MICROBIOLOGY AND MAN

Being an account of the diverse properties and characteristics of microorganisms, a description of the various tools and techniques for their handling, and an inquiry into their subtle relationships to everyday life.

By

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To

M. W. B.

PREFACE TO SECOND EDITION

The first edition of this text, presented six years ago, was designed for students seeking a general knowledge of microbiology. The same design is preserved in this edition. We have found most college sophomores and upper classmen interested in obtaining a better understanding of the role of microorganisms in the scheme of things and positively eager for information concerning their relation to health and disease. This interest serves to motivate them and sharpen their desire for understanding the methods, techniques, possibilities, and limitations of this branch of biological science.

In this edition the taxonomic material is deleted, having proved to serve but little purpose here. New illustrations and six new chapters have been added. In our choice of additional subject matter we have again chosen material that lends itself to the teaching of basic principles. The chapter on bacteriological warfare, for example, is designed to serve as an illustration of how this phase of bacteriology might be developed.

The author wishes to thank the many who have generously permitted the use of illustrative material. Many of the photomicrographs were obtained from Dr. Harry Morton of the Committee on Visual Instruction in Microbiology of the Society of American Bacteriologists.

J. B.

PREFACE

"The proper study of mankind is man."—It is a study of such tremendous scope that it is impossible to deal with it as a whole. We must, perforce, separate it into broad fields, subdivide these, and examine the small parts bit by bit from various points of view and by all the methods at our disposal. But, while so doing, we must be careful not to magnify the importance of the part nor to lose sight of its relation to the whole.

This book tells about the part played by microorganisms in the environment of man and it tells of man himself, for in these pages I have tried to show not only what microorganisms are and how they behave, but also how we know what they are and how we know how they behave.

It would seem that the intellectually curious would like accurate information concerning those living forms too minute to be seen with the naked eye, yet without which life as we know it could not exist—organisms which represent the first living forms to appear on earth; organisms present in the air, water, and soil; organisms capable of transforming atmospheric nitrogen and making it available for plants; organisms that can tear down the plant and animal residues; and organisms producing the deadly diseases of man, animals, and plants.

It would seem that the intensely practical who have no concern with philosophic considerations would want accurate information about those living organisms that determine soil fertility, destroy foods and crops, kill livestock and man, and, when under control or 'domesticated,' may be used in the preparation of bread, dairy products, beverages, various other food stuffs, and many articles of commerce.

It would be fine if the information necessary for an understanding of these microorganisms could come to the student largely through his own observations but, since this is impossible, it is fortunate that there is available a large store of facts and interpretations gathered over a long period of years by countless experienced observers. Upon the author of a text book falls the burden of selecting from this vast store of information what is pertinent to his objectives. We find a score of texts in microbiology designed for the beginning student and as many more for the advanced. Some are written for students who intend to become microbiologists, some for those who specialize in fields which lean heavily upon microbiology: agriculture, home economics, medicine, nursing, and veterinary medicine; and some for those who desire what may be described as a 'cultural' knowledge of the field.

This book is designed as an elementary text for the student who plans to take but one or two courses in microbiology. It is intended to serve

as a basis for an understanding of the part played by microorganisms in everyday life. It is a guide for learning as well as a source book of facts.

To understand the science of microbiology and to grasp its potentialities and limitations, it is necessary to appreciate how the microbiologist works, what tools and techniques he uses, what questions he can answer, and the terms in which his answers are couched. Consequently emphasis is placed on the methodology used to obtain information and on the inferences that can be drawn from the information obtained. Microbiology, thus presented, should sharpen the intellect and discourage the tendency to accept apparently established principles without critical analysis. It should give the student facts, and, what is far more important, a training in methods for obtaining facts. It should prepare him for intelligent action.

For the most part the material in the book is arranged in the conventional manner. The introduction to infectious disease is fairly complete since experience acquired in teaching several thousand students suggests that after they are well grounded in the principles of infection, immunity, and epidemiology, they can deal more intelligently with specific diseases. The order in which the diseases are taken up is based, in general, on the manner of transmission of the infectious agent, and on the site of the disease rather than in accordance with the classification of the microorganism. The section on food, milk, water, and sewage is placed at the last because it is felt that the public health aspects of these subjects cannot be appreciated fully until the student has acquired a background in the principles of infection, immunity, and epidemiology and a knowledge of the diseases involved. In many instances I may have written too much and in others too little. It has been my aim to give meaning to facts and not merely to state them.

I wish to express my appreciation to the following for illustrative material: to Robert M. Coffin of the Department of Fine Arts at Ohio State University for preparing many illustrations and charts and for advice on others, to Dr. Marshall W. Jennison of The Massachusetts Institute of Technology for his photograph of a sneeze, to Dr. Stuart Mudd of the University of Pennsylvania for permission to reproduce his electronic micrograph, to Louis Dublin of the Metropolitan Life Insurance Company for selected charts and tables from *Twenty-Five Years of Health Progress*, and to many others to whom due acknowledgement is made in the text.

It is impossible to acknowledge in detail the help received from others but I want to express my indebtedness to Dr. Casper I. Nelson of the North Dakota State Agricultural College for opening up the field of bacteriology to me and for his continued interest and sympathetic counsel;

to Dr. N. Paul Hudson, Chairman of the Department of Bacteriology at Ohio State University, for his stimulating teaching, his friendly advice, and his conscientious criticism of the manuscript; and to my colleagues: Dr. Grant L. Stahly for criticising the chapters on morphology and physiology, Dr. Oram C. Woolpert for reading the section on common infectious diseases, Dr. W. A. Starin for advice on matters concerning infection and immunity, Dr. Harry Weiser for helpful suggestions on the chapter on milk and dairy products, and Dr. Frank Holtman for similar service in the chapters on food, water, and sewage. And, finally, I am especially and deeply indebted to Dr. Floyd S. Markham for his generous encouragement and constructive criticism.

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Section I

FUNDAMENTALS OF MICROBIOLOGY

Chapter I

BY WAY OF INTRODUCTION

This book deals with living organisms that are too small to be seen with the naked eye. It tells how they were discovered, how they are studied, what they look like, how they grow and reproduce, how they affect our everyday life, and how we can control them. Because these organisms are so small that they can be seen only with the aid of a microscope (Gr. *micro* meaning small and *scope* meaning to view) they are called microorganisms or microbes. The word "germ" (Latin *germen* meaning sprout or bud) is frequently used synonymously with microbe. Its popular connotation is such that it should probably be limited in use to indicate those forms of microorganisms producing disease. The science which deals with microorganisms collectively is called microbiology or, literally, the study of the life of the small.

There are several quite distinct kinds of microorganisms differing in habitat, growth requirements, and morphological details. Some, as for example, the molds, yeasts, and bacteria, are plants; the protozoa are single-celled animals; and the rickettsiae and viruses cannot be placed with certainty in either category. Not only do the microorganisms differ widely, but the methods used in studying them are also somewhat different. Microbiology, consequently, is divided into several sciences each of which is named according to the kind of organism with which it is primarily concerned. The study of molds is called mycology; of protozoa, protozoology; and of bacteria, bacteriology. However, the term bacteriology as used here also includes the study of rickettsiae and viruses and, for practical reasons, of certain molds, yeasts, and a few protozoa.

Microorganisms are associated in the minds of many people with disease. In subsequent chapters we shall try to explain how some of them do cause disease and death, how the body defends itself against them, and how the microbes get from the sick to the healthy. But only a few of the microorganisms produce disease. Most of them are harmless and many are absolutely essential in the economy of Nature. We shall try to relate many common everyday phenomena of the field and stream, the stables, and the home to the activity of helpful microorganisms. The truth of the matter is that for every kind of germ that causes disease, and there are many, hundreds of other kinds are performing functions essential to the

existence of their biological brothers higher up in the tree of life. Indeed, many of the biological and chemical transformations brought about by the lowly microbes are extremely complex and impossible to duplicate even in the most modern laboratory.

DEVELOPMENT OF MICROBIOLOGY

The science of microbiology is young and its span, although historically short, is filled with important and dramatic events. These cannot all be fully appreciated without some knowledge of the science, but today's microbiological thinking may be better understood if we pass in review some of the factors and circumstances attendant upon its birth and development.

Science is never static. It is continually growing, developing, and pushing into new areas. In order to appreciate its possibilities for growth and the circumstances affecting its growth, it is well to recall two factors that greatly influence its development. The first of these might be called the "intellectual climate." At any one moment the knowledge and opinions of the time determine to a large extent the inferences drawn from observations and the applications that may be made from them at that time. This intellectual climate affects the growth of ideas even as our physical climate affects the growth of plants. The second factor influencing the advancement of scientific knowledge has to do with the development of mechanical devices, or tools, and the methods, or techniques. In the development of microbiology the intellectual climate and the available tools and techniques have determined the pace.

No doubt the possibility that there might be animals and plants too small to be seen occurred to the first men who speculated on the nature of the universe. Many of the ancient writers entertained such ideas but, like so many theories based upon pure speculation, these contributed little to a better understanding of the world in which they lived. Proof of the existence of microorganisms had to wait until the chance observations of a Dutch lens grinder by the name of Antony van Leeuwenhoek (1632–1723). There were others, such as the encyclopedic German priest, Anthanasius Kircher, and the ingenious Englishman, Robert Hooke, who preceded Antony van Leeuwenhoek in the field of microscopy; but it was this industrious Dutchman who devoted himself unsparingly to exploring the world with microscopes. Leeuwenhoek was not a professional lens grinder nor even a scientist. He was an amateur in the true sense of the word for he loved to make better and better lenses so as to penetrate deeper and deeper into the mysteries of the commonplace things that lay about him. Like Hooke he examined the flea and the louse but he was not satisfied to see insects made huge only. He took them apart and studied their internal structure under his lens and made many discoveries of the first order.

He carried the work of the great William Harvey to its logical conclusion by demonstrating under the microscope how blood passes from the arteries into the veins through a vast capillary network. Although Leeuwenhoek was not the first to discover spermatozoa in human seminal fluid, he studied the sperm of many species and was much impressed with the fact that the spermatozoa of the ox and of the mouse were so alike in size. He even commissioned one of his sea-going friends to bring him some of the sperm of a whale so that he could compare it with other species. But not often

FIG. 1. Replica of Leeuwenhoek's microscope made by Bausch and Lomb Company. Photograph by Charles Mooney.

did he go far afield to get material to examine, for there were wonders enough in what was close at hand. In wine and beer and vinegar he discovered not only perfectly shaped tiny crystals but also the little globules we now know as yeast cells. He thought perhaps sharp little crystals such as he saw in wine might be responsible for the way pepper bit his tongue so he soaked some pepper corns in water and looked at the juice under his best lens. He found the fluid teeming with minute bodies even smaller than the yeast cells of wine and beer. These were bacteria—he referred to them as "wee beasties" or "animalculae." He found them

in scrapings from his teeth, in pond and rainwater, in the discharges of his own bowels and of the bowels of animals. In fact, almost everywhere he looked he found bacteria and other microorganisms. He was fascinated by their minuteness and the fact that they were alive.

All of these things and many more Leeuwenhoek saw and wrote about in the long chatty letters he sent to his friends in the Royal Society of London where he had been introduced by his great countryman Reignier De Graaf. The gentlemen of the Royal Society, which then included Samuel Pepys, Isaac Newton, Christopher Wren, and Robert Boyle, to name but a few illustrious ones, were impressed and called for more, but, for the most part, they were too busy with their own diverse fields of interest to make microscopes and to observe these wonders for themselves.

Leeuwenhoek did not concern himself with the implications of his discoveries. He was a pioneer in the field of microscopy and of the tremendous significance of what he saw he had no inkling. When he died in 1723 probably no one alive had seen half so much of the microscopic world. Had he lived a hundred years later he would have had followers and imitators everywhere. But the intellectual climate of Leeuwenhoek's day was a stormy one. The brilliant observations and discoveries he made were snowed under by the profusion of discoveries made by others in all parts of Europe and in all fields of science. The answers to "whats" were coming so fast that few tarried long enough to ask or learn "how."

Discoverers of new things deserve much credit but it is to those who stay with a thing, study it under various conditions, and elucidate its nature, its functions, and its usefulness that we are most indebted. Genuine progress is made most rapidly when several individuals attack the same problem, for science is essentially a cooperative endeavor. When two or more investigators become involved, differences of opinion are likely to occur. Then new and more careful experiments must be devised to prove or disprove a point and with each new experiment new facts come to light and new problems are created. New problems call for new tools, new techniques, and new methods of approach. Leeuwenhoek had fine lenses but missed the stimulation of competition because he did not make microscopes for other people and was too suspicious to teach others how to use them. The professional lens grinders either lacked his skill in making lenses of short focal distance or there was no demand for them. At any rate Leeuwenhoek's far-ranging spade work in the field of microbiology was neglected and relatively unproductive until around the close of the eighteenth century when experimental science slowly began to find its legs and make progress.

The problem that gave the first real impetus to microbiology was an old one—the origin of life. Before Aristotle, the great Greek naturalist, and

down through the ages after him, the field of natural history was filled with many bizarre ideas about the origin of living things. In the case of cultivated plants and domestic and some wild animals the answer was fairly simple and obvious. But the more removed from intimate human experience and the smaller in size the species were, the more fantastic were the explanations of their origin. Aquatic plants and animals were thought to be generated by some mysterious force operating on the mud along the banks of lakes and streams where frogs and crabs could be seen crawling up out of the bottom soil. Burrowing rodents and snakes supposedly arose in the same fashion in the forests and fields. It was a common belief that decaying meat generated maggots and that lice and fleas were engendered by sweat. The great chemist, van Helmont (1652), gave this formula for the production of mice: "Place some dirty rags together with a few grains of wheat or a piece of cheese in a dark place and in a few days they will be transformed into mice." Isaac Walton in the *Compleat Angler* notes that pickerel may come into being either from other pickerel or from pickerel weeds!

William Harvey (1578–1667), who demonstrated the circulation of the blood, taught, on the basis of animal dissection and the study of incubating hen's eggs, that all life came from the egg which is produced by the parent species. This idea was generally accepted only insofar as it was easily demonstrable. Probably many people had seen insect eggs but very few had ever seen an insect lay them or watched the young hatch and develop into mature insects. In the popular mind there was no connection between the egg and the adult, and the idea of spontaneous generation, that is, of the generation of life out of dead and inert matter, persisted through the ages. In fact the theory of spontaneous generation was abandoned only after a long and bitter struggle which lasted until late into the nineteenth century. It was upon facts established during the wordy arguments and sweaty experiments that characterized this controversy that modern microbiology was founded—the only experimental science, by the way, that began with attempts to answer the philosophical question of how life originates. In justice to the protagonists of the spontaneous generation theory it should be pointed out that the ubiquity of bacteria, their rapid multiplication rate, and the great resistance of bacterial spores to heat made belief in spontaneous generation difficult to disprove.

The simple experiments performed by an Italian physician, Francisco Redi (1626–1697) won one of the first scientific skirmishes with this theory. Redi put some pieces of meat in an open vessel and covered it with fine gauze to protect the meat from flies. A similar but uncovered jar of meat was also prepared. In both cases the meat decayed, but only in the open vessel did maggots appear and flies emerge. Redi saw flies

hover over the protected meat and even observed them lay eggs on the gauze but there was no spontaneous generation of maggots or flies in the meat beneath. Instructive and conclusive as this experiment was, it did not dispose of the theory of spontaneous generation. It did not convince even Redi himself that forms other than flies could not arise spontaneously. He went on believing, for example, that intestinal worms and gall gnats were born without parents. The life cycles of these creatures were a little too complex to be discovered by anything as simple as tying a bit of gauze over the mouth of a glass jar.

It was some years later (1776) that another Italian, Abbé Spallanzani, first battled the theory of spontaneous generation in the realm of microbiology. He was unconvinced by the reports of Joseph Needham and the French naturalist Buffon that bacteria or animalculae arose spontaneously from the dead substances in boiled mutton broth. Needham's experiments had been carefully performed but Spallanzani improved upon them. Instead of closing the mouths of his flasks of broth with corks, he melted down the necks in a blast lamp and sealed them so that nothing could get into them—not even air. Needham had boiled his flasks for only a few minutes; Spallanzani boiled his for hours. Thereafter, no matter how long he left these flasks of broth about, he found, when he opened them, that the broth was free of any signs of life, microbic or otherwise. Needham and Buffon claimed that Spallanzani had so devitalized the broth by prolonged boiling that it could not support the growth of animalculae. The Italian proved them wrong by opening a flask and allowing it to stand exposed to the air for a day or two. The broth grew cloudy and, when examined under the microscope, was seen to be teeming with bacteria. Spallanzani then thought to settle the argument by showing that microbes came from other microbes just like themselves. This he did by diluting some broth containing bacteria with clean broth to such an extent that when a tiny drop was placed under his microscope lens he could see only one or two bacteria in the whole drop. He watched the microorganisms elongate, then pinch in two and separate. Each of the two resultant organisms proceeded to do the same thing and in a few hours the drop of broth before his lens was crowded with bacteria. This uncomplicated and elementary reproductive process, called binary fission, is employed by all bacteria and many other microbes. Spallanzani was probably the first to observe it. But Spallanzani's work was not hailed for the great discovery it really was. It was forgotten and people went on arguing about spontaneous generation. Thus adherents of the theory forced their opponents to devise better and better experiments and, as the controversy continued, a fairly respectable body of facts about microorganisms and their activities accumulated.

Pasteur (1822–1895) performed many brilliant and telling experiments during his controversy with Pouchet and other proponents of spontaneous generation but his less skillful adversaries had great difficulty in reproducing his results, so that many of them remained unconvinced. They boiled their extracts, infusions, and broths and bacteria still appeared. It seemed ridiculous to them to presume that any living thing could survive such high temperatures, so the microbes that grew in their preparations, they believed, must have been generated spontaneously out of the dead constituents of the fluid in the flasks.[1]

Finally the mystery was solved and the ghost of spontaneous generation laid by John Tyndall, an English physicist. Tyndall's experiments were primarily concerned with the scattering of light by dust particles in the air and he indirectly became interested in the presence of microorganisms on such floating matter in the atmosphere. He observed that open tubes of broth remained free of bacteria when exposed in a closed glass chamber through which a strong beam of light could be passed without revealing the presence of dust particles. He thus concluded that air free of visible suspended matter as shown by the light beam was also free of bacteria.

Tyndall's real contribution, however, came when he ran into difficulty in preparing sterile infusions to put into the tubes of his cabinet. Sometimes his broths remained free of microbes before he exposed them in the chamber and sometimes they did not. After a great deal of painstaking work he discovered that certain bacteria could be killed very readily by heat when they were actively multiplying in broths. On the other hand when they were in a resting state, as they were, for instance, on old dry hay or vegetables, or in the dust of his laboratory shelves, these microbes were remarkably resistant to heat and survived five or six hours of boiling. It was difficult to believe that any living thing could withstand boiling temperatures for so long but here were structures which could be identified under the microscope and whose development could be watched inside the bacterial cell. After producing vegetatively for a number of generations these bacteria in response to a stimulus, the nature of which is as yet unknown, produce within themselves a tough little body called a *spore*.

[1] The science of microbiology has come a long way since Leeuwenhoek turned his lens on pepper infusions and discovered his wee beasties. It has answered many questions about biological phenomena and provided methods and facts that have been most useful in solving practical problems in the fields of agriculture, industry, medicine, and veterinary medicine. But it is still silent when confronted with the question that gave impetus and direction to its early development. It is often said that Pasteur disproved the theory of spontaneous generation. Actually he disproved only the contention that the present day yeasts and bacteria arose from inanimate materials. Neither he nor anyone else has yet succeeded in designing an experiment to answer the basic question of how life did originate.

Many of these spores are highly resistant to heat, drying, sunlight, and the action of strong chemicals, all of which readily destroy the parent cell in its growing stage. When such spores find themselves in a new environment favorable to growth they hatch like an egg and out comes a young bacterial cell just like the parent that originated it.

A second factor in the development of microbiology was man's interest in fermentation and it had been by way of this interest that Pasteur became involved in the controversy on spontaneous generation. The most primitive peoples enjoy fermented drinks but although the transmissibility of the ferments had long been recognized, as witness the Biblical statement about the "little leaven that leaveneth the whole loaf," the cause of fermentation remained a mystery until 1838 when Cagniard de la Tour showed that yeasts were responsible. His work was not accepted and even such men as Liebig, one of the greatest German chemists of the middle of the last century, denied the role of yeasts in fermentation, insisting that it was a purely chemical process. The information gained from observations made during the battle between those who supported the biological theory and those who supported the chemical theory did much to further the knowledge of bacteriology. It was Pasteur who by his brilliant experimentation submitted convincing evidence that alcoholic fermentation was due to yeasts. He also studied several other types of fermentation such as the souring of milk and the manufacture of vinegar and showed that in each instance certain particular microorganisms were responsible for each type of fermentation and that, without them, the fermentation did not take place. Like Leeuwenhoek, he discovered bacteria, yeasts, and molds almost everywhere he chose to look for them. He found them in the air of his laboratory, the cellar, the courtyard, and some, though few, in the clean clear air on mountain tops.

A third and most important factor in the development of microbiology was man's interest in disease. Disease has always been one of the greatest hazards confronting man. Many ancient taboos were based on notions as to its cause and much religious practice has centered about its control. It is interesting to note that as early as 1546 Fracastoro, without any experimental evidence, propounded a theory that diseases were caused by seeds of infection. Two hundred years later Plenciz, still unable to secure adequate experimental proof, advanced the theory that each particular disease was caused by a particular type of organism which caused that disease only and which could be distributed through the air, thus carrying the disease from person to person. During the period from 1840 to 1880 many men sought in microorganisms an explanation of disease. The germ theory of disease which was finally evolved is, like so many other theories, not due to any one man but is rather the result of the accumulated ex-

perience of many. Pasteur, Lister, and Koch are important names in this connection. It was Robert Koch (1843–1910), a German physician, who definitely identified one of these spore-forming bacteria, the anthrax bacillus, as the specific cause of a disease in man and animal. He obtained the bacterium from sick animals, cultivated it outside the animal body for a considerable period of time, and then reproduced all of the typical features of the natural disease by injecting the spores into experimental animals. Thus, for the first time, an important infectious disease was shown to be caused by a microbe. From this point on rapid strides were made in the field of bacteriology and most of the advances tended to emphasize the role of microbes as producers of disease. In the period from 1860 to 1890 which is often called the "Golden Age of Bacteriology," non-medical investigators were unearthing evidence to show that microbes could make for weal as well as for woe. The part they play in soil fertility and in the rotation of essential biological elements is not so dramatic but it is no less important and interesting.

The early discoveries in microbiology helped to clear up so many everyday problems and the application of laboratory findings led to such dramatic results that the practical value of microbiology was immediately recognized. It is little wonder, then, that microbiology developed first as an applied science and that there has accumulated a vast body of information on the relation of microbes to problems in various fields. It may be convenient to think of bacteriology as divided into these branches:

General bacteriology
Soil bacteriology
Dairy bacteriology
Food bacteriology
Industrial bacteriology
Sanitary bacteriology
Veterinary bacteriology
Medical bacteriology
Immunology

Very closely related to and largely dependent upon microbiology are public health and epidemiology. In each branch we find microbiology applied to problems in a special field. The techniques and tools in the various fields vary with the nature of the materials and problems.

Chapter II

GENERAL PROPERTIES OF MICROORGANISMS

From the pragmatic point of view we are primarily interested in what microorganisms do, not in what they look like nor where they may belong in a system of classification. It is, nevertheless, desirable to place them properly in the scheme of things and this problem presents certain difficulties not encountered in dealing with the higher plants and animals. Not only is the microbiologist unable to say with assurance whether certain microorganisms are plants or animals; he is unable even to say definitely whether some of the forms he encounters are living or non-living entities.

The biologist's concept of what constitutes a plant or an animal was obtained from the study of materials readily distinguishable. Trees and grasses, for example, although very different in many respects, show common plant characteristics which distinguish them from cattle or mice. Likewise the biologist's concept of what constitutes living things in contrast to non-living things was gained from observations of forms quite as readily distinguishable. The horse, the ox, and the elm were living; while water, rocks, and the air were non-living. These concepts were based largely on observations of the larger multicellular plants and animals. The development of lenses sufficiently powerful to resolve objects not visible to the naked eye led to the discovery of organisms, each composed of but a single cell showing many of the characteristics of both plants and animals and not fitting into the classification used in grouping the multicellular forms. The further discovery of entities, subsequently named viruses, so small that they could not be seen even with the aid of the most powerful modern microscope but nevertheless displaying many of the characteristics of living things has led to still further difficulties. It is not only impossible to say whether they are plant or animal but equally difficult to fit them into our old scheme of living and non-living.

It might be well to point out that although the concepts of plant and animal, living and non-living, are man-made and probably have no sound biological basis they do serve a useful function. It is pertinent for us to ask: What are the characteristics of plants? Of animals? Of microorganisms? Of viruses?

The cell is considered the structural and functional unit of living things. Plants such as trees and grasses, and animals like ourselves are multicellular. They may contain several billion cells so organized and coordinated, structurally and functionally, that the aggregate functions as a single individual. Many of the lower plants and animals are unicellular. Each cell is so constructed and organized that it is capable of carrying on

all the functions necessary to the existence of the individual and the perpetuation of the species. These single-celled organisms are often referred to as simpler forms. It is doubtful whether this is correct. Such a cell may be more complex than a single cell of a multicellular organism since, as we have said, it carries on all of the functions of growth and reproduction.

THE CELL THEORY

The cell theory or concept states that all living things are composed of cells or products of cells, that cells arise only from preexisting cells, and that the activities of an organism are the sum total of the activities, responses, interactions, and interrelationships of individual cells.[1] This concept of the cell as the structural and functional unit of living things has had a profound influence on biological thinking.

Structure of the Cell

A cell is usually defined in terms of both structure and function. It consists of a unit of protoplasm, limited by a membrane or cell wall. The protoplasm consists of cytoplasm and nucleus.

Protoplasm (*protos* meaning first and *plasma* meaning formed substance): Protoplasm is a greyish semi-fluid substance, the exact nature of which is a biological mystery. Chemical analysis shows that it consists of a mixture of compounds: proteins, fats, carbohydrates, salts, and water. Proteins are the most conspicuous and have been subjected to intensive investigation by various techniques of the biologist, physicist, and chemist. Much has been gathered from X-ray analysis and by the use of the ultracentrifuge. Proteins are crystalline in structure and highly complex in organization. The secret of protoplasm, as yet unrevealed, appears to be associated with the crystalline pattern or organization of its protein. After all, the same atoms of carbon, hydrogen, nitrogen, oxygen, phosphorus, and the like found in protoplasm are also found in quite lifeless substances. But a mixture of proteins, fats, carbohydrates, salts, and water, or of the atoms of which they are composed, does not constitute protoplasm. The whole appears to be greater than the sum of the parts. It is only when these atoms or compounds are properly arranged or organized that they become living protoplasm, and only the Master Chemist, Nature, can convert these lifeless atoms into life.

[1] The cell theory usually but erroneously ascribed to Schleiden (1838) and Schwann (1839) is, in fact, the product of the observations, speculations, and deductions of a number of men. Robert Hooke, Leeuwenhoek, Swammerdam, Malpighi, and Grew all observed the cellular structure of plants and animals during the period from 1665 to 1700. About a hundred years later Wolff again described the cells and formulated, at least in part, the cell theory. For a discussion of the development of this theory see *Biological Symposia*, Vol. I, The Jacques Cattell Press, Lancaster, Pa., 1940.

All protoplasm as it exists in the cells of the various plants, animals, and microorganisms is very similar in appearance and in essential properties. However, it is different in that each species has a protoplasm which is characteristic of that species only; that is to say, the difference between species is due to an inherent difference in the protoplasm.

A cell contains, in addition to those compounds which may be considered normal for any species, other compounds which may serve as reserve food or which may represent by-products of cellular metabolism. Water is present in all protoplasm and the amount is relatively constant for a given species. It exists in two forms, free and bound. Water plays an important role because the chemical reactions that take place in the cells require water as a solvent. It also serves as a carrier in the taking in of food and in the elimination of waste products.

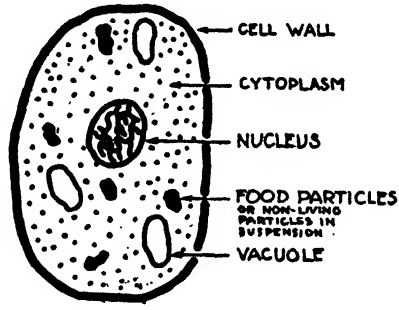

FIG. 2. Diagrammatic drawing of a cell

Cell Membrane: A cell is usually inclosed by a more or less readily distinguished envelop or limiting membrane. The membrane may be carbohydrate or chitinous or it may be merely a concentration of the protoplasm on the surface. While a bacterium also has a membrane, its exact nature has not been determined. It is probably a secretion of the cytoplasm and is quite variable in thickness, which may be altered by external conditions to a considerable degree. The cell membrane serves as a protective device, gives the cell rigidity and shape, and plays a very important role in cellular metabolism.

Cytoplasm: In appearance, cytoplasm is a semi-fluid substance presenting a more or less granular structure. It is a mixture consisting largely of protein and lipoidal substances in a colloidal state. The percentage of carbohydrates is low. The composition of cytoplasm in the bacterial cell varies greatly according to the composition of the medium in which it is grown.

Nucleus: The typical cell contains a nucleus, spherical in appearance,

limited by a membrane, and surrounded by cytoplasm. The nucleus plays a dominant role in regulating the activities of the cell, particularly its physiological and reproductive activities. Not every cell, however, contains a discrete nucleus as will be seen in our consideration of bacteria.

The concept of a cell as a unit of structure consisting of a wall, cytoplasm, and nucleus has to be considerably modified when we consider bacteria and viruses. Perhaps the structural and functional unit should not be thought of in terms of cells but rather in terms of the large protein crystals which appear to be the essential feature of protoplasm. Perhaps these are the basic elements of living things. The answer lies in the future.

CHARACTERISTICS OF LIVING THINGS

Biologically the characteristics of living organisms, be they unicellular or multicellular, are referable to the characteristics of protoplasm. These usually are: Movement, Growth, Reproduction, Metabolism, and Irritability.

Movement: We can recognize two kinds of movement in living things. One is the power to travel from place to place, which we usually associate with animals, and the other is the protoplasmic movement which takes place within the cell and is evidenced by a streaming readily observed in a number of cells when viewed under the microscope.

Growth: Growth is the power or capacity to enlarge or increase in size as a result of changes that take place within the cell. It must be recognized that biologic growth is a quite different thing from growth of crystals.

Reproduction: Reproduction or multiplication is the capacity to increase in numbers. If we were to select any one single characteristic which distinguishes living from non-living things we should say that "the ability to make more of itself out of something which is different" is the essential feature of living things. This involves, of course, growth, reproduction, and metabolism.

Metabolism: Metabolism includes practically all the changes involved in the maintenance of the living state.

Irritability: Irritability or sensitivity is a characteristic of protoplasm. All living things respond to stimuli from the external and internal environments. Physical and chemical agents such as heat and cold and acids and alkalies, profoundly influence protoplasm. It is because protoplasm is irritable; that is, because it responds to stimuli, that we are able to observe and induce changes in the various forms.

CLASSIFICATION OF MICROORGANISMS

Systematic arrangement and classification of data is an essential step in the development of any science, a step that must be taken before principles can be formulated and relationships determined. The present prin-

ciples of classification or taxonomy of plants and animals were laid down by Linnaeus about 1750 to 1760. He arranged all living things into two large groups called *kingdoms*: the plant kingdom and the animal kingdom. The kingdoms he subdivided into *phyla*, and the phyla into *classes*. Further subdivisions are usually recognized, making nine categories in all.

Kingdoms: Plant and Animal. A separate kingdom called Protista has been suggested for bacteria but this designation has not been generally adopted.
Phyla: The plant kingdom is divided into four phyla (sing. phylum); the animal kingdom into eight phyla.
Classes: Phyla are divided into classes. The names of classes end in *etes*, as, for example, *Schizomycetes*.
Orders: Classes are divided into orders. The names of orders end in *ales*, as, for example, *Eubacteriales*.
Families: Orders are divided into families. The names of families end in *aceae*, as, for example, *Bacteriaceae*.
Tribes: Families are sometimes divided into tribes. The names of tribes end in *eae*, as, for example, *Spirilleae*.
Genera: Families are divided into genera.
Species: Genera are divided into species.
Varieties: Species are divided into varieties, strains, and types.

It is expedient to have not only an internationally accepted system of classification but also an internationally accepted system of naming organisms. Plants and animals usually have two names, common and scientific. As a rule, bacteria have only scientific names. The name, usually derived from the Latin, consists of two parts: that of the genus, always capitalized, and that of the species, never capitalized. The names are usually descriptive. The common milk-souring bacterium, for example, is called *Streptococcus lactis*, *Strepto* meaning twisted and *coccus* meaning *berry*. Members of this genus are round in shape and cling together in the form of chains. The species name indicates that it is associated with milk.

Organisms are often named after the person who first described or discovered them. *Salmonella* is named after Salmon and *Pasteurella* after Pasteur.

The classification of higher plants and animals is largely on the basis of morphology, that is, of form and structure. Physiological differences are frequently used to distinguish strains and varieties within the species. Bacterial classification presents a different problem, as we have seen, because the organisms are so small and their morphologic features are so limited. Our present bacterial classification is based on morphology, cellular and colonial characteristics, staining, biochemical activities, disease-producing power, habitat, and chemical composition. These will be discussed in detail in a later section.

Chapter III

HOW WE STUDY MICROORGANISMS: THE MICROSCOPE, PURE CULTURES, ANIMAL EXPERIMENTATION

The Microbe

The Microbe is so very small
You cannot make him out at all,
But many sanguine people hope
To see him through a microscope.
H. Belloc

Microbiology is an experimental science and depends upon special tools and techniques for the facts upon which it is built. What the microbes are, what they look like, what they do, and where they belong in the scheme of things are questions that have been raised since Leeuwenhoek first published his discoveries. The answers cannot be secured by speculation. We must observe, we must gather facts, we must organize the information so gained. Then and only then are the answers forthcoming.

Probably the most important single tool for the microbiologist is the microscope. That microorganisms do exist and that they present certain morphological features could not be proven until microscopes sufficiently powerful to reveal them to the observer had been developed. Seeing is believing. The microscope is the tool which the biologist has used to gain an understanding of the structure and nature of living things. It will be of interest, therefore, to become familiar with this tool, to find out how it works, and what it can and cannot do for us.

THE MICROSCOPE AND MICROSCOPY

The ability of man to increase his natural powers by means of appropriate sorts of apparatus is largely responsible for his progress. Very

early in his history he increased his strength in effect by the use of levers. His eyesight, although adequate for most purposes, is definitely limited so it is only natural that he should have spent considerable time and thought on the development of aids to vision.[1]

In the thirteenth and fourteenth centuries there were a number of men working on simple microscopes developed from magnifying glasses. The

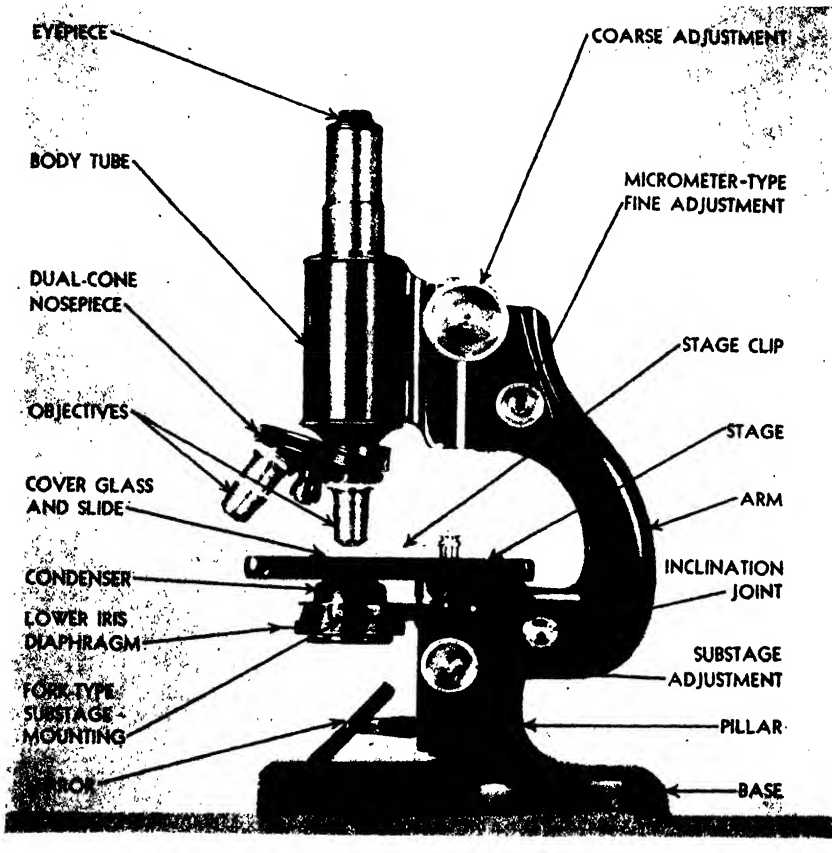

FIG. 3. Mechanical features of the microscope. Courtesy of the Spencer Lens Company

compound microscope was the outgrowth of studies on the telescope and during the late sixteenth and throughout the seventeenth centuries there were several men, among them: Digges (1571), Zacharias (about 1590), Galileo (1623), Hooke (1665), and Kircher, who developed quite elaborate

[1] For a history of the development of the microscope the reader is referred to Erik Nordensköld's *The History of Biology* and Locy's *Biology and Its Makers*.

and complicated compound microscopes. But although compound microscopes had been developed before 1650 and although these instruments gave considerable enlargement or magnification, it was not with them, oddly enough, that the microorganisms were discovered. Leeuwenhoek

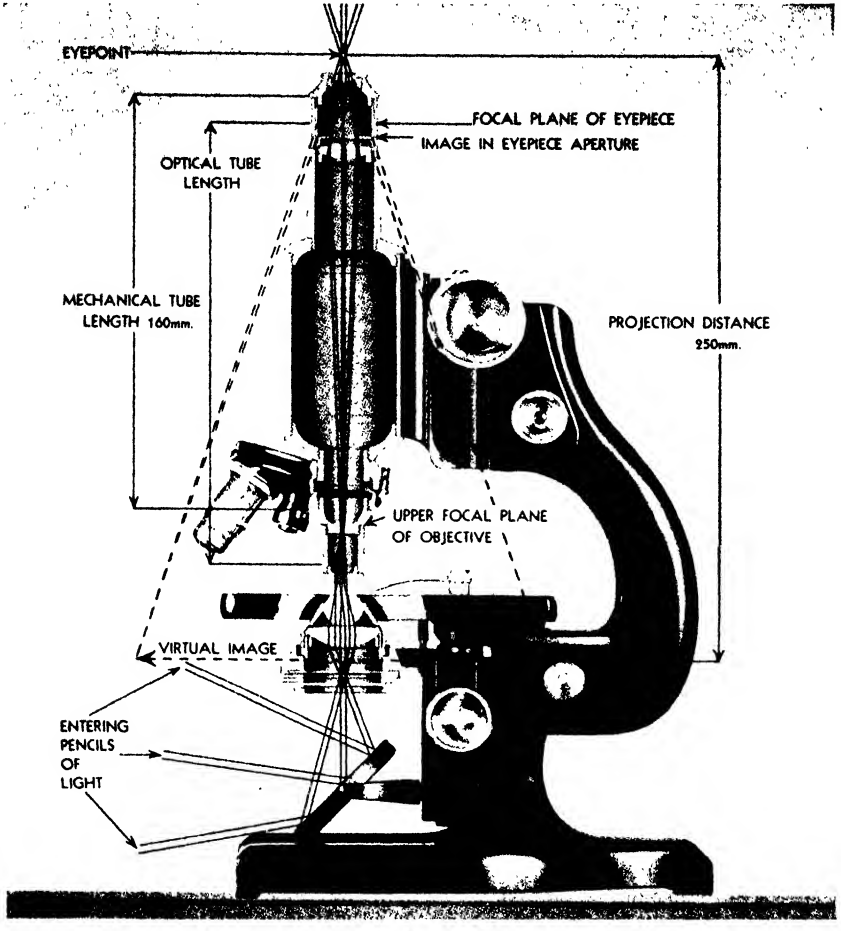

FIG. 4. Path of light rays through the microscope. Courtesy of the Spencer Lens Company

used the simple, not the compound, microscope. The secret of his success was in the precision of his lenses. The modern microscope is the result of the combined efforts of many men. To Abbé, a German mathematician, must go a great deal of credit for developing the formula followed in grinding the lenses used in the objectives. He also developed a system of lenses

for concentrating the light used in viewing bacteria which is known as the Abbé condenser, an integral part of every high-power microscope.

Ordinarily when we think of a microscope we think for the most part in terms of magnification but, important as this is, it is by no means the whole story. Definition and resolving power are equally important. Although the physics of the optics of the microscope cannot be treated adequately here, we shall consider some of the principles that play a part in the problem of making objects visible to the eye.

Magnification: By magnification we mean the enlarging power of a lens. On first thought it might appear that by enlarging an object to the limit of the enlarging power of a lens and then enlarging this image with another lens we could, by constructing a series of lens in tandem, get unlimited magnification and hence see the smallest of objects. Unfortunately this is not true. There are certain difficulties inherent to the process that prevent us from seeing the very small simply by magnifying the whole

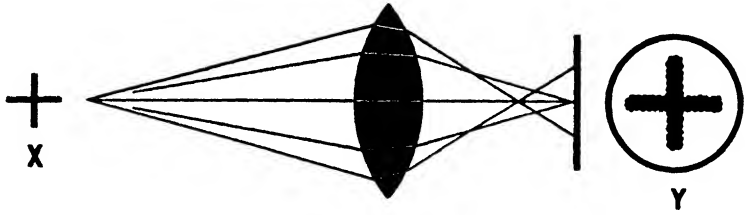

FIG. 5. Spherical aberration. Image *Y* of object *X* appears blurred in outline because rays passing through the edges of the lens are bent more and hence come to a shorter focus than rays passing through the center.

object. The first of these has to do with *definition* and by this we mean the power to see distinctly and clearly. Definition is a matter of contrast. Ordinary magnifying lenses distort both shape and color, phenomena known respectively as spherical and chromatic aberration. We are all familiar with curved mirrors and the distorted and amusing images they give. The aberration produced by magnifying lenses is somewhat analogous.

Spherical aberration is explained by the fact that the periphery of the lens has a greater light bending capacity than the center. Rays from the edges are brought to a focus closer to the lens than are the more central rays and, as a consequence, we obtain a blurred image.

Chromatic aberration is due to the fact that white light is broken up into its spectrum as it is refracted through the lens. The different colors travel at different rates, some are therefore bent more than others, and, as a consequence, the various colors do not come to a focus at the same

point as does the white light. The blues, for example, come to shorter focus than the reds so that the edge of the image presents a fringe of colors and the outline is not clear but fuzzy.

Spherical and chromatic aberration can be corrected for, but even with the best systems of corrected lenses there is still one other factor that limits our ability to see the very minutae. That factor is known as *resolving power.*

Resolving Power: By resolving power we mean the ability to distinguish two points that are very small and close together. Physicists tell us that this property of a lens system or microscope objective is determined by the numerical aperture and the wave length of light. This means that the shorter the light ray, the smaller the object that can be seen. However, no object less than one-half the length of the shortest light ray to which the eye is sensitive can be resolved. This is apparently an absolute limit fixed by the inherent nature of the process. Now the shortest wave length to which the eye is sensitive is about 3700 Ångstrom units or 370

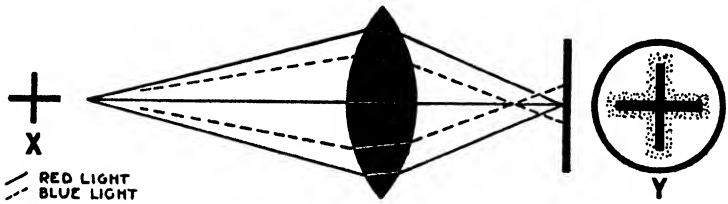

FIG. 6. Chromatic aberration. Image *Y* of object *X* appears with a fringe of colors because the component rays of white light travel at different rates in passing through a lens and come to a focus at different points.

millimicra which, for practical purposes, we can consider 400 millimicra. It follows, therefore, that objects less than 200 millimicra cannot be resolved and hence cannot be seen in detail by ordinary light.

To explain this fact we must remember the wave nature of light. When the rays are brought to a focus they do not come to a definite point but tend to jostle each other forming a sort of cone. The jostling is not so great for the shorter light rays, hence the cone is smaller, appears more as a point, and, in consequence, gives a better resolution.

There is no connection between resolving power, magnification, and definition. Each has its definite effective limits and the microscope, although a tool of great precision, has a limited value. This should be clearly understood because it accounts for many of our difficulties in microbial microscopy.

STAINING

We have said that definition is important. Now it is obvious that the greater the contrast in color between an object and its background, the more clear the outline of the object. Microbes, particularly the bacteria, which have a refractive index very nearly like that of the medium in which they are viewed, are seen with difficulty. When the contrast is increased they are easier to see and for this reason it is advantageous to stain bacteria.

Basic aniline dyes are of great value in staining bacterial protoplasm. Aniline dyes are salts of weak organic acids. The term basic or acid as applied to them does not refer to the alkalinity or acidity of the dye but to the fact that the color-carrying ion is either the positive or negative ion. For instance, sodium salt dye, which contains the basic sodium ion and an acid ion carrying the color, is called an acid dye since the colored acid ion will combine with positively charged particles. The chloride of the organic acid contains the acid ion, chloride, plus the basic colored ion and hence is known as a basic dye. Since bacterial protoplasm is negatively charged it is readily stained by basic dyes.

Weigert and, later, Koch recognized the value of aniline dyes in studying bacterial structure. Certain stains are extremely valuable in studying the chemical structure of bacteria. Perhaps the most useful of these, the Gram stain and the Ziehl-Neelsen stain, bear the names of the men who perfected them. The actual details of staining can best be considered in connection with laboratory methods and directions for their preparation and use will be found in most laboratory manuals.

DARK FIELD ILLUMINATION

No doubt all of us have observed dust particles or motes dancing in a beam of sunlight shining through a small crack into an otherwise dark room. If the room is made light these particles are no longer observed. The eye cannot resolve them nor is the contrast great enough to see them in a normal light. Dark field illumination makes use of the principle illustrated by the observation on the dust particles. The objects are not actually seen but the light striking them is deflected so that it strikes the eye. In actual practice, dark field illumination makes use of a special condenser with a black disk in the center. The light does not come through the condenser directly but is reflected in the form of a hollow cone around this disk and leaves the condenser at a sharp angle. These rays come to a focus at a point where the material under examination is to be placed and then diverge at such a wide angle that none of them strikes the lower lens of the objective. Hence, when looking through a microscope equipped with a dark field condenser, the field appears black. If small objects, such as bacteria, are present in the field of focus some of the light

rays striking them are reflected into the objective and the organisms appear brightly illuminated against a dark background. Dark field illumination is especially useful in studying organisms that are difficult to stain.

ULTRA-VIOLET PHOTOMICROGRAPHY

Photographic plates can be made sensitive to wave lengths of light to which the eye is not sensitive. Barnard has made use of this fact in developing a microscope which uses an ultra-violet light of wave length about one-half as great as the shorter rays to which the eye is sensitive. By using ultra-violet light and a quartz lens system he has been able to photograph organisms much smaller than the smallest resolvable by using ordinary light.

