding decrease of viscerotropism, indicated by a decrease in its ability to produce necrosis of the liver in both man and monkeys.

In 1932, Haagen and Theiler [74] succeeded in cultivating this neurotropic virus of mice in a medium of minced chick embryo and serum-Tyrode solution. They also discovered later that the viscerotropic virus could be cultivated in a medium of mouse embryonic tissue and also in one of chick embryo or mouse testis.

The Army Commission of Reed, Carroll, Lazear and Agramonte also confirmed the opinion of Findlay that the virus was transmitted by the females of the *Aedes aegypti* species of mosquitoes. This solved the mystery of its epidemiology and proved that the disease was not transmitted by fomites. Their classical experiments also showed that the virus was present in the blood of patients during the first three days of illness and that these mosquitoes became infective about twelve days after sucking the blood. It was shown subsequently, however, that if the insects were kept at 37° C., the virus multiplied more rapidly so that their bites became infective within a few days.

The discoveries soon brought about a sharp reduction in the incidence of yellow fever, at least of the urban type, by the screening of patients and the eradication of mosquitoes. But rural, jungle, or sylvatic yellow fever is another problem, since *A. aegypti* is frequently absent, and nothing is known of the vector or the hypothetic permanent reservoir of the virus. This type is ordinarily restricted to the lower animals and is only accidentally transmitted to human beings. Persons who become infected, however, may serve as a source of infection for *A. aegypti* mosquitoes on entering a community where these insects exist. Nothing can be done, consequently, to prevent the spread of "jungle yellow fever" except active immunization of human beings in localities where it prevails. Fortunately, a single subcutaneous injection of the vaccine is apparently highly effective. Another curious situation is the fact that the virus has not invaded the Orient, where susceptible people and susceptible mosquitoes exist in abundance. Both dengue and yellow fevers are carried by *A. aegypti* in the same manner, yet, while dengue virtually belts the tropical world, yellow fever does not go along with it in the Orient.

Laboratory Examinations. Unfortunately, there are no laboratory examinations of proved value as aids in the diagnosis of yellow fever. Of course, the icterus index and van den Bergh tests are of value when jaundice is suspected. Laboratory examinations are also of value in the detection of leptospirosis, especially infectious jaundice, and its differentiation from yellow fever, as previously discussed.

Laboratory examinations, however, are of value in the detection of acquired immunity to the disease, since virus-neutralizing [75] and complement-fixing [76] antibodies, as well as precipitins, occur in the blood. By these methods it has been found possible to detect yellow fever in regions where it was thought to have disappeared. Furthermore, by testing serums from persons of various ages in a community, it has been found possible to estimate at what date yellow fever was last present there. Viscerotomy [77] has likewise proved valuable in this connection. This consists in removing liver tissue postmortem from persons dying less than eleven days after the onset of any febrile illness, regardless of the clinical diagnosis. Because of the characteristic lesions of yellow fever, histologic examinations of such specimens enable one to determine with considerable accuracy whether the disease was yellow fever. Many thousands of liver specimens have been examined and the presence or absence of yellow fever determined in regions where it would have been otherwise impossible.

ACUTE POLIOMYELITIS

Acute poliomyelitis (*Heine-Medin disease*) is due to a filtrable virus experimentally transmissible to monkeys, cotton rats, mice and possibly guinea-pigs. The disease is of worldwide distribution and occurs both sporadically and epidemically. In temperate and colder climates epidemics usually occur during the late summer months. Commonly known as *infantile paralysis,* because of its high incidence among children over one year of age, the disease also occurs among adults. The virus probably enters through the upper respiratory and especially the intestinal mucosa, as well as, possibly, the skin. It is apparently transported to the central nervous system along the dendrites and axones of nerve cells. Healthy carriers play an important rôle in transmission. The virus occurs in the feces and is highly resistant to destruction. Poor sewage disposal may have some bearing on its spread, with the possibility of transmission by flies.

The incubation stage is not definitely known but probably varies from 3 to 18 days. Undoubtedly most cases occur in the abortive form without paralysis or other pathognomonic symptoms. However, the virus, upon reaching the central nervous system, may produce a widespread polio-myelo-encephalo-meningitis resulting in the spinal, progressive ascending, bulbar, meningitic, cerebral, cerebellar, polyneuritic, transverse myelitic or anomalous forms of the disease.

Laboratory Examinations. Unfortunately, there are no characteristic laboratory changes in the disease. Cultures and animal inoculation tests of cerebrospinal fluid, blood, feces or nasopharyngeal washings for the virus are not available for diagnostic purposes. Indeed, there are no laboratory examinations of value in the diagnosis of the abortive form, but the following are usually of helpful diagnostic value in the acute stages of the other forms of the disease:

1. Leukocytosis, due to an increase of the polymorphonuclear neutrophils, occurs frequently. The sedimentation rate of erythrocytes may be increased.

2. The amount and pressure of the cerebrospinal fluid may be increased. The fluid is colorless and water-clear but may be faintly opalescent (ground-glass appearance). A fine coagulum may develop on standing.

3. The total cell count, which should be made as promptly as possible, usually shows from 15 to 50 and sometimes up to 1200 or more cells per c.mm. The latter are usually composed of lymphocytes and monocytes but early cases almost always show polymorphonuclear response in the early stages.

4. The total protein of the spinal fluid may be normal but is commonly slightly increased. The glucose is commonly normal or slightly increased. The chlorides are normal.

5. The colloidal gold test commonly shows a "leutic" (Zone II) or "meningitic" (Zone III) type of curve. Complement fixation tests are of no diagnostic value. The same is true of serum-virus neutralization tests.

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PART THREE

TECHNIC OF LABORATORY EXAMINATIONS

33

BLOOD EXAMINATIONS

COLLECTION OF BLOOD

Cheap diluting pipets, counting chambers and cover glasses are responsible for errors. Pipets with broken tips are especially likely to be inaccurate. It is recommended that only the best apparatus be employed and scrupulous attention be given to technical details.

1. Blood may be obtained from the ball of the middle, or ring, fingers of adults, or from the great toe or heel of infants.

2. Rub the finger briskly or place the hand in warm water to promote good circulation. If cold and clammy, too much squeezing is required, resulting in error.

3. Clean the finger with 70 per cent alcohol or acetone-alcohol and dry. Do not prick a wet finger as the blood will not form a round drop.

4. The "sticker" should have a broad sharp-cutting edge. Do not use a round needle or pin. Simple or spring-type lancets are satisfactory. A Hagedorn needle, or Bard-Parker blade (size 11) pushed through a cork may be employed. Cleanse with alcohol.

5. Hold the ball of the finger tightly between the thumb and index finger until the skin is dark red. Puncture the finger across rather than parallel with the lines of the skin (see Fig. 28, page 453), with a firm, quick stroke deep enough to cause immediate flow of blood.

6. Wipe off the first drop of blood with cotton or gauze.

7. Well up good-sized drops of blood for the hemoglobin estimation, for filling the erythrocyte and leukocyte counting pipets and for making blood smears as indicated.

8. Be sure to fill accurately each pipet with the proper diluting fluid immediately after taking blood (Fig. 50). Mix thoroughly. If the pipets are to be carried any considerable distance, stretch a broad rubber band over the ends to prevent leakage; or, a special device may be employed to prevent the escape of contents.

9. Prepare two or more smears on *perfectly clean, grease-free and polished slides.* For this purpose touch the end of a slide to a large drop of blood and place the slide on a flat surface. Hold a second slide between the thumb and third finger and place one end at a 30 degree angle in the drop of blood. As soon as the blood has spread entirely across the end of the spreader, with a firm, steady motion push the latter to the left (Fig. 51). The smear should be even and

neither too thick nor too thin (Fig. 52). The slower the movement, the thicker the film; the greater the angle, the thicker the film. Avoid all unnecessary pressure because of the fragility of the cells. A good film should be smooth and without waves. The edges should be even and the film should not extend to the edges or end of the slide. Allow to dry in the air. Protect against flies or other insects. The slides should be stained within 24 hours for best results.

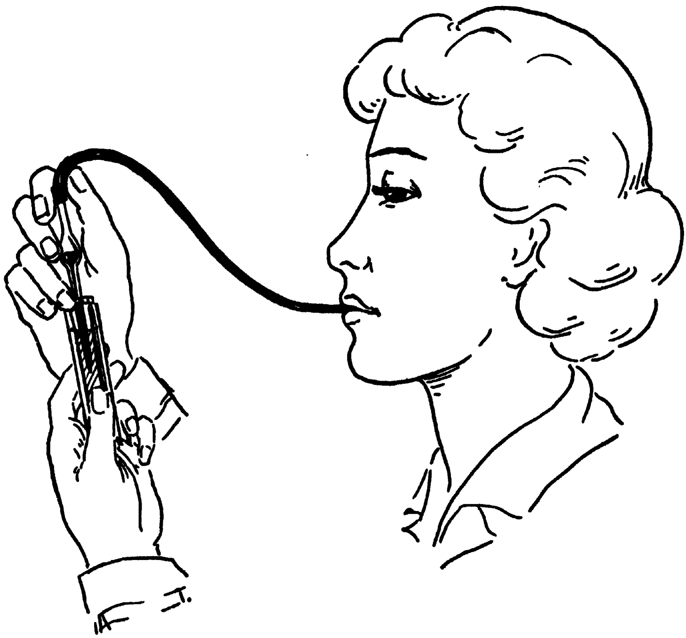


FIG. 50. CORRECT METHOD FOR FILLING BLOOD PIPET WITH DILUTING FLUID.

10. Blood smears may also be made on cover glasses. They should be *perfectly clean and free of grease.* Take up a small drop of blood on one without touching the surface of the skin and place it on the second in such manner that the corners do not overlap. As soon as the blood spreads out between the glasses, draw them apart in a plane parallel to their surface. Dry in the air.

11. Methods for obtaining blood by venipuncture are described on pages 448 to 452.

Methods for Cleaning Apparatus. 1. Pipets and counting chambers should be cleaned immediately or as soon after using as possible.

2. Draw water through pipets until all traces of blood and serum are removed.

3. Without drying, draw through alcohol or acetone.

4. Draw through ether (may be omitted if acetone is used).

5. Draw air through until the pipet is dry (if properly dried the bead in the bulb should be freely movable).

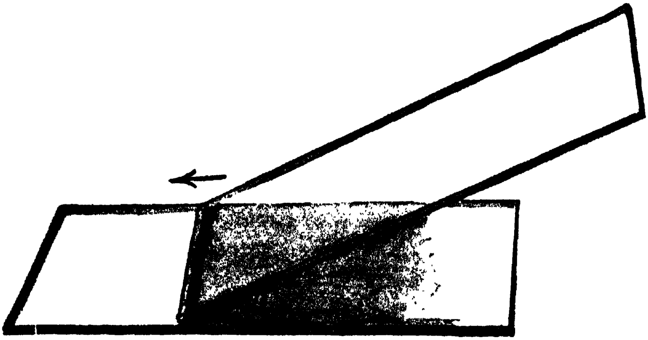


Fig. 51. Method of Preparing a Blood Smear.

Correct angle of the spreader.

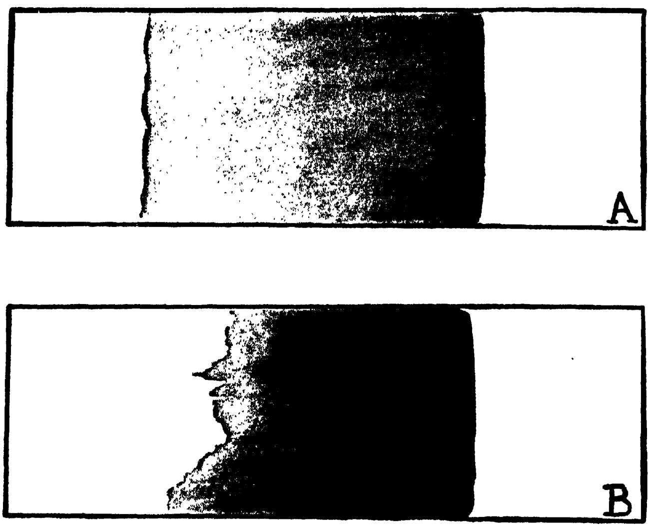


Fig. 52. Blood Smears.

A, a satisfactory thin, evenly spread smear; *B,* an unsatisfactory thick smear.

6. If blood has dried in the stem of a pipet, remove it with horsehair or fine stiff wire and fill pipet with antiformin, nitric acid or bichromate-sulfuric acid cleaning fluid and allow to stand overnight; then clean thoroughly as described.

7. Immediately after use the ruled area, the surface of the slide, and the cover glass of the counting chamber should be cleaned with water and dried with a soft lint-free cloth. If this is not done, the lines will become partly obliterated with debris. If diluted blood has been allowed to remain on the slide, or the

ruling becomes indistinct, it may be necessary to immerse the slide in decinormal sodium hydroxide or in one of the solutions mentioned above for cleaning pipets. The Levy-Hausser chamber may also be washed with alcohol and ether without damage. Do not use xylol or other cement solvents.

METHODS FOR THE ESTIMATION OF HEMOGLOBIN

Tallquist Method. This method, which provides a scale of colors graded to represent the hemoglobin content from 10 to 100 per cent, may be used but is very inaccurate and shows only gross changes. The basis of the scale is 15.8 gm. of hemoglobin per 100 cc. of blood equals 100 per cent. Absorbent paper is supplied in a book accompanying the scale. The method is as follows:

1. Blot a drop of blood with a sheet of the absorbent paper.
2. Set aside for a few seconds and, as soon as the gloss has disappeared, fold the paper back of the drop and compare the color with the scale by allowing the blood-stained portion of the paper to appear at the various holes of the scale.
3. Read the percentage of hemoglobin at the side of the color which it matches best. Compared with more accurate methods the readings are usually too low.

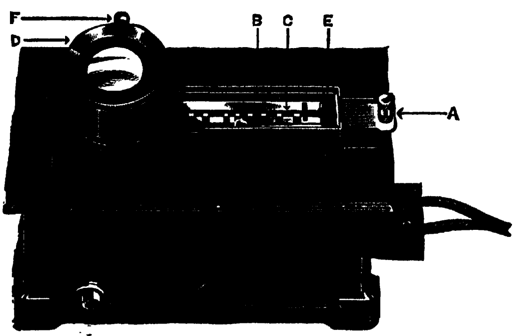


FIG. 53. CLINICAL MODEL OF THE HADEN-HAUSSER HEMOGLOBINOMETER.

A, Movable carrier; *B*, Comparator slide; *C*, Cover glass; *D*, Reading microscope; *E*, Wedge-shaped channel; *F*, Shutter. (From Kolmer and Boerner: *Approved Laboratory Technic,* 4th ed., D. Appleton-Century Company, Inc.)

Haden's Method. This method employs the new Haden-Hausser hemoglobinometer (Fig. 53) in which hemoglobin is converted into acid hematin and the color compared with a permanent color scale of tinted glass.

1. Draw blood to the mark 0.5 of a leukocyte pipet and decinormal hydrochloric acid to 11 (gives a 1:20 dilution). If the hemoglobin is below 50, draw blood to 1 and the acid to 11 (gives a 1:10 dilution).
2. Shake well and allow to stand for thirty minutes.
3. Blow out several drops and allow the blood to run into the channel of the slide at the end of the cover glass. The channel fills by capillarity. A thin uniform film will also extend by capillarity from the dilution channel and cover the

comparator slide above the color standard. This insures a light transmitting surface common to both dilution channel and standard.

4. A color-matching reading is now made according to directions accompanying the instrument and the results expressed in grams of hemoglobin per 100 cc. of blood.

5. For expressing the hemoglobin in percentage, multiply the grams per 100 cc. by factors which vary according to age and sex. Up to fourteen years of age the average factors are the same for either sex as follows: (a) From birth to ten days, average 4.5; (b) from one to five months, average 6.0; (c) from six months to seven years, average 7.5; (d) from eight to fourteen years, average 6.75; (e) from fifteen to nineteen years, average for males 6.3 and females 6.6; (f) from twenty to sixty years, average for males 5.9 and females 6.4; (g) over sixty years, average for both sexes 6.5.

6. For a discussion of hemoglobinometry see page 24.

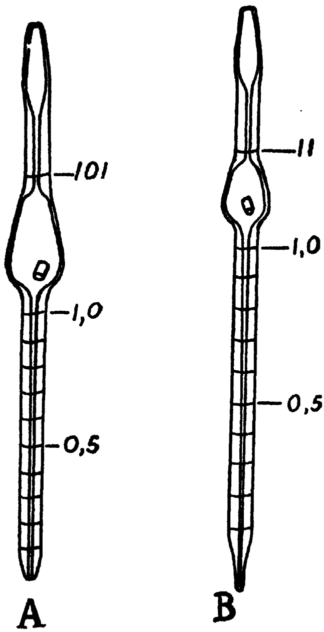


FIG. 54. BLOOD COUNTING PIPETS. *A*, pipet for counting erythrocytes; *B*, pipet for counting leukocytes.

METHOD FOR COUNTING ERYTHROCYTES

1. Draw blood up exactly to the 0.5 mark of the Thoma pipet marked 101 (Fig. 54). If the Trenner automatic pipet is used, draw blood by suction until the stem is nearly full and then discontinue suction and allow the blood to reach the extremity automatically.

2. Immediately draw up diluting fluid to the mark 101, thus making a dilution of 1:200 in either pipet, while rotating the pipet between the thumb and forefinger. Use 0.85 per cent saline solution or Hayem's solution prepared as follows:

Water (distilled)	200.0 cc.
Sodium chloride c.p.	1.0 gm.
Sodium sulfate (crystals)	5.0 gm.
Mercuric chloride	0.5 gm.

3. The diluting fluid should be crystal clear and filtered, if necessary, to be free of artefacts.

4. The ruled area of the Levy-Hausser counting chamber and the cover glass must be carefully cleaned and absolutely free of dust or lint.

5. Place the cover glass in position over the ruled area, using gentle pressure to insure accurate adjustment. The Levy-Hausser chamber is provided with a

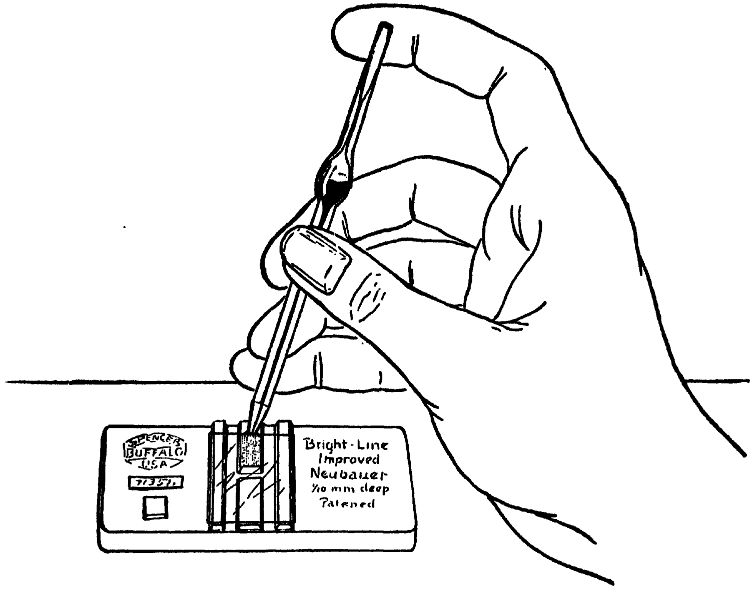


FIG. 55. METHOD FOR FILLING COUNTING CHAMBER BY CAPILLARY ATTRACTION.

pair of clips to prevent any movement during the count. While continuing pressure on the cover glass, slide the centrally placed clip into position simultaneously.

6. Close the tip of the pipet by means of thumb. Sharply kink the rubber tubing over the other end and place the second finger over the kinked tubing. Trenner pipets are more fragile than the Thoma pipets and when filling, cleaning or attaching rubber tubing, the capillary stem should be held between the thumb

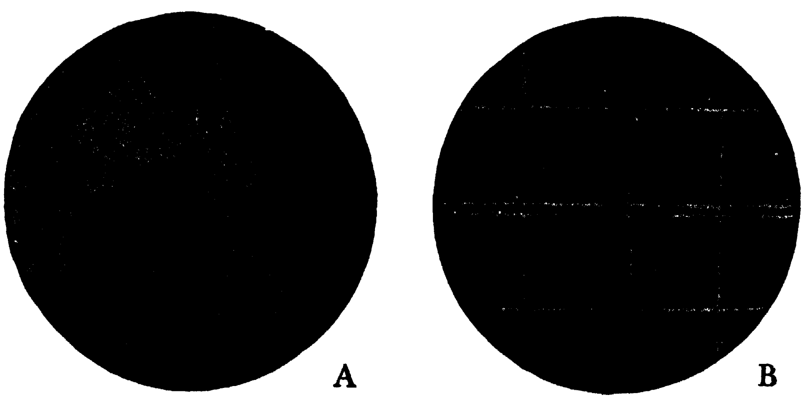


FIG. 56. CORRECT (A) AND INCORRECT (B) FOCUSING IN THE COUNTING OF ERYTHROCYTES.

and forefinger to avoid strain on the bulb. Rotate the pipet well for several minutes, holding in a horizontal position, and finally shake sidewise.

7. Expel the fluid from the stem of the pipet and *without loss of time* touch a drop to the end of the polished surface bearing the ruling, allowing the drop to

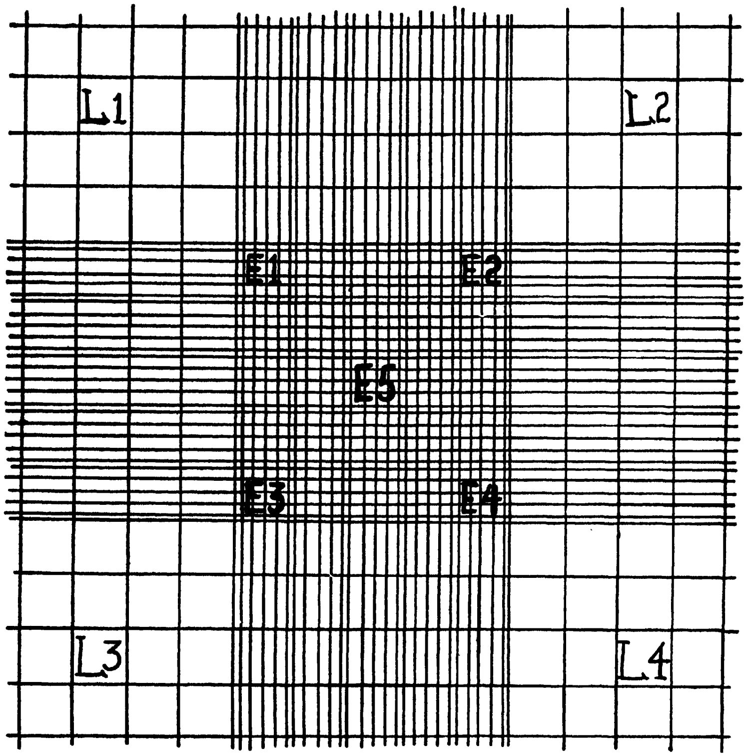


FIG. 57. IMPROVED NEUBAUER COUNTING CHAMBER.

The numbers L_1, L_2, L_3, and L_4 indicate the parts of the slide used in counting leukocytes. The numbers E_1, E_2, E_3, E_4 and E_5, and the areas between the double lines, indicate the areas used in counting erythrocytes.

flow under the cover glass (Fig. 55). The suspension should not flow into the moats on either side, nor should any bubbles form under the cover glass.

8. Allow two or three minutes for the corpuscles to settle.

9. Examine under the high-dry lens of a microscope. Center the light and reduce its volume by lowering the condenser and partially closing the diaphragm for sharp definition.

10. Locate the finding line which leads to the ruled-off area. *Carefully avoid touching the cover glass with the lens, as this would disturb the corpuscles and lead to error in the count.*

11. When correctly focused, the corpuscles are sharply defined and the rulings appear as well-defined *black* lines. When incorrectly focused, the ruled furrows appear as *white* lines, and the corpuscles, which lie above the plane of the ruled surface, are out of focus (Fig. 56).

12. The counting slide will be found to have a number of small squares marked upon it. The size of these small squares is 0.05 millimeter by 0.05 millimeter or 0.0025 sq.mm. When the cover slip is in place there is a chamber formed measuring 0.1 millimeter in depth. Therefore, the small squares are in reality cubes measuring 0.00025 c.mm. (0.05 × 0.05 × 0.1 millimeter).

13. Count all the cells in squares E_1, E_2, E_3, E_4 and E_5 (Fig. 57), covering 80 small squares. Add four zeros to the total, which will give the total erythrocytes per c.mm. of undiluted blood. In counting cells in each square enclosed by double lines, count all cells touching the inner lines of the right and top of the square. Do not count any cells touching the lines on the left and bottom of the square. The difference between the number of cells in any two blocks should not be more than 15 cells. If this is the case, the mixing was not complete or the chamber was dirty.

14. The following *sources of error* should be kept in mind and carefully avoided: (a) Inaccurate dilution due to faulty pipets or technic; (b) too slow manipulation, allowing a little of the blood to coagulate; (c) inaccuracy in the counting chamber, and especially in its depth, due to inaccurate cover glass, faulty manufacture, loosening of parts, etc.; (d) presence of yeasts and other artefacts in the diluting fluid; (e) the delay in filling counting chamber after shaking pipets; (f) uneven distribution of the cells.

15. For the normal number of erythrocytes according to age and sex see page 12.

METHOD FOR DETERMINING THE VOLUME OF ERYTHROCYTES

The determination of the volume of packed erythrocytes in a given amount of blood is a valuable procedure in the diagnosis and differentiation of the anemias. From the erythrocyte count, hemoglobin estimation and cell volume, the volume index, saturation index and mean corpuscular volumes can be determined. Various hematocrit methods for determining the volume of erythrocytes have been described; the method of Wintrobe is as follows:

1. The instrument is a flat-bottomed narrow glass tube 11 cm. in length, of about 2.5 to 3 mm. inside bore (Fig. 58). A centimeter-millimeter scale, commencing at the level of the inside bottom, is etched on the glass.

2. In conducting the examination, 5 cc. of blood is drawn from an arm vein in the usual manner and placed in a dry, clean test tube carrying 6 mg. of ammonium oxalate and 4 mg. of potassium oxalate. With this mixture there is no shrinkage in the volume of packed erythrocytes, and other corpuscular constituents remain unchanged. The mixture is readily prepared by dissolving 1.2 gm. ammonium oxalate and 0.8 gm. potassium oxalate in 100 cc. neutral distilled water. From a buret 0.5 cc. of this solution is measured into each of a series of small test tubes and allowed to dry.

3. The hematocrit tube is filled with blood (about 0.7 cc.) by means of a pipet made from glass tubing drawn out to such length that it can be passed to the bottom of the tube.

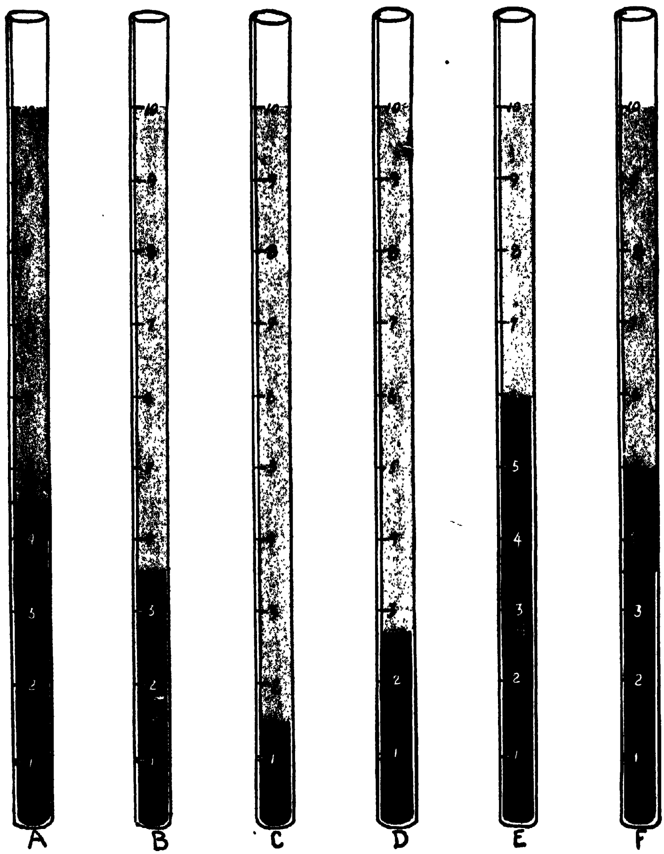


FIG. 58. VOLUME OF PACKED ERYTHROCYTES (Hematocrit Determinations)

A, normal blood; *B,* simple chronic anemia (normocytic); *C,* pernicious anemia (macrocytic); *D,* chlorosis (microcytic); *E,* erythremia (normocytic); *F,* chronic myelocytic leukemia.

4. The tube is closed by means of a rubber stopper and centrifuged long enough for complete packing of the cells, usually for thirty minutes at 3000 revolutions per minute.

5. The cell volume in per cent is determined by dividing the height of the column of packed erythrocytes by the total height of the column of cells and plasma and multiplying by 100.

Color Index. For calculating the color index it is assumed that under normal conditions 5,000,000 erythrocytes per c.mm. of blood carry 100 per cent of hemoglobin. It is calculated as follows:

1. Determine the percentage of hemoglobin.
2. Make an erythrocyte count and multiply the first two figures by 2.
3. Express the results in a fraction: $\frac{\text{per cent hemoglobin}}{\text{erythrocyte figure}} = \text{Color Index}$

Example: 80 per cent hemoglobin; total erythrocyte count 4,500,000 = $\frac{80}{90}$ or a color index of 0.88 which is normally 1.0 (see page 25).

Volume Index. The volume index is an expression of the ratio between the volume of the average erythrocyte in the patient's blood and the volume of the average erythrocyte in normal blood. Calculation of the volume index is, therefore, an indirect method of determining the size of the erythrocytes. For example, in pernicious anemia in relapse the majority of erythrocytes are larger than normal, hence the volume of packed cells will be greater than in the same unit of normal blood with the same cell count. The reverse is true in most cases of so-called secondary anemia. For normal erythrocyte count, 5,000,000 cells has been chosen (therefore the count multiplied by 20 gives per cent), while for normal mean volume of packed cells 43.2 cc. per 100 cc. of blood should be used (therefore the volume found, multiplied by 2.3 $\left(\frac{100}{43.2}\right)$ gives the volume in relation to the normal). The calculation is conveniently made as follows:

$$\frac{\text{cc. packed cells per 100 cc.} \times 2.3}{\text{millions of cells per c.mm.} \times 20} = \text{Volume Index}$$

Example: Patient's red cell count, 1,500,000; patient's packed cells, 18.4 cc. per 100 cc. of blood: $\frac{18.4 \times 2.3}{1.5 \times 20} = 1.41$

Normally the volume index varies from 0.63 to 0.82 for children and 0.80 to 1.00 for adults.

Mean Corpuscular Volume. The mean corpuscular volume of erythrocytes expressed in cubic microns may be determined as follows:

$$\frac{\text{cc. packed cells per 1000 cc.}}{\text{red cell count, millions per c.mm.}} = \text{M.C.V.}$$

Example: 3,600,000 per c.mm.; packed cells, 38.2 per 100 cc. of blood:

$$\frac{382}{3.6} = 106 \text{ cubic microns}$$

Saturation Index. The saturation index is an expression of the ratio between the amount of hemoglobin per unit volume of erythrocytes in the patient's blood and the amount of hemoglobin per unit volume of erythrocytes in normal blood. It is calculated as follows:

$$\frac{\text{Per cent hemoglobin}}{\text{cc. packed cells per 100 cc.} \times 2.3} = \text{Saturation Index}$$

The normal range is 0.9 to 1.2 in absolute terms.

Mean Corpuscular Hemoglobin. This is determined by dividing the hemoglobin in grams per 1000 cc. of blood by the erythrocytes in millions per c.mm. and expressing the results in micromicrograms. The normal for adults is 29 ($\pm$ 2) and for children 26 to 28.

Mean Corpuscular Hemoglobin Concentration. This is calculated by dividing the hemoglobin in grams per 100 cc. of blood by the volume of packed corpuscles in cc. per 100 cc. of blood and multiplying by 100. The results are expressed in terms of grams per 100 cc. The normal range varies from 32 to 34 for children and 34 ($\pm$ 2) for adults.

Example: Hemoglobin, 12.5 gm.; packed cells, 36.2 cc. per 100 cc. of blood:

$$\frac{12.5}{36.2} \times 100 = \text{M.C.H.C. of 34 per cent}$$

METHOD FOR COUNTING TOTAL LEUKOCYTES

1. Draw blood to the mark 0.5 of the Thoma pipet marked 11 (Fig. 54), or fill the stem of the Trenner pipet, as described in the method of counting erythrocytes.

2. Draw up diluting fluid to mark 11, thus making a dilution of 1:20, prepared as follows:

Glacial acetic acid	0.5 cc.
Distilled water	99.5 cc.

A few drops of an aqueous solution of gentian violet or methylene blue may be added to stain the leukocytes slightly.

3. Rotate the pipet well for several minutes, holding it in a horizontal position; finally shake sideways.

4. Blow out several drops.

5. Fill the counting chamber in exactly the same manner as described for the counting of erythrocytes.

6. Allow the cells to settle for at least two or three minutes.

7. Center the light and focus exactly as described for the erythrocyte count.

8. Count the leukocytes in squares L_1, L_2, L_3 and L_4 (Fig. 57), add up and multiply by 50, which gives the total leukocytes per c.mm. of undiluted blood. In counting the cells include those touching the inner lines on the right and top but omit those touching the lines on the left and bottom. The difference between the largest and smallest number of leukocytes in any two squares should not exceed ten.

9. The same errors as those which may occur in erythrocyte counting must be kept in mind and carefully avoided.

10. When the leukocyte count is very high, it may be necessary to use a dilution of 1:100, using the erythrocyte counting pipet, changing the calculation accordingly. When the count is abnormally low, make the dilution 1:10 by drawing blood to the 1.0 mark instead of 0.5.

11. For the normal number of leukocytes according to age and other factors, see page 29.

METHOD FOR STAINING BLOOD SMEARS

Well-prepared films or smears on slides are absolutely essential for accurate results. Wright's stain is recommended, the method being as follows:

1. Completely cover the dried smear with stain for 1 to 3 minutes. This fixes the blood film. The stain is prepared as follows:

Wright's stain powder	0.3 gm.
Glycerin	3.0 cc.
Absolute acetone-free methyl alcohol	97.0 cc.

Add the powder in a dry mortar; grind with a pestle; add the glycerin and grind together thoroughly. Add the methyl alcohol and mix. Allow to stand overnight in a tightly stoppered bottle, then filter and set aside for a few days. Age improves the stain. The glycerin may be omitted when air humidity is high.

2. Add the buffer solution to the stain, drop by drop, until a greenish, metallic scum appears on the top. The stain and buffer solution should cover the slide, but none should run off. Determine the time for staining by trial with a series of slides. This will usually be about two minutes, but varies with each batch of stain. The color of the cells may be varied by changing the *p*H of the buffer solution, which is prepared as follows:

Potassium phosphate (monobasic)	1.63 gm.
Sodium phosphate (dibasic)	3.2 gm.
Distilled water	1000.0 cc.

3. Wash with water until the smear is lavender-pink.

4. Allow the slide to stand on edge until dry.

5. Apply a drop of immersion oil to the smear and examine under the oil-immersion objective.

NORMAL LEUKOCYTES; SHIFT TO THE LEFT

Classification of Normal Leukocytes. 1. Three separate kinds of leukocytes are recognized, namely, (a) lymphocytes from lymphoid tissues; (b) monocytes from the reticulo-endothelial system and (c) granulocytes from bone marrow.

2. The last are so called because of granules in their cytoplasm and are subdivided according to the staining reactions of such into three types: neutrophils, basophils and eosinophils.

3. *Lymphocytes* (Fig. 59) vary in size from about that of an erythrocyte to that of a neutrophil. The nucleus is round and stains deeply with the basic stain. The smaller ones stain more deeply and have a small amount of cytoplasm. The larger ones often stain less intensely and have more cytoplasm, in some of which may be seen several round, reddish-purple azurophilic granules. Occasionally forms with indented nuclei appear. It is generally believed that the large, less deeply staining forms are the younger types which become smaller upon reaching maturity. An increase of these cells is called *lymphocytosis* and a decrease *lymphopenia.*

4. *Monocytes* (Fig. 59) include cells which were formerly called large mononuclear leukocytes and transitionals. They are sometimes known as endotheliocytes. They are the largest type of leukocyte found in the blood (14 to 20 microns). The nucleus is less deeply stained than that of the lymphocytes, is usually indented and at times is horseshoe shaped. The chromatin material in the nucleus has a skein-like appearance. Those with round nuclei are often difficult to distinguish from lymphocytes. There is a wider band of cytoplasm than that of the lymphocytes. The lymphocytes are not usually as large as neutrophils, while the monocytes are usually larger. The chromatin of lymphocytes is more granular in appearance. An increase is called *monocytosis* and a decrease *monopenia.*

5. *Neutrophils* (Fig. 59) are easily recognized by an irregular-shaped and lobulated nucleus, for which reason they are commonly known as "polymorphonuclears." Their average size is about 12 microns. The nucleus may be ribbon, band-like or segmented. The segments vary in number from one to six or seven and are all connected by narrow nuclear bands. The cytoplasm contains numerous fine granules which do not stain definitely either blue (basic) or red (acid) and hence are regarded as neutral or neutrophilic. They may undergo an increase, designated *neutrophilia,* or a decrease, called *neutropenia.*

6. *Eosinophils* (Fig. 59) are granulocytes similar to the neutrophils except for a difference in the size and staining properties of the granules, which are round or oval and large enough to be distinctly outlined. They stain pink to bright red (acid stain) with Wright's stain. An increase is called *eosinophilia* and a decrease *eosinopenia.*

7. *Basophils* (Fig. 59) are granulocytes similar to the neutrophils except that they contain granules which are larger and stain deep purple (basic stain) with Wright's stain. The nucleus is usually without distinct lobulation. The cell itself is slightly smaller than the neutrophil. They are also called "mast cells." An increase is called *basophilia.*

Shift to the Left. It is always advisable to classify the polymorphonuclear neutrophils routinely into (1) the *immature,* nonsegmented or nonfilament, and (2) the *mature,* segmented or filament types (Fig. 60), as recommended by Arneth and especially by Schilling. Normally, in adults, the immature or nonfilamented cells do not exceed 3 to 6 per cent. In acute infections, however, they may be increased without a material increase in the total number of leukocytes. For this reason a differential leukocyte count by this method is frequently more diagnostic than a total leukocyte count alone. An increase of immature cells,

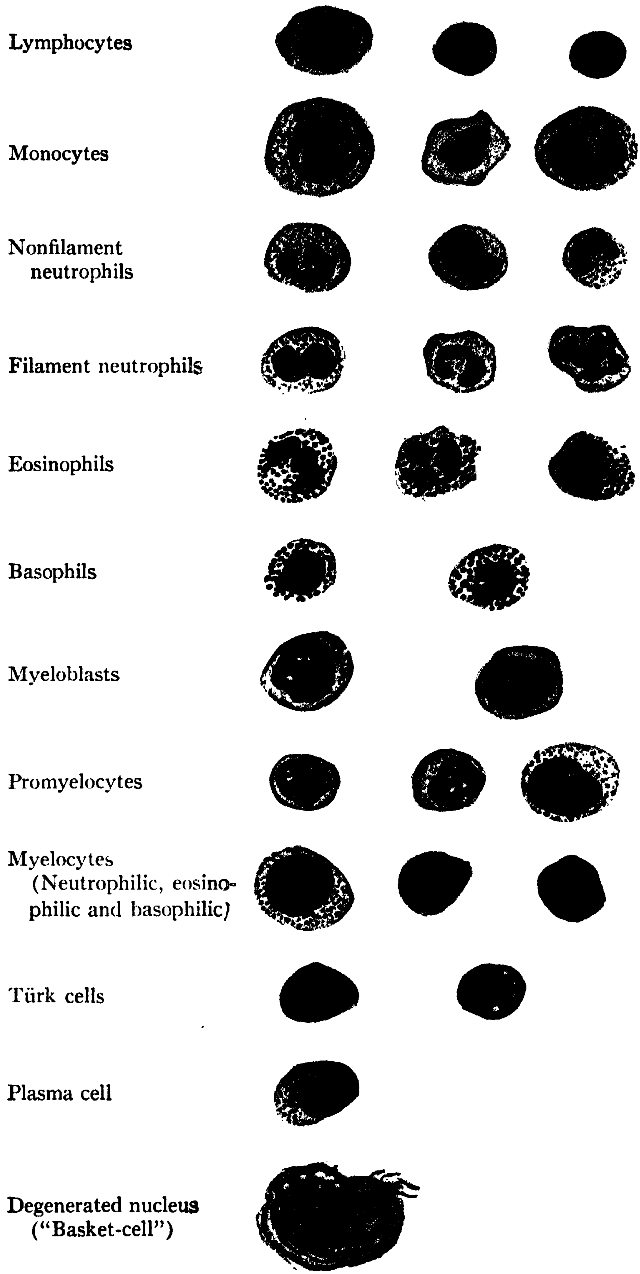


FIG. 59. NORMAL AND ABNORMAL LEUKOCYTES (WRIGHT'S STAIN).

(From Kolmer and Boerner: *Approved Laboratory Technic*, 4th ed., D. Appleton-Century Company, Inc.)

which constitutes a "shift to the left," indicates an increase in the bone marrow output of new cells.

Schilling suggested an index for expressing the degree of "shift to the left" which is obtained by dividing the immature forms (nonfilamented) by the mature (filamented) forms. It is expressed in the form of a fraction as follows:

$$\text{Schilling Index} = \frac{\text{Per cent or number of immature neutrophils}}{\text{Per cent or number of mature neutrophils}}$$

Basophilic or Toxic Granules. Deeply staining basophilic granules may occur in the neutrophils in acute infections with or without leukocytosis. They may occur in either immature or mature cells. Small toxic granules are usually distributed among the pinkish granules. If they are large, few if any normal granules are seen in the protoplasm; large basophilic granules are found only in the acute stages of disease. Basophilic granulation is due to a failure in the development of granules.

ABNORMAL LEUKOCYTES

1. **Lymphoblasts.** This cell is identical with the myeloblast in appearance, although some claim that there are minute differences (Fig. 61). It may be found in the germinal centers of lymph nodes in health.

2. **Young Forms of Lymphocytes.** These are characterized by delicately stained nuclei of a vesicular character. They do not contain nucleoli. They are usually large cells from 10 to 20 microns in diameter. The cytoplasm like that of the mature lymphocyte is lightly basophilic. These young lymphocytes are frequently incorrectly called monocytes because of their delicate nuclear structure. They are found characteristically in infectious mononucleosis. Young lymphocytes may or may not possess azurophil granules.

3. **Türk's Irritation Forms.** The true nature and significance of these cells is disputed. The nucleus stains deeply, is round with irregular markings, and the cytoplasm is intensely basophilic (Fig. 59).

4. **Myeloblasts.** These cells are slightly larger than the polymorphonuclear neutrophils (18 to 20 microns) and possess a deep blue cytoplasm when stained by Wright's method. There are no cytoplasmic granules. The nucleus, which is relatively large, round or slightly oval in shape, is characterized by fine chromatin markings in the form of a fine stippling. To some observers the spaces between the chromatin markings suggests a fine sieve-like character. The nucleus stains bluish red by Wright's method. There are several small nucleoli which are stained light blue (Figs. 59 and 60).

5. **Promyelocytes.** As the myeloblast matures, it acquires cytoplasmic granules. It is then called the promyelocyte (Figs. 59 and 60). At first these granules are of the azurophil variety, so called because with Wright's stain they take a rich blue stain. They are not affected by the oxidase stain of Goodpasture and although they are generally large granules, they vary much in size. The cytoplasm is less basophilic than in the myeloblast. The nucleus stains somewhat more

deeply and the chromatin particles seem somewhat coarser. As this cell grows older, the azurophil granules are replaced by specific granules which become black when treated with Goodpasture's oxidase stain. It is at this point that the cell differentiates into one of the three following specific types of myelocytes: neutrophilic, eosinophilic or basophilic.

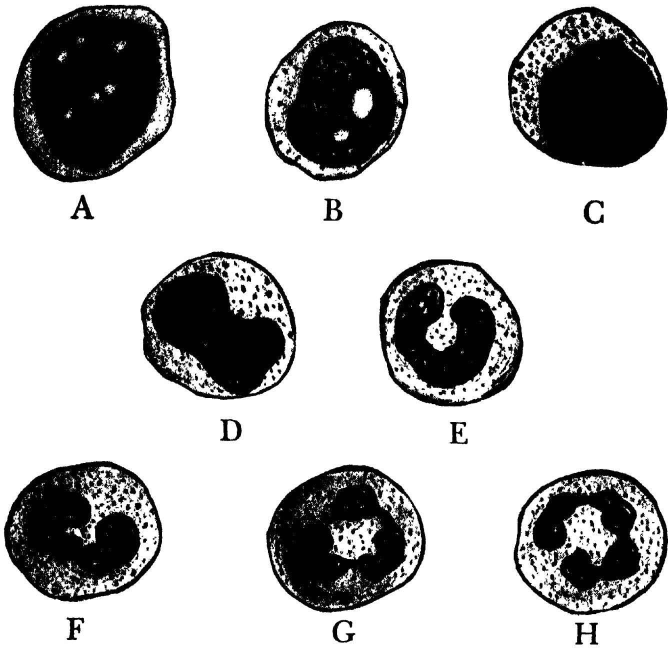


FIG. 60. IMMATURE ("Shift to the Left") AND MATURE NEUTROPHIL LEUKOCYTES.

A, myeloblast; *B*, promyelocyte; *C*, myelocyte; *D*, metamyelocyte; *E*, nonfilamented polymorphonuclear neutrophil; *F*, two-lobed polymorphonuclear neutrophil; *G*, three-lobed polymorphonuclear neutrophil; *H*, four-lobed polymorphonuclear neutrophil. *A, B, C, D* and *E* are immature and *F, G,* and *H* are mature neutrophils.

6. **Neutrophilic Myelocytes.** The cytoplasm has at this stage reached its complete development and acquired phagocytic powers. The cytoplasm is very lightly acidophilic and packed with small, not easily seen, violet granules (Wright's stain). The cell is slightly smaller than the myeloblast (12 to 18 microns). The nucleus is still round but it is more deeply stained, its chromatin markings are coarser and nucleoli are rarely found. Further maturity of the cell is shown not by cytoplasmic changes but by changes in the nucleus. The round form becomes indented (juvenile leukocyte), the indentation extends deeper (stab leukocyte) and finally segmentation occurs (segmented leukocyte). During this process of indentation and segmentation, the chromatin condenses so that it stains more deeply from blue-red to bluish-black; the chromatin masses and nuclear mem-

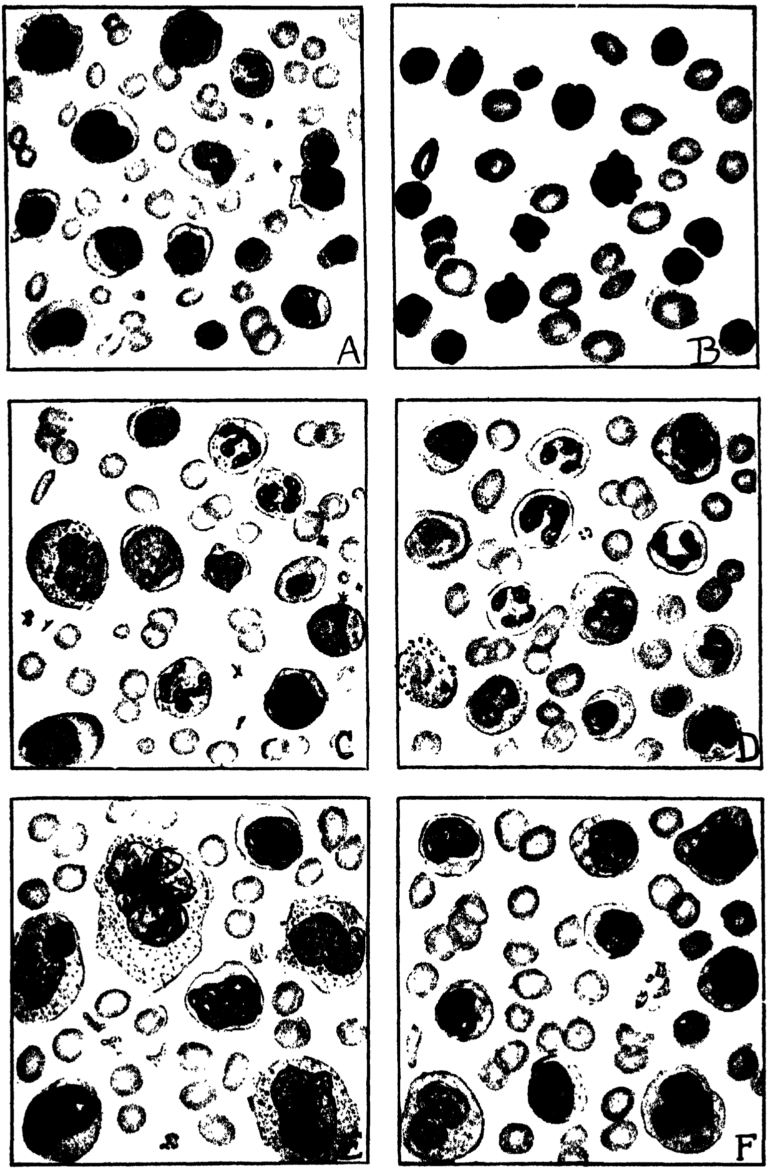


FIG. 61. THE LEUKOCYTES IN THE LEUKEMIAS AND ACUTE INFECTIOUS MONONUCLEOSIS.