ELECTRON MICROSCOPE

The electron microscope operates on a principle analogous to that of the light microscope. It is based on the fact that electrons streaming in a vacuum can be bent or focused by the use of magnetic fields, known as magnetic lens, in a manner similar to the way light rays are bent and focused by a glass lens system. The electron microscope has an electron emitting tube operated at a voltage ranging from 10,000 to 100,000 volts, with higher voltages possible. The wave length of electronic radiations varies with the speed at which the electrons are traveling—the higher the voltage the shorter the wave length. At voltages of 90,000 the wave length is about 50 Ångstrom units. Since resolving power is determined by wave length, it follows that the resolving power of electron microscopes using a wave length of 50 Ångstrom units is many times greater than that of the light microscope which is limited to wave lengths of about 3700 Ångstroms. The electrons are discharged into a vacuum and focused by magnetic fields in much the same manner as light rays are focused by glass lenses. The object to be examined is placed at the point of focus with a fluorescent screen or photographic plate behind it. Electrons travel in a straight line only in a vacuum. If they strike even a few molecules they are deflected or scattered and if they strike dense material they lose their energy and are absorbed. The pattern produced on the fluorescent screen will depend upon the density, the thickness, and the crystalline structure of the object. Electrons not absorbed cause the screen to fluoresce and a sort of shadow picture or outline about three to four inches across is obtained. If the screen is replaced by a photographic plate a permanent image, an electronic micrograph, not a true photograph, however, is obtained.

Electron microscopes are used extensively in research but because of the cost and special problems of operation they are not used for routine bacteriological studies. They cannot be used to observe living organisms,

first, because life is destroyed by the streaming electrons, and second, because the specimen to be observed must be in such an extremely thin layer. They are particularly useful in the study of viruses.

The microscope is the tool *par excellence* of the bacteriologist. Its importance in the development of this science is hard to overemphasize,

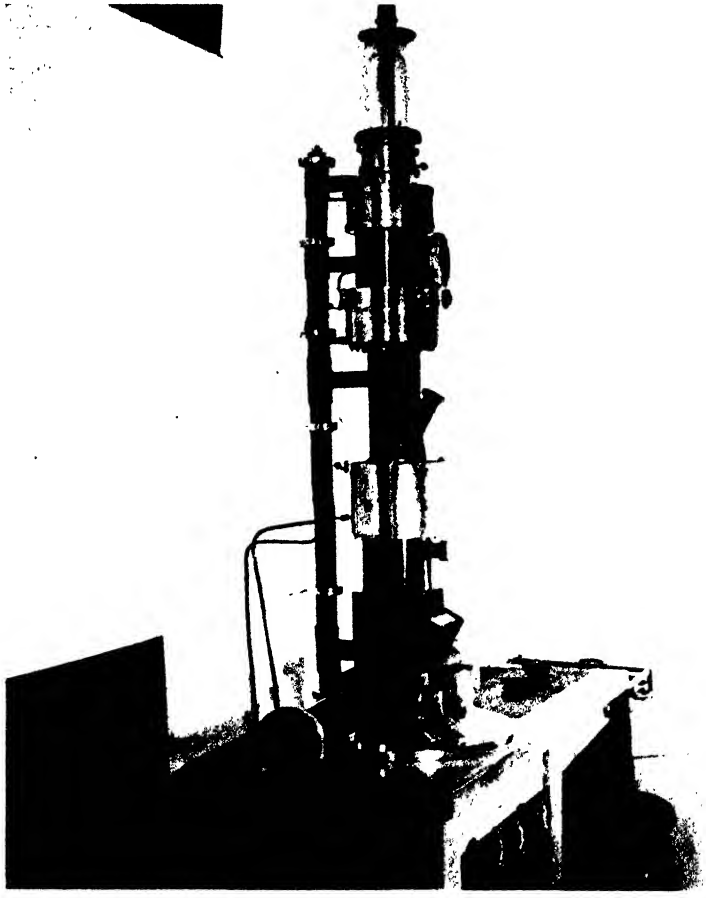

FIG. 7. Photograph of an electronic microscope built by Dr. Albert Prebus, Department of Electrical Engineering, Ohio State University.

but it has definite limitations. Many very important questions cannot be answered by it. You cannot tell how far a frog will jump by looking at it nor can you tell whether a bacterium will produce bubonic plague, sour milk, or fix nitrogen by studying it under the microscope.

At the risk of being obvious it might be well to pause here and note one significant axiom in science: *the answer always comes out in terms of the*

technique used. If we use a foot rule, we can measure the size of a room, not its temperature or utility. When we use a microscope, the answer comes out in terms of size and shape of microorganisms, not in terms of

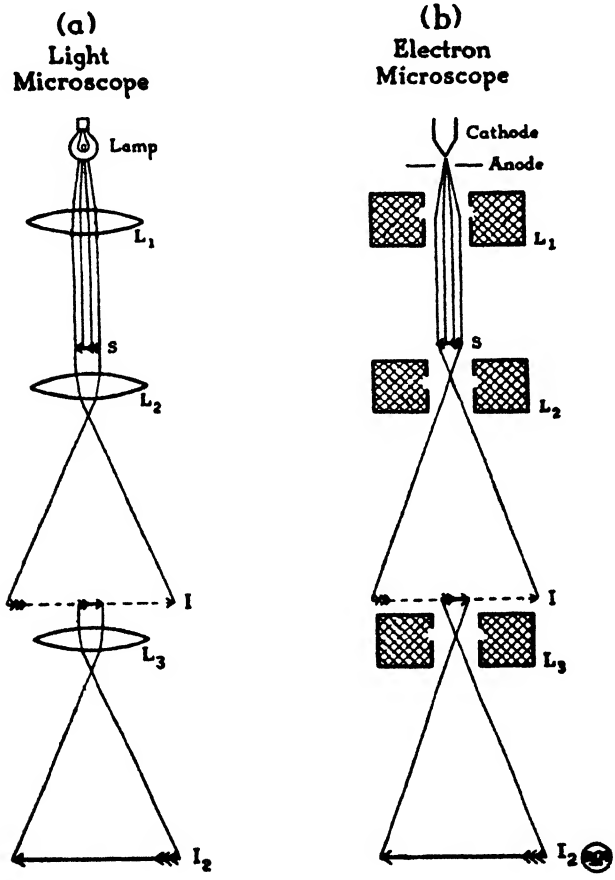

FIG. 8. Comparison of the optics of the compound light microscope to that of the RCA electron microscope. A system of magnetic fields in the electron microscope serve the same function as the series of glass lenses in the compound light microscope. In the diagrams L_1 is the condensing lens, L_2 is the objective lens, and L_3 is the projector lens. S is the object, I the primary image, and I_2 the final highly magnified image.

pathogenicity or fermenting power. This self-evident truth has a very direct bearing upon our ability to use information obtained in the laboratory and upon our ability to transfer facts obtained in one field to problems arising in some other. In order to discover how microorganisms react we

The method consists, in brief, of placing a small amount of the material to be studied in a sterile liquid medium and mixing thoroughly. A small portion of this mixture is removed with a sterile pipette to a second tube of sterile medium, it is again mixed, and the process repeated until a number of dilutions have been made. After a suitable incubation of from twenty-four to forty-eight hours, the organisms will have multiplied until there are millions present in each cubic centimeter. The tubes are now examined. If the dilutions were carried far enough, the tubes containing some of the highest dilutions will show no growth. The highest dilution showing growth will usually contain only the organism present in the greatest numbers in the original material. This method is highly satisfactory for isolating the predominant species but does not allow for obtaining pure cultures of the others.

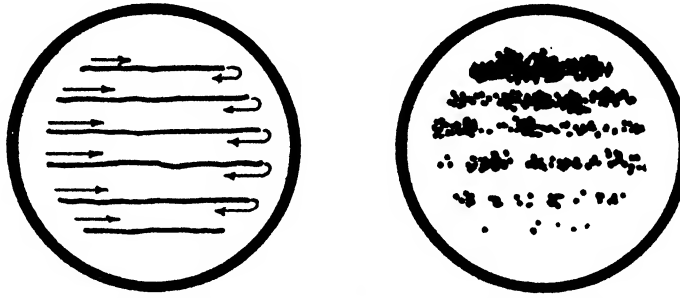

FIG. 9. Illustration of streak plate cultures

Streak Plates and Pour Plates: There are several ways of using solidifiable media in obtaining pure cultures. The two more common are to use streak or pour plates. Streak plates are prepared by spreading the material to be examined over the entire surface of a Petri dish containing the sterile medium. As the inoculating needle is dragged across the surface the organisms are dislodged and each grows into a colony. At the beginning of the streak the organisms are seeded so heavily that the colonies are not separate and pure cultures cannot be obtained, but, as the inoculum becomes less, the colonies are fewer and separated by sufficient space so that they may be "fished out" with a fine wire, transferred to sterile media, and studied in pure culture.

The pour plate technique consists essentially of preparing dilutions according to the dilution method previously described, but the dilutions are made in nutrient agar medium which has been heated to a temperature high enough to liquefy the agar and then cooled to about 43° C, a temperature not high enough to affect the bacteria and not low enough to allow the agar to solidify. After a thorough mixing, the contents of the tubes

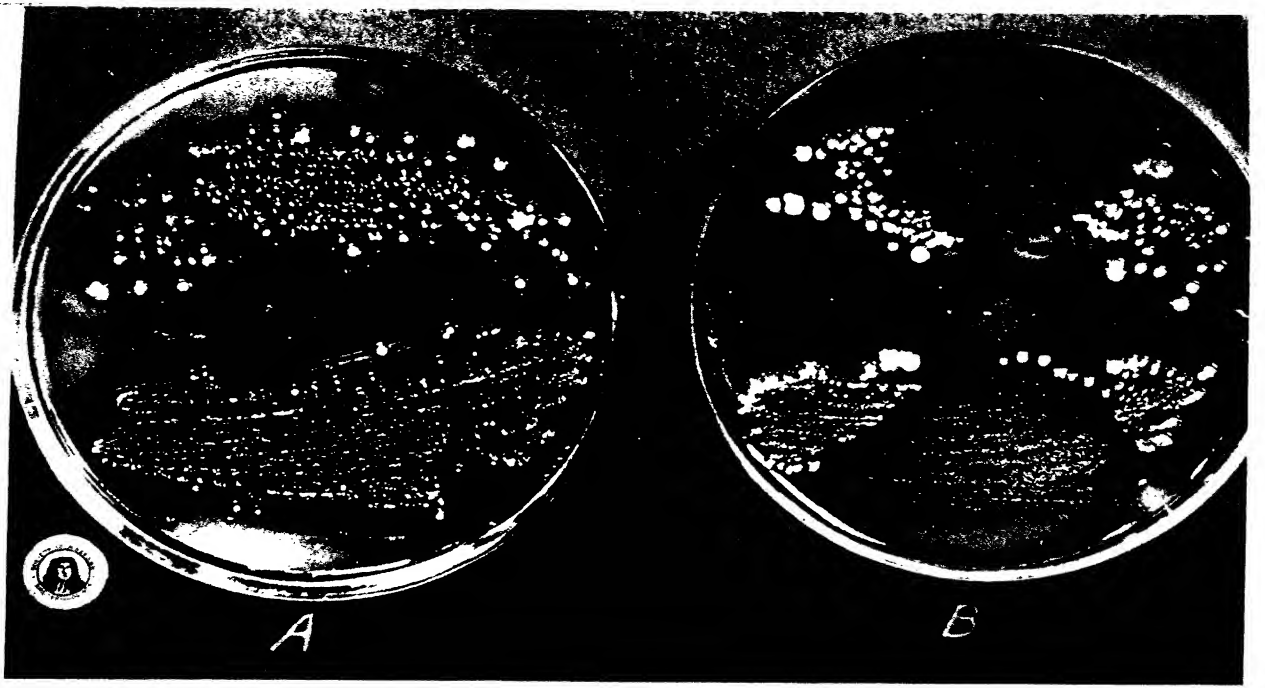

FIG. 10. This is a photograph of a control plate A made by placing a small loopful of sodium chloride solution upon the surface of Bordet-Gengou medium, passing a nasopharyngeal swab back and forth several times through the drop of saline and then streaking the remaining portion of half of the plate with a long, flexible loop. Plate B was made simultaneously by placing a small loopful of penicillin solution on the medium instead of saline solution. The nasopharyngeal swab is rubbed over the surface of the medium through the drop of penicillin solution and the remaining portion of half of the plate is streaked with long, flexible loop. After three days incubation at 37° C the plate A without the penicillin shows the customary heavy growth of the microorganisms usually found in the nasopharynx. The plate B charged with penicillin shows an area in which the contaminating organisms are inhibited. The minute colonies on plate B are a practically pure culture of Hemophilus pertussis.

Courtesy of Dr. W. L. Bradford, Dr. E. Day, Dr. G. P. Berry and the American Journal of Public Health.

are poured into sterile Petri dishes and the agar hardens as it cools. The colonies grow, after suitable incubation, to sufficient size so that they are visible to the eye. Then they can be picked out and isolated by cultivation in suitable media.

The pour plate method is also used for counting the number of bacteria in any given material. One cubic centimeter of milk or other material may be transferred to a tube containing nine cubic centimeters of sterile water, mixed, and one cubic centimeter of this dilution transferred to another nine cubic centimeters of water, again mixed, and so on until any desired dilution is obtained. One cubic centimeter of each dilution

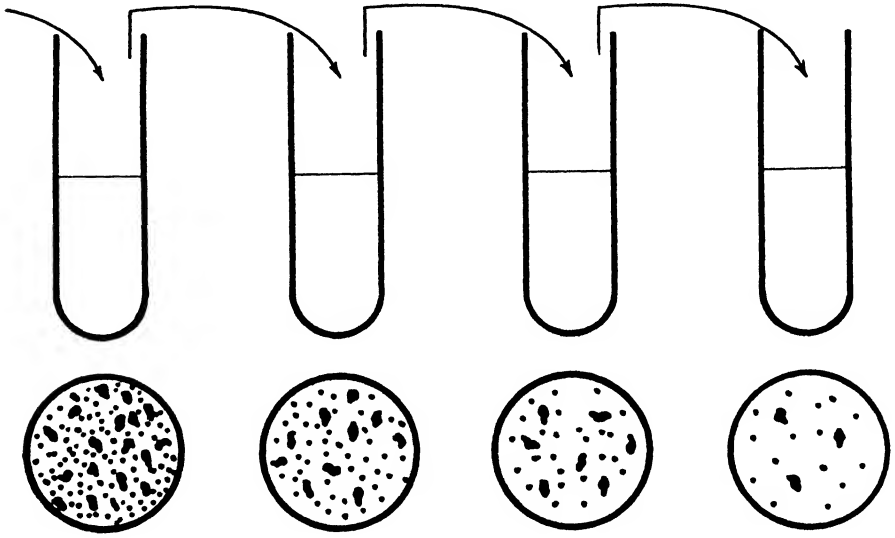

FIG. 11. Pour plate culture. Showing the effect of dilution of the inoculated material on the number and separation of colonies.

may then be transferred to sterile Petri dishes and thoroughly mixed with nutrient agar. After twenty-four to forty-eight hours incubation, the number of colonies multiplied by the dilution gives the number of bacteria in each cubic centimeter of the original sample.

Selective Media: Different species of bacteria differ in regard to the nature of the materials they can use for food, in respect to the acidity or alkalinity at which they grow best, and in their resistance to chemical agents such as disinfectants, dyes, penicillin, and similar substances. By taking advantage of these differences it is possible to concoct media that will inhibit or retard the growth of certain species but allow the growth of others. Such media are said to be "selective."

Differential Media: Sometimes different species grow equally well on a

medium and look alike. In order to distinguish between them it is necessary to take advantage of their physiologic properties. It may happen that one species produces acid from a sugar and the other does not. Consequently if sugar is added to the medium, plus a little dye or indicator, the color of the colonies of the acid-producing bacteria will be different from the color of the colonies of the non-acid producer. Hence the colonies will be readily distinguishable and the different species easily determined. Such media are called "differential media". Frequently media are prepared that are both "selective" and "differential."

Single Cell Isolation: For certain types of experimental work, methods have been developed that involve the picking of a single cell from a drop of a suspension of the organisms. This is achieved by suspending a drop of liquid containing the microorganisms in an especially constructed glass cell which is then placed under a microscope. Single microorganisms are removed from the drop by means of a very fine capillary pipette manipulated by micrometer screws.

Animal Experimentation: Millions of people now alive should give thanks for their existence to research done with experimental animals. Those microorganisms found in nature growing on lifeless media, such as soil, water, decaying plants and animals, and the like, can be readily grown on artificial media in the laboratory. But there are other organisms which are strict parasites; that is, they live in, on, or at the expense of animals or plants. Many of these cannot be grown on artificial media and the investigators must use plants or animals to culture them. Most of our information as to the cause of infectious disease comes from such studies and many of the preventive and curative measures which play such a large part in modern medicine are a direct result of laboratory experimentation on animals.

Tissue Culture: Sometimes bits of animal tissue can be kept alive and growing in nutrient solution. These can be inoculated with viruses and other organisms which will not grow in the absence of living cells.

Chick Embryo: The embryo in the hen's egg has proven an excellent medium for culturing certain viruses, rickettsiae, and bacteria. Some of the organisms grow best in the yolk sac, others on the chorioallantoic membrane. Many vaccines used today including those for yellow fever, Rocky Mountain spotted fever, and typhus fever are grown in the chick embryo.

There are then, as you have seen, a number of tools and techniques available to the microbiologist for his study of microorganisms. Each one is particularly suitable for a particular purpose. The more important are: the microscope, stains, cultural methods, and experimental animals.

Chapter IV

BIOLOGICAL CHARACTERISTICS OF MICROORGANISMS: MORPHOLOGY AND STRUCTURE

There are two ways to approach the study of microorganisms. We can begin with the better known plants and animals and show how they had their beginnings in structurally simpler forms; or we can begin with the most primitive microorganisms and trace their development through to the higher or more structurally complex plants and animals. Either way is beset with difficulties for many facts are wanting.

In tracing the progressive evolution of microorganisms; that is, in beginning with the simpler forms, the question of the cell or structural unit must be considered first. It seems logical to assume that single-celled organisms evolved before multicellular forms, hence the single-celled organisms are considered the more primitive. Some living organisms do not have even an architecturally distinct nuclear body. Since the cells of all the higher forms do have discrete nuclei, those single-celled forms which do not are considered lower in the scale than those that do.

The simplest method of multiplication appears to be simple fission, a process in which a cell enlarges to a certain size and divides into two cells of equal size. Higher forms have special structures set aside that function in the process of reproduction.

The manner of reproduction as well as morphology must be considered carefully in placing living forms properly in the scale of development. However, it is impossible to separate structure from function and the physiological processes involved in metabolism and growth. It is usually assumed, for instance, that an organism able to live on simple salts is more primitive than one that can utilize only such complex foods as the tissues of plants and animals. However, this is a matter of definition and it does not follow that such an organism is more primitive in the sense that it represents a form more simple physiologically. So in classifying the different types of microorganisms both morphologic and physiologic characters have to be considered.

As we have said, the plant kingdom is divided into four large groups called *phyla*, namely:

Phylum I	Thallophyta—plants having neither roots, leaves, nor stems. This group includes all the single-celled plants and many multicellular forms.
Phylum II	Bryophyta—moss-like plants;
Phylum III	Pteridophyta—ferns
Phylum IV	Spermatophyta—the seed-bearing plants; e.g., trees, oats, corn, onions, etc.

The plants which have neither roots, stems, nor branches and are classed in the *Phylum Thallophyta* have been subdivided into *algae* and *fungi*.

Sub-phylum 1. Algae—the algae possess chlorophyll. Some forms are multicellular; some, unicellular. Some do not have a discrete nucleus.

Sub-phylum 2. Fungi—the fungi do not contain chlorophyll. The molds, yeasts, and bacteria belong to this group. The fungi are divided into several classes; i.e.

Class 1. Schizomycetes—fission fungi, bacteria

Class 2. Saccharomycetes—yeasts

Class 3. Phycomycetes—nonseptate, algal-like group having no cross-walls between cells

Class 4. Ascomycetes—spore formation in a sac

Class 5. Basidiomycetes—reproduction by basidiospores

Fungi Imperfecti—heterogeneous group. Either the sexual stages are not present or are not known. They include many important plant pathogens.

Myxomycetes—slime molds, protozoa-like

MOLDS

There are tens of thousands of species of fungi. The term *molds*, which has no taxonomic significance, is used to designate those species commonly associated with food spoilage. Their general appearance is well known to everyone. Who has not seen the soft cottony masses, first white and later brown, black, blue, green, or red, on stale bread, jelly not too well-sealed, meats left exposed, over-ripe and rotting vegetables and fruits, and other food stuffs. Not only are they familiar in appearance but the conditions most conducive to their growth and the type of food they like best is well recognized.

Morphology

The finer details of structure are best studied microscopically. A bit of mold, viewed under a microscope, is seen to consist of long thread-like filaments composed of cells and small highly refractile bodies called spores. The molds differ from bacteria and yeasts in that they are multicellular and their life cycle is, consequently, a bit more complicated.

Asexual Reproduction

The mold spore, when it germinates, produces the long filaments called *hyphae* (singular—*hypha*) which branch to form a mat or network of interlacing filaments called a *mycelium*. Some of the hyphae grow upwards and, after a series of changes involving cell divisions, produce spores. The spore-bearing structures are of two types. In one case the spores are

borne on the outside at the tips or sides of the hyphae, much as seed is found in higher plants, and, in the other, the spores are produced within a sac called a *sporangium*. When they are ripe the sac ruptures and the spores are liberated and germinate on reaching fertile soil, thus completing the vegetative life cycle of the mold.

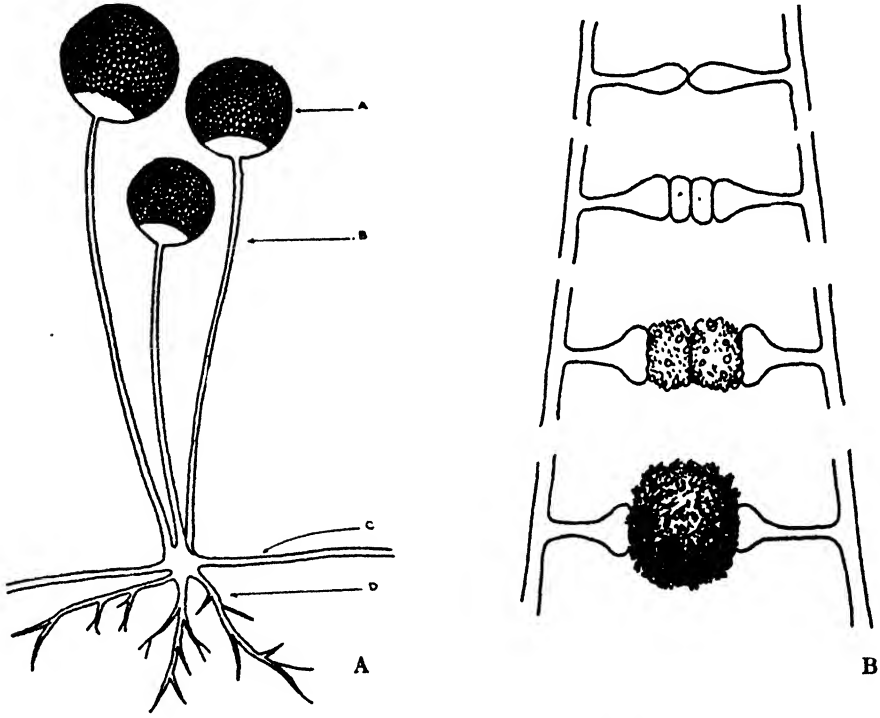

FIG. 12. Fungi. A. Rhizopus: Diagrammatic drawing showing sporangiospores inside the sporangium *A* which is borne on a sporangiophore *B*. The horizontal hyphae *C* are called stolons and the root-like structures *D*, rhizoids.

B. Mucor: Diagrammatic drawing showing successive stages in zygospore formation.

Spores borne inside of a sporangium are said to be borne *endogenously* and are called *sporangiospores*. The stalk bearing a sporangium is called a *sporangiophore*. Sporangia are produced only by the *Phycomycetes*.

The spores which are borne free on the sides or the end of a hypha, that is, which are borne *exogenously*, are called *conidia* (singular—*conidium*). The process of conidiospore formation may take place in two ways. Most frequently the cell at the tip of the hypha rounds up, becomes constricted, and is cut off. The cell next to it goes through a similar process and this

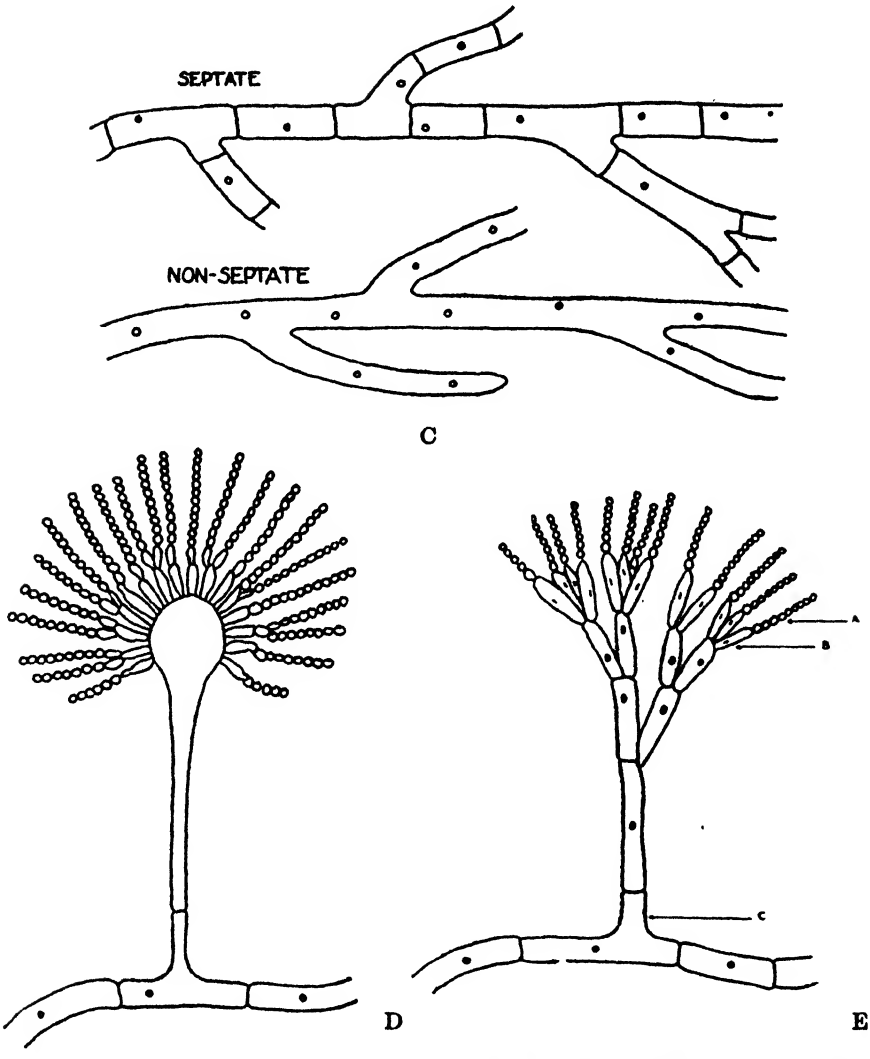

FIG. 12. Fungi. C. Diagrammatic drawing showing septate and non-septate hyphae.

D. Aspergillus: Diagrammatic drawing showing the asexual fruiting structure with conidiospores borne on the conidiophore. The hyphae are septate.

E. Diagrammatic drawing showing the fruiting structure of penicillium. The conidiospores *A* are borne on the conidiophore *B* which arises from a septate hypha.

continues until a chain of spores is produced which then becomes separated from the rest of the organism.

In a less common method a cell rounds up and becomes more or less cut off from the rest of the hypha. This cell then produces a bud which enlarges and buds again and the process continues until a chain of spores is formed, the oldest being next to the original hypha. A cell may form two buds, in which case the chain of spores is branched.

In some cases the walls of the cells composing a hypha may become thickened and the protoplasm be stocked with reserve food supply. Such cells are resistant to unfavorable conditions and can live in a dormant state for long periods. Because of the heavy cell walls that surround them they are called *chlamydospores* (ensheathed spore).

Some species of fungi produce spores called *oidia*. These result from a breaking up of the hyphae into individual cells. Unlike the chlamydospores, the cell walls of oidia are not thickened and the spores are not particularly resistant.

Sexual Reproduction

The methods of sporulation previously described are asexual. Some of the fungi reproduce sexually also and in various ways. In the *Phycomycetes* the spores borne in a sporangium are of two types. One produces a mycelium which grows luxuriantly and has been designated as a plus strain. The other produces a less active mycelium and is called a minus strain. Since the hyphae of the plus and minus strains cannot be distinguished, they are not designated male or female. If the plus and minus hyphae come in contact the ends may swell and fuse and at the point of fusion a spore develops known as a *zygospore*.

Ascopores are borne in a sac or *ascus*. The sequence of events in their life cycle is as follows. Two hyphae of the same mycelium coil together, the ends fuse, and fertilization takes place. The fertilized cell continues to grow, forming a mass of filaments, and the spores are formed from these. In practically every case the number formed is eight. In some cases the hyphae adjacent to the fertilized cells branch, forming a network around the spore sac or ascus. This structure is called an *ascocarp*.

The method of reproduction, the manner in which spores are borne, and the size, shape, color, and the number of spores, all are of importance in classifying the fungi.

Physiology of Molds

Mold physiology has not been studied in as much detail in the laboratory as has bacterial physiology, and consequently not so much is known about it. But the laboratory is not the only source of information. From everyday observation as to where and on what substances molds occur, we can deduce many facts concerning their prevalence and the conditions favoring their growth. For example, molds grow on all manner of mate-

rials: meats, vegetables, leather, clothing, and the like, providing the materials are damp. The amount of moisture necessary for their growth is far less, however, than that necessary for the growth of yeasts or bacteria. Molds, in general, require an abundance of air. This is evident from the fact that they are found growing on the surface of meats and jellies that have been imperfectly sealed and that their growth can be prevented by covering foodstuffs so as to exclude the air.

Molds tolerate a high osmotic pressure. The housewife is well aware of the fact that fruit juice sours rapidly and that the addition of equal parts of sugar prevents souring but does not prevent the growth of molds. The pickling of meats and cucumbers entails the use of salt whose preservative action inhibits or retards the growth of bacteria and prevents souring but does not effectively hinder the growth of molds. The preservation of foodstuffs by the addition of sugar or salt or by drying is based upon the increase in osmotic pressure. The fact that molds may be found growing on meats, pickles, sauerkraut, or jellies which are not being acted upon by bacteria indicates that, in general, molds are more tolerant of high osmotic pressures.

By observing the conditions under which molds grow naturally, we can gain facts which enable us to prevent their growth, if that is desired to prevent spoilage of foods; and we can also set up conditions which enable molds to grow more rapidly, as is desirable in many commercial processes.

Description, Distribution, and Importance of Some Common Molds

Phycomycetes: The *Phycomycetes* are divided into a number of families, one of which, the *Mucoraceae*, contains two genera of importance: *Rhizopus* and *Mucor*. They are often called bread molds and are black. A characteristic feature of this group is the absence of cross walls between the nuclei in the hyphae which are, consequently, known as aseptate hyphae.

Rhizopus nigricans is the mold most frequently found growing on strawberries, potatoes, and sweet potatoes and produces a soft rot. Several other species of *Rhizopus* are important because they ferment grains.

When *Rhizopus* spores germinate, some of the hyphae called *stolons* grow along the surface of the menstruum for a short distance. Where these touch the surface a branched root-like structure called a *rhizoid* is formed which penetrates the substrata. Other hyphae grow on the surface forming a cottony mat of mycelium. At the point where the rhizoids appear, hyphae arise which bear spores endogenously. Because these spores are black, the species is known as *Rhizopus nigricans*.

Mucor also produce decay in vegetables and fruits and grow well on bread and other starchy foods. One species, *Mucor rouxii*, is occasionally used commercially in the production of alcohol since it breaks starch down

to sugar which can then be converted into alcohol by yeasts. The yeasts cannot break down the starch molecules.

Ascomycetes: (Sac-mold). The Ascomycetes have crosswalls separating the nuclei of the cells and, hence, are said to have septate mycelia. The sexual spores are borne within a special cell which, when mature, is called an *ascus*. The more common molds belonging to this class are the *Aspergillus* and the *Penicillium*.

Aspergillus niger is commonly found on starchy fruits, vegetables, and bread. It is rather tolerant of acid and grows best at temperatures somewhat higher than those optimum for the growth of the other molds. The spores are borne on short stalks radiating from the enlarged ends of fertile hyphae.

Aspergillus fumigatus is pathogenic for birds and man and produces a disease which somewhat resembles and is sometimes confused with tuberculosis. It is believed that the normal habitat of this species is grain and that birds become infected by inhaling the spores. An outbreak of aspergillosis of man occurred in pigeon raisers in France. They were masticating feed before forcing it into the pigeon's mouth, and, it is thought, acquired the infection from the grains.

The blue-green molds commonly found on fruits and vegetables are penicillia. Their hyphae are septate. The spore-bearing hyphae branch and end in radiating clusters of cells from which the conidiospores are produced. The appearance of the fruiting structure is brushlike and the name of the genus, *Penicillium*, is derived from the word *penicillus* meaning *brush*.

Two species are of special interest to the dairy industry. *Penicillium roqueforti* is responsible for the characteristic flavor of Roquefort cheese and *Penicillium camemberti* for the flavor of Camembert.

ACTINOMYCETES

Fresh-turned earth, cellars, and some damp woods have a very characteristic musty odor. This is due to a group of microorganisms called the actinomyces. They are very active in breaking down the more resistant plant and animal residues and because of this property play an important role in the economy of nature.

Morphology: The *Actinomycetales* are intermediate between the bacteria and the multicellular fungi. Morphologically they consist of mycelia made up of very fine hyphae. The hyphae display true branching and hence are like the higher fungi but the diameter of the hyphae is in order of that of the bacterial rods. The hyphae do not have cross walls nor demonstrable discrete nuclei. In some species aerial hyphae are formed.

Pigment production is a common characteristic and is greatly influenced by the nature of the medium. Colors range from black to light green or white. Brown is most characteristic but green, blue, yellow, orange are not uncommon.

In reproduction the hypha may break into segments resembling bacterial rods, each capable of starting a new colony. In some species conidia are formed which are not to be confused with true spores. They are produced by the division of hyphae. The segmentation begins at the terminal and proceeds to the base, forming a short chain of conidia. These are somewhat more resistant to heat than are the vegetative segments or the hyphae but are not nearly so resistant as are bacterial spores. The temperature reached in ordinary canning readily destroys the conidia.

Physiologically the family actinomycetaceae are a varied group. The aerobic forms are called *Nocardia*, the anaerobic or microaerophilic are called *Actinomyces*. They are able to grow over a wide range of temperatures, a fact that might be predicted on the basis of their general occurrence in soils. Many species grow well at temperatures ranging from 6°C to 31°C. A few species are thermophylic and grow best at temperatures ranging from 40°C to 70°C. The anaerobic strains are far more exacting and grow best at temperatures ranging from 30°C to 37°C.

While most actinomyces are saprophytic, some are parasitic and a few are pathogens. Diseases due to the actinomyces are called *actinomycosis*. The classic example is lumpy jaw in cattle which is caused by *Actinomyces bovis*. This organism or a closely related one also produces a slowly progressing highly fatal disease of man that may involve the face and tongue, the lungs, and the abdominal region. Fortunately only a few cases of actinomycosis occur yearly.

Another family of this order is the *Streptomycetaceae* which are found in soil and in rotting manure. Several members of this group produce antibiotics such as streptomycin and aureomycin. (See Chapter X, Antibiosis and Antibiotics.)

YEASTS

The microscopic plants known as yeasts are of great economic importance because of the fact that they are capable of breaking down carbohydrates into simpler substances such as alcohol and carbon dioxide. We are most familiar with them in connection with the baking of bread and the production of fermented drinks such as beer and wines. Although some forms do produce diseases of men and animals, they are relatively less important as disease producers than are the bacteria, protozoa, or viruses.

Yeasts have been described as single-celled fungi which do not form

mycelia. They seem to occupy a position intermediate between the bacteria and the molds. Like the bacteria they are single-celled and like the molds they have well-defined nuclei.

Morphology

The yeasts are round, oval, or rod-shaped and considerably larger than bacteria. They have a definite cell wall, thinner in young cells than in old ones. There is a definite nucleus which, though quite small, can be demonstrated by staining. The protoplasm of the young cells is homogeneous but in the older cells vacuoles and granules appear. Volutin or

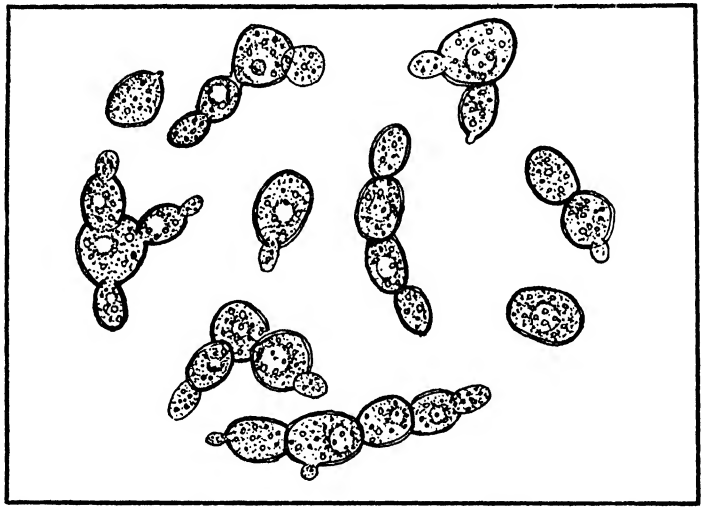

FIG. 13. Diagrammatic drawing of yeast cells showing budding. Redrawn from Sedgwick and Wilson.

reserve food is present in greater amounts in yeasts than in bacteria or molds.

Vegetative Reproduction in Yeasts

Yeasts multiply by budding, fission, and sporulation. Budding is the characteristic method of reproduction in the common bread and beer yeasts. The cell wall apparently becomes softened or weakened and bulges. Protoplasm flows into this bulge or projection and during the process the nucleus divides and one of the nuclei flows into the bulge forming a bud. The bud remains attached to the original cell until it reaches a considerable size and then separates, forming a free daughter cell. Several buds may be produced on the same mother cell. Some of these may, in their turn, bud while still attached to the mother cell and

thus form a chain, or, more often, a cluster of cells. This is somewhat similar to the formation of hyphae by fungi.

Fission is the characteristic method of multiplication in a few yeasts only. It differs from budding in that a wall appears in the middle of the mother cell and forms two daughter cells of equal size.

Sporulation in yeasts is a process of multiplication. When rapidly growing cultures are subjected to an unfavorable environment many of the cells cease budding. Instead, the nucleus divides once or twice forming two or four nuclei within one cell. Each becomes surrounded by cytoplasm and finally a cell wall develops between them. The membrane of the mother cell ruptures and the spores become free. Yeast spores are more resistant than vegetative cells but not nearly so resistant as bacterial spores. They may remain alive for many years if the environment is not favorable for germination. When placed in a favorable environment, the spores germinate into vegetative cells which again reproduce by budding.

Sexual Reproduction

The three methods of multiplication just discussed are vegetative and not sexual. Some yeasts show sexual reproduction. Adjacent cells conjugate and there is a fusion of the two nuclei, followed by nuclear division. The nuclei then separate and become surrounded by cytoplasm and cell walls, thus forming spores.

Classification of Yeasts

Yeasts are usually separated into two groups, the *true* and the *false*. The true yeasts multiply by budding, by formation of endospores, and, in one group, by fission. The false yeasts constitute a group of not closely related organisms all of which are characterized by the negative property of not forming endospores. Only those genera of economic importance will be mentioned here.

True Yeasts:

Saccharomyces: Most members of this genus are active fermenters of carbohydrates and produce a high yield of alcohol and carbon dioxide. Nearly all of the yeasts of economic importance are members of this group. *Saccharomyces cerevisiae* is round or globular in shape and is the yeast used in baking and brewing. *Saccharomyces ellipsoideus* is oval or ellipsoidal in shape. It is found on the skins of grapes and is important in wine and cider making. *Saccharomyces pastorianus* is large and sausage-shaped. It is found in fermenting grains and gives a disagreeable flavor to beer.

Brewers classify yeasts into two groups: *top* and *bottom yeasts.* The top yeasts are active fermenters and produce a high proportion of carbon dioxide. Because of the rapid evolution of carbon dioxide, the yeasts are carried to the top where a foamy scum is produced. The bottom yeasts are not such active fermenters and do not produce so much alcohol. This classification is one of convenience and is not entirely satisfactory. In the brewing industry certain strains of yeasts have been selected and named after the breweries or localities from whence they were isolated. One of the better known strains, for instance, is designated as Carlsbad No. 2.

The *Saccharomyces* has been divided into a number of subgroups on the basis of their sugar fermentation.

False Yeasts:

Torula: The false or wild yeasts are called *torula.* They are of importance in the dairy and brewing industries, chiefly because they bring about undesirable changes. Some species are capable of producing diseases of man and animals.

Upon culture, many of the torula produce pigmented colonies which frequently impart a color to the material in which they are growing. Some are white, some bright red, and some dark red. The red color of "bloody" sauerkraut is frequently due to these yeasts.

BACTERIA

The *Schizomycetes* or bacteria are architecturally the simplest of the microorganisms. They are single-celled, do not have discrete nuclei, multiply by simple fission, and are the smallest of the groups.

Morphology

The bacterial cell consists of a mass of protoplasm surrounded by a limiting membrane. This cell wall or limiting membrane is composed of protein and is usually considered to be modified endoplasm. It confers a certain amount of rigidity to the cell.

Shape and Grouping: The thousands of different kinds of bacteria may be roughly grouped under three basic morphologic types: spheres, rods, and spirals. The cells may appear singly, in pairs, or arranged in characteristic groups.

Cocci: The spherical or nearly spherical bacteria are called cocci (singular coccus) from the Greek word *kokkos* meaning berry. Bacterial cells are commonly surrounded by a gelatinous material that tends to hold the daughter cells together and the characteristic groupings are determined largely by the manner in which the cells divide. In some species, the daughter cells usually remain united in pairs whose adjacent sides are

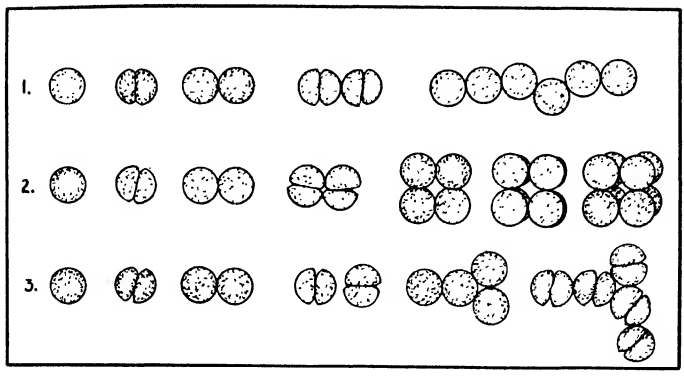

FIG. 14. Forms of division and grouping in the cocci: 1—streptococcus, 2—sarcina, 3—staphylococcus. Redrawn from Greaves and Greaves.

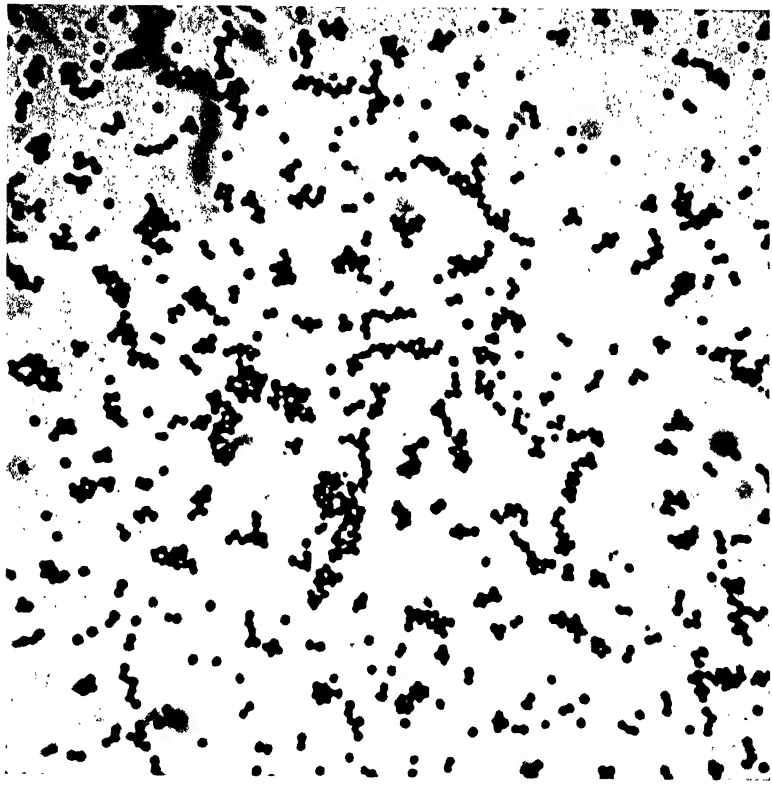

FIG. 15. Photograph of a staphylococcus

flattened, giving each cell a kidney bean shape. In another species, the cells are somewhat flattened and drawn out and look like ovals lying end to end. In most species, the cells are nearly round.

The cocci may divide in any number of planes. When a coccus divides regularly in one plane only and the plane of each successive division is parallel, the cells give the appearance of a chain of beads. This is characteristic of a group called *streptococci* (*strepto* meaning chain). In another group the cocci appear characteristically in pairs and are called *diplococci*.

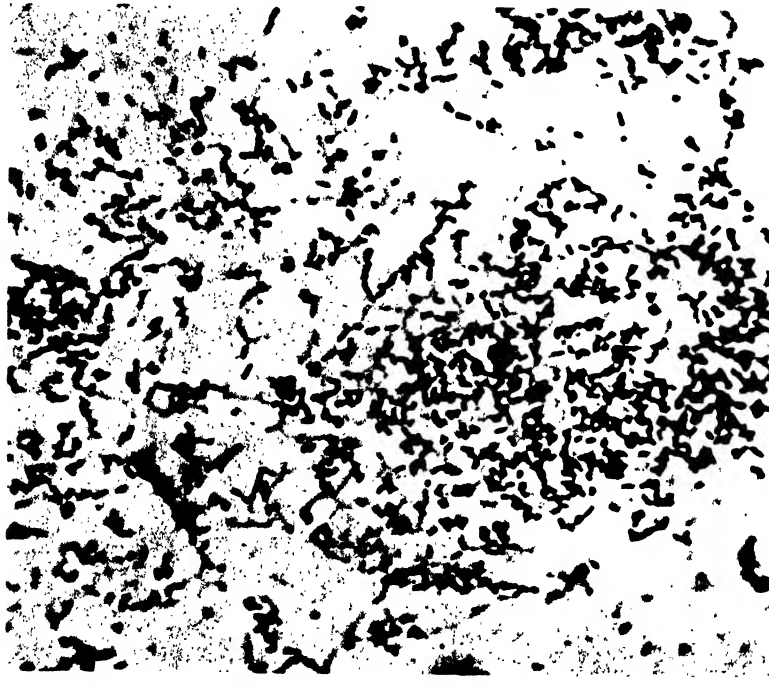

FIG. 16. Photograph of a short non-sporulating rod-shaped bacterium, *Escherichia coli*

If the two daughter cells divide in a plane at a right angle to the original plane of division, the result will be four cells forming a square or tetrad. If these cells divide in a plane perpendicular to the other two planes, the result will be a packet or cube of eight cells, an arrangement characteristic of the *sarcinae*.

If the planes of division are irregular, the cells will be grouped in masses or grape-like clusters and are called *staphylococci* (*staphylo* meaning grape). This arrangement is characteristic of a coccus that is commonly present on the skin and is a frequent cause of wound infections and boils.