A, acute lymphoblastic leukemia; *B*, chronic lymphocytic leukemia; *C*, acute myeloblastic leukemia; *D*, chronic myelocytic leukemia; *E*, acute monocytic leukemia; *F*, acute infectious mononucleosis.

brane become thicker, and the spaces between the chromatin markings become wider (Fig. 59).

7. **Eosinophilic Myelocytes.** The cell develops granules which are much larger than the neutrophilic granules and takes a distinct red, sometimes brownish-red, stain (Wright's stain). The nuclear changes of this cell are similar to those of the neutrophil and the same type cells are formed (Fig. 59).

8. **Basophilic Myelocytes.** In this cell the granules are also large like those of the eosinophil; they stain a deep blue although an occasional metachromatic (red or bluish-red) granule is encountered (Fig. 59). Nuclear maturity occurs as in the other forms of myelocytes. In active bone marrow, cells of varying maturity are seen from the most immature to the mature segmented form. They are best studied in films prepared by smearing bone marrow on a slide and staining in the same manner as blood films.

METHOD FOR DIFFERENTIAL LEUKOCYTE COUNTING

1. Examine a smear with the low-power lens to determine if the leukocytes are well distributed. Look particularly at the edges and end of the smear. If they are not properly distributed, examine another smear.

2. If the slide proves satisfactory, systematically examine with oil-immersion lens by recording each type of leukocyte seen as the slide is moved from one field to another. The Marbel or other blood cell calculator is very convenient.

3. At the same time a special differential count should be made of the neutrophils for the immature forms to ascertain if there is any "shift to the left."

4. The red blood corpuscles should also be examined and any abnormalities noted, especially the number of nucleated cells seen during the count.

5. At least three separate parts of the slide should be examined in order to get an accurate result.

6. The number of cells to be counted and classified should be determined by the total leukocyte count. In routine work the following is recommended by Kolmer and Boerner:

For total counts under 10,000, classify 100 cells.
For total counts of 10 to 15,000, classify 200 cells.
For total counts of 15 to 20,000, classify 300 cells.
For total counts of 20 to 25,000, classify 400 cells.
For total counts over 25,000, classify 500 cells.

7. It is usual to submit a report in terms of the per cent of each type of leukocyte in the count, but a much better plan is to report the actual number of each leukocyte per c.mm. of blood which may be calculated from the total leukocyte count. If, for example, the total leukocyte count is 12,500 with 77 per cent neutrophils, the actual number of these cells per c.mm. of blood would be 125×77 or 9625.

8. The *normal* differential leukocyte count for the *adult* is approximately as follows (Kolmer and Boerner):

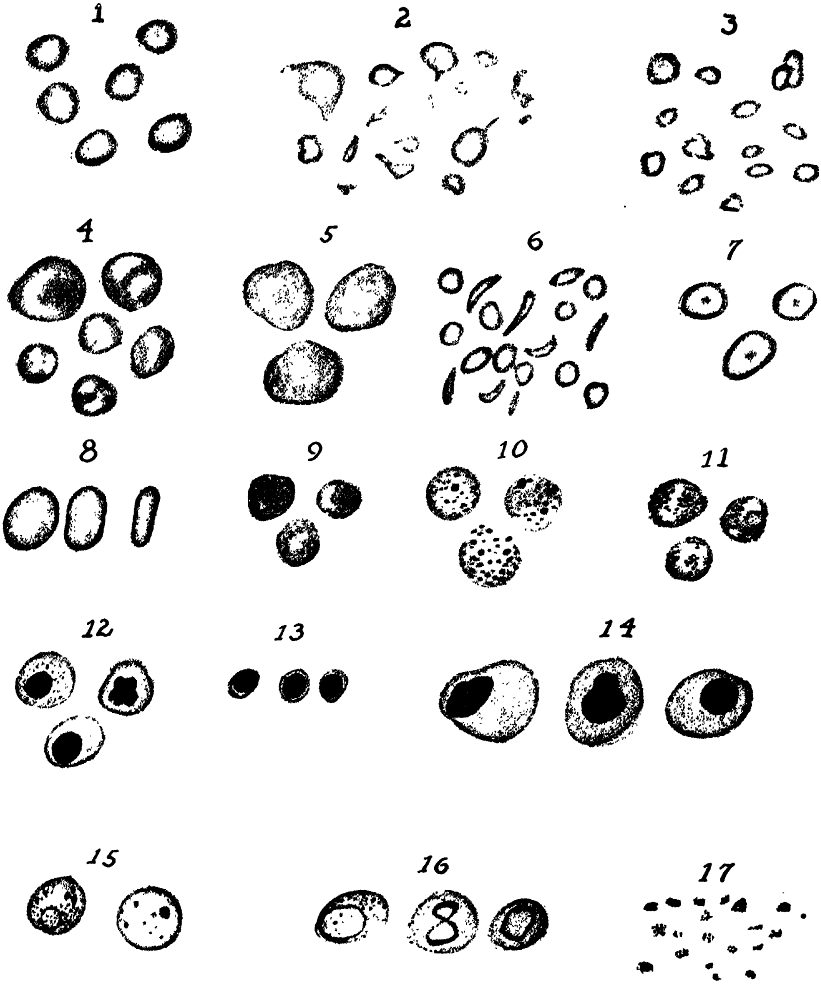


FIG. 62. NORMAL AND ABNORMAL ERYTHROCYTES AND PLATELETS (WRIGHT'S STAIN)

1, Normal erythrocytes (normocytes); 2, Poikilocytes; 3, Microcytes; 4, Macrocytes; three cells show endoglobular degeneration; 5, Megalocytes; 6, Sickle cells; 7, Target cells; 8, Ovalocytes; 9, Polychromatophilia; 10, Basophilic degeneration ("stippling"); 11, Reticulocytes; 12, Normoblasts; 13, Microblasts; 14, Macroblasts; 15, Howell-Jolly bodies; 16, Cabot's ring bodies; 17, Normal platelets. (From Kolmer and Boerner: *Approved Laboratory Technic*, 4th ed., D. Appleton-Century Company, Inc.)

Lymphocytes	1000 to 3000	(20 to 30%)
Monocytes	100 to 600	(2 to 6%)
Neutrophils (immature)	150 to 500	(3 to 6%)
Neutrophils (mature)	2550 to 5350	(50 to 67%)
Eosinophils	50 to 400	(1 to 4%)
Basophils	0 to 50	(0 to 1%)

9. For *children* the figures are approximately as follows (Kolmer and Boerner):

Leukocyte	*Three Months to Three Years*	*Three to Five Years*	*Over Five Years*
Neutrophils	2000 to 7000	3000 to 8000	3000 to 7000
Basophils	0 to 50	0 to 50	0 to 50
Eosinophils	25 to 700	50 to 700	50 to 400
Lymphocytes	4000 to 9000	2500 to 6000	1000 to 3000
Monocytes	25 to 700	25 to 700	100 to 600

NORMAL AND ABNORMAL ERYTHROCYTES

1. The erythrocytes of stained blood smears should also be examined and reported upon, especially in the anemias.

2. Normally they are about 7.5 microns in diameter (Fig. 62). In stained preparations distorted shapes may be seen due to mechanical distortion in preparing smears. In wet preparations they may occur in rouleaux formation and show some crenation.

3. In the anemias with diminished hemoglobin, especially in chlorosis, the central pale area becomes larger and paler, constituting *achromia*. In extreme instances the cells become mere rings (*pessary forms*). In pernicious anemia, however, many of the corpuscles stain deeply and entirely lack the pale center.

4. Abnormal variations in size is called *anisocytosis*. When smaller than normal the cells are called *microcytes* and when larger, *macrocytes* (Fig. 62); extremely large forms are called *megalocytes* (Fig. 62). In congenital hemolytic jaundice the erythrocytes are decreased in diameter but increased in thickness, appearing as deeply stained cells (called *spherocytes*).

5. *Poikilocytes* are corpuscles with abnormal shapes (Fig. 62). They may be caudate, club-shaped, oval or elliptical.

6. *Sickle-shaped* cells are especially numerous in a hereditary type of anemia more commonly seen in Negroes, called "sickle-cell" anemia (Figs. 62 and 63); see page 12. Sickle cell anemia must be distinguished from sickle cell trait. The characteristic phenomenon in sickle cell anemia is observed to take place in the shape of erythrocytes *when a drop of blood is sealed under a coverglass on a slide or in a test tube.*

7. *Polychromatophilia* is the term used for indicating the abnormal affinity of erythrocytes for the basic stains. When present, many erythrocytes will be seen taking the basic blue stain in varying degrees, usually pale to light blue instead of pale pink. The condition is abnormal and is found in anemias where there is active regeneration of erythrocytes (Fig. 62).

8. ***Basophilic degeneration,*** or ***stippling,*** is a condition in which there are many very fine to coarse blue dots or granules present in the erythrocyte (Fig. 63). They are found in cases where erythrocytic regeneration is active and probably represent cells which have undergone a degenerative change before they were fully mature. Their presence in suspected cases of lead poisoning is of diagnostic value (see page 17).

9. *Reticulated erythrocytes* or *reticulocytes* are young cells which, when stained with brilliant cresyl blue, show filaments which are well stained, if the staining is

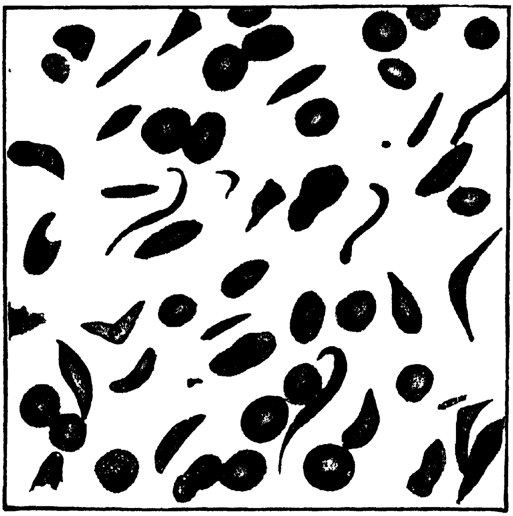


FIG. 63. SICKLE CELL ANEMIA.

done while the cell is still alive (vital staining). Although these filaments take the basic stain they will not stain in the usual dry smear. They are often arranged in skeins or wreaths (Fig. 62) as previously discussed (see page 15).

10. *Nucleated red cells* are immature cells which are thrown into the circulation in severe anemias and leukemias in which there is an active regeneration of bone marrow. The *megaloblast* or *macroblast* is the largest type and has a large, oval, pale-staining nucleus (Fig. 62). The cytoplasm often shows polychromatophilia. Some may closely resemble lymphocytes due to the blue staining of the cytoplasm. These cells are usually present in pernicious anemia, where their presence is of diagnostic value. The *normoblast* (Fig. 62) is of about the same size as a normocyte and has a nucleus more deeply stained than the megaloblast. Occasionally the chromatin is arranged in a manner resembling the spokes of a wheel. Nucleated red cells often show polychromatophilia. The older forms are smaller and the nucleus deeply stained. There may be more than one nucleus or the nucleus may be irregular, lobulated or fragmented. If the nucleus is completely broken up, the fragments may all disappear except for a few. These re-

maining particles are called ***nuclear particles*** or ***Howell-Jolly bodies*** (Fig. 62). The smallest nucleated red is called a ***microblast*** (Fig. 62). It measures less than 5 microns in diameter, has a deeply staining nucleus and is regarded as an older form of normoblast.

Cabot's ring bodies are ring- or figure-eight-shaped structures which stain red or reddish-purple with Wright's stain. They are seen in lead poisoning, pernicious anemia, leukemia and especially in erythroblastic anemia of children (Fig. 62).

METHOD FOR COUNTING RETICULOCYTES

1. Smear across a slide a few drops of a 1 per cent alcoholic solution of brilliant cresyl blue as in preparing a blood smear.
2. Dry in the air.
3. Place a small drop of blood in the center of a cover glass, and place blood side down on the dried stain.
4. Let stand 10 minutes.
5. Examine under oil-immersion lens.
6. Count 1,000 erythrocytes, noting the number containing the bluish strands of reticulum. The thinner the preparation, the easier the count. Report the percentage of erythrocytes which contain reticulum. Normally 0.5 to 1.5 per cent of the cells are reticulated.

METHOD FOR DETERMINING THE SEDIMENTATION RATE OF ERYTHROCYTES

The method of Cutler is as follows: 1. Apply a tourniquet to the arm.

2. Aspirate into a 2 cc. syringe 0.1 cc. of sterile 3 per cent sodium citrate solution.
3. Puncture a cubital vein and draw blood to the 1 cc. mark.
4. Release the tourniquet and withdraw the needle.
5. Draw back the piston of the syringe and gently tilt the syringe backward and forward several times to insure uniform and thorough mixing of the blood and citrate solution.
6. Remove the needle from the syringe and pour the contents into a Cutler sedimentation tube. This is a tube graduated at 1 cc. capacity and marked into fifty 1 mm. divisions with zero at the top.
7. Keep the tube strictly upright at room temperature.
8. Record the drop in the column of erythrocytes in millimeters every five minutes for an hour. A graph may be constructed, and is very convenient, as shown in Figure 64.
9. The normal for men varies from up to 8 mm. and for women up to 10 mm. at the end of an hour, with an almost horizontal line. For normals found with the Westergren and Wintrobe tubes see page 20.
10. The amount of sedimentation occurring during the first 15 minutes, expressed in terms of percentage of total fall of erythrocytes after 24 hours, has been proposed by Rosen as an "index of sedimentation."

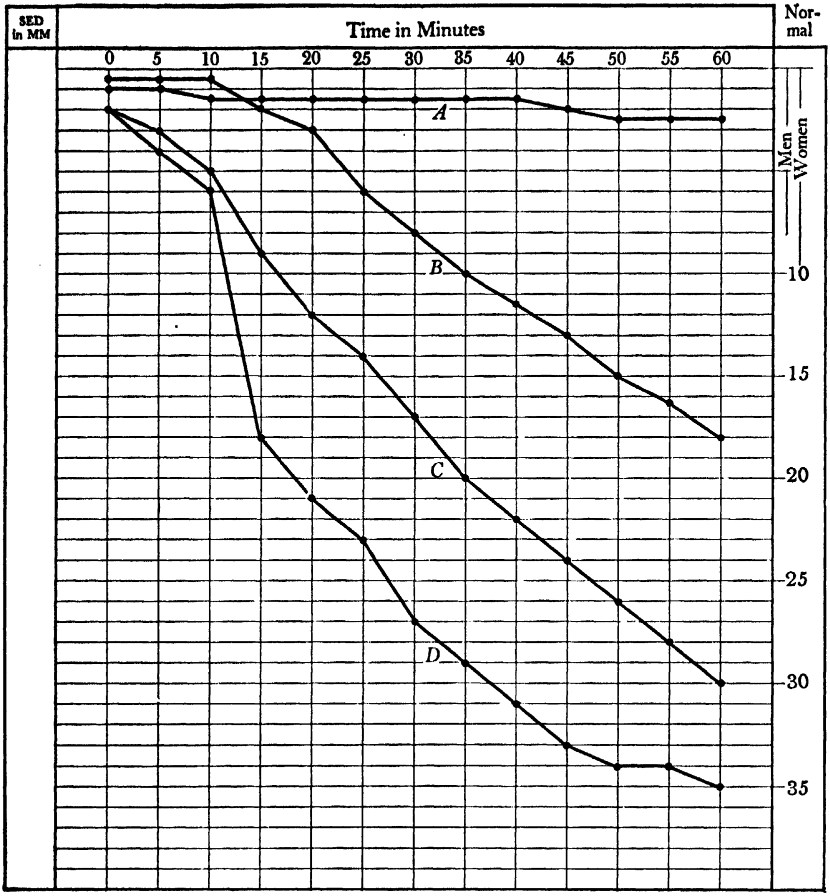


FIG. 64. BLOOD SEDIMENTATION CHART AND GRAPHS.

A, normal; *B*, clinically quiescent pulmonary tuberculosis; *C*, clinically active pulmonary tuberculosis; *D*, acute pyogenic infection.

METHOD FOR DETERMINING THE TONICITY (FRAGILITY) OF ERYTHROCYTES

The method of Sanford is as follows: 1. Prepare a 0.5 per cent solution of sodium chloride by dissolving 0.5 gm. of oven-dried salt in 100 cc. of distilled water.

2. In a series of twelve small test tubes in a rack prepare a series of dilutions of sodium chloride varying from 0.5 per cent in No. 1, to 0.28 per cent in No. 12 as follows:

Tube No.	*0.5% Saline*	*Water*	*% NaCl*
1.	1.25 cc.	0	= 0.5
2.	1.20 cc.	0.05 cc.	= 0.48
3.	1.15 cc.	0.10 cc.	= 0.46
4.	1.10 cc.	0.15 cc.	= 0.44
5.	1.05 cc.	0.20 cc.	= 0.42
6.	1.00 cc.	0.25 cc.	= 0.40
7.	0.95 cc.	0.30 cc.	= 0.38
8.	0.90 cc.	0.35 cc.	= 0.36
9.	0.85 cc.	0.40 cc.	= 0.34
10.	0.80 cc.	0.45 cc.	= 0.32
11.	0.75 cc.	0.50 cc.	= 0.30
12.	0.70 cc.	0.55 cc.	= 0.28

3. Take the tubes in a rack to the bedside of the patient. Obtain 1 or 1.5 cc. of blood from a vein with a small dry syringe and No. 21 needle and at once add 1 drop to each tube.

4. If some time must elapse before the blood can be added, it may be mixed with 5 volumes of a 1 per cent solution of sodium citrate in physiologic saline solution. Mix well. Centrifuge. Discard the supernatant fluid. Add an equal volume of saline solution to the corpuscles (gives a 50 per cent suspension) and add 1 drop to each tube.

5. It is advisable to prepare a similar set of tubes, using the blood of a normal person as a control.

6. Allow the tubes to stand at room temperature for two hours.

7. Read the results, noting the per cent of NaCl in which hemolysis begins and the first tube showing complete hemolysis.

8. Normal blood *begins* to hemolyze in 0.40 or 0.46 per cent sodium chloride (minimal resistance) and is *completely* hemolyzed in 0.30 to 0.36 per cent (maximal resistance); see page 23. When a control is used, a variation of 0.02 or 0.04 may be considered quite definite.

METHOD FOR COUNTING PLATELETS

The method of Orlef is as follows: 1. Immerse the patient's hand in warm water.

2. Wash with soap, water, alcohol and ether. Make a deep puncture in the finger without squeezing.

3. Put five drops of the diluting fluid in a paraffin cup prepared by melting the center of a small cube of paraffin with the heated end of a glass rod. The diluting fluid must be kept in a cool place and filtered every few days; it is prepared as follows:

Sodium metaphosphate	1.0 gm.
Sodium chloride	0.4 gm.
Dextrose	0.1 gm.
Sodium bicarbonate	0.1 gm.
Brilliant cresyl blue	0.15 gm.
Distilled water	100.0 cc.

This solution preserves both platelets and reticulocytes and both counts may be done at the same time.

4. Shake the first drop of blood off the finger.

5. Place a drop of the diluting fluid on the puncture wound, and turn the hand over so that the blood will drop into the paraffin cup. Allow enough blood to fall into the cup to make a dilution approximately 1:5. This will require 1 or 2 drops.

6. Stir with a wooden applicator, the tip of which has been dipped in melted paraffin.

7. Allow to stand 1 to 2 minutes. Stir again and then transfer a drop of this material to a slide with the applicator. (If several preparations are to be done, a heated glass slide placed on top of the paraffin block will keep the fluid from evaporating.)

8. Place a cover glass over the drop and allow to stand for 15 minutes.

9. Examine with oil-immersion lens, counting both platelets and reticulocytes until a total of 1000 erythrocytes has been counted.

10. Make a total erythrocyte count in the usual manner.

11. Calculate as follows:

$$\text{Number of platelets} \times \frac{\text{erythrocyte count}}{1000} = \text{absolute number of platelets}$$
per c.mm. of blood.

12. The normal average number of platelets by this method is about 500,000 per c.mm. of blood (see page 35).

METHODS FOR DETERMINING THE COAGULATION TIME

The *capillary tube method* is as follows: 1. Cleanse a finger and puncture as for a blood count.

2. Fill a capillary glass tube (1.5 mm. in diameter and 3 to 5 cm. long) with blood. The tube will fill readily by capillary attraction if one end touches the drop of blood and the tube is inclined downward. *Note the time.*

3. At half-minute intervals after an interval of three minutes carefully break a small piece off the end of the tube, holding it in such a manner that the broken ends are kept together; then separate the ends slowly and note if fibrin threads span between the ends. When the threads are seen to spread a distance of 5 mm. or more, *note the time.*

4. *The time between the filling of the tube and the appearance of fibrin threads is the coagulation time.* The normal is from one to seven minutes.

The *drop method* is as follows: 1. Cleanse and puncture finger as for blood count (puncture deep to insure free flow of blood).

2. Place several drops on a clean slide (the drops should be about 4 or 5 mm. in diameter). *Note the time.*

3. At half-minute intervals draw a needle through one of the drops. As soon as the needle picks up fibrin threads and drags them along, coagulation has taken place. *Note the time.*

4. The time interval between placing the drop on the slide and the formation of fibrin shreds is the *coagulation time. The normal time is between two and eight minutes.*

The *method of Lee and White* is as follows: 1. With a small syringe, fitted with a gauge 20 needle, puncture a vein at the elbow and collect 1 cc. of blood without using suction. *Note the time.*

2. Remove the needle from the syringe and place the blood in a test tube having a diameter of 8 mm. The test tube should be absolutely clean and rinsed with physiologic salt solution just before the blood is placed in it.

3. Set the tube upright in a rack at room temperature or better in a water bath or glass of water at a temperature of 75° F.

4. At one-minute intervals tilt the tube to see if the blood still flows. As soon as the blood fails to flow and the tube can be inverted, coagulation has taken place.

5. The interval between the time the blood is removed from the vein and the time the tube can be inverted without disturbing the clot, is the *coagulation time*. The normal time is from five to eight minutes.

6. A control test is advised with the blood of a normal person.

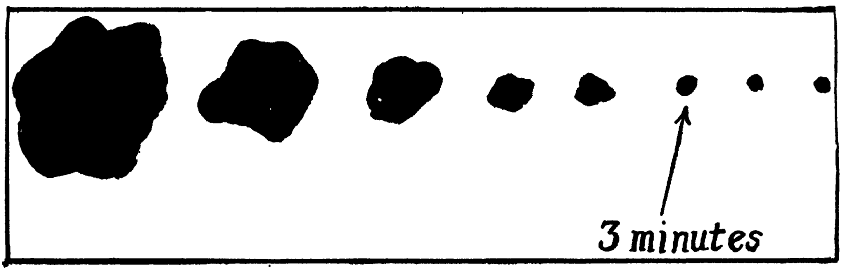


FIG. 65. NORMAL BLEEDING TIME BY DUKE'S METHOD (Natural Size).

(From Kolmer and Boerner: *Approved Laboratory Technic,* 4th ed., D. Appleton-Century Company, Inc.)

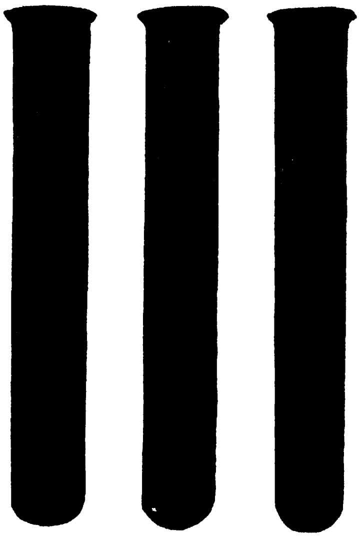
METHOD FOR DETERMINING THE BLEEDING TIME

The bleeding time is the time that it takes the blood to stop flowing from a measured cut in the finger or ear. Duke's method is as follows:

1. Puncture the lobe of the ear or the finger so that the blood flows drop by drop without any resistance.
2. Note the time the first drop appears.
3. Remove with filter paper each drop as it forms, care being taken not to touch the skin.
4. Note the time bleeding stops.
5. The time interval between the appearance of the first drop and the removal of the last represents the bleeding time. Normally it is one to three minutes (Fig. 65).

METHOD FOR DETERMINING THE CLOT RETRACTION TIME

After coagulation has taken place, the clot will contract and express serum. This is called retraction and the phenomenon appears to have some relation to the platelets. If the platelets are present in normal number, retraction occurs; if the platelets are greatly diminished, retraction will be retarded or absent. The test has no relation to the coagulation time even in hemophilia where the retraction is normal. The technic is as follows:



A B C

FIG. 66. BLOOD CLOT RETRACTION.
A, normal or complete retractility; *B*, partial retractility; *C*, poor retractility. (From Kolmer and Boerner: *Approved Laboratory Technic*, 4th ed., D. Appleton-Century Company, Inc.)

1. Secure 2 or 3 cc. of blood by venous puncture.

2. Place in test tube and incubate at 38° C.; observe at the end of each hour for 6 hours and at intervals of 6 to 12 hours for 24 hours.

3. The first evidence of retraction is the separation of the clot from the wall of the tube and then the gradual expression of serum. Normally retraction begins in one hour (Fig. 66).

METHODS FOR DETERMINING THE PROTHROMBIN TIME

The method of Quick for determining the *plasma prothrombin time* is as follows: 1. Rapidly withdraw 4.5 cc. of blood from a vein and add immediately to 0.5 cc. of 0.1 M. solution of anhydrous chemically pure sodium oxalate (1.34 gm. dissolved in 100 cc. distilled water); mix and centrifuge at low speed for five minutes.

2. Transfer 0.1 cc. of the plasma to a dry, clean test tube (13 × 100 mm.); add 0.1 cc. of thromboplastin suspension, mix gently and place in a water bath at 37.5° C. The suspension is prepared by placing 50 mg. of commercial thromboplastin (Maltine; Difco) in 2.00 cc. of 0.85 per cent solution of sodium chloride in distilled water; keep the suspension in a water bath at 50° C. for 10 minutes before using.

3. Add 0.1 cc. of 0.025 M. solution of anhydrous chemically pure calcium chloride (0.26 gm. dissolved in 100 cc. distilled water).

4. Mix quickly and instantly start a stop watch or clock. Tip the tube every few seconds and record the time required for the formation of a firm semi-solid clot.

5. It is necessary to run a duplicate test with normal human plasma.

6. The normal *prothrombin time* is 10 to 25 seconds according to the activity of the thromboplastin employed.

7. The ***prothrombin concentration*** may be expressed as per cent of normal as follows:

$$\frac{302}{\text{prothrombin time} - 8.7}$$

The bedside method of Smith, employing whole blood, is as follows: 1. Suspend 50 mg. thromboplastin in 20 cc. of saline solution and place 0.1 cc. in a small test tube marked at the 1.0 cc. level.

2. Add freshly drawn blood to this mark. Invert the tube to mix and then tilt every few seconds until the blood clots.

3. Run a duplicate test with normal blood.

4. The normal clotting or prothrombin time varies from 25 to 50 seconds, usually 25 to 35 seconds.

5. The patient's blood is compared with the normal control as follows:

$$\frac{\text{Normal control time}}{\text{Patient's time}} \times 100 = \text{Prothrombin time in \% of normal.}$$

METHODS FOR THE DIAGNOSIS OF MALARIA

The *fresh blood method* is as follows: 1. Use perfectly clean and grease-free cover glasses or slides.

2. Puncture the finger or lobe of the ear and take up a small drop of blood by touching same with center of a cover glass.

3. Place on slide so that the blood will spread out in a thin film.

4. If examination is prolonged, seal edges of cover glass with melted vaseline to prevent drying.

5. Examine at once with oil-immersion objective.

6. The best time for examination is six to eight hours after a paroxysm, but the parasites can be found at other times and the examination should be made without any special reference to the occurrence of paroxysms.

The *thin smear method* is as follows: 1. Smears are prepared as for differential leukocyte counts but so thin that the red cells lie flat and well separated.

2. Fix and stain with Wright's or Giemsa's stain in same manner as staining for differential leukocyte counts.

3. Dry and examine with oil-immersion objective.

4. The smears must be well stained for satisfactory results. Unless the nuclei of leukocytes are well stained and have the proper reddish-purple tint due to proper staining of the chromatin, the chromatin of the plasmodia will not be properly stained. Good and poor areas may occur on the same slide.

5. Malaria plasmodia are in the erythrocytes and no object should be considered as a probable plasmodium unless it is so situated.

6. With Wright's or Giemsa's stain the chromatin of the parasite will take on a ruby red color, the protoplasm of the organism a sky-blue (pale blue), the pigment a black or dark brown, and the blood platelets and the nuclei of the leukocytes a reddish purple.

Fig. 67. Plasmodium vivax.

1. Normal-sized red cell with marginal ring-form trophozoite.
2. Young signet-ring-form trophozoite in a macrocyte.
3. Slightly older ring-form trophozoite in red cell showing basophilic stippling.
4. Polychromatophilic red cell containing young tertian parasite with pseudopodia.
5. Ring-form trophozoite showing pigment in cytoplasm, in an enlarged cell containing Schüffner's stippling. This stippling does not appear in all cells containing the growing and older forms of *P. vivax,* but it can be found with any stage from the fairly young ring-form onward.

6, 7. Very tenuous medium trophozoite forms.

8. Three ameboid trophozoites with fused cytoplasm.

9, 11, 12, 13. Older ameboid trophozoites in process of development.

10. Two ameboid trophozoites in one cell.

14. Mature trophozoite.

15. Mature trophozoite with chromatin in process of division.

16, 17, 18, 19. Schizonts showing progressive steps in division ("presegmenting schizonts").

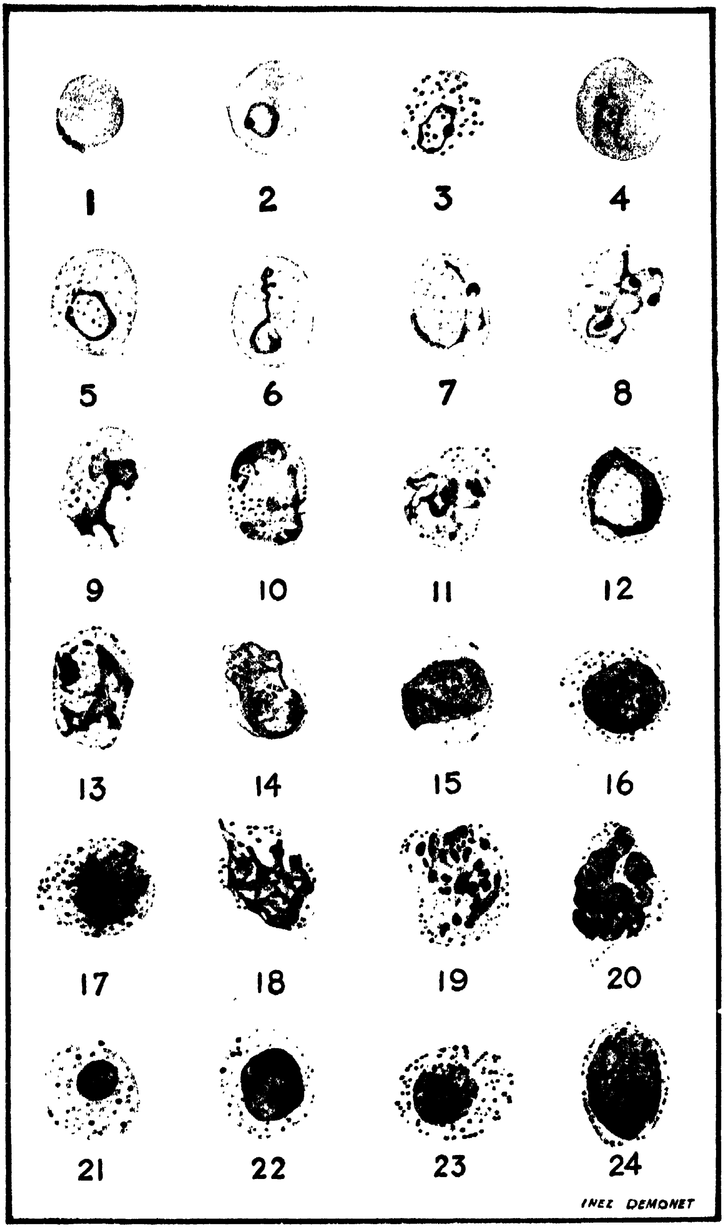
20. Mature schizont.

21, 22. Developing gametocytes.

23. Mature microgametocyte.

24. Mature macrogametocyte.

(From *Manual for the Microscopical Diagnosis of Malaria in Man,* by Aimee Wilcox, Bulletin No. 180, National Institute of Health, 1942, in Kolmer and Boerner: *Approved Laboratory Technic,* 4th ed., D. Appleton-Century Company, Inc.)



See description of this plate on opposite page.

Great care should be exercised to avoid mistaking the blood platelets accidentally superimposed upon red cells for malarial parasites. These platelets are frequently surrounded by an unstained halo. Precipitated stain, dirt, bacteria, etc., may constitute additional sources of error.

Precipitated stain granules may be removed by immersing the slide for a second or two in 95 per cent alcohol and immediately washing with distilled water.

The *thick smear method* is as follows: 1. It is essential to clean the skin carefully with alcohol and gauze in order that the blood be free of dirt, bacteria, dust or other debris. The slides should be perfectly clean.

2. Put on a drop three or four times as large as used for ordinary thin blood smears. Spread by dragging the drop on the surface of the slide with the sticking needle or corner of another slide.

3. The smears should be dried enough to make them adhere, but too much drying will prevent a clear staining of the parasites. In ordinary summer weather it is sufficient to keep the smears overnight in a box with closed lid; or the lid may be removed and the slides dried one to one and one-half hours in an incubator.

4. Dilute 1 part of a good Giemsa stain with 6 parts of *neutral,* or only slightly alkaline, distilled water (*p*H 7.0 to 7.2) just before use. Cover films for about one-half hour. Previous fixation with alcohol and dehemoglobinization are not required.

5. Place the slides in distilled water for about five minutes for partial decolorization. The time required depends upon the dilution of stain, the amount used, and the thickness of the smears. If the background is deep blue and the leukocytes almost black, the preparation is overstained.

6. Drain and allow to dry at room temperature or in an incubator. Examine with oil-immersion lens.

The *concentration method of Bass and Johns* is as follows: 1. Draw 10 cc. of blood from a vein and place in a tube carrying 0.2 cc. of the following solution:

Sodium citrate	5 gm.
Dextrose	5 gm.
Water (distilled)	10 cc.

Dissolve with aid of heat.

2. Divide the blood between two centrifuge tubes and centrifuge at high speed (2500 revolutions per minute) for the proper length of time (about five minutes for a centrifuge with a radius of 18 cm).

3. With a capillary pipet remove the supernatant plasma. Then carefully skim off the grayish layer of leukocytes and parasites and place in a tube about 12 cm. long with an inside diameter of about 0.5 cm. (made from ordinary glass tubing). Add an equal volume of plasma.

4. Mix and centrifuge as before.

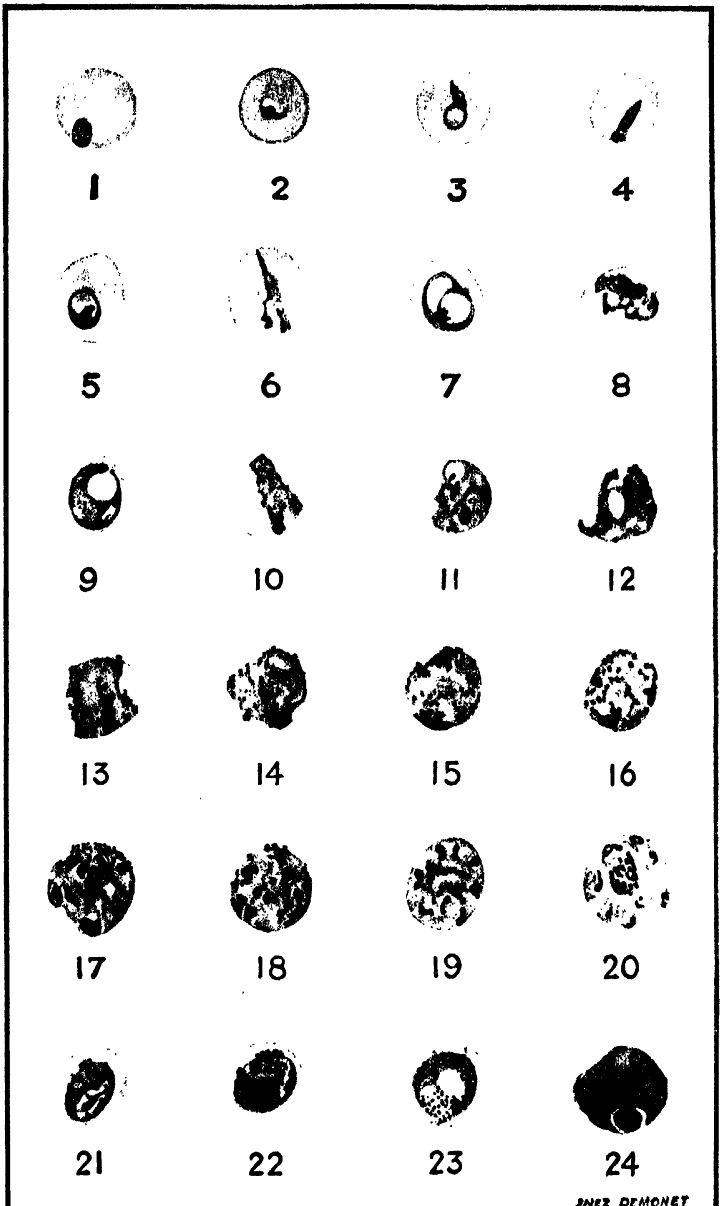
5. With a capillary pipet draw off the "cream." Mix by forcing in and out upon a slide. Then draw into the pipet and seal the tip in a flame. Nick with a file and break off above the blood column.

6. Place this slender tube in the centrifuge and centrifugate again as above.

FIG. 68. PLASMODIUM MALARIAE.

1. Young ring-form trophozoite of quartan malaria.
2, 3, 4. Young trophozoite forms of the parasite showing gradual increase of chromatin and cytoplasm.
5. Developing ring-form trophozoite showing pigment granule.
6. Early band-form trophozoite—elongated chromatin, some pigment apparent.
7, 8, 9, 10, 11, 12. Some forms which the developing trophozoite of quartan may take.
13, 14. Mature trophozoites—one a band form.
15, 16, 17, 18, 19. Phases in the development of the schizont ("presegmenting schizonts").
20. Mature schizont.
21. Immature microgametocyte.
22. Immature macrogametocyte.
23. Mature microgametocyte.
24. Mature macrogametocyte.

(From *Manual for the Microscopical Diagnosis of Malaria in Man*, by Aimee Wilcox, Bulletin No. 180, National Institute of Health, 1942, in Kolmer and Boerner: *Approved Laboratory Technic,* 4th ed., D. Appleton-Century Company, Inc.)



See description of this plate on opposite page.

7. The leukocytes will form a grayish layer upon the surface of the sediment. This and the upper portion of the erythrocyte layer contain the parasites.

8. Nick with a file and break off the capillary tube at a point 1 to 2 millimeters below the bottom of the leukocyte layer.

9. With a capillary pipet, the stem of which will pass inside the capillary tube, remove the small amount of red cells and leukocytes together with a little plasma.

10. Mix well, make smears on slides, and stain with Wright's stain in the usual way.

11. Best results are obtained with estivo-autumnal crescents and adult tertian and quartan parasites. Very young parasites do not concentrate as well, if at all.

Plasmodium Vivax. In benign tertian malaria the erythrocytes are larger than normal and stained pale (Fig. 67). The young parasite is about one-third of the diameter of the infected cell. It somewhat resembles a signet ring, the chromatin mass (staining red) representing the signet or stone and the peripheral (blue staining) cytoplasm, the band with a clear unstained vacuole forming the center.

The growing parasite or *trophozoite* is irregular in shape and may show vacuoles, one or more chromatin masses, and even scattered pigment. The red cell may show Schüffner's dots, which are tiny and pale pink in color.

The segmenting parasite, the *schizont,* fills the cells. It shows 15 to 20 chromatin masses, each surrounded by a mass of blue cytoplasm (*merozoite*). They are arranged irregularly. The pigment is aggregated in a mass which may be seen near the center.

The sexual forms (*macro-* and *microgametocytes*) fill the cell almost completely, are round or oval, having a deep blue cytoplasm, large mass of chromatin and considerable pigment.

In tertian malaria, any of the above forms may be found in a single smear of the peripheral blood, although one of the forms will probably predominate.

Plasmodium malariae. In quartan malaria, the red cells are normal in size and color (Fig. 68).

The young parasite or ring is composed of a large chromatin mass, a central unstained vacuole, and a heavy rim of blue-staining protoplasm. The diameter of the young *trophozoite* is about one-third of the diameter of the red cell.

The growing parasite is elongated or band-like (band forms) made up of blue cytoplasm with irregular and elongated red-staining chromatin masses. The pigment is coarse and scattered throughout. The diseased red cells do not show Schüffner's dots.

The mature parasite, or *schizont,* practically fills the cell, showing 6 to 10 chromatin masses, each surrounded with a blue cytoplasm. Their arrangement resembles a rosette. Coarse pigment is centrally located.

The sexual forms or *gametocytes* are similar to those of *P. vivax.* The pigment, however, is coarser.

In quartan malaria any or all of the above forms may be present in a single smear of the peripheral blood.

Plasmodium falciparum. In estivo-autumnal or malignant tertian malaria the erythrocytes are smaller than normal and may be distorted (Fig. 69).

FIG. 69. PLASMODIUM FALCIPARUM.

1. Very young ring-form trophozoite.
2. Double infection of single cell with young trophozoites, one a "marginal form," the other "signet ring" form.

3, 4. Young trophozoites showing double chromatin dots.

5, 6, 7. Developing trophozoite forms.

8. Three medium trophozoites in one cell.
9. Trophozoite showing pigment, in a cell containing Maurer's spots.

10, 11. Two trophozoites in each of two cells, showing variation of forms which parasites may assume.

12. Almost mature trophozoite, showing haze of pigment throughout cytoplasm. Maurer's spots in the cell.
13. Estivo-autumnal "slender forms."
14. Mature trophozoite, showing clumped pigment.
15. Parasite in the process of initial chromatin division.

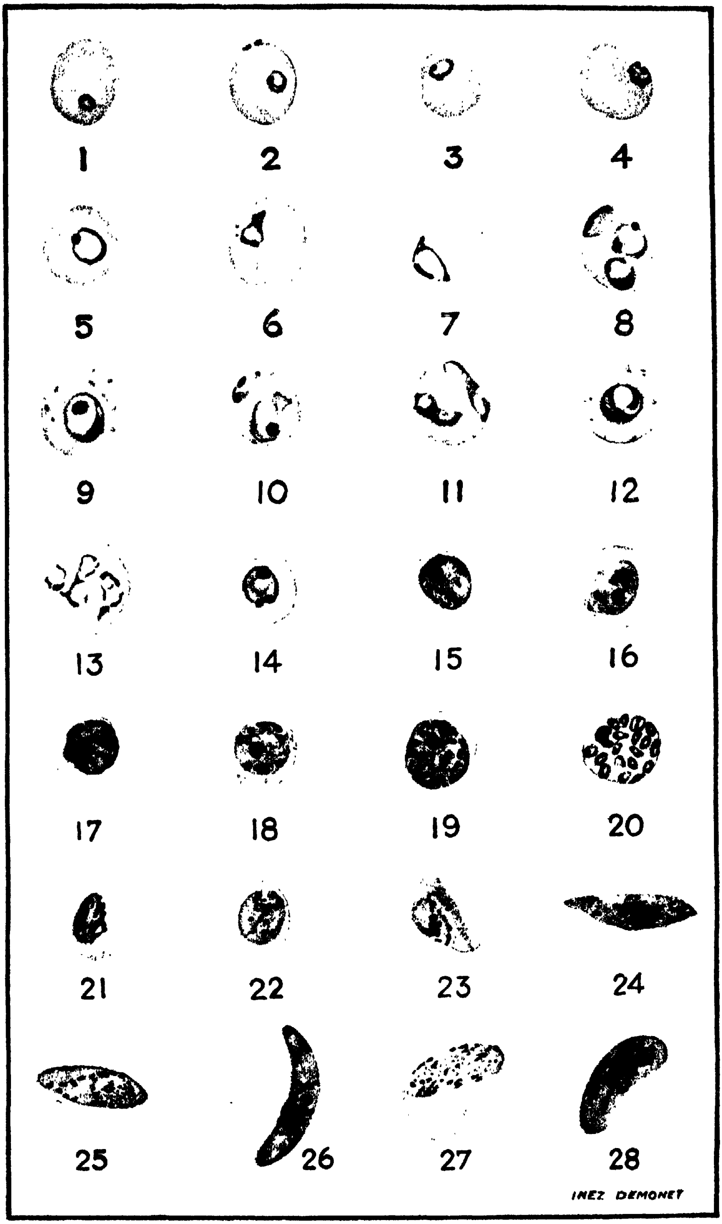
16, 17, 18, 19. Various phases of the development of the schizont ("presegmenting schizonts").

20. Mature schizont.

21, 22, 23, 24. Successive forms in the development of the gametocyte—usually not found in the peripheral circulation.

25. Immature macrogametocyte.
26. Mature macrogametocyte.
27. Immature microgametocyte.
28. Mature microgametocyte.

(From *Manual for the Microscopical Diagnosis of Malaria in Man*, by Aimee Wilcox, Bulletin No. 180, National Institute of Health, 1942, in *Applied Laboratory Technic*, 4th ed., D. Appleton-Century Company, Inc.)



See description of this plate on opposite page.

The young parasites or ring forms are very delicate, with a small chromatin dot, a central vacuole, and a delicate blue-staining peripheral ringlike cytoplasm. It is common for two or more parasites to be seen in the same cell. (This is rarely or never seen in either *P. vivax* or *P. malariae*). Some of the rings may have more than one chromatin dot.

The growing parasite or *trophozoite* and the mature parasite or *schizont* are found only in the peripheral blood in overwhelming infections.

The sexual forms are very characteristic. The female (*macrogametocyte*) is crescent-shaped, pale blue in color with slightly pointed ends. A large mass of chromatin and pigment is arranged ccmpactly near the center. The red cell is deformed and very pale. The male (*microgametocyte*) is crescent or sausage shaped, of a blue-gray color, with considerable chromatin, and pigment near the center arranged more diffusely.

In estivo-autumnal malaria smears of the peripheral blood will show only *the rings* and *the crescents*.

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URINE EXAMINATIONS

COLLECTION OF URINE

1. Single specimens collected at varying times in the day may yield different results, especially in amounts of glucose and albumin; this is a reason for the varying reports frequently obtained from different laboratories examining specimens of urine of the same person collected at different times. Specimens voided two or three hours after a meal are likely to contain most glucose or albumin; those passed first in the morning are least likely to contain them.

2. If any dependable data are desired regarding the quantitative composition of the urine, the examination of the mixed excretion for twenty-four hours is generally necessary. In collecting the urine the bladder may be emptied at any given hour, *e.g.*, 8 A.M., the urine discarded and all the urine voided from that hour up to and including that passed the next day at 8 A.M. saved, thoroughly mixed, accurately measured, and 4 to 8 ounces taken for analysis.

3. In certain pathologic conditions it is desirable to collect both day and night specimens. Urine voided between 8 A.M. and 8 P.M. may be taken as the day sample and that voided between 8 P.M. and 8 A.M. as the night sample.

4. Containers used for collection of urine should be *chemically clean.* Careful cleaning is required particularly in hospital laboratories to avoid the possibility of carrying over traces of albumin and sugar. Traces of syrup in insufficiently washed medicine bottles are sometimes responsible for errors.

5. Contamination with vaginal discharges may account for the presence of albumin and pus; contamination with menstrual discharges may account for the presence of albumin and blood. Both should be carefully avoided, as well as contamination with feces.

6. Urine to be examined for tubercle bacilli may be voided, although there are chances of contamination with smegma bacilli. Urine for other bacteriologic examinations should be collected aseptically, preferably by catheterization into sterile containers (*without a preservative*), as it is almost impossible otherwise to avoid bacterial contamination, especially with *Esch. coli* and staphylococci.

PRESERVATION OF URINE

Decomposition sets in rapidly, especially in warm weather, and greatly interferes with all examinations. An ideal preservative should prevent the growth of bacteria and molds; should not interfere with the accuracy of physical, chemical and microscopical examinations; should be readily soluble, of low cost, and preferably a solid.

1. If a *refrigerator* is available, samples may be kept in it until examined. Samples for pregnancy tests must be so preserved. Avoid freezing.

2. A small piece of *camphor*, sufficient to give a saturated solution, may be used.

3. *Thymol*, if used, should not exceed 0.1 gm. per 100 cc. of urine. An excess may interfere with albumin determinations. It is not as good as formerly surmised and is unsatisfactory when urine contains sugar, acetone or diacetic acid; also when urine is to be examined for phenol and quantitatively for phosphates or magnesium.

4. *Formalin*, in proportion of 2 to 4 drops to the ounce, is the most satisfactory of all, especially for the preservation of the formed elements. An excess may interfere with tests of indican, albumin and sugar, and produce a precipitate.

5. *Boric acid*, 5 grains for each 4 ounces, delays decomposition but may interfere with sugar determinations and precipitate rhombic crystals of uric acid.

6. *Toluol* may be used, especially for specimens to be examined for acetone and diacetic acids. Simply add enough to form a thin layer on the surface. It is a very satisfactory preservative for routine use.

7. *Chloroform* is the least satisfactory and should not be used as it interferes with sugar determinations and microscopic examinations.

PHYSICAL EXAMINATIONS

Amount. See page 47.

Turbidity. See page 51.

Color. See page 51.

Odor. See page 54.

Reaction. Normally, freshly voided urine is acid in reaction, the *p*H ranging from 4.8 to 7.5 with a general average of 6. Twenty-four-hour specimens are less acid than freshly passed urine and may be neutral or even slightly alkaline as a result of standing. Freshly passed urine may be neutral or alkaline as the result of the administration of alkalis, retention with "ammoniacal decomposition," etc. Diet greatly influences the reaction. The urinary acidity may be decreased or the urine may become alkaline for some time after a meal, due to the withdrawal of hydrogen ions from the blood during the secretion of free HCl by the stomach. The occurrence of this so-called "alkaline tide" may be employed as an indirect indication of the occurrence or nonoccurrence of gastric secretion of free HCl.

For ordinary purposes the reaction may be determined with good grades of blue and red *litmus papers* (Squibb's recommended):

Blue turning red: acid
Red turning blue: alkaline
No change in either: neutral
Changes both red and blue: amphoteric

The *total acidity* may be determined by titration according to the method of Folin and Wu as follows: 1. Use a sample of mixed twenty-four-hour urine as fresh as possible and accurately measured.

2. Place 25 cc. in a small flask or evaporating dish. Add 2 drops of 0.5 per cent alcoholic solution of phenolphthalein and 15 gm. of neutral finely pulverized potassium oxalate.

3. Shake vigorously for two minutes.

4. Immediately titrate with N/10 sodium hydroxide solution, shaking after each addition, until the first permanent pink color appears.

5. Read off amount of N/10 sodium hydroxide used.

6. Multiply by 4 to estimate amount required for 100 cc. of urine and report accordingly (normally 25 to 40 cc.).

7. Calculate and report amount required for total twenty-four-hour specimen. Normally 300 to 600 cc. (may be less; depends largely on diet).

There are numerous methods for determining the *hydrogen ion concentration* (or *p*H) of the urine but the simplest method, satisfactory for clinical purposes, is that involving the use of nitrazine paper (phenaphthazine) as follows:

1. With a clean glass rod, transfer a drop of urine to the surface of a strip of nitrazine paper (Squibb) and spread evenly by stroking or leave a small drop on the paper. After one minute compare with the color chart furnished. The paper may be dipped into the urine three consecutive times and the excess shaken off. Compare after one minute.

2. The color comparison chart reads from *p*H 4.5 to 7.5 in 0.5 divisions. It is possible to interpolate between these divisions by estimating the color half-way between them.

✓ **Specific Gravity.** The normal average is from 1.015 to 1.025. Pathologically it may vary from 1.001 to 1.060. If the specimen contains but a small or average amount of sediment, it makes but little or no difference if the specific gravity is determined without mixing in order to use the sediment later for microscopic examination. If, however, there is a large amount of sediment the specific gravity is almost always increased by about 0.002 after thorough mixing.

For ordinary determinations the Squibb urinometer may be used as follows:

1. Fill the cylinder without producing bubbles. The specific gravity may be taken without having first mixed the urine.

2. Float the urinometer so that it does not *touch the bottom or sides.*

3. Make the reading from the bottom of the meniscus.

4. The instrument is usually adjusted for readings at 25° C. For accuracy add 0.001 to the reading for each 3° C. above this temperature and subtract 0.001 for each 3° C. below, although moderate reduction in temperature does not influence the specific gravity as much as increased temperature.

5. For small amounts of urine, dilute with an equal volume of distilled water, mix and take specific gravity. Multiply the last two figures by 2. By this method the specific gravity is usually 0.001 to 0.002 higher. The Saxe urino-pyknometer (Eimer and Amend) may be used if at least 3 cc. of urine is available.

6. In the case of urine containing large quantities of protein, correction should be made for the latter by subtracting 0.003 times grams of protein per 100 cc. from the observed specific gravity.

Total Solids. The amount of solids excreted in the urine may be estimated roughly but accurately enough for most clinical purposes by multiplying the last two figures of the specific gravity of the mixed twenty-four-hour urine by Long's coefficient, 2.66. The product is then multiplied by the number of cubic centimeters of urine voided in twenty-four hours and divided by 1000 which gives the amount of total solids in grams.

QUALITATIVE TESTS FOR ALBUMIN

Normal urine contains a trace of albumin which is too slight to be detected by the simple tests in general use, a large number of which have been described. All depend upon precipitation of the albumin by chemical agents or coagulation by heat. All precipitate both serum albumin and serum globulin and do not differentiate between these two proteins. Most are subject to some error largely due to the precipitation of mucin or other constituents. All require the use of clear specimens, preceded by filtration if necessary, in order to detect small amounts of albumin. As a general rule simple filtration through ordinary filter paper is sufficient unless cloudiness is due to bacteria. Very large numbers of bacteria, especially organisms dissolved in alkaline urine, may yield false traces of albumin. They are difficult to remove, but this may be accomplished sufficiently for testing by centrifuging or by adding about one teaspoonful of purified talc, infusorial earth, or animal charcoal to each 2 or 3 ounces, shaking well and filtering through two thicknesses of filter paper. Some albumin is also removed by adsorption.

Methods for Recording Reactions. A wide diversity of methods for reporting qualitative tests is in use; this accounts in large part for discrepancies in reports from different laboratories. A uniform method and terminology are urgently needed. The following are recommended:

– = *negative*

± = *very slight trace.* Cloudiness or ring can just be seen against a black background.

+ (1) = *slight trace.* Cloud is distinct but not granular; no definite flocculation. Or the ring is sufficiently definite to be seen without a black background.

++ (2) = *moderate trace.* Cloud is distinct and granular without definite flocculation. Or the ring is dense but not wholly opaque when viewed from above. Represents about 0.1 per cent of albumin.

+++ (3) = *heavy cloud.* Cloud is dense with marked flocculation or the ring is heavy, wholly opaque and sometimes curdy. Represents about 0.2 to 0.3 per cent.

++++ (4) = *very heavy cloud.* Heavy precipitate to boiling solid; or very dense ring. Represents 0.5 or higher per cent of albumin; 3 per cent albumin boils solid.

Heat and Acid Test. 1. Boil about 5 cc. of filtered urine in a test tube for one or two minutes. Hold with a test tube holder.

2. Add 3 to 5 drops of 5 per cent acetic acid solution, a drop at a time.

3. A white cloud now disappearing is due to earthly phosphates.

4. A very faint trace of albumin may appear only upon the addition of the acid. Larger traces appear upon boiling and may become heavier upon the addition of the acid. The addition of too much acid must be carefully avoided as this may dissolve faint traces of albumin and give a falsely negative reaction.

Purdy's Test. 1. Fill a thin-walled test tube about two-thirds full with clear urine.

2. Add about one-sixth its volume of a saturated solution of sodium chloride

(to raise the specific gravity and prevent the precipitation of mucin) and 5 to 10 drops of 50 per cent acetic acid.

3. Mix well and boil the upper portion over a Bunsen burner. Rotate or shake gently while heating to prevent cracking of the tube by condensation of steam.

4. A white cloud in the heated portion shows the presence of albumin. A faint cloud is best seen when viewed against a black background (Fig. 70). *Bence-Jones protein* may produce a white cloud which disappears upon boiling and reappears upon cooling.

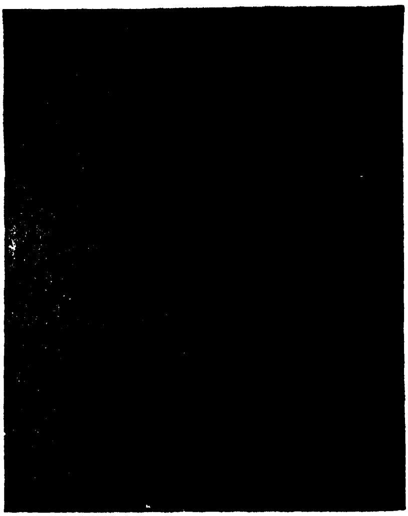

FIG. 70. CLOUD OF ALBUMIN SEEN AGAINST A DARK BACKGROUND.

(From Bass and Johns: *Practical Clinical Laboratory Diagnosis,* Williams & Wilkins Co., in Kolmer and Boerner: *Approved Laboratory Technic,* 4th ed., D. Appleton-Century Company, Inc.)

Roberts' Test. 1. Prepare the reagent by adding one volume of concentrated nitric acid to five volumes of a saturated aqueous solution of magnesium sulfate (U.S.P.).

2. Place a few cubic centimeters of reagent in a test tube, tilt the tube, and introduce the urine with a pipet, allowing the urine to flow gently down the side of the tube so as to overlay the reagent without mixing. If albumin is present, a fluffy, white ring of precipitated albumin forms at the line of contact.

3. Or the test may be conducted with a pipet made of glass tubing with an inside diameter of about 5 mm. Place a few cubic centimeters of the reagent in a test tube. With the pipet take up a small column of urine, about 1 cm. long, wipe excess of urine from the outside, then place the pipet in the test tube carefully, holding the finger firmly over the upper end until the other end touches the bottom of the tube. Release the finger pressure gradually, allowing the reagent to rise in the pipet, forming a clear, distinct layer with the urine.

4. A white ring at the junction of the reagent and urine, by either method, indicates albumin, the thickness and density of the ring showing the amount. No confusing color rings due to indican, iodides, bile pigments or the oxidation products of organic constituents are formed, as is frequently the case when nitric acid alone (Heller's test) is used. A white ring or cloudiness may form above the contact zone, due to urates or mucus, but such things are less sharp, broader, and lie above the albumin ring when both are present.

Exton's Test. 1. Prepare the reagent by dissolving 200 gm. of crystalline sodium sulfate in 800 cc. of water with the aid of heat. Cool to 35° C. and add 50 gm. of sulfosalicylic acid. Dissolve and dilute to 1000 cc.

2. Mix equal volumes of clear urine and reagent in a test tube. Warm the mixture gently; do not boil.

3. If cloudiness does not develop in the cold, albumin is absent. If cloudiness appears and persists or increases on gently heating, albumin is present.

Osgood-Haskins Test. 1. To five volumes of urine in a test tube, add one volume of a 50 per cent solution of acetic acid, followed by three volumes of a saturated aqueous solution of sodium chloride.

2. Heat the mixture gradually to boiling.

3. A precipitate appearing upon the addition of the acid indicates bile salts, urates, or resin acids, etc., whereas a precipitate appearing after the addition of the salt solution suggests *Bence-Jones protein,* or globulin in excess of 0.38 gm. per liter. As the temperature is raised, the precipitate of Bence-Jones protein, if present, will go back into solution; if albumin or globulin are present a precipitate will form. This test has, therefore, the advantage of indicating the presence of Bence-Jones protein as well as albumin and globulin.

QUANTITATIVE TESTS FOR ALBUMIN

Life Insurance Method. This method has been adopted by the Committee on Urinary Impairments of the Association of Life Insurance Directors of America.

1. Prepare *permanent standards* as follows: Dissolve 20 gm. of purest sheet gelatin in 120 to 140 cc. of distilled water at 45° to 55° C. and make up to 200 cc. Add half of the white of an egg and stir it in thoroughly. Heat in a water bath for at least thirty minutes after a temperature of 90° C. has been attained. Filter hot through a Whatman No. 4 paper, yielding a perfectly clear, slightly yellow solution. Immediately before use, add 0.3 cc. of formalin to each 100 cc. of gelatin solution. Formazin, the substance to be suspended in the gelatin, is made up as follows: Dissolve 2.5 gm. of urotropin (hexamethylene tetramine) in 25 cc. of distilled water at room temperature. Add this to 25 cc. of 1 per cent hydrazine sulfate solution also at room temperature. Mix, stopper, and allow to stand at least fifteen hours. Suspend the white amorphous precipitate uniformly by gently inverting the flask several times. Add 14.5 cc. of the formazin suspension to 100 cc. of the 10 per cent gelatin solution, to which the correct amount of formalin has been added, at 45° to 55° C. and mix thoroughly. This produces a turbidity equivalent to that made by an albumin solution of 0.1 per cent, or 100 mg. in 100 cc., when precipitated by three volumes of 3 per cent sulfosalicylic acid. Dilute the stock suspension according to the table on page 996 to make the other standards required.

Pour each standard into a test tube of the same dimensions as those used in making the test with urine. Seal the tubes with waxed stoppers and allow to cool to room temperature. Keep in a well-lighted room. In extremely hot weather, keep in a cool place. If in time they become greenish, exposure to sunlight will bleach them. There is no appreciable change in turbidimetric value in six to eight months and only a slight change in a year. It is best to replace them after eight months.

2. Pipet 2.5 cc. of urine, cleared by filtration or centrifugalization, into a test tube graduated at 10 cc. and add a 3 per cent solution of sulfosalicylic acid in

distilled water to the 10 cc. mark. Invert several times, allow to stand for ten minutes, and compare the turbidity with that of the permanent standards. Record the value of the standard most closely matched as the albumin content of the urine.

Stock formazin suspension equivalent to 100 mg. albumin per 100 cc.	10 per cent Clarified Gelatin	Value of standard made	
		Per cent	Mg. per 100 cc.
25.0 cc.	26 cc.	0.05	50
20.0 "	30 "	.04	40
15.0 "	35 "	.03	30
10.0 "	40 "	.02	20
5.0 "	45 "	.01	10
2.5 "	55 "	.005	5

The Shevky-Stafford Method. 1. Prepare the Tsuchiya reagent by mixing 15 gm. of phosphotungstic acid, 50 cc. of concentrated hydrochloric acid, and 1000 cc. of 95 per cent ethyl alcohol.

2. If the urine contains considerable albumin, dilute 1 cc. with 9 cc. of water. In urines with very scant albumin it is not necessary to dilute. Occasionally a urine is encountered with more than 2.8 per cent albumin, which is the maximum that can be determined with a 1:10 dilution. In such a case dilute 1 cc. of urine with 19 cc. of water.

3. Place 4 cc. of urine (diluted or undiluted) into a special graduated centrifuge tube (A. H. Thomas Co., No. 3007A). Add the reagent to the 6.5 cc. mark. Mix the contents thoroughly by inverting the tube several times, allow to stand exactly ten minutes, and centrifugalize for exactly ten minutes at 1800 rpm. The volume of precipitate is read on the scale in hundredths of a cubic centimeter.

4. Calculate as follows: Grams of albumin per 1000 cc. of urine = cc. of precipitate $\times$ 7.2 $\times$ dilution, where dilution indicates the number of times the urine was diluted before the sample was measured into the tube in the conduct of the test.

Esbach's Method. 1. Prepare the reagent by dissolving 10 gm. of picric acid and 20 gm. of citric acid in 1000 cc. of water. Or, the reagent may be prepared by diluting 100 cc. of trichloracetic acid with 900 cc. of water. The latter is preferred because the effects of the temperature and specific gravity of the urine are reduced.

2. Fill with urine an Esbach-Quick albuminometer to the mark U. Add reagent to the mark R. Close with a rubber stopper, invert several times and set aside in a cool place for eighteen to twenty-four hours.

3. Read off the results according to the markings on the tube which show albumin in grams per 1000 cc.; to express the per cent, divide by 10.

QUALITATIVE TESTS FOR GLUCOSE

Glucose readily reduces the oxide of copper in alkaline solution. When the whitish-blue cupric hydroxide in suspension in an alkaline solution is heated it is converted into insoluble black cupric oxide, but if glucose is present this is reduced

Fig. 1
Negative

Fig. 2
Trace of
Sugar (+)

Fig. 3
1 percent
Sugar (++)

Fig. 4
More Than
1 Percent
(+++)

Fig. 5
More Than
2 Percent
(++++)

FIG. 71. BENEDICT'S QUALITATIVE TEST FOR SUGAR IN THE URINE

(From *Essentials in the Management of Diabetes Mellitus*, Eli Lilly and Company, Indianapolis, in Kolmer and Boerner: *Approved Laboratory Technic*, 4th ed., D. Appleton-Century Company, Inc.)

to insoluble yellow or red cuprous oxide. A large number of tests have been devised on this principle of the detection of glucose in the urine, but that of Benedict is recommended because of its sensitivity, simplicity, and freedom from error. The qualitative reagent does not react with the normal glucose of the urine but detects increases above this level as low as 0.25 per cent. Furthermore, uric acid, creatinine, chloroform, formalin and other aldehydes do not interfere to such an extent as in the case of Fehling's test. If albumin is present in large amounts, it may interfere with the precipitation of copper and should be removed by acidifying with acetic acid, boiling and filtering. Small amounts need not be removed.

Benedict's Test. 1. Prepare Benedict's *qualitative reagent* by dissolving 17.3 gm. of cupric sulfate crystals (U.S.P.) in about 100 cc. of distilled water. Dissolve 117 gm. of sodium carbonate (monohydrated U.S.P) and 173 gm of sodium citrate (U.S.P.) in 700 cc. of water, with the aid of heat, if necessary. Cool to room temperature and pour in the copper solution slowly with constant stirring. When completely mixed make up the volume to 1000 cc. with distilled water.

2. Place 5 cc. of reagent in a test tube. Add 8 drops (0.5 cc.), not more, of urine. Boil vigorously in a water bath for five minutes; then allow to cool. Do not hasten cooling by immersion in cold water. If a large number of tests are to be conducted, the tubes may be placed in a boiling water bath, or a beaker of boiling water, and heated for 5 minutes, then allowed to cool.

3. In the presence of glucose the entire solution will be filled with a bulky precipitate which may be greenish yellow, yellow, or red in color, depending on the amount of glucose present. In the presence of over 0.2 to 0.3 per cent of glucose the precipitate will form quickly. If no glucose is present the solution will remain perfectly clear or will show a faint turbidity due to precipitated urates (Fig. 71).

Fermentation Test. 1. Place 15 cc. of urine in a test tube and add a piece of fresh Fleischmann yeast about the size of a pea; mix gently to emulsify the yeast.

2. Transfer to a fermentation tube; make sure the arm is free of bubbles of air.

3. Place in an incubator for a few hours.

4. A normal urine and a normal urine to which is added a pinch of glucose may be treated in the same manner as negative and positive controls, respectively.

5. A positive reaction due to alcoholic fermentation is indicated by the collection of carbon dioxide gas in the arm.

6. If necessary, guard against gas production by bacterial fermentation by adding a pinch of tartaric acid (advisable if mixtures are incubated more than four hours).

7. By using the Einhorn saccharometer, a *quantitative* test may be conducted, as the graduations on the arm indicate with fair accuracy the percentage of glucose present from 0.1 to 1 per cent.

Rubner's Test for Lactose. Lactose is found occasionally in the urine of women during lactation and in patients who have been on an exclusive milk diet for a long time. It reduces copper solutions, although less actively than glucose, 0.0676 gm. being equivalent to 25 cc. of Benedict's quantitative solution. It is not fermented by yeast.

1. To 10 cc. of urine add 3 gm. of lead acetate (an excess).

2. Shake well and filter into a test tube.

3. Boil the filtrate for a few seconds; add 1 cc. of strong ammonia and boil again.

4. If lactose is present, the solution turns brick-red and a red precipitate develops which is the criterion.

5. This test is not very sensitive but will detect lactose in about 0.3 to 0.5 per cent.

6. Glucose gives a red solution with a yellow precipitate.

7. Lactose does not ferment with yeast although bacteria may hydrolyze it into its constituents, glucose and galactose.

BENEDICT'S QUANTITATIVE TEST FOR GLUCOSE

1. Prepare Benedict's *quantitative reagent* by dissolving with the aid of heat 200 gm. of sodium carbonate (crystallized) or 100 gm. of anhydrous sodium carbonate in 800 cc. of distilled water. Add and dissolve 200 gm. of sodium or potassium citrate (C.P.) and 125 gm. of potassium sulfocyanate (C.P.). Filter if necessary. Dissolve 18 gm. of copper sulfate (U.S.P. crystals) in 100 cc. of distilled water, and pour this solution slowly into the first one, with constant stirring. Add 5 cc. of a 5 per cent solution of potassium ferrocyanide in distilled water, cool at room temperature, and make up to 1000 cc. with distilled water. The copper sulfate should be weighed with extreme accuracy. Exactly 25 cc. of this reagent are reduced by 0.05 gm. of glucose.

2. Dilute 10 cc. of clear urine with 90 cc. of distilled water unless the glucose content is known to be low.

3. Fill a 50 cc. buret with urine (diluted or undiluted).

4. Measure exactly 25 cc. of the reagent into a porcelain evaporating dish, add about 15 gm. of crystalline sodium carbonate or 6 gm. of anhydrous sodium carbonate, along with a small amount of pumice or talc.

5. Heat to boiling over a free flame and keep the mixture boiling vigorously during the entire titration.

6. As soon as the sodium carbonate is completely dissolved, add the urine from the buret, rapidly at first, until a chalk-white precipitate forms and the blue color begins to fade perceptibly. It is then run in a few drops at a time until the last trace of blue disappears from the solution. Half-minute intervals must be allowed to elapse between additions of urine in the final steps of the titration. Water may be added if the mixture becomes too concentrated. The end point must be determined while the solution is still hot; on cooling, the solution tends to regain a bluish-green tint. With urine, the color of the end point tends to be a slight yellowish, or yellowish-green, due to urinary pigments which sometimes results in error in the conduct of the test.

7. The calculation is made as follows: When the urine is diluted 1:10: $\frac{0.050 \times 1000}{N}$ = per cent of glucose in original sample where N is the number of cubic centimeters of diluted urine required to reduce 25 cc. of the reagent. Example: 16.5 cc. required: $\frac{0.050 \times 1000}{16.5} = 3\%$ glucose.

If *undiluted* urine is used the formula to be employed is $\frac{0.050 \times 100}{N}$ where N = number of cubic centimeters of urine required for the reduction. Example: 6.2 cc. required: $\frac{0.050 \times 100}{6.2} = 0.8\%$ glucose.

QUALITATIVE TESTS FOR ACETONE

Lange's Test. 1. Prepare a *fresh* saturated solution of sodium nitroprusside by dissolving several crystals in 1 to 2 cc. of water by gentle heat, having a slight excess of undissolved crystals remaining.

2. Place 5 cc. of filtered urine in a test tube. Add 0.5 cc. of glacial acetic acid and 0.5 cc. of the nitroprusside solution; mix thoroughly.

3. Tilt the tube and carefully overlay the mixture with 1 to 2 cc. of a 28 per cent solution of ammonium hydroxide.

4. A purple or purplish-red ring forms at the line of contact in a few minutes if acetone is present. The ring tends to be more purple or violet in low concentrations, more red-purple in high. Amorphous urates may give a brown or orange ring if present in large amount.

Rantzman's Test. 1. Prepare the reagent by dissolving 37.5 gm. of ammonium nitrate crystals and 2.5 gm. of sodium nitroprusside in distilled water and make up to 100 cc. In a brownish glass-stoppered bottle it will keep for two months.

2. To 3 cc. of urine in a test tube add 1 cc. of the reagent. Mix and overlay with a 28 per cent solution of ammonium hydroxide.

3. If acetone is present, a sharply defined purple or burgundy-red ring appears at the line of contact. The smaller the amount of acetone present, the longer it takes the ring to appear.

Ross Modification of Rothera's Test. 1. Prepare the reagent by mixing 1 gm. of powdered sodium nitroprusside with 100 gm. of powdered ammonium sulfate.

2. Place 1 gm. of the dry powdered reagent in a test tube and add 5 cc. of clear urine. Mix until the powder is dissolved. Then overlay with a 28 per cent solution of ammonium hydroxide.

3. A red-purple permanganate color at the line of contact indicates the presence of acetone.

GERHARDT'S TEST FOR DIACETIC ACID

1. To 5 cc. of urine in a test tube, add a 10 per cent aqueous solution of ferric chloride, drop by drop, until no more phosphates precipitate.

2. Filter and add more ferric chloride solution.

3. If diacetic acid (aceto-acetic acid) is present, a bordeaux-red color develops. A similar color is produced by phenols, coal-tar antipyretics, bicarbonates, salicylates, etc.

OBERMAYER'S TEST FOR INDICAN

This test depends on the decomposition of indican and the subsequent oxidation of the liberated indoxyl to indigo blue, and in the case of some specimens to indigo red.

1. Prepare the reagent by dissolving 2 gm. of ferric chloride in 1000 cc. of concentrated hydrochloric acid (sp. grav. 1.19 or 23.5° Baumé).

2. To 5 cc. of urine in a test tube, add an equal amount of reagent and 1 to 2 cc. of chloroform. Mix by inverting ten times. Allow the chloroform to settle and examine its color.

3. A pale blue to deep blue to violet color indicates the presence of indican, the intensity of the color being proportional to its concentration. If oxidation is slow, a red color due to the formation of indigo red may appear. Iodides may give a red-violet color due to the liberation of iodine. The addition of a few drops of a concentrated solution or a small crystal of sodium thiosulfate will discharge this color. Thymol may produce a violet color which is also discharged by thiosulfate. Bile pigments interfere with the test and must be removed by adding one-fifth volume of 10 per cent calcium or barium chloride solution and filtering. Urotropin and formalin prevent the appearance of indigo blue even when indican is present in large amounts.

TESTS FOR BILIRUBIN

Barium Strip Modification of Harrison's Test. This test is conducted as follows: 1. Thoroughly immerse sheets of Schleicher and Schull filter paper (No. 470) in a saturated aqueous solution of barium chloride; dry in an oven or at room temperature. Cut into strips 3 or 4 inches by ½ inch in size, which keep indefinitely.

2. Insert a strip perpendicularly in the specimen of urine for five to ten seconds (at least ¼ inch of the lower end should be below the surface of the urine).

3. Remove the strip and place it horizontally on ordinary filter paper.

4. At the point where the strip was at the surface of the urine, a yellow or brown line, or zone, is seen running transversely across the strip. Place two or three drops of Fouchet's reagent (25 per cent trichloracetic acid containing 0.9 per cent ferric chloride) directly on this area.

5. A green color indicates the presence of bilirubin. A semiquantitative test may be made by using the color chart of Watson and Hawkinson (Fig. 72).

Methylene Blue Modification of Franke's Test. This test, which is somewhat less sensitive than the barium-strip test, may be conducted as follows: 1. To 5 cc. of prebreakfast urine add 2 drops of 0.2 per cent aqueous solution of methylene blue chloride.

2. If a green color results, add more of the reagent dropwise until the green color is converted to blue. Record the number of drops required. If more than 5 drops are required repeat the test with urine diluted with distilled water and collect for the dilution factor.

3. In 1000 patients with diseases other than hepatitis Gellis and Stokes (J.A.M.A., *128:* 782, 1945) have reported that 74 per cent yielded a blue reaction after the addition of 2 drops of the reagent, 24.3 per cent after 3 drops, and 1.7 per cent after 4 drops. On the basis of these results a test of 5 drops or more was considered a positive reaction.

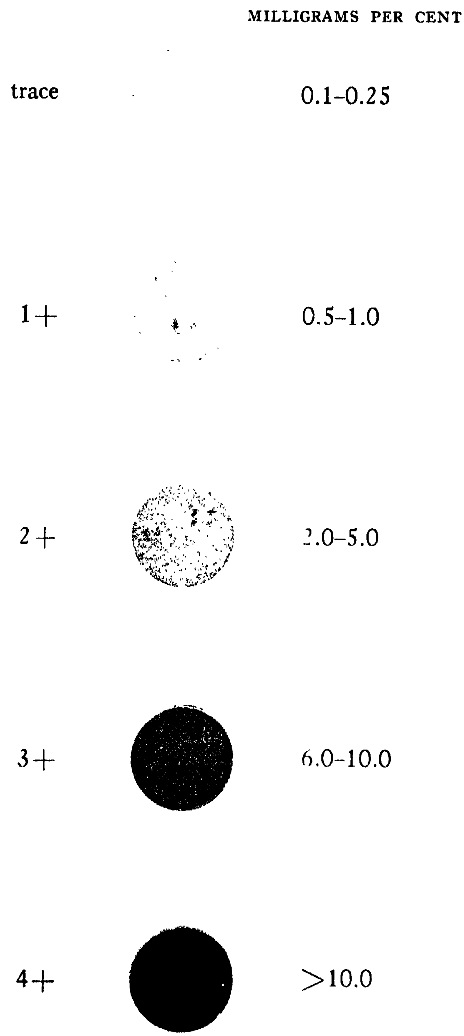

FIG. 72. BARIUM-STRIP MODIFICATION OF HARRISON'S TEST FOR BILIRUBIN IN THE URINE.

(Courtesy of C. J. Watson and V. Hawkinson and the Schleicher & Schuell Co.)

Hammarsten's Test. This is a test for bilirubin, conducted as follows:

1. Prepare a stock reagent by mixing 1 cc. of dilute nitric acid (one part of concentrated acid diluted with three parts of water) with 19 cc. of dilute hydrochloric acid (one part of concentrated acid diluted with three parts of water).

2. Prepare the test reagent by diluting 1 cc. of stock reagent with four parts of absolute ethyl alcohol.

3. Place 2 cc. of the test reagent in a test tube and add a few drops of urine.

4. Or, the test may be conducted with a urinary precipitate prepared as follows: To 5 cc. of acid urine (acidify if necessary) add 5 cc. of a 10 per cent solution of barium chloride. Mix well and centrifugalize. Decant and discard the supernatant fluid. Mix the precipitate with 2 cc. of the test reagent and centrifugalize.

5. A positive reaction is indicated by a green color. This test is sensitive to 1 part of pigment in 1,000,000 parts of urine.

Huppert-Nakayama's Test. This is a test for bilirubin, conducted as follows:

1. Prepare the Nakayama reagent by dissolving 0.4 gm. of ferric chloride in a mixture of 99 cc. of 95 per cent ethyl alcohol and 1 cc. of concentrated hydrochloric acid.

2. To 5 cc. of urine in a test tube add 5 cc. of a 5 per cent solution of barium chloride. Mix thoroughly and centrifugalize.

3. Pour off the supernatant fluid. To the sediment add 2 cc. of the Nakayama reagent. Mix and bring to a boil.

4. A brilliant, deep-green color develops if bilirubin is present. On adding a few drops of nitric acid the color changes to violet or red.

TESTS FOR UROBILINOGEN

Ehrlich's Test. This test, as modified by Wallace and Diamond, is roughly quantitative.

1. Prepare the reagent by dissolving 2 gm. of paradimethylaminobenzaldehyde in 100 cc. of 20 per cent (by volume) hydrochloric acid.

2. To 10 cc. of bile-free, undiluted urine at room temperature, or warmed to 21° to 22° C., add 1 cc. of the reagent. Allow to stand for 3 minutes.

3. If a deep cherry-red color appears, proceed with the test, using 10 cc. amounts of dilutions of urine prepared with tap water at room temperature as follows: 1:10, 1:20, 1:50, 1:100 and 1:200. Add 1 cc. of reagent to 10 cc. portions of each dilution, let stand 3 to 5 minutes, not longer, and read.

4. Express the results in terms of the highest dilution giving a faint but definite pink or cherry color. Normally this is at the 1:20 dilution. Any greater dilution yielding a definite pink color indicates a pathologic amount of urobilinogen. A daily estimation showing positive in greater and greater dilution is especially significant.

Quantitative Ehrlich Test. A quantitative Ehrlich test as modified by Watson may be conducted as follows: 1. Urine collected at any time may be used but it is preferred that the patient empty the bladder at 2 P.M., drink one glass of water and void urine for the test at 4 P.M.

2. After cooling to room temperature, 2.5 cc. are mixed with 2.5 cc. of Ehrlich's

reagent (0.7 gm. p-dimethylaminobenzaldehyde dissolved in 100 cc. distilled water and 150 cc. concentrated hydrochloric acid) in an Evelyn tube.

3. Add 5 cc. of a saturated aqueous solution of sodium acetate and mix thoroughly.

4. In a second Evelyn tube place 2.5 cc. of urine and 5 cc. of the sodium acetate solution and mix thoroughly; slowly add with constant shaking 2.5 cc. of Ehrlich's reagent. This tube serves as a blank for the center setting when the Evelyn photoelectric colorimeter or a comparator block is employed.

5. The total amount of urobilinogen for the two-hour urine is calculated as follows:

$$\left.\begin{array}{l}\text{Conc. of final solution}\\ \text{in terms of mg. urobilinogen}\\ \text{per 100 cc.}\end{array}\right\} \times 4 \times \frac{\text{urine volume}}{100} = \text{Ehrlich units in sample.}$$

$$\text{Example: } 0.15 \times 4 \times \frac{90}{100} = 0.54 \text{ Ehrlich units}$$

EHRLICH'S DIAZO TEST

1. Prepare reagent No. 1 by dissolving 1 gm. of sulfanilic acid in 200 cc. of water and 10 cc. of concentrated hydrochloric acid.

2. Prepare reagent No. 2 by dissolving 0.5 gm. of sodium nitrite in 100 cc. of water.

3. In a test tube, mix 10 cc. of reagent No. 1 with 0.1 cc. of reagent No. 2.

4. Add an equal volume of urine. Mix and overlay with 1 or 2 cc of 28 per cent solution of ammonium hydroxide.

5. A positive reaction is indicated by the development of eosin pink to deep crimson red color at the line of contact. On shaking, a distinct pink color is imparted to the foam (essential feature). The color is a pure pink or red; any trace of yellow or orange is a negative reaction. A doubtful reaction should be considered negative.

BENZIDINE TEST FOR HEMOGLOBIN

This test is very sensitive, provided the reagents are satisfactory. Different lots of benzidine vary greatly in sensitivity, and hydrogen peroxide solution rapidly deteriorates. For this reason it is always advisable to set up a positive control using water with an extremely small amount of blood added.

1. Prepare a saturated solution of benzidine in glacial acetic acid. The benzidine labelled "For blood tests" should be employed.

2. Mix equal parts of this solution and hydrogen peroxide in a test tube.

3. Place a few cubic centimeters of urine in a test tube and add an equal amount of the mixed reagents. A positive reaction is indicated by the development of a green to deep blue color.

1. Prepare *urease paper* as follows: Transfer 30 gm. of jackbean meal to a 200 cc. flask, add 100 cc. of dilute ethyl alcohol (30 cc. of 95 per cent ethyl alcohol diluted to 100 cc.) and 1 cc. of acetate buffer. Stopper and shake vigorously for 15 minutes. Transfer to centrifuge tubes, close the mouths with tinfoil, and centrifugalize for 30 minutes. Transfer the supernatant to a flat-bottomed dish and take up at once strips of filter paper (Schleicher and Schull's No. 597). Suspend the papers and allow to dry overnight in an incubator at 37.5° C. Cut

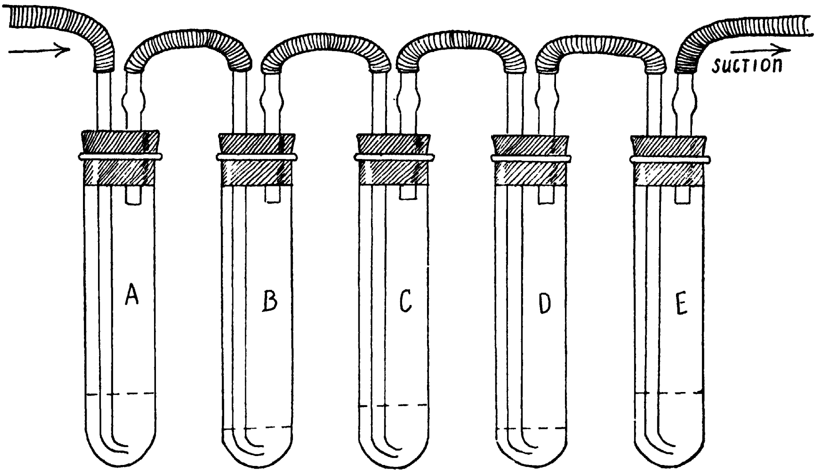

FIG. 73. AERATION APPARATUS.

into pieces about 1 × 2.5 cm. and preserve in wide-mouthed dark glass bottles. Urease so prepared will retain its activity for at least six months.

2. Prepare *acetate buffer* by dissolving 15 gm. of crystallized sodium acetate in a 100 cc. volumetric flask with 50 to 75 cc. of water. Add 1 cc. of glacial acetic acid, dilute to volume, and mix.

3. Prepare five aeration tubes, 200 by 25 mm. with inlet and outlet tubes. two-holed stoppers, and rubber tubing connections for aeration as shown in Figure 73. Mark the tubes A, B, C, D and E in order in which the stream of air will pass through.

4. In tube A place 20 cc. of dilute sulfuric acid; in tube B 10 cc. of ammonia-free water, two drops of acetate buffer and a piece of urease paper; in tubes C and D 25 cc. of 0.02 N sulfuric acid and 5 drops of methyl red indicator; in tube E 5 cc. of urine and 5 drops of caprylic alcohol.

5. Insert stoppers tightly making sure that the inlet tubes reach nearly to the bottom of each tube. Add 5 drops of caprylic alcohol and exactly 0.5 cc. of urine to tube B. Stopper quickly. Allow to stand 20 minutes or longer, occasionally shaking the tube to free the urease from the paper.

6. Attach the train of tubes to a suction pump and draw air through all the tubes for one minute.

7. Shut off the suction and add to B and D 10 cc. of potassium carbonate solution (90 gm. dissolved in 100 cc. of distilled water). Stopper quickly and begin suction slowly, gradually increasing to a moderate rate. Aerate for at least 30 minutes; one hour may be required if too small a stream of air is used.

8. When the aeration is complete, turn off the suction before disconnecting the tubes (center ones first).

9. Titrate the acid remaining unneutralized in tubes C and E with 0.02 N sodium hydroxide (prepared by diluting 20 cc. of tenth normal solution with 80 cc. of distilled water).

10. The number of cubic centimeters of 0.02 N acid neutralized in tube C, multiplied by 0.056, gives the percentage of ammonia nitrogen plus urea nitrogen. The acid neutralized in tube E, multiplied by 0.0056, gives the percentage of ammonia nitrogen. The difference between the two gives the percentage of *urea nitrogen;* this figure multiplied by 2.14 gives the percentage of *urea.* To obtain the percentage of *ammonia,* multiply the percentage for tube E by 1.1216.

QUALITATIVE TEST FOR CALCIUM

The following test is frequently helpful in the rapid detection of tetany due to hypoparathyroidism.

1. Prepare the Sulkowitch reagent by dissolving 2.5 gm. of oxalic acid crystals and 2.5 gm. of ammonium oxalate crystals in 145 cc. of distilled water; add 5 cc. of glacial acetic acid.

2. In a test tube mix equal parts of clear urine and reagent.

3. If there is no precipitate the total serum calcium is probably reduced to between 5 to 7.5 mg. per 100 cc. A fine white precipitate indicates a normal range of serum calcium (9 to 11 mg.). A milky precipitate indicates an increase of calcium (may be observed after the ingestion of calcium in powder or large amounts of milk).

MICROSCOPIC EXAMINATION OF SEDIMENTS

As far as possible, specimens should be examined within six hours after voidance. Unless kept at a low temperature, twenty-four-hour specimens should have a preservative added. Alkaline specimens cloudy with phosphates and obscuring other elements may be slightly acidified with dilute acetic acid to redissolve them. Highly acid specimens containing heavy sediments of urates obscuring other elements may be slightly warmed to redissolve them. If centrifuging is not employed, the sediment should be allowed to collect by gravity (preferably in a conical container) and examined before other tests are conducted. Centrifuging, however, is required for the examination of small amounts of sediment and is advised routinely.

1. Secure sediment by centrifuging at least 15 cc. for three to five minutes

or by allowing the urine to stand at least six to twelve hours in a cool place for settling by gravity (preferably in a conical container).

2. Remove a drop of sediment by means of a pipet and place on a slide. The pipet may be a piece of tubing drawn to a blunt point and fitted with a nipple. Eight may be prepared at one time by using slides of ordinary window glass, 4 by 8 inches, divided by painted lines into 8 compartments. The stage of the microscope may be extended by a wooden table, but this is not absolutely necessary.

3. Cover glasses are not essential for ordinary examination but are advisable for high-power examinations.

4. The examination must be completed before drying takes place.

5. Examine with low power and with oblique illumination obtained by swinging the mirror a little out of the optical axis. *Too strong illumination and too great magnification are common sources of error.*

6. The frequency of occurrence of the various constituents observed should be noted as well as their mere presence. The terminology used may be: "occasional," "few," "many," "very many," etc. A uniform technic of examination and of reporting should be followed so that the results of different examinations may be uniform and comparable. The same amount of urine should be centrifugalized at the same speed for the same length of time in each case. The supernatant urine should be poured off to the same degree of completeness, and approximately the same thickness of drop examined.

Casts and Cylindroids. Casts vary greatly in size but in almost all instances their sides are parallel and ends rounded or broken off squarely. They may be straight or curved, long or short, but the diameter is usually uniform throughout the length. Casts have been classified according to their microscopic characteristics as hyaline, granular, epithelial, blood, pus, fatty, and waxy (Figs. 74 and 75). The finding of casts in the urine is very important, for their presence usually indicates some form of kidney disorder, especially if albumin is also present. Mucous threads, with or without deposits of crystals about them, may simulate true casts. They are usually rough-edged, larger, tapering, or frayed at the ends (Fig. 75). Sometimes they are pale, ribbon-like structures, too long to be casts, with variable widths and small diameters.

Leukocytes and Pus. These are round, mono- or polynucleated cells, ordinarily colorless (Figs. 75 and 76). Normal numbers of leukocytes usually occur as isolated scattered cells. When pus is present, the leukocytes usually occur in clumps. Adding a drop of dilute acetic acid to the sediment brings out the nuclei.

Erythrocytes. In fresh urine these cells appear as biconcave disks becoming compact and crenated in concentrated acid urine (Figs. 75 and 76), and swollen, colorless, disintegrating, faint shadows in dilute alkaline urine. Where less than 12 erythrocytes are present per high-power field, the benzidine reaction is likely to be negative.

Epithelial Cells. A few squamous cells are usually present. In certain pathologic states they may be greatly increased in number and since the different parts of the urinary tract are lined with different types of epithelium, the number and type of cells present may possess diagnostic value. They are usually recorded as squamous or pavement, transitional, small round or polyhedral and caudate cells (Fig. 77).

Spermatozoa. These are usually identified by the characteristic oval heads and long tails (Fig. 77).

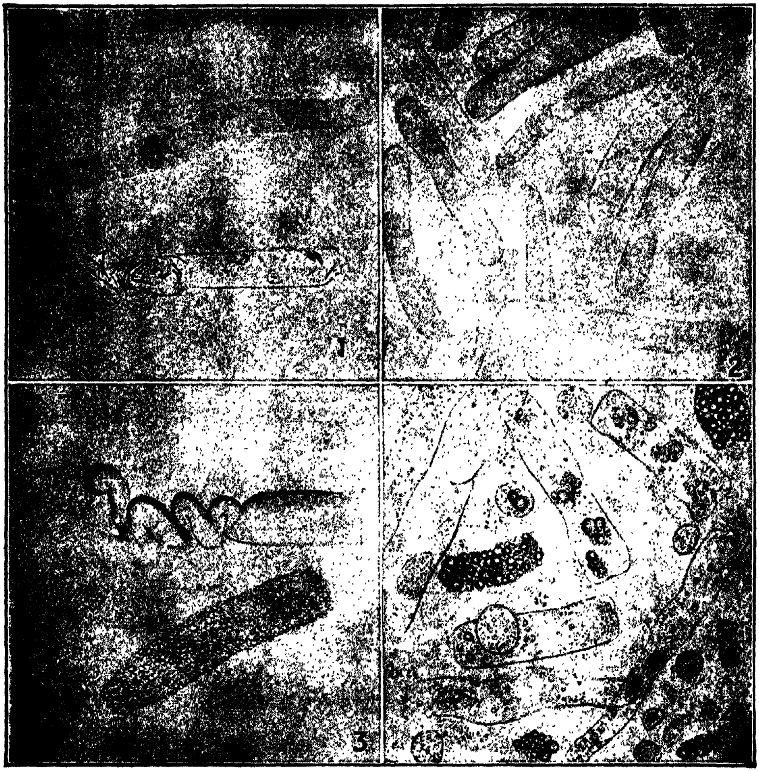

FIG. 74. URINARY CASTS.

1. Hyaline casts (after Riedert). 2. Hyaline and finely granular casts (after Todd and Sanford). 3. Waxy and granular casts (after Riedert). 4. Granular and fatty casts (after Riedert). (From Kolmer and Boerner: *Approved Laboratory Technic,* 4th ed.. D. Appleton-Century Company, Inc.)

Crystals. The presence and kinds of crystals are influenced by many factors including the metabolic activities of the body, fermentation and decomposition occurring in the bladder or in the urine after voiding. Only rarely do they possess clinical significance. Crystals may occur as definite structures or as amorphous deposits having a granular, structureless appearance; the latter can be partially identified by solubility or microchemical tests conducted on the slide while under microscopic observation.

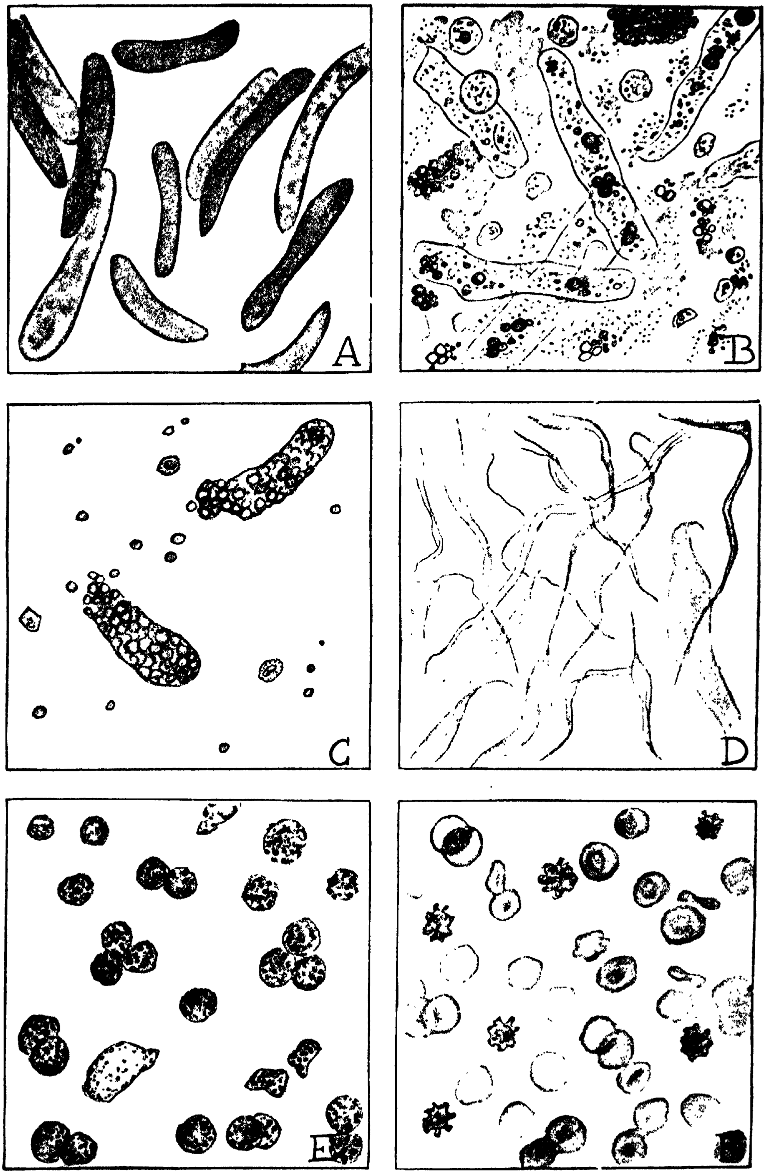

FIG. 75. URINARY CASTS, PUS AND BLOOD.

A, hyaline and finely granular casts; *B*, coarsely granular and fatty casts; *C*, blood casts; *D*, mucous threads and cylindroids; *E*, pus cells; *F*, erythrocytes.

In acid urines one may find amorphous, pinkish sediments of urates; brownish, wedge-like, "whetstone," or dumbbell crystals of uric acid (Fig. 78); small dumbbell or square "envelope" crystals of calcium oxalate (Fig. 78); refractile, colorless, six-sided plates of cystine; yellowish, small spheroids of leucine or fine needles of tyrosine (Fig. 78); and brownish needles or prisms of hippuric acid.

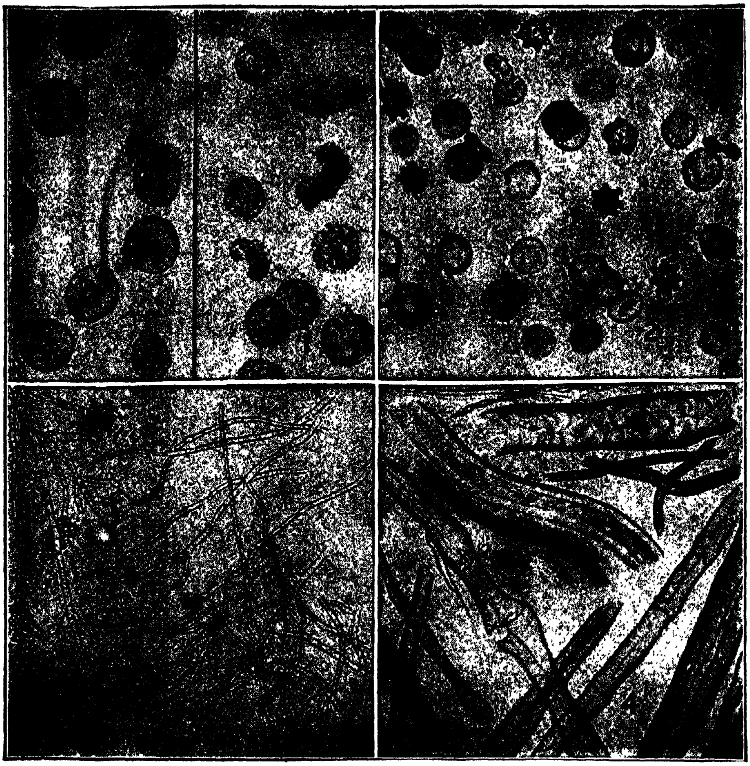

FIG. 76. URINARY LEUKOCYTES, ERYTHROCYTES, MOLDS AND ARTEFACTS.

1. Leukocytes (after Todd and Sanford). 2. Erythrocytes (after Todd and Sanford). 3. Molds (after Riedert). 4. Artefacts (after Riedert). (From Kolmer and Boerner: *Approved Laboratory Technic,* 4th ed., D. Appleton-Century Company, Inc.)