Bacilli or Bacteria: The rod-shaped organisms are called bacilli (singular bacillus) or bacteria (singular bacterium). Both terms are commonly used for all three basic forms, but technically refer to the cylindrical or rod-shaped organisms only. The rods may be short or long, slender or thick, uniform or irregular in shape, and the ends may be squared, pointed, or rounded. The size and shape is fairly constant for each species. All rods divide at right angles to the long axis and the daughter cells may appear separate, in pairs, or in long chains. The rod-shaped organisms are

FIG. 17. Photograph of *Bacillus subtilis* stained by Gram's method. The colorless spots in the cells represent the unstained spores.

divided into two groups: those that form spores and are placed in the family *Bacillaceae*, and those that do not and are placed in several other families.

Spirilla: The spiral organisms are called spirilla (singular spirillum). The individual cell may be long and composed of several spirals or so short that it looks like a curved rod or the comma on a printed page. In fact, the organism that causes Asiatic cholera looks so much like a comma that it is called the "*comma bacillus.*" Since it happens to be a very motile

organism and gives the appearance of constant vibration, it is also known as "*comma vibrio.*"

Bacterial Nucleus: The question as to whether a bacterium contains a nucleus is a much disputed one. There are four possibilities: namely, 1. that a bacterium has no nucleus, 2. that a bacterium does have a nucleus but that it is too small to be seen in most forms, 3. that a bacterium has a diffuse nucleus, and 4. that the bacterial cell is all nucleus.

The controversy is one which cannot be readily settled. Much of the confusion comes from our slavery to terms. A nucleus is usually defined

FIG. 18. Photograph of the diphtheria bacillus, *Corynebacterium diphtheriae.* This organism is pleomorphic and stains unevenly.

as a part of the cell which is morphologically distinct and which bears the determiners of heredity. It is composed of chromatin and plays a part in regulating the activities of the cell. This definition, it will be noted, is in terms of structure and of function. Although recent studies with the electronic microscope and special stains suggest that some bacteria have structures comparable to the classical nucleus, in terms of structure alone it is doubtful whether a bacterium has a nucleus. In terms of function, a bacterium must have a nucleus since there is something in the bacterial cell which governs heredity and which regulates the activity of the cell.

Whether the structures revealed by the electronic microscope represent a true nucleus can be determined only when the function of these bodies in the life history of the bacterium is more fully understood. Whether the

FIG. 19. Picture of *Vibrio schuylkilliensis* taken at 100 kilovolts with an R.C.A. high-voltage research microscope. Note the flagella, the long hair-like appendages. Courtesy of Drs. Polevitzky, Anderson, Mudd and the *Archives of Pathology*.

nucleus is discrete or diffuse is, from the functional standpoint, quite immaterial.

Whether bacteria have genes depends again upon definition. If genes are thought of as the determiners of heredity, then bacteria may be said to have genes since they do transmit their characteristics to their progeny. However, if genes are defined as entities located on the chromosomes

which, in turn, are in the nucleus, then bacteria may not have genes since chromosomes are not determinable.

Spores: One family of true bacteria, namely the *Bacillaceae,* produces spores. The spore is, essentially, condensed protoplasm and appears as a highly refractile body within the cell. In an actively growing culture the cells grow to a certain size and divide to form two cells, which in turn grow and again divide. This process may take place for twenty to fifty generations and then the cells, instead of dividing, may produce spores. Sporulation is not a method of multiplication in the bacteria since each cell produces only one spore. The spore may be looked upon as the bacterial cell in a resting stage. Its resistance to physical and to chemical agents is of special interest. The spores of many species withstand boiling in water for four to six hours, and the spores of a few have been boiled for sixteen to twenty hours without being killed. This is particularly important in connection with canning and other sterilizing processes.

Some species of bacteria have characteristic inclusions within the cell. These may be of several kinds.

Metachromatic Granules: Within some bacterial cells there are granules which have the property of taking up stains readily. Not only do they stain more heavily than the rest of the protoplasm but they may appear a different color when certain complex stains are used. Because these granules may stain a color different from that of the stain, they are called *metachromatic granules.* They may be polar, that is, situated in each end of the cell, or they may be arranged in rows. In either case they are of some value in identification as, for example, in the case of the organism producing diphtheria. The function of these granules is not known, but it is not believed that they represent any specialized structure. They are called *Babes-Ernst granules* after the men who first described them.

Acid-Fast Granules: The tubercle bacillus sometimes shows granules which have the staining properties of the tubercle bacillus, namely, acid-fastness.

Other Inclusions: Fat globules, glycogen, sulfur granules, and a substance called volutin may be present in different species. These are probably products of metabolism or are reserve food and are not an essential part of the cell.

Flagella: Many bacteria possess flagella. These are protoplasmic threads that whip back or forth enabling the organism to swim. All bacteria capable of independent motion have them. They are arranged in characteristic fashion about the cell. Bacteria that do not have flagella are said to be *atrichous* (without hair). Some bacteria have a single flagellum in one end. These are said to be *monotrichous.* Those that have a single flagellum at each end are said to be *amphitrichous.* Those

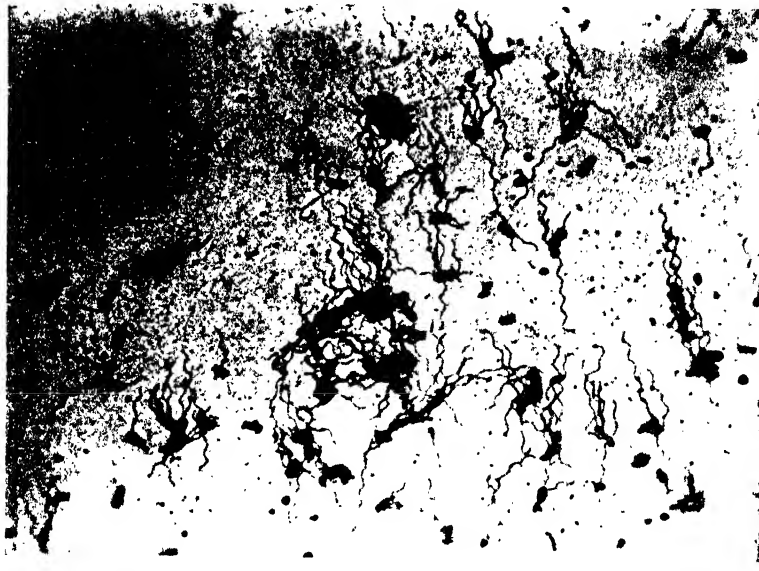

FIG. 20. Photograph of *Salmonella typhosa*, the typhoid bacillus, showing flagella. (×950) Courtesy of Wallace and Tiernan Products Company.

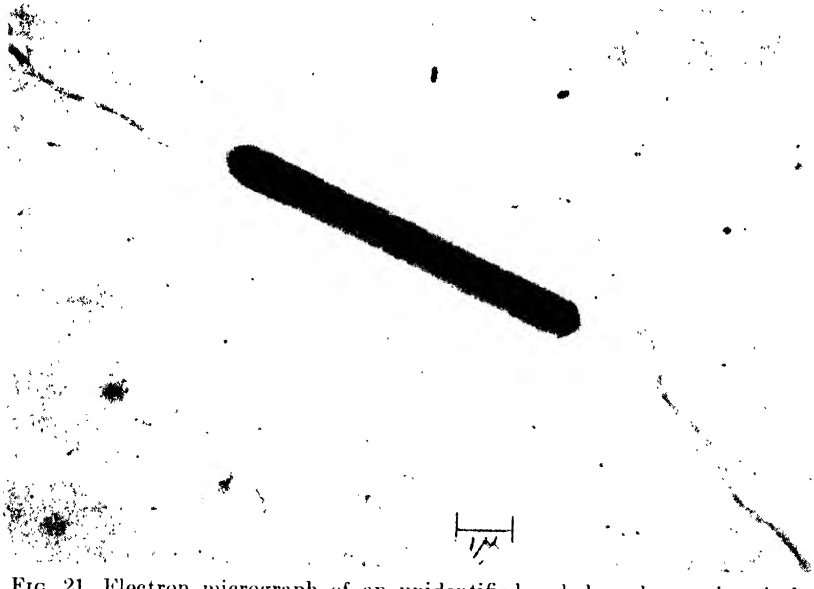

FIG. 21. Electron micrograph of an unidentified rod-shaped organism isolated from pond water. Each flagellum appears to be composed of numerous fine threads loosely united to make a compound locomotor organ.

Courtesy of Dr. W. G. Hutchinson and Miss M. R. McCracken and the Journal of Bacteriology.

with a tuft of flagella at one end are called *lophotrichous*, and those with flagella on all sides are called *peritrichous*. The position of the flagella is a constant characteristic of each species and hence has some value in identification. The flagella are many times as long as the cell but are so thin that they cannot be seen under the microscope except when stained by special methods.

Size of Bacteria

Bacteria are so small that the ordinary units used in measurement are not suitable for measuring them. The unit used by microbiologists is

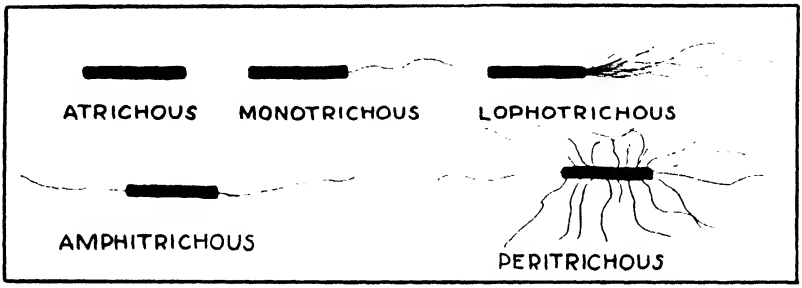

FIG. 22. Diagrammatic drawing of rod-shaped bacteria showing the various arrangements of flagella.

he micron. It is usually indicated by the Greek letter μ, pronounced *mu*. This table shows the micron in relation to other units of the metric system.

1 centimeter	=	10 millimeters
1 millimeter	=	1000 micron or μ
1 micron or μ	=	1000 millimicron
1 millimicron	=	10 Angstrom units

The cocci measure on the average about 0.8 to 1 μ in diameter. Such figures convey very little idea as to the actual size of the bacteria because it is difficult to realize just how small these units are. Under the oil immersion objective of the microscope, which magnifies the object about one thousand times, a coccus appears about as large as the dot over the letter *i*. If a boy five feet three inches were enlarged one thousand times, he would be over a mile high and would cover an area of 160 acres when he lay down. It is not difficult to see how such a Colossus could harbor a great many organisms the size of a dot or of the cross-section of a pin.

The rod-shaped forms are more variable: some quite small, measuring about 0.2 μ in width and 0.5 μ in length and other larger forms about 0.5 μ to 1.0 μ in diameter and 2 to 3 μ in length. A few species are considerably longer, measuring 8 to 10 μ in length.

Capsules: Many bacteria are surrounded by a gummy material called a capsule. In some species this appears to be protein; in a great many others it is carbohydrate in nature. In some species the cell is surrounded by a mucinous sheath causing great numbers to stick together and form a sort of jelly-like mass called a *zooglea.* The formation of capsules appears to be related to the environment in which the organisms are grown. In some species a high sugar content in the medium stimulates capsule formation, others form capsular material only when grown in animals. In several

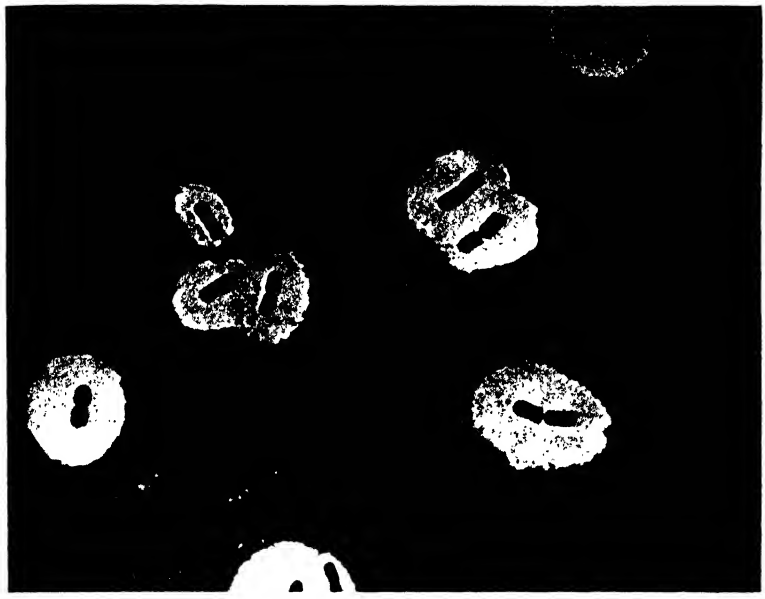

FIG. 23. Photograph of bacteria stained to show capsules. Courtesy of Wallace and Tiernan Products Company.

species, and especially in the pneumococci, the organisms responsible for lobar pneumonia, the capsule is of particular importance since it is related to the disease-producing capacity of the organism. Special staining methods are used to reveal the presence of capsules.

Reproduction

Reproduction in bacteria is asexual. They multiply by simple fission. A bacterial cell grows to a certain size and divides, forming two cells of equal size. Under favorable conditions the generation time, that is, the time it takes for a cell to grow and divide to form two cells, is from fifteen to thirty minutes for most bacteria. This manner of reproduction makes for an extremely rapid increase in numbers. Assuming a generation every

twenty minutes or three every hour and beginning with one bacterium, we should have at the beginning of the second hour, eight bacteria, at the beginning of the fifth hour, over four thousand, and, if this continued unhampered,—as is not, happily, the case,—at the beginning of the second day there would be about 500,000,000,000,000,000,000! So long as bacteria continue to multiply there are no old cells. All the cells are about the

FIG. 24. This electron picture shows *Clostridium tetani* cells from a twenty-four hour culture. In the cells of this young culture the protoplasm is homogeneous within its clear cell walls. Stages of cell division and peritrichous flagella are shown. Courtesy of Dr. S. Mudd and Dr. T. F. Anderson and the Journal of the American Medical Association.

same age and none much more than the average generation time, that is, from fifteen to thirty minutes. Of course, there are some few that cease to multiply and some that do so slowly, but a bacterial culture does not represent successive generations of parent and offspring as is the case with organisms having a life cycle.

The diameter of the streptococcus responsible for the souring of milk is about one micron. If unpasteurized milk is allowed to stand for forty-eight hours in a warm room it will contain about two billion organisms in

each cubic centimeter (a cubic centimeter is about twenty drops). Suppose we could arrange these streptococci in a row and so closely that there was no space between them. They would make a chain a mile and a quarter long.

Of course bacteria do not continue to multiply at this rate for more than a few hours. If they did, it would be but a matter of a few days until the seas were full and the earth covered to a considerable depth. The factors that control bacterial growth will be considered in connection with bacterial physiology.

Occurrence

Where are bacteria found? They are ubiquitous. They are found growing in soil, water, and in and on the bodies of plants and animals. They are found in the polar regions as well as in the warmer parts of the earth. They grow wherever there is food and water. They are found floating in the air but do not multiply there. The numbers found in any place depend upon how favorable the conditions are for their growth.

Classification

Man strives for orderliness. Housewives, farmers, shop-keepers, manufacturers, and librarians group and arrange their materials for convenience and intelligent handling. The scientist, likewise, in order that he may deal more efficiently and intelligently with his materials strives for orderly arrangement and grouping. To achieve this end he must needs have some system of classification that takes into account the nature and distinguishing features or characteristics of his materials.

In general, the classification of plants and animals is based upon morphology since it is believed that similarity in form and structure indicates relationship by descent. When the classification of bacteria was attempted, it was soon found that morphology alone was inadequate, and at present other features such as staining reactions, physiology, pathogenicity, habitat, and serology are also considered. The weight that should be given to any one of these is a matter of opinion and there is no good agreement among the bacteriologists as to just which one most accurately indicates relationship by descent.

For practical purposes bacteria are frequently grouped in an empirical fashion. Just as one might group spades, rakes, hoes, sprinklers, and fencing under garden supplies, having in mind only the use to which the various things were put; so the bacteria are frequently lumped into groups that fix nitrogen, produce acids, or grow best at high temperatures, without taking natural relationships into consideration too.

A committee, working in connection with the Society of American Bacteriologists, is attempting to formulate rules of classification and nomenclature and to list and classify all the bacteria that have been named. This formidable task was originally under the direction of David Bergey and the publication containing the information is known as Bergey's *Manual of Determinative Bacteriology*. The classification is the work of a committee and has not been officially adopted by the Society of American Bacteriologists.

The rules and general principles of bacterial nomenclature are similar to those used by the botanist and zoologist. Each organism has two names, consisting usually of two Latin words. The first word of the name indicates the genus and is always written with a capital letter. The second indicates the species and is not capitalized. The bacterium causing anthrax is named, for example, *Bacillus anthracis*. Some authors capitalize the second word if it is derived from a proper noun but this is not the rule.

Descriptive terms are frequently used to designate the species, as, for example, *Streptococcus lactis*, in which instance the generic name refers to a morphologic characteristic and means chain cocci or round forms, and the species epithet refers to a physiological property, namely, lactose fermentation.

A most difficult problem is to designate suitable criteria for determining a species. There is no absolute rule nor is there much agreement as to what constitutes a species, nor as to what constitutes a genus, for that matter. Consequently some workers classify as varieties within the species, organisms which others consider separate species.

Then too the problem of variation or dissociation complicates the picture, for bacteria vary with respect to the features used in classification. But in spite of the obvious difficulties and in spite of the fact that no absolute and fixed classification can be made, the problem is not hopeless. Considering everything, a fairly satisfactory classification has been worked out.

The class Schizomycetes is divided into orders, each order is divided into a number of families, each family into a number of tribes, each tribe into a number of genera, and each genus into a number of species. The classification is based on morphology, physiology, staining reactions, habitat, disease-producing properties, and chemical composition.

VIRUSES

The discovery that many diseases of plants, animals, and man are caused by specific agents too small to be seen with a microscope but displaying many characteristics of living things was bound to excite the biologist. What are they? What do they look like? Where and how

did they originate? Are they alive? Where do they belong in the scheme of things? These were some of the questions immediately raised. The fact that viruses were found to be the cause of hundreds of diseases gave them an importance not attached to bacteria when they were first discovered. The problem was intensely practical as well as academic in character and as a consequence we find the plant pathologist, the veterinarian, and the groups interested in diseases of man, each engaged in investigating viruses, according to the methods at his disposal. From 1900 until the present there has accumulated a vast body of knowledge relative to the viruses and to the nature and control of virus diseases. Knowledge of the nature of the viruses themselves has had to wait for the development of suitable tools and techniques. The biologist is greatly indebted to the chemist for his recent contributions in this field.

The economic importance of these studies can be gauged from the fact that viruses may produce destructive diseases of nearly all commercial crop plants. Potatoes, tomatoes, tobacco, celery, corn, wheat, beans, peas, sugar beets, cotton, roses, and cherries, to mention but a few, are all subject to attack. As a matter of fact, flax seems to be the only plant of commercial importance which has not yet been shown to harbor at least one virus.

Animals, both wild and domesticated, are attacked by viruses, some of which are mild, others deadly. Hog cholera, foot and mouth disease of cattle, dog distemper, and rabies are a few of the more important diseases produced. Some species of fish are attacked by viruses.

Man is subject to many virus diseases, some almost always fatal, others extremely mild. Infantile paralysis, yellow fever, smallpox, influenza, the common cold, and even such diseases or conditions as warts and fever blisters are due to these agents.

Insects, too, are subject to virus diseases although only a few have been studied from that angle.

Bacteria, many of which are parasites, are parasitized in turn by viruses called bacteriophages.

It is little wonder, in view of the economic and public health importance of virus diseases, that many investigators have studied them intensively and that much has been learned; and, in view of the difficulties encountered in studying them, it is also little wonder that there are many questions as yet unanswered.

The Discovery of Viruses

The discovery of viruses came about in a way entirely different from the discovery of bacteria. Leeuwenhoek's discoveries were made in a haphazard manner. He was merely curious to see what his microscope

would reveal. The viruses were discovered when Iwanowski, in 1892, attempted to find the cause of tobacco mosaic. That bacteria, fungi, and protozoa are the causes of many diseases had been well established by 1890. Iwanowski searched and searched for a microorganism associated causally with tobacco mosaic but failed to find one. He did discover, however, that the juice from mosaic-diseased plants which had been passed

FIG. 25. Bacteriophage. Bacteriophage may be demonstrated by preparing pour plates of bacteriophage and susceptible bacteria. In this photograph the irregular dark areas show where the phage has eaten away the bacteria.

through a filter so fine that it held back fungi, protozoa, and bacteria, contained an agent capable of producing mosaic disease in the plant host. Was the agent a poisonous lifeless compound or was it a living agent? Many examples of the former were known—snake venom, bacterial toxins, the poisons of mushrooms, and so on. All of these were capable of producing disease but none produced diseases that were transmissible. If

an animal is injected with enough rattle snake venom, it dies, but material taken from it will not produce death when injected into another animal. There is no increase of poisonous principle. But Iwanowski could transmit the tobacco mosaic disease in series, and therefore the agent with which he was dealing multiplied in each plant. Little attention was paid to these findings until Beijerinck rediscovered the virus in 1898. He arrived at the conclusion that since it could pass through filters, it was in solution

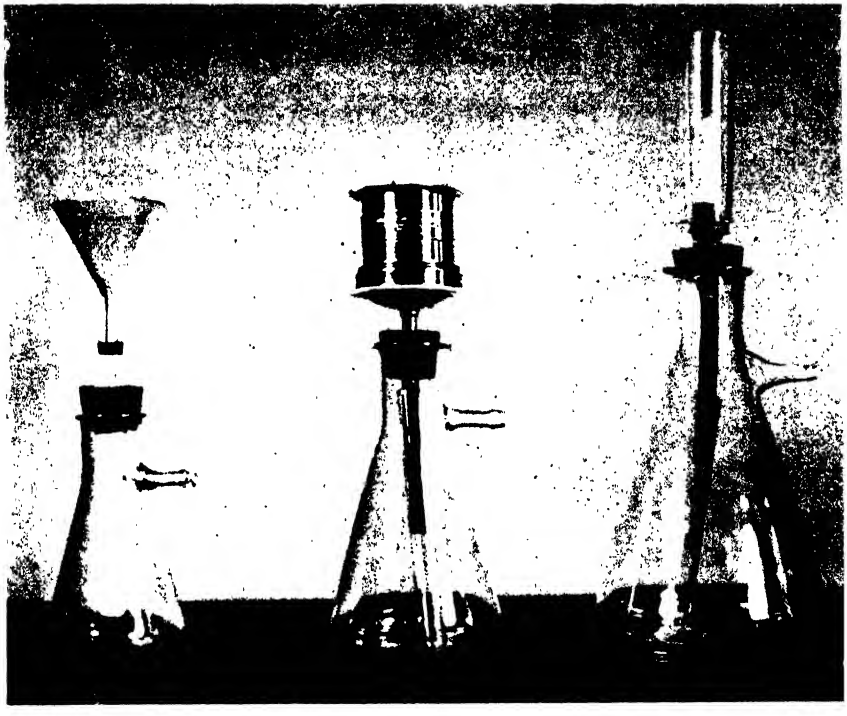

FIG. 26. Chamberland filter made of unglazed porcelain, Seitz filter made of asbestos pads held in metal holders, and Berkefeld filter made of diatomaceous earth. Filters may be obtained in various types, sizes, and grades of porosity.

and since it increased during its sojourn in plants, it was living. He therefore called it a *contagium vivum fluidum.* The same year Loeffler and Frosch discovered that foot and mouth disease is due to a similar agent. And all of these discoveries paved the way for a search for the cause of those many diseases which could not be shown to be due to fungi, protozoa, or bacteria The search still continues and new viruses are being found constantly.

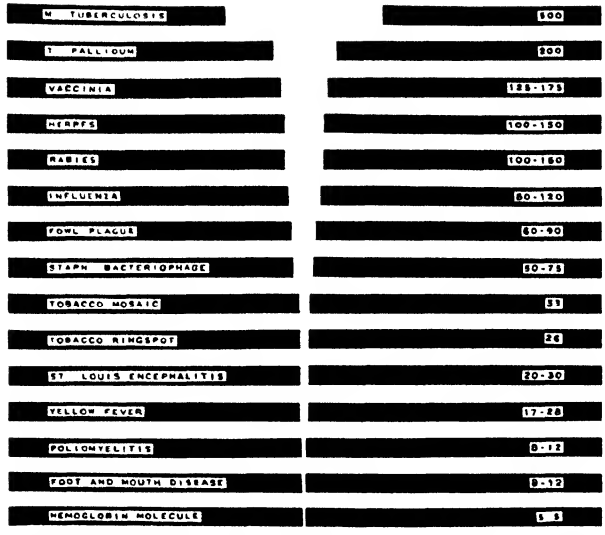

FIG. 27. Diagram showing the size of various viruses as compared to bacteria and to the hemoglobin molecule. The number on the bar is the diameter in millimicrons as estimated by filtration studies and the gap in the bar represents the relative diameters.

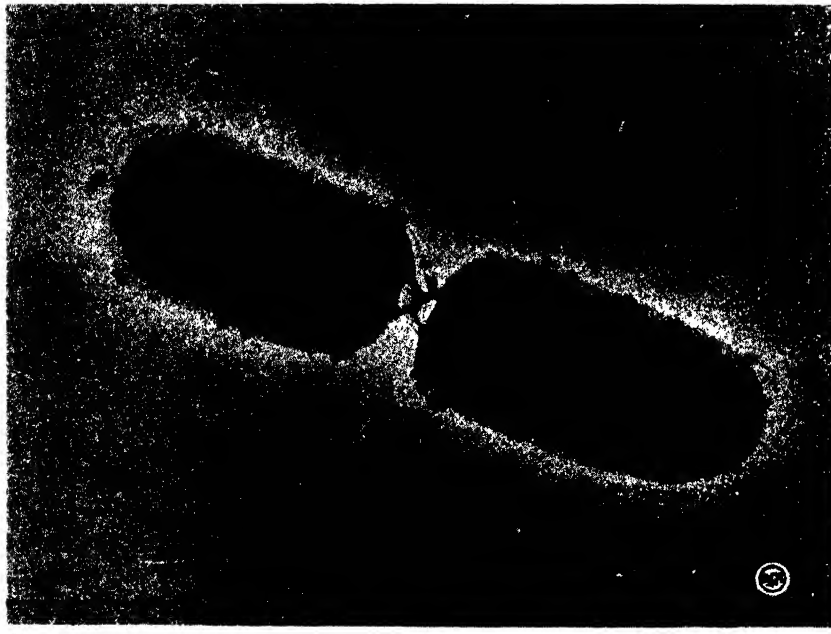

FIG. 28. This electron micrograph illustrates the relative size of *Escherichia coli*, one of the smaller bacteria, and one of the bacteriophages that attack it. There are nineteen adsorbed phage particles visible on the edges of the two bacterial cells.

Courtesy of Drs. Luria, S. E., Delbrück, M., and Anderson, T. F. and the Journal of Bacteriology.

Properties of Viruses

The properties ascribed to viruses reflect the tools and techniques used in their study, and the incompleteness of the data attests to the fact that we do not have adequate methods.

Physical Properties: Size: The outstanding physical feature of a virus is its size. This has been determined largely by two quite independent

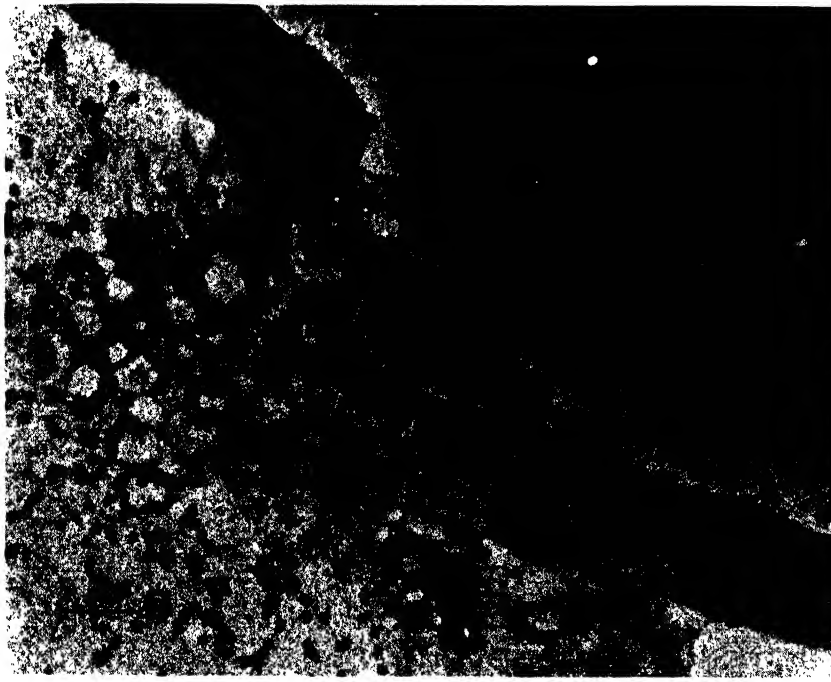

FIG. 29. After being adsorbed, bacteriophage multiply rapidly within the bacterial cell and the cell bursts. In this electron micrograph there can be seen part of an intact cell, the "ghosts" of two cells that have lysed, and many phage particles.

Courtesy of Drs. Luria, S. E., Delbrück, M., and Anderson, T. F., and the Journal of Bacteriology.

methods, filtration and sedimentation. Filters consisting of collodion membranes of graded pore size have been prepared. By using a series of these it is possible to determine the smallest pore which will allow the passage of the virus and thus to determine the size of the particle. The second method depends upon calculations based on the rate of settling during high speed centrifugation. Results obtained by the two methods agree and show that viruses range in diameter from about 10 millimicra to nearly 200 millimicra. In size they are a heterogeneous group.

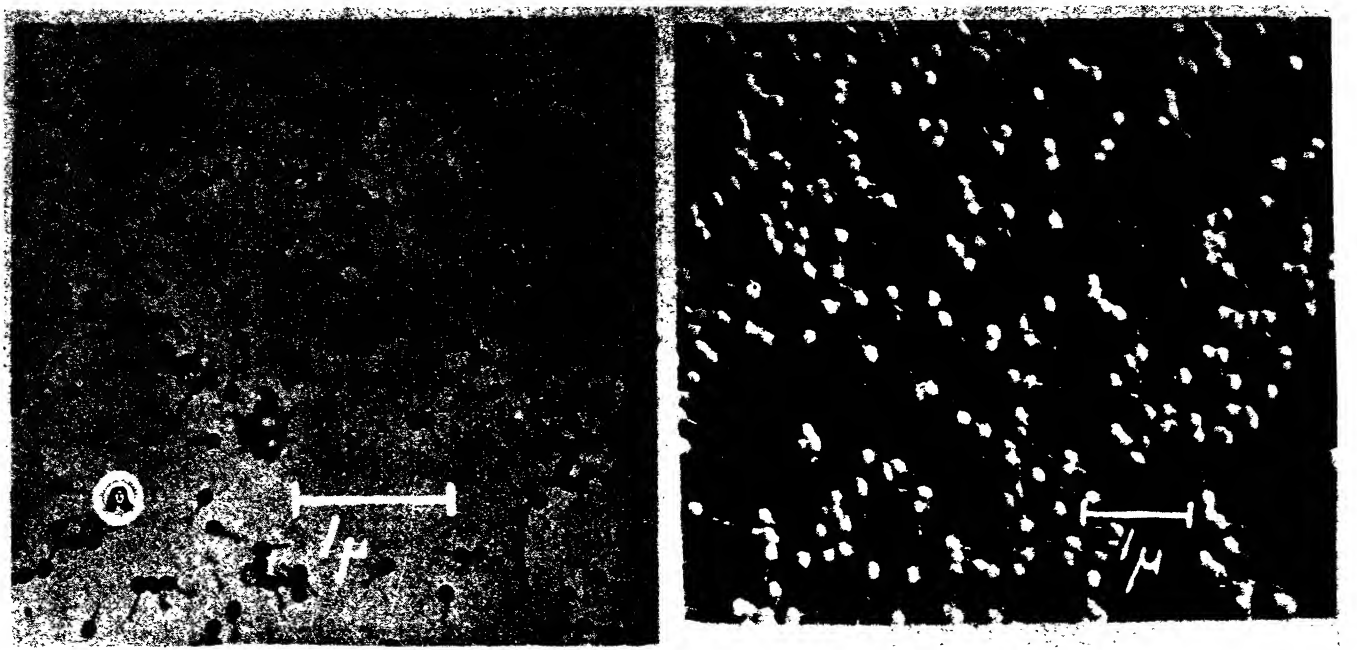

FIG. 30. These two electron micrographs show the difference between the ordinary electron micrograph of *Escherichia coli* bacteriophage and the electron micrograph using the shadow-casting technique. Note the tails on the phage particles.

Courtesy of Dr. G. Gordon Sharp

Resistance: The viruses react to chemical agents in much the same manner as do bacteria. They are rather readily inactivated by strong acids and alkalies and the common disinfectants. For an unknown reason many animal viruses are far more resistant to glycerine than are bacteria and survive in fifty per cent concentrations for years. Conse-

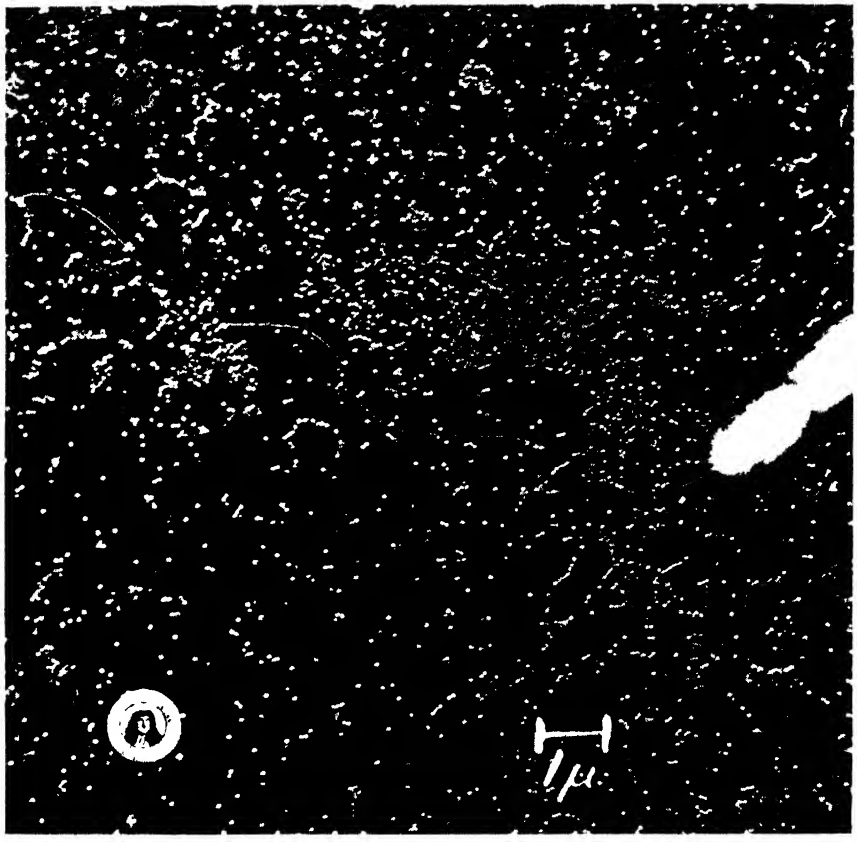

FIG. 31. An electron shadow micrograph of rabbit papilloma virus. A bacterium with a terminal, segmented flagellum appears on the right and establishes the order of magnitude in size of the virus particles.

Courtesy of Dr. G. Gordon Sharp

quently they are often preserved in glycerine. The heat resistance of viruses is not great. They are readily destroyed by boiling for a few minutes. Longevity, that is, the ability to survive apart from their natural habitat, is extremely variable. Some, the cucumber mosaic virus, for example, become inactive after aging for three to four hours; whereas tobacco mosaic virus will live in dry leaves for thirty years or more.

Crystalline Virus: A second feature which sets some viruses apart from other infectious agents is their crystalline structure. Some, but by no means all viruses, have been crystallized. The theory that viruses represent living infectious fluid led many workers to attempt to crystallize the active principle. In 1935 Stanley, following methods applied in the purification of proteins, announced that he had succeeded in isolating from the juice of plants infected with tobacco mosaic a protein of large molecular size which showed all of the properties displayed by the virus. A little later crystalline virus was isolated by sedimentation in ultra-centrifuges. The crystals of tobacco mosaic appear to be two dimensional instead of three dimensional, as are true crystals, and on that account have been called paracrystals. Following Stanley's work, Bawden and Pirie isolated the virus of tomato bushy stunt disease. This virus consists of nucleoprotein and the crystals are three dimensional. Other workers have shown that the virus crystals are present within the cells of plants. The tobacco mosaic virus protein is remarkable in that its molecular weight is said to be 17,000,000. Because of this tremendous weight, virus molecules have been called *macro*molecules (*great* molecules). The fact that viruses can be crystallized is significant because it shows that they can be obtained in a chemically pure state and that an infectious agent may be a macromolecule and not a cell in the traditional sense of the word. Theories concerning the origin of the viruses will be considered later.

Viruses Are Parasites: The third distinguishing feature of viruses is that they cannot be grown, that is, will not increase or multiply, unless they are in living cells. For all we know, they are strict parasites. In this they behave like many microorganisms, as, for example, the rickettsias, some protozoa, and some of the multicellular organisms. Because viruses cannot be grown apart from living cells which are protein, their separation and isolation from the cellular protein of the host is a great step toward a better understanding of them.

Viruses Change: A fourth characteristic property of viruses is their tendency to variation. The evidence for this is the fact that strains may be selected which produce diseases different from those produced by the parent strain. Although a tobacco plant inoculated with tobacco mosaic virus almost always displays symptoms indistinguishable from those displayed by the plant from which the virus was taken, it does happen, very occasionally, that a quite different disease may result. Strains of virus differing according to the species of plants they attack or the severity of the disease they cause may be artificially selected by a number of means. These strains do, however, show a great similarity to the parent strain.

In the case of animal virus we may likewise select strains differing in respect to their disease-producing capacity. When the virus which pro-

duces smallpox in man is introduced into the calf, it causes cowpox. If transferred serially through several calves and then reintroduced into man, it no longer produces the deadly smallpox but a very mild infection which confers an immunity to smallpox. Smallpox vaccine is smallpox virus which, during its sojourn in the calf, has undergone a loss of its power to produce smallpox.

The bacteriologist is continually at work trying to induce a similar variation of other viruses, hoping that these mild strains may be used as vaccines for severe diseases. He has succeeded with the virus of yellow fever but not with the virus of infantile paralysis.

It is an accepted fact among plant pathologists that new virus diseases are continually appearing. Plants are attacked which never before suffered from a particular virus disease. The most plausible explanation for the appearance of many new plant diseases is that a virus which has been attacking other species has undergone a change resulting in a new strain with the capacity for growing and producing disease in the plant in question.

The fact of variation or mutation in viruses is, then, of great practical importance because it accounts for the appearance of many new diseases and because the investigator can, in some instances, take advantage of the production of non-virulent strains to prepare vaccines capable of protecting animals and men against the more deadly strains.

The Nature and Origin of Viruses

In considering the nature and origin of viruses the characteristic features already mentioned should be recalled: viruses are extremely small, they are protein in nature, some have a crystalline structure, none can grow apart from living cells, and they have a tendency to vary or mutate.

Before or rather instead of attempting to answer the question "Are viruses living?" it might be well to point out that the concepts of living or non-living are man-made and have no biological validity. They are polar concepts like male and female, positive and negative, health and disease, good and bad. One has meaning only in terms of the other. There is no way in which we can set up an experiment to determine whether or not a virus is alive. But we can determine the attributes of other living things and decide whether viruses have these same characteristics. In light of our present information all we can say is that viruses display some features which would place them with the living microorganisms and others which would tend to place them with non-living. Perhaps we may find that there are degrees of life just as there are degrees of health or goodness and that life is not an all or none phenomenon. Viruses may have a place in a scale rather than on one side of an artificial line.

The theories advanced to account for viruses may be grouped into two broad categories. The first includes the theory that viruses are living microorganisms differing from bacteria mainly in respect to size. The fact that they react like microorganisms to heat or chemicals, that they multiply, that they produce disease and induce immunity, and that they show a tendency to variation but, in general, breed true is in accord with such a supposition. The fact that some can be crystallized is not in accord, although it may not rule out such a possibility.

If viruses are microorganisms, they cannot represent the most primitive forms even though they do lack the degree of morphologic organization found in bacteria; and the fact that they are parasitic on higher forms rules out the probability that they represent the connecting link between living and non-living forms from the evolution standpoint. Hence the suggestion has been made that viruses may have arisen by a process of "retrograde" evolution in which they descended from protozoa or bacteria that had become adapted to a parasitic existence within cells and had subsequently lost their power to live independent lives. We find many examples of the latter both in microorganisms and in higher forms.

Organisms must have food for energy and growth. They must also have the mechanisms necessary for the utilization of food and for the regulation of growth and reproduction. The reactions by virtue of which cells carry on most of their activities are enzyme reactions. It has been postulated that as bacteria became adapted to a parasitic existence within the cells of plants or animals they lost many of the enzyme systems necessary for maintaining an independent existence but continued able, somehow or other, to utilize the host cell as a source of food and energy. As these bacterial parasites became more and more highly specialized there was a progressive loss of enzymes and of size, since enzymes occupy space. Theoretically this process could be continued until a point is reached at which the parasite consists of only a portion of its former self—a portion which, however, retains the property of regeneration and multiplication, and this, then, is the virus. Such a hypothesis accounts for the size of viruses and for their absolute dependence upon host cells for multiplication. Furthermore, it may be postulated that different kinds of bacteria, or perhaps protozoa, could undergo such a progressive retrograde evolution and that the resulting viruses might be quite different from one another, thus accounting for the fact that viruses are a heterogeneous group in so many respects. It has been further suggested that the damage to the host cells by the virus is due to their interference with the normal activities of the cell, the virus utilizing energy and materials necessary for the cell. This, also, is in accordance with the facts at hand.

The second category holds the theory that viruses are non-living chemical

substances, describing them as autocatalytic enzymes or "wild" genes. According to this view, a virus is not, in itself, an organism. It is probably an enzyme which has the power or property of altering the metabolism of the parasitized cell in such a manner that the cell produces more of the enzyme. Such an enzyme could be called autocatalytic but just how such an autocatalyst originated would be difficult to say.

The supposition that viruses are "wild" genes is based on the fact that genes are protein, that they play a part in the control of inheritance, and they are duplicated or multiply, in the cells. One might suppose that a gene could by chance be introduced into a cell of a different species and that if the cell proved a suitable environment it could not only survive but actually influence the activities of the cell and be duplicated in the process.

These various theories concerning the nature and origin of viruses serve a useful purpose in that they suggest further investigations which may add new facts and bring about a better understanding of these fascinating agents.

Chapter V

BIOLOGICAL CHARACTERISTICS OF MICROORGANISMS: PHYSIOLOGY

"I sometimes think that never blows so red
The rose as where some Buried Caesar bled;
That every Hyacinth the Garden wears
Dropt in her Lap from some once lovely Head"

All organic matter is continuously changing. An organism grows and dies and decays. The elements which went to make it up are never destroyed but are used over and over again. The same nitrogen and carbon atoms that were a part of the dinosaurs have been a part of countless plants and animals in the succeeding ages and will be the building stones for new forms of life so long as life exists. In this ever-changing procession there is a continuous construction and a continuous destruction of organic compounds. Green plants build them up from the carbon dioxide of the air, from water, and from the minerals of the soil; energy for this synthesis coming from the sun. Animals feed upon plants or upon other animals which have, in their turn, fed upon plants, and obtain the nutrients and energy necessary for life from them. They are destructive, as are most bacteria, in that they must break down compounds of plants or animals in order to obtain food and energy. However, this destruction or breaking down is just as essential in the economy of nature as is the constructive process. If it did not take place, the earth's surface would soon be covered with the remains of dead plants and animals, the elements would be locked up in them, and life could not continue.

"The *physiology* of an organism," says Buchanan, "may be defined as including all the inter-relationships between the organism and its environment. This will include all of the effects of environment upon the organism and all the effects of the organism upon its environment." The chain of chemical reactions involved when microorganisms feed upon plant and animal residues and reduce the more complex substances to simpler ones which may again be used by plants is a part of the physiological process of the microorganism. It is one of the features of microbiology of greatest practical importance. We want to know enough about the effects of the environment upon the organism so that we may provide favorable conditions for those organisms which act upon their environment in such a way as to bring about the changes we desire and we also want to know how to

provide unfavorable conditions for those organisms bringing about undesirable changes. Knowledge of how microorganisms do what they do and of what factors influence their activities is essential if we would deal intelligently with them and control them consciously. It must be remembered that the microorganisms are living things and that they obey biologic laws. They react in general to the same influences as do the higher forms. This need not surprise us since we have already seen that they are made up largely of protoplasm and that it is the essential component of all living things.

ENZYMES

Enzymes play an all important part in the functioning of biological forms, high and low. The word itself comes from a Greek word *en* meaning in and *zyme* meaning yeast. Because they are the essential stuff of all biological processes we shall consider them first and in some detail. Enzyme is a general term for a class of substances which have certain characteristics in common.

First, enzymes are the products of living cells. All cells produce enzymes and by virtue of them are able to prepare foodstuffs in an appropriate form, build up complex materials from simple compounds, and make available the energy necessary for all the activities of the cell.

Second, enzymes are biochemical catalysts. A catalyst is a substance that changes the rate of a chemical reaction, usually by increasing it. It is not used up in the process but apparently acts over and over again and may be recovered unchanged at the completion of the reaction. Enzymes have much this same property and are even more active than inorganic catalysts.

Third, many enzymes can carry on their activities quite apart from the living cell. This feature led a good many of the early workers to dispute Pasteur's theory that fermentation was a biological process dependent upon living cells.

Fourth, enzymes are protein in nature or are linked to proteins. They all contain nitrogen. A few have been crystallized and found to contain about fifteen per cent nitrogen.

Fifth, in general, enzymes are soluble in water. We should probably expect this to be true since water is a constituent of all cells. It plays a part in the chemical reactions involved in metabolism and in the transport of food stuffs through the cell wall.

Chemically, enzymes are rather unstable substances. They are amphoteric in nature, that is, they may behave like acids or like bases. This characteristic may determine their activity as adsorbing agents and account

for the fact that they act more strongly at certain hydrogen-ion concentrations than at others.[1]

Factors Affecting Enzyme Activity: Temperature is one of the factors governing the activity of enzymes. The speed of chemical reactions is affected by temperature, being approximately doubled with every ten degree Centigrade rise. The speed of chemical reactions involving enzymes increases as the temperature rises until an optimum is reached. This lies between 30° to 45° C. for most enzymes. Most do not act at temperatures greater than about 70° C., and none are active at the temperature of boiling water.

Many enzyme reactions involve the liberation of heat. In the decomposition of farmyard manure or hay, the heat may be sufficiently great to destroy the microorganisms bringing about the reaction.

Enzymes are profoundly affected by the acidity or alkalinity of the medium in which they are acting. Each has its optimum pH and any increase in acidity or alkalinity tends to retard the action, or, if great enough, to inactivate the enzyme completely. An instance of this is found in the following example. The reaction of the stomach is highly acid and that of the intestines alkaline. The enzyme pepsin which acts on protein in the stomach acts only in an acid medium such as found in the stomach,

[1] Hydrogen-ion Concentration: The acidity or alkalinity of a solution depends upon the relative proportion of the free or dissociated hydrogen ions (H) and hydroxyl ions (OH). If the H and OH ions are equal, the solution is considered neutral. Pure water is such a solution for, although it is very slightly ionized, the hydrogen and hydroxyl ions are always equal. For every hydrogen ion liberated there is a hydroxyl ion liberated—$HOH \rightarrow H \rightleftarrows OH$.