Alkaline urine may contain white amorphous phosphate deposits; "coffin lid" or feathery crystals of ammonium magnesium phosphate known as triple phosphates (Fig. 78); spheres or dumbbells or amorphous deposits of calcium carbonate (Fig. 78); and dark yellow or brown "cockle-burr" crystals of ammonium urate (Fig. 78).

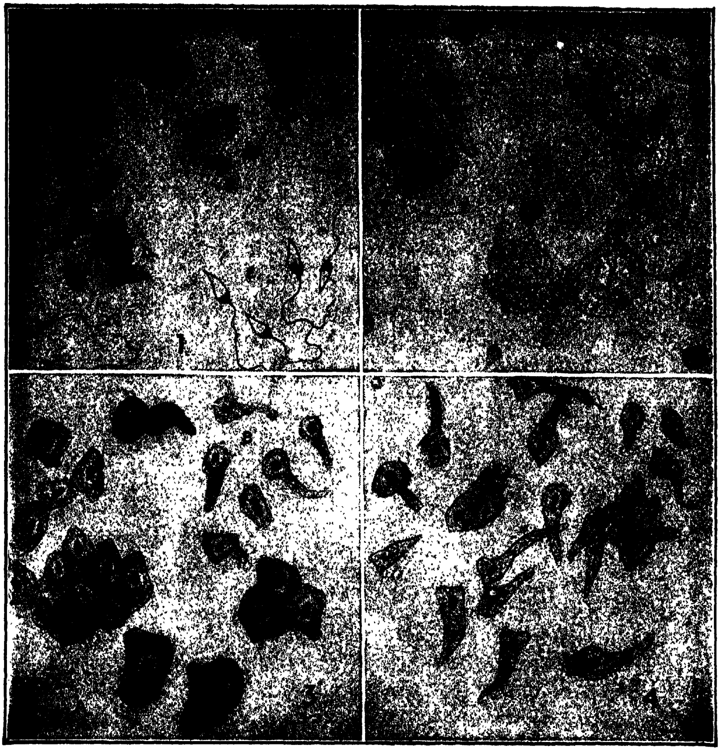

FIG. 77. URINARY EPITHELIUM AND SPERMATOZOA.

1. *A,* vaginal cells; *B,* urethral cells; *C,* renal cells; *D,* cells from the pelvis of kidney; *E,* spermatozoa (all after Riedert). 2. Squamous and pus cells (after Todd and Sanford). 3. *A,* bladder cells; *B,* urethral cells (after Todd and Sanford). 4. Cells from pelvis of kidney (after Todd and Sanford). (From Kolmer and Boerner: *Approved Laboratory Technic,* 4th ed., D. Appleton-Century Company, Inc.)

ADDIS SEDIMENT COUNT

This test is based upon the principle that the number of casts and cells in the urine varies with the type and extent of kidney disease. It is of the utmost importance that the patient clearly understand the conditions under which the specimen must be secured, and it is advisable that, in order to prevent errors, the directions be furnished in writing.

In order that the specimen may reach the laboratory in a suitable condition, the urine should be passed directly into a wide-necked bottle which has been thoroughly cleansed, rinsed with distilled water, dried in the inverted position, and finally rinsed out with a clean solution of formaldehyde, allowed to drain

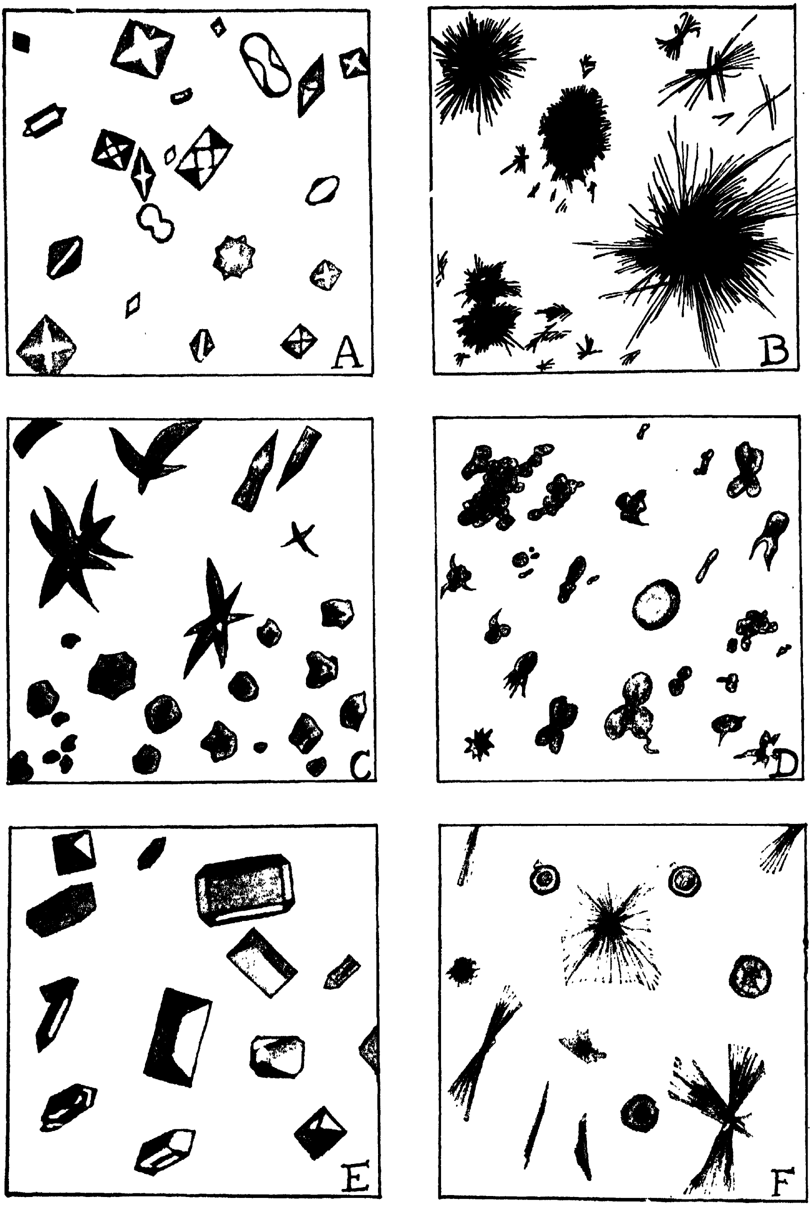

FIG. 78. URINARY CRYSTALS.

A, calcium oxalate; *B*, calcium urate; *C*, uric acid; *D*, ammonium urate; *E*, triple phosphates; *F*, leucine and tyrosine.

out for a short time before the bottle is closed with a rubber stopper. In women, catheterization is essential and it is important that the bladder be *completely emptied.*

1. The patient is instructed to take breakfast as usual, including coffee, tea or milk as desired, *but must abstain from all fluids thereafter* during the day and night until the urine has been collected in the morning. In other respects, the diet is unaltered, except that specific instructions are given not to take more fruit than is customary. The time of collection may run from any convenient hour in the evening until the patient arises in the morning, covering at least 12 hours. The patient is asked not to void during the afternoon of the day on which the collection is begun. The first voiding is discarded, all others being passed directly into the container as above described, care being taken to empty the bladder as completely as possible. The time when the collection is begun and ended must be recorded on the bottle.

2. Any uratic precipitate is dissolved by immersing the bottle in warm water; phosphatic turbidity is cleared by the addition of acetic acid, avoiding an excess.

3. Measure the volume (to within $\pm$ 2 cc.), return the specimen to the collecting bottle, stopper, and mix well by repeated inversion.

4. Transfer 10 cc. to a graduated centrifuge tube with a special narrow tip and centrifuge 5 minutes at 1800 r.p.m.

5. Decant the supernatant urine and remove the remainder with a capillary pipet.

6. Thoroughly mix the sediment by repeatedly drawing it up and blowing out in the capillary pipet.

7. Place 1 drop of mixed sediment on each cell of a blood counting chamber and count the casts and other formed elements, using a high dry lens. In normal urines where casts are few, 10 such drops are usually counted. Where the concentration of formed elements is heavy, however, the sediment (1 cc.) should be diluted with 4 cc. of normal saline solution and at least 2 drops counted.

8. Since in the usual form of blood counting chamber the ruled area is composed of 9 large squares, each of which is 1 sq. mm. in area, and since the chamber is 1 mm. deep, the volume contained over the total area is 0.9 c.mm. or 0.0009 cc. If 10 areas are counted, the volume of urine represented is 0.009 cc.

Assuming that 90 casts were found in this volume and that the volume of urine (or 1 per cent sodium chloride solution) in which the sediment was mixed was 0.9 cc., then $90 \times \frac{0.9}{0.009} = 9000$ = number of casts in 0.9 cc.

But, as the casts in 10 cc. are all (presumably) concentrated in the 0.9 cc. volume, the 9000 represents the number of casts in 10 cc. of urine.

If the total volume of urine in 12 hours was 300 cc., then the total number of casts in 12-hour urine is:

$$9000 \times \frac{300}{10} = 270{,}000$$

The following general formula applies to the quantitative determination of all the formed elements: number counted times volume in cc. in which sediment was mixed, divided by volume in cc. in which count was made, multiplied by volume in cc. per 12 hours, divided by 10, equals number in 12-hour urine.

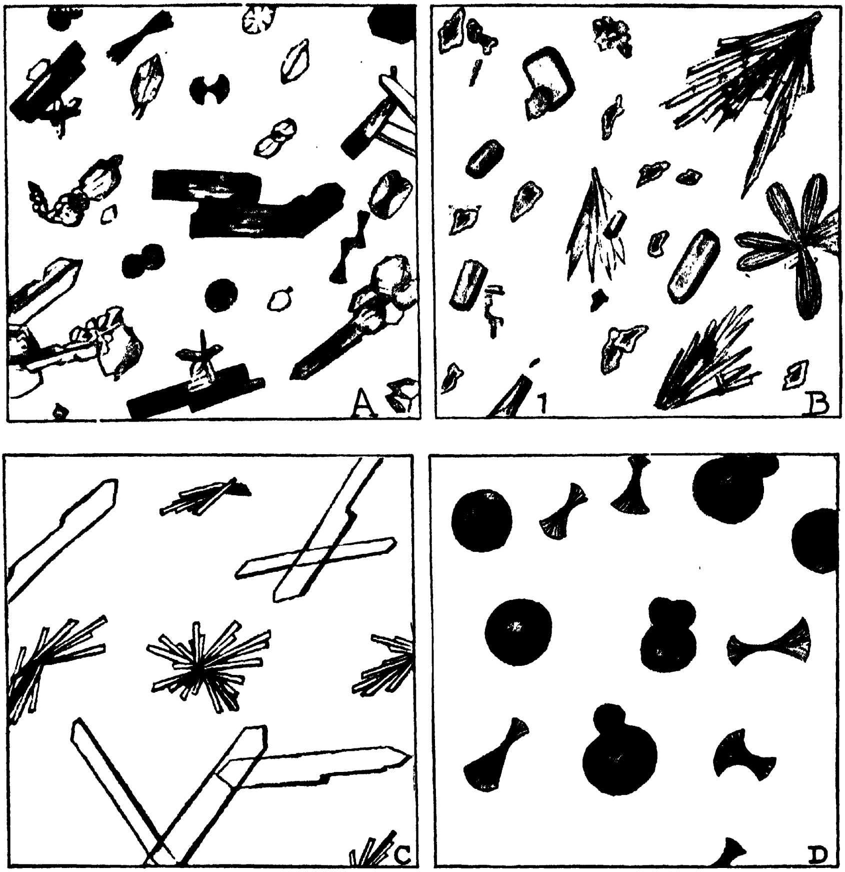

FIG. 79. TYPES OF URINARY CRYSTALS OF THE SULFONAMIDE COMPOUNDS.
A, sulfathiazole; *B,* sulfapyridine; *C,* sulfanilamide; *D,* sulfadiazine.

SULFONAMIDE CRYSTALS

When microscopic or macroscopic hematuria occurs during treatment with the sulfonamide compounds and especially with sulfathiazole or sulfapyridine, it is important to examine the urine for the crystals of these compounds. The latter usually occur as crystals of free and acetylated compounds, especially the latter. *Fresh urine should be employed,* sediment being secured by centrifugalization. The crystals produced by sulfathiazole, sulfadiazine, sulfapyridine and sulfanilamide are shown in Figure 79.

1. Collect morning urine in a clean container. It should be kept in a refrigerator until used. In case of considerable delay, about 4 grains of boric acid may be added to 4 ounces of urine.

2. Filter through paper or centrifugalize.

3. Administer two doses of 10 cc. each intravenously about six hours apart to a female rabbit which should be not less than seventeen weeks old and weigh not less than 1500 gm. One dose of 15 cc. is probably preferred. The urine should be warmed to room temperature before injection. Rabbits should be obtained from a reliable dealer. They should be separated at weaning and kept in individual cages for three to four weeks before use. Otherwise, mature nonpregnant rabbits not less than six months old and weighing not less than 2500 gm. may be used. They should have had a litter and been isolated for at least three or four weeks preceding the test. Such mature rabbits should receive two injections of 15 cc. each of urine.

4. Forty-eight hours after the first injection anesthetize the rabbit and examine the ovaries.

5. In a negative reaction the ovaries remain small in size and show no changes. In a positive reaction the ovaries show numerous corpora haemorrhagica and corpora lutea. If one ovary appears negative, the other must be examined, but if one is positive the other need not be inspected. Some rabbits are refractory and fail to show the characteristic ovarian changes even when injected with the urine of well-advanced pregnancy. This appears to happen more frequently in young than in older rabbits. Therefore, if the ovaries appear small and poorly developed, it is advisable to repeat the test with a fresh animal.

6. Negative rabbits can be used again almost immediately. Rabbits showing positive reactions may be used again after an interval of ten to fourteen days.

35

BLOOD CHEMISTRY EXAMINATIONS

COLLECTION OF BLOOD

1. With a sterile syringe, blood is usually taken from a vein at the elbow. It may also be taken from a finger if a micromethod of analysis is to be conducted. Methods are described and illustrated in Chapter 17 (see page 448).

2. Specimens are usually taken in the morning, before breakfast, to obviate changes due to the ingestion of foods. Protein-free filtrates should be prepared within an hour or two for changes take place in the blood rapidly, especially at summer temperatures. This is especially true in the case of glucose determination where a considerable decrease of glucose, due to naturally occurring glycolytic enzymes, takes place. If necessary, specimens may be kept in the refrigerator at temperatures near 0° C. if, after being drawn, they are immediately chilled in ice water.

3. In most instances whole blood or plasma is employed. Consequently, it is necessary to use an anticoagulant. For this purpose lithium oxalate is probably better than either sodium or potassium oxalate. However, sodium oxalate is generally used. A convenient method of preparing oxalate tubes is to pipet into each 0.5 cc. of a hot saturated solution of sodium oxalate, prepared by adding about 8 gm. to 100 cc. of water, heating to boiling, and allowing the excess salt to sediment. With constant rotation at low heat (*below* 80° C.) evaporate the solution, leaving the dry oxalate deposited about the sides of the tubes. Stopper the tubes with rubber stoppers. The deposited oxalate goes into solution quickly when blood is added. Drying at temperatures over 80° C. converts part of the oxalate into carbonate, with consequent production of blood clots when the tubes are used.

4. Sodium fluoride in amount of 0.060 gm. per 10 cc. of blood may be added for the preservation of glucose whenever the analysis cannot be made immediately. Under the circumstances, however, the blood cannot be used for the determination of urea nitrogen because fluoride inhibits the activity of urease. It is also advisable to add 0.001 gm. of thymol for each cubic centimeter of blood.

5. After adding about 10 cc. of blood to a tube, the latter should be inverted several times in order that the anticoagulant may come in contact with all of the blood; never shake.

6. When a specimen is to be sent to a distant laboratory for analysis, whole blood cannot be shipped as it would have decomposed before reaching its destination. Instead, the blood is drawn in the usual manner and a protein-free blood filtrate prepared as described below, except that 7 volumes of 0.25 per cent benzoic acid are substituted for the 7 volumes of distilled water customarily used. This filtrate will keep for a considerable period of time.

7. In some blood chemistry determinations serum, instead of whole blood or plasma, is employed. For this purpose blood is placed in a dry sterile test tube. Allow the tube to stand for a short time until a clot has formed, chill in ice water and then place in a refrigerator. When the clot has contracted, gently free the top part from the tube if it sticks, then centrifugalize. Transfer the serum by means of a pipet, or by pouring, to a clean, dry tube. In order to avoid hemolysis which ruins specimens for some determinations, syringe, needles and tubes must be clean and dry. If a centrifuge is not available, clear serum may be obtained by placing the tube containing the blood nearly horizontal, thus forming a long, slanting surface. After clotting is complete, chill thoroughly, then place blood tube upright in the refrigerator over night. In the morning pour off the clear serum from the side of the tube opposite the slant.

TECHNIC OF COLORIMETRY

Colorimetry is the quantitative comparison of the color intensity of an unknown solution with that of a standard solution derived from a known quantity of the material under examination. It is based upon Beer's law which states that light, in passing through a colored solution, is absorbed in direct proportion to the concentration of colored substance. The intensity of observed color is therefore directly proportional to the thickness of the colored substance and inversely proportional to the thickness of layer or depth of the column of solution traversed by the beam of light during the examination.

Visual colorimeters vary greatly in principle and design. They may be (1) the block or rack comparator; (2) the dilution colorimeter or (3) the comparison colorimeter. The latter is the type mostly employed. Some method is used to vary the thickness of one solution while the thickness of the other remains constant. This thickness or "depth" is varied until the color intensity is the same in both solutions. The plunger and cup or Duboscq colorimeter is employed most often as follows:

1. North daylight may be used but a "daylight" lamp is preferred.

2. The colorimeter must be standardized before use by the person using it. Frequent checks must be made to be sure that the instrument remains in proper adjustment throughout the day. To standardize, first see that the plungers are clean and are screwed firmly into their sockets. Place the empty, dry cups in their holders, being sure always to use the same cup on the same side, since the thickness of the glass cup bottom varies. Run the carriers up until the plunger just rests in contact with the bottom of its cup, then adjust the verniers, as may be necessary, to read zero. With the cups still in position, adjust the mirrors (or, in some types, the light source) so that the amount and quality of light are equal in both halves of the field.

3. When beginning work, or when changing from one colored solution to another, rinse the cups carefully, first with distilled water, then with a small amount of the solution to be used in the respective cups. The plungers should be wiped with a damp cloth, then with a dry one.

4. Place the unknown solution in the left cup, the standard solution in the

right. Never fill the cups so full that they will overflow. Keep all solutions and reagents from contact with the mirrors; Nessler's solution is especially destructive to the silver backing. Rack the standard to the specified setting (usually a multiple between 10 and 25 mm. of the concentration of standard actually employed). Adjust the cup containing the unknown to match the color of the standard. Make the reading. Make sure that bubbles have not collected on the bottoms of the plungers. Do not delay readings. Avoid eye strain by resting the eyes frequently by looking up from the eyepiece. Suspend a large sheet of filter paper in front of, and in line with, the eyes as they are raised from the instrument; this rests the eyes and improves their color-matching ability. The retina soon tires when color matching is done.

5. In general terms the calculation may be made as follows:

$$\text{Unknown} = \frac{\text{Reading of standard in mm.}}{\text{Reading of unknown in mm.}} \times \begin{matrix}\text{concentration}\\ \text{of standard}\end{matrix} \times \text{dil. of unknown}$$

If, for example, in the determination of urea nitrogen the standard solution contains 0.10 mg. of nitrogen in a 25 cc. volume, is set at 10 and the unknown reads 16.8, the amount of nitrogen in 100 cc. of blood is determined as follows:

$$\text{Concentration of unknown} = \frac{10}{16.8} \times 0.10 \times 200 = 11.9 \text{ mg.}$$

6. After use, rinse and dry plungers and cups.

PREPARATION OF PROTEIN-FREE BLOOD FILTRATE
(Haden's Modification of the Folin-Wu Method)

A protein-free blood filtrate is used for the determination of glucose, urea nitrogen, total nonprotein nitrogen, creatinine, uric acid and plasma chlorides.

Ten cubic centimeters of blood are usually sufficient. The fact that the protein precipitation is done volumetrically not only permits the use of all of a small sample of blood, but gives a filtrate which, regardless of the initial quantity of blood used, is itself 10 per cent blood. Thus, no matter what amounts of blood be taken at first, 10 cc. of the filtrate corresponds to 1 cc. of blood, 5 cc. of filtrate to 0.5 cc. of blood, and so on. This latter fact considerably simplifies the calculations.

1. Transfer one volume of oxalated blood to a 100 cc. Erlenmeyer flask. Add 8 volumes of N 12 sulfuric acid. Mix, by rotating the flask, and let stand about 2 minutes.

2. With a pipet, add 1 volume of a 10 per cent aqueous solution of sodium tungstate. Stopper and shake thoroughly.

3. Filter through a Whatman No. 1 paper into a 50 cc. Erlenmeyer flask. Any grade of ammonia-free filter paper is satisfactory. If the first portion of the filtrate is not clear as water, it may have to be returned to the filter.

If the precipitation is performed properly, little or no foam is produced on shaking the final mixture. The appearance of foam and a cloudy filtrate are evidences of the use of an acid solution of incorrect strength or the use of an inferior grade of sodium tungstate. If the filtrate is brown, an insufficient amount of acid is present. In such a case the sample may be saved by the cautious addition of 10 per cent sulfuric acid, adding it drop by drop, shaking vigorously after each addition, and allowing the mixture to stand for a few minutes before adding more until coagulation is complete, and refiltering. Centrifugation may be u ed instead of filtration and is advantageous when the sample is small, as more protein-free solution is obtained.

DETERMINATION OF BLOOD GLUCOSE (Folin-Wu)

1. Prepare a 0.25 per cent solution of benzoic acid by dissolving 2.5 gm. in 800 to 900 cc. of water, using heat. Cool and dilute to 1000 cc.

2. Prepare a *stock solution of glucose* by dissolving 1 gm. of best grade glucose (carefully weighed) in 100 cc. of the benzoic acid solution. Two working standards are prepared from this stock solution. *Standard No. 1* is prepared by diluting, in a volumetric flask, 1 cc. of stock glucose solution with benzoic acid solution to 100 cc. (2 cc. = 0.2 mg. of glucose). *Standard No. 2* is prepared by diluting, in a volumetric flask, 2 cc. of stock glucose solution with benzoic acid solution to 100 cc. (2 cc. = 0.4 mg. of glucose).

3. Prepare an *alkaline copper solution* by dissolving, in a liter flask, 40 gm. of pure anhydrous sodium carbonate in about 400 cc. of distilled water. Add 7.5 gm. of tartaric acid and when the latter has dissolved, add 4.5 gm. of crystallized copper sulfate. Mix and accurately make up to the liter mark with distilled water which gives a solution of proper strength for the test.

4. Prepare a *molybdate-phosphate solution* by placing 35 gm. of molybdic acid and 5 gm. of sodium tungstate in a liter beaker. Add 200 cc. of 10 per cent sodium hydroxide solution and 200 cc. of distilled water. Boil vigorously for 20 to 40 minutes. Cool, dilute to about 250 cc. with distilled water, and add 125 cc. of concentrated (85 per cent) phosphoric acid. Dilute to 500 cc. with distilled water.

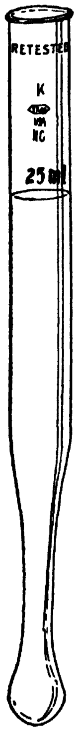

FIG. 80. FOLIN-WU BLOOD GLUCOSE TUBE.

5. Into three separate Folin-Wu blood glucose tubes (Fig. 80), place 2 cc. of protein-free blood filtrate, standard glucose solution No. 1 and standard glucose solution No. 2, respectively. To each of the three tubes add 2 cc. of the alkaline copper solution. Mix by allowing a bubble to enter the bulb.

The surfaces of the mixtures must now have reached the constricted parts of the tubes and must not lie above these parts. Tubes either too large or too small in capacity should be discarded; the surface of the mixture should lie within the constriction.

6. Transfer the tubes to a boiling water bath and heat for 6 minutes; then transfer to a cold water bath and allow to cool, without shaking, for 2 or 3 minutes.

7. Add to each tube 2 cc. of the molybdate-phosphate solution. Mix, by tapping, and allow to stand 2 minutes. The cuprous oxide dissolves rather slowly if a large amount is present; but the whole, up to the quantity given by 0.8 mg. of dextrose, usually dissolves within 2 minutes.

8. When the cuprous oxide is dissolved, dilute the resulting blue solutions to the 25 cc. mark with a 1 + 4 dilution of the molybdate-phosphate reagent, and mix each tube thoroughly by inverting several times, using care to insure thorough mixing, as the greater part of the color is developed in the bulb of the tube.

9. Compare in the colorimeter, using the standard which more nearly approximates the unknown.

10. In making the calculation using the weaker standard, that is, No. 1 containing 0.2 mg. of glucose, the reading of the standard, usually 20 mm., multiplied by 100 and divided by the reading of the unknown, gives the glucose in milligrams per 100 cc. of blood. When standard No. 2 containing 0.4 mg. of glucose is used, substitute 200 for 100 in the preceding.

MICROMETHOD FOR THE DETERMINATION OF BLOOD GLUCOSE (Folin)

1. Prepare a *sulfate-tungstate solution* by adding 10 gm. of anhydrous sodium sulfate and 15 cc. of a 10 per cent solution of sodium tungstate to a 500 cc. volumetric flask about one-half full of distilled water. After dissolving, add distilled water to 500 cc.

2. Prepare a *sulfuric acid solution* by filling a 100 cc. volumetric flask to the mark with N 12 sulfuric acid. Remove 4 cc. Add 2 gm. of anhydrous sodium sulfate. Dissolve and add water to 100 cc.

3. Prepare a *potassium ferricyanide solution* by dissolving 1 gm. in 250 cc. of distilled water. Keep in a brown bottle away from light. Use from a small brown bottle. Prepare fresh when it gives an appreciable blue colored blank with the ferric iron gum ghatti solution.

4. Prepare a *sodium cyanide-carbonate solution* by dissolving 8 gm. of anhydrous sodium carbonate in 40 to 50 cc. of distilled water in a 500 cc. volumetric flask. With a cylinder, add 150 cc. of freshly prepared 1 per cent sodium cyanide solution. Dilute very carefully to 500 cc. with distilled water and mix.

5. Prepare a *ferric iron gum ghatti solution* by filling a liter cylinder with distilled water. Suspend 20 gm. of gum ghatti on a copper screen, just under the surface of the water, and leave overnight. Remove the screen and strain the solution through a double layer of a clean towel.

Dissolve, with the aid of heat, 5 gm. of anhydrous ferric sulfate in 75 cc. of 85 per cent phosphoric acid and 100 cc. of distilled water. Cool and add to the gum ghatti solution. Prepare a 3 per cent solution of potassium permanganate in distilled water, add to the iron-gum solution in small amounts and shake until a pink color remains which persists for at least one-half hour. This solution keeps indefinitely.

6. Prepare a *standard stock solution of glucose* by dissolving 1 gm. of benzoic

acid in about 300 cc. of hot distilled water. Weigh out exactly 1 gm. of chemically pure anhydrous glucose on a watch glass. Rinse the glucose through a funnel into a volumetric liter flask with the aid of the warm benzoic acid solution. Add about 400 cc. of distilled water, cool to room temperature, make up to 1000 cc. with distilled water, and mix. Transfer to a clean, dry, glass-stoppered bottle. The dilute working standard may be prepared by diluting, with distilled water, 1 cc. of this stock solution to 100 cc.

7. Place 4 cc. of the sulfate-tungstate solution in a clean, dry centrifuge tube.

8. Prick a finger with a lancet and collect exactly 0.1 cc. of blood with a special, previously cleaned, Folin micro blood pipet. Transfer at once (before coagulation has had time to occur) to the solution in the centrifuge tube. Rinse the pipet two or three times by suction and stir a little with the pipet. Let stand for about 15 minutes, or longer if convenient.

9. Add 1 cc. of the sulfuric acid solution, mix, by stirring, and centrifuge for about 5 minutes.

10. Transfer 2 cc. of the water-clear supernatant solution and 2 cc. of distilled water to a test tube which is graduated at 25 cc.

11. In a similar tube place 4 cc. of the standard dilute glucose solution.

12. To each tube add 2 cc. of the 0.4 per cent solution of potassium ferrocyanide and then, to each tube, add 1 cc. of the cyanide-carbonate solution.

13. Heat both tubes together in a beaker of boiling water for 8 minutes, cool, and to each tube add 5 cc. of the ferric iron gum ghatti solution.

14. Dilute the contents of each tube to 25 cc. with distilled water and mix.

15. Make the color comparison in the usual manner with the standard set at 20 mm.

16. Calculate as follows:

$$\frac{20}{\text{Reading of unknown}} \times 100 = \text{mg. glucose per 100 cc. of blood}$$

DETERMINATION OF PLASMA CARBON DIOXIDE CAPACITY
(Van Slyke and Cullen)

In this method it is not necessary to use anaerobic precautions, but the exposure of blood and plasma to air should be held within reasonable limits.

1. Draw about 10 cc. of blood into a syringe with as little stasis as possible and discharge into the bottom of a tube containing about 30 mg. of potassium oxalate. Stopper tube tightly with a cork and invert gently several times to mix the oxalate. Centrifuge blood as soon as possible and remove plasma to another tube. After separation of the plasma, it can stand several hours if necessary. Just before the analysis, transfer plasma to a dry separatory funnel and blow *alveolar* air several times into the funnel through a bottle of beads (to remove excess moisture). Stopper funnel quickly and rotate so as to expose a large area of the plasma to alveolar air for two minutes, after which it is set upright in a rack or holder until used in the test.

2. If the apparatus (Fig. 81) is kept clean, with well-greased stop-cocks, the only preparation necessary is to test for leaks. This test should be routine and

should never be omitted. It is performed by sealing both capillaries above the top stop-cock with mercury, closing it, and drawing a vacuum to the 50 cc. mark. The mercury is then allowed to rise to the top cock and to strike it gently. A

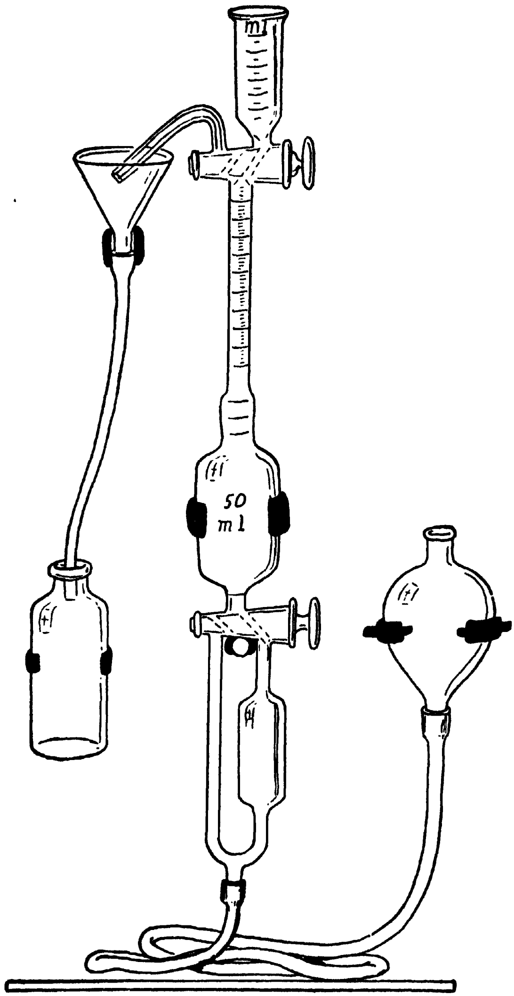

FIG. 81. VAN SLYKE VOLUMETRIC APPARATUS.

sharp click should be produced. A muffled or soft click is an indication of a leakage of air.

3. Fill apparatus with mercury, including both capillaries above the top stop-cock. Introduce a drop of *caprylic alcohol* into the cup and allow it to run

down into the capillary. Then add 2 cc. of 1 per cent lactic acid to the cup. Draw up 1 cc. of plasma into a Mohr type pipet (one which is *not* calibrated to deliver to the tip), place the tip of the pipet against the bottom of the cup and allow exactly 1 cc. of plasma to run slowly underneath the lactic acid. Remove pipet carefully, close bottom stop-cock and open top cock into cup. Then by means of bottom cock, allow liquids in cup to flow slowly into apparatus until a total of 2.5 cc. has been introduced. The plasma is thus rinsed in with the acid. Close top cock and seal bore and capillary above it with about 0.2 cc. mercury, introduced from above. Draw level of mercury (not of aqueous solution) to 50 cc. mark, and close lower cock. Remove from stand and shake apparatus for 2 minutes. Replace and allow to drain. Draw aqueous layer (but no gas) as completely as possible into bulb below lower stop-cock, and then allow mercury to rise slowly through other arm of cock until it reaches graduated portion. Hold top surface of mercury in levelling bulb $\frac{1}{13}$ of way between bottom of aqueous layer and its top (just above surface of mercury in apparatus), and close lower cock. Avoid oscillations. Read volume of gas to thousandths; read temperature and barometric pressure. Apply correction tables to be found in reference books, or, in emergency, subtract 0.12 cc. from volume at 760 mm. (volume read times barometric reading in mm. divided by 760). If necessary to use only 0.5 cc. of plasma, use only half volumes of reagents, and multiply gas volume found by 2 before applying correction tables.

4. The results give the cubic centimeters of carbon dioxide, reduced to 0° C., and 760 mm. of mercury pressure, bound as bicarbonate in 100 cc. of plasma; that is, the combining power of the plasma in "volumes per cent."

DETERMINATION OF BLOOD UREA NITROGEN
(Van Slyke and Cullen)

1. Prepare a stock *standard nitrogen solution* by dissolving 4.716 gm. of pure, dry ammonium sulfate in 0.2 N sulfuric acid and make up the volume to 1000 cc. This solution contains 1 mg. nitrogen per cc. For use, dilute 10 cc. of this stock solution to 100 cc. with 0.2 N sulfuric acid. This solution contains 0.1 mg. nitrogen per cc.

2. Prepare *Nessler's solution* by dissolving 22.5 gm. iodine in 20 cc. of water containing 30 gm. of potassium iodide. After solution is complete, add 30 gm. of metallic mercury and shake vigorously so as to break up the mercury into globules, cooling the mixture from time to time by immersing the flask in cold water. Continue until the supernatant has lost all color due to iodine. Decant the supernatant from the excess mercury and test for free iodine by adding a drop or two of the solution to 1 cc. of a 1 per cent starch solution. If the starch test for iodine is negative, add a few drops of an iodine solution of the same concentration as that above, until a faint excess of iodine can be detected by the starch test. Dilute the double iodide solution to 200 cc. with distilled water and mix well. Prepare accurately a 10 per cent sodium hydroxide solution from a saturated solution of sodium hydroxide which has been allowed to stand until all carbonates have settled out. To 975 cc. of this 10 per cent hydroxide solution, add the entire

solution of potassium mercuric iodide prepared above. Mix thoroughly and allow to settle.

3. Prepare *acetate buffer solution* by dissolving 15 gm. of crystallized sodium acetate in a 100 cc. volumetric flask with 50 to 75 cc. of distilled water. Add 1 cc. of glacial acetic acid, dilute to volume, and mix.

4. Prepare a *5 per cent gum acacia solution* by adding 3 cc. of the potassium mercuric iodide solution made for Nessler's solution to each 100 cc. of a 5 per cent solution of gum acacia in distilled water. Keep the gum solution in a tall cylinder where the precipitate which forms can settle out.

5. Place 5 cc. of the blood filtrate in a test tube graduated at 25 cc.; to a similar tube add 1 cc. of the diluted standard nitrogen solution containing 0.1 mg. nitrogen and dilute to 5 cc. with distilled water. To both standard and unknown add 2 drops of the acetate buffer mixture and a piece of urease paper (for method of preparation see above). Stopper and set aside for 30 minutes at room temperature, during which time the tubes are frequently shaken to set free the urease from the paper. At the end of this period add 1 cc. of gum acacia solution to each tube and dilute to about 20 cc. with distilled water. Then, to each tube add 2 cc. of Nessler's solution, dilute to the 25 cc. mark, and compare in the colorimeter.

6. The protection against precipitation, afforded by the gum acacia, is of limited duration and therefore the reading in the colorimeter must be made at once upon the addition of the Nessler's solution to the unknown. No precipitation or change will occur in the standard; consequently, a series of unknowns may be read against the same standard, provided each unknown is Nesslerized and diluted individually to the 25 cc. mark just before matching.

7. The reading of the standard, usually 10 mm., multiplied by 20 and divided by the reading of the unknown, gives the urea nitrogen in milligrams per 100 cc. of blood. Multiplying this figure by 2.143 gives the milligrams of urea per 100 cc. of blood.

DETERMINATION OF BLOOD NONPROTEIN NITROGEN
(Folin and Wu)

1. Prepare a *sulfuric-phosphoric acid digestion mixture* by mixing 300 cc. of phosphoric acid (about 85 per cent) with 100 cc. of concentrated sulfuric acid. Transfer to a tall cylinder, cover well to exclude ammonia, and set aside for sedimentation of calcium sulfate. This sedimentation is very slow, but in the course of a week or so the top part is clear, and 50 to 100 cc. can be removed by means of a pipet. If this cannot be done, rapid centrifugalization will yield a perfectly clear solution. To 100 cc. of the clear acid mixture add 10 cc. of a 6 per cent copper sulfate solution and 100 cc. of distilled water.

2. Prepare a *stock nitrogen solution* as described on page 1021. Dilute 10 cc. up to 100 cc. with 0.2 N sulfuric acid (1 cc. contains 0.1 mg. nitrogen).

3. Prepare Nessler's solution (page 1021).

4. Introduce 5 cc. of protein-free blood filtrate corresponding to 0.5 cc. of blood, into a dry 200 by 25 mm. *pyrex glass* test tube graduated at 35 cc. Add 1 cc. of the sulfuric-phosphoric acid digestion mixture and boil vigorously over a

micro-burner until the characteristic dense acid fumes begin to fill the test tube (usually 3 to 7 minutes). If the test tube is held in a slightly inclined position and the heating begun by applying the flame of the burner at the side of the tube and just below the top of the contained mixture, no bumping will occur; as the mixture begins to boil, the flame can be applied lower down, and finally, under the bottom of the tube. Unless this method of heating is followed, bumping is likely to be troublesome and may even result in the loss of a part or all of the preparation with inaccurate results.

5. When the sulfuric acid fumes are unmistakable, cut down the flame so that the contents of the tube are just visibly boiling, and close the mouth of the test tube with a small watch glass or funnel.

6. Continue the heating very gently for 2 minutes from the time the fumes begin to be unmistakable, even if the solution has become clear and colorless at the end of 20 to 40 seconds. If the oxidation is not visibly finished at the end of 2 minutes, the heating must be continued until the solution is nearly colorless.

7. Allow the contents to cool for 70 to 90 seconds and then add 15 to 25 cc. of distilled water; cool further, approximately to room temperature, and add distilled water to the 35 cc. mark. Occasionally a heavy white precipitate forms, probably silicates; this may settle out, or can be removed by filtering or centrifuging, *after* Nesslerization, and just before reading against the standard.

8. At the same time that the unknown has been prepared in this manner, a standard for comparison is made as follows: Place 3 cc. of the standard nitrogen solution, containing 0.3 mg. of nitrogen, in a 100 cc. volumetric flask; add 2 cc. of the sulfuric-phosphoric acid digestion mixture, and then about 50 cc. of distilled water.

9. Then add to the unknown 15 cc. and to the standard 30 cc., respectively, of the Nessler's solution; fill the standard to the mark with distilled water; mix each thoroughly by inverting several times and compare in the colorimeter. It is essential that the unknown and the standard should be Nesslerized at approximately the same time.

10. The reading of the standard (usually 20 mm.), multiplied by 30, and divided by the reading of the unknown, gives the nonprotein nitrogen in milligrams per 100 cc. of blood.

DETERMINATION OF BLOOD PROTEINS

(Andersch and Gibson's Modification of the Method of Wu and Ling)

This method determines the total serum protein and serum albumin separately. The serum globulin is determined by subtracting the albumin from the total protein. The albumin-globulin ratio may then be expressed.

1. Prepare a *standard tyrosine solution* by dissolving 0.2 gm. of pure dry tyrosine in 1000 cc. of 0.1 N hydrochloric acid.

2. Prepare the *phenol reagent* of Folin and Ciocalteu by placing 100 gm. of sodium tungstate and 25 gm. of sodium molybdate together with 700 cc. of distilled water in a 1500 cc. Florence flask. Add 50 cc. of 85 per cent phosphoric acid and 100 cc. of concentrated hydrochloric acid. Connect with a reflux con-

denser by means of a cork or rubber stopper wrapped in tinfoil and boil gently for 10 hours. At the end of the boiling period add 10 gm. of lithium sulfate, 50 cc. of water, and a few drops of bromine. Boil the mixture without the condenser for about 15 minutes to remove the excess bromine. Cool, dilute to 1000 cc., and filter. The finished reagent should have no greenish tint, as this means the presence of blue reduction products which will reduce the range of true proportionality.

3. Collect blood in a dry clean tube and separate the serum as described on page 1015.

Determination of Total Protein. 1. Dilute 1 cc. of serum with 9 cc. of 0.9 per cent solution of sodium chloride (1:10). Transfer 1 cc. of 1:10 serum to a 15 cc. centrifuge tube, add 4 cc. of distilled water and 1 cc. of 20 per cent solution of trichloracetic acid. The precipitate is centrifuged out and the supernatant discarded. Dissolve the precipitate in 0.5 cc. of 10 per cent solution of sodium hydroxide and heat in a boiling water bath for 30 minutes. Place 2 cc. of the standard tyrosine solution into a centrifuge tube similar to the one containing the unknown. Both tubes should be accurately graduated at the 10 cc. mark. Dilute both standard and unknown to the 5 cc. mark with distilled water, add 1 cc. of the phenol reagent and 3 cc. of a saturated solution of sodium carbonate in distilled water. Make up both to the 10 cc. mark with distilled water, mix, and compare in the colorimeter after standing for 30 minutes.

2. The reading of the standard, usually 10 mm., multiplied by 6.4 and divided by the reading of the unknown, equals the grams of total protein per 100 cc. of serum.

Determination of Serum Albumin. 1. To 1 cc. of serum add 4 cc. of distilled water and 5 cc. of saturated ammonium sulfate solution. Place stoppered tube in an incubator at 37° C. for 15 minutes and then filter through a fine filter paper (Whatman No. 42). If the filtrate is not clear, return to the paper. To 2 cc. of the filtrate in a centrifuge tube accurately graduated at 10 cc., add 3 cc. of distilled water and 1 cc. of a 20 per cent solution of trichloracetic acid. Centrifugalize, pour off the supernatant fluid, dissolve the precipitate in 0.5 cc. of a 10 per cent solution of sodium hydroxide, heat in a boiling water bath for 30 minutes, and then develop the color and compare against a standard in the same manner as described above for total protein.

2. The reading of the standard set at 10 mm., multiplied by 2.58 and divided by the reading of the unknown, equals the grams of serum albumin per 100 cc.

Determination of Serum Globulin. Subtract the grams of serum albumin per 100 cc. of serum from the grains of total protein per 100 cc. of serum.

Determination of Fibrinogen. 1. to 1 cc. of *plasma from oxalated blood* in a 15 cc. centrifuge tube, add 2 cc. of distilled water and 1 cc. of a saturated solution of ammonium sulfate. Mix, let stand for a few minutes, and then centrifugalize to throw down the precipitated fibrinogen (fibrin). Pour off the supernatant fluid completely. Dissolve the fibrin precipitate in 0.5 cc. of a 10 per cent solution of sodium hydroxide and heat in a boiling water bath for 30 minutes. For the standard, place 2 cc. of the tyrosine solution in another centrifuge tube. Dilute both standard and unknown to the 5 cc. mark, add to each 1 cc. of the phenol reagent, and then 3 cc. of a saturated solution of sodium carbonate. Make up both to the 10 cc. mark with distilled water, mix, and let stand for 30 minutes. Compare in the colorimeter, with the standard set at 10 mm.

2. The reading of the standard, multiplied by 0.52 and divided by the reading of the unknown gives the grams of fibrinogen per 100 cc. of plasma.

METHODS FOR THE DETERMINATION OF BILIRUBIN

Icterus Index (Bernheim). Since this test is simply a measurement of the color of the serum, it is evident that even the slightest trace of hemoglobin will vitiate the results. Consequently, it is of the utmost importance that hemolysis be avoided. The needle and syringe used in the collection of blood should be entirely dry. The blood should be allowed to clot in a dry tube, protected from the light, and then centrifugalized so as to secure clear serum. Since carrots in the diet may impart a yellow color to the serum (carotenemia) which gives a high index, they should be excluded from the diet during the day preceding the test, and the blood should be drawn before breakfast to avoid chyle.

1. Prepare a 1:10,000 *solution of potassium dichromate* by dissolving 0.1 gm. of chemically pure potassium dichromate in about 500 cc. of distilled water in a 1000 cc. volumetric flask. Add 4 drops of concentrated sulfuric acid and dilute to the mark with distilled water. Keep in a dark glass bottle in the dark. This is the standard against which the color of the serum is matched.

2. Accurately dilute 1 cc. of clear serum with 0.9 per cent solution of sodium chloride in distilled water until its color approximately matches the standard. This dilution may be 1:2, 1:5, 1:10, or even more.

3. Place the standard in one colorimeter cup and set it at 15 mm. Place the serum, undiluted or diluted as necessary, in the other cup and match against the standard set at 15 mm.

4. The reading of the standard, divided by the reading of the serum, multiplied by the dilution, gives the icterus index. The normal by this method is from 4 to 6.

Van den Bergh Method (Modification of Gibson and Goodrich). This quantitative method supersedes the older direct, indirect, and quantitative van den Bergh reactions. It has been found that the three types of direct van den Bergh reaction, thought due to differences in the chemical or physical character of the bilirubin in the blood, are in reality due to its concentration. The present method compares the color of diazotized serum or plasma with a standard solution of diazotized bilirubin.

1. Prepare a *sulfanilic acid solution* by dissolving 1 gm. of sulfanilic acid in 15 cc. of concentrated hydrochloric acid and dilute to 1000 cc. with distilled water.

2. Prepare the *diazo reagent* immediately before use by mixing 25 cc. of the sulfanilic acid solution with 0.75 cc. of a freshly prepared 0.5 per cent solution of sodium nitrite in distilled water.

3. Prepare a *bilirubin standard* by dissolving 0.010 gm. of pure bilirubin in 10 to 20 cc. of distilled water with the aid of a few drops of a 10 per cent solution of sodium hydroxide; make up to 100 cc. with distilled water. Add 50 cc. of freshly prepared diazo reagent, 100 cc. of a saturated solution of ammonium sulfate, and 400 cc. of 95 per cent ethyl alcohol. When the red color has developed, add 100 cc.

of concentrated hydrochloric acid and let stand at least 3 hours before using. If kept in the refrigerator this standard will keep 3 months. Smaller proportionate amounts may be used in preparing this standard.

4. Serum or plasma from oxalated blood may be used but there must be no hemolysis and the test should be done as soon as possible after the blood is collected.

5. To 2 cc. of serum or plasma in a 15 cc. centrifuge tube, add 1 cc. of freshly prepared diazo reagent and mix. The appearance of a red color at this point indicates a positive "*direct*" reaction. Add 2 cc. of saturated ammonium sulfate solution, 8 cc. of alcohol, and mix. When the red color has developed, add 2 cc. of concentrated hydrochloric acid, mix, and let stand several minutes. Centrifugalize (or filter), pour the clear supernatant into a colorimeter cup and compare with the bilirubin standard.

6. The reading of the standard, multiplied by 10, divided by the reading of the unknown, equal milligrams bilirubin per 100 cc. of serum or plasma. One "unit" of bilirubin is 1 part in 200,000 or 0.5 mg. per 100 cc. By this quantitative test, normal serum contains from 0.2 to 0.5 units of bilirubin (0.1 to 0.26 mg.) per 100 cc.

METHOD FOR THE DETERMINATION OF THE SULFONAMIDES
(Marshall and Litchfield, modified)

The methods for sulfanilamide, sulfapyridine, sulfathiazole, sulfaguanidine, and sulfadiazine are the same except for the preparation of standards and the calculations. The procedure is based on the diazotization of the drug and subsequent coupling to form a colored compound which is compared colorimetrically with a standard treated in the same manner.

1. Prepare a *sodium phosphate and ammonium sulfamate solution* by dissolving 13.8 gm. of monobasic sodium phosphate and 0.5 gm. of ammonium sulfamate in water and make up to 100 cc. Kept in a dark bottle in a refrigerator, this reagent will keep at least 6 months.

2. Prepare a 0.4 per cent *solution of dimethyl-a-naphthylamine* by dissolving 0.4 gm. in 100 cc. of 95 per cent ethyl alcohol. Kept in a dark bottle in the refrigerator, this reagent will keep at least 6 months.

3. Prepare a *stock sulfanilamide solution* by dissolving 0.1 gm. of pure sulfanilamide crystals in distilled water and make up to 1000 cc. Do not use tablets. Prepare a *working standard* by diluting 10 cc. of the stock solution with 90 cc. of distilled water (0.01 mg. sulfanilamide per cc.).

Method for Free Sulfanilamide. 1. Lake 1 volume of oxalated blood with 15 volumes of distilled water. Let stand 15 minutes. Otherwise, laking may be accomplished by using a 0.05 per cent aqueous solution of saponin; in this case laking will be complete in 1 to 2 minutes. To the laked blood add 4 volumes of a 15 per cent aqueous solution of trichloracetic acid. Filter after standing 5 minutes.

2. To 10 cc. of filtrate add 1 cc. of a 0.1 per cent aqueous solution of sodium nitrite (freshly prepared each day) and mix. After 3 minutes, add 1 cc. of the sodium phosphate-ammonium sulfamate solution, mix, and let stand 2 minutes.

Add 5 cc. of the dimethyl-a-naphthylamine solution and, after standing 10 minutes, compare in the colorimeter with a standard prepared at the same time and treated in the same manner. For blood, two standards will cover the range of concentrations ordinarily encountered. The weaker standard consists of 2 cc. of the working standard solution (total 0.02 mg. sulfanilamide), 2 cc. of trichloracetic acid solution, and 6 cc. of distilled water. In the stronger standard use 5 cc. of working standard (0.05 mg. sulfanilamide), 2 cc. of trichloracetic acid, and 3 cc. of distilled water.

3. Set the standard at 10 and calculate as follows: $\frac{\text{Reading of unknown}}{\text{Reading of standard}}$ $\times$ 4 if the weaker standard is used, $\times$ 10 if the stronger is used = mg. per 100 cc. of blood.

Method for Total Sulfanilamide. Treat 10 cc. of blood filtrate prepared as above with 1 cc. of a 2N hydrochloric acid in a boiling water bath for 1 hour. Cool and adjust the volume to 10 cc. with distilled water. Proceed as for free sulfanilamide except that a 2 molal sodium phosphate, 0.5 per cent ammonium sulfamate solution (27.6 gm. to 100 cc.) is used.

Method for Acetylsulfanilamide. The difference between the total and the free gives the amount of conjugated acetylsulfanilamide.

Sulfapyridine Determinations. The technic is exactly the same as for sulfanilamide. The same sulfanilamide standards are used. However, since sulfapyridine has a lower color-producing value than the sulfanilamide in the standard used (0.8 compared with 1.0), the results must be multiplied by the factor 1.25 to give the true values.

Sulfathiazole Determinations. The reagents, procedures, and calculations are exactly the same as those used in the sulfanilamide determinations except for the standard.

Since the color developed by sulfathiazole differs considerably in shade from that of sulfanilamide, the stock standard must be made by dissolving 0.1 gm. of pure sulfathiazole crystals in distilled water and making it up to 1000 cc.

Sulfaguanidine Determinations. The reagents, procedures and calculations are exactly the same as for sulfanilamide except for the standard used. The stock standard should be prepared from pure sulfaguanidine crystals.

Sulfadiazine Determinations. The reagents, procedures, and calculations are exactly the same as those used in the sulfanilamide determinations except for the standard which is prepared by dissolving 0.1 gm. sulfadiazine crystals in 500 cc of 1/100 sodium hydroxide solution and adding distilled water to 1000 cc.

COPPER SULFATE METHOD FOR MEASURING SPECIFIC GRAVITIES OF WHOLE BLOOD AND PLASMA *

This method, described by Phillips, Van Slyke, Dole, Emerson, Hamilton, Archibald, Stanley and Plazin, makes it possible with three or four drops of blood,

* From Phillips, R. A.; Van Slyke, D. D.; Dole, V. P.; Emerson, K., Jr.; Hamilton, P. B., and Archibald, R. M.: *Copper Sulfate Method for Measuring Specific Gravities of Whole Blood and Plasma,* by permission of the United States Navy Research Unit at the Hospital of the Rockefeller Institute for Medical Research.

a medicine dropper, and small bottles of copper sulfate solution to determine the specific gravity of the blood, and from it the hemoglobin content within 10 per cent. By examining in a like manner the serum or plasma from the same blood one can determine also the plasma protein concentration and increase the accuracy of the hemoglobin estimation to ± 2 per cent.

The results can be used as follows:

1. To assist in ascertaining the results of hemorrhage.
2. To estimate decrease in plasma volume indicated by hemoglobin increase, and to decide whether the plasma volume decrease is due to loss of water (dehydration of cholera, dysentery, exposure), or to loss also of plasma proteins (extravasation in burns, trauma, etc.).
3. To assist in deciding whether blood replacement therapy requires administration of saline solution or plasma or whole blood.
4. To follow the results of such therapy and decide whether it has been adequate, and when it must be repeated.
5. If the number of cases exceeds the amount of blood and plasma available, the method will assist in deciding which cases must receive it and which may be able to do without.
6. Besides these acute conditions, the method will assist in diagnosing the different types of anemia and in detecting various pathological conditions, partially summarized in a later section, in which the plasma proteins become diluted or concentrated.

Principles. The technic consists of letting drops of plasma or whole blood fall into a graded series of solutions of copper sulfate of known specific gravity, and noting whether the drops rise or fall in the solutions. Each drop, on entering the solution, becomes encased in a sack of copper-proteinate, and remains as a discrete drop without change of gravity for 15 or 20 seconds, during which its rise or fall reveals its gravity relative to that of the solution. The size of the drops does not have to be constant, hence no special pipet is needed for delivering the drops. No temperature correction is needed, because the temperature coefficient of expansion of the copper sulfate solutions approximates that of blood and plasma. This method is capable of measuring gravities to ± 0.00005, which is more than ten times the accuracy required. The copper sulfate solution automatically cleans itself after each test, because within a minute or two after the test is completed the material of the drop settles to the bottom as a precipitate. The standard $CuSO_4$ solutions are prepared by dilution of a saturated solution, hence a balance is not needed to weigh the $CuSO_4$.

For accurate work, viz., gravities within ± 0.0002, a series of copper sulfate solutions graded at intervals of 0.001 in specific gravity are used; twenty solutions cover the plasma range 1.015 to 1.035 and forty cover the whole blood range, 1.035 to 1.075. For rougher work with gravities accurate to ± 0.001, sixteen solutions at intervals of 0.004 suffice to cover the entire range of blood and plasma.

Reagents. *Saturated copper sulfate solution:* Four pounds of "fine crystals," or pulverized, copper sulfate are placed in a 4 liter bottle. About 2500 cc. of distilled water is added and the bottle is stoppered and shaken vigorously for a total of 5 minutes, which need not be continuous. (Three minutes has been found sufficient, even at 0° C., to saturate this solution if the sulfate is well pulverized.) As soon as the shaking is finished the temperature of the solution is taken to the nearest half degree Centigrade and is recorded. (It will be a little cooler than the water was before the saturation, because the saturation process absorbs heat.) After taking the temperature the solution is *immediately* decanted off the crystals and is filtered, to remove fine suspended crystals, through cotton or dry filter paper into a clean dry 4 liter bottle. The solution is at once used to make up a stock solution of gravity 1.100. (It is preferable not to let the saturated solution stand long before using, as if it cools some of the copper sulfate may crystallize and change the concentration.) The undissolved sulfate can be used again.

Stock copper sulfate solution of gravity 1.100: The volume of saturated solution indicated in Table 1 is measured in a 500 cc. graduated cylinder and poured into a 1 liter volumetric flask. The upturned cylinder is allowed to drain into the flask for 30 seconds. The flask is then filled to the mark with water and is inverted several times to mix the solution. The mixing results in a contraction, so that the meniscus now falls below the mark. The flask is let stand for a minute until the solution drains down from the neck. Then enough additional water is added to bring the volume to 1 liter, the solution is mixed, and then poured into a clean, dry, 4 liter bottle. The same 1 liter volumetric flask is used to prepare 3 more liters of the stock copper sulfate solution of gravity 1.100. Each time before the flask is used again it is rinsed with water and the rinsings are discarded.

The saturated solution, the stock solution and the standard solutions next described must be prepared at within 5° C. of the same temperature. The coefficients of expansion of the saturated and stock copper sulfate solutions are slightly but definitely greater than that of water, so that if, for example, the saturated solution and stock solution were prepared at 35° C., and the standard solutions at 20° C., the standards would have more copper sulfate than intended, enough to increase the gravity by about 0.001.

Once prepared, the standard solutions may be used at any temperature within ± 15° or 20° of the temperature at which they were made up.

The accuracy of the stock solution of gravity 1.100 can be checked by weighing 100 cc. in a volumetric flask, and then weighing 100 cc. of distilled water at the same temperature in the same flask. The copper sulfate solution should weigh 1.1000 times the weight of the water. This check is desirable because the accuracy of the method depends on the accuracy of the stock solution.

Preparation of standard solutions in 100 cc. portions: The standard solutions are prepared in 100 cc. portions when 4-ounce bottles are available for storage.

For the standard of 1.075 gravity, 74 cc. of stock solution of gravity 1.100 are measured from the buret into the 100 cc. flask, the flask is filled to the mark with water, and the solution is mixed and transferred to a labeled 4-ounce bottle, which is stoppered to prevent evaporation.

To prepare the standard of gravity 1.074, the 100 cc. flask is rinsed once with water and the buret is refilled from a 250 cc. Erlenmeyer flask containing the stock solution. Then 73 cc. of the stock solution are measured into the volumetric flask and diluted to 100 cc.

The same procedure is carried through for preparation of the entire series down to 1.015, which covers the extreme ranges for blood and plasma. If gravities on ascitic fluid and transudates are desired, the series is extended to 1.008. For each standard the number of cc. of stock solution *less by 1* than the number indicated in the second and third decimal places of the desired gravity is measured into the rinsed 100 cc. flask and diluted to the mark.

If there were no contraction when the stock solution is mixed with water one would dilute 75 cc. of the stock to 100 cc. to get a gravity of 1.075, etc. However, there is a contraction which is empirically corrected for by taking 1 cc. less of the stock. It happens conveniently that the same 1 cc. correction serves for the entire range, 1.075 to 1.008, over which its use yields gravities correct within $\pm$ 0.0003.

The LaMotte Chemical Products Company supplies a copper sulfate specific gravity outfit which consists of 16 standard solutions with specific gravity intervals of 0.004, covering the range from 1.016 to 1.076. These are contained in special screw-cap bottles mounted in movable racks, all compactly arranged in a carrying case of special design. With this outfit a test requires only two minutes. No special pipet is required and temperature correction is unnecessary. With a few drops of blood it is possible to determine the specific gravity of blood, and from it the hemoglobin content. By examining the serum or plasma in the same manner it is possible to determine the total protein concentration.

Technic. 1. Tourniquets should not be applied for more than 1 minute. Longer application may force so much fluid out of the blood that the concentration is measurably increased. In conditions of shock, capillary blood may contain 30 to 40 per cent more cells than venous blood; hence in such conditions capillary blood cannot serve as a sample of circulating blood.

2. *Whole blood* may be delivered directly from the syringe and needle in which the blood was drawn into the copper sulfate solutions. If, however, the blood is transferred to a test tube containing anticoagulant, *the cells and plasma must be thoroughly mixed immediately before a sample is drawn* into a medicine dropper for the gravity test. For this purpose, the tube containing the blood is (a) inverted 10 times, or (b) a glass rod with a mushroom end is raised and lowered through the blood 10 times, just before the sample is drawn into the dropper. Gross error in hemoglobin estimation could result if the blood sample were taken from blood in which partial settling of the cells had occurred.

Plasma gravity is best determined on plasma from blood which is treated with heparin, 0.2 mg. per cc. blood, as anticoagulant, since the heparin exerts no measurable effect on the gravity results. Almost equally good is Heller and Paul's mixture of 3 parts ammonium oxalate and 2 parts potassium oxalate, *if the amount used does not exceed 1 mg. per cc. of blood.* Sodium citrate cannot be used as anticoagulant. In effective concentrations it exerts too great an effect on the gravities.

3. The drop of serum, plasma or whole blood is delivered from a height of about 1 cm. above the solution from a medicine dropper, or from a syringe needle. It is preferable to use small drops for the reason that they permit more tests before the standard solution must be changed. Therefore, a medicine dropper with a fine tip is preferable to a coarse one. Greasing the sides of the tip with vaseline also reduces the size of the drop, especially if the vaseline is mixed with

a little caprylic alcohol. When the drop is delivered it is convenient to steady the dropper on the edge of the bottle (Fig. 82).

The delivered drop breaks through the surface film of the solution and penetrates 2 to 3 cm. below the surface; within 5 seconds the momentum of the fall is lost and the drop then either begins to rise, or becomes stationary, or continues to fall. The gravity of the drop relative to the solution does not change appreciably until the drop has been immersed in the solution for another 10 or 15 seconds, and there is ample time to note its behavior during this interval. If the drop is lighter than the test solution it will rise, perhaps only a few millimeters and may begin to sink immediately afterward. If the drop is of the same gravity as the standard test solution it will become stationary for this interval and then fall. If the drop is heavier it will continue to fall during the interval. *In summary, the behavior during the 10 seconds after the drop has lost the momentum of its fall into the solution indicates whether the drop is lighter or heavier than the test solution; if it rises at all during this period it is lighter than the standard.*

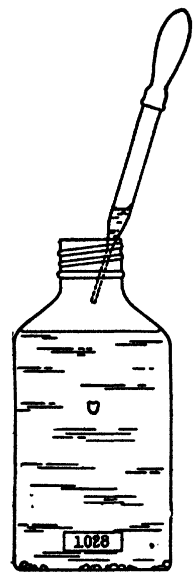

FIG. 82. BOTTLE FOR DETERMINATION OF SPECIFIC GRAVITY OF BLOOD OR PLASMA BY COPPER SULFATE METHOD.

(From Phillips, R. A.; Van Slyke, D. D.; Dole, V. P.; Emerson, K., Jr.; Hamilton, P. B., and Archibald, R. M.: *Copper Sulfate Method for Measuring Specific Gravities of Whole Blood and Plasma*, by permission of the United States Navy Research Unit at the Hospital of the Rockefeller Institute for Medical Research.)

4. For general work it may suffice to determine the gravities to ± 0.001. For this only 16 standard solutions with gravity intervals of 0.004 covering the range from 1.016 to 1.076 will be needed. An error of 0.001 in plasma gravity affects plasma proteins by 0.3 gm. per 100 cc.; additive errors of 0.001 in the gravities of both plasma and whole blood affect hemoglobin results by 5 per cent.

Calculations. Line charts for the conversion of plasma and whole blood gravities to plasma protein concentrations, hemoglobin concentrations and hematocrit percentages have been prepared by standard methods, and are given in Figures 83 and 84. The calculations are made by laying a straight edge or stretched thread as directed on the charts. The brackets on the scales indicate normal ranges.

Corrections to Observed Gravities for the Effects of Addition of Oxalates or Removal of Fibrinogen. Addition of oxalates raises the gravity of whole blood and of plasma; on the other hand, removal of fibrinogen by clotting when no anticoagulant is used yields serum, which has a gravity lower than that of plasma. These effects are so small that for clinical studies the errors introduced may ordinarily be neglected, and figures for plasma proteins and hemoglobin may be calculated with sufficient accuracy by applying the observed gravity values of blood and plasma or serum directly to the line charts. However, if more than 1 mg. of oxalate mixture per cc. of blood is used, or if the greatest precision is desired, corrections are applied as follows

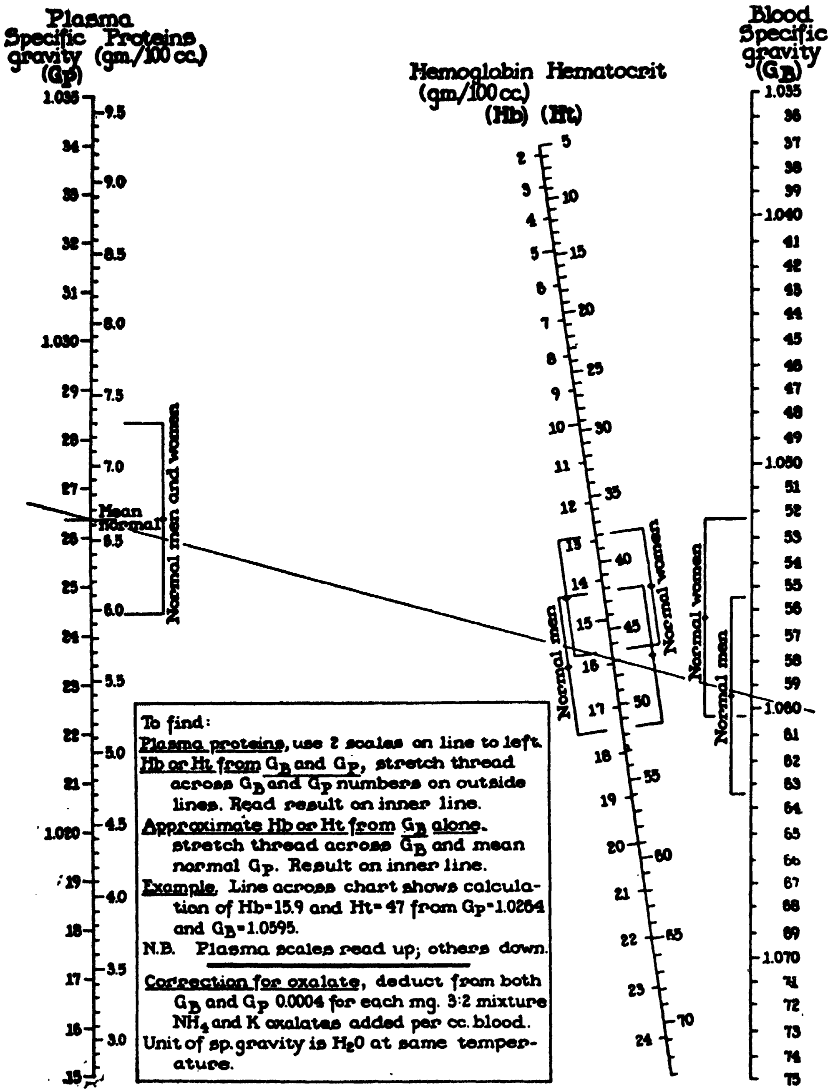

FIG. 83. LINE CHART FOR CALCULATING PLASMA PROTEINS, HEMOGLOBIN AND HEMATOCRIT FROM GRAVITIES OF PLASMA AND BLOOD.

(From Phillips, R. A.; Van Slyke, D. D.; Dole, V. P.; Emerson, K., Jr.; Hamilton, P. B., and Archibald, R. M.: *Copper Sulfate Method for Measuring Specific Gravities of Whole Blood and Plasma,* by permission of the United States Navy Research Unit at the Hospital of the Rockefeller Institute for Medical Research.)

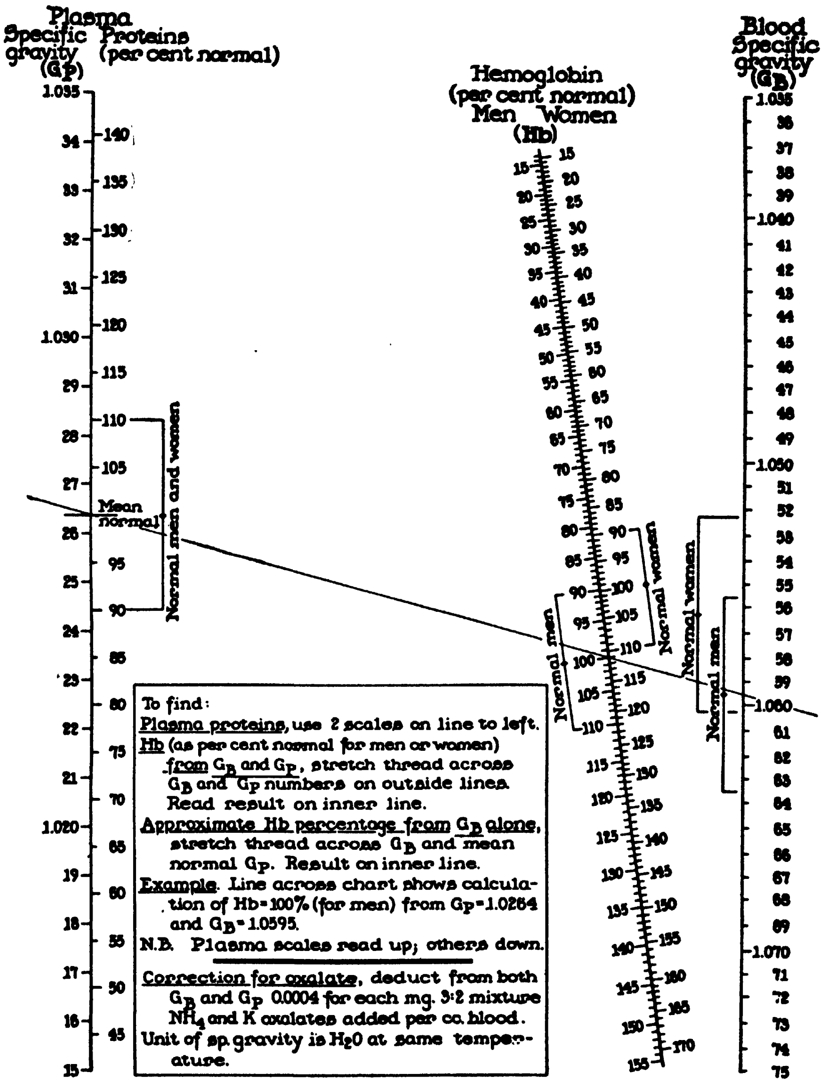

FIG. 84. LINE CHART FOR CALCULATING PERCENTAGES OF NORMAL PLASMA PROTEINS AND HEMOGLOBIN FROM GRAVITIES OF PLASMA AND BLOOD.

(From Phillips, R. A.; Van Slyke, D. D.; Dole, V. P.; Emerson, K., Jr.; Hamilton, P. B., and Archibald, R. M.: *Copper Sulfate Method for Measuring Specific Gravities of Whole Blood and Plasma,* by permission of the United States Navy Research Unit at the Hospital of the Rockefeller Institute for Medical Research.)

Corrections for Added Anticoagulants. No corrections are needed if heparin, 0.1 or 0.2 mg. per cc. of blood, is used. For each mg. of the ammonium-potassium oxalate mixture added per cc. of blood subtract 0.0004 from the observed G_B and the observed G_P. If a tube with 5 mg. of oxalate receives the expected 5 cc. of blood, the correction to G_P and G_B is therefore 0.0004. Neglect of the 0.0004 correction would lead to an overestimation of the plasma proteins by 0.1 mg. per 100 cc. of plasma, and of hemoglobin by 0.1 gm. per 100 cc. of blood, errors which may usually be neglected. If, however, the volume of blood placed in the tube is less than 5 cc. the oxalate concentrations will be greater, and the corrections to G_B and G_P will be as follows: for 4 cc. of added blood —0.0005; for 3 cc., —0.0007; for 2 cc., —0.0010; for 1 cc., —0.0020.

Total Plasma Protein Concentration from Specific Gravity Data. The formula and the nomogram scale for calculating plasma proteins are based upon Moore's and Van Slyke's data, which were chiefly based on pathologic plasma. For *normal* human plasma, it is now found that 376 is a more accurate factor than 343, and mean normal plasma proteins are slightly over 7.0 gm. per 100 cc.

Cc. of Saturated Copper Sulfate Solution to Be Diluted to 1 Liter to Give the Stock Solution of Specific Gravity *

Temperature in °C. or °F. refers to the temperature of the saturated solution at the time of saturation (end of shaking for 5 minutes).

Temperature °C.	°F.	cc.	Temperature °C.	°F.	cc.	Temperature °C.	°F.	cc.
10.0	50.0	578	20.0	68.0	488	30.0	86.0	425
10.5	50.9	573	20.5	68.9	484	30.5	86.9	423
11.0	51.8	568	21.0	69.8	480	31.0	87.8	420
11.5	52.7	563	21.5	70.7	477	31.5	88.7	417
12.0	53.6	558	22.0	71.6	473	32.0	89.6	414
12.5	54.5	553	22.5	72.5	469	32.5	90.5	412
13.0	55.4	548	23.0	73.4	466	33.0	91.4	409
13.5	56.3	543	23.5	74.3	463	33.5	92.3	406
14.0	57.2	539	24.0	75.2	460	34.0	93.2	403
14.5	58.1	534	24.5	76.1	456	34.5	94.1	401
15.0	59.0	529	25.0	77.0	453	35.0	95.0	398
15.5	59.9	525	25.5	77.9	450	35.5	95.9	395
16.0	60.8	521	26.0	78.8	447	36.0	96.8	392
16.5	61.7	516	26.5	79.7	445	36.5	97.7	390
17.0	62.6	512	27.0	80.6	442	37.0	98.6	387
17.5	63.5	508	27.5	81.5	439	37.5	99.5	384
18.0	64.4	504	28.0	82.4	436	38.0	100.4	381
18.5	65.3	500	28.5	83.3	434	38.5	101.3	379
19.0	66.2	496	29.0	84.2	431	39.0	102.2	376
19.5	67.1	492	29.5	85.1	428	39.5	103.1	373
20.0	68.0	488	30.0	86.0	425	40.0	104.0	370

* From Phillips, R. A.; Van Slyke, D. D.; Dole, V. P.; Emerson, K., Jr.; Hamilton, P. B., and Archibald, R. M.: *Copper Sulfate Method for Measuring Specific Gravities of Whole Blood and Plasma,* by permission of the United States Navy Research Unit at the Hospital of the Rockefeller Institute for Medical Research.

36

FUNCTIONAL EXAMINATIONS

UREA CLEARANCE TEST FOR KIDNEY FUNCTION

1. Prepare the patient and collect the two specimens of urine and the specimen of blood (oxalated) as described on page 163.
2. Determine the blood urea nitrogen (B), as described on page 1021.
3. Measure each specimen of urine and divide each by the time, in minutes, to obtain the *urine output per minute.*
4. Determine the urea nitrogen in milligrams per 100 cc. (U) in both specimens of urine as described on page 1003. They serve as a check on each other, as under the conditions of the test the blood urea nitrogen does not change to any extent during one hour.
5. The urea clearance equals the number of cubic centimeters of blood cleared of urea per minute. If the quantity of urine voided is more than 2 cc. per minute, the formula used is that for "maximal clearance" as follows:

$$\frac{\text{U (urine urea N, mg. per 100 cc.)}}{\text{B (blood urea N, mg. per 100 cc.)}} \times \text{V (cc. urine per minute)}$$

If the quantity of urine excreted is less than 2.1 cc. per minute, the square root law is applied and the formula for "standard clearance" must be used, as follows:

$$\frac{\text{U (urine urea N, mg. per 100 cc.)}}{\text{B (blood urea N, mg. per 100 cc.)}} \times \sqrt{\text{V (cc.}}\text{ urine per minute)}$$

The values for the square root of V are shown in the following table (Todd and Sanford):

V	$\sqrt{V}$	V	$\sqrt{V}$	V	$\sqrt{V}$	V	$\sqrt{V}$
0.2	0.45	0.7	0.84	1.2	1.1	1.7	1.3
0.3	0.55	0.8	0.89	1.3	1.14	1.8	1.34
0.4	0.63	0.9	0.95	1.4	1.18	1.9	1.38
0.5	0.71	1.0	1.00	1.5	1.23	2.0	1.42
0.6	0.78	1.1	1.05	1.6	1.27	2.1	1.45

PHENOLSULFONPHTHALEIN TESTS FOR KIDNEY FUNCTION

Intramuscular Method. 1. Administer 1 cc. (6 mg.) of a sterile solution of phenolsulfonphthalein intramuscularly and collect the two specimens of urine as described on page 169.

2. In a graduate or volumetric flask dilute each of the specimens of urine with water to about 800 cc. Add about 5 cc. of 10 per cent sodium hydroxide solution or enough to bring out the maximum purplish-red color; add water to each to 1000 cc. and mix thoroughly.

3. Prepare a standard by diluting exactly 1 cc. of 0.6 per cent phenolsulfonphthalein solution with about 800 cc. of water. Alkalinize with sodium hydroxide solution to obtain the maximum color and dilute to 1000 cc. with water.

4. Filter the diluted and alkalinized specimens of urine and compare with the standard in a colorimeter. The Dunning colorimeter consists of thirteen sealed ampules containing standard color solutions of different percentages, an open ampule in which the unknown specimen is placed, and a small box in which the specimen is compared with the standards. It is very satisfactory for office work because the physician need not make his own standard solution. The colors remain satisfactory for over a year with very little fading when kept in the dark.

5. Otherwise the Duboscq colorimeter may be used with U set at 10 mm. If V equals the volume of urine in cc. to which it was diluted and S the standard, the percentage of dye excreted equals $\frac{SV}{100}$.

Fractional Method (Chapman and Halsted). 1. The patient empties the bladder and drinks 600 cc. of water. Discard the urine.

2. Within an hour inject 1 cc. (6 mg.) of sterile phenolsulfonphthalein solution *intravenously.*

3. Have the patient void urine exactly 15 and again 30 minutes after the dye is injected.

4. Prepare a standard by diluting exactly 1 cc. of 0.6 per cent phenolsulfonphthalein solution to 1000 cc. with distilled water made alkaline by the addition of sodium hydroxide solution.

5. Determine the dye content with both specimens of urine as follows: Add to each specimen a 10 per cent solution of sodium hydroxide slowly and with stirring until the maximal red color is obtained. Dilute the alkaline urine to a volume between 100 and 1000 cc. so that the color of the standard is approximated.

6. Compare in a colorimeter (Duboscq preferred) as described above. The calculation is exactly the same: Percentage of dye excreted $= \frac{SV}{100}$.

BROMSULFALEIN TEST FOR LIVER FUNCTION

1. Inject the dye intravenously and collect the two specimens of blood as described on page 209.

2. Determine the amount of dye in the two specimens by using the Rosenthal colorimeter supplied by Hynson, Westcott, and Dunning. For this purpose equal amounts of the serum from each specimen of blood are pipetted off into three small test tubes of the same diameter as the color tubes of the colorimeter. To one of the tubes add a drop of 10 per cent sodium hydroxide solution. To the others may be added a drop of 5 per cent hydrochloric acid solution if the serum is colored with hemoglobin. Place the tubes in the colorimeter side by side and

match by placing the standard tubes behind the tubes without the alkali. The percentage strength of the dye is marked on the standard tube.

The determination may be made also as follows: 1. Prepare a solution of hydrochloric acid by adding 5 cc. of concentrated acid to 95 cc. of distilled water.

2. Dissolve 10 mg. of bromsulfalein (Hynson, Westcott, and Dunning) in 80 cc. of distilled water made alkaline with 0.25 cc. of 10 per cent sodium hydroxide solution and dilute with water to 100 cc. This represents a 100 per cent standard. Prepare a series of standard tubes of uniform diameter, ranging from 5 to 100 at intervals of 10, diluting the above solution with alkalinized water. The standards may be sealed and will keep for several months in the dark. The numbers on the tubes denote amounts of dye retained as per cent of amount injected. The dilutions required are as follows:

Tube	*Dye*	*Alk. Water*
5	0.25 cc.	4.75 cc.
10	0.50 "	4.50 "
15	0.75 "	4.25 "
20	1.00 "	4.00 "
30	1.50 "	3.50 "
40	2.00 "	3.00 "
50	2.50 "	2.50 "
60	3.00 "	2.00 "
70	3.50 "	1.50 "
80	4.00 "	1.00 "
100	5.00 "	0 "

3. Transfer clear serum to two small test tubes uniform with the standards. To one of these, add 1 or 2 drops of 10 per cent sodium hydroxide solution and to the other 3 drops of 5 per cent hydrochloric acid solution.

4. Compare with the standards in comparator in artificial light, using a white background. Place the acidified tube before the standard and the tube with water in front of the alkalinized tube. Read at once to the nearest standard tube. The results are expressed as per cent retention at 30 minutes.

The bromsulfalein test is invariably positive, and readings are unsatisfactory, when marked jaundice is present. Since the dye is expensive, it is suggested that its use be dispensed with if an appreciable degree of jaundice exists.

GALACTOSE TEST FOR LIVER FUNCTION

1. Administer galactose and collect five specimens of urine at hourly intervals thereafter as described on page 207.

2. Test each specimen for sugar by the Benedict qualitative method (page 997).

3. Mix the specimens giving positive reactions, measure and determine the total amount of sugar excreted by the Benedict quantitative method (page 998). An excretion of more than 3 gm. of reducing sugar may indicate intrahepatic jaundice, although less than 6 gm. may not be significant.

The test may be conducted also as follows: 1. Measure the total volume of the urine samples in cc.

2. Divide this volume by 100 and with a Mohr pipet transfer this amount to a 250 cc. cylinder. Dilute carefully with distilled water to 150 cc. and mix thoroughly.

3. Determine the amount of sugar present in 2 cc. of the diluted urine by the Benedict quantitative method (page 998). As galactose gives only 80 per cent as much reduction as glucose, the result must be multiplied by 1.25 to obtain galactose. If the Duboscq colorimeter is used, set the unknown at 25 and calculate as follows: $\frac{7.5S}{100}$ = grams of galactose in total specimen.

When the fasting urine contains sugar, or it is desired to perform the test on a diabetic patient, a portion of the urine should be fermented with washed yeast suspension. Consider carefully the dilution of the urine or use other appropriate calculations.

CEPHALIN FLOCCULATION TEST (HANGER) FOR LIVER FUNCTION

1. Prepare a stock solution of cephalin and cholesterol by adding 5 cc. of U.S.P. anesthetic ether to a 1 unit vial of Bacto cephalin-cholesterol mixture (Difco Laboratories). Stopper and shake thoroughly. This stock solution is stable if kept in a refrigerator.

2. Add, with stirring, 1 cc. of the stock ether solution to 35 cc. of freshly distilled water which has been heated to 65–70° C. Heat the mixture slowly to boiling and allow to simmer until the volume is reduced to 30 cc. Cool to room temperature. This emulsion must be freshly prepared on the day used.

3. Place 0.2 cc. of the patient's serum in a clean test tube. Add 4 cc. of 0.85 per cent sodium chloride solution and 1 cc. of the cephalin-cholesterol emulsion.

4. Shake thoroughly, stopper with cotton and allow to stand undisturbed at room temperature.

5. Prepare a control tube containing 4 cc. of saline solution and 1 cc. of cephalin-cholesterol mixture. This tube should show a pearly opalescence without a trace of flocculation.

6. Make readings at 24 and 48 hours. The results are graded −, ±, +, ++, +++, and ++++ on the basis of the degree of flocculation. A ++++ reaction is one showing complete flocculation with a clear supernatant fluid. A ± reaction is one showing a very slight degree of flocculation that can be detected by comparison with the control. The reaction should not be regarded as negative unless there is no flocculation at the end of 48 hours.

THYMOL TURBIDITY TEST (MAC LAGEN) FOR LIVER FUNCTION

This test as modified by Shank and Hoagland may be conducted as follows: 1. Prepare the *thymol-barbital buffer reagent* by placing 1.03 gm. sodium barbital, 1.38 gm. barbital, 3.0 gm. powdered thymol crystals and 500 cc. distilled water in a 1000 cc. Erlenmeyer flask; heat to the boiling point; mix thoroughly and allow to cool to room temperature (solution becomes turbid); add a small quantity of powdered thymol crystals and mix thoroughly; stopper the flask and stand overnight at room temperature; mix thoroughly and remove excess crystals by filtration; the clear solution, which keeps indefinitely at room temperature, is employed.

2. The test is conducted by adding 0.05 cc. of serum to 3.0 cc. of the reagent in a 10 x 75 mm. cuvette. The contents of the cuvette are thoroughly mixed and after 30 minutes turbidity is determined in the Coleman Junior spectrophotometer at a wave length of 650 mu. The galvanometer is adjusted to 100 per cent transmission with a blank containing 3.0 cc. of reagent.

3. Turbidity is expressed in units derived from a standard curve prepared by the use of barium sulfate suspensions. The turbidity standard is prepared by diluting 3.0 cc. of 0.0962 N barium chloride solution to volume in a 100 cc. volumetric flask by the addition of 0.2 N sulfuric acid at 10° C. A 10-unit turbidity standard is prepared by adding 1.65 cc. of 0.2 N sulfuric acid to 1.35 cc. of the barium sulfate suspension in a 10 x 75 mm. cuvette. Similarly, a 20-unit standard is prepared by adding 0.3 cc. of 0.2 N sulfuric acid to 2.7 cc. of the barium sulfate suspension. At room temperature there is some tendency for the barium sulfate to settle out. For this reason cuvettes should be well shaken just before readings are made in the spectrophotometer. If a cuvette containing 3.0 cc. of distilled water is used as a blank, there is a straight line relationship between the optical density of various dilutions of the barium sulfate standard at 650 mu.

BASAL METABOLIC RATE

1. Any of the basal machines made by reliable manufacturers may be used. Detailed directions and data, by which the basal metabolic rate may be determined, are supplied. The authority of standards used may often be open to question since they may be only the averages of various accepted standards. The calculation is somewhat longer, but the machine becomes no less useful if the actual oxygen consumption is determined in cc. and converted to calories by the factor—one liter of oxygen is equivalent to 4.825 calories—and compared to any standard desired. The amount of oxygen consumed may be read from the kymograph after consulting the book concerning the standardization of the kymograph sheet and making necessary corrections for temperatures and pressure.

2. The preparation of the patient and the numerous factors influencing the results of the test are discussed on page 175. Although it is ideal to take the machine to the bedside, this is often impossible. The determination may be made in the physician's office. Under these circumstances the patient may make the trip in the morning with as little exercise as possible and rest in the prone position for one hour during which time the metabolism will return to the basal level. The emotional state of the patient is a much more important factor. Anxiety, anger, hurry, etc., may readily elevate the metabolic rate into the pathologic zone. A calm and reassuring manner, patience and tact on the part of the physician are essential. Only well-trained technicians should be entrusted with the test.

Routine instructions are supplied by the manufacturers. The following supplementary data are important: (1) Take the patient's temperature which should be normal; (2) take the pulse rate before and after the test and one or two hours previous if possible (this aids in deciding on the results of the test); (3) thoroughly accustom a "new" patient to the test by one or more trial periods; (4) make two records of oxygen consumption with a rest period of 5 to 10 minutes between (they should agree within 5 per cent); (5) watch the patient for restlessness or tenseness which, in addition to a pulse rate above the apparent normal, indicates that the patient is not under basal conditions.

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EXAMINATIONS OF GASTRIC CONTENTS

Routine examinations of the gastric contents yield information on the secretory, digestive and motor functions of the stomach, as discussed in Chapter 10. They are chiefly concerned with the secretory function in response to test meals, and the different kinds commonly employed, along with the collection of stomach contents for laboratory examinations, are described in Chapter 10. In this connection it should be remembered, however, that the gastric contents may include a variety of swallowed materials and others that are regurgitated from the small intestines. Thus pus, blood, bacteria, tissue fragments, etc., from the nose, mouth, pharynx, and esophagus may find their way into the stomach. Similar materials and secretions and excretions of the liver, gallbladder and duodenum may be regurgitated through the pylorus, and with reversal of peristalsis, the fecal contents of the jejunum and ileum may likewise gain entrance.

PHYSICAL EXAMINATIONS

Macroscopic. Physical examinations of the stomach contents include measurements of the *amounts* of residuum removed before a test meal, as well as the amounts of the latter recovered during a period of one or more hours thereafter; also the *color, layering, odor*, etc., as well as the presence or absence of *foods* eaten previously, as discussed in Chapter 10.

Microscopic. As a general rule, microscopic examinations of the residuum and recovered test meals do not afford much information of clinical value. Occasionally, however, the findings are unexpected as, for example, the presence of ova of parasites or the latter themselves. Microscopic examinations are best carried out on the unfiltered residuum of the fasting stomach, or the unfiltered specimens obtained after a test meal.

These examinations are conducted by mixing a drop of the residue remaining on the filter with a drop of Gram's iodine solution, covering with a cover glass, and examining microscopically with a lowered substage to reduce the illumination. If gastric washings are examined, allow them to stand in a conical glass and examine the sediment.

If gross particles of *tissue* are found, they should be fixed in 4 per cent formalin and sections prepared for histologic examination. Normally, only a few *epithelial cells* are found. Groups of these cells entangled in mucus are commonly seen in chronic gastritis. *Pus cells* may be found in phlegmonous gastritis and ulcerated cancers. Epithelial cells, deeply stained with bile, probably originate from the bile ducts or gallbladder. *Erythrocytes* are usually degenerated and difficult to recognize; if easily recognizable, they are usually due to the trauma of the tube passage.

Yeasts and *sarcinae* stain yellow and brown with the iodine solution. If present in large numbers, they usually indicate retention and fermentation. Budding forms of yeast are not seen ordinarily.

Food remnants in the residuum usually indicate retention. Starch granules after the Ewald meal are numerous; when stained with the iodine solution they appear blue or red, depending on their stage of digestion. Striated muscle fibers and connective tissue are easily recognized. Fat globules, unstained by the iodine solution, may be stained by running under the cover glass a drop of Sudan III; neutral fat globules stain red or yellow.

Normally, only a few *bacteria* swallowed with nasopharyngeal secretions are present in the residuum. Large numbers of staphylococci or streptococci may occur in infective gastritis, as discussed in Chapter 15. The Boas-Oppler bacilli, when present, indicate gastric retention from any cause but are especially likely to be found in advanced gastric carcinoma. They belong to the lactic acid-producing group. They occur as long, nonspore-forming, Gram-positive bacilli growing in long jointed chains or clumps, and when present, lactic acid is detectable in the gastric contents.

Flagellates may be found in the early anacid stage of gastric carcinoma before lactic acid production is marked; they originate in the intestines. Other *animal parasites* and *ova* may be found, due to contamination of the food by faulty personal hygiene or regurgitation from the small intestine into the stomach.

Crystals of fatty acids, bile acids, cholesterol, calcium oxalate, etc., may be found but have no clinical significance.

TÖPFER METHOD OF CHEMICAL ANALYSIS

The method of Töpfer consists in the administration of a test meal of the Ewald type (see page 241) followed by its removal one hour later.

1. Measure and record the volume of the sample. Strain through gauze or cheesecloth and place 10 cc. of the coarsely filtered fluid in each of three beakers or porcelain dishes labelled No. 1, No. 2 and No. 3. If sufficient contents are not obtained, use 5 cc. and calculate accordingly. Normally 50 to 100 cc. are recovered.

2. Prepare a phenolphthalein indicator by dissolving 0.05 gm. of phenolphthalein in 100 cc. of 50 per cent ethyl alcohol.

3. Prepare Töpfer's reagent by dissolving 1 gm. of p-dimethylaminoazobenzene in 100 cc. of 95 per cent ethyl alcohol.

4. Prepare alizarin red indicator by dissolving 1 gm. of sodium alizarin monosulfonate in 100 cc. of water.

5. Prepare a 0.1 N solution of sodium hydroxide.

Total Acidity. This includes free hydrochloric acid, hydrochloric acid combined loosely with protein food (combined hydrochloric acid), organic acids (chiefly lactic acid), and acid salts. It is determined as follows:

1. To the 10 cc. sample of filtered gastric contents in beaker No. 1, add 1 drop of phenolphthalein indicator (which is colorless in the presence of acid).

2. Add 0.1 N sodium hydroxide solution from a buret until a faint pink is produced which persists for 2 minutes.

3. The number of cubic centimeters of 0.1 N sodium hydroxide solution used, multiplied by 10, gives the number of cubic centimeters of 0.1 N hydroxide necessary to neutralize 100 cc. of gastric fluid. This value is reported as expressing the total acidity. It can be converted into terms of hydrochloric acid by multiplying by 0.00365, which is the equivalent value of 1 cc. of 0.1 N sodium hydroxide in grams of hydrochloric acid.

Free Hydrochloric Acid. 1. To sample No. 2 add 2 to 4 drops of Töpfer's reagent and titrate with 0.1 N sodium hydroxide until the initial red color becomes salmon pink. If there is an initial yellow color on adding the indicator, no free acid is present.

2. The number of cubic centimeters of sodium hydroxide solution used, multiplied by 10, gives the value for 100 cc. of the gastric juice. Occasionally Töpfer's reagent gives a red color in the absence of hydrochloric acid, due to a large increase in the organic acids, especially when lactic acid is over 1 per cent and albumoses are present.

3. In case the amount of gastric juice is small, the same specimen may be used to determine the total acidity. After the end point is reached for free hydrochloric acid, add 2 drops of phenolphthalein indicator and continue the titration with 0.1 N sodium hydroxide until the persistent pink end point of total acidity is reached. The number of cubic centimeters of hydroxide used in the determination of free hydrochloric acid, plus the additional cubic centimeters necessary to complete the titration with phenolphthalein, is multiplied by 10, giving the value of the total acidity.

Free Acidity. This includes hydrochloric acid in the free state, organic acids and acid salts, but does not include the combined hydrochloric acid. It is determined as follows:

1. To sample No. 3 add 1 to 3 drops of sodium alizarin sulfonate indicator.

2. Titrate with 0.1 N sodium hydroxide solution. As the hydroxide is added, the initial tinge of yellow changes to red. The end point is indicated by a distinct violet color.

3. The number of cubic centimeters of hydroxide used, multiplied by 10, gives the free acidity value. Töpfer states that alizarin is sensitive to all acidity except combined hydrochloric acid.

Combined Hydrochloric Acid. This value is obtained by subtracting the value obtained for free acidity from that of the total acidity. Cases are seen where there is no free hydrochloric acid but much combined acid, indicating that acid has been secreted but has combined with the food protein.

Organic Acids and Acid Salts. This value is obtained by subtracting the value of free hydrochloric acid from that of the free acidity.

REHFUSS METHOD OF FRACTIONAL CHEMICAL ANALYSIS

The fractional method of Rehfuss consists in giving a retention meal for supper the previous evening. After a 12-hour fast, the fasting residuum is removed. The tube is left in place and a test meal of the Ewald or clear fluid type is given. Samples are then withdrawn every 15 minutes for one or more hours until the stomach is empty. Each specimen is examined for total and free acidity; other tests may be made when indicated. This method follows the cycle of gastric digestion and secretion, allowing the plotting of curves, as shown in Chapter 10, which permits better clinical interpretation, especially of acid secretion. Measure and strain the residuum and each fraction separately through gauze or cheesecloth. *The reagents are the same as in Töpfer's method except that 0.01 N sodium hydroxide is used instead of 0.1 N.*

Total Acidity. 1. Place 1 cc. of the filtrate and 15 cc. of distilled water in a porcelain evaporating dish.

2. Add one drop of phenolphthalein indicator.

3. Titrate with 0.01 N sodium hydroxide solution until a faint pink, lasting for 2 minutes, indicates the end point.

4. The number of cubic centimeters of 0.01 N sodium hydroxide required to neutralize 1 cc. of the sample, multiplied by 10, gives the number of cubic centimeters of 0.1 N hydroxide required to neutralize 100 cc. of the gastric contents.

Free Hydrochloric Acid. 1. Place 1 cc. of gastric filtrate and 15 cc. of distilled water in a porcelain evaporating dish.

2. Add 1 or 2 drops of Töpfer's reagent; if, on adding the indicator, there is an initial yellow color, no free acid is present.

3. Titrate with 0.01 N sodium hydroxide solution until the initial red color becomes salmon pink (the end color is more definitely yellow than orange).

4. The number of cubic centimeters of 0.01 N sodium hydroxide required, multiplied by 10, gives the number of cubic centimeters of 0.1 N hydroxide required to neutralize the free hydrochloric acid in 100 cc. of the gastric contents.

SAHLI METHOD FOR FREE HYDROCHLORIC ACID

This method requires more time but gives more accurate results because of a sharper end point. It is based on the liberation of iodine from the reagent employed in the presence of free hydrochloric acid. The iodine is titrated with sodium thiosulfate, using a starch indicator.

1. Prepare Sahli's reagent, which is a mixture of equal parts of a 48 per cent aqueous solution of potassium iodide and an 8 per cent aqueous solution of potassium iodate.

2. Prepare a 0.01 N solution of sodium thiosulfate.

3. Prepare a 1 per cent aqueous solution of starch.

4. Place 1 cc. of the strained sample and 10 cc. of distilled water in a porcelain evaporating dish.

5. Add 1 cc. of Sahli's reagent, mix and allow to stand for 5 minutes.

6. Titrate with 0.01 N solution of sodium thiosulfate until only a faint yellow color of the liberated iodine remains.

7. Add 0.5 cc. of the soluble starch solution. The mixture turns blue. Continue the titration until the blue disappears.

8. The total number of cubic centimeters of 0.01 N sodium thiosulfate used

in the titration of 1 cc. of gastric juice is equivalent to the number of cubic centimeters of 0.01 N sodium hydroxide necessary to neutralize the free hydrochloric acid in 1 cc. of gastric contents. This value, multiplied by 10, represents the number of cubic centimeters of 0.1 N sodium hydroxide necessary to neutralize 100 cc. of stomach contents.

QUALITATIVE TESTS FOR LACTIC ACID

Lactic acid is a product of carbohydrate fermentation by bacteria and yeasts. Normally it is not present at the height of digestion. Small amounts may be introduced with the test meal unless arrowroot cookies are used, as discussed in Chapter 10. It is more often present in stagnation of the gastric contents associated with deficient hydrochloric acid.

Uffelmann's Test. 1. Prepare the reagent by adding 10 per cent ferric chloride solution to a 1 per cent aqueous phenol solution until an amethyst color develops.

2. To 5 cc. of reagent, add 5 cc. of strained gastric juice. To another 5 cc. portion, add a few drops of dilute hydrochloric acid as a control.

3. Lactic acid produces a canary-yellow color. The reagent will detect 0.01 per cent of lactic acid. Hydrochloric acid discharges the amethyst color, leaving the solution colorless. If the gastric juice contains much free hydrochloric acid, the value of the test is decreased. Other organic acids give results similar to lactic acid.

MacLean's Test. 1. Prepare the reagent by dissolving 5 gm. of ferric chloride in a mixture of 100 cc. of saturated aqueous solution of mercuric chloride and 1.5 cc. of concentrated hydrochloric acid.

2. Place 5 cc. of water in a test tube as a control. In another tube place 5 cc. of gastric contents. To each add 5 drops of reagent.

3. A reddish color indicates the presence of lactic acid.

Strauss' Test. 1. Prepare a 10 per cent aqueous solution of ferric chloride.

2. Place 5 cc. of strained gastric contents in a small separatory funnel. Add 20 cc. of ether and shake thoroughly. Let stand until the ether layer has separated, then run out the layer of gastric juice and all but the final 5 cc. of ether.

3. To this ether extract add 20 cc. of distilled water and 2 drops of the ferric chloride solution. Shake the mixture gently.

4. When lactic acid is present in a concentration of 0.05 per cent, a slight greenish color develops. If the concentration is 0.1 per cent, or higher, the color is an intense yellow, due to ferric lactate.