The ionization of water is a reversible reaction and the hydrogen and hydroxyl ions are formed at the same rate at which they recombine to form water. This rate is a constant and is called the *ionization constant*. Conductivity measurements have shown that in one liter of water the free H and OH ions weigh 0.000,000,000,000,01 or 10^{-14} grams. Since the H and OH ions are equal there are 10^{-7} gram moles of H ions and 10^{-7} gram moles of OH ions per liter.

The acidity of a solution depends upon the concentration of H ions and can be expressed in terms of the hydrogen-ion concentration. A neutral solution has an H ion concentration of 10^{-7}. To simplify the expression it has been agreed that the base 10 and the negative sign of the exponent be omitted and that the symbol pH be used. Thus a hydrogen-ion concentration of 10^{-7} is written pH 7, which is considered neutrality. By definition, pH is the logarithm of the reciprocal of the hydrogen-ion concentration.

Since the pH number represents the logarithm of a fraction, the smaller the pH number, the larger the fraction. The difference of 1 pH unit represents a ten-fold change in hydrogen-ion concentration. The lower the number, the more acid the solution; the higher, the more alkaline.

The acidity or alkalinity of a solution can also be expressed in terms of the total amount of acid or alkali present. This is determined by titration with standard acid or alkali and referred to as *titrable acidity* or *alkalinity*. Titrable acidity depends

while the enzyme trypsin, found in the intestine, acts only in an alkaline medium such as found in the intestine.

This sensitivity of the enzymes to the acidity of the medium makes it possible to control certain reactions by changing the pH. Since microorganisms carry on their metabolism by means of enzymes, their growth may be restricted by providing conditions too acid or too alkaline for the enzymes to act. This is what we do when we pickle, make sauerkraut, silage, and so on. When bacteria break down sugars the acids produced may be sufficient to lower the pH of the medium to a point where the enzymes no longer function. This accounts in part for the rapid dying of certain bacteria when grown in a medium containing a fermentable sugar.

A number of chemicals act quite specifically to inhibit or block the action of enzymes. Cyanides, for example, act on the enzymes that bring about oxidation and thus interfere with respiration. The heavy metals such as mercury inactivate or inhibit the action of the enzyme which hydrolyzes sucrose. It seems probable that many of the dyes which inhibit bacterial growth in dilutions as high as 1 to 500,000 exert their action on some special enzyme system necessary to growth; for if the inhibitors be removed by appropriate means, the bacteria will grow, thus indicating that the enzyme itself was not destroyed but was merely inactivated.

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upon the number of replacable ions and not upon the number present in solution. 100 c.c. of normal hydrochloric acid and 100 c.c. of normal acetic will neutralize the same amount of sodium hydroxide. However, the pH of hydrochloric acid is much greater because it is almost completely ionized; whereas acetic acid is only slightly ionized.

The following table illustrates normality, pH, and the concentration of ions expressed as moles per liter.

FRACTION OF NORMALITY	pH	HYDROGEN IONS (MOLES PER LITER)	HYDROXYL IONS (MOLES PER LITER)
N HCl	0.0	10^{-0}	10^{-14}
0.1 HCl	1.0	10^{-1}	10^{-13}
0.01 HCl	2.0	10^{-2}	10^{-12}
0.001 HCl	3.0	10^{-3}	10^{-11}
0.0001 HCl	4.0	10^{-4}	10^{-10}
0.00001 HCl	5.0	10^{-5}	10^{-9}
0.000001 HCl	6.0	10^{-6}	10^{-8}
Neutrality	7.0	10^{-7}	10^{-7}
0.000001 NaOH	8.0	10^{-8}	10^{-6}
0.00001 NaOH	9.0	10^{-9}	10^{-5}
0.0001 NaOH	10.0	10^{-10}	10^{-4}
0.001 NaOH	11.0	10^{-11}	10^{-3}
0.01 NaOH	12.0	10^{-12}	10^{-2}
0.1 NaOH	13.0	10^{-13}	10^{-1}
N NaOH	14.0	10^{-14}	10^{-0}

How Enzymes Are Classified and Named: Enzymes may be separated into two general groups, those that appear in the medium surrounding the growing organisms and those that can be liberated only by disrupting the cell walls. The former are called *exo-* or *extracellular* enzymes because they appear outside the cell. They are also called *hydrolases* because the reactions with which they are concerned involve the addition of water. The latter are called *endo-* or *intracellular* enzymes because they are within the cell. They are also called *desmolases* or oxidation-reduction enzymes because the reactions with which they are concerned involve energy changes. Such a distinction or grouping is worth while because of the differences as to the types of reactions which each group induces.

From the point of view of the microorganisms, the enzymes have very different functions. Bacteria, yeasts, and molds require food in solution. However many of the food stuffs needed by these organisms are not readily soluble nor easily diffusible through the cell membranes. Extracellular enzymes secreted into the surrounding medium break down the fats, carbohydrates, and proteins by a series of hydrolytic changes involving the chemical addition of water and resulting in the production of compounds which are diffusible and therefore can be absorbed by the cell. These are *hydrolytic reactions* induced by *exoenzymes* or *hydrolases* and yield very little energy. The reaction involved in the actual building up of the protoplasm within the cell requires considerable energy which the organism gets from changes induced in the absorbed food by *endoenzymes* or *desmolases*.

Specificity of Enzymes: An enzyme that attacks fat does not attack carbohydrates or proteins and one attacking carbohydrates does not attack fats or proteins. In general, enzymes are named by attaching the suffix *ase* to the name of the compound acted upon or according to the type of reaction induced. Thus enzymes that act on proteins have been called *proteases*, those that act on fats, *lipases*, and those that act on carbohydrates, *carbohydrases*. Enzymes bringing about oxidation and reduction are called *oxidases* or *reductases*, and those inducing hydrolysis, *hydrolases*. Enzymes involved in respiration are called *desmolases* and include reductases and oxidases. Hydrolases include carbohydrases.

The specificity of enzymes goes beyond the general class of substances such as fats, carbohydrates, and proteins. Enzymes that hydrolyze glucose do not hydrolyze maltose. The enzyme lactase which acts on milk sugar or lactose does not attack starch, and so on. There is a specificity for the type of chemical linkage but not necessarily for each compound.

Enzymes and Bacterial Classification: Inasmuch as enzymes are usually specific with respect to the type of compound they break down or build up and inasmuch as some organisms possess certain enzymes and hence can hydrolyze compounds which other organisms not possessing these

enzymes cannot hydrolyze, it follows that organisms can be separated and, to a certain degree, classified by determining which enzymes they possess. The so-called physiological or fermentation reactions so widely used in the classification of bacteria are nothing more nor less than a determination of the enzymes possessed by a given organism by growing it in a medium containing known compounds and then making the appropriate determinations for changes occurring in them.

Adaptive and Constitutive Enzymes: Bacterial enzymes are, as a rule, produced regardless of whether the specific substrate is one upon which the enzymes can act. However, occasionally a bacterium produces an enzyme only when material upon which it can act is present. *Escherichia coli-mutabile,* for example, does not generally produce the enzyme lactase; but if this organism is grown in a medium containing the sugar lactose, it will, after repeated transfers, acquire the ability to ferment it. Such an enzyme appearing as a specific response to a particular substance is called an *adaptive enzyme.* One produced whether the substance upon which it acts is present or not is called a *constitutive* enzyme.

How Enzymes Act: There are a number of theories as to how enzymes act. None is entirely satisfactory and all are somewhat involved and will not be considered here. For discussions the reader is referred to Topley and Wilson, *Principles of Bacteriology and Immunity;* Anderson's *Bacteriological Chemistry; Annual Review of Biochemistry,* Stanford University Press; C. H. Werkman, *Dissimilation of Carbohydrates; Bacteriological Reviews,* Volume 3, No. 2, 1939.

BACTERIAL METABOLISM

What Type of Food Do Bacteria Need?

The type and variety of materials which bacteria can use for food is infinitely greater than that utilizable by the higher plants or animals. All of the higher plants require essentially the same elements and simple compounds which, with the aid of energy derived from sunlight, they use to build up plant protoplasm. The higher animals get their energy from the breakdown of relatively complex substances and, in addition, must be supplied with more than twenty amino acids. In contrast to plants on the one hand and animals on the other, both of which follow a general pattern with respect to food requirements, the bacteria, although they require essentially the same elements, are characterized by great diversity with respect to their food requirements. They do however fall into two rather clearly defined groups: the autotrophic and the heterotrophic.

Autotrophic Bacteria: Some bacteria are able to use simple inorganic compounds such as carbon dioxide and ammonia, nitrates and nitrites as a source of carbon and nitrogen. The energy needed for growth can be

obtained in one of two ways. Some, the *photosynthetic autotrophic* bacteria, contain pigments, such as *bacteriopurpurin*, which function as does chlorophyll in plants. These can utilize the sunlight as a source of energy. Others, the *chemosynthetic* bacteria, get energy from the oxidation of simple inorganic compounds or elements, such as sulfur, iron, or hydrogen. These bacteria are called autotrophic because they are self-sufficient in that they are not dependent upon compounds built up by living things. They are widely distributed in soils.

Heterotrophic Bacteria: For the most part bacteria, like animals, obtain energy by the dissimilation of complex organic compounds. The heterotrophic bacteria vary widely as to the types of compounds they require as food. Some are facultative autotrophs and can use inorganic as well as organic substances. Some require not only complex organic compounds but also "accessory growth" substances. Since this group is not self sufficient but depends upon compounds built up by other living things, it is said to be *heterotrophic.* To it belong all parasites and pathogens and many of the saprophytes.

From the evolutionary point of view it would seem reasonable to place the chemosynthetic autotrophs first since they can grow on simple salts, photosynthetic autotrophs next, and then the heterotrophs. The latter can be arranged in a graded series beginning with facultative autotrophs and ending with fastidious parasites which require accessory growth substances.

Bacterial Respiration

The term respiration has been defined in different ways depending upon the concept of the mechanism involved and has consequently changed from time to time as our information has become more exact. Originally respiration referred to the breathing of animals; later to the taking up of oxygen and the giving off of carbon dioxide and water. When it was realized that the process involved energy, the term came to refer to reactions which involved oxidation of materials with the release of energy. In all these early concepts oxygen played an important part and respiration was thought of essentially as an oxidation process. The discovery by Pasteur of an organism which did not grow in the presence of air led to a still further modification of the concept of respiration until the term has now come to mean any chemical reaction carried on by cells which involves the release of energy for use by the cells. Oxygen is not essential although oxidation is involved.

Bacteria may be divided into four groups on the basis of oxygen requirements or of the type of reaction by which they obtain energy. *Obligate aerobes* are bacteria which grow in the air or in the presence of free oxygen

and are said to be aerobic. *Obligate anaerobes* cannot grow in the presence of air or free oxygen and are said to be anaerobic. *Facultative anaerobes*, although they multiply most rapidly in the presence of air or free oxygen, can grow in the absence of free oxygen. *Microaerophilic organisms* are, as the name implies, lovers of small quantities of free air or oxygen. They do not grow in the total absence of air but neither will they grow unless the oxygen tension has been reduced.

To find the reasons that some organisms are able to grow only in the presence of oxygen or air and others only in its absence brings us to a consideration of the nature of the reactions by which bacteria obtain their energy, that is, to respiration. Because during this process one substance is oxidized and some other substance, consequently, reduced, respiration is an oxidation-reduction reaction. The types of substances which can be oxidized and reduced by virtue of the enzymatic equipment of the organism determine, by and large, whether the organism is an anaerobe or an aerobe.

Oxidation may or may not involve oxygen but it must involve the transfer of hydrogen or electrons. In a biological oxidation-reduction, hydrogen is transferred from one substance called a *donator*, which is thereby oxidized, to another called an *acceptor*, and in the process energy is released. When a substance is oxidized, electrons or hydrogen may be removed or oxygen may be added. When it is reduced, electrons or hydrogen may be added or oxygen removed. The withdrawal of electrons may take place without involving the addition of oxygen, and the addition of electrons may take place without involving the element hydrogen.

To put it in another way:

Oxidation may be defined as:	Reduction may be defined as:
1. addition of oxygen	1. removal of oxygen
2. removal of hydrogen	2. addition of hydrogen
3. removal of electrons	3. addition of electrons

In ordinary respiration oxygen acts as a hydrogen acceptor. However, and this is where enzymes are important, oxygen does not readily combine with hydrogen which is chemically linked and enzymes are necessary to act in some way to speed up the reaction. The oxygen is then said to be activated, a term which expresses the concept but does not explain it. Enzymes which activate atmospheric oxygen are called *oxidases*. If the hydrogen acceptor is a dye or some simpler substance, the enzyme which activates it is called a *reductase*. Otherwise, the enzyme activating the transfer of hydrogen is called a *dehydrogenase*.

Biological oxidation-reduction reactions are divided into three types, the following description of which is taken from Anderson's *Bacteriological Chemistry:*

"Type I. The hydrogen acceptor is atmospheric oxygen, that is, direct oxidation

occurs, as is the case with the production of acetic acid in the vinegar fermenting or the action of many molds and of the mycobacteria on sugar.

Type II. The hydrogen donator and acceptor are the same molecule, giving rise to an intra-molecular fermentation. As an example one may take the conversion of glucose $C_6H_{12}O_6$ into 2 molecules of lactic acid 2 $C_3H_6O_3$. Apparently no hydrogen or oxygen is required from the outside, the new compound resulting from a rearrangement of the distribution of the hydrogen and oxygen within the molecule. Actually the process is not as simple as this since the final effect is brought about by a whole series of intermediate reactions. What it really amounts to is that a single carbon compound is sufficient for the growth of the organism.

Type III. The hydrogen donator and acceptor are different compounds, resulting in inter-molecular fermentation. Type I is a special case of Type III in which the acceptor is oxygen. Examples of this type are the anaerobic fermentations at the expense of the oxygen of fumarates, nitrates, sulfates, or similar highly oxidized substances."

In the light of the fact that bacteria may obtain energy from oxidation-reduction reactions of a number of types, let us consider the respiration of the four groups of bacteria previously mentioned. But before doing so, let us note one more thing. When oxygen is used as a hydrogen acceptor, peroxide is usually formed. This is more or less toxic to bacteria and, if it were allowed to accumulate, would soon reach sufficient concentration to be destructive. However peroxide is broken down to water and free oxygen by an enzyme called *catalase* and some, though not all bacteria, produce catalase. While hydrogen peroxide will decompose without the intervention of catalase, the reaction is very much slower. This can be readily demonstrated. Hydrogen peroxide bubbles very slowly when it is poured on the intact skin. If poured in a wound or taken into the mouth as a gargle, it foams because the tissue cells, with which it comes in contact, contain the enzyme catalase that speeds up the breakdown of the hydrogen peroxide.

McLeod, after studying the relation of catalase to the oxygen requirements of bacteria, first advanced the following hypothesis which explains the situation, at least in part: obligate anaerobes do not produce catalase and are sensitive to hydrogen peroxide. In the presence of air the free oxygen acts as a hydrogen acceptor and hydrogen peroxide is formed. In other words, it is not the oxygen itself which is toxic to anaerobes but the hydrogen peroxide formed from it.

Some aerobic bacteria do not produce hydrogen peroxide and hence can grow in the presence of free oxygen even though they do not produce catalase. In the case of some, both catalase and hydrogen peroxide are produced. The catalase acts on the hydrogen peroxide so that it does not accumulate in toxic amounts.

Microaerophilic bacteria do not produce catalase and do produce hydrogen peroxide. However, they are relatively resistant to it and are able

to tolerate the amount that accumulates in the presence of a small amount of air. These organisms can be grown only when the amount of air or oxygen is reduced.

Another explanation, based on different grounds and with much to recommend it, is that anaerobes will grow in the presence of air providing a suitable reducing substance such as sodium thioglycollate, for instance, is present. Such a substance lowers the oxidation-reduction potential and experimental evidence suggests that it is the oxidation-reduction potential rather than the oxygen itself which is important.

Bacterial Metabolism Defined: Bacterial metabolism is the total of the chemical changes produced by cellular activities in the building up of new protoplasm, the repair of protoplasm, and the breaking down of the substances used by the cell. In general physiology it is customary to refer to the building up process as *anabolism* and the tearing down one as *katabolism.* In recent years the students of bacterial metabolism have introduced two other terms to designate these processes, namely, *assimilation* and *dissimilation.*

Assimilation refers to the conversion of food into cellular material and the parts of the compounds which are so used are said to be assimilated. This process has not been studied intensively because of the inherent difficulties involved.

Dissimilation refers to the breaking down of compounds which provide the energy used in assimilation. It has been possible to study dissimilation intensively because it lends itself to controlled experiment and it is desirable because the microbial processes of economic importance are, in the main, dissimilations.

Fermentation and Putrefaction: These terms are defined in various ways by different workers. Fermentation (to boil) is often used to designate the incomplete breakdown of carbohydrates which results chiefly in the production of acids, alcohols, carbon dioxide, and water. Putrefaction is often used to indicate the incomplete breakdown of proteins. Since the products that result are often vile smelling compounds this term carries with it the connotation of offensive odor. The term dissimilation includes both putrefaction and fermentation.

How Microorganisms Feed

The process of digestion, that is, the chemical conversion of raw food into a form which can be assimilated or used for energy, differs according to the organism.

Single-celled animals such as the amoeba engulf or ingest the raw food and digestion by means of intracellular enzymes takes place within the cell. The waste products are excreted.

Since bacteria, yeasts, and molds have rigid cell structure they cannot engulf particles. Their food must be in solution. Enzymes excreted by the cell into the surrounding medium dissolve the food which can then be absorbed through the cell membrane and metabolized. Such digestion is said to be extracellular because the enzyme reactions take place outside the cell. They are largely hydrolytic in character and yield very little energy, which, since it is liberated outside the cell, cannot be used in the building up of protoplasm although, as previously mentioned, it may be liberated as heat and actually be detrimental to the organism.

The cell membrane, which is semipermeable, exercises a selective action allowing certain substances to pass through it and keeping others out. The mechanism by which this is accomplished is highly complex and not clearly understood.

The amount of food needed by a single bacterium or yeast is, of course, extremely little, although in relation to what larger organisms use, it is relatively great. Rahn has calculated that bacteria consume about twice their own weight in sugar per hour. However, many organisms, particularly the anaerobes, do not as a rule utilize more than a small part of the total energy in the molecules they attack since few are able to dissimilate them completely. It is their inefficiency in this respect that makes them so efficient as chemical engines. When an organism uses only a fraction of the energy of any molecule, it requires a great many molecules to produce the necessary energy for the organism's growth and multiplication. One molecule of glucose yields 673 calories when it is completely oxidized to carbon dioxide and water. An organism that cannot completely ferment it but can break it down only as far as lactic acid obtains only 21 calories of this total. Thus yeasts which convert glucose to alcohol obtain only a fraction of the total energy in the molecule. The aerobic organisms are far more efficient in that they can oxidize the glucose more nearly completely, but as converters of sugar to some of the intermediate compounds they are not satisfactory.

The Size of Microbial Cells Again: The smaller the organism the greater the ratio of its surface to the volume of its mass. Salle has compared the ratio of the area to the weight of an average bacterium and of man and finds that it is 200,000 times greater for a bacterial cell than for man. Many of the reactions involved in bacterial metabolism are surface reactions and this proportionately tremendous amount of surface accounts for the rapidity with which microorganisms act upon materials in which they are growing. The streptococcus responsible for the souring of milk has a diameter of about 0.7 micron. At the end of forty-eight hours incubation there are about two billion organisms per milliliter and the number in a

quart of milk would have a surface of over thirty square feet and would weigh less than a gram.

GROWTH PHASES

Nature is characterized by an ever present struggle for existence that serves to keep the number of any one species in check. Were any species allowed to multiply at its theoretical maximum rate, in a very short time that species would cover the earth. A bacterium which divides once every twenty minutes could give rise to 5,000,000,000,000,000,000,000 descendants in twenty-four hours. Of course, bacteria never do continue to increase at any such rate for many factors play a part in keeping their numbers in check. It has been observed in experimental cultures that multiplication does not continue at a uniform rate but goes through a regular series of what may be called "phases" in the life of the culture.

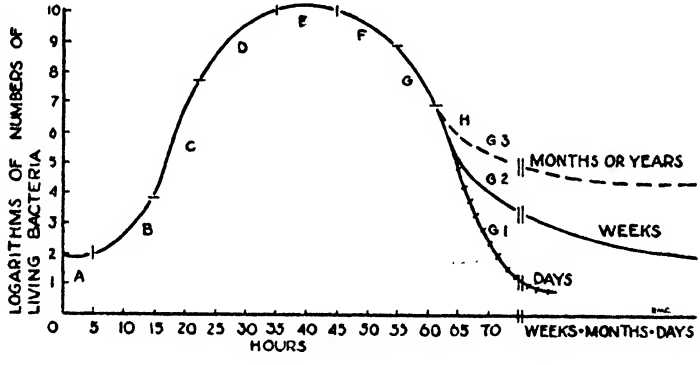


FIG. 32. Growth curve

Bacterial Growth Curve

If we inoculate a tube or flask of culture broth with a few bacteria, determine the number at regular periods of time, and then plot the logarithms of their numbers against the time, we get a characteristic curve which shows the rate of increase at any time interval. Numerous workers have plotted growth curves for various bacteria and, in general, the curves show a similar pattern. Such curves have been arbitrarily divided into several sections and each given a descriptive name. In the accompanying hypothetical curve these sections or phases are designated by letters and may be described briefly as follows:

A. *Initial Stationary Phase:* Bacteria when first introduced into a suitable culture medium do not begin to multiply immediately. There is a period in which they appear to be taking up water, increasing in size, and perhaps becoming adjusted to their new environment, in which no multi-

plication occurs. There may actually be a decrease due to cells dying. This period, commonly called the initial stationary phase, varies from minutes to hours or longer depending upon the species of organism used and the conditions of the experiment.

B. *Accelerated Growth Phase:* In due course of time, the organism begins to divide. The rate, at first, is slow but becomes faster with each generation until the average generation time for the species in question is reached. Some workers designate A plus B as the *lag phase.*

C. *Logarithmic Growth Phase:* When the generation time reaches a constant minimum level, the rate of increase likewise becomes constant. The logarithmic growth phase represents the period of greatest activity during which materials in the medium are being broken down and built up into bacterial cells most rapidly. Many microbiological processes are designed to keep organisms in this phase.

D. *Negative Growth Acceleration Phase:* After a period of time the rate of growth begins to decrease, at first slowly, then more rapidly until it again becomes stationary. As in the accelerated growth phase, the time required to complete this phase is subject to the influence of the environment and the species of organism.

E. *Maximum Stationary Phase:* When the stationary phase is reached the number of viable bacteria has reached its maximum. There is no further increase in numbers although for a while some cells are dividing, others dying, and the rate of multiplication and of dying approximately equal. Many workers have become interested in this phenomenon. Bail's findings, abstracted from Topley and Wilson, are of particular interest. He finds that in a particular medium each species reaches a constant concentration of living organisms which he has called the M-concentration. It differs with each species and particular set of conditions. If the bacteria in a certain medium have reached the M-concentration point, and more bacteria of the same species are introduced, some will die off until the M-concentration is again reached.

There are many theories and much speculation as to the reasons for bacteria ceasing to increase. The two common explanations are that the food becomes depleted or that toxic by-products of metabolism limit further growth. Both may play a part but are by no means the whole explanation, for if a culture which has reached its M-concentration is centrifuged and the cells packed on the bottom, further growth may take place in the supernatant until the M-concentration is again reached. This shows rather clearly that lack of food or the presence of toxic products in the culture does not account for the cessation of growth. Furthermore, if a culture which has reached the M-concentration is heated until most of the bacteria are killed, the survivors will begin to multiply and again reach the M-con-

centration. There is no evidence that physical crowding as such is the limiting factor so the concept of "biological space" has been introduced in an attempt to explain the observed facts.

Whatever the explanation, the fact that bacteria do reach a point in their culture cycle where they no longer increase in number is of tremendous practical importance. In the maximum stationary phase there is little activity and consequently little degradation or digestion of the constituents of the medium. In any biological process where microbial breakdown of organic matter is desired, as in soil or in the trickling filters or activated sludge tanks used in sewage disposal, it is essential that the organisms be kept from reaching the maximum stationary phase. They must be kept in the logarithmic growth phase where the greatest activity takes place. In nature the protozoa which feed on bacteria undoubtedly keep their numbers down and prevent them from reaching the M-concentration. As a consequence the presence of protozoa in the soil or on trickling filters increases the rate of bacterial decomposition of the organic matter present.

F. *Accelerated Death Phase:* The number of viable bacteria in a culture remains at the M-concentration for a period varying from about an hour to several days or more, after which a slight decline begins. The rate of decline increases progressively, each generation time becoming longer, until a constant rate is reached, the viable number then decreasing rapidly. This period of constant decrease has been called the *logarithmic death phase.*

G. *Logarithmic Death Phase:* In some cases death follows a logarithmic course, proceeding as indicated by the portion of the curve marked *G 1.* This is particularly true if a fermentable carbohydrate is present and the acidity produced is sufficient to kill the organisms. Under other conditions there may be periodic spurts of multiplication followed by periods in which the rate of dying is greater than the rate of increase. The course of death may then be indicated by the portion of the curve marked *G 2.* In such an experiment it may take several weeks before the culture reaches sterility. In many instances the decline does not follow a logarithmic course at all; but the population, after a rather sharp reduction in numbers, remains relatively constant with sporadic increases and decreases. Steinhaus and Birkeland followed cultures of several different organisms for as long as two years, after which time plate counts showed that there were as many as one million living bacteria per cubic centimeter. In such a culture the curve plotted assumes the shape indicated by the portion marked *G 3.* They have suggested that this long period of slow and almost imperceptible decline in numbers interrupted by occasional increases be called the *senescent phase.*

The foregoing discussion is only a brief resumé of the culture cycle of

bacteria and is intended to show roughly what happens to their numbers when bacteria are seeded in liquid culture media. That there are limiting factors to growth is evident since they continue to multiply at their maximum rate for a short period only. The lack of food and the presence of toxic substances resulting from metabolism are not sufficient to explain the decline. Biological space also appears to be important.

Morphological changes occur during the various phases of the growth cycle. Cells taken from an actively growing culture are in general large, uniform in shape, and stain evenly. As the culture reaches its stationary phase the cells become more irregular in shape, smaller in size, and stain unevenly. And, as the aging progresses, these changes become even more noticeable. In old cultures many pleomorphic cells of bizarre shapes may appear.

Chapter VI

MICROORGANISMS AND THE CARBON AND NITROGEN CYCLES

Life is a series of chemical changes involving the building up and the tearing down of chemical compounds, simple and complex, organic and inorganic. Of these the carbohydrates are of tremendous importance not only as a source of food for man and animal, a subject which need not concern us here, but because when the microorganisms utilize them as food, the end product is largely carbon dioxide and water, the carbon thus being released in a form which can again be used by plants. In fact plants are absolutely dependent upon this microbial waste product for their continued existence. This interdependence of one form of life upon other forms, the balance which exists between living forms, and the orderliness of the universe is a subject upon which the biologist likes to meditate.

A complete picture of what microorganisms do in the transformation of organic matter is difficult because in some cases the chemistry of the microbial process is so involved and in many others so incompletely worked out. All we can do is indicate some of the changes and the organisms responsible. In considering the nutrition of microorganisms in Nature, it must also be understood that carbohydrates and proteins are metabolized simultaneously and many complex chemical reactions involved. For convenience here, however, we shall consider the carbohydrate metabolism and protein metabolism separately.

CARBON CYCLE

In nature most of these microbial changes are brought about in the soil. Wood, straw, and other cellulosic plant residues are there attacked by a variety of microorganisms belonging to the bacteria, fungi, actinomyces, and even protozoa. These organisms are never in pure cultures, hence it is often difficult to determine just what species is responsible for certain changes. Several growing in association may bring about changes of which no one alone is capable.

Cellulose Fermentation: In the first stages of the decomposition of the more complex polysaccharides, fungi and bacteria play a predominating role. The fermentation of the polysaccharide cellulose involves the addition of water, is brought about by the extracellular enzyme *cellulase*, and yields the dissaccharide *cellobiose*. This is further hydrolyzed by the enzyme *cellobiase* to the monosaccharide *glucose*, the fermentation of which

will be considered later. Many molds and bacteria are able to utilize cellulose in the form of filter paper, straw, and the like as a sole source of carbon. The bacteria belong to the genera *Cellulomonas*, *Cytophaga*, *Cellovibrio*, *Cellfalcicula*, *Clostridium* and *Actinomyces*.

In the digestive tract of herbivorous animals, protozoa are thought to play an important part in converting the cellulose to glucose which can be used by the animal. Much information on this subject is lacking. The wood-eating termites cannot digest cellulose but harbor protozoa which break it down to a utilizable form. How important the microbial digestion of cellulose in the intestinal tract of man may be is not known.

Hemicellulose Fermentation: Hemicelluloses differ from celluloses in several ways but their chemistry is even less well known. Bacteria, in general, do not readily attack them but the molds and actinomyces do. In natural fermentations hemicellulose is decomposed more rapidly than cellulose, indicating that it is more easily attacked. It is hydrolyzed by the enzyme *cytase* and sugar such as a *xylose* is split off which is further fermented by a number of organisms.

Pectin Fermentation: Between two adjacent cells of plants there is a layer called the *middle lamella* which cements the two together. The middle lamella is composed of pectic substances or *pectose*, the calcium salt of pectic acid. As fruits ripen, it disintegrates allowing the cells to separate and the fruit to become soft and mushy. It is the pectin, derived from pectose, which confers the jellying properties to fruit juices.

Bacteria producing diseases of plants, such as, for example, *Erwinia carotovora*, bring about their destruction by digestion of the middle lamella. Molds, such as *Penicillium* and *Rhizopus*, so commonly associated with and responsible for the rotting of fruits, secrete the enzyme *pectinase*. The appearance of decaying fruits is related to the action of this enzyme on the middle lamella.

Retting of Flax: The fibers of flax from which linen is made and those of hemp are held together by pectoses or pectic substances. An old method for retting flax is to submerge the plants or leave them exposed to heavy dews. Where the moisture is sufficient, bacteria from the soil attack the middle lamella, dissimilate the pectose, and the bundles of bast fibers can then be easily separated by mechanical means. In retting by the dew method, fungi play a dominant role and in the submerged method, bacteria such as *Clostridium butyricum* or *Clostridium pectinovarum* and *Clostridium felsineum* bring about the desired changes.

The retting of flax, hemp, or jute is a good illustration of a process worked out in the school of experience and scientifically explicable on sound biological principles. It should be added that a better understanding of the

biology and chemistry of the process has led to greatly improved methods of retting.

Lignin: The wall of young plant cells is composed largely of cellulose which, as the tissue matures and becomes woody, is displaced by lignin, the substance to which wood owes much of its strength and resistance. When wood, straws, or other plant residues decompose, the more easily digested substances as starches, cellulose, hemicellulose, and the like are broken down first while the lignin, being more resistant to the attack of the microorganisms, remains. Lignin thus tends to accumulate. It is subject to attack chiefly by the wood rotting fungi but decomposition is relatively slow. Humus, which plays such an important role in determining soil fertility, is composed largely of lignin and microbial bodies. It is thought that a ligno-protein is formed.

Waxes and Gums: Waxes and gums, resins, fats, and oils are also subject to microbial attack and although the degradation may be slow, if these substances were not eventually broken down the soil would become unproductive.

Starch Fermentation: Starch is the most common reserve food material stored in plants. Seeds of cereals contain fifty to sixty-five per cent by dry weight and potato tubers as much as eighty per cent. Tubers of dahlias, artichokes, and a few other plants contain a substance similar to starch called inulin. Starch is dextrorotary and on hydrolysis yields dextrose, whereas inulin is levorotary and yields fructose. The enzyme or system of enzymes in the former case is *diastase* or *amylase* and in the latter *inulinase.* A number of bacteria, including the pneumococci, produce inulinase. Inulin fermentation is consequently used in the identification of these organisms.

As mentioned before, starch is stored as reserve food in plants but it is insoluble and cannot be transported as such. Plants, however, contain the enzyme diastase that hydrolyzes starch to sugar which is soluble and can be transported and used for food. The fact that diastase brings about this cleavage of starch is the basis for the practice of malting in the making of beer.

Fermentation of starch may be brought about by many molds, and a few bacteria, but not by the common yeasts.

Fermentation of Sugar: The fermentation of sugars by microorganisms is a highly complex affair. End products depend upon the enzymatic equipment of the microorganisms, the kind of sugar, and the conditions under which the fermentation is carried on. There are a great many different kinds of sugars, each of which is fermented by specific enzymes. Since different species of organisms have different enzyme systems, it is in

many instances possible to differentiate and identify bacteria by placing them in various sugar broths and observing what type of fermentation takes place. Of course widely different species of organisms may have some enzymes in common and hence ferment the corresponding sugars.

Disaccharides: These are twelve carbon sugars having an empirical formula $C_{12}H_{22}O_{11}$. The more common are sucrose, maltose, and lactose (milk sugar). On hydrolysis by the enzyme sucrase, or invertase, sucrose yields one molecule of glucose and one of fructose. Maltose is hydrolyzed by the enzyme maltase to two molecules of glucose. Lactose is hydrolyzed by the enzyme lactase and yields one molecule of glucose and one of galactose. The sugars glucose, fructose, and galactose are six carbon sugars and have the same empirical formula $C_6H_{12}O_6$. They differ in configuration and in their properties.

Monosaccharide Fermentation:[1] The breakdown of cellulose, hemicellulose, inulin, starch, pectose, lignin, and the like is accomplished by the addition of water, that is, by hydrolysis. The enzymes responsible for these changes are extracellular. The changes are hydrolytic, take place outside the cell, and hence yield little energy. In the case of the fermentation of the monosaccharides the situation is quite different. These sugars are fermented inside of the cell. The reactions are not hydrolytic but oxidative and may yield a considerable amount of energy. The fermentation of these sugars yields mainly organic acids, alcohols, and carbon dioxide and water. A number of intermediate compounds are formed and the amount of energy liberated depends upon the stage to which the sugar is broken down or upon the degree of oxidation. This, in turn, depends upon whether the reactions are carried on aerobically, in which case the end product is carbon dioxide and water, or anaerobically, in which case the end products may be alcohols: ethyl and butyl, or the organic acids: acetic, butyric, and lactic. Other alcohols, acids, and acetone may also be formed. The enzymes involved are known collectively as *desmolases.*

Glucose Fermentation: The fermentation of glucose may take several courses and result in several different products. The energy released will depend upon how much is left in the resulting compounds. When the enzyme zymase, which is in effect a system of enzymes, attacks glucose only a part of the energy is released. The reaction may be expressed in this equation:

$$C_6H_{12}O_6 = 2\ C_2H_5OH + 2\ CO_2$$

Sugar plus zymase equals ethyl alcohol plus carbon dioxide
Energy yield, 17 calories

[1] For a good review of this topic the reader is referred to C. H. Werkman, *Dissimilation of Carbohydrates,* Bacteriological Reviews, 1939, vol. 2, no. 2, and C. G. Anderson, *An Introduction to Bacteriological Chemistry.*

Glucose may also be broken down to lactic acid by several enzymes and a slightly larger amount of energy be released.

$$C_6H_{12}O_6 = 2\ C_3H_6O_3$$

Sugar plus enzymes equals lactic acid
Energy yield, 21 calories

The complete oxidation of glucose may be represented by the following equation:

$$C_6H_{12}O_6 + 6\ O_2 = 6\ CO_2 + 6\ H_2O$$

Sugar plus oxygen plus enzyme equals carbon dioxide and water
Energy yield, 673 calories

Thus it can be seen that organisms bringing about a complete oxidation obtain much more energy than those only partially oxidizing the sugars. Glucose may be fermented in a number of other ways but the total energy released is always the same so long as the final products are the same. The process is infinitely more complex than the equations seem to indicate, involving a number of intermediate compounds not mentioned here.

NITROGEN CYCLE

The reactions by which bacteria obtain their nitrogen result in a liberation of the nitrogenous compounds essential to the life of plants. The dry residue of bacteria on chemical analysis shows that about ten per cent of the cell consists of nitrogen and about sixty-five to seventy-five per cent of nitrogenous compounds, such as globulins, albumens, and nucleoproteins. These are a part of the protoplasm and the enzyme systems of the cell. The microbial proteins are essentially similar to those of other organisms in that they are made up of the same amino acids and yield the same compounds on degradation. As in the case of plants and animals, the amino acids comprising the proteins are arranged so as to give a different molecular configuration in each species. That is to say, proteins are *species specific*, a fact of paramount importance in connection with immunity to disease. Although bacteria contain the same amino acids as do plants and animals, they differ from them as to the nature of the nitrogenous materials which they can use as a source of nitrogen. Moreover bacteria are not all alike with respect to their nitrogen metabolism. Some use elemental nitrogen from the air, others simple salts, still others certain amino acids only, and so on.

The decomposition of proteins yields little energy, and bacteria, in general, do not obtain enough for growth from this process. They use the nitrogen for structural purposes and get the energy necessary to bring about the synthesis of protoplasm from carbohydrates. There are a few notable exceptions, for some bacteria do get energy from the oxidation of ammonia and some from nitrites.

Protein molecules are so large that they cannot pass through the bacterial cell walls. Therefore the first stages of degradation are extracellular. A similar situation pertains in the animal body for there digestion takes place in the digestive tract and the liberated amino acids are absorbed and then enter the blood stream.

The dissimilation of proteins is often referred to as putrefaction and is associated with the bad odors present in the later stages and due primarily to the decomposition of amino acids. These are broken down inside the cell, intracellular enzymes being responsible for the production of the foul-smelling compounds.

As in the case of carbohydrate metabolism, the nitrogen metabolism of bacteria does not exist apart from the general metabolism which involves all the reactions of the organism. For our purposes, it is easier to follow separately the series of reactions involved.

Nitrogen is the key element in the amino acids which are the building stones of protoplasm. It is an essential part of the great cycle of life and is used over and over again. The cycle may begin anywhere but, since the primary source of nitrogen is the air, let us trace its journey from the air through its various stages and so back to the air.

About eighty per cent of the air by volume is nitrogen. It is estimated that the total atmospheric nitrogen over an acre of land is between 145,000 and 150,000 tons. Our concern with it is not due then to its scarcity in nature but to the fact that plants, animals, and most microorganisms cannot utilize it in the free or uncombined form. It is present in such large quantities in the free state just because it does not readily combine with other elements. Only under the influence of powerful agencies, such as electrical discharges or chemicals, or by the action of certain soil bacteria does nitrogen combine. One of the many unexplained mysteries of nature is how bacteria can, at ordinary temperatures and pressures, cause nitrogen to enter into combination with other elements when it takes such tremendous temperatures and pressures to bring about a combination in the laboratory. The taking of nitrogen from the air and the fixing of it by artificial chemical methods need not concern us here although it is an important process in the manufacture of commercial nitrates.

Nitrogen Fixation

Although some nitrogen is fixed by lightning, the bulk of the nitrogen consumed by plants is fixed by two groups of microorganisms: the symbiotic nitrogen-fixing bacteria, and the free living nitrogen-fixers.

The Symbiotic Nitrogen-Fixers: The better known symbiotic nitrogen-fixers belong to two genera, *Rhizobium* and *Mycobacterium*. The rhizobia live in a symbiotic relationship with the leguminous plants. They pene-

trate the root hairs and stimulate the formation of nodules which teem with bacteria. Although the exact mechanism by which the reaction takes place is not known, it may be said, in brief, that these bacteria obtain salts and carbon compounds from the plant and convert free nitrogen from the air in the soil to a form which may be used not only by the bacteria but also by the plant. Hence the term *symbiotic* relationship, that is, a relationship of mutual benefit to the plant and to the bacterium. Although rhizobia can be readily grown in the laboratory on artificial media and occur free in the soil, they have never been shown to fix nitrogen under these conditions.

The symbiotic nitrogen-fixers belong to six or more species, each of which is capable of producing nodules in a group of plants. There is considerable host specificity in this group of organisms and the species are separated on their ability to establish themselves in certain hosts.

Rhizobium leguminosarum produces nodules on peas, vetch, and lentils; *Rhizobium trifolii*, on clover; *Rhizobium phaseoli*, on beans; *Rhizobium meliloti*, on sweet clover and alfalfa; *Rhizobium japonicum*, on soy beans; and *Rhizobium lupini*, on lupins.

There is considerable difference in the efficiency of different strains of the same species, some strains fixing more nitrogen than others. Commercial producers of inocula take advantage of this in selecting strains for propagation.

The *Mycobacter* are acid-fast bacteria found in the nodules of the leaves of certain tropical plants of the family *Rubicaceae*. These organisms have not been studied as extensively as the rhizobia but they too are thought to fix nitrogen symbiotically.

The Free Living Nitrogen-Fixers: Of the considerable number of bacteria capable of fixing some nitrogen, only a few are of economic importance. Some of these are anaerobes, others aerobes. The principal anaerobe is *Clostridium butyricum*, the principal aerobe, *Azotobacter chroococcum*.

Mechanism of Nitrogen Fixation:

Anaerobic: Although the exact mechanism of nitrogen fixation by *Clostridium butyricum* is not clear, Winogradsky, to whom we owe much of our knowledge of the phenomenon, suggests that nascent hydrogen is split from glucose and combines with free nitrogen to form ammonia. The nitrogen, that is, acts as a hydrogen acceptor and is reduced, the amount fixed being directly proportional to the amount of glucose fermented.

It is usually assumed that nitrogen fixation by microorganisms is an endothermic reaction or one which requires energy, and that, since nitrogen fixation takes place in media containing fermentable carbohydrates, the energy is obtained from the exothermic breakdown of these organic compounds. A consideration of the thermodynamics involved suggests that

nitrogen can combine with hydrogen and water to form ammonium hydroxide and release energy. If oxygen is used in place of water even more energy is released.[2]

Clostridium butyricum and related species are present in soils in variable numbers, depending upon how favorable conditions are for their growth and for the growth of other kinds of organisms which may suppress them. If these other organisms produce ammonium salts, *Clostridium butyricum* will use this nitrogenous compound instead of fixing free nitrogen.

FIG. 33. Electron micrograph of *Azotobacter vinelandii*, one of the nitrogen-fixing bacteria. Note the flagella that surround the cell.

Courtesy of Dr. A. W. Hofer, and Dr. R. F. Baker and the Journal of Bacteriology

Aerobic Nitrogen-Fixers: Probably the most important aerobic nitrogen-fixer is *Azotobacter chroococcum*. When grown in a medium containing no nitrogeneous compound but an ample supply of carbohydrate, it is capable of utilizing the free nitrogen of the air. When grown in the presence of air and nitrates, it reduces the nitrates to nitrites and fixes little free nitrogen. If grown in the absence of air in a medium containing nitrates, it reduces these to ammonia and again fixes only a small amount of nitrogen.

[2] *Bacterial Metabolism*, Margery Stephenson, 1939, Longmans, Green, and Company.

Achromobacter radiobacter grows in the presence or absence of air and reduces nitrates to free nitrogen. There is an interesting relationship existing between this organism and *Azotobacter chroococcum.* When they are grown together the amount of nitrogen fixed by the *Azotobacter chroococcum* is greatly increased and depends upon the amount of nitrate present. If large amounts are present, little nitrogen is fixed; if little is present, large amounts are fixed.

Thus we see that the fixation of nitrogen in soils is conditioned by a number of factors for, even though the proper organisms are present, if the soils contain large amounts of nitrates, the nitrogen-fixers may actually reduce the amount of nitrogen present. If, however, the soils are lacking in nitrates, these organisms may fix free nitrogen and thus increase the amount available for plants.

In addition to the organisms discussed, there is evidence that some of the molds and a considerable number of other species of bacteria can fix small amounts of nitrogen. Even yeasts, under certain conditions, fix some but it is doubtful whether the amount is sufficient to be of any importance. The nitrogen fixed by molds may be of significance. Some of the blue green algae, too, are efficient nitrogen fixers.

The nitrogen fixed can be traced through several other changes. Some of it is used by the fixing bacterium to build protoplasm and enzymes, some may be liberated or accumulated as nitrates, in which case it may serve as a source of nitrogen for other bacteria that use it for building their protoplasm or reduce it to nitrogen and thus set it free again. Some is used by plants to build their protoplasm. And so it is that through the agency of the bacteria the nitrogen of the air becomes a part of living organisms.

Protein Decomposition and Putrefaction

The proteins, whether they be microbial, plant, or animal and whether they are decomposed by the enzyme systems of animals or of microorganisms, go through a similar series of stages and similar fractions appear during their decomposition. The hydrolysis of proteins to their component amino acids follows this course: proteins to proteoses to peptones to polypeptids to amino acids. The first stages are extracellular. Polypeptids and amino acids, however, are taken into the cells of the bacteria and are then used for the building of new protoplasm or are further changed to ammonia, nitrites, or nitrates.

A great many bacteria are proteolytic, that is, capable of hydrolyzing proteins. Many common anaerobes are very active in decomposing plant and animal tissues. Some, as for example, *Clostridium histolyticum*, are pathogenic, bringing about a rapid digestion of the living flesh and causing

gangrene. These bacteria were responsible for many of the infections following war wounds which had become contaminated with soil. Some spore-forming bacilli, such as *Bacillus subtilis*, some non-spore-forming bacilli, such as *Proteus vulgaris*, and many cocci are active proteolytically.

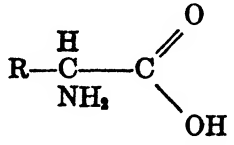
Protein decomposition may be readily demonstrated in the laboratory by inoculating a suitable medium in a petri dish with the organism in question, and, after about forty-eight hours, flooding the plate with mercuric chloride. The intact protein will combine with the mercuric chloride to form a white precipitate in the medium whereas the cleavage products will not. If the organism has broken down the protein, a clear zone will be evident adjacent to the colonies.

Milk protein or casein is readily attacked by many organisms and broken down to more soluble and more stable compounds.

We have said on several occasions that the early stages in the breakdown of proteins are extracellular and are carried out by exoenzymes. These are formed only by growing bacteria. When bacteria which are ordinarily able to utilize protein are placed in a medium containing only intact proteins they do not grow. If a little nitrate or some amino acids are added they grow readily. The addition of small amounts of nitrogen in such compounds initiates growth, the growing cells then begin secreting exoenzymes which can digest the proteins in the medium, and the growth thus started goes on independent of the amount of nitrate or amino acids introduced into the medium.

Amino Acid Metabolism

There are nearly thirty amino acids differing considerably in many respects but similar in that the next to the last carbon atom of the chain has an amino group linked to it and the last carbon atom has an oxygen and a hydroxy group. The general structural formula for the amino acids may be written:



It is to these groupings that the amino acids owe many of their properties, for example, the fact that they are *amphoteric*, behaving as alkalies or acids; and that they are *buffers*. They may be acted upon by a number of organisms and in different ways. The following summary of these changes is taken almost entirely from Anderson's *Introduction to Bacteriological Chemistry* to which the reader is referred for a more complete discussion.

A. Decarboxylation to give the amine:

$$R \cdot CH_2 \cdot CH \cdot NH_2 \cdot COOH \rightarrow R \cdot CH_2\,CH_2 \cdot NH_2 + CO_2$$

It will be noted that the resulting amine has one less carbon atom than the amino acid.

In the process of decarboxylation tyrosine is converted to tyramine. arginine to putrescine, lysine to cadaverine, and histadine to histamine, Many of the amines are poisonous. They are called ptomaines and were formerly thought to be the principal cause of "food poisoning." It is now known that ptomaines are of little importance in "food poisoning" which is due to bacteria that produce infections when growing in man or powerful toxic substances when growing in foods.