BENZIDINE TEST FOR OCCULT BLOOD

Provided the reagents are satisfactory, this test is a very sensitive one. Different lots of benzidine vary greatly in sensitivity and hydrogen peroxide solution rapidly loses its strength. For this reason it is always advisable to set up a positive control, using water with an extremely minute amount of blood added, such as would adhere to the tip of an applicator.

1. Prepare a saturated solution of benzidine labelled "For blood tests" in

glacial acetic acid. Keep in a brown bottle in a dark place. Or, prepare the reagent just before use by dissolving the crystals picked up on the point of a knife blade in 5 cc. of glacial acetic acid with the aid of gentle heating.

2. To 3 cc. of the reagent, add 2 cc. of the gastric contents and mix thoroughly. Add 1 cc. of hydrogen peroxide solution (usually 3 per cent).

3. If blood is present, a green to deep blue color, depending on the amount of blood, will form on adding the peroxide. Too much benzidine solution or too much peroxide interferes with the sensitivity and accuracy of the test.

4. A confirmatory test may be conducted as follows: (a) If fat is present, render the gastric specimen slightly alkaline with sodium carbonate or sodium hydroxide solution. (b) Extract in a separatory funnel with an equal amount of ether. (c) Discard the ether extract. (d) Render the residue acid with acetic acid and extract with ether. (e) Evaporate the ether extract to dryness, using a water bath which has been heated to boiling and the flame then extinguished. (f) Add 1 cc. of water, stir to dissolve the residue, then add a few drops of benzidine solution and a drop or two of hydrogen peroxide. (g) The development of a green to deep blue color indicates a positive reaction.

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FECES EXAMINATIONS

Diseases of the gastro-intestinal tract and disturbances of digestion are among the most common ailments physicians are called upon to diagnose and treat. Considerable assistance is given by an examination of the feces, provided practitioners possess adequate information concerning the composition of the feces in health and variations caused by disease. Due to the comparatively simple dietary of infants, the relatively rapid passage of the intestinal contents, and the absence of intestinal decomposition under ordinary conditions, the results of the analyses of their stools are much easier to interpret than is the case with adults, in whom one has to contend with a more complicated dietary and take into account a large number of underlying factors, including the influence of diet, personal habits, the effects of former illnesses, etc.

GENERAL MACROSCOPIC EXAMINATIONS

Much information of helpful diagnostic value is frequently obtained by taking into account the *amount, form* and *consistency, color, odor, reaction* and the presence or absence of an excess of *mucus,* as discussed in Chapter 11. Indeed, physicians should conduct these examinations themselves more frequently than is generally customary, instead of relying solely on the reports of laboratory technicians.

Normally, the feces of adults are slightly alkaline when freshly passed. An acid reaction is much less frequent and is commonly due to a vegetable diet. The stools of infants are usually acid. The reaction is readily determined by testing with red and blue litmus and Congo red papers; also by adding a few drops of a 1 per cent alcoholic solution of phenolphthalein to a watery suspension. If alkaline, a pinkish color is produced.

MACROSCOPIC EXAMINATIONS FOR HELMINTHS

1. Place the entire stool specimen in a suitable receptacle and add a sufficient amount of tap water to make it fluid.

2. Thoroughly mix water with the stool and pass through a suitable screen (No. 20) to remove fluid. If the fecal matter has been properly broken up, most of it will pass through, leaving the worms or segments on the screen.

3. The material from the screen is now transferred to a clean shallow glass dish or tray (preferably with a black bottom) containing salt solution or tap water. Against this black background the parasite may be seen easily with the unaided eye.

4. The parasite or segment may now be placed on a microscopic slide, covered with a cover glass, and examined with the naked eye or low power scope. In examining tapeworm segments and flukes, it is desirable to cover with a second slide in place of a cover glass. By pressing the two together, the specimen may be flattened, making it less opaque and thus better revealing the anatomic structure. Clearing the specimen with carbo-xylol (25 per cent phenol crystals and 75 per cent xylol) will also aid.

5. The specimen is examined best for internal structure by transmitted light. For details of the cuticle, or in the examination for scolices of tapeworms (to show hooklets, suckers, etc.) direct illumination or a combination of the direct illumination and transmitted light will be found helpful and advantageous.

6. Examine carefully for the presence of the head or scolex of a tapeworm. The head is very tiny, about the size of a pin head (1 mm.), the neck and adjacent segments scarcely larger than a heavy thread. They may be easily overlooked if great care is not exercised. *The finding of the head is of paramount importance to the physician since the worm will continue to grow as long as the head remains in the intestine.*

GENERAL MICROSCOPIC EXAMINATIONS

1. Prepare a thick suspension by rubbing up a portion about the size of a walnut in water. This gives a uniform mixture more representative than selecting small bits at random.

2. Place a drop on a slide and cover with a large cover glass for general examination (No. 1).

3. Place a drop on a slide with 1 or 2 drops of 30 per cent acetic acid for muscle, leukocytes and pus (No. 2).

4. Place a drop on a slide with 1 or 2 drops of Sudan III for fats (No. 3).

5. Place a drop on a slide with 1 or 2 drops of Lugol's solution for starches (No. 4).

6. Examine each microscopically with low and high power with the light well cut down, as is done in the examination of urinary sediments.

7. In this general examination the following may be looked for:

(a) *Vegetable fibers* and *hairs*.

(b) *Connective tissue*, consisting of colorless or yellowish threads which swell and become gelatinous in the acetic acid preparation.

(c) *Muscle*. If striations are visible, digestion is imperfect. If the nuclei are visible, pancreatic function is absent or deficient.

(d) *Elastic tissue*, which generally accompanies connective tissue; outlines more definite with branching; more distinct in the acetic acid preparation.

(e) *Starch*. If undigested, the granules are blue on the slide treated with Lugol's solution; reddish if partially digested.

(f) *Neutral fats*. Stain red with Sudan III solution; also globules of fatty acids.

(g) *Leukocytes* and *pus*, which are seen best in the acetic acid preparation. A few are normal; an excess occurs in dysentery and in other inflammatory states masses of pure pus may be seen. In bacillary dysentery, *macrophages* may be found consisting of large mononuclear phagocytic cells with large vesicular nuclei, frequently containing remnants of ingested leukocytes and erythrocytes. They show varying degrees of necrosis and may present only circular or oval rims with granular debris ("ghost cells"). These macrophages may be mistaken for amebae but can be differentiated by lack of motility and by the character of the nuclei.

(h) An excess of *eosinophils* may be found in the mucus in the discharges of intestinal allergy.

(i) *Mucus,* especially in mucous colitis, dysentery and other diseased states.

(j) *Erythrocytes,* which are seen best in the untreated slide.

(k) *Epithelial cells,* which show all stages of disintegration and are often unrecognizable. A marked excess of recognizable cells may occur in diseased states.

(l) *Crystals,* which ordinarily have but little significance: (1) Slender, needle-like crystals of fatty acids and soap; (2) triple phosphates; (3) calcium oxalate from vegetables; (4) Charcot-Leyden crystals, especially in parasitic infestments; (5) yellowish or brown needles or rhombic crystals of hematoidin after intestinal hemorrhages, etc. Cholesterin crystals and calcium bilirubinate are occasionally found, especially in cases of cholelithiasis.

MICROSCOPIC EXAMINATIONS FOR INTESTINAL PROTOZOA

The intestinal protozoa include *Endamoeba histolytica, Balantidium coli, Chilomastix mesnili, Trichomonas hominis,* and *Giardia lamblia.* They may be present as motile trophozoites or cysts.

Collection. Cold feces, collected in the usual manner, may be used in examinations for cysts but when examinations for trophozoites are indicated, the method of collection may be as follows:

1. The specimen should be collected directly in clean, covered receptacles (bedpans, swabs in test tubes containing 0.5 cc. of warm saline solution, syringes, bottles or droppers) preferably sterilized by heat. These receptacles should not be sterilized by chemical disinfectants as protozoa in the vegetative stage are easily killed and quickly undergo autolysis in the presence of only small amounts of such chemical agents. If the receptacles are not properly cleaned and sterilized, there is always the possibility of introducing free-living protozoa into the specimen and thus confusing the findings.

2. Specimens should be kept in the original receptacles used for collection until examined. *All specimens should be examined as soon as possible after collection,* since protozoa degenerate rapidly and the possibility of an accurate diagnosis diminishes as the time between collection and examination of the specimen increases. *If a delay in examination occurs the material should be kept at or near 37° C.,* as all protozoa are quite sensitive to chilling and are rapidly killed by temperatures of 45° C. or higher. Since drying also affects them, the specimen should have its original moisture when presented for examination.

3. If the lesions are in the rectum or sigmoid, specimens may be obtained by means of the proctoscope or sigmoidoscope. These are more likely to yield protozoa

than feces passed in the regular way. However, because of the attending discomfort and probable pain to the patient, these methods should be used only after it has been demonstrated that the passed feces are negative.

4. It is practically impossible to find protozoa in a stool after an oil cathartic or following a barium meal. Specimens collected by means of an enema are also unsatisfactory. Therefore, in the event any of the above has been used, examinations for protozoa in the feces should be delayed for at least 72 hours.

General Method. 1. The portions of a stool most likely to contain parasitic protozoa are those showing blood or mucus. In formed stools, small flecks of mucus or mucus and blood can always be found on the surface of the specimen. In semiformed or liquid stools, if the specimen is examined carefully, mucus and also blood may be found.

2. Formed stools usually contain only cysts or precysts of protozoa. Semiformed and liquid stools will ordinarily contain only trophozoites (vegetative forms) and it is these types of stools that afford the best opportunity for detecting a protozoan infestment. If the patient is not already passing such a stool, and it is necessary to rule out the possibility of a protozoan infestment, a saline cathartic may be given with the examination of the second or third liquid stool.

3. Warm a clean slide so that it feels comfortable when touched to the back of the hand. Then secure a small amount of mucus, or mucus and blood, by means of a wire loop or wooden applicator and thoroughly emulsify it in 1 drop of warm normal saline solution on the middle of the slide.

4. Take a clean No. 1 cover glass between the thumb and forefinger of the right hand, contact the slide with one edge of the cover glass near the drop, but not touching it, push the cover glass along the surface of the slide until its edge contacts the drop, rock it slightly from side to side to allow a portion of the fluid to come under the edge of the cover glass, and then let the cover glass drop from between the fingers allowing it to fall on the slide. The fluid portion of the drop on the slide will then automatically be drawn by capillary attraction under the cover glass, while the solid particles will be excluded. This method insures a thin, even preparation of not too great a density and insures even apposition of the cover glass to the slide. The preparation is now ready for examination, but, if it is to be kept on a warm stage for any period of time, it should be ringed with vaseline.

Zinc Sulfate Flotation Method. In formed or semiformed stools, where cysts are more likely to be found, the zinc sulfate centrifugal flotation method may be used as follows (if cysts are not found in ordinary preparations):

(1) Prepare a fecal suspension by emulsifying 1 part stool (about the size of a pecan) in 10 parts lukewarm water. (2) Strain 10 cc. of this emulsion through two layers of wet cheesecloth into a small test tube or centrifuge tube. (3) Centrifugalize for 45 to 60 seconds at approximately 2500 r.p.m., pour off the supernatant fluid, and add 2 or 3 cc. of water. Then break up the sediment and repeat the above, centrifugalizing and discarding the supernatant fluid three or four times. (4) After pouring off the last supernatant fluid, add 3 to 4 cc. of 33 per cent aqueous solution of zinc sulfate, break up the packed sediment and add enough zinc sulfate solution to fill the tube about one-half inch from the rim.

(5) Centrifugalize the tube for 45 to 60 seconds at top speed. (6) Remove several loopfuls of the material floating on the top surface film to a clean slide. Add 1 drop of iodine stain and a cover glass. The iodine stain is prepared by dissolving 4 gm. of potassium iodide and 2 gm. of iodine crystals in 100 cc. of water. After finding trophozoites or cysts and studying them in the fresh, wet, unstained mount, the nuclear details can be clearly visualized and studied microscopically if the cover glass is raised at one edge and a drop of the iodine stain is thoroughly mixed with the contents under the cover glass. For special staining methods, including methods for staining smears on slides, consult laboratory manuals; do likewise for culture methods, although these and animal inoculation tests are not usually employed for routine diagnostic purposes.

The trophozoites of *E. histolytica* must be differentiated from those of *Esch. coli*. For this purpose, consult laboratory manuals. Differentiation is based upon (1) size and color; (2) differentiation of ectoplasm and endoplasm; (3) granularity of endoplasm and presence of cell inclusions; (4) the visibility of the nucleus, location when in motion, and size; (5) type of motility (active, sluggish, progressive or nonprogressive); (6) pseudopodia (single or multiple, clear or granular); (7) flowing of endoplasm into pseudopod (slow or explosive) and (8) presence or absence of erythrocytes or bacteria in the endoplasm (Fig. 85).

Tissue cells derived from the patient, or ingested food, may at first glance appear as amebae, but careful examination of them will easily establish their true nature. Macrophages may be found containing phagocytized erythrocytes, but examination reveals their typical nuclear structure and ameboid movement is not observed. Epithelial cells are pale in color and have nuclear characteristics which easily differentiate them. Vegetable cells, such as starch granules, pollen granules, yeast cells, or other cells of this type, have a certain definiteness of outline and structure that should lead to no confusion. However, yeast cells such as *Blastocystis hominis*, may be confused with cysts of amebae. The presence of budding forms and their particular structure should cause no difficulty in differentiating them.

MICROSCOPIC EXAMINATIONS FOR THE OVA OF HELMINTHS

General Method. The laboratory diagnosis of the majority of the helminths is accomplished by finding ova or embryos in the feces. The finding of even one typical ovum is sufficient to establish a diagnosis (Figs. 85 and 86).

Direct Smear Method. 1. Place a drop of tap water on a clean microscopic slide.

2. Take a small portion of feces from the specimen and thoroughly emulsify in a drop of tap water.

3. Cover with a cover glass and press gently to produce a thin preparation.

4. Treat a second portion in a similar manner, substituting a drop of iodine solution for the tap water. The iodine-stained preparation is used to avoid the missing of cysts of intestinal protozoa, such as amebae, in the routine examination for ova.

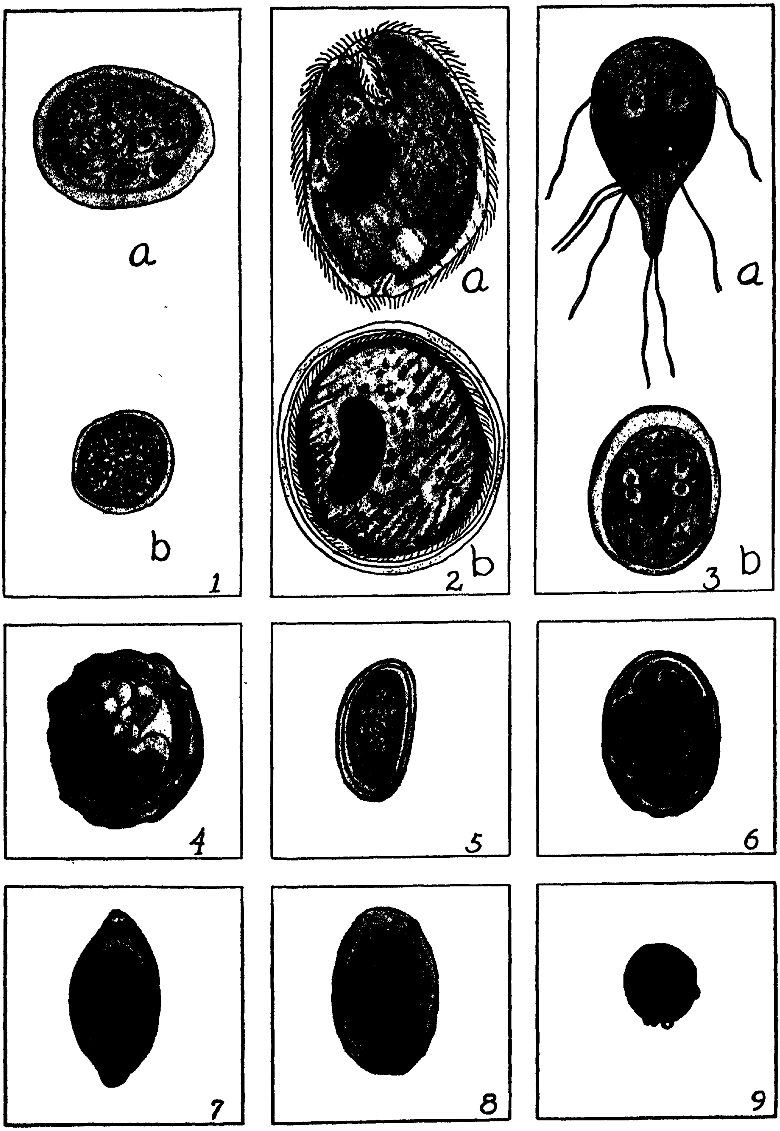

FIG. 85. TROPHOZOITES, CYSTS AND OVA OF ANIMAL PARASITES.

1, trophozoite (a) and cyst (b) *E. histolytica;* 2, trophozoite (a) and cyst (b) of *Balantidium coli;* 3, trophozoite (a) and cyst (b) of *Giardia lamblia;* 4, ovum of *Ascaris lumbricoides;* 5, ovum of *Oxyuris vermicularis* (*Enterobius vermicularis*); 6, ovum of human hookworm; 7, ovum of *Trichiuris trichiura* (*Trichocephalus trichiuris*); 8, ovum of *Strongyloides stercoralis;* 9, ovum of *Taenia saginata.*

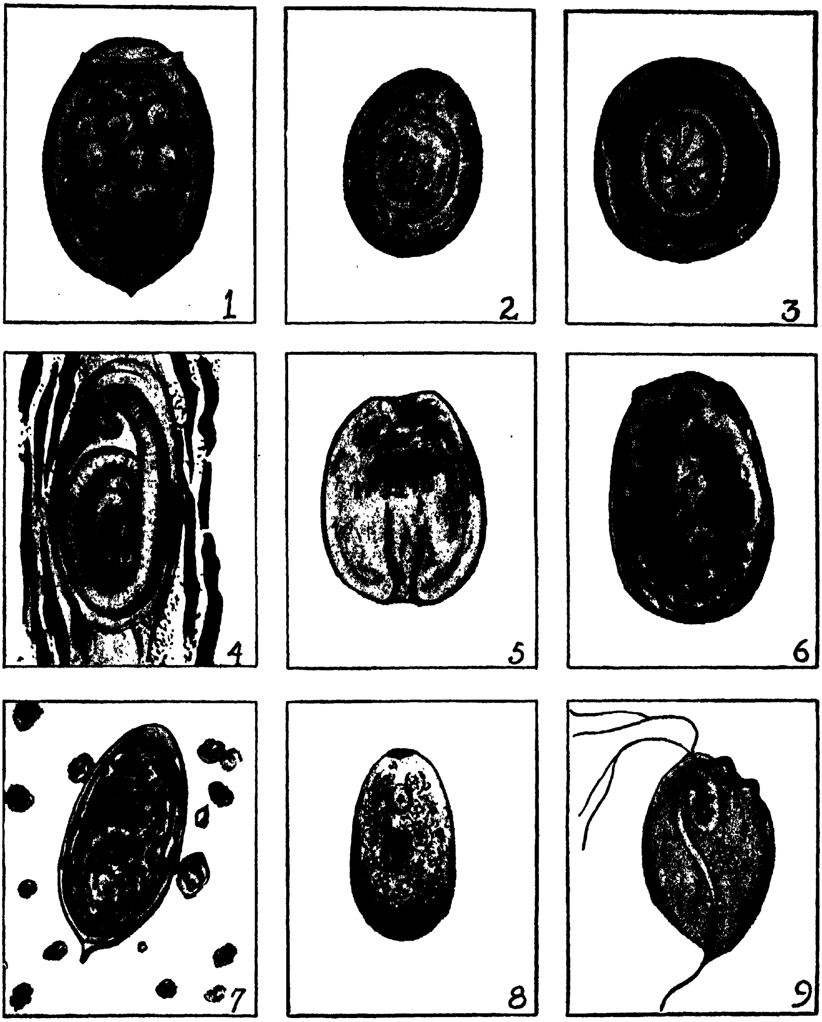

FIG. 86. OVA OF ANIMAL PARASITES.

1, ovum of *Diphyllobothrium latum;* 2, ovum of *Hymenolepis nana;* 3, ovum of *Hymenolepis diminuta;* 4, encysted larvum of *Trichinella spiralis* in muscle; 5, a scolex of *Echinococcus granulosis* from a liver abscess; 6, ovum of *Paragonimus westermani* in sputum; 7, ovum of *Schistosoma haematobium* in urine; 8, ovum of *Fasciolopsis buski;* 9, *Trichomonas vaginalis.*

5. Examine under microscope, using low-power ocular and objective.

6. If ova are not found, a concentration method of examination should be employed as follows:

Shearer's Concentration Method (Benbrook Modification). 1. Place about 1 or 2 gm. of feces in a small amount of water to produce a suspension.

2. Coarse particles may be removed if necessary by straining.

3. Fill a test tube or centrifuge tube nearly half full of the fecal mixture or suspension.

4. Add to the above an equal quantity of sugar solution prepared by dissolving 1 pound of sugar in 12 ounces of water. Dissolve the sugar in the water by immersing the bottle in hot water. Add phenol as a preservative to a concentration of 1 per cent.

5. Mix by slowly inverting the tube several times.

6. Centrifuge the tube containing the mixture for about three minutes at moderate speed, 1500 to 2000 r.p.m. Centrifuging may be omitted if the tube is allowed to stand twelve to twenty-four hours.

7. Remove the tube from the centrifuge to a test tube holder without shaking.

8. Lift off the surface layer of fluid (which now contains the ova) from the tube by means of a headed glass rod prepared as follows: Heat one end of a six-inch length of 5 mm. glass rod until it is soft enough to be "headed" against a cold metal object. The head portion should be just slightly less in diameter than the inside of the tubes used. A heavy glass rod, slightly smaller than the inside of the tube, may be used in place of the headed rod. The rod should be slowly lowered into the tube and the instant full contact is made with the liquid, withdraw the rod quickly, bringing with it a large drop.

9. Transfer the drop from the rod to a microscope slide by gently rotating the rod in the center of the slide. A second or third drop may be added to the first to obtain sufficient material to fill in under a micro cover slide.

10. Carefully lower a cover glass on the drop without pressure.

11. Examine the slide under the low power of the microscope. For best results, bright illumination should be obtained by adjusting the mirror and condenser and then modified by closing the diaphragm opening. The microscope should be vertical, not inclined, and the cover glass area should be searched in a systematic manner. A mechanical stage is recommended.

Brine Flotation Method of Kofoid and Barber. 1. A large fecal sample is thoroughly mixed with about twice its volume of saturated solution of table salt in a paraffined pasteboard cup or small beaker.

2. A lightly compressed circular disk of No. 1 or No. 0 steel wool about one-eighth to one-quarter inch thick is then placed in the cup and pushed to the bottom. This carries down all coarse particles.

3. The fluid is allowed to stand for one hour, during which time the ova rise to the surface.

4. Finally, the surface film is looped off with a wire loop about one-half inch in diameter, placed on a slide, and examined without a cover glass. The objective should be focused on the surface of the fluid.

Test	Neutral Fats	Fatty Acids	Soaps
Microscopic (No. 1)	Round or irregular globules; highly refractile or minute needles.	Sheaves of large needles or short, delicate, curved needles which occur in such thick masses that the shape of the individual crystals can seldom be determined.	Needles arranged in clusters or fans or in short plump crystals or scales. In amorphous form as gnarled bodies everted like the pinna of the ear. Soap crystals are comparatively coarse, as a rule in thick, short needles or flakes, but are distinguishable from those of fatty acids.
Microscopic (No. 2) (Sudan III)	Stained.	Light orange crystals not stained.	
Microscopic (No. 3) (Scharlach R)	Stained.	Crystals not stained; globules stained.	Not stained.
Ether Solubility	Dissolved.	Dissolved.	Not dissolved.
Water Solubility	Not dissolved.	Not dissolved.	Sodium and potassium soaps dissolved. Calcium and magnesium soaps not dissolved.
Heat Solubility	Melted.	Melted.	Not melted.

BENZIDINE TEST FOR OCCULT BLOOD

1. In order to avoid erroneous results, the patient should be on a meat-free diet for not less than 72 hours before the collection of the specimen. This properly includes abstinence from fish as well as from broths and soups made from meat stock.

2. Since occult blood in feces may be unevenly distributed, it is advisable to mix the specimen thoroughly before conducting the test.

3. Prepare the reagent freshly as required by adding the amount of benzidine (labelled "For blood tests") picked up on the point of a knife blade to 5 cc. of glacial acetic acid and warm gently to effect solution.

4. Prepare a thin suspension of feces in about 5 cc. of water. Shake with 5 cc. of ether to remove fat. Discard the ether extract. Acidify the residue with acetic acid and again extract with 5 cc. of ether. Evaporate the ether extract to dryness, using a water bath which has been heated to boiling and the flame turned off. Add 1 cc. of water, stir to dissolve the residue, then add a few drops of benzidine reagent and a few drops of hydrogen peroxide solution. A green to deep blue color indicates a positive reaction.

5. The test may also be conducted by smearing a little of the feces on a slide. Pour over it the reagent made by dissolving a knife-tip of benzidine dissolved in 2 cc. of glacial acetic acid, to which is then added 1 to 1.5 cc. of peroxide solution. In a positive reaction, the smear turns blue without any misleading green tints from the reagent.

QUALITATIVE TEST FOR UROBILIN (SCHMIDT)

1. Rub up a small amount of feces in a mortar with a saturated aqueous solution of mercuric chloride.

2. Transfer to a shallow white dish and let stand for 6 to 24 hours.

3. The presence of urobilin is indicated by a deep red color imparted to the particles of feces containing the pigment. If unaltered bilirubin is present, a green color is produced through its oxidation to biliverdin.

DETECTION OF FATS

Neutral fats, fatty acids and soaps are best detected and differentiated by quantitative chemical methods. Otherwise, useful data are sometimes obtained by rubbing up a small portion of feces on a slide with 36 per cent solution acetic acid, applying a cover glass and heating over a flame until the preparation shows bubbles (No. 1). Duplicate slides are prepared in the same manner to one of which Sudan III solution is allowed to flow under the cover glass (No. 2) and to the second a solution of Scharlach R (No. 3). Both stains are saturated solutions in equal parts of 70 per cent alcohol and acetone. The slides are examined microscopically. Additional tests may be conducted and the results interpreted as shown in the table on page 1055.

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SPINAL FLUID EXAMINATIONS

PHYSICAL EXAMINATIONS

A method for the *collection* of cerebrospinal fluid is described and illustrated in Chapter 14. It is generally advisable to determine the *pressure* before and after the collection of fluid for examination (page 314). The *color* should be recorded, (page 323) as likewise its appearance, from the standpoint of *transparency*. Normally, the spinal fluid is colorless and perfectly clear (resembling distilled water) if no blood has gained access to it during collection. Pathologic fluids may also be perfectly clear as, for example, in tuberculous meningitis and syphilis. In the absence of accidental puncture of a vein during collection any departure from perfect clearness is abnormal; alterations in transparency may be recorded as faintly opalescent, distinctly opalescent, faintly turbid, markedly turbid or purulent.

Normal spinal fluid does not form *coagula* or sediments. Under pathologic conditions, coagula may form on standing and may be recorded as numerous and small, "cobweb" or "pine tree" (as in tuberculous meningitis), or as heavy and sunken, as in the acute suppurative meningitides.

METHOD FOR COUNTING TOTAL CELLS

Whenever possible the total cell count should be made immediately after the collection of fluid while the cells are in suspension and before coagula have formed. If there is no excess of fibrin, so that coagulation does not occur, counts made some hours later, or next day, are fairly accurate, provided the fluid is shaken well to secure an even resuspension of cells. Spinal fluids containing visible amounts of blood are unfit for total cell counts because of the presence of leukocytes resulting in counts that are too high. Traces of blood too small for naked eye detection also increase the count very slightly but probably not to the point where the error seriously interferes with diagnosis.

1. The Levy counting chamber with the *Fuchs-Rosenthal ruling* is recommended. When the cover glass is on, it has a depth of 0.2 mm. with a capacity of a trifle more than 3 c.mm.

2. Draw diluting fluid to the mark 1 in a leukocyte-counting pipet; draw spinal fluid to the mark 11. The diluting fluid is prepared as follows:

Crystal violet	0.2 gm.
Glacial acetic acid	10.0 cc.
Water (distilled)	90.0 cc.

Filter. Should be crystal clear and free of artefacts.

3. Shake well, as in leukocyte counting, and discard 2 or 3 drops.

4. Fill the chamber, as in leukocyte counting, and wait five minutes for the cells to settle.

5. Count all of the cells (erythrocytes are hemolyzed) in the entire ruled-off area and divide by 3.5 to give the number of cells for each cubic millimeter of spinal fluid. The error incident to this calculation is practically balanced by the opposite error due to dilution.

6. If the Fuchs-Rosenthal chamber is not available, the ordinary leukocyte counting chamber may be used. In this case count the cells in the entire ruled-off area (9 large squares, or 0.9 c.mm.); divide by 8 and multiply by 10. This calculation compensates for the dilution factor and gives the total cells per cubic millimeter of fluid.

METHOD FOR DIFFERENTIAL CELL COUNTING (CYTODIAGNOSIS)

1. Centrifuge fresh specimen of fluid. Pour off supernatant fluid and make thin smears of sediment on slides; or tease out coagula on slides. Dry in air.

2. Stain with Wright's stain in the same manner as blood smears.

3. Count and tabulate cells (lymphocytes, polymorphonuclears and endothelial cells) and determine the number or percentage of each variety per 100 cells.

4. Normally, only lymphocytes and occasional endothelial cells are found. In acute suppurative meningitis due to the pneumococcus, meningococcus, streptococcus, etc., polymorphonuclear cells predominate in the acute stage. In tuberculous meningitis, small lymphocytes predominate (usually). In acute anterior poliomyelitis, polymorphonuclears early; later small lymphocytes (usually). In syphilis (paresis, tabes, etc.), small lymphocytes predominate. In meningisms (serous meningitis or acute meningeal congestion), endothelial cells predominate.

QUALITATIVE TESTS FOR PROTEIN

A large number of tests have been devised for the detection of an increase of protein in spinal fluid. Most of these have been for the detection of the globulins, but practically all react to some extent to serum albumin. Some were originally considered specific for syphilis of the central nervous system but none are pathognomonic for syphilis or any other disease; they merely detect an increase of protein (mostly globulins) which is always pathologic unless the cerebrospinal fluid contains sufficient blood to yield positive reactions. None of these tests, therefore, are applicable to cerebrospinal fluids containing macroscopic amounts of blood. Heavy bacterial contamination may likewise yield falsely positive reactions.

Pandy's Test. 1. Prepare the *reagent* by placing 10 cc. of pure phenol in a bottle (melt crystals by standing bottle in hot water) and adding distilled water to 100 cc. Shake vigorously and place in incubator for several days. Carefully pipet off supernatant fluid (6.3 per cent phenol) or use it direct from the bottle without disturbing the layer of undissolved phenol.

2. Place about 1 cc. of the reagent in a small test tube. Add 1 drop of spinal fluid.

3. If there is an increased amount of protein, a bluish white ring or cloud is formed immediately. By this test normal spinal fluids often show a very faint trace of globulin which should not be mistaken for a positive reaction. A 5 per cent solution of phenol is stated by Binkley and Johnson to give fewer falsely positive reactions.

Ross-Jones' Test. 1. Prepare the *reagent* by placing 85 gm. of Merck's purified neutral ammonium sulfate and 100 cc. of distilled water in an Erlenmeyer flask; heat to boiling until all of the salt is dissolved; cool slowly and filter.

2. Place 2 cc. of the reagent in a small clean test tube; carefully overlay with 1 cc. of the fluid to be tested which should be centrifuged if not perfectly clear.

3. The appearance of a clear-cut, thin, grayish white ring at the point of contact—within a few seconds—indicates a positive reaction. Normal fluid may develop a ring after 5 minutes.

Tryptophane Test for Tuberculous Meningitis. This simple test is said to be of aid in the diagnosis of tuberculous meningitis.

1. Place 2 or 3 cc. of spinal fluid in a large test tube.

2. Add 15 to 18 cc. of concentrated hydrochloric acid and 2 or 3 drops of 2 per cent formalin (1 cc. of formalin and 19 cc. distilled water).

3. Shake the tube and let stand for 5 minutes.

4. Carefully and slowly overlay with 2 cc. of 0.5 per cent solution of sodium nitrite.

5. Allow to stand for 2 or 3 minutes.

6. In tuberculous meningitis a violet ring develops at the juncture of the first and second layers (positive reaction). A negative reaction is shown by a brown ring or the absence of any colored ring.

Levinson Test for Tuberculous Meningitis. This is based on the principle that a characteristic ratio may be obtained between the alkaloidal precipitate formed by sulfosalicylic acid and the metallic precipitate formed by mercuric chloride in tuberculous meningitis. While positive reactions are indicative of this infection, they are not specific.

1. Into each of two small test tubes of uniform length and width, place 1 cc. of cerebrospinal fluid.

2. To one add 1 cc. of a 3 per cent solution of sulfosalicylic acid (CP) in water, and to the second, 1 cc. of a 1 per cent solution of mercuric chloride (CP) in water.

3. Shake the tubes, stopper and let stand at room temperature for 24 hours; measure the height of the precipitates in millimeters.

4. Under *normal* conditions the sediment in both tubes is very slight. In all *suppurative meningitides,* the height of the sediment in the sulfosalicylic acid tube is very heavy, often being three times the size of the sediment occurring with mercuric chloride. In *tuberculous meningitis* (rarely in other conditions) the opposite occurs, the precipitation with mercuric chloride being usually three times as high as that obtained with sulfosalicylic acid.

The two precipitates are of a different character: that of the acid is heavy and compact and starts to form immediately, while that of the chloride is light, feathery, and forms slowly. Sometimes the precipitate does not come down into a compact sediment, as small floccules may adhere to the walls of the test tube. Under these conditions it is advisable to shake the tubes gently 2 or 3 hours before making the final readings. For diagnostic purposes it is not the amount of protein thrown down in the two precipitates, but the relative height of the sediments in millimeters in the two tubes.

If no precipitate forms, use a 2 per cent mercuric chloride solution and a 6 per cent sulfosalicylic acid solution.

TESTS FOR GLUCOSE

Qualitative Test. Since blood glucose will give positive reactions, spinal fluids containing macroscopic amounts of blood are unfit for testing.

1. In a test tube place 0.5 cc. of Benedict's *qualitative* reagent and add 1 cc. of cerebrospinal fluid.

2. Boil for one or two minutes and allow to cool.

3. A change of color to turbid greenish-yellow is a normal reaction for the normal sugar of spinal fluid. No color change shows an absence of sugar and is pathologic. See page 910 for determinations in relation to the treatment of influenzal meningitis.

4. If the fluid contains an increase of protein and an absence of sugar, the color of the reagent may be changed to a deep purplish-violet or pinkish-violet (the biuret reaction with copper).

Quantitative Test (Lyttle and Hearn). The reagents required are the same as those used in the Folin-Wu method for the precipitation of blood proteins and determination of blood sugar, described in Chapter 35.

1. Four volumes of spinal fluid are added to 14 volumes of distilled water and to this mixture 1 volume of 10 per cent sodium tungstate is added, followed by 1 volume of 0.66 N sulfuric acid. Shake and allow to stand for 10 minutes, then filter. The glucose determination is carried out on 2 cc. of this filtrate in exactly the same manner as in determining blood glucose (see page 1017).

2. Where the lower standard has been used, the reading of the standard, usually 20 mm., multiplied by 50 and divided by the reading of the unknown, equals milligrams of glucose per 100 cc. of spinal fluid. When the higher standard is used, substitute 100 for the 50 above.

COLLOIDAL GOLD TEST

In preparing the *reagent* the glassware should be chemically clean and should be rinsed inside and out with double-distilled water before using. Pyrex glass is not essential except in the flask used for boiling. Quicker heating without wire gauze may thus be attained and is important. It is also better to store the completed reagent in pyrex but not absolutely necessary. The reagent may be prepared by the following method:

1. Prepare a 1 per cent solution of gold chloride as follows: Place an ampule

of Merck's gold chloride (15 grains) in warm water and remove the label and all paste. Rinse thoroughly in double-distilled water before breaking. Take care that none of the chloride is lost in breaking. Place the halves of the ampule, with their contents, in beaker containing 97.2 cc. of double-distilled water and stir thoroughly to insure complete and even solution of the chloride.

2. Prepare a 1 per cent solution of sodium citrate, CP, in double-distilled water by dissolving 1 gm. (accurately weighed) in 100 cc.

3. Pipet 10 cc. of the gold chloride solution into 950 cc. of double-distilled water in a 2000 cc. Erlenmeyer flask.

4. Heat, as rapidly as possible, to between 90° and 95° C. (*no higher and no lower*).

5. Remove thermometer from flask as soon as it records 92° C.

6. Without removing from the flame, add 50 cc. of the citrate solution.

7. As soon as the solution comes to a boil again add quickly 0.77 cc. of hydrogen peroxide (or 10 drops from a standard 1 cc. pipet). When drawing up the peroxide into the pipet, take care to exclude the many small bubbles that form in a freshly opened bottle. The hydrogen peroxide should be CP (10 volumes, 3 per cent). It should be a freshly opened bottle. *Never* use one that has been standing, uncorked, at room temperature for any length of time.

8. Remove from flame, cool, and store in a dark place at room temperature. Cork tightly with a tinfoil-covered stopper.

9. *Do not shake the flask during procedure.*

10. When the solution begins to re-heat after the addition of the citrate, a slight bluish color begins to appear which gradually changes to the standard color during 3 minutes of boiling. No further change takes place thereafter. The addition of the peroxide, with its immediate color change, gives the required sensitivity. The solution must not be boiled after the peroxide is added. Titration is not necessary if the final color change (brownish-yellow shade of burgundy red with no bluish tinge) is right.

The *test* is conducted as follows: 1. Place 10 chemically clean test tubes in a rack.

2. In the first tube place 0.9 cc. of a 0.4 per cent sodium chloride (CP) solution in double-distilled water and in each of the remaining 9 tubes place 0.5 cc. amounts of the saline solution.

3. To the first tube add 0.1 cc. of the spinal fluid and mix thoroughly.

4. Remove 0.5 cc. of the mixture from tube No. 1 and place in the second tube, thoroughly mixing as before.

5. Remove 0.5 cc. from tube No. 2 and carry to the third; continue this transfer until the tenth tube is reached; from the latter discard 0.5 cc. after mixing.

6. Add to each tube 2.5 cc. of the gold chloride reagent.

7. Mix thoroughly by rotating of the tubes and set aside at room temperature for 24 hours. Read results.

8. It is always advisable to include a control tube carrying 0.5 cc. of the saline solution and 2.5 cc. of the reagent. It should show no color change. If a color change occurs, the reagent is too sensitive and should be discarded. It is

also advisable to include a test conducted with paretic spinal fluid as a positive control. It should show a Zone I curve of precipitation. If this does not occur, the reagent is unsuitable.

9. The readings depend upon any change of color which takes place in the tubes. For convenience, these color changes are recorded and reported by number (see page 337), thus:

0—no color change
1—slight violet tinge
2—bluish red
3—blue
4—light, transparent pink or blue (almo decolorized)
5—complete decolorization

10. The recordings may be made in the form of curves (Zone I or paretic [Fig. 20, facing page 336], Zone II or luetic, Zone III or meningitic), as shown in Figure 21 on page 337.

COLLOIDAL MASTIC TEST (CUTTING)

This test depends on the precipitation of mastic in colloidal suspension as determined by a clarification of the reagent and the production of precipitates. It is highly probable that in spinal fluid the substance producing the reaction is the same as that producing the colloidal gold reaction, although its nature is unknown. The reagent is much simpler and easier to prepare, although the reactions are less sensitive than the colloidal gold reaction. The reaction, while less sensitive, is also less subject to technical errors.

The *reagent* is prepared as follows: Dissolve 10 gm. gum mastic (USP) in 100 cc. of absolute ethyl alcohol. For use, dilute 2 cc. with 18 cc. of absolute ethyl alcohol, mix thoroughly, and pour rapidly into 80 cc. of double-distilled water.

The *test* is conducted as follows: 1. Dissolve 1.25 gm. (accurately weighed) sodium chloride (CP) in 100 cc. of double-distilled water. Remove 1 cc. with a pipet and add 1 cc. of a 0.5 per cent solution of potassium carbonate in double-distilled water (alkaline-saline solution).

2. Arrange six small test tubes in a rack.

3. Place 1.5 cc. of alkaline-saline solution in the first tube and 1 cc. in each of the remaining five tubes.

4. Add 0.5 cc. of spinal fluid to the first tube, mix thoroughly and transfer 1 cc. to the second tube.

5. Transfer 1 cc. from the second tube to the third, and so on until the fifth tube, from which 1 cc. is discarded. The sixth tube is used as a control.

6. To each tube add 1 cc. of mastic reagent, mix well and allow to remain at room temperature for twelve to twenty-four hours; or in the incubator for six to twelve hours.

7. A positive reaction is indicated by the formation of a heavy precipitate which settles, leaving the supernatant fluid clear.

8. A negative reaction shows no precipitation in any tube (00000).

This test, devised by Guillain, Laroche and Lechelle, is similar in many respects to the mastic test. It is not specific for neurosyphilis but gives practically the same results as the more complicated colloidal gold test.

The *reagent* is prepared as follows: Dissolve 1 gm. sumatra benzoin resin in 10 cc. absolute ethyl alcohol. Let stand 48 hours and filter. Keep the filtrate in a tightly stoppered bottle. This is a stock solution from which the colloidal benzoin reagent is freshly prepared for conducting the test as follows: Add 0.3 cc. of the stock benzoin solution, drop by drop, shaking constantly, to 20 cc. of double-distilled water. Heat to 35° C. in a water bath, stirring constantly.

The *test* is conducted as follows: 1. Place 16 small test tubes in a rack.

2. In the first tube place 0.25 cc. of 0.01 per cent solution of sodium chloride (CP) in double-distilled water. Place 0.5 cc. in the second tube, 1.5 cc. in the third, and 1 cc. in each of the remaining tubes.

3. Next add cerebrospinal fluid: 0.75 cc. to the first tube; 0.5 cc. to the second and third tubes. From the third tube transfer 1 cc. of the thoroughly mixed dilution of spinal fluid to the fourth tube, and so on, until the fifteenth tube is reached from which, after mixing, discard 1 cc. The sixteenth tube is used for control. The dilutions thus range from 3:4 in the first tube to 1:16,384 in the fifteenth tube.

4. Finally, add 1 cc. of the benzoin reagent to each tube and mix by shaking. Allow the tubes to stand for from eighteen to twenty-four hours.

5. The reaction will vary from no change in the mixture to complete precipitation of the benzoin, with absolute clearing of the supernatant fluid. The degree of reaction in each tube is reported: 0, no precipitation; 1, slight precipitation, with partial clearing; 2, more than half precipitated, fluid still cloudy; 3, complete precipitation, water-clear fluid. A curve may be plotted, or the figures representing the degree of reaction may be set down for each tube. Precipitation in the first six tubes indicates cerebral involvement (Zone I or paretic curve); precipitation beginning with the seventh tube indicates involvement of the meninges, or spinal cord (Zone II curve). The test is not as sensitive as in the colloidal gold test, and is not as definite in its reaction in multiple sclerosis.

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BACTERIOLOGIC EXAMINATIONS

The general principles and collection of materials for bacteriologic examinations have been considered in Chapter 15. Emphasis has been placed upon the importance of proper methods for preparing smears and cultures (including the use of proper culture media) because, by errors in these particulars, the results of bacteriologic examinations are sometimes rendered practically worthless.

Needless to state, many bacteriologic examinations require special skill and experience and the use of special methods for the isolation and identification of micro-organisms. The methods described herein are the simpler procedures adapted for the use of practicing physicians and dentists in their own laboratories. Relatively simple and inexpensive equipment suffices for their conduct. Appropriate culture media may be readily purchased from laboratories. For those physicians and dentists who prefer making their own, the Difco media, marketed by the Difco Laboratories, Detroit, Michigan, can be highly recommended.

PREPARATION OF SMEARS

1. The examination of stained smears of pus, sputum and other exudates is usually of value in bacteriologic examinations and diagnosis; in some instances it constitutes the chief means of diagnosis as in gonorrhea, Vincent's angina, fusospirochetal gingivitis, acute contagious conjunctivitis, tuberculosis, leprosy, etc.

2. Slides are preferred to cover glasses as they are less breakable, more easily handled, and readily filed. They should be clean and not too badly scratched.

3. Smears may be prepared with sterile cotton swabs or with flamed stiff wire loops. At least two should be prepared on the same or separate slides.

4. It is important to have smears neither too thick nor too thin. They need not be larger than 1 cm. in diameter if the material is scanty.

5. *Vigorous rubbing should be avoided,* as the cells may be broken up making intracellular examinations difficult or impossible. This is especially important in examinations for gonococci and meningococci, or when making a differential count of cells for cytodiagnosis. *The swab should be rolled on the slide* and should not cover the same area twice.

6. Cerebrospinal fluid and other transudates and exudates poor in cells may be first centrifuged and smears prepared of the sediment.

7. In preparing smears of cultures, place a loopful of water on a slide; with a sterile wire transfer a small amount of culture to give an opalescent suspension; spread into a thin layer. The water should be essentially sterile, preferably distilled, and not more than 3 days old.

8. Allow smears to dry in the air or with the aid of gentle heating. A slide may be dried by holding it *with the fingers* above a Bunsen flame, since a degree of heat bearable by the fingers will not "cook" or harm the smear.

9. Do not use the filthy and dangerous method of covering a thick wet smear of pus with another slide.

PREPARATION OF CULTURES

1. It is extremely important and essential to use a culture medium adapted to the growth of the suspected micro-organism. This is particularly true of those requiring special growth factors. In general terms, tubes of glucose hormone broth, slants of Loeffler's serum medium, and slants or Petri plates of blood agar are suitable for most routine purposes.

2. Whenever possible it is advisable to secure material and make cultures with sterile cotton swabs prepared of wooden applicators. Sterile wires may not secure sufficient material. In inoculating a tube of broth the swab end of the applicator, which has not been contaminated by the hands, may be broken off in the tube. If a solid medium is employed it is important not to break the surface. It is essential that solid media contain moisture.

3. In transferring bacteria from a culture to a tube or plate of medium, precautions must be taken to prevent contamination by outside bacteria. Since the time of Koch, the wire needle or loop has been generally used. These needles are made of platinum or nickel-chromium steel (nichrome, or stainless steel) about 0.025 inch in thickness. Steel is better and also less expensive. The wire needles are sterilized in the flame by heating to red heat. The lower part of the handle should also be sterilized.

4. The tubes should be held almost parallel with the table top to avoid air contamination. Remove the plugs (do not flame) and hold them between the third and fourth fingers of the right hand; now flame the ends of both tubes (but not too long, as cracking may occur); transfer the material; reflame the ends of the tubes and replace the stoppers. When making smears replace the plugs before spreading the material on the slide. It is not necessary to flame the stoppers before replacing them. If they are flamed, however, be sure to hold the test tube end of the plug low down in the flame to prevent the loose cotton held by the fingers from catching fire. Be sure that the plugs are inserted so deeply that they will not become loosened. Label properly, preferably with gummed labels, as pencil markings may be rubbed off.

5. When a large amount of fluid material is to be transferred, use a sterile pipet with a cotton plug. When the material is very infectious, attach a piece of rubber tubing to the pipet with mouthpiece or use a Pasteur pipet fitted with a rubber bulb. As soon as the culture has been made, place the pipet in a jar containing a disinfectant. If material is accidentally taken into the mouth, rinse thoroughly with water, then 40 to 50 per cent alcohol, and again with water.

6. If a Petri plate is to be inoculated, raise the cover at one side just high enough to admit the wire or swab, keeping the plate as completely covered as possible to prevent contamination from the air.

BLOOD CULTURES

1. See page 342 for a discussion of the general principles of blood cultures. As a general rule, the inoculation of 100 to 200 cc. of glucose hormone broth in a flask with 5 to 15 cc. of blood is satisfactory.

2. Boil a 25 cc. all-glass syringe and gauge No. 18 or 20 needle for at least 3 to 5 minutes for sterilization.

3. With alcohol, thoroughly cleanse the skin over a prominent vein and the surrounding area.

4. Paint with tincture of iodine and leave on for 2 or 3 minutes.

5. Be sure not to contaminate the inside of barrel or shaft of plunger when assembling the syringe. Touch only the hub of the needle in attaching it to the syringe.

6. Apply tourniquet above the elbow, not too tightly. If the vein does not distend well, have patient open and close the hand vigorously several times and then keep the fist clenched.

7. Sponge off the iodine with alcohol.

8. Puncture the skin with needle *a little to one side of the vein* and parallel to it; then enter the vein from that side, about half an inch above the skin puncture.

9. Withdraw the desired amount of blood, loosen the tourniquet and have patient open fist.

10. Withdraw needle quickly, then press an alcohol-soaked pledget firmly over the puncture.

11. Open flask and flame mouth thoroughly with alcohol lamp, holding the syringe near but not in the flame at the same time.

12. Insert needle into flask and force blood directly into culture medium. Withdraw needle from flask without touching sides of flask with either needle or blood.

13. Flame neck of flask again, replug, and incubate aerobically at 37° C. Apply dressing to the arm.

14. In some instances it is advisable to withdraw 20 cc. of blood with the inoculation of two flasks of medium (10 cc. each), one of which should be incubated anaerobically.

EXAMINATION OF CULTURES

1. For smears of culture on solid media, place a small loop of water on the clean slide. With the needle add a minute amount of growth to the water. Mix, spread and dry in the air. From fluid media spread small loop of culture onto the slide; no water is needed. When the smear is perfectly dry "fix" it by passing the slide back and forth through the flame three times (do not overheat) and allow it to cool before staining.

2. In the case of Petri dishes, remove the cover and place it right side up on the table.

3. Examine the plate with unaided eye or hand lens and ring off selected colonies with wax pencil on bottom of plate.

4. Or, select colonies with the aid of the lower part of the microscope.

5. With a sterile needle, carefully remove portions of selected colonies to fresh media and prepare smears for staining.

6. In the case of broth cultures, including those of blood, open the tube or flask, flame the neck, remove one or more loopfuls to a slide, flame the neck and cotton stopper, and replace the latter with all precautions against contamination.

7. Stain the smears by the method of Gram.

STAINING METHODS

Stains are most conveniently kept in dropping bottles or bottles provided with a rubber stopper and nipple with a short dropping pipet attached. The staining is generally done by putting the stain on the slide but, for some purposes, a staining dish is employed. Only sufficient stain to cover the smear should be used in the interests of economy. It should not be spread with the tip of the bottle as contamination of the stain may result.

Loeffler's Alkaline Methylene Blue. 1. Prepare the stain by dissolving 0.4 gm. methylene blue in 30 cc. ethyl alcohol (95 per cent). To 70 cc. distilled water, add 0.07 cc. of a 10 per cent solution potassium hydroxide. Mix and add the alcoholic solution of methylene blue.

2. Make thin smear of material to be examined on slide.

3. Dry in air and fix with gentle heat.

4. Cover smear with stain and allow to stand for one or two minutes; heat slightly if deep staining is desired.

5. Wash with tap water, blot and examine with oil-immersion lens.

Methylene blue does not stain very intensely and there is little danger of overstaining. It is a good stain to use when studying the morphology of organisms and is generally used in the examination of cultures for diphtheria bacilli.

Gram's Stain. This is the most important of all bacteriologic stains.

1. Prepare the stain by dissolving 0.7 gm. crystal violet in 10 cc. ethyl alcohol (95 per cent). Add 40 cc. of 1 per cent aqueous solution of ammonium oxalate.

2. Prepare Gram's iodine solution by dissolving 1 gm. iodine and 2 gm. potassium iodide in 300 cc. distilled water.

3. Prepare a thin, even smear on a slide, dry in the air, and pass through flame for fixation.

4. Apply stain for 1 minute. Pour off excess stain but do not wash.

5. Apply the iodine solution for 1 minute. Wash in water.

6. Apply decolorizer (equal parts of 95 per cent ethyl alcohol and acetone) several times until no further traces of stain can be washed out of the preparation (½ to 2 minutes). Wash in water.

7. Counterstain with a 1:10 dilution of Ziehl-Neelsen's carbol-fuchsin for 30 seconds. Wash in water. Blot and dry with the aid of mild heat.

8. Gram-positive organisms are stained violet. Gram-negative organisms are stained pink or red. Gram-ambophile organisms give a variable result.

Acidfast Stain (Ziehl-Neelsen's Carbol-Fuchsin). 1. Prepare stain by dissolving 0.8 gm. basic fuchsin in 10 cc. ethyl alcohol (95 per cent). Add 90 cc. of a 5 per cent solution of phenol.

2. Prepare acid alcohol by diluting 3 cc. hydrochloric acid with 97 cc. ethyl alcohol (95 per cent).

3. Prepare smears of material on slides, air-dry, and fix by heat.

4. Apply carbol-fuchsin and heat gently until steam appears over the surface. Allow to steam for 5 minutes. Wash in water.

5. Decolorize with acid alcohol to a faint pink or until no more color is removed. Wash in water.

6. Counterstain with Loeffler's methylene blue for 30 seconds. Wash in water and dry with mild heating.

7. Acidfast organisms (tubercle and lepra bacilli) are stained red in a blue field; non-acidfast organisms are stained blue.

Carbol-Fuchsin (General Bacterial) Stain. Staining smears with a 1:10 dilution of carbol-fuchsin is excellent for general purposes.

1. Prepare a thin smear on a slide and dry in the air. Fix with heat.

2. Dilute 1 cc. of Ziehl-Neelsen's carbol-fuchsin with 9 cc. of water. Cover smear and stain for about 1 minute. Wash in water.

3. Blot, dry with mild heat and examine.

Spore Stain (Dorner's Method). 1. Prepare a thin smear and fix with heat.

2. Apply Ziehl-Neelsen's carbol-fuchsin and heat gently until steam appears over the surface. Allow to steam for 5 minutes. Wash in hot tap water.

3. Rinse rapidly with 95 per cent ethyl alcohol.

4. Apply Loeffler's alkaline methylene blue for 2 to 5 minutes. Wash with water, blot and dry with mild heat.

5. Spores are red and the cell body is blue.

Nigrosine Method for Spirochetes. 1. Prepare solution by adding 10 gm. nigrosine to 90 cc. distilled water; boil in flask for 30 minutes and add 0.5 cc. formalin (40 per cent). Filter twice through double filter paper. Store in small sealed test tubes.

2. On a slide, mix a loopful of material with a loopful of the nigrosine solution. Prepare a smear and allow to dry.

3. Spirochetes are not stained but are demonstrated negatively as unstained spirals on a smoky background.

India Ink Method for Spirochetes. Like the nigrosine method, a drop of material is mixed with a drop of India ink and the mixture spread on a slide. When dry, examine for white spirals against a dark background.

Neither of these methods, however, is as valuable or as reliable in the examination of lesions for *T. pallidum* as the darkfield method.

DARKFIELD EXAMINATIONS FOR SPIROCHETES

This method is particularly valuable in examinations for spirochetes, especially with reference to the detection of *T. pallidum* in chancres. It may also be used in the diagnosis of Vincent's angina, "trench mouth" or fusospirochetal gingivitis and pulmonary spirochetosis, although the spirochetes in these diseases, along with associated fusiform bacilli, are readily stained with dilute carbolfuchsin. In relapsing fever the method is also applicable to examinations for spirochetes in the blood although errors may occur due to artefacts of fibrin, etc. This is particularly true in darkfield examinations of the blood for *Lept. icterohaemorrhagiae.*

The illumination principle of this method is comparable to that which causes dust particles to be illuminated in a ray of sunlight. A cover glass preparation (ringed with vaseline) of material under examination is prepared, using thin slide and cover glass, and is placed on a specially prepared microscope, a darkfield condenser replaces the ordinary condenser, a "funnel stop" is placed in the oil-immersion objective, especially intense light is used, and immersion oil is placed between the slide and the condenser as well as on top of cover glass. All highly refractile objects, including spirochetes and bacteria, will be seen as bright objects against a black background.

DIAGNOSIS OF SYPHILIS

1. Lesions should be cleansed of surface crust, detritus, pus, and surface organisms by gauze or cotton applicator. If the lesion has received any local treatment with a germicidal agent, examination should be deferred until all germicide has been removed and a saline pack has been applied to the lesion for a day or two.

2. Secondary lesions are merely cleansed and abraded.

3. A fresh slide-cover glass preparation may be made from an accessible lesion by merely touching the slide to tissue juice and immediately placing the cover glass over this moist drop. Vaseline placed around the edge will prevent drying. If the patient is not accessible, the fluid may be collected in a capillary pipet (see Fig. 32 on page 534) and brought to the laboratory.

4. Examine immediately on darkfield microscope for characteristic morphology (Fig. 87) and motion of *T. pallidum.* The saprophytic *T. refringens* of the genitalia is easily distinguished. *T. microdentium* of the mouth, however, cannot be reliably differentiated from *T. pallidum.*

5. As previously stated, "artefact spirochetes" may result in errors in the case of those unfamiliar with the appearance of blood and pus under darkfield illumination. Wavy filamentous structures may occur which may be mistaken for spirochetes. Forms given off by erythrocytes may also falsely suggest spirochetes.

DIAGNOSIS OF GONORRHEA

1. Materials submitted for examination for the gonococcus (*N. gonorrhoeae*) are usually purulent secretions from the genital tract of both sexes, the vagina of children, and the conjunctivas (especially of infants).

2. Well-prepared smears stained by the method of Gram and carefully examined are still relied upon mainly for bacteriologic diagnosis. There is no specific differential stain for the gonococcus.

3. Typical gonococci are gram-negative. Duplicate smears stained with Loeffler's methylene blue are advisable for morphology but should not be relied upon alone for identification.

4. They are usually arranged in pairs, with adjacent sides flattened or slightly concave, resembling a pair of kidney beans. They are not encapsulated.

5. In the pre-acute stage, before the exudate becomes profuse, the organisms may be extracellular but become intracellular (Fig. 87) during the acute stage when the exudate is at its height. At this stage it is common to find many organisms gathered within one leukocyte while other cells in the immediate neighborhood have none. In gonococcal conjunctivitis the organisms may occur in or upon epithelial cells.

6. For the interpretation of stained smears see page 397. Observe whether diplococci are intra- or extracellular, or both. Also observe any other bacterial forms present, noting for each whether gram-negative or gram-positive and whether coccus or bacillus; also the relative numbers and kinds of tissue cells present.

7. The cultural demonstration of the gonococcus is superior to direct smear examinations in cases of chronic gonorrhea in both sexes and in all cases in the female, especially when material for examination is taken from the cervix. For cultures, specimens of representative material should be collected and directly inoculated on plates of moist chocolate agar with cultivation in 10 per cent carbon dioxide in a closed jar at 37° C. The gonococcus must be identified by colony form and the oxydase reaction.

DIAGNOSIS OF CHANCROID

1. The best method of bacteriologic examination consists in aspirating an unopened bubo and preparing smears and cultures of the pus. When buboes are not present, material may be scraped from the base of the ulcer or from beneath its overhanging edge with a stiff wire.

2. Stain the smears by the method of Gram.

3. The presence of extremely small gram-negative (*H. ducreyi*) with no capsules and no spores is usually sufficient for diagnosis. They have a tendency to occur in short chains and in parallel rows. In smears of pus they are often intracellular. They stain more deeply at the poles.

4. The bacillus is difficult to cultivate; the method of Teague and Deibert is recommended: (a) Bleed a rabbit aseptically from the heart and distribute 1 cc. amounts in small sterile test tubes. Allow to clot, heat for 5 minutes at 55° C. and keep in refrigerator; (b) with sterile stiff iron wire, bent at one end, secure pus by gently rubbing over the base of the ulcer or under its undermined edge and then pick up a bit of pus from the dressing; (c) transfer to a tube of the clotted blood and distribute in the serum; (d) incubate at 37° C. for 24 hours; (e) stir the serum with a platinum loop and prepare a smear; stain by the method of Gram.

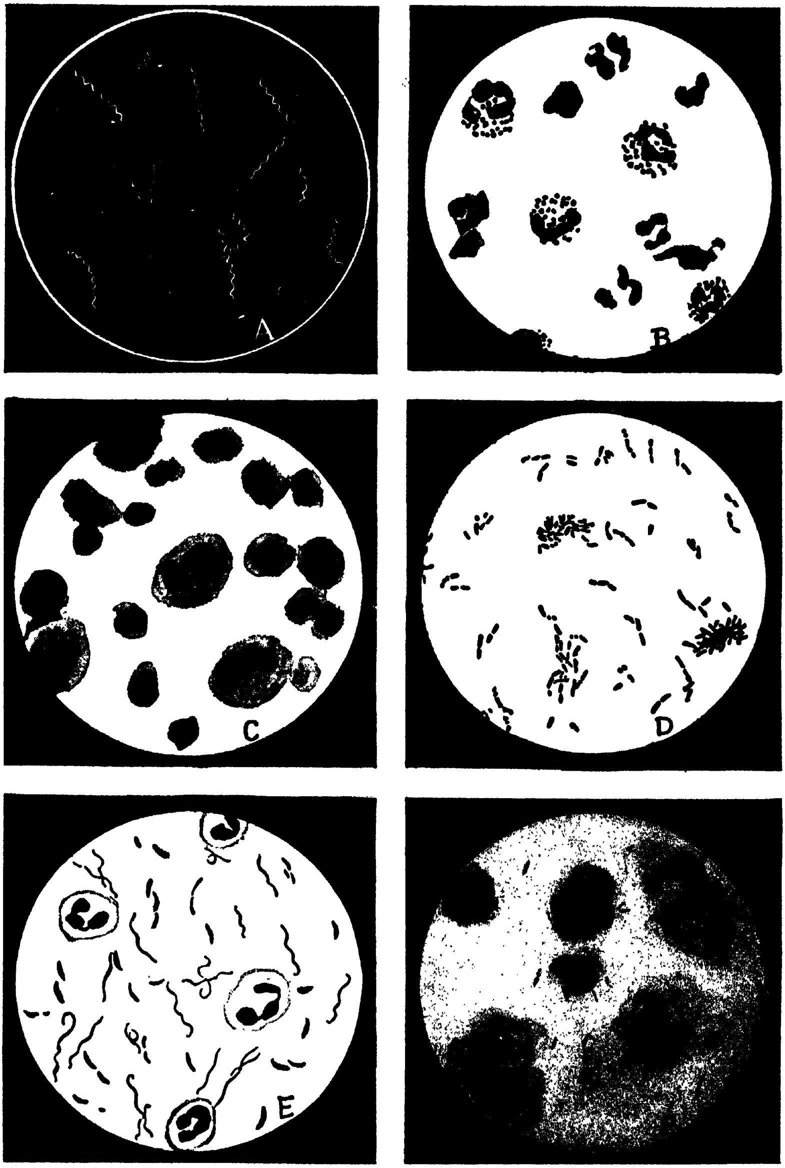

FIG. 87. BACTERIOLOGIC EXAMINATIONS.

A, *T. pallidum* (darkfield examination of a chancre); *B*, Gonococci in urethral pus; *C*, "Donovan Bodies" in pus in granuloma inguinale; *D*, *C. diphtheriae* in smear of throat culture; *E*, *B. fusiformis* and *Bor. vincentii* in Plaut-Vincent angina and fusospirochetal gingivitis; *F*, Tubercle bacilli in sputum.

DIAGNOSIS OF GRANULOMA INGUINALE

1. Carefully cleanse a granulomatous area near the periphery of the lesion with saline solution to remove the pus and then scrape the surface with the edge of a scalpel to obtain tissue cells. Blood and pus should be avoided as much as possible. As the procedure may be painful, it may be necessary to anesthetize the surface with a few drops of procaine or cocaine solution. The scrapings should be deep enough to include many tissue cells, as superficial scrapings are usually unsatisfactory.

2. Place the tissue material between two microscopic slides. While one is held stationary, move the other circulatory or laterally with firm pressure in order to deposit the cells evenly on the surface of the slides. Fibrotic tissue sometimes obtained from a cicatrical type of lesion is not satisfactory for preparing the spreads; such tissue is better examined by preparing sections for histologic study.

3. Stain the smears with Wright's or Giemsa stains and examine the cells for the intracellular bodies. The organisms, *Donovani granulomatis* or "Donovan bodies," which occur in cells are coccoid, diplococcoid with or without a closed safetypin appearance, straight or slightly curved rods with either homogenous or metachromatic staining (Fig. 87); they may appear noncapsulated, with or without a clear, nonstaining halo, or they may be encapsulated.

DIAGNOSIS OF DIPHTHERIA

1. The importance of properly prepared smears and cultures is discussed on page 341.

2. An immediate presumptive diagnosis can sometimes be made on the basis of the morphology and staining features of what few diphtheria bacilli may be observed in a direct smear, but here they will be confusedly mixed with many other micro-organisms of mouth or wound flora. Smears should be stained with Loeffler's alkaline methylene blue. Negative results, however, have no value. Presumptive positive results should be confirmed by cultures.

3. A rapid method consists of applying a sterile serum-swab to the involved area, returning it to the serum tube, and incubating for a few hours at 35 to 37° C. Transfer to other media and examine a smear stained with Loeffler's alkaline methylene blue, prepared by gently rolling the swab out into a thin film. Sterile serum swabs are prepared by placing sterile swabs into sterile tubes containing a few cubic centimeters of sterile serum. Some such swabs are made to contain 2 per cent potassium tellurite to attain blackening from growth of diphtheria bacilli.

4. Cultures are usually made on slants of Loeffler's blood serum medium. Slants of blood agar are next best. Cultures should be incubated at 35 to 37° C. for 18 to 24 hours. At higher temperatures the bacilli become smaller and atypical. Cultures may be examined as early as 8 to 12 hours but the bacilli are larger than usual, more solid, and more difficult to recognize.

5. Prepare smears and stain with methylene blue. Be sure to pass the loop lightly over the entire surface of the slant in order not to miss isolated colonies.

The smear will often show a pure or almost pure culture, owing to the fact that in the first 18 to 24 hours the bacilli tend to outgrow other organisms.

6. Diphtheria bacilli are slender, straight or slightly curved rods (Fig. 87) occurring in three principal morphologic types, which Wesbrook and others have subdivided into many subvarieties:

(a) *Granular* or *beaded* types, which are most typical and likely to be most virulent.

(b) *Segmented* or *barred* types, encountered less frequently but likewise likely to be virulent.

(c) *Solid* types, which may be long or short and are likely to be less virulent. These bacilli are readily mistaken for *C. pseudodiphtheriticum* and diphtheroid bacilli.

7. Any of these, and especially the granular and segmented types, may have swollen ends producing the typical club-shaped bacilli. They are not encapsulated, do not form spores, and are nonmotile.

8. It is usual and characteristic of diphtheria bacilli to lie at various angles to one another, forming V or Y shapes which, when clumped, give the appearance of Chinese letters. They do not occur in palisade formation or in chains.

9. With experience, *C. diphtheriae* may be readily identified by these means; but it is not always possible to differentiate safely the solid types from *C. pseudodiphtheriticum* and diphtheroid bacilli. Sugar fermentation and guinea-pig virulence tests are sometimes required.

DIAGNOSIS OF PLAUT-VINCENT ANGINA AND FUSOSPIROCHETAL GINGIVITIS

Plaut-Vincent Angina. 1. Smears are required for diagnostic purposes, as the organisms are anaerobic and difficult to cultivate. These should not be too thin and should be made on glass slides. Swabs accompanying cultures on Loeffler's blood serum for diphtheria bacilli may be used for preparing smears and this is a good routine practice as Plaut-Vincent angina may be mistaken clinically for diphtheria.

2. Dry in air.

3. Fix by passing through flame 4 times.

4. Cover with carbol-fuchsin diluted 1:10 with water; heat gently and stain for 2 minutes. Stain second slide by method of Gram.

5. Wash in water and dry.

6. Examine with oil-immersion lens for fusiform bacilli and spirochetes. The former are gram-negative (variable), long, slightly curved with pointed ends and show faintly staining granules. The latter are large, wavy spirals (Fig. 87).

7. Both organisms are also readily seen in wet preparations with high dry or oil-immersion objectives or by darkfield examination.

Fusospirochetal Gingivitis. 1. The material should be collected with care, especially from gingival pockets, with suitable instruments or after expression by pressure.

2. Prepare several smears. Dry in the air. Fix with heat. Stain with 1:10

carbol-fuchsin for 2 or 3 minutes. Wash with water, dry, and examine with oil-immersion lens for spirochetes and fusiform bacilli.

3. A few spirochetes and fusiform-shaped bacilli, resembling *B. fusiformis*, are to be found in most mouths and do not alone constitute evidences of infection; but the presence of large numbers is regarded as pathologic. *Leptotrichia buccalis* is frequently found in the mouth and may be mistaken for fusiform bacilli. They occur as long, gram-positive bacilli or filaments. They do not grow under ordinary aerobic conditions.

EXAMINATIONS OF SPUTUM

Sputum, to be examined for tubercle bacilli, pneumococci, streptococci or other micro-organisms producing pneumonia, may be collected as described in Chapter 9 on page 228.

Tubercle Bacilli. 1. Pour the sputum into a Petri dish and pick up with sterilized platinum wire small white or yellow caseous particles; if none are present, choose for examination some of the thicker yellowish or greenish portions.

2. Make at least two smears on glass slides. They should be thin and uniform: never heavy and unevenly distributed. Material may be put on the upper half of a slide and squeezed out with another slide, continuing the rubbing until the sputum is evenly distributed; then the slides should be separated.

3. Stain with Ziehl-Neelsen carbol-fuchsin for acid-fast organisms.

4. The tubercle bacilli will appear as red, solid or vacuolated, straight or slightly curved rods (Fig. 87); other bacteria and cells are stained blue. At least two smears should be examined before a negative report is given and five minutes or more devoted to the examination of each.

5. The average number of tubercle bacilli per field may be recorded according to the following scheme of Gaffky as modified by L. Brown:

No. 1—only 1 to 4 in whole preparation
No. 2—only 1 bacillus on an average in many fields
No. 3—only 1 bacillus on an average in each field
No. 4—about 2 to 3 bacilli on an average in each field
No. 5—about 4 to 6 bacilli on an average in each field
No. 6—about 7 to 12 bacilli on an average in each field
No. 7—about 13 to 25 bacilli on an average in each field
No. 8—about 50 bacilli on an average in each field
No. 9—about 100 or more bacilli on an average in each field
No. 10—enormous numbers in each field.

The following may be *sources of error:* (a) Scratches in the slides may retain the stain and be mistaken for acid-fast bacilli. (b) Incomplete decolorization. (c) There may be tubercle bacilli in the carbol-fuchsin washed off from former specimens if the stain is used repeatedly. (d) Acid-fast bacilli may be present in stale distilled water used for washing slides; also in vaseline and in milk bottles used for collection of specimens. (e) Wood fibers, food particles and crystals may retain the fuchsin and resemble tubercle bacilli, although the latter are usually readily differentiated by careful study of morphology.

6. If tubercle bacilli are too few to be found in this manner, the following *concentration method* may be employed:

(a) Prepare a digesting fluid by dissolving 20 gm. sodium hydroxide, 1 gm. potassium alum and 0.01 gm. bromthymol blue in 500 cc. water.

(b) Prepare an acid solution by diluting 25 cc. concentrated hydrochloric acid with 75 cc. water (about 2.5 N).

(c) Mix 3 to 5 cc. sputum with 1 to 4 parts of digesting fluid. Shake well. Incubate at 37° C. for 30 minutes.

(d) Adjust to *p*H 7 with the acid solution, using an indicator with a range of 6.0 to 7.6 (yellow to blue); *p*H 7.0 equals light bluish green.

(e) Centrifuge at top speed for 5 minutes.

(f) Remove supernatant fluid. Prepare smears of the sediment and stain with Ziehl-Neelsen's carbol-fuchsin for acid-fast bacilli.

7. *Guinea-pig inoculation* is the most certain method of establishing diagnosis. Suspend in sterile saline solution the sediment obtained by the concentration method and inject this subcutaneously or intramuscularly into the thigh of a young guinea-pig (250 gm.). Autopsy of animal at its death several weeks later, or, if it lives, at 6 weeks, will reveal generalized tuberculosis, particularly apparent by caseation of glands, spotted liver and large, spotted spleen, which may be confirmed by finding acid-fast bacilli by direct smear or culture examinations.

8. Cultures of sediment may also be made on slants of the Petragnini medium and incubated at 37° C.

Pneumococci. For this purpose a small sample of expectorated sputum, as free as possible of saliva, should be collected in a Petri dish or wide-mouthed bottle. It should be examined within an hour; if this is not possible it should be kept on ice. If typing is to be done, the sample should be collected before beginning treatment with a sulfonamide compound (which interferes with this test).

1. Smears are best prepared of blood tinged ("rusty") portions of sputum on slides.

2. Dry in air; fix by gentle heating and stain by the method of Gram.

3. The pneumococcus is gram-positive and occurs in pairs or, less frequently, in short chains (must be differentiated from streptococcus). The adjacent ends of the cocci are usually rounded while the opposite ends are more pointed or lancet-shaped. A well-marked capsule is present which may be retained in cultures on suitable media. Type III pneumococcus may often be identified by very large capsules. Special capsule stains may be employed.

4. With a platinum loop, streak out one or two blood agar plates with material to be examined. Incubate 24 to 48 hours. Examine colonies; prepare smears and stain by method of Gram.

5. Colonies on blood agar at the end of 48 hours' incubation are usually smooth and circular with the edges sharply raised from the surface of the medium and surrounded with greenish zones of incomplete hemolysis. Prepare smears of colonies and stain by Gram method.

6. Another method for the rapid isolation of pneumococcus from sputum is as follows: (a) Inoculate a mouse intraperitoneally with 1 cc. of the emulsion of washed sputum. (b) Usually in from 4 to 48 hours, the animal becomes ill and

succumbs (the time varies according to numbers and virulence of pneumococci present). (c) When ill, kill the mouse or immediately after death, remove the peritoneal exudate aseptically with a capillary pipet. Prepare smears and stain by Gram method. Inoculate glucose hormone broth; also prepare smears and culture of heart blood. The balance of the peritoneal exudate may be used for typing.

Neufeld Method of Typing Pneumococci. 1. Use the following typing serums (rabbits) supplied in capillary tubes (each with enough for one test), or in small bulk bottles: A mixture (types 1, 2 and 7); B mixture (types 3, 4, 5, 6, 8); C mixture (types 9, 12, 14, 15, 17); D mixture (types 10, 11, 13, 20, 22, 24); E mixture (types 16, 18, 19, 21, 28) and F mixture (types 23, 25, 27, 29, 31, 32).

2. Divide three clean slides into halves by wax pencil and label halves "A, B, C, D, E and F."

3. Place tiny fleck of sputum or mouse peritoneal exudate in center of an area, with small loop.

4. Add typing serum (about five times as much as specimen used).

5. Add large loopful of methylene blue stain. Mix thoroughly and apply cover glass.

6. Let stand for 5 minutes (prepare other slides while waiting).

7. Examine under oil-immersion objective for dark-blue diplococci, surrounded by unstained area with definite outline. Only a small indistinct capsule can be seen around the pneumococci mixed with heterologous antiserums. Large distinct halo surrounds the pneumococci which have been mixed with their type antiserums (Fig. 88). If none of the mixtures is found positive at first examination, yet pneumococci have been shown to be present by stained smears, re-examine these slides from time to time over a period of 30 minutes.

8. Positive mixtures having been determined, repeat the test procedure with each serum contained in that mixture, until the positive type or types have been determined.

DIAGNOSIS OF LEPROSY

1. Since the initial lesion is often an ulcer of the mucosa of the nose, prepare smears and stain by the method of Ziehl-Neelsen. If desirable, the patient may be given 60 grains of potassium iodide beforehand to produce coryza and increase the nasal secretions.

2. Prepare smears of a skin lesion with a safety razor blade or scalpel and stain by method of Ziehl-Neelsen.

3. If possible, remove a portion of lesion by biopsy and place in 4 per cent formalin. Prepare paraffin sections and stain for acid-fast bacilli.

4. Leprosy bacilli are acid-fast and gram-positive. They are rather long, slender and usually straight with pointed ends. Decolorization should not be carried too far as they are more easily decolorized than *Myco. tuberculosis.*

5. In nasal smears the bacilli are apt to be packed in cells (lepra cells) (Fig. 88) while in sections of nodules they are found chiefly in the skin, packed in characteristic lepra cells (foam cells) and in endothelial cells.

6. Lepra bacilli are rarely demonstrable in the anesthetic type of nerve leprosy.

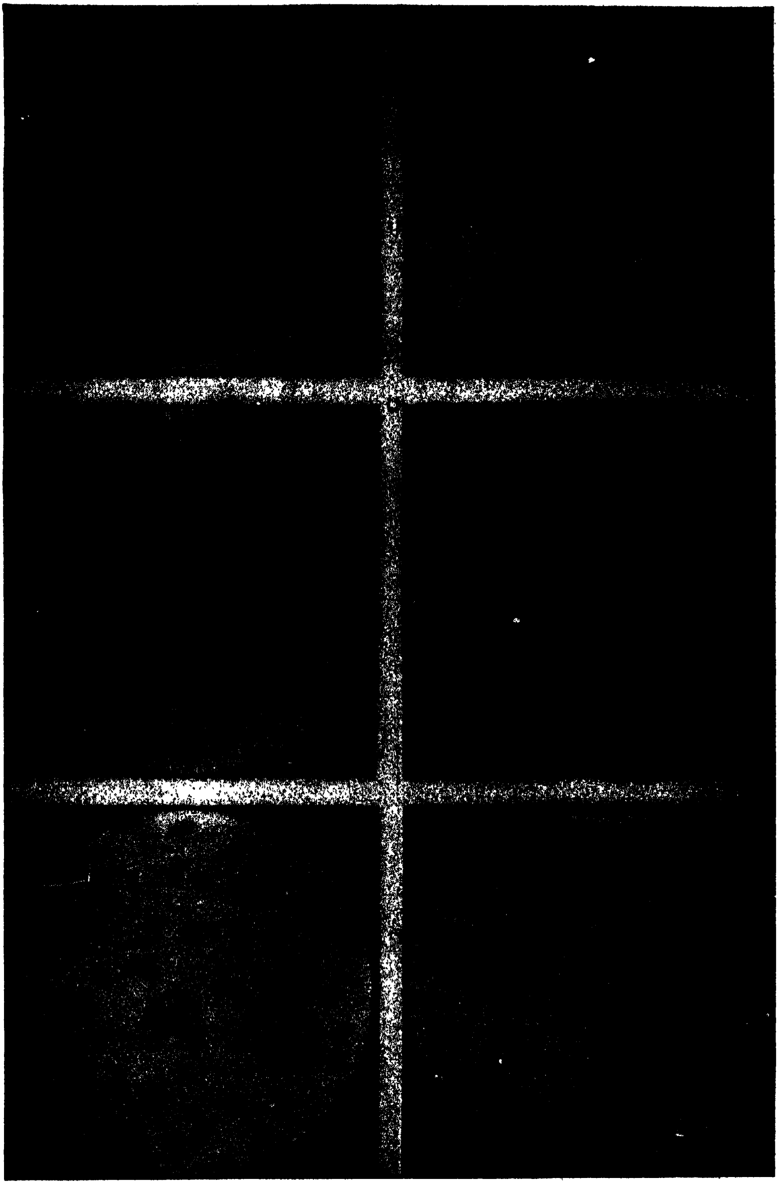

FIG. 88. BACTERIOLOGIC EXAMINATIONS.

A, Neufeld method of typing pneumococci; *B*, *Myco. leprae* in smear of nasal mucosa; *C*, Meningococci in smear of spinal fluid; *D*, Hemolytic streptococci in smear of spinal fluid; *E*, *K. pneumoniae* in smear of spinal fluid; *F*, Anthrax bacilli in smear of cutaneous lesion.

EXAMINATIONS OF SPINAL FLUID IN MENINGITIS

Acute meningitis may be caused by various micro-organisms with special reference to meningococci, *H. influenzae*, hemolytic streptococci, pneumococci, *K. pneumoniae*, etc., as discussed in Chapter 15, page 348; also by the virus of acute lymphocytic chorio-meningitis for which there are no direct or practical means of bacteriologic diagnosis. Subacute and chronic meningitis is usually due to *Myco. tuberculosis* or *T. pallidum.* There are no practical bacteriologic examinations for the latter.

General Technic. 1. Spinal fluid is collected with precautions against contamination, as described on page 314.

2. As a general rule, in all cases of acute meningitis, it is advisable to make a blood culture at the same time by inoculating a flask of 100 to 200 cc. of glucose hormone broth with 5 to 10 cc. of blood.

3. The gross appearance (color, opacity, coagula, etc.) of the spinal fluid should be noted.

4. Smears and cultures should be made as promptly as possible and especially in the case of suspected meningococcal meningitis. In preparing cultures it is advisable to transfer at least 1 cc. amounts of fluid to slants or plates of blood agar; otherwise several loopfuls of sediment.

5. If the fluid is distinctly turbid, smears and cultures may be made directly. If opalescent or but faintly turbid, at least 5 to 10 cc. should be centrifuged at high speed and smears and cultures prepared of the sediment.

6. Smears should be stained by the method of Gram with thorough decolorization. A duplicate smear should be stained with Loeffler's alkaline methylene blue. In suspected *tuberculous meningitis* smears may be prepared of sediment but are better prepared by carefully teasing out a portion of coagulum on a slide. They should be stained with Ziehl-Neelsen's carbol-fuchsin or by the special method of fluorescent microscopy.

Meningococcal Meningitis. 1. In smears of spinal fluid, the meningococcus is usually arranged in pairs bearing a close resemblance to the gonococcus in morphology. When thoroughly decolorized they are gram-negative organisms but, unless well decolorized, may appear gram-positive with the possibility of being mistaken for the pneumococcus. They are not encapsulated.

2. Early in meningococcal meningitis the number of organisms present in smears may be very few and largely extracellular, requiring careful examination. Later they become more numerous and largely intracellular (Fig. 88). As a general rule, the finding of intracellular and extracellular gram-negative, coffee-bean shaped diplococci in spinal fluid justifies the immediate presumptive diagnosis of meningococcal meningitis.

3. On blood agar plates incubated at 37° C. for 48 hours, the colonies are small, moist, convex, elevated, colorless, transparent, circular and nonhemolytic.

4. Prepare smears and stain by the Gram method. Gram-negative diplococci of varying size and shape are usually meningococci.

5. The following *presumptive slide agglutination test* may be conducted for confirmatory purposes: (a) On a clean glass slide place 1 drop of polyvalent

antimeningococcus serum diluted 1:10; on a second, 1 drop of normal horse serum 1:10 and on a third, 1 drop of normal saline solution. (b) With a small platinum loop mix a portion of suspected colony in each of the three drops. (c) Allow to stand at room temperature for 5 to 15 minutes and examine. (d) Agglutination on the first slide is sufficient for a presumptive diagnosis of "meningococcus, type undetermined." (e) Allow the films to dry and stain by the Gram method to verify morphology, staining characteristics and agglutination.

Influenzal Meningitis. 1. Smears of spinal fluid or sediment show numerous gram-negative bacilli, mostly extracellular. They are usually very small, short, and almost coccal. The bacillus, however, is highly pleomorphic and may occur in spinal fluid in such long threads or filamentous forms as to suggest a mycotic infection (Fig. 89). The bacilli are not encapsulated and do not form spores (Fig. 90).

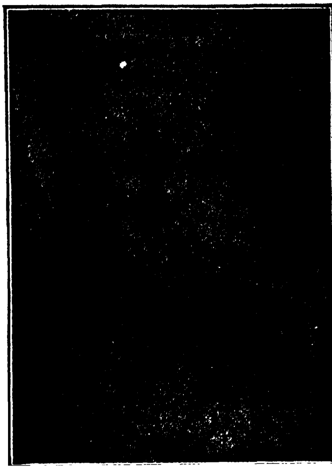

FIG. 89. HEMOPHILUS INFLUENZAE.

Forms from R type colony. (From *Zinsser's Textbook of Bacteriology,* 9th ed., Appleton-Century-Crofts, Inc.)

2. Being a strict parasite, *H. influenzae* requires for cultivation the presence of accessory substances or "growth factors," of which two, X and V, have been distinguished. The X factor, which is thermostable, is associated with hematin and with less well-defined substances present in fruits, potato and other vegetables containing iron. The V factor, which is thermolabile, is found in blood, potato and yeast and resembles vitamin C.

FIG. 90. HEMOPHILUS INFLUENZAE.

Smear from culture of organism from meningitis; growth on chocolate agar, 24 hours. (From *Zinsser's Textbook of Bacteriology,* 9th ed., Appleton-Century-Crofts, Inc.)

3. Satisfactory media are chocolate agar, fresh blood agar (human or rabbit blood preferred) and Avery's sodium oleate agar (pH 7.2).

4. Cultivate 24 hours at 37° C. The colonies which emulsify readily, are pinpoint in size, smooth, circular, transparent and homogeneous and have entire edges.

5. Prepare smears and stain by Gram method. Transplant colonies to slants of plain and chocolate agar. *H. influenzae* will not grow on the former.

6. It is a difficult organism to identify until considerable experience has been gained. In spinal fluid, however, it is readily identified by these characteristics.

Streptococcal Meningitis. Smears of spinal fluid or sediment show the presence of gram-positive cocci in chains of variable length. They are nonencapsulated and are usually extracellular (Fig. 88). On blood agar plates the colonies are usually surrounded by zones of hemolysis. Short chains of streptococci in direct smears of spinal fluid cannot always be differentiated from pneumococci. Cultural studies may be required. They are not bile-soluble.

Pneumococcal Meningitis. Smears of spinal fluid or sediment show the presence of numerous gram-positive diplococci with capsules. They are usually extracellular. Pneumococci may also occur in short chains and require differentiation from streptococci. They are bile-soluble. The type of pneumococcus may be determined by the Neufeld reaction (page 1076) conducted with the sediment of spinal fluid or cultures.

K. Pneumoniae Meningitis. Smears of spinal fluid or sediment show the presence of short, plump bacilli. They are gram-negative and encapsulated, occur singly or in short chains, and are mostly extracellular (Fig. 88). The bacilli grow well on ordinary media and produce no hemolysis on blood agar plates. Final identification may require fermentation tests for acid and gas production with the various sugars.

Tuberculous Meningitis. Smears of sediment or of coagulum stained for acid-fast bacilli require prolonged and thorough examination as but few tubercle bacilli are present. Failure to find them in smears does not exclude the disease. In the presence of the usual signs and symptoms, the diagnosis is usually justified under these conditions if the spinal fluid is comparatively clear, shows a pleocytosis due to an increase of lymphocytes, a "cob-web" or "pine-tree" coagulum, a strongly positive Pandy reaction and a reduction in chloride. Heavy coagula and sediments may be examined by a concentration method (page 1075). Cultures on the Petragnini medium are advisable. Guinea-pig inoculation tests (page 1075) should be conducted routinely unless tubercle bacilli are found in smears, which suffice for diagnostic purposes.

DIAGNOSIS OF ANTHRAX

1. The "malignant pustule" or anthrax of the skin produced by *B. anthracis* may be mistaken clinically for a simple furuncle or carbuncle.

2. It is usually encountered among workers in hides, hair and wools but may be derived from shaving brushes or other articles.

3. Great care must be exercised in handling material, cultures, etc. Be sure to disinfect pus, glassware, etc., thoroughly before discarding.

4. *Septicemia may occur and blood cultures should be included routinely.*

5. Anthrax of the bronchi may occur ("woolsorter's disease") in which case the bacillus occurs in the sputum.

6. Anthrax of the intestinal mucosa may also occur, the bacillus appearing in the feces.

7. Prepare smears and cultures of the lesion and particularly of any blister fluid that may be obtained. Blood or plain glucose agar slants or plates may be employed. Avoid rough manipulation of the lesion.

8. Stain smears by Gram method. The presence of scattered large bacilli with

square ends is suspicious. Occasionally short chains are seen (Fig. 88). The bacilli are gram-positive if not decolorized too long. They may be encapsulated.

9. Incubate the cultures for 24 hours and examine. Growth is rapid. The colonies are dull, grayish-white, flat and spreading with irregular borders which, when viewed under the lower power of the microscope, have a Medusa-head appearance due to filamentous interlacing chains of bacilli. Examine smears stained by Gram method; spores are produced.

10. Examine for motility; *B. anthracis* is not motile.

11. Inoculate a Loeffler blood serum slant and incubate 24 to 48 hours. *B. anthracis* produces no liquefaction or but very slight.

12. If in doubt, inoculate a young guinea-pig subcutaneously or intraperitoneally with 1 cc. of a 24-hour broth culture or with a suspension in saline solution. *B. anthracis* usually produces a fatal septicemia in 12 hours to 2 or 3 days with the presence of organisms in the heart blood, spleen, liver and other organs. Prepare smears of blood and spleen; stain by Gram method; also prepare cultures on plain or blood agar. If an early diagnosis is desired, the suspected material may be inoculated subcutaneously into guinea-pigs, mice or rabbits, without waiting for the isolation of pure culture.

EXAMINATIONS OF WOUNDS

Principles. All traumatic wounds, including war wounds, are invariably contaminated with bacteria, aerobic and anaerobic, from the soil, clothing, skin or air. Dead or devitalized tissues furnish excellent culture media for bacterial growth. If more than six hours elapse without definitive surgical aid, the contaminating micro-organisms proliferate and produce infection. The immediate aim of treatment, to be followed by such surgical restoration as indicated, is the prevention or limitation of infection. Bacteriologic examinations of wound smears and cultures determines these procedures. Debridement is the first step by the surgeon in limiting infection, by removing all of the devitalized tissues and foreign substances which would provide nidus of infection. By this procedure, bacteria are greatly diminished in number, but are not eradicated; at least most of the culture materials for bacterial growth are removed. Primary suture is not done except in quiet periods of warfare and in hospitals where the patient may be retained for careful observation; otherwise, wound suture may lead to enclosure, in an imperfectly debrided wound, of harmful micro-organisms, especially of the gas gangrene group. Delayed primary suture may be done if the cultures, taken 18 to 48 hours after debridement, show no micro-organisms; if hemolytic streptococci or staphylococci are present, suture is not considered. The presence of an additional micro-organism per two fields (including a few anaerobes) does not contraindicate suture. Considerable numbers of micro-organisms of any kind indicate delay of suture. Secondary suture is undertaken when the micro-organisms, on two successive counts, are few and the culture has shown an absence of hemolytic streptococci and staphylococci.

Smear Method. 1. No smears are taken while hemorrhage exists. Smears should not be taken within 2 hours after the application of Dakin's or other antiseptic solution. Smears need not be taken earlier than 12 hours after the infliction of the wound, since up to that time few bacteria will be found. Smears are made of the wound secretions every other day, or daily, as the time of secondary closure occurs, in such a way that an appropriate estimate of the number of bacteria contained in the wound can be made by their examination.

2. Smears are taken with a platinum loop from different parts of the wound, choosing areas most likely to harbor bacteria, such as crevices, necrotic bone, foreign bodies, or deep sinuses; do not take from bleeding points, smooth muscle, or clean areas, and also avoid the skin adjacent to the wound. With a small platinum loop, small amounts of secretion are picked up and smeared on slides in such a way that about the same area is covered by the different loopfuls of secretions—with practice a uniformity of technic is attained to provide comparable bacteria counts.

3. Allow smears to dry, fix with heat, and stain with Loeffler's alkaline methylene blue or according to the method of Gram (preferred).

4. The number of bacteria per field (oil-immersion objective) is counted. If the average number exceeds 50 or more per field, more accurate counting is not necessary for the wound still contains too many bacteria to warrant closure or relaxation in local therapy. Gradually, as the wound improves, fewer and fewer bacteria will be found in the daily smears and, when the number has dropped below 50 per field, careful counting may give an index to daily variation. Eventually the number will decrease to only one micro-organism per 5, 10 or 20 fields. The daily counts may be charted to provide a curve which will show the surgeon, at a glance, the numerical progress of the bacterial infection.

Culture Method. 1. Where no bacteria can be found in smears, cultures of the wound secretions should be made on blood agar plates (without glucose) as the absence of bacteria in smears does not mean that the wound is completely sterile. Cultures are required particularly when the period of secondary closure approaches and especially in hemolytic streptococci or staphylococci have been found previously. Suture of a wound is not carried out if hemolytic streptococci or staphylococci of any kind are present; hence, frequent cultures on blood agar plates are made during the progress of treatment. If the smears show a great many bacilli resembling the ordinary anaerobes, anaerobic cultures also may be made. However, because of the length of time required for working out the anaerobes in the laboratory, the surgeon is not concerned about this as a guide for his program of therapy.

PREPARATION OF AUTOGENOUS VACCINES

1. The method employed in making primary cultures of infected tissues is very important in order to secure the organism or organisms responsible for infection. *Faulty methods may result in securing only saprophytes or contaminating organisms which, if employed, are likely to defeat the purpose of vaccine therapy at the outset.* It is particularly important to use a good culture medium. Tubes of glucose hormone broth or slants of blood agar are recommended for routine use.

If material is collected on swabs, they should be sent promptly to the laboratory for the preparation of cultures.

2. Incubate the cultures for 24 to 48 hours. Examine smears stained by the method of Gram. If a pure culture is found, inoculate media for the preparation of vaccine. If a mixed culture is found, secure each micro-organism in pure culture by plating on blood agar. Whenever possible, select smooth (*S*) colonies for the preparation of a vaccine. In mixed infections a selection must be made of the micro-organisms to be employed. Do not use spore-forming bacilli (like *B. subtilis*) or saprophytes (like *Serratia marcescens,* diphtheroids, etc.). Organisms of secondary infection should be included.

3. The organism or organisms chosen for the preparation of the vaccine may be cultivated on slants of blood agar for 2 or 3 days. In the case of staphylococci and streptococci, however, cultivation in a broth medium for 4 or 5 days at 37° C. is recommended in order to incorporate into the vaccine any toxins produced. While the vaccine is being prepared subcultures of the organism or organisms employed may be subjected to final identification.

4. If a solid medium like blood agar is employed, add 2 or 3 cc. of sterile saline to each tube and emulsify the bacteria by shaking or by agitation with a platinum loop. The suspension should be quite heavy. Avoid breaking up the medium. If a broth culture is employed, do not centrifuge. In either case transfer the suspension to a sterile flask or bottle containing glass beads and shake thoroughly to break up clumps.

5. Dilute the suspension to the desired bacterial content. For this purpose a nephelometric method is recommended. If the suspension has been prepared with saline solution, a nephelometer (McFarland) may be prepared as follows: (a) Prepare a 1 per cent aqueous solution of CP sulfuric acid and a 1 per cent aqueous solution of CP barium chloride; (b) to a series of 10 pyrex glass test tubes of uniform size, add increasing amounts of the barium chloride solution, starting with 0.1 cc. in the first tube, increasing the quantity by 0.1 cc. in each succeeding tube, so that 1.0 cc. is added in tenth tube; (c) then add to each tube enough sulfuric acid solution (9.9 cc. to 9.0 cc., respectively) to bring the total volume to 10 cc. Seal hermetically and label serially from 1 to 10.

If broth cultures are employed the nephelometer is prepared in the same manner except that the 1 per cent solution of barium chloride is prepared with broth instead of water in order to impart the color of the latter to the tubes.

The density of the suspensions in these tubes corresponds approximately to from 300 million organisms per cubic centimeter for the first tube, to 3000 million per cubic centimeter for the tenth tube, increasing by 300 million bacteria for each succeeding tube 1 to 10.

Since autogenous vaccines are usually made up to carry a total of approximately 1000 million per cubic centimeter, a nephelometer showing this numerical concentration can be prepared of one tube by adding 5 cc. of sterile saline solution to 5 cc. of commercial triple typhoid-paratyphoid vaccine (2000 million per cc.) and sealing in a pyrex glass test tube. For vaccines prepared of broth cultures, the nephelometer may be prepared in the same manner except that 5 cc. of sterile broth are used instead of saline solution.

Place a measured quantity (1.0 cc. or more, depending on density) of the bacterial suspension in a test tube of the same diameter and color as those used for the standard. Dilute by adding a measured amount of sterile saline to the density of one of the standards; shake well during the process. If the broth nephelometer is used, dilute with sterile broth instead of saline solution.

6. Dilute to the desired numerical strength and sterilize with tricresol in final concentration of 0.5 per cent. The latter is preferred to sterilization by heat. A 5 per cent aqueous (saturated) solution of phenol is employed. Proceed as per the following example: 20 cc. of vaccine containing 1000 million per cc. is desired; the suspension contains 2,700 million per cc. $= \frac{1000 \times 20}{2700} = 7.0$ cc. of suspension to be used.

In a sterile vial or bottle containing a few glass beads place 7.0 cc. of the suspension, 2 cc. of 5 per cent phenol and 11 cc. of sterile saline solution.

If the vaccine is to be a mixed one, prepare separate vaccines of each organism in this manner and mix equal parts of each.

Stopper with a sterile rubber cap, mix, and place in the incubator at 37° C. for 24 hours.

Test for sterility by removing 0.5 cc. with a sterile syringe and needle and place in a flask of at least 50 cc. of suitable broth. Do not use tubes of broth as the vaccine must be diluted beyond the bacteriostatic threshold of the phenol. Incubate 24 to 48 hours. If sterile, the vaccine is ready for administration. As a general rule, the vaccine is found sterile unless spores are present. Some physicians prefer having vaccines dispensed in ampules. In this case the designated doses, like 0.1, 0.2, 0.3, 0.4 cc., etc., are placed in sterile 1 cc. ampules with a sterile syringe and the volume in each brought up to 1 cc. by adding the necessary amounts of a 0.1 per cent solution of phenol in saline solution. Each ampule is then sealed in a flame and properly labelled.

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SEROLOGIC EXAMINATIONS

Methods for the collection of blood and the preparation of serums are described in Chapter 17. Antibodies and antigens in relation to serologic examinations are briefly discussed in the same chapter. Serologic tests are also conducted with spinal fluid in the diagnosis of syphilis; a method for its collection has been described in Chapter 14.