B. Deamination: Deamination results in the removal of the nitrogen grouping and may be (a) reductive, (b) hydrolytic, (c) desaturative, or (d) oxidative.

The enzymes which hydrolyze amino acids with the production of hydroxy acids and ammonia are *desaminases.*

(a) *Reductive* to give *Saturated Acids:*

$$R \cdot CH_2 \cdot CH \cdot NH_2 \cdot COOH \xrightarrow{H_2} R \cdot CH_2 \cdot CH_2 \cdot COOH + NH_3$$

This reaction takes place only under anaerobic conditions and may be due to a wide variety of bacteria.

(b) *Hydrolytic* to give *a-Hydroxy-acids*

$$R \cdot CH_2 \cdot CH \cdot NH_2 \cdot COOH \xrightarrow{H_2} R \cdot CH_2 \cdot CHOH \cdot COOH + NH_3$$

(c) *Desaturated* to give *Unsaturated Acids*

$$R \cdot CH_2\ CH \cdot NH_2 \cdot COOH \rightarrow R \cdot CH = CH \cdot COOH + NH_3$$

(d) *Oxidative to give a-Keto-acids:*

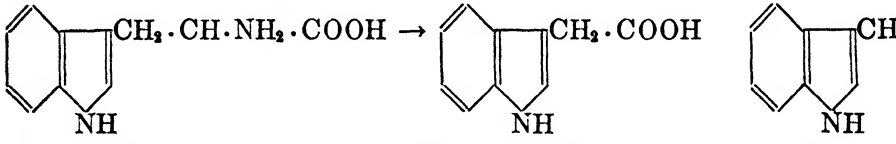
$$R \cdot CH_2 \cdot CH \cdot NH_2 \cdot COOH \xrightarrow{+(O)} R \cdot CH_2 \cdot CO \cdot COOH + NH_3$$

The a-keto-acids are readily decomposed by many organisms, particularly by yeasts. Since oxygen is involved, the reactions are favored by aerobic conditions.

It will be noted that in contrast to decarboxylation which yields carbon dioxide, the deamination reactions all yield ammonia and the resulting compounds have the same number of carbon atoms as the original amino acid. These compounds may be further decarboxylated to yield aldehydes which in turn may be oxidized to acids or reduced to alcohols. The production of higher alcohols from amino acids is frequently due to yeasts, occasionally to certain bacteria and molds.

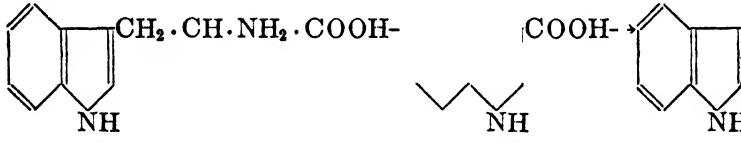
Hydrocarbons: Amino acids may give rise to hydrocarbons if the deamination is followed by decarboxylation. Thus, for example, glycine ($CH_3 \cdot NH_2 \cdot COOH$) when acted on by anaerobic bacteria may yield methane. The production of this inflammable gas during the digestion of sewage may be of considerable importance since it can be used commercially as a source of heat or power.

The decomposition of tryptophane is of particular interest since the amino acid appears to be utilized by many bacteria and the remaining compounds are particularly vile smelling. Feces owe much of their characteristic odor to them. Under anaerobic conditions the changes are as follows:



Tryptophane Indol-acetic acid Scatole

Under aerobic conditions the following changes occur:



Tryptophane Indol-carboxylic acid Indole

The production of indole which depends upon decarboxylation and can be accomplished only by those bacteria possessing the enzyme carboxylase is of considerable value in identifying and classifying closely related organisms.

Nitrification: Ammonia, released from amino acids during the process of ammonification, is further oxidized to nitrous acid by bacteria, particularly by the autotrophic *Nitrosomonas*. These organisms derive energy from this process: $NH_3 + 3\,0 = H\,NO_2 + H_2O + 79{,}000$ calories. The nitrous acid does not remain as such but immediately combines with the bases in the soil to form nitrites which are further oxidized to nitric acid by another group of autotrophic bacteria called the *Nitrobacter*. This reaction also yields energy, but not so much: $H\,NO_2 + O = H\,NO_3 +$ 21,600 calories. The nitric acid also combines with bases in the soil to form nitrates which are then available for plants.

Denitrification: Nitrates can be and frequently are reduced by bacteria to nitrites and these further reduced to free nitrogen. Denitrification

takes place under anaerobic conditions. Under aerobic conditions many organisms will oxidize nitrogen compounds to produce nitrates. In the absence of free oxygen these same organisms use the oxygen of the nitrates and nitrites as a hydrogen acceptor. This is known as denitrification and results in the loss of nitrates and the lowering of soil fertility. In water-logged soils or soils that are too compact, denitrification is favored. Tillage tends to counteract it by increasing the amount of oxygen in the soil.

Protein decomposition by microorganisms takes place wherever conditions are suitable. We see it on every hand in the spoilage of foods and the rotting of plant and animal remains. From the microbial standpoint these reactions are but a part of their attempt to obtain food. They cannot utilize intact protein as such and many use only a few of the fractions. Of these the amino acids are the more important. We have seen how they may be decomposed in different ways. Not all of the amino acids can be used by the bacteria, nor do all bacteria require them. Most do, however, and certain amino acids appear to be essential for the growth of the heterotrophic bacteria which seem unable to synthesize some of them from simpler compounds.

Decay, protein decomposition, or proteolysis results in the production of more stable and more soluble compounds. As each successive stage is reached there is less energy remaining in the system. And so the microorganisms are continually acting upon nitrogen, whose primary source is the atmosphere, building it into more complex compounds and breaking it down into simpler ones, as it appears again and again in the cycle of life. This cycle may be constructed as follows:

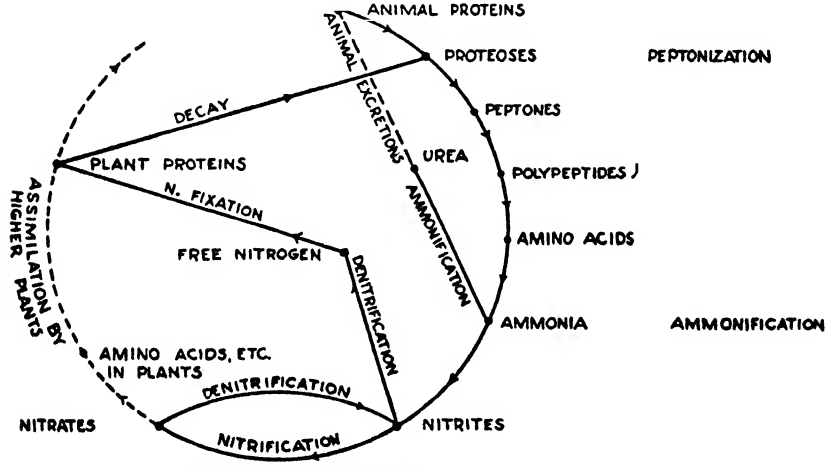


FIG. 34. The nitrogen cycle. Changes brought about by bacteria are represented by solid lines.

METABOLISM OF OTHER ELEMENTS

We could construct similar cycles for sulfur, phosphorus, potassium, and calcium. Microorganisms require these for growth and as a result of their activities bring about transformations which may leave them in a form utilizable by plants.

While we have considered the carbon and nitrogen metabolisms separately, it must always be remembered that in nature as in the soil, for example, the reactions involving carbon, nitrogen, sulfur, potassium, calcium, phosphorus, and other elements occur simultaneously. Add to that the facts that the same organisms may oxidize a compound under one condition, reduce it under another with entirely different fractions resulting; that two organisms, neither one of which can break down certain compounds, can, when growing together, do so; that in nature microorganisms are never in pure culture but that there is not only a vast number of species of bacteria present at any one time and place but also molds, algae, and protozoa; and that the amount of air, the moisture, and temperature profoundly affects each one of these organisms. How vastly complex this biological laboratory of Nature is.

SUMMARY

Life is a series of chemical changes. Existing compounds are being torn down into simpler ones and new combinations are being built up continuously, energy for this work coming originally from the sun. The plants and a few bacteria, however, are the only living forms which can utilize sunlight as a source of energy. All animals, the yeasts, molds, and most of the bacteria depend upon the oxidation of compounds for energy.

In order to obtain all the energy in a compound it is necessary to carry the oxidation to completion. This is true whether we are dealing with coal and oil fuels for machines, or foodstuffs for microorganisms. For example, when a molecule of the sugar, glucose, is completely oxidized 673 calories are released. If it is broken down to lactic acid, only 21 calories are released. The microorganisms may be considered quite inefficient for in many instances they do not carry the breakdown of compounds to completion and hence do not get all the energy in the system. But it is because they do not, for instance, break down carbon compounds to carbon dioxide and water that they are so important from the industrial standpoint. The breakdown or dissimilation of complex molecules such as fat, starch, or protein takes place in a series of stages and at any stage different new compounds appear. The enzymes necessary to break down these compounds are more or less specific and the microorganisms may not possess

all of the enzymes necessary to bring about complete dissimilation. For protein the steps in this dissimilation may be illustrated schematically: protein to proteose to peptone to polypeptid to amino acid to ammonia to nitrites and nitrates. Finally the nitrate may be reduced and free nitrogen liberated. These are essentially the stages through which the nitrogen in the soil, milk, sewage, and so on passes. It should be noted that at each stage the system has less energy than at the preceding one.

Chapter VII

BACTERIAL HEREDITY AND VARIATION

It is a common observation that although children resemble their fathers and mothers and the progeny of animals and plants is like the parent stock, no two individuals are exactly the same. Like begets like but there is a tendency to variation. Were there not, there would be no evolution.

The principles governing the inheritance of resemblances and differences are embraced by the science of genetics. By cross-breeding and studying the progeny of such matings, the geneticist has been able to formulate principles the practical application of which has made it possible to produce plants and animals vastly superior to the parent stock. These studies have contributed greatly to a better understanding of the evolutionary process.

Before considering the genetics of bacteria, however, it is well to recall that the higher plants and animals are multicellular, have demonstrable nuclei, and reproduce sexually. The geneticist has confined himself almost wholly to a study of these plants and animals and, as a consequence, has formulated his principles and explanations in terms of these forms. His methods depend largely upon the examination of individuals, although considerable information has, of course, been obtained by cytological studies and by the application of statistical methods to populations.

When scientific studies on inheritances in plants and animals were begun, man had the benefit of knowledge accumulated over thousands of years. He was familiar with the gross morphologic features: the size, shape, and color, of the different species, and knew that each species remained relatively constant: that cows begat cows, pigs begat pigs, and that if wheat were planted, wheat would be harvested. Now the microorganisms found in any bit of soil or drop of water are as mixed a lot as are the plants and animals inhabiting one of our states. Just as a bird's eye view of a community reveals men, plants, and animals living in close association; so a microscopic view of a bit of soil reveals bacteria, protozoa, and fungi struggling for existence in a microcosmos. But the early bacteriologists had no way of knowing whether these microorganisms represented different species or a single species occurring in different forms. Their tools were crude, their techniques inadequate, and it was some time before much was learned about the constancy, stability, and limits of variability of the different species.

If daily microscopic examinations are made on a sample of milk, the first organisms to be seen in large numbers will be the streptococci. As the

milk ages, large and small bacillary forms, both sporulating and non-sporulating, become more evident, and, finally, molds appear. Similar successions of species will occur in broth inoculated with various types of materials. It is little wonder that the early workers were confused when confronted by such forms and, in the light of their knowledge of the life cycles of fungi, postulated complex life cycles for bacteria. One investigator is said to have remarked that no matter what bacterium he started with, he always wound up with *Rhizopus nigricans*. The notions of variability of bacteria culminated in the enunciation of the doctrine of "pleomorphism" by Nägeli in 1877. He suggested that all bacteria belonged to a single species whose individuals existed in different forms.

The development of the "pure culture" technique by Koch made it possible to separate and study species. It soon became evident that the various forms observed were different species in a succession, not successive stages in the life cycle of a single species, and that the species were stable. As a consequence, the doctrine of "monomorphism" then developed. Adherents to this doctrine would not admit that variation was possible. If new forms appeared, they said that they were due to some sort of degradation and should be classed as involution forms, or suggested that the cultures had become contaminated. And, in many instances, both explanations were true.

During the period from about 1880 until about 1915, the idea of "monomorphism" dominated, but evidence continued to accumulate that bacteria do undergo two types of variation, designated as *temporary* and *stable*. The temporary variations are those which are not transmissible or which do not appear in subcultures. Ageing, drying, or increasing the acidity of the cultures tends to produce bizarre or aberrant shapes called "involution" forms. These variations appear to be due to the influence of changed environmental conditions and the organisms revert to their original state when grown in an ordinary environment.

The stable variations are those that are transmissible and hence appear in subcultures. It is with these we are most concerned.

BACTERIAL VARIATION OR DISSOCIATION

The terms, *variation, dissociation, and mutation*, have been introduced to designate variation of the transmissible type.

Bacterial variation is recognized by changes which occur in the characteristic features of size; shape; spore formation; staining reaction; presence or absence of flagella, capsules, or granules; type and color of colony; physiological activities such as the power to ferment various sugars; disease-producing properties; and antigenic composition. It might be well to note in passing that these are the characteristics upon

which our classification of bacteria is based and that much of the difficulty in identifying and classifying them arises because these variations do take place.

Morphologic Variation

Variation in size and shape is quite common in bacilli, cocci, and spirilla. They may also vary in respect to staining reactions, species that are normally gram-positive becoming gram-negative and those that are acid-fast, losing this property. Species that normally produce spores may be changed to nonsporulating strains. Motile bacteria may lose their flagella and hence become nonmotile. Encapsulated bacteria may lose their power of producing capsules. But, while numerous changes do occur, there is a definite limit to variation, and it is usually associated with the loss of morphologic features rather than the gain. Bacteria that are normally non-flagellated do not dissociate to produce flagellated variants, nor do non-spore formers produce spore-forming variants. Such variations in morphology as the absence of flagella in normally flagellated bacteria or the absence of capsules in normally encapsulated forms are associated with changes in the type of colonies, in pathogenicity, and in antigenic composition. It seems likely that all morphologic variation of the stable type is associated with physiologic differences, but this has not been proven yet.

Colonial Variation

When streak plates are made from pure cultures, the colonies have a characteristic size, color, outline, and appearance. Colonies of motile and of encapsulated organisms are commonly smooth, round, and shiny, although a careful observation of such plates will usually reveal a small percentage that are rough, wrinkled, and dull. The smooth colonies are designated as *S* and the rough as *R* types. Considerable attention has been paid to the *S* to *R* transformations or dissociations and it has been found that some but not all of the colonial changes are causally related to changes in cellular morphology. Others are associated with such changes, but the causal relationship is not apparent. The characteristic features of the *S* and *R* types have been outlined by Jordan and Burrows as follows:

"Smooth (S) Type	Rough (R) Type
(1) Broth cultures uniformly turbid	(1) Sedimental deposit in broth cultures; supernatant fluid clear
(2) Suspensions in salt solution (0.8% NaCl) stable and remain cloudy	(2) Suspensions in salt solution clump spontaneously and settle out
(3) Flagellated species usually motile	(3) Motility reduced or absent
"Smooth (S) Type	Rough (R) Type
(4) Capsulated species show capsules	(4) Capsules absent

(5) Somatic and flagellar or type specific antigens present; flocculent agglutination	(5) Only somatic antigens present; agglutination granular in type
(6) Pathogenic species generally virulent	(6) Virulence greatly reduced or absent
(7) Biochemically active	(7) Biochemical activity reduced
(8) "Normal" morphology	(8) Tendency toward abnormal forms"

Cultures showing the *S* to *R* type of variation usually yield intermediate types and, occasionally, minute colonies designated by Hadley as *G* colonies. Some workers have suggested that these come from filterable or virus-like forms of bacteria since they were first observed in cultures of filtrates presumably free from large bacterial cells. This interpretation is as vigorously denied by many workers and the significance of these colonies remains unknown.

Although the smooth colony appears to be normal for most bacteria, there are two notable exceptions. In the case of virulent streptococci and anthrax bacilli, the rough types appear to be normal and are the more virulent.

The enzyme systems associated with assimilation and dissimilation are also subject to variation, but, since it is not always easy to identify such changes, the isolation of physiological variants is often a matter of chance. For convenience we may consider physiological variations under two headings: those associated with changes in the fermentative capacity of an organism and those associated with changes in the virulence or the disease-producing capacity of the organism. Both are of great importance and the bacteriologist uses them to advantage in various ways.

The capacity of an organism to ferment carbohydrates or break down protein, fix nitrogen, produce acid, reduce nitrates, or act on any of a number of substances is not constant. Strains which lack these capacities or which are more active than the parent culture can often be isolated. Just as the plant or live stock breeder selects his strains for desirable characteristics, so the bacteriologist can take advantage of microbial variation to select strains that fix more nitrogen; produce better flavors in butter, cheese, wine, or beer; or give higher yields of acids, alcohols, and a number of other products of microbial activity.

Variations in properties associated with the capacity of an organism to produce disease are likewise of great practical importance, being basic principles underlying the production of vaccines and of other products used in the treatment and prevention of disease.

In general, the virulence of pathogens which are encapsulated is associated with the capsule, and when a bacterium loses its capacity for producing capsules, it loses its virulence too. Such is the case with the pneumococcus, the cause of pneumococcus pneumonia. A possible ex-

planation for this association is that the capsule protects the virulent organism from destruction by the phagocytes of the body.

Another type of dissociation or variation associated with morphologic changes is found in species possessing flagella. Normally the flagellated forms produce smooth colonies, the non-flagellated, rough ones. However, in the case of *Proteus vulgaris* the flagellated organism is so highly motile that the normal colony is not discrete but spreads and has a film-like appearance. The variant is non-flagellated, hence nonmotile, and so produces discrete colonies. This type of dissociation, first observed on the non-virulent *Proteus vulgaris*, is called an *H* to *O* transformation (the letter *H* standing for the German word *Hauch* meaning breath or tinge, and *O* for *ohne Hauch* meaning without breath or tinge) and is associated with the virulence and with the antigenic structure of the organism.

Still another type of variation associated with virulence is concerned with the capacity of the organism to produce toxins. For example, *Corynebacterium diphtheriae*, the cause of diphtheria, produces a potent toxin responsible for most of the disease symptoms. Smooth, *S*, strains are virulent and produce toxins while *R*, rough, strains are relatively non-virulent. In some instances organisms isolated during the acute stages of a disease are in the *S* phase and those isolated during convalescence or from carriers, in the *R* phase.

There are several other toxic substances produced by pathogenic bacteria: *hemolysins* which break down red blood cells, *leucocidins* that destroy white blood cells, *necrotoxins* that destroy tissue, and *enterotoxins* that induce intestinal disturbances. Bacteria producing such toxins vary in their ability to do so and variant strains may be isolated which do not produce any toxic substances.

Antigenic Variation

Associated with the loss of flagella or of capsules or with the change from the *S* to the *R* phase is the loss of certain antigenic factors. In general the *S* and *H* phase organisms produce antigens specific for the strains in addition to antigens specific for the group or species, whereas the *R* or *O* phase organisms produce only the group or species antigens.

Antigenic variation may be stimulated or induced in a number of ways. In general, when a pathogenic organism such as the pneumococcus is cultivated on artificial media it loses its capsule and becomes non-virulent. When such strains are passed in series through mice, they become more virulent with each successive passage until they have again reached their full virulence and at that time they are again encapsulated. The capsular material of the pneumococcus is composed of polysaccharides and each of

the thirty-two or more *types* has a specific or different polysaccharide which is its identifying characteristic.

Normally when an encapsulated pneumococcus loses its capsule and later regains it, the new capsule is identical with the one previously produced, but it has been shown that under certain conditions the organisms may change in *type*. Griffith found that when a Type I pneumococcus which had lost its capsule or was in the *R* phase was injected into a mouse along with heat-killed Type II organisms in the *S* phase, or encapsulated, the Type I organisms were converted to Type II. It appears then that some substance present in heat-killed Type II encapsulated pneumococci was capable of transforming Type non-encapsulated pneumococci to Type II. Dawson and Sia brought about this same conversion by growing a Type I in the presence of its own antiserum and heat-killed encapsulated cells.

Avery, MacLeod and McCarty, in a series of brilliant investigations, succeeded in isolating and identifying the transforming substances in Type III as nucleic acids of the desoxyribose type. Although found in the encapsulated cells only, this desoxyribonucleic acid is not present in the capsule but is in the cell itself. When it is added to a suitable broth in a dilution of one part in 600,000,000, non-encapsulated pneumococcal cells growing therein become Type III. Desoxyribonucleic acids capable of bringing about similar changes in types have also been isolated from Type II and Type I.

As previously stated, the desoxyribonucleic acid becomes part of the cell. It conditions the synthesis of the specific capsule but is not a part of the capsule. The transformation is permanent. Once it has taken place, the pneumococci, even when grown in media devoid of the transforming principle, continue to produce it for it can be isolated from such cells. The desoxyribonucleic acid becomes an inherent part of the converted cells. Somehow the nucleic acid is reduplicated and, since it conditions the production of the polysaccharide capsule but is not a part of the capsule, it behaves like a gene. In other ways it behaves like a virus. That is, it acts on susceptible cells only, it is reduplicated and hence transmissable, and it brings about changes in the activity within the cell.

Another instance of such conversion or transmutation was reported by Holtman. He noted that the organism of "contagious abortion" in cattle, Bang's bacillus, did not produce a substance known as the Forssman heterophile antigen, whereas strains of this same organism isolated from a horse did. It seemed that the Bang's bacillus, perhaps, had acquired the ability to produce the heterophile antigen when grown in the horse. To test this possibility, he grew strains taken from cows in a medium containing heterophile antigen and found that such a conversion did take place and

also that the Bang's bacillus continued to produce heterophile antigen thereafter when grown in media devoid of it.

Transmutations of the type produced in the pneumococcus and Bang's bacillus are not like most bacterial variations, in that they apparently represent the acquisition of the power to produce something new and different and not merely the increase or decrease in an organism's ability to produce a substance or lose a character. Just what interpretation should be placed on these findings is not clear. They appear to represent a sort of "inheritance of an acquired character" though not in the Lamarckian sense. Are these isolated cases or does the same thing occur in other species? The answer will have to await further experiemental evidence.

Mechanism of Bacterial Variation

Although the fact of bacterial variation is well known and the factors that tend to bring it about are pretty well agreed upon, the interpretation to be placed on the facts and mechanisms involved is not at all clear.

In the case of higher plants and animals, variation may come about in a number of ways. It may be the result of gene mutation, that is, of some change in the gene itself. It may be due to a recombination of genes, as in cross-fertilization when a new combination of genes in the offspring produces a character possessed by neither of the parents. Or variations may be due to chromosomal aberrations, that is, to the loss or gain of genes resulting from unequal division or from a change in the order of their arrangement.

In attempting to interpret bacterial variation we must remember that bacteria multiply by simple fission, that they do not have a demonstrable or discrete nucleus, that they apparently have no sexual stage, and, consequently, no cross-fertilization. Whether they have genes depends upon our definition. If we think of genes solely as determiners of heredity, we may say that bacteria do have something that functions like genes in the higher forms. If we think of genes as entities present in linear arrangement in chromosomes located in a nucleus, then, since a bacterium cannot be shown to have either nucleus or chromosomes, we cannot say that it possesses genes.

But, if we accept for the moment the hypothesis that bacteria do have genes and that their exact location with respect to chromosomes is of little importance, we may explain variation in a number of ways. (1) It may be assumed that as the cell grows and prepares to separate into two daughter cells, there is an unequal distribution of genes, a few cells perhaps receiving an extra gene and an equal number having one gene less than normal.

(2) There may be a rearrangement of the genes within the cell. It is

known from the study of genetics that the position of a gene with reference to other genes is important.

(3) The genes themselves may mutate. In higher plants and animals this is a reversible process. If it is in the case of bacteria, a characteristic lost and later regained, is explained. These suggested explanations, based on principles of the genetics of higher plants and animals, may or may not be valid for bacteria.

(4) Some observations suggest that there may be some sort of fusion or conjugation. Whether this represents an interchange of gene material cannot be determined from microscopic study and, as yet, there is no evidence of cross-breeding.

Selection and Variation

The appearance of variants in bacterial cultures which have been subjected to drying, to the influence of X-rays, to high salt concentrations, or to other agencies known to induce variation may be accounted for in a number of ways. These agencies may actually cause changes in the genetic composition of the organism, they may act as selective agents, or they may function in both ways.

It is well known that mutation occurs in higher plants and animals and one might expect it to occur in a bacterial population. If it did the chances of detecting such a mutant would be almost impossible unless the character in question had survival value, as, for example, the ability to utilize some component of the medium which the other cells could not or to resist such destructive elements as the white blood cells. If a mutation of that kind occurred, the rapidity of bacterial multiplication is so great that in a short time most of the cells in that particular culture would be the progeny of the mutant.

The appearance of the so-called "drug-fast" strains of protozoa and bacteria can perhaps be explained on the assumption that the chemical in question acts as a selective agent. It has frequently been observed that organisms grown in the presence of toxic compounds acquire a resistance to the particular compound; and that strains isolated from persons or animals undergoing drug treatment may be much more tolerant of the drug than were those isolated before the treatment began. This occurs in many infections treated with specific chemotherapeutic agents. It is indeed a rather common occurrence in staphylococcus, streptococcus, pneumococcus, and gonococcus infections treated with sulfa drugs. It occurs less frequently when these infections are treated with penicillin. Drug-fast strains of tubercle bacilli and gram negative rods appear very rapidly when infections due to these bacteria are treated with streptomycin or when the organisms are grown in media containing it. Strains a thousand-fold more

resistant than the parent strain are not uncommon. It has also been found that some types of bacteria when grown in the presence of streptomycin not only become resistant to it but actually require it for growth. For them streptomycin acts like a growth-promoting substance or a vitamin. The development of "drug-fastness" is of practical importance because the toxicity of the drug for the body tissues may be so great that it may not be safe to increase the dosage sufficiently to cope with the more tolerant strains of the disease-producing agent.

LIFE CYCLES

A number of students of bacterial variation have postulated a rather complex life cycle involving several types of vegetative reproduction followed by sexual reproduction.

Some of the observed facts of variation may be explained on the assumption that each stage in the life cycle requires a different environment and that the environment will determine which stage the organism will maintain. Thus there are three commonly occurring life cycle stages, or cyclostages, each of which produces a characteristic colony. There are smooth, *S*, rough, *R*, and very minute colonies like dewdrops in appearance, the *G* colonies. The *G* forms arise primarily from the *R* stages and are filterable. They have also been called *gonidia*. These theories must be considered as theories only, for the presence of life cycles in bacteria has never been satisfactorily proven.

Chapter VIII

HOW THE ENVIRONMENT AFFECTS MICROORGANISMS: PHYSICAL AGENTS

Microorganisms may be stimulated, retarded, induced to produce variants, or killed by the various components of their environment. This is not surprising since they are, in effect, batteries of enzymes and enzymatic reactions are profoundly influenced by physical and chemical agents. Although it is not always possible to separate the various physical components of the environment nor to differentiate between physical and chemical effects, let us, for convenience, consider physical agents under these headings: Temperature, Moisture, Pressure, Surface tension, Sunlight, Radiant energy emanations, and Supersonic waves.

TEMPERATURE

Since the biologic reactions by means of which living organisms carry on their functions are chemical reactions and since the rate at which they take place is dependent upon temperatures, it must follow that temperature greatly influences the activity of all living forms. The higher animals have elaborate regulating mechanisms which enable them to maintain relatively constant temperature within the body even though that of the surroundings varies considerably. And, since the temperature within their bodies is constant, the chemical reactions involving their growth proceed at about the same rate in winter and summer.

The lower animals, the plants, and the microorganisms have no such temperature regulating mechanisms and so we find that rate of growth in these forms varies with the temperature of the surroundings. Trees and other plants grow faster in summer than in winter. The microorganisms react as do the plants, and just as a few plants grow best in the Arctics, many in the temperate zone, and others in the tropics, so a few bacteria grow best at relatively low temperatures, many at intermediate, and others at high temperatures. For each species the temperature at which growth takes place most rapidly is known as the *optimum growth temperature.* As this temperature is increased or decreased, growth is retarded until a point is reached where it ceases. The highest temperature at which growth takes place is called the *maximum growth temperature,* the lowest, the *minimum growth temperature,* and the number of degrees between the minimum and the maximum, the *growth temperature range.* This range is narrow for some species and wide for others, a fact which has considerable

bearing on many such microbiological problems as food spoilage and disease transmission.

On the basis of optimum growth temperatures, bacteria can be grouped into three classes: *psychrophiles* or those organisms that grow best at a temperature below 14° C.; *thermophiles* or those that grow best at temperatures above 45° C.; and *mesophiles* or those having an optimum growth range between 40° and 20° C.

The psychrophilic or cold-loving organisms are commonly found in cold springs, deep lakes, and the ocean. They are all saprophytic and some of them are of considerable importance in cold storage plants where they may be the cause of decomposition in meat, poultry, and other foods.

The thermophilic or heat-loving bacteria are commonly found in hot springs or decaying manure, although they may be present in a number of other places. None produces disease and our principal interest in them is in connection with the dairy and canning industries. Because of their ability to grow at temperatures as high as 65° C. they may increase very rapidly during long periods of milk pasteurization and, while there is no evidence that they produce products injurious to man, they do reduce the quality of the milk. They may be bothersome in connection with plate counts on milk because they grow out in the form of pin-point colonies easily mistaken for other types of organisms which should have been and doubtless were destroyed by pasteurization. This may confuse the dairy inspector or plant operator checking the thoroughness of processing and the sanitary quality of the milk.

The thermophiles do considerable damage in the canning industry since they may survive the processing temperatures and grow rapidly during the period of cooling, producing a condition in the can known as "flat souring."

Although some of the spore-forming thermophiles can withstand boiling for several hours, not all are so resistant. On the other hand, the *thermoduric organisms* are resistant to high temperatures, although they cannot grow when the temperatures are above 40° to 45° C.

The majority of bacteria are mesophiles and grow best at temperatures higher than 20° C. and less than 40° C. The saprophytes commonly found in soil and water grow most rapidly at room temperatures ranging from 20° C. to 28° C. and those associated with animals usually grow best at the temperature of their animal host. Thus bacteria responsible for infections in man grow best at body temperature which is about 37° C.

The temperature at which an organism grows most rapidly is not necessarily the one allowing for the greatest number of organisms in a given culture nor the one permitting the greatest physiological activity.

Cold

There is a natural tendency to associate freezing with purification, probably because putrefaction does not occur in frozen materials and consequently they do not undergo changes resulting in the liberation of foul smells. While it is true that freezing prevents microbial growth, bacteria, in general, are remarkably resistant to low temperatures. When cultures are frozen, the bacteria go into a state of "suspended animation" in which they show no signs of life and, although there is a gradual decline in numbers, many remain viable for long periods.

It must not be supposed, however, that freezing has no effect on bacteria for during the process many are destroyed. It may be that the formation of ice crystals plays a part in the actual killing of individual cells, for the slower the freezing and the larger the ice crystals, the greater the destruction of bacteria. There is less killing when bacteria are frozen in the dried state than when they are frozen in solutions. In fact, one of the best ways to preserve bacteria or viruses is by rapid freezing and drying *in vacuo*, as utilized in the recently developed Cryochem Process.

Numerous workers have subjected bacteria to alternate freezing and thawing and although the plate counts do indicate a progressive reduction in numbers they find living organisms even after repeating the process twenty to thirty times. Temperatures below 0° C. seem to have little additional effect. In fact, bacteria subjected for several hours to temperatures as low as −250° C. still remained viable. It is practically impossible to sterilize a culture by freezing.

The gradual decrease in the number of bacteria suspended in frozen foods and water undoubtedly results in some purification but it cannot be relied upon. Tests have shown that typhoid bacilli placed in ice-cream were alive after several years although there was a considerable reduction in numbers.

Heat

Fire is the purifier *par excellence*. While microorganisms may survive extremely low temperatures, there is a limit to the amount of heat they can tolerate. The determination of this limit for any organism and of the factors influencing destruction by heat is of considerable practical importance. Canning, pasteurization, and a number of procedures and practices depend upon accurate knowledge of just what degree of heat is necessary to destroy undesirable organisms without damaging the material in which they are contained.

The *thermal death point* is usually defined as the lowest temperature necessary to kill all the organisms in a given population in a specified

period of time, usually ten minutes unless otherwise stated. The term *point* is not a happy choice since all the bacteria in a population do not succumb simultaneously. Some die off at a considerably lower temperature than is necessary to kill all and the number of survivors decreases in an orderly fashion, the rate depending upon the intensity of the heat. It might be more accurate, since killing at a given temperature is a function of the length of exposure, to use the term, *thermal death time.*

How Heat Kills Bacteria: There is such a close relationship between the temperature necessary to destroy enzymes and that necessary to destroy bacteria that many workers attribute the effect of heat on bacteria to the inactivation or destruction of enzymes. At lower temperatures there may be the destruction of certain specific enzymes without which the bacterial cell cannot grow in a medium ordinarily satisfactory. Evidence for this is that such heat injured cells will grow in special media.

There is an equally good correlation between the temperature necessary to kill bacteria and that necessary to coagulate proteins. Consequently many investigators have concluded that death is due to coagulation or denaturation of the protein. Since enzymes are probably protein, the second explanation does not rule out the first.

Several factors influence the rate of death or the thermal death point of bacteria: time, moisture, the character of the medium, the kind of organism, and the number present.

Time: The length of exposure to any destructive agent is important. Lower temperatures for a longer time may be as effective as higher temperatures for a shorter time, a fact put to use in the canning and dairy industries.

Moisture: The thermal death point depends upon the amount of moisture present, for, within limits, the greater the amount of moisture the lower the temperature necessary to kill bacteria. If heat killing is due to coagulation of protein, this is to be expected, since there is a direct relationship between the amount of moisture and the temperature of coagulation. This table, taken from McCulloch, illustrates the relationship.

Temperature at Which Egg Albumin Coagulates in the Presence of Various Amounts of Moisture . . .

Water, %	*Temperature of coagulation*
0	160–170° C.
6	145° C.
18	80–90° C.
25	74–80° C.
50	56° C.

It should be noted that the moisture content of most bacteria runs well over seventy per cent and that a number of bacteria have a thermal death point of less than 50° C.

The thermal resistance of spores is considerably greater than that of vegetative cells. Although earlier workers attributed this to a lower water content of spores, recent investigators doubt this explanation since they find very little difference between the water content of spores and of vegetative cells. Whatever the explanation may be, moist heat is far more destructive than dry heat at the same temperature.

The Character of the Medium: The acidity or alkalinity as well as the composition of the medium has considerable influence on the thermal death point. Any change in pH from the neutral point increases the destructiveness of heat. That is why it is much easier to sterilize acid fruits or vegetables than those whose reaction is more nearly neutral.

Constituents of the medium may also serve to protect the bacteria.

The Kind of Organism: Species of bacteria vary widely in their resistance to heat. Most of the pathogenic organisms are readily destroyed at temperatures of 50° to 60°C. Many of the saprophytes are killed at relatively low temperatures although some are able to withstand temperatures approaching that of boiling water. The spore-formers have two thermal death points, one for the vegetative cell and one for the spore. Some spores will withstand boiling for ten to twenty hours. The spores of *Clostridium botulinum*, the causative agent of botulism, remain viable after boiling five to six hours.

Number of Organisms. The number of organisms in a population influences the thermal death time. The greater the number, the higher the temperature or the longer the time necessary to sterilize.

Practical Methods of Sterilization by the Application of Heat

The nature of the material to be sterilized will determine how heat may best be applied.

Burning: Incineration is obviously a thoroughly effective method of destroying all microorganisms but its application is limited. In the laboratory, inoculating needles are heated red hot in a Bunsen burner before and after transferring bacteria. This kills all the microorganisms. Contaminated articles such as clothing or toys are sometimes burned. The carcasses of diseased animals may be disposed of by incineration. In fact, it is essential when death is due to a disease such as anthrax whose spores survive in the soil for fifteen to twenty years.

Dry Heat: Laboratory glassware is commonly sterilized by the use of dry heat, a temperature of 160° to 170° C. being maintained for one to two hours. Higher temperatures char the cotton usually used as plugs. Dry heat, although effective as a sterilizing agent, cannot be applied to culture media nor to other organic substances. Of several dis-

advantages, its principal one is lack of penetrating power. This is probably why the "fire guns" developed for disinfecting poultry houses cannot be depended upon, under practical conditions, to kill the disease-producing organisms responsible for bacillary white diarrhea of chickens.

Boiling: Although boiling for a few minutes coagulates the common proteins and kills most forms of microorganisms, spores of a number of bacterial species can resist boiling for six hours and a few survive boiling even for twenty hours, a fact very difficult to explain and one responsible in part for the difficulty in disproving the theory of spontaneous generation. It had been assumed that boiling for a few minutes killed all living forms, and when growth was obtained after such treatment, the natural assumption was that life had originated spontaneously.

Fortunately only a few bacteria pathogenic for man produce spores. Most of the pathogenic bacteria, protozoa, and viruses are destroyed by a few minutes of boiling or ten to twenty minutes at temperatures from 58° to 60° C. Clothing contaminated with bacteria that produce typhoid, pneumonia, diphtheria, and tuberculosis may be rendered sterile by boiling for a short time. Dishes and glasses so contaminated may also be disinfected by boiling. It should be remembered that the temperature ordinarily used in dishwashing is, of course, far below boiling and is not in itself sufficient to kill all the bacteria. The addition of soaps and washing powders greatly increases the germicidal effect of the process.

It is common practice to sterilize syringes and surgical instruments by boiling for ten minutes. Sodium carbonate, borax, and other chemicals are frequently added to enhance the action of the heat and to prevent corrosion. It should be remembered that this treatment does not kill the spores of such bacteria as *Bacillus anthracis*, *Clostridium tetani*, or *Clostridium welchii*.

Intermittent Sterilization or Tyndallization: Tyndall, while working on the problem of "spontaneous generation" in 1877, studied bacterial spores and noted how much greater their resistance to heat was than that of the vegetative cells. On the strength of his observations, he developed a method for destroying bacteria which is variously known as *fractional*, *intermittent*, or *discontinuous sterilization*, or as *Tyndallization*. It is based on the observation that while bacterial spores are extremely resistant to boiling, they will germinate when incubated under proper conditions and the resulting vegetative cells are readily destroyed. In practice the material to be sterilized is heated to boiling for five to ten minutes or subjected to flowing steam for fifteen minutes to an hour in a special (Arnold) sterilizer. It is then removed from the heat, incubated for several hours or over night as the case may be, and again subjected to heat. The vegetative cells originally present are killed by the first heating. The surviving spores

germinate during the incubation period and are killed in the subsequent heatings. The process has to be repeated three or four times to insure sterility. Tyndallization is used to sterilize materials not sufficiently resistant to withstand temperatures high enough to destroy the spore forms at one heating.

Steam Pressure Sterilization: When steam is put under pressure the temperature can be raised to a degree sufficient to kill all forms of bacteria, including the most resistant spores, in a few minutes. The killing agent in steam sterilization is temperature and the exact time of exposure necessary to destroy all bacteria is difficult to determine since such a factor as the age of the culture may influence the process. Tests show, however, that dried cultures of spore-bearing organisms are rendered sterile in five minutes when exposed to saturated steam at 107° C. or 225° F., temperatures acquired at a pressure of about four pounds.

The Autoclave: To apply steam under pressure a type of sterilizer known as an autoclave is used. It is indispensable in a bacteriology laboratory and in hospitals and is used for sterilizing culture media, surgical supplies, dressings, gowns, and solutions. Essentially it consists of a specially constructed cylindrical or rectangular double jacketed container, fitted with a steam tight door, provided with a means of admitting steam under pressure, and equipped with pressure and temperature gauges, a safety valve, and a means of drainage. A number of types and sizes are available.

The care and operation of an autoclave require considerable judgment if satisfactory results are to be secured. Packing is particularly important when bundles of dressings or hospital linens are to be sterilized. Special attention must be paid to driving out all the air and replacing it with saturated steam and to packing the materials in such a way as to allow complete penetration by the steam.

Since temperature is the destructive factor in steam pressure sterilization, it is obvious that the time necessary to sterilize depends upon how long it takes to bring the materials in the autoclave up to the required temperature. Liquids in tightly closed flasks heat up slowly and considerable time must be allowed to bring them up to the killing temperature. The usual practice is to autoclave media and small packs of surgical dressings or instruments for fifteen minutes at fifteen to twenty pounds pressure. This gives a maximum temperature of 115° to 121°C. If the autoclave is packed full, it may be necessary to heat for as much as forty-five minutes or even longer to insure sterilization. The all too common practice of observing the pressure gauge without paying attention to the temperature in the center of the pack has led to many failures.

Steam, as we have mentioned before, owes much of its effectiveness to its penetrating power. It penetrates porous materials by a process of

condensation and not by the relatively slow process of adsorption as is the case with dry heat. Superheated steam, however, behaves more like dry heat and is less effective than saturated steam. That is why it is absolutely essential that all the air in the autoclave be replaced by saturated steam if sterilization is to be effective.

Pasteurization is a special case of destruction of disease-producing organisms by heat and will be considered in the section on milk.

MOISTURE

Although the amount of moisture present may vary tremendously, complete desiccation is unknown in nature. When microorganisms are subjected to drying, a series of physical and chemical changes takes place which may or may not result in their death. If it does, death may be due to an increase in the osmotic pressure brought about by an increase in the solutes about the cell, to denaturation of the protoplasm, to destruction of enzymes, to oxidation, or to a combination of all these as well as to unidentified factors. The inherent qualities of the cell, the presence of spores, the character of the medium, and the rate of drying are factors that influence resistance of microorganisms to desiccation. Reports on the length of time pathogenic organisms will survive when placed on glass slides, on wood, or on clothing show rather wide variation between species.

Some unpublished data by Koser and Birkeland indicate that even though organisms may survive for days and sometimes for weeks when placed on telephone mouth pieces, the majority die during the process of drying. When broth cultures of scarlet fever streptococci were placed on the telephone mouth pieces or on glass slides, 65 to 80 per cent could not be recovered after fifteen minutes and, at the end of four hours, 90 to 98 per cent were dead. The remaining few persisted for as long as ten to fourteen days. Similar results were obtained with the pneumococcus and diphtheria bacillus.

PRESSURE

Bacteria and viruses are capable of withstanding pressures of 2000 to 5000 atmospheres and bacterial spores will survive 20,000 atmospheres. Killing accomplished by mechanical pressure is probably due to denaturation of the bacterial protein.

SURFACE TENSION

Since many of the chemical reactions involved in bacterial growth and reproduction are surface reactions, surface tension depressants such as soaps interfere with them. It is difficult to separate their physical effects and chemical actions but it is usually assumed that they modify the perme-

ability of the cell wall, disturb the rate of diffusion, and alter the concentration of substances which may be soluble in them.

RADIATIONS

Radiations of various kinds possess definite germicidal powers, the effect produced being proportional to the intensity of the adsorbed rays, for only those are effective. Since many substances will adsorb the rays, the effectiveness of radiations as germicidal agents is greatly influenced by the character of the material harboring the microorganisms and by the particular components of the cell which absorbs the rays. (For a more complete discussion of this subject, see Topley and Wilson, and Zinsser and Bayne-Jones.)

Sunlight: Sunlight, popularly associated with health, does, under ideal conditions, possess considerable germicidal power; but its intensity is variable and its penetrating power so small that it is doubtful whether its beneficial effects are due, ordinarily, to its direct action on infectious agents. We have come to think of sunlight in connection with cleanliness, well-being, fresh air, open spaces, and healthful conditions and the lack of it, in our cities, in connection with poverty, overcrowding, malnutrition, filth, and a low order of sanitation.

Killing of bacteria by sunlight depends upon the wave length and intensity of the rays and the length of exposure. These factors also determine its limitations. When sunlight is passed through a prism, it is separated into its component rays of varying wave lengths. The rays of the visible spectrum, which range from 3970 Ångstrom Units in the violet zone to about twice that length in the red, have been shown to have little effect on bacteria. It is the short rays, lying between 2540 and 2800 Ångstrom Units, that are adsorbed and have the highest germicidal power. However, atmosphere, smoke, dust, and window glass present effective barriers against anything shorter than about 3100 Ångstrom Units, so that very few of the short rays reach us except under ideal conditions. In fact, ordinary electric light has about the same germicidal properties as sunlight passed through window glass.

Although direct sunlight will rapidly destroy pathogenic bacteria if they are exposed in thin films, the presence of organic matter often protects them from the effective rays. The tubercle bacillus, protected by the sputum in which it is usually found, will survive several days' exposure to direct sunlight.

As an inhibiting agent sunlight is very effective, particularly if an ample supply of oxygen is present; for under such conditions many bacteria produce hydrogen peroxide in sufficient concentration to retard or prevent their

growth. The drying due to heat adsorbed may also play a part in inhibiting their growth and perhaps in their destruction.

The ultraviolet light generated by mercury vapor lamps is effective but, like sunlight, lacks penetrating power.

Photodynamic Action: The effect of light in conjunction with certain dyes is an interesting phenomenon. Some dyes sensitize microorganisms to light so that they are readily killed by exposures which ordinarily would have little effect. This photodynamic action depends upon oxygen for its effect and the active rays are those absorbed by the dye. When certain viruses are mixed with methylene blue in a concentration of 1 to 100,000 and exposed in thin layers to sunlight or to ordinary electric light, inactivation occurs in a few minutes.

Radiant Energy Emanations:

a. *Electricity:* Bacteria and viruses are negatively charged. When electricity is passed through a suspension of these organisms, they migrate to the positive pole at a rate dependent upon the magnitude of their electrical charge, a species characteristic. When electricity is passed through a bacterial suspension, killing does occur. It is due in part, if not entirely, to heating and to the liberation of chlorine resulting from the dissociation of chlorides. If bacteria could be electrocuted, the sterilization of milk and biologicals would be a simple matter. The "electropure" process of pasteurization depends upon passing electricity through milk. However, there is some doubt as to its effectiveness.

b. *Cathode Rays:* Cathode rays generated by the Coolidge type of electron tube are destructive to bacteria. At present their use is of greater academic interest than practical importance.

c. *X-Rays:* X-rays or Roentgen rays stimulate the rate of dissociation of bacteria or kill them depending upon intensity and length of exposure. Like all other emanations, they lack penetrating power and are not particularly effective killing agents unless the material to be sterilized is exposed in thin layers.

d. *Radium:* Although conflicting reports have been given on radium, it can be said with certainty that it is not very destructive to most bacteria.

Supersonic Waves: Sound waves whose frequencies are above the audible range are called supersonic waves or vibrations. Vibrations of 200,000 or more cycles per second produced by piezo-electric crystals and high frequency oscillators have a destructive and disrupting action on microorganisms. This has been attributed to the liberation of dissolved gases and is called *cavitation*, but precisely what happens is not known. Supersonic vibrations have been used in a limited way in the sterilization of milk and in the production of vaccines. Not enough is known about their applicability as yet to warrant any prediction as to their usefulness.

Chapter IX

HOW THE ENVIRONMENT AFFECTS MICROORGANISMS: CHEMICAL AGENTS

There is perhaps no phase of bacteriology about which there is so much popular interest and at the same time so much ignorance as that concerning the effect of chemical agents on bacteria. The germ-conscious public is continually exploited by commercial interests who have far more powerful advertising than disinfectants. Aside from the money wasted, there is a real danger in placing confidence in worthless compounds for it often leads to the neglect of more adequate measures. Too few people understand how disinfectants act, what factors influence their action, and just what can be expected of them.

Chemicals may increase, retard, or inhibit microbial growth; or they may actually destroy the microorganisms. We shall here concern ourselves only with those that inhibit growth or destroy. The terms usually employed in describing the action of chemical agents on microorganisms are often used so loosely that, for the sake of precision, a few definitions are in order.

An *antiseptic* is a substance that prevents sepsis; that is, that retards, opposes, or inhibits microbial growth. The term is synonymous with *bacteriostatic*. The term *preservative* means essentially the same, but is used mainly in connection with food.

A *disinfectant* is a substance that frees from infection. Strictly speaking, it is a chemical agent destroying the disease-producing bacteria with which it comes in contact. Actually the term is used with reference to any agent, be it chemical or physical, which destroys disease-producing organisms It is synonymous with *germicide*, *fungicide*, and *bacteriocide*. It should be noted that the essential difference between an *antiseptic* and a *disinfectant* is that the former implies inhibition and the latter, destruction. The same chemical agent may, depending upon its concentration, act as a disinfectant or as an antiseptic. Indeed in very weak dilutions, it may act as a stimulating substance.

The term *sterilization* implies the elimination of all living forms. Sterilization may be accomplished by physical and chemical destruction of the microorganisms or by their removal by filtration.

A *deodorant* is an agent that destroys or masks offensive odors. It may be antiseptic and destroy the offensive compound by chemical action, or it may simply mask one smell by another less offensive. The real danger in the use of deodorants is that they give a false sense of security or cleanliness.

THE PHENOL COEFFICIENT

Since Koch's early work on the effect of chemicals on bacteria and the discovery that phenol is a powerful disinfectant, there has been a vigorous search for other chemicals that might be just as effective and less toxic. Methods were developed which made it possible to compare one chemical disinfectant with another and in 1903 Rideal and Walker suggested one using phenol as a standard of comparison and noting the results in terms of a coefficient.

TABLE 1

Data Used in Determining Phenol Coefficient

	DILUTION	5 MINUTES	10 MINUTES	15 MINUTES
Phenol control	1:90	0	0	0
	1:100	+	0	0
	1:110	+	+	0
Test disinfectant	1:250	0	0	0
	1:300	0	0	0
	1:350	0	0	0
	1:400	+	0	0
	1:450	+	+	0
	1:500	+	+	+

The *phenol coefficient* of a disinfectant is obtained by dividing the highest dilution of the test disinfectant by the highest dilution of phenol which sterilizes a given culture under standard conditions of time and temperature. The following technique used by the Food and Drug Administration of the United States Department of Agriculture and described in Circular 198 issued by that department is considered official. A series of test tubes is prepared, each containing 5 c.c. of varying dilutions of the test disinfectant and the phenol control respectively. One half of a cubic centimeter of a twenty-two to twenty-six hour culture of a test organism, *Salmonella*, unless otherwise stated, is added to each of the tubes. After five, ten, and fifteen minutes incubation, samples are transferred to tubes containing a suitable medium for growth and these are incubated for forty-eight hours. . Results are recorded as in Table 1, plus signs indicating growth, and zero signs, no growth.

The phenol coefficient is then calculated by dividing the highest dilution of the test disinfectant which kills in ten minutes but not in five—in this case, 1:400—by the highest dilution of phenol which kills in ten minutes but not in five—in this case, 1:100. This particular test disinfectant, then,

has a phenol coefficient of *4*, which may be interpreted to mean that it is four times as powerful as phenol.

While *Salmonella* is the standard test organism and may be assumed to have been the one used, unless others were named, it is true that different results are obtained when other organisms are used. Since *Staphylococcus aureus* is nearly always present in wounds and is a common cause of infections, it is acknowledged that this organism should also be included in the test and it is a common practice to determine the phenol coefficient of a disinfectant using *Staphylococcus aureus* in addition to *Salmonella*. A number of chemicals show a considerable difference in killing power when tested against gram-positive and gram-negative bacteria and representatives of both groups should be used in the tests.

The phenol coefficient, determined on broth cultures of organisms, gives little information as to the effectiveness of the chemical applied to living tissue in which pathogenic organisms are growing. Several methods have been developed recently for testing disinfectants under conditions simulating those actually encountered. While the details vary, the highest dilution of the disinfectant toxic for living tissue cells is compared with the highest dilution toxic for bacteria and the result expressed as a *toxicity index*.

WHAT TO LOOK FOR IN A DISINFECTANT

The ideal disinfectant has not yet been produced. The problem is so complex that a disinfectant satisfactory for some purposes may be worthless for others. When choosing one, it is well to consider just how adequately particular needs will be met.

Germicidal Power: It is obvious that high killing power is a characteristic of a good disinfectant. However, bacteria differ in their sensitivity to chemicals and a satisfactory disinfectant for one species may be of little value against another.

Stability: The stability of a chemical in the presence of organic matter is of considerable importance. Most disinfectants combine readily with the organic matter present and are used up or form inert compounds so rapidly that it is difficult to maintain an effective killing concentration. For example, chlorine gas, although the most valuable chemical for disinfecting drinking water, which naturally contains little organic matter, is not so valuable as a wound disinfectant because it combines with the tissue itself. Oxidizing compounds, such as potassium permanganate ($KMNO_4$) and hydrogen peroxide (H_2O_2) are rapidly rendered ineffective for the same reason. A compound should be so stable that it does not decompose when standing. This is another weakness of hydrogen peroxide.

Homogenicity: Disinfectants, such as mercuric chloride ($HgCl_2$), which may be obtained in crystalline form, are pure but many of the organic compounds such as the coal tar derivatives are not and different batches may vary in germicidal power.

Solubility: A disinfectant should be soluble in water or tissue juices. If its killing power is very high, its solubility need not be, for not much of it will be needed; if it kills only in low dilutions, its solubility should be correspondingly high.

Selective Toxicity: The ideal disinfectant should be toxic to bacteria and non-toxic to the tissue cells of man and animal. Now any number of compounds are highly toxic to bacteria but nearly all of them are about as toxic or even more so to tissue cells, which might be expected since they act on protoplasm. However, there are a few compounds that are relatively non-toxic for man, and that may be injected into the blood stream or taken by way of the mouth in sufficient quantities to be effective against certain disease-producing organisms. They are quinine, used in the treatment of malaria; arsphenamine, used in the treatment of syphilis; and sulfanilamide and its derivatives, effective in a number of bacterial diseases. It is doubtful whether their effectiveness is due to their direct toxicity on the microorganisms.

Penetrating Power: A disinfectant should be able to penetrate into the tissue. Most compounds do not reach any appreciable depth and consequently are of little value in the treatment of any but the more superficial wounds or infections.

Cost: The cost of a disinfectant is of considerable importance. An ideal one must be sufficiently inexpensive to be within the reach of all. Of course, the purpose to which the disinfectant is to be put must be considered in this connection. A disinfectant inexpensive enough to be used in the treatment of wounds may be too expensive to be used in the disinfection of barns.

Deodorizing Powers: A disinfectant which deodorizes is to be preferred, but should not be confused with a deodorant that does not disinfect.

Miscellaneous: The ideal disinfectant will not damage fabrics nor corrode metals.

The cleansing power of a disinfectant, although not absolutely necessary, is of some importance.

And to this list, might be added two more desirable characteristics: the ability to neutralize toxic products of bacteria which counteract the defensive mechanisms of the body, and the ability to stimulate defensive mechanisms, such as phagocytosis.

FACTORS AFFECTING DISINFECTION

Although a voluminous literature has accumulated on the subject of disinfection, the process is so complex that it is not possible to write equations for the chemical reactions involved. We do not know just how microorganisms are destroyed, but we know a great deal about the factors that

FIG. 35. Cup plate method of testing disinfectants. Melted nutrient agar is seeded with the test organism, poured into a plate, and allowed to harden. A portion of the agar is removed with a cork borer. The well left is then filled with the disinfectant to be tested and the plate incubated. The effectiveness of the disinfectant in preventing growth of the organism can be gauged by the width of the halo or clear zone around the well.

influence the process and, in order to use disinfectants and antiseptics intelligently, they must be recognized.

Time: Destruction of microorganisms by physical or chemical means is a function of time. It requires time for the chemical to penetrate the material harboring the organism and time for it to kill the organism after

it has made contact. This often accounts for the failure of oral disinfectants which cannot be held in the mouth or throat long enough to penetrate the mucosa. A perfectly satisfactory germicide for disinfecting body discharges may be useless when applied to infected tissue for it may be impossible to keep it there long enough to complete the destruction of the microorganisms present.

Temperature: The rate of chemical reactions bears a direct relationship to temperature, being approximately doubled with every ten degree rise. There has been considerable work on the influence of temperature on the effectiveness of disinfectants and the results obtained indicate that although there is a strong resemblance between simple chemical reactions and disinfection, the laws governing monomolecular reactions are not generally applicable to the influence of temperature in biological processes. The temperature coefficient of disinfection is high. It varies with different chemicals, being several times higher for phenol than for mercuric chloride, and is not constant over a wide range of temperatures. In general the increase in velocity is from two to five fold for every ten degree Centigrade rise between 25° and 45° C.

Concentration: Not only does a disinfectant require time for action; it also requires a certain definite concentration for killing in any given period of time. Because of the dilution which may take place, it is not always possible to keep this concentration when the disinfectant is in contact with tissue.

Chemical Nature of the Disinfectant: The chemical nature of the disinfectant determines its mode of action on bacteria and the reactions between it and the menstruum. In general, disinfectants act by oxidation, hydrolysis, or chemical combination.

Oxidation: Hydrogen peroxide, potassium permanganate, and chlorine are powerful oxidizing agents. However, they act not only on the bacteria but on all organic matter present and consequently the uses to which they may be put are pretty much limited by the amount of organic matter. If there is a great deal, they may be completely used up in its oxidation.

Hydrolysis: Strong acids and alkalies cause a digestion of the bacterial cell. Such agents are usually too destructive to body cells to find wide application as tissue disinfectants.

Chemical Combination: A number of chemicals, particularly the salts of the heavy metals such as mercuric chloride, form a chemical union with the bacterial protoplasm bringing about a coagulation or precipitation. Some chemicals apparently alter the permeability of the bacterial cell wall and so interfere with its function. It is probable that many act on specific enzyme mechanisms.

There appears to be a definite relation between the chemical structure of organic compounds and their toxicity for bacteria. For example, in

the case of sulfur-containing compounds such as prontosil and sulfanilamide, it appears that only those derivatives in which the sulfur has a valence of six are effective as chemotherapeutic agents.

Nature of the Substance to be Disinfected: Two things must be considered here: the nature and numbers of the organisms, and the nature of the material harboring the organism.

Nature of Organism: The chemical composition and such morphologic features of the species as spores and capsules profoundly influence the effectiveness of a disinfectant. Even strains within the species may vary in resistance. There is ample evidence to suggest that constant contact with a toxic agent leads to the development of tolerant or resistant strains. (See Variation, page 96.)

The number of organisms present also influences the process of disinfection. It requires a longer period of time or a higher concentration of disinfectant to sterilize a culture containing two million organisms per cubic centimeter than one containing two thousand.

Nature of Material Harboring the Organism: It is rather obvious that the problem of disinfecting materials such as body discharges, water, or clothing is much different from that of destroying disease-producing organisms in living tissue such as the throat. Human tissue cells are, in general, as or more susceptible to disinfectants than the bacteria which invade them and do not regenerate so rapidly. Consequently disinfectants which readily kill bacteria may be worse than useless when applied to infected tissue because they may destroy the tissue cells and thus encourage infection by interfering with the natural defensive mechanisms of the body. That is why disinfectants valuable in preventing bacterial infection in fresh wounds are so ineffective in combatting deep-seated infections.

The presence of organic matter, either as living tissue or in some other form, interferes with the action of many powerful disinfectants. Protein precipitants such as mercuric chloride cannot be depended upon to destroy bacteria in sputum or other material containing appreciable quantities of protein. They combine with the protein, often precipitating it around the bacteria and thus actually protecting them.

The presence of salts may greatly interfere with the action of those disinfectants that must be ionized to be effective. Thus, sodium chloride greatly reduces the efficiency of mercuric chloride whereas it has no such effect on phenol, because it is the mercury ion of mercuric chloride that is toxic, while, in the case of phenol, it is the whole molecule.

TYPES OF DISINFECTANTS

The disinfectants, antiseptics, and preservatives may, for convenience, be grouped as follows: (1) Acids and alkalies, (2) Salts of heavy metals, (3) Halogens, and (4) Complex organic compounds.

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Acids

The preservative and antiseptic properties of acids have been recognized from time immemorial and acids are still depended upon for the preservation of foods. As disinfectants they have a more limited value. The antiseptic or germicidal power of the mineral acids is approximately proportional to their degree of dissociation. In the case of hydrochloric and sulfuric acid, the germicidal power is proportional to the concentration of free hydrogen ions and not to their normal strength. Nitric and fluoric acid seem to have an additional effect due to the anion.

The germicidal power of organic acids is dependent upon the nature of the whole molecule or organic radical and not upon the hydrogen ion liberated. It is specific for each acid and does not depend on the degree of dissociation. Many of the organic acids, although slightly dissociated and therefore weak acids, are far more powerful germicides than the highly dissociated strong mineral acids.

When acids are added to a culture, they exert an effect on the medium as well as on the bacteria. We have previously seen that bacteria have an optimum hydrogen-ion concentration for growth. Now when dilute acids are added to a medium, they may act as preservatives for they may increase the hydrogen-ion concentration to a point where bacteria can no longer grow. If the acids are strong enough to raise the hydrogen-ion concentration further, they may act as disinfectants, for the acidity may be sufficient to destroy the bacteria. The buffering capacity of the medium will determine the amount of acid necessary to raise the hydrogen-ion concentration, a fact well illustrated in the preservation of foods by the use of benzoic acid. Acid foods may be preserved by the addition of much less benzoic acid than is needed for alkaline or neutral foods.

Alkalies

The germicidal action of bases such as potassium and sodium hydroxide (KOH and NaOH) is due mainly to the OH ion and is consequently proportional to the degree of dissociation. The germicidal action of bases having a heavy metal cation such as barium hydroxide $Ba(OH_2)$ is due mainly to the whole molecule or to the toxic metallic cation and is not proportional to the degree of dissociation.

In the case of both acids and alkalies, the action on bacteria may be due in part to physical forces such as osmotic pressure and surface tension.

That strong alkalies are effective germicides is shown by Table 2.

Selective Action of Strong Alkalies: In general, strong alkalies are more effective against the gram-negative bacteria and some of the animal viruses than against gram-positive bacteria or protozoa. The acid-fast organisms

such as the *Mycobacterium tuberculosis* are very resistant, a characteristic utilized in obtaining pure cultures from sputum.

Potassium hydroxide in the form of lye has been found to be one of the most satisfactory disinfectants for use in barns. McCulloch found that lye in a dilution of 1:800 killed all strains of *Brucella abortus*, the causative agent of Bang's disease, in ten minutes or less. Lye in a concentration of 5% is often recommended as a disinfectant against the anthrax bacillus.

Sodium hydroxide has been found effective against a large number of bacteria as well as such viruses as those of foot and mouth disease and hog cholera.

Lime, quick lime, slaked lime, and *whitewash* are strong germicides and very valuable for disinfecting buildings or fecal material. Fresh lime is CaO. When about two parts of CaO are added to one part of water, $Ca(OH)_2$ or

TABLE 2

*Effect of Concentration of Hydroxyl Ions in Destruction of E. coli**

(After Meyers, 1928)

pH OF SUSPENSION	SUSPENSION AT 250° C.		MEDIUM OF EXPOSURE
	At start	After 10 minutes	
8.0	1,510,000	1,270,000	KH_2PO_4-NaOH
9.1	1,510,000	730,000	KH_2PO_4-NaOH
10.0	1,510,000	25,000	Na_2HCO_3-NaOH
11.1	1,510,000	0	Na_2HPO_4-NaOH
12.1	1,510,000	0	NaH_2PO_4-NaOH
13.0	1,510,000	0	NaH_2PO_4-NaOH

* Taken from McCulloch.

slaked lime is formed. This, diluted with about eight parts of water, is known as milk of lime or, if still further diluted, as whitewash. Though not so effective in the same concentrations as NaOH or KOH, lime is nevertheless a very valuable disinfectant for barns and for fecal material. It should be remembered that when lime, CaO, is exposed to air, it is converted to $CaCO_3$, calcium carbonate, and is practically valueless as a germicide.

Sodium carbonate is not a particularly good germicide although when used in hot water it is more effective. *Sodium bicarbonate*, although recommended and widely used in the sterilization of instruments, is not in itself a germicide. It is used to prevent corrosion.

Trisodium phosphate and *sodium metasilicate* are widely used for washing glassware and in the dairy industry. They are more effective germicides than sodium carbonate and when used in hot water are of considerable value.

In general, it may be said that the germicidal power of the common alkalies is not noticeably affected by the presence of organic matter. This is of considerable importance and they are commonly used in circumstances where organic matter is present. Their germicidal action does not increase as rapidly with rises in temperature as does that of most other disinfectants.

The Effect of Salts on Bacteria

Salts are of considerable interest in connection with bacterial growth, food preservation, and disinfection. Their effect depends upon their concentration and chemical nature. The mode of action is complex and may be on the medium or on the bacterium itself.

Osmotic Pressure: All salts, if present in high enough concentration, are growth inhibiting even though they may not have any direct effect on the bacteria. Different bacterial species vary in their behavior toward high salt concentrations. Some, such as the meningococcus, are very sensitive; while others, as the halotrophic bacteria, are capable of growing in concentrations of ten to twenty per cent of sodium chloride. The osmotic effect is important in food preservation.

Oxidation: Some of the complex salts containing oxygen may be effective oxidizing agents and active germicides.

Reduction: The germicidal power of sulfates and ferrous salts seems to be related to their reducing activities.

Molecular Action of Salts: In the case of the salts of organic acids, it is probable that the whole molecule is toxic to bacteria.

Ionic Action: Salts of heavy metals owe their germicidal power to the toxicity of the heavy metal cation. In other salts, the anions may be the more toxic. Regardless of which ion is toxic, the germicidal action of any salt is greatly affected by the presence of other salts. Bichloride of mercury owes its toxicity to the mercury ion, and salts such as sodium chloride, which depress the ionization of bichloride of mercury, greatly interfere with its germicidal action.

Salts of Heavy Metals

A number of salts of heavy metals are so toxic to bacteria that they are effective germicides. Some of these are simple salts and others are complex salts of organic acids.

Mercury: There is something almost mystical about mercury. In ancient times it was regarded as a potent drug and the afflicted were plied with it internally and externally until their hair fell out and their teeth loosened. During the sixteenth century it was widely prescribed in the treatment of syphilis.

Bichloride of mercury, $HgCl_2$, or corrosive sublimate as it is commonly called, is a potent disinfectant. In dilutions of 1:1000 it kills most bac-

teria in a short time and in dilutions of 1:500 kills the spores of the common spore-bearers in less than one hour. Dilutions as high as 1:50,000 or even 1:100,000 are definitely inhibitory to many species. However, the value of bichloride of mercury as a disinfectant is distinctly limited in spite of its high inhibiting power. It is highly poisonous to animals, corrosive to instruments, and, most significant of all perhaps, a protein precipitant, combining to form insoluble compounds which may actually protect the organisms against the penetration of the disinfectant. For this reason bichloride of mercury is of little value for the disinfection of sputum or other body discharges.

Many attempts have been made to prepare organic compounds of mercury which would have the high germicidal power of bichloride of mercury and, at the same time, none of its undesirable features. The theory on which some of these have been synthesized is that the undesirable properties of bichloride of mercury are due to the mercury ion and that if the mercury is incorporated in the organic molecules where it can exert its effect as a part of the whole molecule, it will lose its irritating effect but retain its germicidal power. A number of compounds of this sort have been put on the market. These are some of the more widely used:

Mercurochrome, 220 soluble, is the sodium salt of dibromoxy-mercuri-fluorescein. Experimental evidence as to its disinfecting power is somewhat conflicting. It is more effective in acid than in neutral or alkaline solutions, is relatively non-toxic or non-irritating to skin or wounds, but lacks penetrating power and is adversely affected by organic matter. It is commonly used in two to five per cent solutions and, in these concentrations, is probably very much overrated as a skin and wound disinfectant.

Metaphen is 4, nitro- 5 hydroxy-mercuri-orthocresol and contains about fifty-six per cent mercury. It has a high inhibiting power. Reports on its value as a skin disinfectant are somewhat conflicting but on the whole indicate that it is effective. On the other hand, the results obtained when it is used as a chemotherapeutic agent and injected intravenously in the treatment of experimental diseases in which bacteria are in the blood stream indicate that it is not only of no value as a curative agent but probably hastens the death of experimental animals.

Merthiolate is sodium ethyl mercuri-thiosalicylate. Experiments show that its toxicity for animals is very low, its germicidal power high. It has been recommended for the sterilization of instruments in dilutions of 1:1000 and as a skin disinfectant in dilutions from 1:5000 to 1:30,000.

Merphenyl nitrate is a double salt of phenyl mercuric hydroxide and phenyl mercuric chloride in which the mercury is said to be present as the cation and not as the anion. This compound, according to Stark and Montgomery (Journal of Bacteriology, 1935: 29: 6) inhibits *E. coli* in

dilutions of 1:2,500,000 and *Staphylococcus aureus* in dilutions of 1: over 300,000,000. Its high inhibiting or bacteriostatic power is attributed to the fact that the mercury in merphenyl nitrate bears a positive charge and hence combines readily with bacteria which are all negatively charged.

Silver Salts: Silver in the form of simple salts or more complex colloidal compounds is widely used as an antiseptic and disinfectant. The silver ion is remarkably effective in sterilizing water, even in unusually high dilutions, some reports published indicating that ionic silver in a concentration of only one part to 20,000,000 will effectively sterilize drinking water. The mechanism of this so-called "oligodynamic" action is not clear, but it has been suggested that the silver ion catalyzes the formation of hydrogen peroxide which is the agent of destruction. It seems probable that the effect of heavy metals and, indeed, of other toxic ions or molecules may be one of inactivation of certain enzymes necessary for multiplication of the organism, and not of direct killing of the organisms. Evidence for this is found in the statements of many workers that organisms may be reactivated and rendered capable of growth by the chemical removal of the toxic agent. This may explain why silver compounds have more bacteriostatic than disinfecting action. Regardless of what the mechanism may be, the germicidal power of silver compounds seems to be related to the free ions and not to the total amount of silver present.

Silver nitrate, although both irritating and corrosive when concentrated, is fairly well tolerated in one or two per cent solutions. It was introduced by Credé in 1881 as a prophylactic measure for the prevention of gonococcal infection in the eyes of the newborn. A one per cent solution instilled into the eye immediately after birth destroys the gonococci, thus preventing an infection which usually results in blindness. It is a procedure that should be followed invariably and is required by law in most states.

Silver salts of organic acids, such as citric and lactic, have been recommended for use in the treatment of wounds and diseases of mucous membranes. The former is less irritating than the latter. Both have definite antiseptic and disinfecting powers in concentrations that can be tolerated by the tissue.

Silver protein mixtures in a colloidal state, such as *protargol,* are relatively strong disinfectants but are also irritating; while the silver protein compounds such as *argyrol, silvol,* and *neosilvol,* are mild disinfectants and less irritating. These are often used in concentrations of from two to twenty-five per cent in the treatment of infections of the eye. In more dilute solutions, they are often used for irrigating the nasal passages and sinuses.

Salts such as sodium chloride and proteins such as blood interfere with the germicidal power of all the silver preparations.

Warnings have been issued recently against the too promiscuous use of silver compounds in the treatment of colds and other upper respiratory infections because of the danger of producing silver staining of the cutaneous tissue and teeth, a condition known as *"argyria"*.

Gold Salts: Gold salts have, in general, not been found to be satisfactory, although a complex gold salt known as *sanocrysin* has been used in the treatment of tuberculosis, particularly in Europe. Results obtained with this compound in the United States have not been encouraging.

Copper Salts: Copper, like silver, by virtue of its oligodynamic action exerts a powerful inhibiting influence on microorganisms, particularly on algae. Copper sulfate in a concentration of two parts per million is widely used as an algicide in water impounded for drinking purposes and in the treatment of swimming pools that are exposed to sunlight. Concentrations of 1:400,000 will kill typhoid bacilli in twenty-four hours. It is effective as an algicide in concentrations tolerated by the fish.

Copper sulfate in the form of *Bordeaux mixture*, and copper carbonate are widely used as fungicides and are very satisfactory in the prevention of certain plant diseases.

Zinc Salts: Zinc salts, which are less active than copper or silver salts, are frequently incorporated in mouth washes or gargles. As disinfectants they have little value and are desirable chiefly for their astringent action.

Arsenicals: The use of arsenic in the treatment of disease dates back to ancient times. It is extremely toxic to men and animals and is an effective insecticide. Its toxicity for bacteria is relatively low considering its high toxicity for animals. The brilliant researches of Paul Ehrlich, the German chemist, and his synthesis of organic arsenicals which proved effective in the treatment of trypanosomal infections and the development of arsphenamine (606), so successful in the treatment of syphilis, is one of the most fascinating chapters in the history of man's conquest of disease.

A number of other organic arsenicals have been prepared which are relatively non-toxic for man but effective in the treatment of protozoan diseases. *Stovarsol* or *Acetarsone* is effective in the treatment of amoebic dysentery. *Bayer 205* is effective in the treatment of trypanosomiasis, known as African sleeping sickness. Various others, closely related chemically to arsphenamine, are effective in the treatment of spirochetal infections such as syphilis, yaws, and Vincent's angina.

The arsenicals seem to be specific for protozoa and to have no effect on infections due to the true bacteria, a fact which leads many workers to feel that the *treponema* are perhaps more closely related to the protozoa than to the bacteria.

Organic Compounds

Formaldehyde: Formaldehyde is an acrid gas that possesses high germicidal power. It has been much used in the past as a fumigant and for "terminal disinfection," which is disinfection of the premises following death from an infectious disease. This procedure has been largely discarded as both useless and expensive. *Formalin*, a 37 to 40 per cent aqueous solution of formaldehyde, is commonly used for the preservation of tissues, as a hardening agent for specimens, and in embalming. Used in concentrations of 1:10 to 1:20 it shows marked germicidal properties. It also detoxifies toxins and inactivates viruses. But although formalin is an active bacteriocide, it is not commonly used in connection with living tissue because of its irritating properties. It is very effective for the disinfection of tuberculous sputum and has been recommended for the sterilization of such articles as shaving brushes which might be contaminated with anthrax bacilli. It is effective against anthrax spores, a 5% solution destroying them in two hours, and a concentration of 25% in ten to thirty minutes.

Formaldehyde shows a high temperature coefficient and is about ten times as effective at body temperature as it is at 0° C. Organic matter and salts have little effect on its germicidal powers.

As a fungicide, formaldehyde is very useful. It destroys soil and pathogenic fungi and is commonly used in the treatment of seeds and potato tubers infected with *scab* and *rhizoctonia*.

Formalin quickly deodorizes fecal material. It is destructive to flies and other insects and for this reason is often incorporated in fly poison. It is not particularly poisonous for humans but is irritating to the eyes and throat.

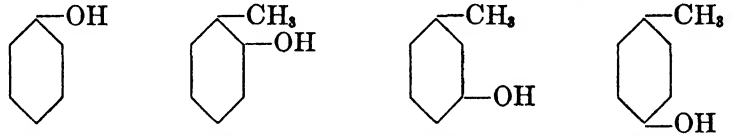
Phenols. The coal-tar derivatives, the phenols (carbolic acids) and the cresols, are active germicides. Since the classical researches of Lister on surgical infection and the consequent development of antiseptic surgery, carbolic acid has been a popular disinfectant. In dilutions varying from 1:80 to 1:110 it destroys most bacteria in ten minutes. A five per cent solution will destroy the spores of most bacterial species in a few hours although it is said that the spores of anthrax bacilli will survive a twenty-four hour exposure. Five per cent phenol is also an effective disinfectant for fecal material and sputum. As a wound disinfectant a two per cent solution is sometimes used.

The mode of action of phenol is not clear but it seems to be the whole molecule that acts, not the ions. Its action is, therefore, not affected by the presence of salts, and organic matter interferes but little. There is some question as to the effect of alcohol on its germicidal properties, some of the early workers believing that ethyl alcohol reduced its effectiveness, whereas some of the later reports show that the presence of 20 per cent to 30 per

cent ethyl alcohol definitely increases it. Phenol is more effective in acid than in neutral or alkaline solutions. The temperature coefficient of phenol is high, an increase from 2° to 40° C. increasing its effectiveness fourfold. Soaps of all kinds definitely reduce its germicidal power. Phenol also has a local anaesthetic action and for that reason is sometimes incorporated in oral antiseptics.

As a class the filterable viruses appear to be resistant to bacteriocidal concentrations of phenol. A five-tenths per cent concentration is frequently used as a preservative for virus material and for biologicals such as vaccines and antisera.

Cresols: The cresols are stronger germicides than phenol. That they are closely related chemically is seen from the following formulae.



Phenol Ortho Cresol Meta Cresol Para Cresol

They differ in the position of the OH group on the benzine ring and have a CH_3 group. A mixture of the three, known as *tricresol*, is commonly used. Since the cresols, which are not very soluble, readily form emulsions in the presence of alkalies and soaps, they are used in combination with them. *Lysol*, one of the most practical disinfectants for use in the home or hospital, is a mixture of tricresol and a linseed oil soap and is about five times as powerful as phenol. In a two to five per cent solution it is a very effective disinfectant for instruments and contaminated glassware, desks, or floors. Organic matter does not interfere with the germicidal power of cresols and consequently they are useful for disinfecting sputum and feces, or any other organic material. The temperature coefficient of the cresols is high. It requires about five times as high a concentration to kill at 2° C. as it does at 40° C. Phenols and cresols rapidly lose their germicidal power on dilution.

An excellent disinfectant for farm buildings is a kerosene emulsion, prepared by dissolving a half pound of laundry soap in a half gallon of boiling salt water, adding two gallons of kerosene and about a pint of crude carbolic acid or cresol, and diluting with about nine parts of water. This kerosene emulsion without the phenol or cresol is recommended for the destruction of mites and other parasites.

Other coal tar products such as creosote are used to preserve fence posts, piles, railroad ties, and telephone poles against the wood-destroying fungi.

Hexylresorcinol, S. T. 37, is a powerful disinfectant and a surface tension reductant. The commercial preparation, S. T. 37, which contains one part

of the crystalline chemical in a 1:1000 solution of aqueous 30 per cent glycerine, destroys all vegetative cells of the pathogenic bacteria in fifteen seconds. In five seconds contact *in vivo* it destroys many of the protozoa, such as the trypanosomes. Its name, S. T. 37, refers to the surface tension value expressed in dynes. Although hexylresorcinol is both a powerful disinfectant and a surface tension reductant, it is doubtful whether the two properties are directly related. It is nontoxic and non-irritating, and is frequently used in infections of the urinary tract and oral cavity, and in wounds. It is a local anaesthetic which may account, in part, for the relief it gives in cases of sore throat. S. T. 37 is also prepared in the form of *Sucrets* or sugar candy. As these slowly dissolve in the mouth, the antiseptic is liberated. Particles of organic matter interfere with its antiseptic value.

TABLE 3*

The Phenol Coefficients of Various Alcohols.

ALCOHOLS	PHENOL COEFFICIENTS
Methyl	0.026
Ethyl	0.040
Propyl	0.102
Butyl	0.273
Amyl	0.78
Hexyl	2.30
Heptyl	6.80
Tetyl	21.00

* Tilly, F. W., and Schaffer, J. M., Relation between chemical constitution and germicidal activity of monohydric alcohols and phenols. Jour. Bact., 12, 303, 1926.

Alcohol: In spite of the fact that Koch in his early work and numerous bacteriologists since him have all shown that ethyl alcohol is a weak disinfectant, the layman and too many trained men place considerable confidence in it.

Alcohol is fairly effective against vegetative forms in concentrations of fifty to seventy-five per cent; in lower concentrations, it is practically valueless. In concentrations greater than eighty per cent, it is not germicidal but acts as a preservative. It is a protein coagulant and its action is greatly reduced by the presence of proteins. Whiskey with its alcoholic content of forty to forty-five per cent cannot be considered a disinfectant.

Alcohol is also a dehydrating agent. A possible explanation for the failure of ethyl alcohol to disinfect in the high concentrations is that it must penetrate in order to be an effective germicide and can do so only

in the presence of water. In concentrations greater than seventy-five to eighty percent its dehydrating action is so pronounced that it removes the water from the cell and is consequently unable to penetrate.

Ethyl alcohol in a concentration effective against vegetative forms is practically inert against bacterial spores, probably because it cannot penetrate. Spore-forming organisms have been cultured from supplies of alcohol used in clinics and *Clostridium perfringens* infections have occurred where seventy percent alcohol was depended upon to sterilize surgical instruments.

Alcohol reduces the effect of phenol and formaldehyde but enhances that of bichloride of mercury. Its own germicidal power is greatly increased by the addition of small amounts of sodium hydroxide, probably because that prevents the coagulation of protein.

The germicidal power of the various alcohols increases with the increase in their molecular weight. Table 3 shows the phenol coefficients of various alcohols for *Salmonella*.

Halogens

The halogens: chlorine, bromine, iodine, and fluorine, in the free state are highly toxic to bacteria and to other microorganisms, and increasingly toxic in the order of their listing. They are so active chemically that they do not exist in the free state in nature. They combine readily with protoplasm of all sorts, are toxic to tissues, and corrosive to metals, characteristics which determine the uses to which they and their compounds may be put.

Chlorine, either in the form of a gas or combined, is the most widely used of all chemical disinfectants. It is used almost to the exclusion of all other chemicals for the purification of drinking water. It is widely used as a disinfectant for utensils in the dairy industry and on the farm. It is valuable for the disinfection of glassware in restaurants, soda fountains, and beer parlors, and is an important disinfectant for certain types of wounds.

The chloride ion is not toxic, so highly ionized salts, such as sodium chloride, are not disinfectants. The germicidal power of chlorine compounds depends upon the chlorine liberated and may be due either to direct chlorination or to oxidation. Chlorine may unite directly with some part of the bacterial cell to form a toxic chloro-product or it may inactivate the organism without actually destroying it. The action is reversible and chlorine-inactivated organisms incapable of growth may be "activated" by the addition of a dechlorinating agent.

Chlorine is assumed to accomplish its bleaching action and probably

part of its germicidal action by liberating oxygen. The following reactions illustrate how the oxygen is liberated.

1. $HOH + Cl_2 \rightleftarrows H \cdot Cl + H \cdot O \cdot Cl$
2. $H \cdot O \cdot Cl \rightarrow H \cdot Cl + O$

The hypochlorous acid is unstable and breaks down to form hydrochloric acid and nascent oxygen.

When chlorine combines with certain types of compounds such as the phenols, ammonia, or amino acids, the resulting compounds are also highly germicidal, a feature that makes chlorine particularly valuable as a disinfectant for water and sewage. When most disinfectants combine with or act on organic matter, the resulting compounds are nontoxic.

Liquid chlorine, which is the gas compressed to a liquid state, is irritating when wet but relatively inert when dry. When added to drinking water in amounts sufficient to leave a residue of 0.2 parts per million, it destroys all water-borne pathogens. It has largely replaced such chlorine compounds as the hypochlorides for the disinfection of drinking water, swimming pools, and sewage. (For the effect of chlorine on taste and color, see the section on Water and Sewage.)

Chlorine gas has been used by some investigators for the treatment of upper respiratory infections, but, although beneficial results have been reported, its use is very limited and its value doubtful. It is also very difficult to handle.

Hypochlorites: The hypochlorites, sodium and calcium, are popular disinfectants when liquid chlorine is not practical. Calcium hypochlorite, also known as chlorinated lime, "hypo," bleaching powder, or "bleach" is prepared by chlorinating calcium oxide of lime.

The reactions that take place when calcium hypochlorite is added to water are complex. For example:

1. $2\ Ca \cdot O \cdot Cl_2 \rightarrow Ca\ (Cl \cdot O)_2 + Ca \cdot Cl_2$
2. $Ca\ (Cl \cdot O)_2 + 2\ H_2CO_3 \rightleftarrows 2\ H \cdot Cl \cdot O + Ca\ (H \cdot CO_3)_2$
3. $2\ H \cdot Cl \cdot O + 2\ H \cdot Cl \rightarrow 2\ H_2O + 2\ Cl_2$
4. $2\ H_2O + 2\ Cl_2 \rightarrow 2\ H \cdot Cl + O_2$

Calcium hypochlorite contains about thirty-five per cent of available chlorine when freshly made up but loses it upon standing. Some samples of commercial hypochlorites examined by various workers had as little as five per cent chlorine, many others less than twenty. Such products obviously cannot be depended upon as disinfectants.

We have seen that the addition of acids to chloride of lime makes it more unstable and that under such conditions chlorine is rapidly liberated. The addition of alkalies to chloride of lime or to any of the other hypo-

chlorites stabilizes them. Most of the liquid hypochlorites in the market contain sodium hydroxide and sodium hypochlorite, potassium hydroxide and potassium hypochlorite, or a mixture of these. Such compounds, of of which Zonite is an example, are said to be more stable and to retain their germicidal power longer than the unstabilized hypochlorites. The chlorine content varies with the different preparations.

Dakin's Solution: Neutral and relatively non-irritating sodium hypochlorites were prepared by a number of methods and widely used in the treatment of war wounds in 1914–1918. Dakin's Solution is the best known. These solutions exerted their action so rapidly that they had to be renewed constantly to be effective. To overcome this difficulty a number of more complex compounds which liberate their chlorine slowly have been prepared, the most important being: Chloramine T, Dichloramine T, and Azochloramide. Recent investigations indicate that Azochloramide is remarkably effective in treating experimental infection due to organisms of the gas gangrene group. Azochloramide apparently destroys the toxins and at the same time appears to promote the healing of tissues.

Bromine in the gaseous state or in solution is a powerful germicide with properties similar to chlorine.

Iodine: Metallic iodine, only slightly soluble in water, is readily soluble in aqueous potassium iodide and in alcohol. Of a number of iodine preparations, *tincture of iodine*, which is seven per cent iodine and five per cent potassium iodide in ninety-five per cent alcohol, is the most commonly used of all the skin disinfectants. In spite of the fact that it is irritating, corrosive, and does not have as high a phenol coefficient as a number of other disinfectants, it still remains one of the most dependable for prophylactic treatment of wounds and for skin disinfection. A number of workers recommend tincture of iodine made in fifty to seventy per cent alcohol as less irritating, less dehydrating, and just as effective.

Lugol's solution, five per cent iodine dissolved in ten per cent aqueous potassium iodide, is less irritating than the tincture but also less penetrating and hence not so valuable for most purposes.

Iodoform ($C \cdot H \cdot I_3$), used less now than formerly, is not a very strong disinfectant. In the presence of wounds it decomposes to liberate iodine which is the active agent.

Colloidal Iodine: Chandler prepared an aqueous suspension of iodine from a solution of sodium iodohypoiodite which contained acacia as a protective colloid. This "colloidal iodine" suspension when diluted with water forms a saturated aquecus solution that is relatively non-corrosive, non-irritating, and, at the same time, highly bacteriocidal. It has been found useful in the treatment of internal parasites of poultry.

As a disinfectant or antiseptic for the mucous membranes of the mouth and throat, tincture of iodine has a limited use because of its unpleasant taste and irritating properties. One to three per cent iodine solutions in glycerine are said to be very effective disinfectants for the surfaces of the mucous membranes and are well tolerated by the tissues.

Iodine is also fairly effective against fungi.

Fluorine: The fluorides are not as powerful bacteriocides as are the other halogens. Sodium fluoride has, in the past, been used to control the growth of molds in breweries but because fluorine causes mottling of teeth, the practice has been largely discontinued. It is used effectively as an insecticide, particularly in the control of the ectoparasites of poultry.

Dyes: The early workers in bacteriology noted that some of the coal tar dyes, even in high dilutions, inhibited the growth of bacteria. In general, it has been found by Churchman and others who have worked on the action of dyes on bacteria that gram-positive bacteria are more sensitive than gram-negative to the action of aniline dyes. Some dyes exert a more or less specific action on different species and have been employed as selective agents in the isolation of bacteria from such materials as sputum and feces and for the separation of different strains of the same species in the laboratory. Thus the tubercle bacillus may be cultured in a concentration of crystal violet or of other dyes which inhibit the growth of other organisms present in the sputum. Various media containing one or more dyes have been prepared and found very satisfactory for the isolation of this organism.

Huddleson has used the selective action of dyes to separate the goat, hog, and bovine strains of the organism producing undulant fever in man and contagious abortion in cattle.

Various dyes acting as hydrogen acceptors are reduced to the colorless base by the action of bacteria. The rate of reduction is dependent upon the rate of hydrogen liberation and this depends upon the number of bacteria present and multiplying. If methylene blue is added to a test tube containing milk and the mixture incubated, the number of bacteria present in the milk may be estimated by following the color change.

Crystal violet, methylene blue, eosin, gentian violet, malachite green, brilliant green, and acriflavine are some of the more common dyes employed as bacteriostatic agents in selective media and as disinfectants for wounds and more deep-seated infections.

In general, it may be said that these dyes, though extremely valuable aids in the separation of bacteria, are not so effective as disinfectants, for they are probably as toxic or more toxic to tissue cells. They may be inhibiting to bacteria in concentrations well tolerated by the body cells. As a disinfectant for burns a one per cent aqueous solution of gentian violet

has been found satisfactory. Dyes have been used with varying success as disinfectants or antiseptics for the urinary tract.

Soaps

From the practical point of view the germicidal action of soaps is of particular interest since they are so widely used for the washing of the skin, clothing, household utensils, dishes, and glassware. The literature on the subject, which dates from the work of Koch, is difficult to interpret because of the many factors involved in the testing of germicidal power. It was formerly believed that the germicidal power of soaps was due almost entirely to their alkalinity but more recent investigations show that the fatty acids from which they are made play an important part. The surface tension depressing qualities probably alter the surface relationship between bacteria and the medium in such a way as to increase the con-

TABLE 4

Killing Strength of Various Soaps in Two and One-half Minutes at 20° C.

SOAP	PNEUMOCOCCUS	STREPTOCOCCUS	MENINGOCOCCUS	GONOCOCCUS
White floating	1:320	1:320	1:640	1:1,280
Perfumed toilet	1:320	1:160	1:640	1:640
Laundry	1:320	1:80	1:640	1:640
Coconut oil	1:320	1:320	1:640	1:1,280
Olive oil	1:320	1:160	1:640	1:640
Phenol	1:50	1:50	1:100	1:100

centration of the disinfectants sometimes added to soaps. Thus soap increases the effectiveness of bichloride of mercury. Soaps have far less germicidal effect in hard than in soft waters. The hydrogen-ion concentration of the water or medium likewise affects it.

Soap does not, of course, sterilize the skin, although it does remove large numbers of organisms and dirt and should be used before applying the true disinfectants. Soaps display a selective action for different species of bacteria, a fact of considerable practical importance. The staphylococci, universally present on the skin, are relatively resistant. The typhoid bacilli and related organisms associated with intestinal disturbances are also resistant. This is of considerable importance since these organisms contaminate the hands of persons suffering from such infections and of others who, although not ill, may be harboring and excreting them. They are probably removed by a thorough washing of the hands, a task not often performed. The organisms that produce gonorrhea, the streptococci

responsible for many wound infections and child-bed fever, and the pneumococci are readily destroyed by soaps.

Table 4 taken from Walkers data, shows the dilutions of soap necessary to kill various organisms.

It must be remembered that soaps are far more germicidal at higher temperatures. When used in strong hot solution they destroy the ordinary bacteria and are excellent for cleaning eating and cooking utensils. They should never be depended upon as disinfectants when there is a question of destroying any but the most susceptible bacteria.

Soaps probably neutralize bacterial toxins in wounds and thus contribute to healing. Medicated soaps are of little additional value since they cannot exercise effective germicidal action in the time in which they are in contact with the organism. It is better practice to use a good cleansing soap and follow through with a suitable disinfectant than to depend upon medicated soap.

Soaps, then, although not active germicides are valuable aids in preventing infection because of their cleansing powers.

CHEMOTHERAPEUTIC AGENTS

Sulfonamides

The compounds considered as disinfectants are, almost without exception of little value when injected. In contrast, the chemical compounds called chemotherapeutic agents may have little effect on microorganisms in the test tube but when injected or taken by way of mouth may exert a profound effect on infections caused by certain microorganisms. Quinine has long been used as a specific for malaria and in 1910 Ehrlich discovered in arsenicals a dramatically effective treatment for syphilis. An intensive search for chemicals that would be equally effective in the treatment of bacterial infections followed but it was not until 1935 that the search was rewarded. Then came the discovery that sulfanilamide exerted a pronounced effect on hemolytic streptococcus infections. Shortly thereafter sulfapyridine and sulfathiozole, which are effective against other gram-positive organisms, were developed. The sulfonamides are effective against staphylococci, hemolytic streptococci, pneumococci, the gram-negative cocci that cause gonorrhea and meningitis, and a few of the gram-positive anaerobes. Sulfonamides given orally prevented the spread of infection and greatly reduced the mortality from war wounds. However, studies by the army medical services on healing gave no evidence that the local application of sulfonamides prevented infection. In fact, there is some evidence that it actually delayed healing in the wounds.

Chapter X

ANTIBIOSIS AND ANTIBIOTICS

"There is no such thing as accident in scientific discovery."—Louis Pasteur

The most dramatic developments in microbiology in the past ten years have been in the field of antibiotics. Antibiotics are defined as chemical substances that are produced by microorganisms and that have the capacity to inhibit growth or to destroy bacteria and other microorganisms.[1]

The observation that microorganisms growing in mixed cultures may be antagonistic to one another is by no means a recent one. In fact, Tyndall, in 1876, observed that bacteria producing a green pigment would inhibit the mold *Penicillium*. In 1777, Pasteur and Joubert noted a natural antagonism between the anthrax bacillus and some other bacteria. This led to the discovery of the antibiotic, pyocyanase, which is produced by *Pseudomonas aeruginosa*. Many other observations of naturally occurring antibiosis were made in the next thirty years but not enough was known and the intellectual climate was not appropriate for an appreciation of these chance discoveries. The great advances in immunology had captured the imagination of most of the workers and, as a result, the immunologic approach to prevention and therapy dominated the field. Effort was concerted on trying to produce a vaccine or antiserum for each infection and not on the field of antibiosis. Another factor might be mentioned. Antibiosis, while of academic interest, did not at that time give promise of immediate practical application. However, observations continued to be made and information was accumulating.

In 1929 Fleming[2] made the "chance discovery" that staphylococci growing on blood agar plates were inhibited by a mold that had accidentally contaminated the plate. The mold was later identified as *Penicillium notatum*

[1] The sulfa drugs and other agents of therapeutic value produced by chemical means are called chemotherapeutic agents. Those produced by living organisms such as fungi and bacteria are called antibiotics. However, there is little reason to distinguish them on such a basis because some of the antibiotics have been synthesized in the laboratory and, in all probability, many others will be.

[2] Some one has said that a real scientist is one who sees what is there when he isn't looking for it and does not see what isn't there when he is looking for it. Almost every laboratory worker had noticed that certain microorganisms interfered with or inhibited the growth of others but he had not gotten the implication. Fleming had been interested in agents that would inhibit staphylococcus and it seems likely that this interest had sharpened his perception so that he grasped the significance of the picture presented to him. Others failed to see what was there because they were not looking for it. In science "lucky accidents" happen only to those who are keen enough to grasp their meaning.

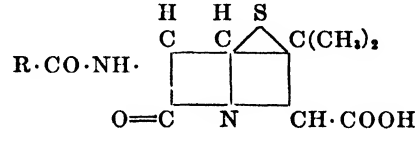
and the antibiotic it produced was named penicillin. Penicillin proved to be unstable and difficult to produce in useful concentrations and although it was demonstrated to be non-toxic to animals, little interest was shown in it until about 1938 when Florey, Chain, Heatley, and their associates at Oxford concentrated sufficient penicillin to test its value in disease. Clinical trials indicated that it might be a great value in the treatment of infection.