PRETRANSFUSION BLOOD TESTS

Determination of Blood Groups. As far as a determination of the major blood groups is concerned, the only reagents required for the test are serums from blood groups A and B. The test may be conducted as follows:

1. Divide a slide down the middle with a wax pencil. Mark the left side A and the right B.
2. Place 1 drop of A serum in the center of the A side of the slide and a drop of B serum in the center of the B side. One drop of each is sufficient for the test.
3. Puncture the finger or the ear lobe of the person to be tested and transfer a minute drop of blood, by means of a clean applicator, to the drop of A serum, mixing to a smooth suspension of the cells. With a fresh applicator, transfer a like drop to the B serum and mix thoroughly. *Never use the same applicator for both serums.*
4. Allow to stand for 5 minutes, occasionally rolling or tilting the slide to insure thorough mixing. If it is difficult to distinguish between true agglutination and rouleau formation, stir again with the applicator, as rouleaux will be broken up to a smooth suspension thereby, but true agglutination will be unaffected. If definite agglutination has occurred at the end of five minutes, a reading and report may be made. If there is no agglutination, cover each mixture with a cover glass and examine at intervals, making a final reading at the end of 30 minutes. Sometimes a final reading must be deferred for 60 minutes in the case of group AB blood; in this case it is advisable to ring the preparations with vaseline to prevent evaporation.
5. The readings are made as follows (Fig. 91): (a) No agglutination by either A and B serums = group O; (b) agglutination by B serum but not by A serum = group A; (c) agglutination by A serum but not by B serum = group B; (d) agglutination by both A and B serums = group AB.
6. Occasionally there will be doubt as to the agglutination in weakly reacting blood. It is then advisable to use a suspension of erythrocytes in saline solution. One drop of the blood to be typed in 0.5 cc. of saline solution tested in the same manner as whole blood will almost invariably always give a reaction which can

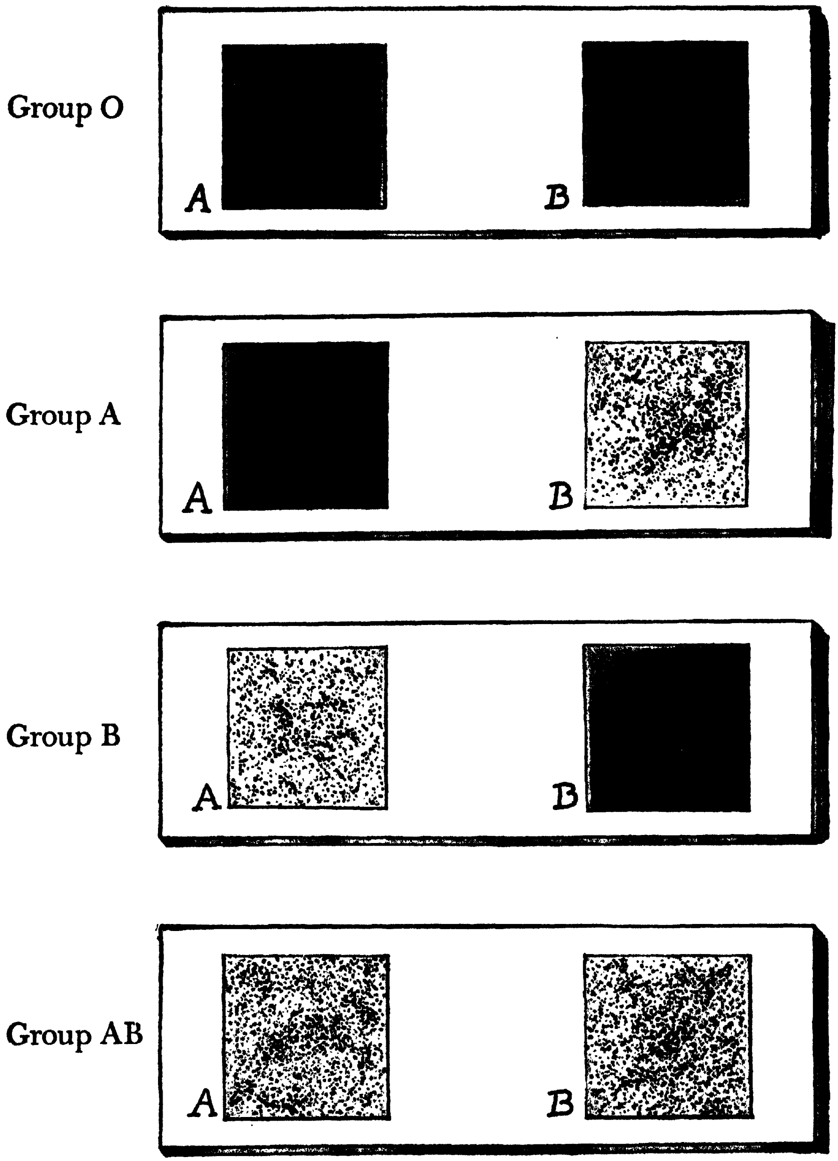

Fig. 91. Blood Grouping According to the Landsteiner or International Classification.

be read clearly, even if it is necessary to cover the mixtures with cover glasses and use the low-power magnification of the microscope to determine the presence or absence of agglutination.

Cross Typing. It is always advisable to cross-type or match the serum of the patient with the erythrocytes of the donor and the serum of the donor with the erythrocytes of the patient. Occasionally the bloods of donor and patient (recipient), even though clearly of the same major blood group, will not match, that is, there will be some agglutination of the donor's cells by the patient's serum or of the patient's cells by the donor's serum. This can be avoided by making a final selection of a donor by the cross-typing test conducted as follows:

1. Collect 1 or 2 cc. of the prospective donor's blood and the same amount of the recipient's blood in separate small test tubes and label each respectively DS (donor's serum) and RS (recipient's serum). After each has clotted, break up the clots and centrifuge to secure the sera.

2. At the same time collect 1 drop of donor's blood in 1 cc. of saline solution in a small test tube and label DC (donor's cells). Collect 1 drop of recipient's blood in 1 cc. of saline solution in a second small test tube and label RC (recipient's cells). Mix each to an even suspension.

3. Divide a clean slide, as for the typing test, and mark the left side $\frac{DS}{RC}$ and the right side $\frac{RS}{DC}$.

4. Place 1 drop of donor's serum on the left side and 1 drop of recipient's serum on the right, using fresh capillary pipets for each transfer.

5. Mix 1 drop of recipient's cells with the donor's serum and 1 drop of donor's cells with recipient's serum, using fresh capillary pipets for each transfer.

6. Allow to stand at room temperature, occasionally rolling or tilting the slide to insure thorough mixing. It is advisable to cover each with a cover glass and examine with the low-power magnification of the microscope. Examine at intervals. Any agglutination evident within 30 minutes should disqualify the donor and another should be tried until one is found giving no trace of agglutination. This is especially true when there is any grade of reaction between donor's cells and recipient's serum. When permissible, it is better to make the final reading at the end of one hour; in this case the preparations should be ringed with vaseline to prevent evaporation.

Rh Typing. 1. Place a small test tube 0.5 cc. of physiologic saline solution containing 1 per cent sodium citrate.

2. Prick a finger or the lobe of an ear and drop *two full freely falling drops of blood* into the tube. This gives about a 10 per cent suspension which is essential for avoiding occasional false negative reactions.

3. Mix the corpuscle suspension and place one drop on a clean glass slide. Next to this place one drop of Lederle anti-Rh serum. With a glass rod, mix the two drops over an area of about three-quarters of an inch.

4. Tip the slide back and forth once and leave undistributed for three minutes. In a dry, warm atmosphere, it may be advisable to protect the mixture against too rapid evaporation by covering the slide with a large Petri dish.

5. At the end of this time pick up the slide and give it two or three gentle rotations. While continuing to hold the slide, and with a very slight motion, observe the mixture for three more minutes. *Oblique lighting on the specimen increases the visibility of the clumps and facilitates accurate reading of the reaction.*

6. In positive reactions, agglutination is clearly visible in six minutes. False negative reactions may occur if the corpuscle suspension is too thin (under 5–10 per cent).

7. Because of the rapidity of the test, no more than ten specimens should be examined at any one time.

8. It is advisable to test at the same time a known Rh— blood and a known Rh+ blood as positive and negative controls.

HETEROPHIL ANTIBODY TEST FOR INFECTIOUS MONONUCLEOSIS (Davidsohn)

This test is based on the agglutination of washed sheep corpuscles by the heterophil agglutinin in the serums of individuals with infectious mononucleosis.

1. Prepare a 2 per cent suspension of washed sheep erythrocytes in normal saline solution in the same manner as for the Kolmer complement fixation test (page 1094). The sheep blood should not be less than 24 hours old and not older than about one week, because later the corpuscles tend to become agglutinated too easily. The suspension must be prepared from cells washed on the same day.

2. Secure blood and separate the serum. Heat the serum in a water bath for 30 minutes at 56° C.

3. Place 11 small clean test tubes in a rack.

4. Place 0.4 cc. of normal saline solution in No. 1 and 0.25 cc. in each of the remaining tubes.

5. Add 0.1 cc. serum to tube No. 1. Mix and transfer 0.25 cc. to No. 2 and so on to the last tube from which 0.25 cc. is discarded after mixing.

6. Add 0.1 cc. of the 2 per cent suspension of washed sheep corpuscles to each tube.

7. Set up a control carrying 0.25 cc. saline solution and 0.1 cc. of corpuscle suspension.

8. Shake the test tubes and leave at room temperature for 2 hours after which the reading is made. When time permits, it is advisable to repeat the reading after an overnight incubation in the refrigerator. The titer is then usually 1 to 2 dilutions higher. The final dilutions of serum range from 1:7 to 1:7168, as shown in the table on page 1089.

9. The results are read after shaking the test tubes. The shaking is continued until the entire sediment is suspended. The test tubes in which, after shaking, the cells remain in the form of a single clump are read as 3 plus. Those in which the cells break up into distinctly visible clumps and the fluid is clear and transparent, are called 2 plus. The reading of the 1 plus agglutination is best carried out by means of a low-power objective of the microscope. The test tube is placed horizontally on the stage after the tube has been shaken and in this way one can determine the end point of the agglutination with the greatest

accuracy. However, after one acquires some experience, one can determine the end point fairly accurately with the naked eye. It is usually 1 to 2 dilutions lower than the titer determined with microscope.

10. Normally, human serums seldom agglutinate washed sheep corpuscles in final dilutions higher than 1:7 to 1:14. The finding of a titer 1:56 or higher in a person who has not had an injection of normal horse serum, or horse immune serum, in the recent past and who presents clinical and hematologic manifestations of infective mononucleosis, indicates the presence of the disease. A titer of 1:224 or higher may be considered positive even if there is a history of administration of horse serum, unless the patient is suffering at the time of examination from serum disease or has had a recent attack of it.

Tubes	Saline cc.	Serum cc. Dilution	Serum Dilutions	2% Sheep Cells cc.	Titer (Final Dilutions of Serum)	
1	.4	.1	1:5	.1	1:7	Shake tubes well; keep at room temperature for 2 hours and read.
2	.25	.25 of 1:5	1:10	.1	1:14	
3	.25	.25 of 1:10	1:20	.1	1:28	
4	.25	.25 of 1:20	1:40	.1	1:56	
5	.25	.25 of 1:40	1:80	.1	1:112	
6	.25	.25 of 1:80	1:160	.1	1:224	
7	.25	.25 of 1:160	1:320	.1	1:448	
8	.25	.25 of 1:320	1:640	.1	1:896	
9	.25	.25 of 1:640	1:1280	.1	1:1792	
10	.25	.25 of 1:1280	1:2560	.1	1:3584	
11	.25	.25 of 1:2560 *	1:5120	.1	1:7168	
Control	.25					
12	.25			.1		

* Discard .25 cc. from last tube.

COLD HEMAGGLUTINATION TEST FOR PRIMARY ATYPICAL PNEUMONIA

1. Collect 5 cc. of blood from the patient in a sterile test tube. Separate the serum from the clot after the tube has stood at room temperature for one to three hours.

2. Collect 5 cc. of blood from a Group O individual and place in a centrifuge tube with 1 cc. of 3.8 per cent solution of sodium citrate. Mix, add 5 cc. of saline solution, and centrifuge. Discard the supernatant fluid and wash the corpuscles two more times with saline solution. After the final washing prepare a 1 per cent suspension of the erythrocytes in saline solution.

3. Place 8 small test tubes in a rack. In the first tube place 0.8 cc. of saline solution and 0.5 cc. in the remaining seven tubes.

4. To tube No. 1 add 0.2 cc. of serum (which need not be inactivated); mix thoroughly with a 1 cc. pipet, and make serial dilutions (1:5, 1:10, 1:20, 1:40,

1:80, 1:160, 1:320) and discard 0.5 cc. of diluted serum from the next to the last tube. The last tube contains only saline solution and is the corpuscle control.

5. To each tube add 0.5 cc. of the 1 per cent suspension of erythrocytes. This doubles the dilutions of serum so that they will be 1:10 to 1:640.

6. Shake the tubes to mix the erythrocytes thoroughly and place them in a refrigerator at 4° C. overnight.

7. Examine the tubes immediately on removal from the refrigerator, holding them in a nearly horizontal position over a well-illuminated white background. Shake each tube gently but quickly until all erythrocytes in the bottom have been dislodged. Record the titer in terms of the highest dilution showing agglutination. Reversibility of the reaction may be demonstrated by incubation in a water bath at 37° C. for one hour.

KAHN STANDARD FLOCCULATION TEST FOR SYPHILIS

1. Separate serum from the clot and centrifuge if necessary until entirely free of erythrocytes. Inactivate the serum in a water bath (56°C.) for 30 minutes.

2. For each serum arrange 3 clean test tubes (7.5 cm. in length and 1.2 cm. outside diameter). Place tubes in a rack, three deep.

3. Mix Kahn antigen by placing 1 cc. of antigen in a mixing vial and the required amount of 0.9 per cent saline solution (as indicated by titer on antigen bottle) in another vial, then pour the saline solution into the antigen and quickly pour from one vial to the other 12 times. Allow to stand for 10 minutes. The antigen suspension is reliable for only one-half hour after dilution. An antigen prepared of 0.1 per cent cardiolipin, 1.0 per cent lecithin and 0.025 per cent cholesterol has been found to behave like Kahn antigen both in its titration with saline solution and in its reactions with serum (*Am. J. Clin. Path. 18:* 364, 1948).

4. Shake the antigen well to insure an even mixture and then pipet antigen (use 0.25 cc. Kahn pipet) into the bottom of the tubes in the following amounts:

Back tube—0.0125 cc.
Middle tube—0.025 cc.
Front tube—0.05 cc.

5. Add 0.15 cc. of inactivated serum to each tube.

6. Set up the following controls at the same time the test is conducted. The same amounts of antigen are used:

No. 1—Add 0.15 cc. of 0.85 per cent saline solution to antigen in each of the three tubes.

No. 2—Add 0.15 cc. of known positive serum to antigen in each of the three tubes.

No. 3—Add 0.15 cc. of known negative serum to antigen in each of the three tubes.

7. The rack of tubes is shaken vigorously for 10 seconds to insure thorough mixing of the ingredients. Allow to stand for about 5 minutes (preferably not less than 3 and not more than 7 minutes) at room temperature.

8. Shake all tubes for 3 minutes.

9. Add saline solution to all the tubes in the following amounts:

Back tube—0.5 cc.
Middle tube—0.5 cc.
Front tube—1.0 cc.

10. Shake all tubes sufficiently to thoroughly mix the saline solution and antigen-serum mixtures.

FIG. 92. TYPES OF REACTIONS IN KAHN TEST.

(From *Kahn Test,* Williams & Wilkins Co., in Kolmer and Boerner: *Approved Laboratory Technic,* 4th ed., D. Appleton-Century Company, Inc.)

11. The tests are read immediately and again in 15 minutes (Fig. 92). The final result is the average of the first and second readings. A negative reaction shows homogeneous cloudiness with no visible floes. A ++++ reaction shows complete flocculation in a water-clear fluid; +, ++, and +++ reactions show degrees of flocculation and cloudiness of fluid varying from negative to ++++.

KLINE DIAGNOSTIC FLOCCULATION TEST FOR SYPHILIS

1. Prepare slides as follows: Microscope slides 2 x 3 inches as purchased are rubbed on both sides with Bon Ami paste (prepared by breaking up a cake of Bon Ami in a small quantity of hot water). As soon as the paste is dry (about 5 minutes) it is completely removed from the slide with a soft muslin cloth. For convenience, the slides covered with paste may be stuck to each other, allowed to dry, and cleaned at any time.

On clean slides for the heated serum tests, 12 paraffin rings, each with an inside diameter of 14 mm., are mounted as follows: A piece of soft iron wire

(No. 28) is wound twice tightly about a test tube (about 15 mm. in outside diameter) forming a double loop and leaving a double shaft about an inch in length. The two shafts are then twisted together to within a quarter of an inch of the free end. After removing the looped wire from the test tube, a piece of linen thread (No. 12) is started from the free end of the shaft after being fastened there by a single twist of the free ends. Three long turns are made, reaching the loop which is then tightly wound with the thread. The winding is continued up the shaft to the free end where it is fastened between the two ends of the wire by twisting them. The loop is then bent at right angles to the shaft. It is then reshaped by working the loop against the bottom of the test tube mentioned above. The shaft is then inserted into the handle of a teasing needle or into a straight hemostatic forceps. The paraffin rings are made by dipping the instrument into smoking paraffin (about 120° C.), draining quickly at one point and transferring the remainder to the glass slide. Paraffin ring makers smaller or larger than standard should not be employed. If the paraffin is too hot, it will spread too much and the chambers will be too small. Care should be exercised also that the wire end of the loop is sufficiently long to prevent transfer of paraffin on the bottom of the wooden handle to the glass slide.

2. An emulsion of antigen (supplied by the LaMotte Chemical Products Co., Towson, Baltimore, Md.) is prepared as follows: (a) Pipet 0.85 cc. of distilled water (*p*H about 6) into a small bottle; (b) holding the bottle at an angle, allow 1.0 cc. of a 1 per cent solution of cholesterol (Pfanstiehl) in absolute ethyl alcohol, pipetting with the left hand, to run along the side of the neck of the bottle while shaking or rotating the container fairly vigorously; (c) rotate the bottle gently from the neck for 20 seconds; (d) holding the bottle at an angle pipet 0.1 cc. of antigen against the side of the neck from a finely graduated pipet; (e) promptly stopper the bottle (preferably with a glass stopper) and shake vigorously for 1 minute; (f) add 2.45 cc. of 0.85 per cent solution of sodium chloride (CP or Merck) in distilled water (*p*H about 6) quite rapidly; (g) stopper and shake less vigorously than previously for 1 minute. The emulsion is best kept in a refrigerator (8° to 12° C.) and is satisfactory for use for at least 48 hours after preparation.

Just before use, place 1 cc. in a narrow test tube (12 mm. inside diameter) in a water bath at 35° C. (beaker of water in usual laboratory air incubator at about 37° C.) for 15 minutes.

The LaMotte Chemical Products Co. also supplies cardiolipin-lecithin antigen for this test. It is diluted and sensitized with a 1 per cent solution of cholesterol in the same manner as described, but the mixture is not heated at 35° C. for 15 minutes.

3. Inactivate clear serum (centrifuge if necessary to remove corpuscles) in a water bath at 56° C. for 30 minutes.

4. Into a ring pipet 0.05 cc. of heated serum.

5. Add 1 drop (about 0.008 cc.) of the antigen emulsion from a Wright capillary pipet or with a syringe fitted with a No. 26 needle.

6. Rotate the slide on a flat surface for 4 minutes.

7. Examine the results at once through the microscope at a magnification of

about 120 times (low-power 16 mm. objective, eye piece 12) with the light cut down as for the study of urinary sediments and reported in terms of pluses according to the degree of clumping and the size of the clumps (Fig. 93).

Any spilling from the chamber makes the reaction therein unsatisfactory and the serum concerned should be re-tested.

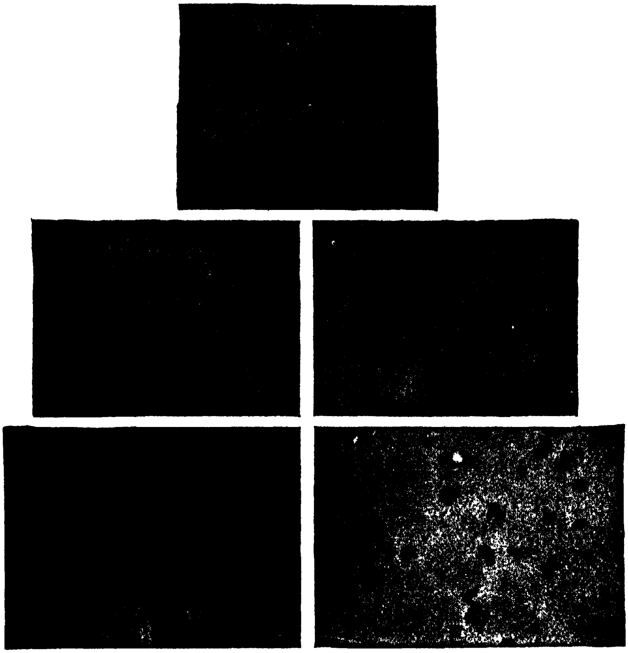

FIG. 93. THE KLINE MICROSCOPIC PRECIPITATION REACTION.

A, negative reaction; B, positive (+); C. positive (++); D, positive (+++); E. positive (++++). (Kolmer and Boerner: *Approved Laboratory Technic,* 4th ed., D. Appleton-Century Company, Inc.)

Occasionally there is atypical clumping with very strongly positive serums. Such irregular feathery clumping is not at all like the uniformly distributed small clumps of doubtful reactions with which they could be confused. It is advisable to dilute such serums with saline or negative serum, using one part of the serum in question and 1, 3 and 15 parts of saline or negative serum. The results with one or more of the diluted mixtures will be typical of the strongly positive reaction.

KOLMER COMPLEMENT FIXATION TEST FOR SYPHILIS

The *simplified test* is conducted with single amounts of serum or spinal fluid; the *quantitative* test employs five amounts of serum or spinal fluid. Both are equally satisfactory for diagnostic purposes but the latter is particularly useful when the test is being employed as a serologic guide in treatment. The technics herein described are the regular methods but for purposes of economy both tests can be conducted with one-fifth amounts of all reagents, with equally satisfactory results (for technic see *Am. J. Clin. Path. 12:* 109, 1942). Both of the tests may also be conducted with one-half volumes. The amounts of complement, antigen and other reagents required are in this way reduced by half. Some accuracy, however, may be sacrificed by using these reduced quantities, since the relative effects of measuring errors are increased.

General Technic. 1. Prepare *saline solution* by dissolving 8.5 gm. of dried, chemically pure sodium chloride and 0.1 gm. of pure magnesium sulfate in 1000 cc. of freshly distilled water. Filter through paper and sterilize in an Arnold sterilizer for 1 hour before use (not essential if immediately used).

2. Prepare *sheep corpuscle suspension* (indicator antigen) as follows: (a) In a clean quart-sized Mason jar place 30 cc. of a 10 per cent solution of sodium citrate in saline and 2 cc. of formalin. At the abattoir have the jar almost filled with *fresh* blood, screw on the top, mix well, and keep in a refrigerator (ordinarily fit for use for about 2 weeks). Or collect blood by bleeding from the external jugular vein of a sheep. (b) Filter a small quantity of blood through cotton or gauze into a graduated centrifuge tube. Add 2 or 3 volumes of saline solution, mix and centrifuge at high speed. Remove the supernatant fluid, add saline solution, mix by inverting several times, and centrifuge. Repeat once more for a third washing. Remove supernatant fluid and dilute 2 cc. of the corpuscle mass with 98 cc. of saline solution (2 per cent suspension). If the supernatant is not colorless on the third washing, the corpuscles are too fragile and should not be used. Place the corpuscle suspension in a refrigerator when not in use. Always shake before using to secure an even suspension, as the corpuscles settle to the bottom of the flask when allowed to stand.

3. Prepare *antisheep hemolysin* by giving a rabbit 5 or 6 intravenous injections of 5 cc. of a 10 per cent suspension of washed sheep corpuscles every 5 days. Bleed the rabbit 7 to 9 days after the last injection if a preliminary titration gives a unit of 0.5 cc. of 1:4000 or higher. Separate the serum and preserve with an equal part of best-grade neutral glycerine. Keep in a refrigerator.

4. Prepare *complement serum* as follows: (a) Under light ether anesthesia, remove 4 or 5 cc. of blood from the hearts of each of 3 or more large, well-nourished, nonpregnant guinea-pigs that have fasted for 12 hours. Number the tubes and allow firm clots to form. At the end of half-hour, break up the clots, and centrifuge. The serums may be pooled but it is advisable to test each separately and pool only those that are satisfactory. The technic of *pre-testing complement* is as follows: (a) Prepare a 1:30 dilution by diluting 0.2 cc. of serum with 5.8 cc. of saline solution; (b) in a series of 6 test tubes place 0.8, 0.6, 0.4, 0.8, 0.6 and 0.4 cc. respectively; (c) in each of the first three tubes place 0.5 cc. of diluted antigen carrying the test dose; (d) add sufficient saline solution to make the total volume 2.0 cc. in each tube, mix and place in a refrigerator at 6° to 8° C. for 15 to 18 hours, follow by water bath at 37° C. for 10 minutes; (e) add 0.5 cc. of a dilution of hemolysin carrying 2 units of 0.5 cc. of 2 per cent

suspension of washed sheep corpuscles; (f) mix and place in a water bath at 37° C. for 1 hour. All complement serums showing complete and sparkling hemolysis in all tubes are satisfactory. Otherwise, serums showing an equal degree of incomplete hemolysis in both the antigen tubes and tubes carrying no antigen may be used, but any serum showing a greater inhibition of hemolysis in the presence of antigen than observed in the tubes carrying no antigen, are unsatisfactory and should not be included in the pooling of serums. Guinea-pig serums found unsatisfactory may prove satisfactory at a later bleeding. *Keep the complement in a refrigerator when not in use.*

Commercially supplied dehydrated or dried complement serum is satisfactory. It should be restored to its original volume by dissolving in the prescribed amount of buffered diluent or distilled water. When *improved* Kolmer antigen (*Am. J. Clin. Path. 18:* 731, 1948) is employed, it is unnecessary to pre-test the complement. With this antigen it is, likewise, unnecessary to use egg albumin in spinal fluid tests for the prevention of prozone and falsely positive reactions.

5. *Titrate hemolysin* as follows: (a) Prepare a stock 1:100 dilution by diluting 2.0 cc. of glycerinized hemolysin with 94 cc. of saline solution and 4 cc. of 5 per cent phenol (will keep in the refrigerator for several months); (b) prepare a 1:1000 dilution by diluting 0.5 cc. of the 1:100 dilution with 4.2 cc. of saline solution; (c) in a series of 6 test tubes prepare dilutions ranging from 1:3000 to 1:10,000 as follows (usually a sufficient range):

No. 1—0.5 cc. hemolysin (1:1000) + 1.0 cc. saline (= 1:3000)
No. 2—0.5 cc. hemolysin (1:1000) + 1.5 cc. saline (= 1:4000)
No. 3—0.5 cc. hemolysin (1:1000) + 2.0 cc. saline (= 1:5000)
No. 4—0.5 cc. hemolysin (1:3000) + 0.5 cc. saline (= 1:6000)
No. 5—0.5 cc. hemolysin (1:4000) + 0.5 cc. saline (= 1:8000)
No. 6—0.5 cc. hemolysin (1:5000) + 0.5 cc. saline (= 1:10,000)

(d) Mix the contents of each tube thoroughly and transfer 0.5 cc. of each to six test tubes respectively; add 0.3 cc. of 1:30 complement (dilute 0.2 cc. of complement serum with 5.8 cc. saline solution), 0.5 cc. of 2 per cent sheep corpuscle suspension and 1.7 cc. saline solution. Mix each tube and incubate in a water bath at 37° C. for 1 hour. Read the unit of hemolysin. The *unit is the highest dilution of hemolysin giving complete sparkling hemolysis* in this period of time.

Two units are used in the tests. For example, if the unit is 0.5 cc. of 1:6000, two units equal 0.5 cc. of 1:3000. Prepare just enough hemolysin for the complement titration and the tests to be conducted. *Keep hemolysin and corpuscle suspension in the refrigerator when not in use.*

6. *Titrate complement* as follows: (a) Use the 1:30 dilution employed in the hemolysin titration; (b) in a series of 5 test tubes place 0.3, 0.35, 0.4, 0.45 and 0.5 cc. respectively; (c) dilute antigen so that the dose employed is 0.5 cc. of the same dilution as used in the complement fixation tests; (d) add 0.5 cc. of diluted antigen to each tube and sufficient saline to make the total volume 2.0 cc. in each tube; (e) mix and place tubes in a water bath at 37° C. for one hour; (f) add 0.5 cc. hemolysin (2 units) and 0.5 cc. of 2 per cent corpuscle suspension to each tube; (g) mix and place in water bath for one hour when the reading is made.

The smallest amount of complement just giving complete sparkling hemolysis is the *exact unit*. The next higher tube (0.05 cc. more) is the *full unit*. In conducting the complement fixation tests *two full units* are employed and diluted so as to be contained in 1 cc. as per the following example:

Exact unit:	0.3 cc.
Full unit:	0.35 cc.
Dose (2 full units):	0.7 cc.

To calculate the dilution to employ so that 1 cc. contains the dose of 2 full units, divide 30 by the dose: $\frac{30}{0.7} = 43$ or 1 cc. of 1:43 dilution of serum.

In preparing the dilution use *cold* saline solution. If all tubes show complete hemolysis take 0.3 cc. (No. 1) as the exact unit, as less complement falls below the absolute minimum and is likely to give incomplete hemolysis and prove unsatisfactory.

7. In the *simplified* tests it is not necessary to remove natural antisheep hemolysin from the serums to be tested. In *quantitative* tests this is advisable in order to secure reactions of maximum sensitivity as follows: (a) To each specimen of blood add a few drops of washed sheep corpuscle mass; (b) thoroughly mix with a wooden applicator (one for each specimen) and place in a refrigerator for at least 15 minutes; (c) centrifuge and separate the serums to labelled test tubes; (d) inactivate the plain or absorbed serums by placing the tubes in a water bath at 56° C. for *20 to 30 minutes*. Previously heated serums should be reheated for 5 minutes at 56° C. on the day of testing. No preparation of *spinal fluids* is required except centrifuging to remove blood corpuscles and other particulate debris. Heat all spinal fluids received through the mail, or stored for three or more days, at 56° C. for 15 minutes to remove thermolabile anticomplementary substances.

Simplified Test. 1. Arrange 2 test tubes (8.5 by 1.5 cm. outside diameters) for each *serum* and place 0.5 cc. of saline solution in No. 2. Add 0.2 cc. inactivated serum to each tube.

2. For each *spinal fluid* arrange 2 test tubes and place 0.5 cc. in each.

3. To the No. 1 tubes add 0.5 cc. of *improved* Kolmer antigen so diluted with saline solution as to carry the dose stated on the label. Calculate the amount of diluted antigen required. In preparing the dilution place the required amount of saline solution in a small flask and add the required amount of antigen drop by drop, shaking the flask after each addition of antigen. An antigen prepared of 0.03 per cent cardiolipin, 0.05 per cent lecithin and 0.6 per cent cholesterol may be diluted in the same manner and used in dose of 0.5 cc. of 1:150 dilution (*J. Ven. Dis. Inform. 29:* 166, 1948).

4. Allow the tubes to stand at room temperature for 10 to 30 minutes. If a longer interval elapses place the racks in a refrigerator.

5. Add 1 cc. of complement (2 full units) to all tubes.

6. Include the following controls: (a) *Antigen control* carrying 0.5 cc. of diluted antigen, 0.2 cc. saline solution, and 1 cc. (2 full units) of complement; (b) *hemolytic system control* carrying 0.7 cc. saline solution and 1 cc. of comple-

ment; (c) *corpuscle control* carrying 2.2 cc. saline solution. Controls of known positive and negative serums are optional but advisable, especially in the case of inexperienced workers.

7. Mix the contents of all tubes by shaking gently and place in a refrigerator at 6° to 8° C. for 15 to 18 hours.

8. Place tubes in a water bath at 37° C. for 10 minutes (not longer).

9. To all tubes, except the corpuscle control, add 0.5 cc. hemolysin carrying 2 units.

10. To all tubes add 0.5 cc. of 2 per cent corpuscle suspension (shaken up).

11. Mix the contents of each tube by gentle but thorough shaking and place in a water bath at 37° C. *Watch the serum, antigen and hemolytic system controls and 10 minutes after these show complete hemolysis (usually 25 to 30 minutes) remove the tests and make the readings.* In the case of those tests in which these controls are not completely hemolyzed, continue the water bath incubation for a total of one hour which frequently permits the reading of tests with serums and spinal fluids which are slightly anticomplementary.

12. The reactions may be reported as positive, doubtful, or negative. Or they may be reported as follows: strongly positive (++++ (4) or +++ (3) in the first tube); weakly positive (++ (2) or + (1) in the first tube); doubtful (± in the first tube); negative (— in the first tube).

Slightly *anticomplementary reactions* may be safely reported as follows: 4 ± = positive; 4 1 = positive; 4 2 = doubtful; 3 1 = doubtful; 3 ± = doubtful; 2 2 = negative; 1 1 = negative; 1 ± = negative; ± ± = negative. But it is always advisable to repeat the tests, especially in the case of doubtful reactions. Anticomplementary serums may be retested after they have been prepared by a modified Sachs method.

Quantitative Test. 1. For each *serum* arrange six test tubes and place in them the following amounts of saline solution respectively: 0.9, 0.5, 0.5, 0.5, 2.0 and 0.5 cc. To the first tube add 0.6 cc. of inactivated serum. Mix and transfer 0.5 cc. to No. 2 and 0.5 cc. to No. 6 (serum control). Mix No. 2 and transfer 0.5 cc. to No. 3. Mix No. 3 and transfer 0.5 cc. to No. 4. Mix No. 4 and transfer 0.5 cc. to No. 5; mix No. 5 and discard 2.0 cc.

2. For each *spinal fluid* arrange 6 test tubes and place 0.5 cc. of saline solution in Nos. 2, 3, 4, 5 and 6. Add 0.5 cc. of spinal fluid to Nos. 1, 2, and 6. Mix No. 2 and transfer 0.5 cc. to No. 3; mix No. 3 and transfer 0.5 cc. to No. 4; mix No. 4 and transfer 0.5 cc. to No. 5; mix No. 5 and discard 0.5 cc.

3. The balance of the test is conducted in exactly the same manner as the simplified test.

4. Reactions may be interpreted as follows:

(a) *Very strongly positive* when *complete fixation* (+ + + +) occurs in the third and fourth or fifth tubes. Examples 44444; 4444—; 4442—; 444— —; 344— —.

(b) *Strongly positive* when *complete fixation* (+ + + +) occurs in the second tube. Examples 4431—; 442— —; 342— —; 44— — —.

(c) *Moderately positive* when *complete fixation* (+ + + +) occurs in first tube only. Examples: 431— —; 42— — —; 4— — —.

(d) ***Weakly positive*** when ***partial fixation*** occurs in one or more tubes. Examples: 321— —; 21— — —; 1— — — —.

(e) *Doubtfully positive* when the reaction is ± in the first tube. Example: ± — — — —.

(f) *Negative* when there is complete hemolysis in all tubes. Example: — — — — —.

The method of recording and reporting a complement fixation test by this method is according to the following examples:

Quantitative reaction	= strongly positive (442 — —)
Serum 0.2 cc.	= + + + +
Serum 0.1 cc.	= + + + +
Serum 0.05 cc.	= + +
Serum 0.025 cc.	= —
Serum 0.005 cc.	= —
Serum 0.2 cc. (control)	= —

Quantitative reaction	= strongly positive (42 — — —)
Spinal fluid 0.5 cc.	= + + + +
Spinal fluid 0.25 cc.	= + +
Spinal fluid 0.125 cc.	= —
Spinal fluid 0.0625 cc.	= —
Spinal fluid 0.03125 cc.	= —
Spinal fluid 0.5 (control)	= —

Positive reactions may also be reported in *Kolmer units* in which the potency of any serum or spinal fluid is determined according to the formula S = 4D as used in the Kahn quantitative serum test, where S is the serum or spinal fluid potency in terms of units and D is the highest dilution giving a positive reaction. If a serum or spinal fluid gives a +, ++, +++ or ++++ reaction in the first tube only, it is considered as containing 1, 2, 3 or 4 units respectively; a positive reaction of any degree in the second tube = 8 units; a positive reaction of any degree in the third tube = 16 units; a positive reaction of any degree in the fourth tube = 32 units; a positive reaction of any degree in the fifth tube = 160 or more units. In this case still higher dilutions of serum may be tested if desired.

Slightly *anticomplementary reactions* may be safely reported as follows: 4 4 4 1 — — 2 = positive; 4 4 1 — — 1 = positive; 4 4 1 — — ± = positive; 3 2 — — — ± = positive; 4 4 1 — — 3 = doubtful; 3 2 — — — 1 = doubtful; 2 1 — — — ± = doubtful; 3 — — — — ± = doubtful; 1 — — — — ± = negative; 1 — — — — 1 = negative; — — — — — 1 = negative; 2 — — — — 2 = negative. But it is always advisable to repeat the tests, especially in the case of doubtful reactions. Anticomplementary serums may be retested after preparing them by a modified Sachs method.

Preparation of Reading Standards. (1) Heat tubes of hemoglobin solution (saved from the hemolysin titration) in a water bath at 56° C. for five minutes; (2) prepare a 1:6 dilution of 2 per cent corpuscle suspension by adding 5 cc. of saline solution to 1 cc. of suspension; (3) prepare reading standards by mixing the hemoglobin solution and cell suspension in the following proportions:

Corpuscle Suspension cc.	Hemoglobin Solution cc.	Equivalent Complement Fixation %	Record
3.0		100	++++
1.5	1.5	50	+++
0.75	2.25	25	++
0.3	2.7	10	+
0.15	2.85	5	±
	3.0	0	—

Modified Sachs Method for Anticomplementary Serums. 1. Heat 0.5 cc. of serum at 55° to 56° C. in a water bath for 15 minutes. If the serum has been previously heated and is being retested, reheat for 5 minutes.

2. Add 4.1 cc. of accurately titrated N/300 hydrochloric acid and mix thoroughly.

3. After standing 1/2 hour at room temperature, centrifuge thoroughly, save the supernatant fluid and discard the sediment.

4. To the supernatant fluid add 0.4 cc. of 10 per cent sodium chloride solution. The acid is fixed by the precipitate of globulin; hence neutralization is unnecessary. This gives a 1:10 dilution of original serum ready for testing.

The *simplified test* is conducted as follows:

1. Arrange two rows of 3 test tubes each (the rear tubes are serum controls).

2. Place 0.5 cc. of saline solution in third tube of the first row and 0.5 cc. in each of the three tubes of the rear row.

3. Place 1 cc. of prepared serum (1:10) in the first tube of the front row and 1 cc. in the first tube of the second row.

4. Place 0.5 cc. of prepared serum (1:10) in the second tube of the front row and exactly the same amount (0.5 cc.) in the second tube of the rear row.

5. Place 0.5 cc. of prepared serum (1:10) in the third tube of the first row; mix and transfer 0.5 cc. to the third tube of the rear row.

6. The three tubes of each row now carry 0.1, 0.05 and 0.025 cc. of serum respectively.

7. Add improved Kolmer antigen (0.5 cc. of proper dilution) to each tube of the *front* row.

8. Mix the contents of all tubes and allow to stand at room temperature for 10 to 30 minutes.

9. Complete the test in the usual manner.

10. On completion of the test all of the tubes of the rear row should show complete hemolysis. However, the first tubes carrying 0.1 cc. and sometimes the second tubes carrying 0.05 cc. of serum, of both rows, may show slight inhibition of hemolysis. With negative serums the corresponding front tubes show the same degree of inhibition of hemolysis and if the degree of inhibition is slight, a negative report may be rendered. With positive serums inhibition of hemolysis is much more marked in the tubes of the front row. It is advisable to report reactions as positive, doubtful, or negative.

The *quantitative test* is conducted as follows:

1. Arrange two rows of 5 test tubes (the rear row are serum controls and receive no antigen).

2. Place 0.5 cc. of normal saline solution in tubes 3 and 4, and 2 cc. in tube 5 of both rows.

3. To both rows add 1 cc. of prepared serum to tube No. 1 and 0.5 cc. to tubes Nos. 2 and 3. In each row mix No. 3 and transfer 0.5 cc. to No. 4; mix No. 4 and transfer 0.5 cc. to No. 5; mix No. 5 and discard 2 cc.

4. Add improved Kolmer antigen (0.5 cc. of proper dilution as used in the regular test) to each tube of the front row and 0.5 cc. of saline solution to each tube of the rear row. Allow to stand at room temperature for 10 to 30 minutes when 2 full units of complement are added to all tubes of both rows and the balance of the test completed in the usual manner.

5. The tubes of the front and rear rows carry 0.1, 0.05, 0.025, 0.0125 and 0.0025 cc. of serum respectively.

6. On completion of the tests the readings are made in the same manner as in the simplified test.

Method for Anticomplementary Spinal Fluids. 1. Heat the spinal fluid for 15 minutes in a water bath at 56° C.

2. Arrange two rows of five test tubes each and number 1 to 5.

3. Place 0.5 cc. saline solution in tubes Nos. 2, 3, 4 and 5 of both rows.

4. In both rows add 0.5 cc. spinal fluid to Nos. 1 and 2. In both rows mix No. 2 and transfer 0.5 cc. to No. 3; mix No. 3 and transfer 0.5 cc. to No. 4; mix No. 4 and transfer 0.5 cc. to No. 5; mix No. 5 and discard 0.5 cc.

5. Add 0.5 cc. of proper dilution of improved Kolmer antigen to each tube of the first row.

6. Add 0.5 cc. of saline solution to each tube of the second row.

7. Add 1 cc. of diluted complement (2 full units) to all tubes of both rows, mix well, place in refrigerator for 15 to 18 hours at 6° to 8° C., and complete the test in the usual manner.

8. Interpret the reactions according to the following examples:

First row:	4	4	4	1	—	
Second row:	4	1	—	—	—	Positive
First row:	4	3	2	—	—	
Second row:	4	1	—	—	—	Positive
First row:	4	1	—	—	—	
Second row:	1	—	—	—	—	Positive
First row:	4	4	2	—	—	
Second row:	4	2	1	—	—	Negative
First row:	4	2	—	—	—	
Second row:	4	1	—	—	—	Negative
First row:	3	2	—	—	—	
Second row:	3	1	—	—	—	Negative

As discussed in Chapter 16, agglutination tests are of value as aids in the diagnosis of many diseases, especially typhoid and paratyphoid fevers, brucellosis, tularemia, infectious jaundice, typhus fever, Rocky Mountain spotted fever, etc. Macroscopic or microscopic tests may be conducted but the former are preferred.

Antigens are prepared by making an even suspension of young cultures of the micro-organisms, so diluted with saline solution that news print can just be read through the final test tube suspension. For macroscopic tests with antigens of motile bacilli two antigens are generally employed. One is prepared with formalin, designated *H or flagellar antigen* which gives large flaky agglutination. The second is prepared with alcohol, designated *O or somatic antigen* which gives small granular agglutination. Stock antigens may be prepared and kept in heavy, sterile suspensions and diluted to proper strength as required for test.

Macroscopic Test. 1. Place a series of 10 small test tubes in a metal rack.

2. Add 0.9 cc. of saline solution to No. 1 and 0.5 cc. in each of the remaining tubes.

3. Add 0.1 cc. of serum to No. 1 and mix (= 1 cc. of 1:10 serum).

4. Transfer 0.5 cc. from No. 1 to No. 2; mix and transfer 0.5 cc. to No. 3 and so through the remaining tubes to No. 9; mix No. 9 and discard 0.5 cc. Tube No. 10 receives no serum and is the antigen control.

5. The series may extend to additional tubes if necessary to extend beyond titer of the serum.

6. Add 0.5 cc. of bacterial antigen to all tubes; mix thoroughly.

7. The final serum dilutions are now 1:20 in tube No. 1 to 1:5120 in tube No. 9.

8. The optimum incubation is 50° to 55° C. in a water bath; 37° C. or even room temperature is advisable for large-clumping antigens of motile bacilli when 50° to 55° is impossible. For small-clumping antigens 37° C. is not desirable.

For H antigens the incubation may be 2 to 4 hours at 50° to 56° C., 8 hours at 37° C., or 24 hours at room temperature.

For O antigens the incubations should be 8 to 24 hours at 50° to 55° C.

9. A preliminary reading of agglutination may be made at the end of 2 hours, and a final reading at the end of 24 hours.

10. The control should show no clumping. The negative (−) tubes show no clumping. The positive tubes show clumped and sedimented organisms. The degree of agglutination should be recorded separately for each tube as follows:

+ + reaction = completely clumped and sedimented.
+ reaction = half clumped and sedimented.
± reaction = less than half clumped and sedimented.

11. The titer of the serum is the highest dilution which shows complete (+ +) agglutination. Notation should be made whether agglutination is floccular or granular in type.

Microscopic Test. This test is not generally employed except in the conduct of the Widal test for typhoid or paratyphoid fever.

1. Place 1 drop of serum in a watch crystal or small test tube. Add 9 drops of saline solution and mix (= 1:10 dilution).

2. Transfer 5 drops to a second tube; add 5 drops of saline solution and mix (= 1:20 dilution).

3. Similarly third, fourth, or more dilutions may be prepared if necessary.

4. Place 1 loopful of each dilution on separate cover glasses.

5. Add 1 loopful of living broth culture of organisms to each of the serum dilutions and mix. The final dilutions are now 1:20, 1:40, 1:80, etc.

6. Place each cover glass over a ringed concave slide for hanging drop preparations.

7. Similarly, prepare a control having only saline solution and broth culture.

8. Let stand for 1 hour at room temperature.

9. Examine each slide with the high-dry objective for loss of motility and clumping of the bacteria, compared to the control.

10. Record as positive the highest titer giving microscopic clumping.

Rapid Slide Agglutination Test. This test is presumptive only and is generally employed as an aid in the final identification of typhoid bacilli, meningococci, typing of hemolytic streptococci, etc., recovered in cultures.

1. Prepare a 1:50 dilution of a known high titer antiserum.

2. Place 1 drop of this on a slide. Place another drop of saline solution nearby.

3. Emulsify a loopful of bacteria from a blood or plain agar culture with each drop.

4. Let stand at room temperature for 15 to 60 minutes.

5. Observe under low-power of the microscope for the presence or absence of clumping in the mixture of bacteria and antiserum, and the smoothness of the emulsion (absence of clumps) in the saline solution control.

TESTS IN PNEUMOCOCCUS PNEUMONIA AND MENINGITIS

Recovery from pneumococcus pneumonia, meningitis, and other infections is largely dependent upon the presence in the body of adequate amounts of type-specific antibody. In the presence of inadequate amounts of the latter the soluble specific capsular polysaccharide of the infecting pneumococcus is not neutralized and may occur in the serum, cerebrospinal fluid or urine. Under the conditions, tests for this type-specific polysaccharide or its antibody are of value in relation to gauging the severity of infection and treatment with penicillin and/or sulfadiazine, with special reference to pneumonia and meningitis.

Skin Test. This test, as described by Francis (*J. Exper. Med. 57:* 617, 1933), is conducted by injecting intradermally 0.1 cc. of a sterile protein-free solution carrying 0.01 mg. of the type-specific capsular polysaccharide. Positive reactions, which develop within 15 to 30 minutes, occur at about the time of recovery and are apparently the result of interaction between antibody and the polysaccharide. Therefore they are indicative of the presence of adequate amounts of antibody and of good prognostic import. Negative reactions, however, are indicative of severe infections and the presence of inadequate amounts of antibody in the blood and tissues, with the need for the administration of adequate amounts of sulfadiazine and type-specific immune serum.

Precipitin Test. The precipitin test for the detection of the type-specific polysaccharide in the blood serum, urine or cerebrospinal fluid (in meningitis) is usually employed. It may be conducted as follows:

1. Place 0.2 cc. of clear rabbit type-specific antipneumococcus serum in a small test tube.
2. With a fine capillary pipet, carefully overlay with 1 cc. of perfectly clear spinal fluid, urine or blood serum.
3. A positive reaction consists in the formation of a white ring of precipitate at the line of contact. If occurring within ten minutes at room temperature, it is indicative of a severe infection with the presence of free type-specific polysaccharide in the fluid being tested. The significance of a positive reaction, therefore, is just the opposite to that of a positive skin reaction in relation to prognosis and treatment.

PRECIPITIN TEST IN MENINGOCOCCUS MENINGITIS

The presence of meningococcus polysaccharide in the cerebrospinal fluid is also indicative of a severe infection and the need for adequate treatment with penicillin and/or sulfadiazine. A test for the polysaccharide may be conducted as follows:

1. Place 0.2 cc. of perfectly clear polyvalent or type-specific antimeningococcal serum in a small test tube.
2. With a fine capillary pipet, carefully overlay with 1 cc. of perfectly clear centrifuged spinal fluid.
3. A positive reaction is indicated by the formation of a white ring of precipitate at the line of contact. If occurring within ten minutes, at room temperature, it is usually indicative of a severe infection with the need for adequate sulfadiazine and serum therapy.

TESTS FOR HEMOPHILUS INFLUENZAE INFECTIONS

Hemophilus influenzae belonging to type B is also known to produce a type-specific soluble polysaccharide. Recovery from severe infections with the bacillus, as in influenzal meningitis and pneumonia, is due largely to specific antibody supplemented by sulfadiazine and streptomycin or penicillin therapy. As shown by Alexander and her colleagues (*J. Pediat. 20*: 673, 1942), whether or not adequate amounts of antibody are present in the blood may be determined by the capsular swelling or skin tests. The presence of the polysaccharide in the cerebrospinal fluid, blood or urine is indicative of severe infection with inadequate amounts of antibody in the body and may be detected by a precipitin test.

Capsular Swelling Test. This test may be employed not only for a determination of the specific type of *H. influenzae* producing infection but also for the detection of antibody in the blood serum in relation to prognosis and specific therapy. It may be conducted with concentrated suspensions of nasopharyngeal mucus, sputum or cerebrospinal fluid if sufficient numbers of the bacilli are present. Otherwise, suspensions of the bacilli harvested from four to six-hour cultures in Levinthal broth and preserved with 0.4 per cent formalin should be employed.

1. For purposes of typing the test is conducted with type-specific anti-influenzal serum in the same manner as that used for typing pneumococci, as described on page 1076.

2. For the purpose of determining whether or not there is an excess of free antibody, the test is conducted with a 1:10 dilution of the patient's serum. Capsular swelling is regarded as indicative of the presence of sufficient antibody in the blood.

Skin Test. This test is conducted by injecting 0.1 cc. of a 1:5000 dilution of type B *H. influenzae* polysaccharide intracutaneously (Alexander). A positive reaction, indicative of an excess of antibody in the patient, is of the immediate type developing within five to ten minutes and remaining for approximately thirty minutes. It is characterized by a wheal with extrusion of pseudopods.

Precipitin Test. This test may be conducted with blood serum, urine or cerebrospinal fluid as follows:

1. Place 0.2 cc. of clear type B anti-influenzal serum in a small test tube.

2. With a fine capillary tube, carefully overlay with 1 cc. of perfectly clear fluid to be tested.

3. The appearance of a white ring of precipitate at the line of contact within ten minutes, at room temperature, is believed to indicate a severe infection with insufficient antibody in the body for the neutralization of the excess polysaccharide shown in the cerebrospinal fluid, serum or urine being tested.

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