Stimulated by this early promise but, because of the pressure of war, confronted with seemingly unsurmountable difficulties in producing it in sufficient amounts, the British workers, Florey and Heatley, enlisted the aid of American workers. The subsequent developments in penicillin research are an impressive illustration of imaginative and tireless cooperation between British and American governmental agencies and private pharmaceutical houses.[3]

During this period brilliant investigations on antibiosis were also being conducted by Dubos and by Waksman[4]. General interest was aroused and literally thousands of higher plants and microorganisms were investigated. Hundreds were shown to produce substances antagonistic to some other microorganism. Only two of the several hundred antibiotics found in the higher animals, plants, fungi, actinomyces, and bacteria have been widely used in medicine These are penicillin and streptomycin.

Penicillin

Penicillin is produced by *Penicillium notatum* and *Penicillium chrysogenum*. There are at least four penicillins differing in antibacterial potency and other properties. The general formula of penicillin as given by Kavanagh (Advances in Enzymology, 1947) is:



It is the *R* radicle in the penicillins that differs, the basic structure is the same.

The virtue of penicillin lies in its almost complete lack of toxicity for man and its effectiveness against many of the most deadly infections due to bacteria. The following taken from Keifer's report in *Advances in Military Medicine* states the benefits that have resulted from its use.

[3] For the story of the development of penicillin the reader is referred to *Advances in Military Medicine*, Vol. II, Little Brown and Co., 1948. See chapter 52 by Kiefer and chapter 53 by Raper.

[4] *Microbial Antagonisms and Antibiotic Substances*, Selman A. Waksman, Commonwealth Fund, 1947.

"(1) It has reduced the death rate of staphylococcal infections with bacteremia from 75 to 10 per cent and has lowered the total number of days of illness in these infections immeasurably.

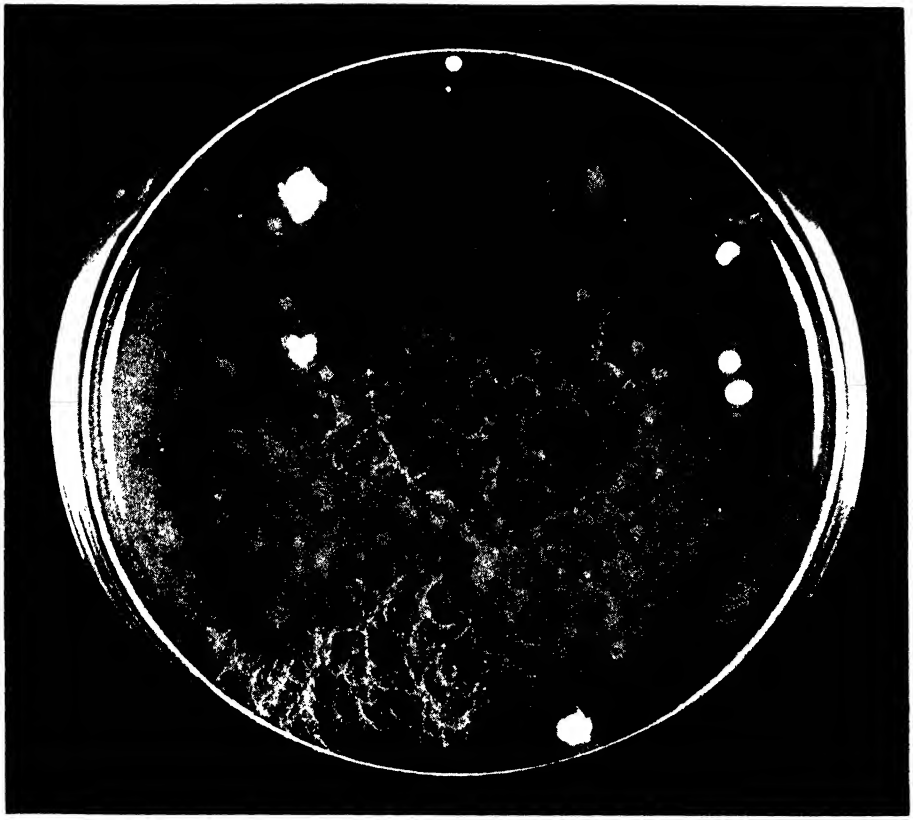


FIG. 36. This photograph shows microbial antagonism in an agar plate seeded with a 1:10,000,000 dilution of soil and incubated at 28° C for five days. A major portion of the medium is covered with a Bacillus mycoides type of growth, but several other types of organisms are growing in and upon the medium. One colony, an actinomycete colony, about one millimeter in diameter, has a clear zone around it about four millimeters in diameter. Two other colonies have larger, more irregular zones of inhibition of certain organisms.

Courtesy of Dr. G. Albert Zelner

(2) It is the most effective agent available for the treatment of hemolytic streptococcus infections.

(3) It is as efficacious in cases of lobar pneumonia as the sulfonamides, and under certain conditions it is the agent of choice.

(4) It is the most powerful therapeutic agent available for the treatment

of venereal diseases such as gonorrhea and syphilis, and has been adopted by the Army and Navy as the standard method of treatment of these diseases.

(5) In infected wounds and burns, penicillin when combined with good surgical treatment is an effective weapon in limiting infection and accelerating healing.

(6) When combined with antiserum and surgical débridement it is helpful in the treatment of gas gangrene.

(7) It is the best drug available for the treatment of such diverse diseases as pyogenic meningitis, bacterial endocarditis and mastoiditis, empyema, lung abscess and bronchiectasis, acute and chronic osteomyelitis, and anthrax.

There are still other diseases in which it shows great promise in experimental infections in animals, but opportunities for extensive clinical trial have not been available.

In essence, it can be said that penicillin is the most remarkable of all the chemotherapeutic agents. It is truly extraordinary that this substance should have such a powerful effect on so many different infectious agents and yet be nontoxic."

Streptomycin

Streptomycin, discovered by Schatz, Bugie, and Waksman in 1944, is produced by *Streptomyces griseus*. While more toxic than penicillin, it is effective against many organisms that are not inhibited by penicillin. Streptomycin inhibits the gram-negative rod, *Pasteurella tularensis*, for instance, and is effective in the treatment of tularemia. It also inhibits the growth of the tubercle bacillus and is useful in suppressing tuberculosis. In importance, streptomycin is second only to penicillin.

Other Antibiotics

Three other antibiotics that differ from penicillin and streptomycin in several major characteristics may be mentioned. First is polymyxin, obtained from *Bacillus polymyxa*. Its activity is chiefly against gram-negative rods. Experimentally and in a few clinical trials it shows promise.

Another is chloromycetin which is effective experimentally against the rickettsia.

The third is aureomycin, isolated from *Streptomyces aureofaciens*, which shows activity against rickettsia, viruses of the lymphogranuloma-psittacosis group, and a variety of gram-negative and gram-positive bacteria.

Most of the other antibacterial agents are either powerless against infections although they may be active in the test tube, or are too toxic to be of general value. Several have been found to be of value for local applications.

Mode of Action of Antibiotics

One of the outstanding features of the antibiotic agents is their specificity. In dilutions as high as one part in two or more million they will inhibit completely the growth of certain bacteria and, even in concentrated solutions, fail to inhibit other bacteria. The same specificity is found in the chemotherapeutic agents such as sulfa drugs and in some of the dyes. It is far less true for disinfectants.

By and large, the gram-negative cocci, the gram-positive cocci, and the gram-positive rods are sensitive to the action of antibiotics; while the gram-negative rods, the viruses, and the rickettsiae are resistant. Streptomycin is active against some gram-negative bacteria and shows a wider coverage than does penicillin. The reason an antibiotic fails to inhibit one kind of bacterium and not another is not clear but it seems that differences in the susceptibilities of the bacteria are related to differences in their essential metabolisms.

The mode of action of chemotherapeutic and antibiotic agents is essentially alike and is referable to the physiological economy of the cell. It accounts for the high degree of specificity, it is associated with the toxicity for the host, and it is involved in the problem of "drug fastness" and the more recently discovered "drug requiring" strains of sensitive organisms. The implications are basic and fundamental.

Early workers noticed that the bacteriostatic concentration of sulfonamides was reduced by peptone, pus, tissue extracts, or yeast extract. Woods, in 1940, showed that the active agent in yeast extract was para-aminobenzoic acid (PABA). An agent or organism that counteracts another agent or organism is called an "antagonist". Para-aminobenzoic acid is said to antagonize sulfonamides since it counteracts their inhibiting effect. The important factor in the relationship between a biologically active substance and its antagonist is the relative and not the absolute concentration of the two. One unit of para-aminobenzoic acid will counteract or antagonize about 25,000 units of sulfanilamide and from 500 to 1000 units of other sulfonamides. The ratio differs for the different systems.

The chemical structure of para-aminobenzoic acid and sulfanilamide is strikingly similar and this suggested that compounds structurally related to essential metabolites compete with these and "block" their utilization by the cell. This is the essence of the "Woods-Fildes" hypothesis and, while it does not explain all instances of inhibition, experimental evidence shows that it is essentially correct. It has proven a very fruitful hypothesis and has been strengthened by the work of numerous investigators, particularly McElwain. (Advances in Enzymology, Vol. 7, 1947, page 409.)

A crude analogy often used to express the concept is that of the lock and

key. Structurally similar keys can be inserted into the keyhole but only the proper one will open the lock. Structurally similar compounds may be absorbed by the cell, but only the proper one can function in the physiological economy of the cell.

Figure 37 illustrates the chemical similarities between two essential metabolites and some of their antagonists or inhibitors.

Not only has the Woods-Fildes hypothesis explained the action of bacteriostatic substances but it now appears that, in general, it explains many problems of animal metabolism. Biologically active substances such as the amino acids and vitamins required by a species can be blocked by suitable antagonists, many of which are chemical analogues of the essential compounds.

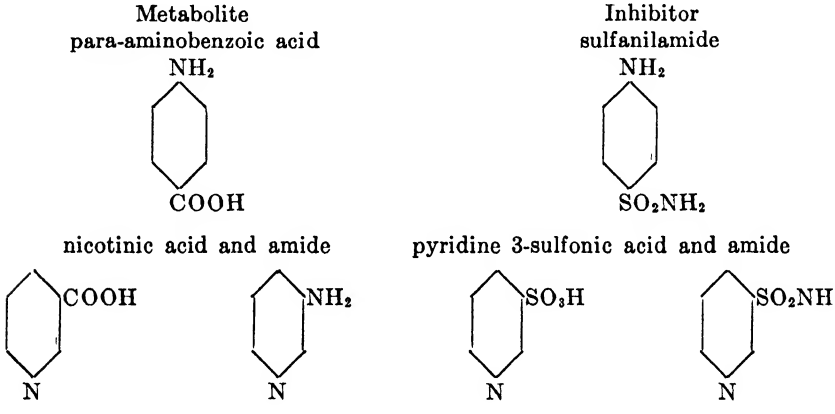


FIG. 37

"The mechanism of "blocking" or antagonism is not always clear. It has been suggested by Fildes that antibacterial substances may operate in one of three ways: by oxidizing a substance that the cell requires in reduced form, by combining with an essential metabolite and producing a biologically inactive product, or by competing for an enzyme system associated with the utilization of the essential metabolite. The latter seems to be particularly important.

It follows from this hypothesis that bacteria requiring a large number of amino acids and vitamins which they cannot themselves synthesize are more vulnerable to the action of a variety of blocking agents than are bacteria that synthesize their own. And it is interesting to note that the gram-negative bacteria that, in general, have few requirements and can manufacture all of their amino acids and vitamins from simple salts are far less susceptible to most antibiotics than are the gram-positive bacteria that cannot synthesize all of their amino acids and vitamins.

The mode of action of antibacterial agents may also be interpreted as follows: the antibacterial agents effect their inhibitory action by interfering with energy yielding mechanisms and thus preventing the bacteria from growing, by interfering at some point in the pathway by which bacteria synthesize cell materials, and by interfering with cell division apparently by cutting in and blocking some mechanism involved in cell division. Penicillin appears to function in some such fashion.

Drug Fastness

The appearance of "drug fast" or resistant strains in cultures of bacteria growing in the presence of an antibacterial agent occurs with such regularity as to be a general rule. Species that are usually susceptible produce strains a thousand or even ten thousand times more resistant.

One observation made by Landy may explain this phenomenon, at least in part. The staphylococci are inhibited by sulfa compounds, presumably because these interfere with the utilization of the para-aminobenzoic acid essential for their growth and which they do not synthesize in sufficient amounts. Sulfa resistant strains were shown to synthesize para-aminobenzoic acid and their capacity to do so is directly related to their resistance to the sulfa drug.

Drug fastness is specific for each type of antibacterial agent. The sulfa resistant strains obtained by growing a bacterium in the presence of sulfa drugs are no more resistant to penicillin or streptomycin than is the sulfa susceptible strain. Likewise, streptomycin resistant strains are no more resistant to penicillin or to sulfa drugs than was the susceptible parent strain. This is to be expected if the different agents act on different enzyme systems. However, it is perfectly possible to get a strain of bacteria that is resistant to sulfa compounds, to penicillin, and to streptomycin.

Of singular significance to chemotherapy is the further observation by Miller and Bohnhoff that not only did the meningococcus produce streptomycin resistant strains but that some strains actually came to require streptomycin for growth. When mice are inoculated with ordinary meningococci, they die from the resulting infection. If they are treated with streptomycin they live. When mice are inoculated with streptomycin resistant strains and then treated with streptomycin, they die from the infection. However, when mice are inoculated with streptomycin requiring strains, they do not die of the infection because normal mice do not have the streptomycin essential for the growth of such strains. If these mice are treated with streptomycin, the organism will then grow and produce an infection that will kill the mice.

Other workers have also reported on the appearance of streptomycin requiring strains of other organisms, including the tubercle bacillus.

The practical importance of this cannot be overlooked. If, when a patient is treated, the infectious agent produces strains a hundred to a thousand times more resistant, the dosage of the drug will have to be increased accordingly to get the desired therapeutic value. Most agents are too toxic to allow for any such increase. Furthermore, secondary cases due to the resistant strain will also be resistant, as has been shown to be the case with sulfa resistant strains of the gonococcus. If strains that require the antibacterial agent are produced during the course of treatment, the drug will be worse than useless because their growth will be stimulated by the drug. There is, fortunately, no danger that such strains will be propagated because they will be unable to grow in persons not under treatment with the antibacterial drug in question.

It was reported recently that strains of the tubercle bacillus isolated from a patient undergoing treatment with streptomycin grew luxuriously in media containing a thousand times as much streptomycin as was necessary to prevent the growth of normal strains. This patient's infection exhibited rapid progression during the period of streptomycin treatment. How general the possibility may be for the development of strains requiring antibacterial agents inhibitory to the parent strain remains to be seen.

Perhaps the most acceptable explanation for drug fastness is based on mutation and natural selection. Bacteria, like other organisms, have a genetic mechanism which governs their heredity. Each characteristic, such as the capacity to produce the enzyme necessary to utilize a certain substance or to produce a substance, is subject to genetic laws. The genes involved are subject to mutation and mutation is constantly taking place. Therefore individual cells that have an enzyme mechanism capable of utilizing the materials in which they find themselves will be the ones that multiply. Those that do not will die. Any mutation with survival value in a particular environment will determine the characteristics of succeeding generations.

The rate of multiplication in bacteria which reproduce every fifteen to thirty minutes and increase by a geometrical progression is so rapid that in a short period, according to our way of reckoning time, the entire surviving population will consist of individuals derived from those that had an advantage.

All environments in which bacteria grow are highly selective and natural selection takes place constantly. The disease-producing bacteria that grow in the body are constantly mutating and the mutations that have survival value will predominate as the infection proceeds. When the environment of the body is changed by introducing a sulfa drug or antibiotic, the individual organisms that have the capacity to grow in the presence of the drug will propagate and the others will not. If one bacterium out of

every hundred thousand is able to grow in the presence of the drug, that is the one that will determine the character of the future generations. There is nothing mystical about the phenomenon of drug fastness or about the development of drug resistant and drug requiring strains.

It is of interest to point out that while para-aminobenzoic is an essential metabolite for staphylococci, clostridia, and several other organisms, it is inhibitory to rickettsiae. It inhibits growth in rickettsiae just as sulfa drugs inhibit growth in bacteria requiring para-aminobenzoic acid, and has been used with some success as a therapeutic agent in some rickettsial infections.

The singular lack of effect antibiotic agents have in virus diseases raises some interesting questions as to how viruses are duplicated by the host cell or how they multiply therein.

In summary it may be said that the development of sulfonamides and antibiotics is among the foremost contributions of science. The basic information acquired concerning their mode of action is likely to prove of even greater importance because it points a way to a better understanding of the essential physiology of microorganisms, animals, and man.

Section II

INFECTION AND RESISTANCE

Chapter XI

AN INTRODUCTION TO INFECTIOUS DISEASE

PARASITISM

In order to grow and reproduce, a species must find a suitable temperature and sufficient food and must also be more or less able to withstand the attacks of other species. All living forms are growing in association with other living forms and each species must adjust to its biological as well as to its physical environment in order to survive. These associations and adjustments between species are of particular interest to the biologist who distinguishes them on the basis of the resulting benefit or harm.

Parasitism is a term used to designate an association wherein one organism, known as the *parasite* (Gr. *para* meaning beside and *sitos* meaning food), lives in, on, or at the expense of another organism known as the *host.* More specifically, it connotes an association wherein the host is damaged although by no means all such associations are harmful to the host. Another term, *commensalism,* is frequently used to indicate a type of association wherein the parasite is benefited and the host neither benefited nor harmed. Such a relationship exists between man and the billions of bacteria inhabiting his skin and intestinal tract. There are also associations wherein both organisms are benefited, a type already referred to as *symbiosis.*

In the economy of nature, parasitism is a universal phenomenon. Animals are parasitic on plants and on other animals. In this sense man is also parasitic for he lives on both plants and animals and, in turn, is parasitized for he serves as host for innumerable microorganisms and, sometimes, for larger forms of life.

It is customary to consider animal parasites such as the protozoa and the metazoa in *parasitology* and plant parasites such as the bacteria in *bacteriology.* Fundamentally the nature of the association is similar, but the differences in methods of study and materials make such a division convenient.

Origin of Parasites: It is of interest to note that all parasitic organisms have their more individualistic free-living relatives. Parasitism seems to be a later adaptation and organisms so adapted have lost many of the characteristics necessary for independent existence and developed others which make their survival as parasites more certain.

Bacteria show all degrees of parasitism. Some are strict or obligate parasites and are not capable of leading independent existences in nature; others live in the soil or on the body and invade living tissue only under exceptional circumstances. How these adaptations came about is a subject for speculation. First it must be remembered that there is no sharp line of demarcation between a parasitic and a saprophytic organism. A bacterium, such as the staphylococcus or the colon bacillus, that lives in constant association with the animal body but exists on dead cells, body secretions, or excretions, is just as truly a saprophyte as one that lives in the soil or water. If, as might occasionally happen, such an organism produced variants that could invade living tissue, we would have true parasites.

Living forms, of course, have "defensive mechanisms" which tend to resist invasion by other organisms; and, in order to become parasitic, a bacterium must be able to overcome these defensive mechanisms of the host. Countless bacteria possess the enzymatic equipment necessary to utilize animal or plant tissue as food—in fact, such tissues can be used in the laboratory as an excellent medium—but only a few are capable of growing in these same tissues in the living host. This ability of certain bacteria to overcome host defenses or to adapt themselves to certain conditions plays an important part in the development of parasitism.

The science of *immunology* which deals with the defensive mechanisms of the host has contributed greatly to our knowledge of human disease and has made it possible to control some of the most deadly scourges of mankind. It has also proven a useful tool for the investigation of many biological phenomena.

When a parasite proliferates in living tissue, the tissue reacts to combat the invasion and it is this reaction of the tissue to the parasite that is called disease. But, although communicable diseases are always due to parasites, and although disease is usually a phase of parasitism, it is not necessarily so. A rattlesnake or black widow spider are not parasitic on man, yet they may produce disease by virtue of the venom in their bite. Likewise, some species of bacteria produce disease, not because they are parasitic, but because they elaborate powerful poisons. *Clostridium botulinum*, for instance, elaborates its toxin when growing in such materials as improperly canned vegetables and, when this toxin is ingested, the disease, botulism, results. *Corynebacterium diphtheriae* lives a parasitic life on the tissues of the throat and, although it does not invade the living tissue to any extent, it does elaborate a powerful poison or toxin which is absorbed by the tissue and is responsible for the symptoms of diphtheria.

From the biological point of view, parasitism is of benefit to the parasite. The fact that disease may result is accidental and is not to the benefit of

the parasite; for, if the disease is severe enough to kill the host, the parasite perforce perishes.[1] The tubercle bacilli in the human body at the time of death are themselves doomed. There are numerous instances of plant viruses which destroy certain hosts so rapidly that they cannot maintain themselves in these plants. If they did not have other hosts in which they produced milder diseases, they could not survive.

The viruses of plants and animals represent an extreme case of parasitism. Insofar as is known, none is capable of multiplying in the absence of living tissue. How they developed, what they are, from whence they came—these are unsolved mysteries of biology.

DISEASE

We cannot think of disease except in terms of health—disease and health are polar concepts. A healthy organism is one so well-adjusted to its environment that it is capable of carrying on all of the functions necessary to its maintenance, growth, and multiplication with the least expenditure of energy. All organisms are capable of withstanding a certain degree of insult and there is a range of easy tolerance within which they can function without too great a strain. When this range of easy tolerance is passed, the organism may be said to be *diseased* or in a *pathic* state. *Disease, then, is a process, not a thing and represents the response of the body to injury or insult.*

Disease may be on different levels. A cell may be diseased and this may or may not affect the organ. An organ may be diseased and this may or may not affect the survival of the host. The range of easy tolerance and the ability to withstand or resist harmful influences varies considerably not only between different species and between individuals of the same species, but also between the organs of individuals of the same species.

The methods of preventive medicine have a two-fold purpose: (1) to increase the tolerance of the individual, and (2) to prevent the disease-producing agents which are capable of overcoming his resistance from gaining entrance to the body.

Theories of Disease: Man is an egocentric animal much concerned with his aches and pains. Throughout his history he has been afflicted with all

[1] It is an axiom in parasitology that a successful parasite does not jeopardize the survival of the host. This principle would appear to hold in political and economic affairs too, for no economic system so toxic or destructive that it jeopardizes the survival of its hosts can itself survive. It might be well for the opposing forces of capital and labor to study the elementary principles of parasitism for they might come to recognize the fact that a symbiotic relationship is not only desirable but the only one which makes it possible for both groups to profit. Many economic systems have been so destructive to the host that they could not have lasted for long except for a continual supply of fresh hosts. The economist calls this "Imperialism" . . .

manner of diseases. He has been the victim of plagues and pestilences which have wrought such havoc that, at times, they have threatened to extinguish him. Being a rational creature, too, it is natural that he should seek to explain the cause of his sufferings.[2]

The early theories of disease are interesting and many of the remedies proposed, though they seem fantastic to us in the light of our knowledge, were perfectly rational. The fault lay in major premises which were not based on experiment or controlled observation and deductions which were colored by superstition and fear.

The Demonic Theory is one of the earliest and most widespread. Most primitive peoples believed that disease was due to evil spirits. The logical control measure then was to exorcise the demon, for if we concede the validity of demons as the cause of disease, what better way to prevent or cure it than to entreat or entice the evil spirits away or, failing that, to make such terrifying noises or nauseating stenches that the devils would look for more pleasant surroundings? The notion that bad tasting or vile smelling medicines are potent agents and work miraculous cures has carried over to the present time, and we still find our modern friends demanding and our pharmacist supplying all manner of compounds whose greatest value is the psychological one of a hideous stench or a horrible taste.

The wearing of charms, lucky pieces, and fetishes as a protection against bedevilment is a relic of superstition often encountered at the present time in one form or another.

Humoral Theory: We have previously mentioned the importance of the "climate of opinion" on the inferences drawn from observations and on the explanations of natural phenomena. The concept of harmony, the

[2] Thus William Boghurst, a London apothecary, writing of the Great Plague of London in 1665, gave the following explanation of its cause: ". . . plague or pestilence is a most subtle, peculiar, insinuating, venomous, delcterious exhalation arising from the maturation of the ferment of the faeces of the earth extracted in the aire by the heat of the sun and difflated from place to place by the winds and most tymes gradually but sometymes immediately aggressing apt bodyes. . . ."

nothing-to-excess ideal, was a pervading influence in Greek thought. It carried over to their concept of disease and from Hippocrates (460–395 B.C.) comes the humoral theory of disease and health. This holds that there are four essential humors: phlegm, blood, yellow bile, and black bile, and that these humors must be in balance or harmony to insure health. Disease is the result of humoral disharmony; that is, of too little or too much of one or more of the humors. The old practice of blood-letting or phlebotomy arises as a logical consequence of this concept of the cause of disease. Three common expressions in daily use are reminiscent of the time when the humoral theory was in good standing. *Sanguine* originally denoted fulness of blood and is associated with confidence and hopefulness—qualities believed to attend such a condition. *Melancholy*, from black bile, describes the low-spirited state accompanying too much of that humor. *Phlegmatic*, which now means sluggish or indifferent, originally meant an overabundance of phlegm.

The Pythogenic Theory: About one hundred years ago, Murchison, an Englishman, propounded a theory, based upon an appreciation of the existence of bacteria and some observations but no experimental proof, that disease was due to dirt or filth. Decaying vegetable and animal matter in addition to affording an excellent breeding place for disease-producing organisms was considered capable of engendering them. This theory still makes its influence felt in modern sanitation, and, although we no longer believe that dirt or filth can engender disease, we know that it is frequently associated with certain types of pathogenic microorganisms.

Other theories have been advanced to account for specific diseases. Malaria, a disease caused by a protozoan, was thought to be caused by *bad air*, hence its name, *mal-aria*. At the present time many farmers believe damp hot weather to be the cause of black stem rust of wheat, basing their belief on the observed fact that rust develops during such weather. The great lesson is to learn to distinguish between the specific cause or etiology of a disease and the contributory causes.

The Germ Theory of Disease, like so many theories, is the result of the cumulated experiences and observations of many, rather than the work of any one man. The belief in the existence of living forms too small to be seen is old, but it was not until Leeuwenhoek, in childlike curiosity, turned his simple lenses on scrapings from his teeth and pepper infusions that such forms were actually seen. Fracastorius, as early as 1546, had suggested that infectious disease was caused by a *contagium vivum* and Plenciz, in 1764, advanced a theory that each disease was caused by a specific microbe, but these theories were unsupported by evidence and it was not until nearly two hundred years after Leeuwenhoek proved the existence of microbes that their relationship to infectious disease was

established. The previous discovery that microbes were responsible for fermentation, putrefaction, and decay no doubt suggested this possibility.

Davaine, in 1850, found rodlike organisms in the blood of cattle dead from anthrax and succeeded in experimentally transmitting the disease to healthy cattle. Pasteur, in 1865, showed that pébrine, a very destructive disease of silkworm, was due to a protozoan. Previous to this there had been other isolated observations on bacteria present in infectious processes, but proof that they were responsible for the process is largely the work of a German country doctor, Robert Koch (1843–1910). The methods Pasteur and his workers had developed for the cultivation of bacteria were not suitable for the isolation of pure cultures. Koch, a methodical and painstaking worker, concerned himself with detailed studies of these microbes and to him we owe the development of methods which make isolation of pure cultures possible. The need for such methods became evident when it was realized that many different kinds of bacteria were present at the same time in healthy and diseased tissues. Ambitious but not too critical workers were obtaining various bacteria from all manner of conditions and naming them as the cause of this or that disease. Koch laid down his now-famous *Postulates* which he said must be fulfilled before a specific organism could be said to cause a specific disease. These postulates had, in essence, been laid down previously by Henle, a teacher to whom Koch owed a great deal. Koch's Postulates, which have been stated in a number of ways, are briefly:

1. The parasite must be present in all cases of the disease.
2. The parasite must be isolated in pure culture.
3. The specific disease in question must be reproduced with the pure cultures.
4. The same parasite must be present and recoverable from the experimental animal.

To these is now added a fifth rule or dictum which states that certain immunological relationships must be demonstrated.

In many cases all of these postulates cannot be fulfilled for various reasons. Some organisms cannot be grown on laboratory media. Many infectious diseases of man are limited to man and as yet no suitable experimental animal is available although the bacteriologist, wittingly or unwittingly, has frequently served as such. Much information has been gained from the study of accidental laboratory infections.[3]

[3] "Koch's Postulates" might well supplant the mental gymnastics which pass for thinking in connection with economic, sociologic, and political problems. When Koch announced the discovery of the tubercle bacillus and gave his proof, the scientific world tested his theory, recognized its worth, and even those who had previously held quite different notions were obliged to accept his view. But they did so. Had they been politicians they would have tried to controvert his statements by referring to his beard, his nationality, the past glory of some race or nation, and

Types of Disease: It is necessary to distinguish between the various types of disease to which man and animals are subject and the bacteriologist has a list of terms, more or less descriptive, which have come into use for this purpose. For convenience, diseases are placed into two main groups: noninfectious and infectious.

Noninfectious diseases may be due to various agencies and may be grouped on that basis as follows. There are those due to physical agents; for example, contusions, concussions, bruises, broken bones, sunstroke, frost bite, lacerations, and such. There are those due to chemical agents, chemical poisons of all kinds—an almost unlimited list. There are those due to a lack of proper food, a vitamine deficiency or a deranged metabolism. Scurvy and pellagra fall in this group. And some diseases are congenital.

Infectious Disease: An infectious disease is a disease due to the presence of a living organism. Examples are tuberculosis, malaria, typhoid fever, syphilis, and the common cold.

A *contagious* disease denotes an infectious disease which is transmitted by direct contact or one which we call "catching." The term, *communicable* disease, is to be preferred.

A *communicable* disease is one which is transmitted naturally from one person to another. Thus tuberculosis is both an infectious and a communicable disease. Tetanus is an infectious disease but is not communicable, since one individual does not contract it from another infected individual.

Endemic, Pandemic, and Epidemic Diseases: (*demos*—people) The infectious diseases vary in intensity and distribution in different communities and at different times. In some instances climate and season play an important role in determining the number of cases. When the number of cases of a disease is relatively constant, the disease is said to be *endemic.* When there is an unusual number of cases, the disease is said to be *epidemic.* An epidemic on a world-wide scale is called a *pandemic.* A disease like influenza may be endemic, a small number of cases being constantly present in the population; it may become epidemic in certain years; and it may, as it did in 1917 and 1918, reach pandemic proportions.

Chronic and Acute Diseases: Not all bacteria produce the same type of disease nor are all the disease-producing bacteria equally potent. A disease which is characterized by a sudden onset and a short period of rather severe illness with the subsequent death or recovery of the patient

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would have wound up with the conclusion that he was altogether wrong. It might be that if we were to apply the impartial and objective method of the scientist to such problems as tariffs, wages, and states rights, instead of appealing to patriotism, passion, prejudice, hatred, fear, and ignorance; the forces which threaten the existence of our civilization could be controlled.

is said to be *acute*. A disease in which the onset of the symptoms is gradual and which is characterized by prolonged illness is said to be *chronic* (Gr. *chronos*—time).

Infection

Infection is a process in which microorganisms invade the body tissues of other organisms, such as plants, animals, or man, multiply there, and cause a reaction harmful to that host. Before a microorganism can act as an infectious agent it must find suitable conditions for growth and multiplication within the body tissues of other plants or animals, which is to say that the tissues of the host must present the proper oxygen tension, an oxidation-reduction potential of such magnitude that the invading organism can synthesize its own proteins and other constituents, and necessary food elements in a form in which they can be utilized. Moreover, the infectious agent must be able to withstand those substances, produced by the host, which would prevent the invading parasites from establishing themselves.[4]

Infection is to be distinguished from *contamination* in that the latter term refers to the presence of organisms in or on inanimate materials or objects such as food, water, instruments, and the like, or to their mere presence on hands, wounds, or other parts of the body. Thus we speak of contaminated instruments, water, and clothing. A wound may be con-

[4] When the scientist discovers new truths or attempts to state new principles or concepts he frequently finds difficulty in choosing terms which are adequate. Two choices are open: he can coin new words or he can apply old and familiar terms. He does both. It often happens that after the investigator has made a discovery and applied certain terms to it, subsequent discoveries show that his choice was not a happy one. The new concept may prove to be relative and not absolute, or it may be found that an entirely different mechanism is involved than was originally supposed. Since it is practically impossible to discard an old term once it has been accepted, it has become customary to broaden its meaning so as to cover the new facts. Such a broadening, of course, does violence to the derivation of the words but the student will find it easier to discuss the concepts of the fields of bacteriology and immunology when he realizes that these sciences are full of such terms. Pasteur and his pupils discovered that when dead or attenuated organisms were injected into an animal, they protected it against the subsequent injection of living virulent organisms and introduced the term *immune* to denote such a resistance. Immune and immunity are absolute, not relative, terms, meaning freedom or exemption from (*immunis*—exempt). Subsequently it was discovered that the injection of dead or attenuated strains of an organism did not always protect the animal against subsequent infections but that it did result in the animal's showing a higher degree of resistance. The term immunity has been kept, however, its meaning broadened to include all degrees of resistance, and we speak of *absolute* immunity and *relative* immunity. There are many other concepts which, in spite of the terms used to denote them, are relative, never absolute. Pathogenicity, virulence, susceptibility, and immunity are all relative terms.

taminated by the presence of an organism but it is not until the organism invades that the wound becomes infected.

Pathogenicity

Microbes can be divided into two broad groups: saprophytes that live on dead organic matter, and parasites that live on or at the expense of higher living forms.

Parasites capable of infecting the body and producing disease are called *pathogens* (*pathos*—suffering and *gen*—producing) and their ability to produce disease or their pathogenicity is variable. No pathogens are capable of producing disease in all animals or under all conditions. Indeed they are remarkably specific. Many bacteria or viruses which produce disease in man are not pathogenic for animals; and, in general, animal pathogens are not pathogenic for man. There are, of course, many exceptions and in the control of disease it is very important to know whether the pathogen dealt with has more than one host.

The pathogenic bacteria may, for convenience, be divided into two groups: the "*opportunists*" and the "*true*" pathogens. Among the "opportunists" are those microorganisms living in constant association with the host, either on the skin or the membranes of the upper respiratory tract. Whether they live on living cells or are saprophytes living on dead tissues and debris is not always easy to determine. They may invade the tissues and incite disease when the natural barriers are broken by some other organism or when the natural resistance of the host has been lowered as by disease, exposure, or malnutrition. For example, the staphylococci are normal inhabitants of the skin and probably incapable of penetrating when it is intact but if the skin becomes bruised or is cut, they may enter the deeper tissues, multiply, and give rise to infections such as boils or the so-called "stitch" abscesses. Streptococci are abundant in the throat where they live without causing any injury but, if the resistance of the individual has been lowered, for instance, by an attack of measles, they may infect the lungs and produce broncho-pneumonia. These organisms invade the body tissue only when a special opportunity is offered by some preliminary infection or injury and are often referred to as "*secondary* invaders." They are secondary in time but not in importance.

The second group, or *true* pathogens, are capable of invading under normal conditions of host resistance and only rarely live in association with the body without producing disease. Diphtheria and typhoid bacilli are exceptions for they may multiply in immune individuals, called "*carriers*," without giving rise to disease. It is impossible to draw a clear line of demarcation between the "opportunists" and the "true" pathogens,

but, in general, diseases caused by opportunists are not communicable and do not result in immunity.

Virulence

The disease-inciting power of a pathogen is known as its virulence and depends upon its ability to invade and multiply in the living tissue or to produce a toxin or poisonous substance. Most pathogens do both. Some, however, are like the diphtheria bacillus which does not invade the tissues to any appreciable extent but localizes and secretes a potent toxin to which the main symptoms of the disease are due.

Virulence is an inherent property of a pathogen, differing with the different species and with the various strains within each species. It is usually associated with the S, smooth, colonial type rather than the R, rough, and often with the capsules. The carbohydrate capsule of the pneumococcus probably serves to protect it; for non- or avirulent strains which are not encapsulated are readily disposed of by the phagocytic cells whereas the encapsulated virulent strains proliferate freely, relatively unaffected by the phagocytes.

Pathogens are, as a rule, most virulent when freshly isolated from the living tissues that are their normal environment and tend to become less so when grown on artificial media. This fact is of much practical significance. Close contact with infected persons and their fresh body discharges is very likely to result in infection unless adequate precautions are taken.

The virulence of cultures that have lost their potency can frequently be restored by passage through animals. The pneumococcus, for instance, gains in virulence if passed through a series of mice and loses when grown on artificial media. Sometimes passage through an animal alters the organism so that its virulence for its usual host is decreased; this is the case with the smallpox virus and is the principle underlying the making of smallpox vaccine. Some species of pathogens retain their virulence for years in the laboratory, others may loose it after one or two transfers.

HOW BACTERIA PRODUCE DISEASE

The injurious substances or poisons produced by bacteria appear to be of two sorts: those related to their invasiveness whose action appears to be directed, so to speak, against the defensive mechanisms of the host, and those which destroy tissue or impair its capacity to function and which may or may not promote the pathogen's chances of survival.

The substances which seem to be associated with the ability of an organism to invade or permeate living tissue are: hemolysins, leucocidins, fibrinolysins, coagulases, and hyaluronidase, the Duran-Reynals or "spreading factor."

Hemolysins: A great many bacteria, not all of which produce disease, secrete a substance capable of breaking down red blood cells. It is known as hemolysin and is thermolabile and filterable. Its presence is easily demonstrated by streaking the bacteria in question on blood agar and observing for changes. These will be of two general types: the reddish color of the medium around the colonies will become clear and uncolored or it will be replaced by a zone of greenish coloration. The former reaction, called *Beta-hemolysis*, is characteristic of the hemolytic streptococci and of some strains of staphylococci; while the latter, called *Alpha-hemolysis*, is characteristic of the green-producing viridans streptococci and the pneumococci.

The hemolysins produced by different bacteria are species specific; that is to say, the hemolysin produced by the streptococcus is different from that produced by the staphylococcus, even though its action on red blood cells is similar. Hemolysins are frequently named after the bacteria producing them: *streptohemolysins* are produced by streptococci, *staphylohemolysins* by staphylococci. Hemolysins are antigenic and during the course of infection antibodies are formed which inactivate them. These antibodies are also specific and those formed against streptohemolysin do not inactivate staphylohemolysin, a fact which has a bearing on the specificity of resistance following recovery from infection.

Hemolysins produced by bacteria are not to be confused with the hemolysins produced when red blood cells of one species are injected into another species. The former are antigens and from bacteria, the latter antibodies and from the host.

Leucocidins: As the name implies, leucocidins are substances which destroy leucocytes or white blood cells. Since leucocytes have the power of engulfing and digesting bacteria and are vital in the defense against bacterial invasion, it would seem that the production of leucocidin by a bacterium should greatly enhance its invasive capacity. Leucocidins are produced by a number of bacteria, particularly the pyogenic or pus-producing cocci, the streptococci, staphylococci, and pneumococci. Leucocidins are easily destroyed by heat, and, like the hemolysins, are species specific and antigenic.

Coagulases and Fibrinolysins: The deposition of fibrin and the clotting of blood are usually considered as part of the defense of the body against disease for, when bacteria gain entrance into the tissue, these substances aid in walling them off and in preventing their spread through the blood stream. Coagulase, a substance which hastens the formation of blood clots, is produced by many staphylococci and may account in part for their virulence and for some of the characteristic features of infections produced by them. The formation of thrombi or blood clots in vessels, for instance,

is a characteristic feature of staphylococcic infections. Another is their tendency to remain localized which may be causally related to their production of coagulase.

The streptococci, in particular, produce a substance called fibrinolysin which dissolves the blood clot. Since the formation of the clot tends to prevent the spread of organisms through the blood vessels, it seems that its dissolution would remove a barrier to invasion and that a bacterium equipped, as it were, with the power to dissolve blood clots, would not remain localized. This is the case in general with streptococci for they are known for their invasiveness and their tendency to produce generalized infections such as blood poisoning.

Fibrinolysin seems to be associated with virulence and also with host specificity. In general, streptococci isolated from human infections produce fibrinolysins which will dissolve the clots of human blood but not of horse blood, and strains isolated from infections of the horse produce fibrinolysins which dissolve the clots of horse blood but not of human blood. Fibrinolysin is antigenic and antifibrinolysins probably play a part in the resistance which follows streptococcic infections.

Hyaluronidase: Many bacteria, including the streptococci, staphylococci, pneumococci, and some of the spore-forming anaerobes associated with gas gangrene, produce a substance that affects the permeability of the tissue. Extracts of leeches contain enormous amounts of it and it is also found in some animal tissue, notably the testicular. This substance has been called the "spreading factor" or Duran-Reynals factor and has been identified with the enzyme hyaluronidase which hydrolyses hyaluronic acid.

Hyaluronic acid is a high molecular weight polysaccharide. It is produced by many bacteria where it is found in the capsular substance and it is a constituent of normal animal tissue responsible for viscosity. When bacteria or other particulate agents get into tissue or fluids, such as the synovial fluid or the vitreous humour, the hyaluronic acid, because of its viscosity, prevents their spread. However, if hyaluronidase is secreted by the bacteria, hyaluronic acid is broken down, the compound loses its viscosity, and the bacteria can spread more freely through the tissue.

Hyaluronidase or the "spreading factor" can be demonstrated readily by injecting bacteria, vaccinia virus, or inert particles such as india ink or similar substances either with or without the enzyme into the skin of animals. Under the influence of the enzyme these materials will readily diffuse into the surrounding tissue, whereas in its absence they will remain localized.

There is a decided relation between an organism's ability to produce this agent and its invasiveness. The spermatozoa, for instance, have hyaluronidase and it plays a part in the process of fertilization by digesting the

viscous mucoid material surrounding the ovum and thus allowing the entrance of the sperm. The exact relationship of hyaluronidase and invasiveness or virulence of bacteria is not clear but the enzyme appears to play an important role in at least some primary infections and is probably responsible in part for the severity of some mixed infections.

Toxins: In a few diseases the principal damage appears to be due to poisons secreted by the bacteria; in others, to poisons liberated when the organisms die and disintegrate. Bacterial poisons of these types are called toxins and, although not directly associated with invasiveness, are responsible for virulence. Broadly speaking, there are two types: exotoxins and endotoxins.

Exotoxins: The exotoxins are powerful poisons which are secreted by or diffuse out of the bacterial cell. They are water-soluble proteins that appear in the surrounding medium and can readily be separated from the cells by filtration. They are easily destroyed by heat and are detoxified by formaldehyde. Exotoxins are antigenic and stimulate the production of equally potent antitoxins which neutralize them. They are also specific; the exotoxin produced by one species being different from that produced by any other species. They are the most potent poisons known. Compared to them a chemical poison such as strychnine is relatively innocuous. They are far more powerful than snake venoms and only a few of the plant poisons, such as ricin and abrin, approach them in potency.

Of the few diseases due to bacteria which produce exotoxins, the more important are diphtheria, tetanus, botulism, scarlet fever, gas gangrene, and one type of dysentery. In these diseases, bacteria-free filtrates of broth cultures which contain the toxin will produce all or most of the symptoms of the disease. For example, when filtrates of the diphtheria bacillus are injected, they will produce symptoms like clinical diphtheria and filtrates of the tetanus bacillus will produce the symptoms of tetanus. The exotoxins produced by an organism may consist of more than one fraction and these may act in different ways. Some are neurotoxins, affecting nerve tissue primarily; some may affect other tissue. The injection of killed and washed cells of bacteria capable of producing exotoxins when living does not produce any appreciable damage. The exotoxins differ from chemical poisons in that they do not produce their effect immediately but require an incubation in the animal body of several hours or days.

Endotoxins: The endotoxins are poisonous intracellular substances. They do not diffuse into the surrounding medium and are liberated only when the cell is disrupted. The endotoxins are not particularly toxic. They are poor antigens and do not stimulate the production of effective antitoxin. They are relatively heat stable and are not affected by formaldehyde. The injection of endotoxins from bacteria does not produce the

symptoms of the disease characteristic of that species. For example, the injection of dead typhoid bacilli, either intact or disrupted, does not produce the symptoms of typhoid fever, although they are poisonous. Our knowledge of endotoxins is very incomplete but they are probably of considerable importance in the production of disease.

The endo- and exotoxins are, then, quite different substances although there is some over-lapping of characteristics. From the practical point of view perhaps the greatest difference is that those who recover from diseases due to bacteria which produce exotoxins are usually immune to a second attack. Immunity to such diseases can also be artificially induced by injecting suitable preparations of modified toxins, such as diphtheria toxoid, or of effective antitoxins, as diphtheria antitoxin. Although immunity may result from an infection by bacteria producing endotoxins, effective antitoxins for such infections have not yet been prepared.

Capsules: Capsules are definitely associated with the virulence of the pneumococci and of some other bacteria. The non-encapsulated strains are not virulent and the encapsulated strains are. The polysaccharide capsular material is non-toxic and does not seem to have any destructive effect on the body tissues. It probably acts only as a protection for the bacteria against the body defenses.

Miscellaneous Factors: The mere presence of bacteria in living tissue, the mechanical pressure produced when they are multiplying, the utilization of oxygen and the production of acids, gases, and other metabolic products not clearly defined, probably play some part in the disease picture. However, in the case of many or most pathogenic bacteria it is not clear as to just what factors their virulence may be due nor by what means they induce disease. Herein lies a challenge.

Chapter XII

HOW THE BODY DEFENDS ITSELF AGAINST INFECTION

FIRST LINES OF DEFENSE

There are a number of natural barriers to infection which constitute what may be referred to as the "first lines of defense." Some are mechanical, some physiological in nature. A convenient way of considering them is according to the routes by which infectious agents may enter the body; namely, the skin, digestive tract, respiratory tract, eyes, and genital tract.

The intact skin is probably the most important barrier against infection and few bacteria or viruses are capable of penetrating it. It serves as a mechanical barrier against organisms and there is evidence that it actually destroys certain types. Hair follicles and sweat glands are vulnerable spots in this armor.

The oral and intestinal mucosa, although probably not so impervious as the skin, also form a barrier to prevent the bacteria from entering the deeper tissues. When foreign bacteria are planted in the mouth they disappear rapidly, although the natural flora of the mouth is both varied and rich. When they are eaten or drunk in food and water, they pass into the stomach where they are subjected to the action of the gastric juice whose high acidity reduces their numbers. However, many, protected by the food and water mixture, escape its action and travel into the upper portion of the intestine where the acidity is less and conditions more favorable to them. As they progress downward, secretions become more alkaline and the natural flora increase in number and kind. The lower intestines are a veritable culture tube and the limiting factor to bacterial growth is the antagonistic action of other organisms. The secretions there appear to have little growth-inhibiting effect.

Air-borne bacteria encounter a number of obstacles that tend to prevent their entrance into the lungs. Many are caught on the moist surfaces of the torturous passages of the nose or become entangled in the cilia of the trachea and bronchi whose waving motion keeps bacteria and other particles continually moving upward and away from the lungs. Many are expelled by coughing and sneezing. The secretions of the nose contain an enzyme-like substance known as lysozyme which is destructive to many gram-positive bacteria, although relatively harmless to the gram-negative.

The eyes are protected by the mechanical action of tears and by the lysozyme in them. Lysozyme is an enzyme belonging to the class carbohydrases. Some species of bacteria contain a polysaccharide readily

hydrolysed by lysozyme. The inhibiting and destructive effect of lysozyme on sensitive bacteria is apparently referable to its action on this polysaccharide.

The flushing action of the urine tends to protect the urethra from infection.

These mechanical barriers imposed by the skin and mucous membranes, the anatomical structure of the nasal passage, and the outward flow of the various secretions are sufficient to prevent invasion of the deeper tissues by a number of bacteria, particularly the "opportunists," and doubtless keep out many of the true pathogens. However, bacteria may get through the skin and into the deeper tissues or blood stream by way of the hair follicles and sweat glands, scratches or wounds, or by the bite of insects. They may grow on the surface of the oral and intestinal mucosa and produce sufficient damage to the tissues so that they can invade, or they may actually be carried into the deeper tissues, lymph, or blood stream by wandering leucocytes. When bacteria do get into the deeper tissues or blood stream they encounter what we might call the second lines of defense.

SECOND LINES OF DEFENSE

These involve the inflammatory processes, phagocytic cells, and the screening action of the lymph nodes, lungs, spleen, and liver.

Inflammation: Inflammation is a characteristic response to local injury. Although it may be stimulated by mechanical or chemical means, it is more commonly caused by bacteria or their poisons. When bacteria, such as the staphylococci, get into the deeper tissues and begin to multiply, they produce poisons which damage or kill the neighboring cells. As these disintegrate, various substances are liberated, among them histamine, and subsequent events are partly due to its action on the small blood vessels.

The first stage in the inflammatory process is a dilatation of the capillary walls, resulting in an increase in the amount of blood in the region, and a diffusion of fluid into the tissue spaces. This accounts for the reddening and swelling.

There is evidence of the liberation of a substance as yet unidentified which stimulates the mobilization of phagocytic white blood cells. These migrate through the capillary walls surrounding the wound and begin engulfing and digesting the staphylococci and dead tissue cells. The phagocytes thus present a barrier which prevents the staphylococci from invading the surrounding tissue. Many are destroyed by the leucocidin produced by the staphylococci and this accounts for the creamy color and consistency of the pus characteristic of such infection. Not only is the mobilization of leucocytes stimulated by infection, but the actual number in the blood stream usually increases. Normally there are from 5000 to 8000 per cubic

millimeter. During infections due to the pyogenic cocci the number may increase to 16,000 to 30,000, producing a condition known as *leucocytosis.* In many virus diseases there is a decrease in the number of leucocytes, a condition known as *leucopenia.*

While the process of phagocytosis is going on, other cells, notably the lymphocytes, are busily engaged in the process of repair.

If the local inflammatory reaction is not prompt enough to isolate the staphylococci, they will spread through the *lymphatic* capillaries and may reach the lymph nodes which drain the region. The lymph nodes act as screens and collect many bacteria which are then engulfed and digested by the "fixed" phagocytic cells. If the bacteria entering the lymph nodes are too numerous or too virulent, some may escape digestion and continue to multiply, causing a swelling and soreness of the node.

When bacteria get into the blood stream, a few are attacked by wandering phagocytic cells, the so-called *polymorphoneuclear leucocytes,* and some are destroyed. Of far more importance, however, are the "fixed" phagocytic cells which line the capillaries of the spleen and liver. Their action can be shown experimentally for when bacteria or other particulate substances such as india ink, for instance, are injected into an animal's blood stream, the majority will disappear rapidly, that is, in a few minutes. By appropriate means it can be shown that the bacteria are screened out in several organs, particularly in the spleen and liver where the fixed phagocytic cells engulf and digest them. If virulent bacteria are injected they may not all be taken up or they may multiply within the phagocytic cells, kill them, and produce a fatal infection.

The process of inflammation, the collecting and screening out of bacteria by the lymphatics and in the spleen and liver, and the digestion of bacteria by phagocytic cells are normal defensive mechanisms. There are other defensive reactions which depend upon previous contact with the infectious agents and these constitute the third line of defense. They are called immunological reactions.

THIRD LINES OF DEFENSE

Immunological Reactions and Resistance to Infection

Infectious diseases show two characteristic features that cannot be explained on the basis of the defense mechanisms previously discussed. The first is the course of a non-fatal infection, which is rather definite. There is an incubation period during which the organisms multiply freely. This is followed by a period of illness, and, finally, by a period of convalescence and recovery. The second characteristic feature of an infectious disease is the period of immunity following recovery.

In some diseases, such as diphtheria, the infectious agent remains local-

ized and produces a potent toxin to which the major symptoms of the disease are due. For a time the normal body defenses seem powerless against it. In other diseases, such as typhoid fever, the infectious agent proliferates freely for a time, indicating that again the normal body defenses are not adequate. Now if, as in the case of diphtheria, the toxins continued to be as damaging to the tissue; or, if, as in the case of typhoid fever, the bacilli continued to proliferate at the same rate, death would inevitably ensue.

But everyone knows that such is not the case, for in these diseases about ninety per cent of the patients recover. Why do the toxins cease to poison and why are the bacilli eventually destroyed? It is evident that during the course of the infection some mechanism other than those normally present in the body defenses enters the picture and enables the body to neutralize specific poisons or to destroy specific species of bacteria. The appearance of such a defense mechanism explains two characteristic features of the infectious disease: first, the fact that recovery follows a period during which the body has seemed unable to cope with the infectious agent; and, second, that after such a recovery there is a period of immunity to a second attack.

And such is the case, for when the mechanical and non-specific lines of defense are penetrated and the infectious agents or their poisons gain access to the deeper tissues, the body produces a series of substances which have the power to kill or inactivate the bacteria and to neutralize their toxic products. These reactions on the part of the body are called immunological reactions because they were first studied in connection with immunity. Fundamentally, however, they are physiological reactions for they are concerned with ridding the body of foreign proteins and the net result is not always beneficial to the body. Indeed, it may be disastrous as in the case of anaphylaxis. These reactions manifest themselves only under certain conditions and only as a specific response to a specific agent.

When egg protein or any other protein is taken into the digestive tract, it is acted upon by the digestive enzymes and broken down to amino acids which are absorbed through the walls of the digestive tract and pass into the blood stream where they are used in the building up of protein. But if this same egg protein or other protein is injected directly into the blood stream or is introduced into the body in some other way than by the natural route, an immunological reaction occurs. The foreign protein introduced into the blood stream soon disappears and, in due course of time, other substances appear in the blood serum which will react with it.

This can be proven experimentally as follows: the blood serum of a normal rabbit will not produce a precipitate when mixed with egg protein but if a rabbit has been injected with egg protein and, after a suitable

length of time, bled; its blood serum mixed with some of the egg albumin will now produce a precipitate. We know that this precipitation appears as a direct result of the foreign protein and that the serum of the injected animal has something in it which is not present in the serum of the normal animal. These substances which appear in the serum as the result of parenteral introduction of protein are called *antibodies* and any substance that will stimulate their formation is called an *antigen*. The one is defined in terms of the other.

The antibodies will react only with homologous or very closely related antigen. If the red blood cells of sheep are injected into rabbits, the rabbit serum will react with sheep red blood cells but not with those taken from the cow or horse. The protein of each species is species specific and antigenically different from that of any other species. The antibodies produced are all different.

When bacteria, viruses, or other parasites, or their products invade the tissue, the body reacts as it does towards any other foreign protein and produces antibodies.

Antigen-Antibody Reactions: Antibodies are recognized by what they do and have been given names more or less descriptive of their visible manifestations. Thus we have antitoxins, agglutinins, precipitins, hemolysins, opsonins, and so on, named according to the demonstrable changes produced on or in the antigen by the antibody.

The Precipitin Reaction: In 1897 Kraus showed that filtrates of bacterial cultures produced a precipitate when mixed with homologous antiserum and that this reaction was specific. Later it was found that any foreign protein could be used as an antigen and that serum from animals injected with it would always give a precipitate when mixed with the same protein. Kraus called the substance present in the serum a *precipitin*.

The precipitin reaction has been used extensively in the study of the relationships of plants, animals, and bacteria. Because of its sensitivity and specificity it affords a means of distinguishing and identifying the proteins of the various species and strains. It has found a place in legomedicine where it has proven useful in the identification of blood and spermatozoa, for instance.

Antitoxins: Antitoxins are substances which neutralize the poisonous action of toxins. They are produced only in the animal body and only in direct response to the introduction of toxins produced by bacteria, plants, or animals. Antitoxins neutralize toxins in the test tube as well as in the animal body. Their action is specific. Diphtheria antitoxin has no effect on tetanus toxin, nor does the antivenom produced against rattlesnake venom have any effect on cobra venom. Antitoxins are thermolabile proteins.

The discovery of antitoxin by Behring and Kitasato in 1890 was one of the great events in science and forms the foundation of our knowledge of immunological reactions. In 1889 Kitasato proved that the diphtheria bacillus exerts its effect by virtue of its toxin and in the same year Roux and Yersin proved that the tetanus bacillus, likewise, exerts its effect by virtue of a toxin it produces. While studying the action of toxins on animals, Behring and Kitasato noted that the blood serum of rabbits and mice immunized against tetanus was capable of neutralizing the action of tetanus toxin and that the injection of the serum from such animals into normal animals conferred an immunity on them. This discovery pointed the way to serum therapy which has proven so dramatically successful in the treatment of diphtheria and some other diseases. It also explains some of the characteristics of diseases due to bacterial toxins, such as recovery and the immunity that follows.

As previously mentioned, the diphtheria bacillus localizes on the tissue in the throat and while growing there elaborates a toxin which is absorbed and is responsible for the major symptoms of the disease. The normal defenses are powerless against this toxin. However, it is antigenic and after a few days a kind of antibody which is called an antitoxin is elaborated by the body as a direct response to the presence of the diphtheria toxin antigen. Whether the outcome of the disease will be death or recovery depends upon the speed of antitoxin production. If antitoxin is produced rapidly enough, and it usually is, the toxin will be neutralized and its damaging action stopped. If antitoxin in sufficient quantity is not produced soon enough the toxin will do irreparable damage and death will ensue. If the patient recovers, the antitoxin produced probably remains in his blood serum for life and if the diphtheria bacillus should again establish itself, it will have no damaging effect, for the antitoxin in the blood will neutralize the toxin as fast as it is formed.

Antitoxins have no curative power in the sense of repairing damage already done. They merely prevent additional damage and so, although they are used as curative agents, they are in reality, preventive agents.

The principal diseases due to bacteria which produce toxins are: tetanus, diphtheria, scarlet fever, gas gangrene (*Cl. welchii*), and one type of bacillary dysentery. In these diseases the degree of immunity is directly related to the concentration of antitoxin in the blood. The specific antitoxins are considered in connection with the diseases.

Neutralizing or Protective Antibodies: There are antibodies which will protect against infection by such agents as the viruses. Basically they are no different from the other types of antibodies but the only way in which they can be recognized is by their protective action. If the virus of infantile paralysis, poliomyelitis, is introduced into the brain of a monkey

and the disease ensues and the monkey recovers, it is thereafter immune to reinfection. If its serum mixed with an infective dose of virus is incubated and introduced into susceptible monkeys, infection will not occur. The blood of monkeys recovered from poliomyelitis contains a substance or antibody which protects against infection. It is specific, like all other antibodies, and is used extensively in the laboratory in the identification of the virus.

The Agglutinin Reaction: If an animal is immunized against typhoid bacilli and its serum mixed with a suspension of these organisms, the bacilli may be observed to lose their motility, clump together, and settle to the bottom of the tube. This clumping or agglutination is due to an antibody called an *agglutinin.* The serum from a person who has recovered from typhoid fever will likewise agglutinate a suspension of typhoid bacilli. But the reaction is specific for the typhoid bacillus; the serum will not aggluti-

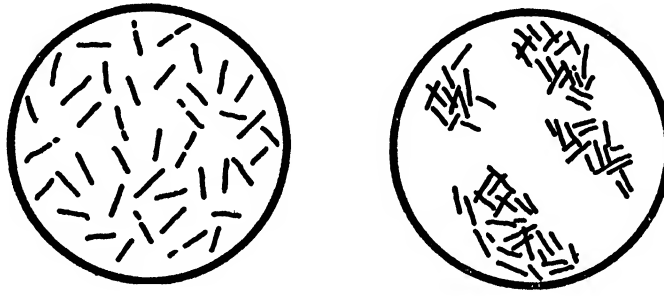


FIG. 38. Illustrating microscopic agglutination reaction. Redrawn from Greaves and Greaves.

nate any other organism, such as *Brucella abortus.* This specificity has made the agglutination reaction useful in the identification of bacteria and in the diagnosis of disease. Agglutinins are undoubtedly important in the defense against infection for by clumping the bacteria they make them more likely to be phagocytized or filtered out by the spleen and liver. In many infections there is a direct correlation between the concentration or the titre of the agglutinins in the blood and resistance.

Lytic Reactions: Bacteriolysins: Early investigators noted that the germicidal power of blood from different animals was not the same for various bacteria, that the active principle was present in the serum, which was just as effective as whole blood, that if the serum was aged or heated to 56° C. for a half hour it lost its activity, and that the killing power of the serum was specific. The serum from an animal immunized against a certain organism was far more destructive than normal serum to that particular organism.

Pfeiffer attempted to find out what happened to cholera bacilli when they were introduced into the intraperitoneal cavity of guinea pigs previously immunized. He observed that the bacilli first lost their motility, then swelled up, and then disintegrated. This is known as the *Pfeiffer Phenomenon.* It was also noted that fresh immune serum reacted the same way towards the bacilli when the two were mixed in the test tube.

When such serum is heated to 56° C. for a half hour or allowed to age, it loses its activity. However, if a drop or two of fresh guinea pig serum, which showed no killing power because it had been taken from an animal which had not been immunized, were added to it, the killing power of the inactivated serum was restored. This observation lead to the discovery of a component of all normal serum which was called *alexin* by Buchner, a term taken from the Greek and meaning *to ward off*, and to which Ehrlich later gave the name, *complement.*

Complement: Complement is a thermolabile component of all serum. It is necessary in the lytic reactions, for cells will not dissolve or disintegrate in the presence of immune serum unless it is there. If antigen is mixed with its homologous antiserum in the presence of complement, the complement is bound and is not found free in the mixture. When heterologous antiserum is used, the complement is not bound but remains free. This fact forms the basis of the *complement fixation* reaction.

Complement is non-specific, that is, the complement of serum will react with any antigen-antibody complex regardless of its source or kind. The complement titre of serum cannot be increased by immunization and its role in body defense is a much debated point.

Hemolysins: The body also produces lysins which cause the red blood cells to dissolve. Much of our knowledge of the lytic reactions has been obtained from studies of these substances, known as hemolysins. When red blood cells are injected into an animal of a different species, that animal produces an hemolysin which is specific for the cells injected and which will not act against the cells of another species. Hektöen *et al.* have shown that it is possible to immunize a rabbit with mixtures of cells from a chicken, duck, turkey, cat, dog, goat, sheep, pig, horse, cow, and man and that the rabbit will produce antibodies against each and everyone of these antigens. The red blood cells of each species are antigenically specific.

Phagocytes and Immunity: According to the *humoral theory of immunity* developed by Ehrlich and his school, the antibodies already discussed were the essential elements in the defense against bacteria and their poisonous products.

At about this same time, that is around 1890, Metchnikoff and his students were pointing out the importance of certain cells in the defense against infection. And, just as Ehrlich explained the formation of anti-

bodies on the assumption that they were a by-product of cell nutrition, so Metchnikoff built up his theory of *cellular immunity* as a result of his studies on the manner in which some of the single-celled organisms obtained their food. The amoeba engulfs or ingests foreign materials, digests those suitable for food, and expels those that are not. In the higher forms the capacity for intracellular digestion is limited to a group of cells to which Metchnikoff gave the name *phagocytes* or "devouring cells." In a study of the natural immunity of dogs to anthrax, it was found that the blood serum, which should contain the antibodies, had little effect on the anthrax bacilli, but that the white blood cells or leucocytes readily engulfed and digested them. This capacity for devouring and digesting bacteria was much greater in animals that had been immunized than in untreated animals. Metchnikoff further showed that the power of phagocytosis was not limited to the wandering leucocytes but was also possessed by other cells. He divided the phagocytes into two groups: "wandering" and "fixed." The former includes the polymorphonuclear leucocytes of the blood to which he gave the name *microphages* and certain larger cells of the tissues called *macrophages*.

In recent years the phagocytic cells have been subjected to intensive study and the observations of Metchnikoff confirmed and extended.

The macrophages belong to or are derived from what is now spoken of as the *reticulo-endothelial* system. They are found in the lining of the blood capillaries, in the lymph nodes, in the bone marrow, the liver, "Kupffer cells," the spleen, and other organs. They have been given various names and may be differentiated histologically by the size and shape of the nucleus and by staining methods. They act as scavengers and defenders, engulfing and digesting dead and damaged cells as well as bacteria.

The Mechanism of Phagocytosis: There are three steps or stages involved in the phagocytosis of bacteria: first, the bringing together of the phagocyte and the bacterium, second, the actual engulfing of the bacterium, and, third, the intracellular digestion of the bacterium.

Certain substances, including some bacteria, have a power, known as *chemotaxis*, of repelling or attracting leucocytes. Those that repel are said to be negatively chemotactic and those that attract, positively chemotactic. The repelling action is associated with the electrical charge carried by the bacterium and probably linked with its ability to invade. Avirulent streptococci seem to attract leucocytes, while virulent streptococci repel them.

After the leucocyte has engulfed the bacterium, it may and usually does digest it. However, some bacteria, notably the tubercle bacilli, are resistant to the digestive enzymes of the leucocytes. Such organisms may be transported to some other part of the body by the leucocyte and liberated

there upon its death. In such a rare instance the wandering phagocyte actually serves to spread the infectious agent.

If leucocytes are washed and thus freed from blood serum and then brought in contact with bacteria, they will engulf only a very few and those only after prolonged contact. When the serum from an animal that has recovered from an infection or from one that has been immunized is mixed with the leucocytes and homologous bacteria, the process of phagocytosis is enhanced. Evidently there is something present in the serum that stimulates the process.

Opsonins and Bacteriotropins: A substance which enhances phagocytosis is called an opsonin. The choice of the term, which means *to prepare food for*, is obvious: opsonins are antibodies that act upon the bacteria in such a way as to make them more readily taken up by the leucocytes. They are specific, which is to say that opsonins or tropins, as they are sometimes called, that act on one species are not effective on another. They may be removed from the serum by adsorption with bacteria.

Wright, to whom we owe much of our information concerning opsonins, developed a technique for measuring the content of these antibodies in serum. It is known as the *opsonic index*.

Specificity of the Antigen-Antibody Reaction: We have previously emphasized the remarkable specificity of the antigen-antibody reactions. Now, it so happens that the antibodies produced against some bacteria will react not only with them, but also with closely related species. However, this does not invalidate our concept of specificity, for a bacterial cell has been shown to contain a number of proteins which are antigenically distinct. The organisms of the colon-typhoid-dysentery group have been intensively studied and the antigens have been mapped. Each cell consists of a patchwork of proteins, antigenically and chemically distinct, and even the flagella, in the flagellated forms, are composed of several different antigens. When cross-reactions occur, that is, when the antibodies of one species agglutinate another species, it is because the two species possess a common antigen. By appropriate means, such as the agglutinin or precipitin adsorption technique, it is possible to tell whether the organisms are antigenically similar or whether they contain only a few antigens in common. This technique is very valuable in determining relationships and hence in classification of bacteria and diagnosis of disease.

Haptenes: The specificity of proteins may be altered by linking simple substances to them. Such substances are called *haptenes* or "partial" antigens. When injected alone into animals, haptenes do not stimulate the production of antibodies. When the protein-haptene complex is injected, antibodies are produced which will react not only with the protein-

haptene complex but with the haptene alone. Thus the haptene acts as an antigen in that it reacts with the antibody in the test tube although it will not fulfill the requirements when injected into an animal. In a great many instances the specificity of immunological reactions is due to haptenes.

Of particular interest are the polysaccharides. In the case of many bacteria, particularly the pneumococci, it is the polysaccharide which confers the *type* specificity. The bacterial proteins of each *type*, although antigenically different from those of other species, are apparently immunologically identical but the polysaccharide of each *type* is serologically distinct. In many diseases the therapeutically effective antibodies are those which react with the polysaccharides and not those which react with the proteins.

Antigen-Antibody Reactions and Diagnosis: Since antibodies are produced only in direct response to the presence of antigens within the body and since they are specific, it follows that the presence of antibodies for any bacterium or its toxin is proof that the body has been exposed to this antigen. The diagnosis of many diseases of animals and man and tests for immunity depend upon finding such antibodies in the blood serum.

To cite a few examples: cattle, when infected with the organism of contagious abortion or Bang's bacillus, produce antibodies which will agglutinate this organism in the test tube. The well-known *Bang Test* consists of mixing serum from the cow with a suspension of Bang's bacillus and observing for agglutination. Similarly, chickens may be tested for *pullorum infection*, or bacillary white diarrhea, by mixing their blood with a suspension of *Salmonella pullorum*, the bacterium which causes this disease. In man the agglutination reaction is widely used in the diagnosis of undulant fever, tularemia, typhoid fever, and several other diseases. To detect syphilis several different types of serological reactions are used. They are known by the names of the men who developed them and the Wassermann, Kline, and Kahn reactions are the most common. The details of the tests differ considerably but all depend upon the detection of antibodies for the *Treponema pallida*, the cause of syphilis, by the use of suitable antigen.

In virus diseases the protective antibodies may be detected and identified by injecting mixtures of antiserum and virus into suitable animals.

The presence of antibodies of the antitoxin type can be detected by several methods. In the case of diphtheria or scarlet fever, the toxin produces a local swelling and reddening when injected in small amounts into the skin of susceptible persons. However, if antitoxin is present, the toxin will be neutralized and no local reaction will occur. This is the basis

for the *Schick Test* for immunity to diphtheria and the *Dick Test* for immunity to scarlet fever.

Hypersensitivity

A most curious phenomenon is that known as hypersensitivity. We have seen that the animal body produces antibodies which serve a protective function against infectious agents and their poisons. Paradoxical though it may seem, this same defensive mechanism is responsible for a great many ills and, sometimes, for death. The terminology used in describing the various manifestations of hypersensitiveness is a bit complicated and confusing and the explanations put upon them by different workers not always in accord. However, for our purposes we can generalize and outline some of the more salient points.

The term *hypersensitivity* or *hypersensitiveness* is used to designate a state in which the body shows a heightened reaction to a subsequent introduction of substances which provoked little or none when first introduced. It is an immunological response depending upon the antibody-antigen reaction and may manifest itself in widely different ways. The same substance may produce immunity or hypersensitivity, depending upon the amount and the manner in which it is introduced.

The term *anaphylaxis*, meaning *against protection*, in contrast to *prophylaxis*, meaning *prevention* or *protection*, is used by some writers to describe severe forms of hypersensitiveness in experimental animals and by others to describe any severe or fatal form whether it occurs in man or animal. Much of our information about hypersensitiveness and allergy comes from experimental studies on anaphylaxis in animals.

The Relation of Anaphylaxis and Immunity: The repeated injection of such harmless proteins as egg, wheat, milk, or animal serum into an animal will produce no ill effects, providing the injections are spaced less than ten days apart. Antibodies to the protein will appear in the blood stream and if the antigen is again introduced ten to twenty days after the first series of injections the animal will show no untoward response. If the antigen used is a toxin, the animal will be able to tolerate a far greater dose than it could have before receiving the series of injections. It will be *immune.*

Under certain conditions the injection of harmless proteins may lead to the anaphylactic state and the animal may die of shock. This is true when the second injection is delayed and is not made until ten to twenty days after the first one. Thus the introduction of the same protein may, in one case, lead to immunity and, in another, to hypersensitivity. In the first case the animal is said to be *immunized:* in the second, to be *sensitized.* In the second case, the first injection is called the "sensitizing dose" and

the second the "shocking dose." The immediate administration of adrenalin prevents shock and for this reason it is included in many antisera.

Facts Concerning Hypersensitiveness: Hypersensitiveness is specific and sensitized animals can be shocked only by the introduction of homologous antigen.

Hypersensitiveness may be produced by the inhalation of antigen, as in hay fever; by its ingestion, as in certain food allergies; or by its contacting the skin, as in allergies to wool or silk.

Animals, recovered from anaphylactic shock, are immune or refractory for a variable period of time after which they may again become hypersensitive.

Animals or persons may be desensitized by injecting the antigen in high dilutions or by injecting in sites such as the skin or muscle which permit a slow adsorption only.

Hypersensitiveness may be transferred passively. The injection of antibodies into normal animals or persons may confer either immunity or hypersensitivity. The transfer of serum from an allergic to a normal person confers a local hypersensitivity at the site of the injection and the subsequent injection of antigen causes a pronounced local reaction called the *Prausnitz-Küstner* reaction.

Hypersensitiveness may be local or general. The symptoms depend upon the location of the sensitized or shocked tissue.

The prominent symptoms of anaphylaxis are due to contraction of the smooth muscle and its characteristic manifestation in each species may be explained by differences in the relative amount of smooth muscle. Pieces of smooth muscle, such as strips of the uterus or intestines, from sensitized guinea pigs will react on contact with the antigen *in vitro*. This is the basis of the *Schultz-Dale* techniques widely used in thestudy of anaphylaxis.

Hypersensitiveness does not depend upon the inherent toxicity of the antigen. In fact, egg and milk protein are among the most potent sensitizing agents.

An Explanation of Hypersensitiveness: What presumably happens when the body becomes hypersensitive is this. When the antigen is first introduced into the body it becomes attached to certain cells of the macrophage system. Being protein it does not diffuse into the cell and hence cannot be acted upon by intracellular enzymes. In response to the foreign protein, the cells produce substances which diffuse out to react with it and which are recognized as antibodies. More are produced than are required to counteract the antigen. Some cover the surface of the cell. Others become detached and are found free in the circulating blood stream. If antigen is again introduced, it will combine with the antibodies free in the blood stream and the animal will show no ill effects. However, if not

enough are free, it will combine with the attached antibodies and throw the cell into what is known as *shock*. There is evidence that when the antigen combines with the antibodies attached to the cell, histamine or a histamine-like substance is liberated and it is to this substance that the symptoms of shock are due. Histamine is broken down by the enzyme, histaminase, and its use has been suggested in the treatment of some types of allergy.

The difference between hypersensitivity and immunity seems to be in the concentration of free-circulating antibodies. This is probably why hypersensitive animals become immune when the concentration of free-circulating antibodies is increased and is the basis of the inoculation treatment for allergies such as hay fever.

Allergy: The term allergy, which means *altered reaction*, is commonly used to denote milder forms of hypersensitiveness in man. Food and drug idiosyncrasies, asthma, hay fever, and such manifestations are described as allergic reactions. It has been estimated that about ten per cent of the population suffers from some form of allergy, indicated frequently by respiratory disturbances, gastro-intestinal disturbances, skin eruptions, and, perhaps, by migraine headaches. There seems to be a relation between age and the various types of disturbance. Infants are more apt to show intestinal disturbances; adults, disturbances of the upper respiratory tract.

It appears that the tendency to develop a hypersensitivity such as allergy is genetically determined because allergic reactions are more common in children of allergic parents. Children do not, however, inherit an allergy to a particular substance but, rather, a tendency to become allergic. This may be due to an inherited defect in absorption or metabolism. The development of any specific allergy depends upon sensitization.

Non-protein Allergies: Many individuals become hypersensitive to non-protein substances such as iodine, formaldehyde, sulfonamides, or aspirin. It has been suggested that in drug idiosyncrasies the non-protein substances may react with the proteins of the body to produce a complex which acts as a foreign protein. The drugs serve as haptenes which confer a specificity to this complex.

In many instances reactions similar to protein allergies are produced by physical agents such as sunlight, heat, or cold. It seems likely that these agents, although not antigens, cause the liberation of the histamine or histamine-like substance which is responsible for the symptoms in protein allergies. Desensitization can often be accomplished by graded exposures to the offending agent. Thus a gradual accustoming to cold baths or showers may desensitize persons who are "hypersensitive" to sudden changes in temperature.

Hypersensitiveness and Disease Symptoms: There is evidence that in

many diseases such as rheumatic fever, tuberculosis, and syphilis, the principal symptoms are due not to the innate toxicity of the causative agent or its secreted poisons but to a peculiar hypersensitive or allergic reaction of the host tissue to that particular protein.

OUTLINE OF IMMUNITY

Everyone knows that some species of animals are free from diseases which are deadly to others, and that some people are less often attacked or suffer less severely when attacked by certain diseases than do their fellows. There are several reasons for this. In many instances it is a question of exposure. Animals and men may be free from a certain disease, even though they are experimentally susceptible, because they do not serve as hosts for the insects which transmit it or because their customs or habits are such that they do not come in contact with the infectious agent. Domestic rabbits are free from tularemia, even though they are susceptible, because they are free from the parasites that transmit it in wild rabbits; and Jews do not suffer from trichinosis or pork tape worms, not because they are resistant, but because their customs do not allow the eating of pork. Likewise groups, not exposed to the insect vectors, are free from malaria, plague, or typhus. The climatic, geographic, occupational, seasonal, sex, and age distributions of many diseases can be explained on the basis of risk or chances of becoming infected.

In other instances, freedom from disease is dependent upon immunity for, though their exposure may be the same, some species or individuals suffer less and are less often attacked by some diseases than are others. Two types of immunity from disease may be distinguished: natural and acquired.

Natural Immunity

Natural immunity is an inherent characteristic. It is genetically determined and does not depend upon a conditioning of the body cells by contact with the infectious agent. It may be species, racial, or individual. If species, it may be absolute or relative; if individual, it is always relative. To illustrate—cattle and man are immune to hog cholera, hogs are immune to yellow fever, sheep are immune to diphtheria. These instances represent an *absolute immunity* or lack of susceptibility and are a species characteristic.

Some breeds or races of sheep are more resistant to anthrax than are others and some races of men are more resistant to tuberculosis than are others. These instances represent differences in the degree of resistance and, hence, a *relative immunity.*

Natural immunity is an innate characteristic and although it can be decreased by fatigue, malnutrition, lowering of temperature, and the like

it cannot be increased for the normal healthy animal or person. Just what mechanism is involved is not known although in special cases it is suspected that the temperature of the host may be a factor. In other cases it may be a question of anatomical structure or of some physiological feature. It is not dependent upon specific antibodies.

Races or groups that have been in contact with a disease for generations are, in general, more resistant than are groups experiencing it for the first time. Thus the negro appears to be more resistant to yellow fever than the white man but less resistant to tuberculosis. Indians suffered far more from smallpox and scarlet fever than did the colonists. How important natural selection has been is difficult to say. It seems that it might be important in diseases of children and young adults, probably not in diseases that attack in middle and old age. If the disease is to act as a selective agent it would have to be one that acted before the age of reproduction were past.

Acquired Immunity

The fact that persons or animals who have recovered from certain diseases less often suffer second attacks suggests that they have become more resistant as a direct result of their first attack. This resistance is known as *acquired immunity*. It is relative and varies for different diseases. After some diseases it is strong and not readily overcome; after others, weak. It is usually of long duration, lasting for years or for life, although in some diseases as in the common cold, for example, the immunity produced lasts only a few months. Acquired immunity depends upon specific antibodies and the introduction of these into normal animals confers an immunity upon the recipient. Acquired immunity may be of two kinds, *active* or *passive*, and may be acquired *naturally* or *artificially*.

Active Immunity: Active immunity is acquired when the host cells produce antibodies or become more reactive as a result of their experience with antigen. It is called active immunity because the body takes an active part in the production of antibodies. When antigens, such as bacteria or viruses, invade the body naturally as in disease or in inapparent infection, the immunity is said to be *naturally acquired*. They may also be introduced purposely by inoculation, in which case the immunity is said to be *artificially* acquired.

If the antigen used to produce immunity artificially is a living agent, it is called a *vaccine;* if killed, it is called a *bacterin*. The procedure used to induce active immunity artificially is called *vaccination*. The term comes from the Latin word *vacca*, meaning cow, and refers to the cowpox virus used in immunizing against smallpox. In honor of Jenner, who developed the method, Pasteur introduced the term vaccination for any active immunization and it is frequently so used.

The term *immunization* is broader and refers to either active or passive immunity.

Two terms commonly confused are *vaccine* and *serum*. Vaccines are antigens. Serum is the fluid constituent of blood separated from the cellular components by coagulation. It contains specific antibodies only when taken from immunized animals. The term *antiserum* or *immune serum* should be used when referring to such sera.

The preparation of a suitable antigen to be used in producing active immunity is of considerable practical importance. In general the antigens used are: living virulent cultures, living attenuated cultures, living virulent cultures plus antiserum, killed cultures, toxin, toxoid, or toxin-antitoxin.

Living Virulent Cultures: For obvious reasons it is not practical to use virulent organisms unless they can be introduced by a route which does not lead to infection or in numbers too few to produce disease. Their use as immunizing agents is largely limited to experimentation in animals.

Living Attenuated Cultures: The virulence of some organisms can be lowered by animal passage, by aging, drying, and by growing on special media. The best-known example of such an organism is smallpox vaccine. This virus loses its virulence for man when passed through calves but retains its immunizing power.

Living Virulent Cultures Plus Antiserum: Some pathogens, particularly viruses, can be inactivated by antiserum and used safely as immunizing agents. In other instances, the antiserum can be injected with or previous to the introduction of the virulent virus. The presence of specific antibodies affords a temporary protection from disease and the presence of antigen, even though inactivated, stimulates the cells to produce antibodies which persist.

Killed Cultures: In typhoid fever and a few other diseases active immunity can be induced by injecting killed cultures. In many other diseases their introduction, although it may stimulate the production of antibodies, does not induce immunity to infection. The organisms may be killed by physical agents such as heat or supersonic waves or by chemical agents, and the manner in which they are killed seems to affect their immunizing power.

Toxin: In diseases such as diphtheria, scarlet fever, and tetanus, in which the principal symptoms are due to exotoxins, active immunity can be induced by injecting suitable preparations of the toxins.

The toxin may be first introduced in dilutions high enough to be tolerated and, by giving a series of injections of increasing concentrations, an active immunity may be induced. Because of the large number of injections required this method is not practical.

Toxoid: Toxins may be detoxified without destroying their power of inducing antitoxins and when so treated are called toxoids. Precipitation

with alum or treatment with formaldehyde is commonly used in detoxifying.

Toxin-antitoxin: Toxin which has been inactivated by antitoxin is an effective immunizing agent and large amounts can be introduced with safety. The greatest objection to its use lies in the fact that the antitoxin is from horse or other animal serum which is also antigen and persons immunized with toxin-antitoxin may become hypersensitive to the horse protein.

Passive Immunity: Immunity conferred by the introduction of antibodies is called passive immunity because the cells of the recipient do not take part in the production of antibodies but become immune as a result of the introduction of preformed antibodies. Passive immunity may be acquired *naturally* or *artificially.*

Naturally acquired passive immunity is acquired congenitally. In some animals and in man, antibodies pass through the placental membrane from the mother to the fetus and the infant is born with antibodies in its blood stream. Since its cells have not come in contact with the antigen and therefore have not produced the antibodies, its immunity was acquired passively.

Antibodies may also be secreted by the mother in milk, particularly in the colostrum, and thus may be acquired by the nursing infant. It is of interest to note that in cattle, whose anatomical structure is such that antibodies cannot pass from the mother to the fetus, the colostrum of the dam is particularly rich in antibodies which are rapidly absorbed by the newborn calf. Calves that get colostrum seldom contract a disease called *White Scours* prevalent among those who do not get it.

Although the antibodies acquired from the mother disappear from the circulation of the infant during the first year, they undoubtedly account for the resistance of infants to many infections.

Passive immunity may be acquired *artificially* by the introduction of antibodies which may be obtained from immunized animals, persons who have recovered from infection, convalescents, or persons who have had inapparent infections.

The essential differences between active and passive immunity may be summarized as follows:

Active	*Passive*
Induced by the introduction of antigens.	Induced by the introduction of antisera.
Takes several weeks to produce.	Takes effect immediately.
Lasts for months, years, or life of individual.	Lasts only a few weeks.
Used as preventive measure.	Used as therapeutic measure.

When the therapeutic value of diphtheria antitoxin was discovered in 1890 many bacteriologists believed that it was only a question of time before effective antisera could be produced for most or even all bacteria. Unfortunately that sanguine hope could not be realized. Again, when effective methods were developed for producing active immunity, hopes were raised and again were not realized. There are only a few diseases: diphtheria, scarlet fever, tetanus, Shiga dysentery, and some types of pneumococcus pneumonia, for which there are effective antisera. There are several for which there are effective vaccines, but as yet none has been developed for tuberculosis, syphilis, the common cold, poliomyelitis, and a number of other common infections.

Infection Immunity: In some diseases, immunity lasts only as long as the individual is infected. This is called infection immunity. It is often said that the only immunity to syphilis is syphilis, for infected persons are resistant to reinfection but become susceptible again when cured.

Chapter XIII

HOW INFECTIOUS DISEASES SPREAD

"I have six honest serving-men,
They taught me all I know.
Their names are Who and What and When
And Where and Why and How."

How Is Infection Transmitted? From the standpoint of the control of infectious disease the most important type of practical information is a knowledge of how the infection spreads from person to person. Just to know that yellow fever is transmitted by the bite of the mosquito is sufficient to indicate how the disease can be controlled. When we know that *Eberthella typhosa* can produce typhoid fever only if swallowed and that it can leave the body only in the excreta, we can logically deduce that to prevent typhoid we must keep food and water from being contaminated with feces or urine from infected patients. So the student interested in the spread of infectious disease asks these questions about the infectious agent:

1. What is its portal of entry?
2. What is its mode of exit?
3. How long can it live away from the host?
4. Can it carry on an independent existence and live a saprophytic life?
5. Is the disease limited to man or are animals also affected?

PORTAL OF ENTRY

The portal of entry or the route by which the infectious agent gets into the body is often the decisive factor in determining whether an infection will be produced. In general, every pathogen has a particular route by which it naturally enters the body and many will not infect unless they do enter by that route. The anthrax bacillus, for example, naturally infects by way of the skin and rarely by other routes. The typhoid bacillus will not infect if rubbed on the skin but readily invades and produces infection when swallowed. The organisms that produce gonorrhea and syphilis naturally enter by way of the skin and mucous membranes and set up local infections. The nose and mouth are the two great portals of entry into the body and ninety per cent of the organisms that get into the body get in by way of them.

In general, the easiest way to infect experimental animals is to inject the infective agent into the blood stream but in some infections, as for example, in experimental poliomyelitis, monkeys cannot be infected in that manner.

A very few organisms, as for example, *Pasteurella tularensis*, the causative

agent of tularemia, seem to be capable of invading and producing infection almost anywhere. *Pasteurella tularensis* can enter by way of the skin, eyes, mouth, nose or by the bite of insects, and, consequently, is highly infective.

MODE OF EXIT

It is obvious that parasites which can maintain themselves only in the human body must be able to leave a host as well as to get into one. They need "their exits and their entrances."

The mode of exit of a parasite depends largely upon the region of the body in which the organism tends to localize. In some infections, as in the case of the staphylococcus which produces boils, it remains localized in the skin. Such an infectious agent can be transferred to a new host only by more or less intimate contact with the lesion or its discharges.

Many infections remain localized in the upper respiratory tract and are transmitted by coughing and sneezing or by contaminated objects or foods. Such is the case with diseases like tuberculosis, diphtheria, scarlet fever, and whooping cough.

In diseases like typhoid fever, amoebic dysentery, and bacillary dysentery, the organisms are present in the intestinal tract in great numbers and leave the body in the intestinal discharges. Since the entrance to the gastro-intestinal tract is by way of the mouth and since there is often an intimate connection between sewage and drinking water, it is not surprising to find that water plays an important part in the transmission of such infections.

In a number of diseases of man, such as malaria, yellow fever, and typhus fever, the inciting agent is confined to the blood stream and internal organs. Since the blood stream is a "closed system" the parasite could not escape were it not for the fact that mosquitoes, lice, ticks, and other blood-sucking insects carry it from host to host. The "hitch-hiker" may or may not parasitize its insect vector.

SOURCES OF INFECTION

Man

The "pythogenic" theory of disease or the notion that disease germs were generated in filth proved untenable when it was realized that not a single one of the organisms that incites communicable diseases of man carried on a natural existence apart from the bodies of men or animals. It is true that air, food, soils, and water act as a means of conveyance for these organisms but they are not primary sources.

To be sure there are a few infectious diseases of man caused by naturally saprophytic bacteria, yeasts, and molds as, for example, the tetanus bacilli and the anaerobes responsible for gas gangrene. These saprophytes may

accidentally produce infection when introduced into wounds but their physiology is such that they live habitually as harmless saprophytes in the soil or in the intestinal tract of animals. The infections they produce are not communicable in the ordinary sense of the word. In general, the true pathogens are adapted to a parasitic existence and are incapable of living apart from the host for any length of time.

Fortunately for man, the pathogens, with the exception of the anthrax bacilli and the soil anaerobes, are not spore-formers and consequently die rapidly when exposed to sunlight and drying. The length of time one can

FIG. 39. High-speed photograph of a sneeze. Courtesy of Dr. Marshall W. Jennison.

survive is influenced by the character of the medium in which it is eliminated. The tubercle bacillus will remain alive for months. It is the most resistant of the lot, probably because it is protected from external influences by its waxy coating. Then too, it is eliminated in sputum which protects it from drying. The typhoid bacillus will remain alive for a few weeks in water. The gonococcus dies in a few hours. A few of the common pathogens, notably the diphtheria and typhoid bacilli and some of the streptococci, will multiply in milk, a fact of considerable importance from the public health standpoint.

Infections Are Transmitted by Contact: Since, under ordinary conditions,

pathogens survive apart from their hosts for only a limited time, it is evident that their transmission depends either upon direct contact with infected persons or with their fresh secretions and excretions. Since most infections of man are limited to man, it follows that man is the main source and reservoir of human infection and thus is his own worst enemy. He is the true source of the infectious agent whether the disease be one transmitted by direct contact or more indirectly through the medium of food and water, or by an insect vector.

Infected individuals may be separated into several groups:

The typical case is an important source of the infectious agent because the number of organisms eliminated is apt to be large and of a virulent type. However, such a case is easily recognized and control measures may be instituted, particularly when the disease is so severe that the individual has to be confined to his bed. It is not so easy to exercise control measures for diseases like gonorrhea and syphilis for in these the infected persons can still be up and about and working.

The atypical case. In every outbreak of disease there are always persons who have mild cases or whose symptoms are not the usual ones associated with the infection. Such cases frequently go unrecognized and because the persons afflicted are not so seriously ill, they are more likely to be about and to spread the infection by contact.

The carriers or those harboring the germs but showing no symptoms of the disease may be divided into two groups: *convalescent carriers* or those who have had the disease and recovered but who still harbor the infectious agent, and *healthy or contact carriers* who may harbor and discharge the virulent germs but show no evidences of illness. Such carriers are immune themselves but do transmit the infection.

Carriers are very important factors in the spread of infection. They are not ill so they are difficult to confine and they are usually unaware of their state and so do not exercise any special precautions. About fifteen per cent of the cases of typhoid fever become carriers for a time and about four per cent remain carriers for as long as they live. It has been estimated that an active case of diphtheria is ten times as dangerous to contacts as is a carrier. However, the number of carriers in the population is so much greater than the number of cases, that most cases of diphtheria are due to carriers. The carrier problem is particularly acute in typhoid fever, diphtheria, scarlet fever, and some of the other common diseases.

Biologically the carrier state represents an instance in which the pathogen and host have become adjusted to each other.

Animals As a Source of Infection

While the usual source of the more common human infectious diseases is man, animals are the source of some diseases. Glanders, for example,

is transmitted by direct contact with diseased horses; rabies, by the bite of a mad dog. Undulant fever is usually acquired from an infected cow, and bubonic plague and endemic typhus from rats.

Insect Vectors

If the infectious agent is numerous in the blood, bloodsucking insects can pick it up while feeding and transmit it to other hosts upon which they also feed. In some cases the infectious agent multiplies in the insect and is transmitted or transferred from parent to offspring. Thus it can maintain itself in nature without the intervention of an animal host and its elimination is extremely difficult. This is true of tularemia. The infectious agent increases in the wood tick and the progeny of infected ticks are infected.

There is sometimes an extrinsic incubation period, that is, a period during which the infected insect cannot transmit the infectious agent. In yellow fever the insect is not infectious until about ten days after it has taken up the virus. The period of incubation may be shortened by raising the temperature and lengthened by lowering it.

WHAT FACTORS DETERMINE INFECTION?

Why is it that at any time in any community a certain number of individuals show evidences of infection? Why, in any outbreak, do certain members of a family succumb to the infection while others have mild cases or escape entirely? Why is it that, if bacteria and viruses are the specific cause or etiology of disease, all of us do not contract tuberculosis or diphtheria since these organisms are so universally present? How can we account for the fact that some diseases are more prevalent in certain age groups? Why do certain diseases show a periodicity, that is appear, tend to disappear, and then reappear at rather definite intervals?

It is obvious that contact between a pathogen and a susceptible host does not necessarily lead to infection. Several factors condition the result of such an encounter.

Susceptibility of the Host

Age: It is a well-known fact that infants and young children suffer in relatively greater numbers than adults from certain infections, such as scarlet fever, whooping cough, diphtheria, and measles. The child may, for anatomical and physiological reasons be inherently more susceptible and he does not have the immunity which adults have acquired as a result of previous contact with the pathogen. The child's opportunity for exposure to the infectious agent is probably greater because of his play habits, his eating habits, his diet, his sanitary habits—or lack of them, and his constant association with a susceptible population in his own age group.

Sex: Some diseases show a difference in their sex distribution, the attack rate being higher for males usually. Opportunity for infection and anatomical and physiological differences may be responsible.

Race: There is considerable variation in the incidence of disease in the different races even though they may live in the same community. Jewish people are more resistant to tuberculosis than are Irish. The incidence of various diseases in the different racial groups may differ, not because of any genetically determined resistance but rather because of habits of living peculiar to the race.

Occupation: Occupation plays an important role in infection for several reasons. It may determine exposure, it may be of such a nature that it predisposes to infection by lowering resistance, and by determining economic status more or less it affects nutrition, living conditions, and so on.

Nutrition: Although all the indirect evidence points to the fact that malnutrition lowers resistance to disease and that lack of certain vitamines predisposes to infection, it is difficult to assess definitely the importance of nutrition.

Past History: Under this heading can be listed the influences of past infections on the resistance of the body at any given moment. Individuals who have had previous experience or contact with a disease frequently show a greater resistance than those encountering it for the first time. The reasons for this are discussed in the section dealing with defensive mechanisms.

Climate and Season: Many diseases show a geographic and climatic distribution. How much this distribution is influenced by climate and how much by associated factors such as nutrition, sanitation, and different modes of living is difficult to determine. The various elements are so interrelated that it is not possible to separate and evaluate each by itself.

THE PATHOGEN—VIRULENCE AND DOSAGE

The dosage or number of organisms necessary to infect depends upon the virulence of the organism in question. Dosage or virulence can be determined by injecting or exposing experimental animals to varying numbers of organisms. The smallest number which will bring about infection is called the *Minimum Infective Dose* or the *M.I.D.* and the smallest number which will produce death is called the *Minimum Lethal Dose* or *M.L.D.* These vary with the route by which the organisms are introduced and with each species of animal. In the case of some infections a single organism will produce disease. In others hundreds of thousands will be required.

In some organisms the virulence remains constant; in others it can be changed by passage through animals, by aging, and by cultivation on

special media. There may or may not be a relation between the M.I.D. and the M.L.D. In a disease such as rabies in which the mortality in man is one hundred per cent, they are, of course, the same.

Some organisms are highly infectious and highly virulent: some are highly infective and not particularly virulent: some are not very infective but have a high virulence: and some are neither particularly infective nor particularly virulent.

SUMMARY

To illustrate the relation of the several factors which determine the outcome when a pathogenic organism comes in contact with a potentially susceptible host, Theobald Smith suggested the following equation:

$$D = \frac{N.V.}{R}$$

D represents the probability of *D*isease, *N* the dosage or *N*umber of organisms, *V* their *V*irulence and *R* the *R*esistance of the host. When the product of *V* times *N* is greater than *R*, the result is disease. When less, it is not. There is, of course, no way in which we can measure virulence or resistance in numerical terms, but the equation illustrates the situation.

A fact always to be remembered is that bacteria, viruses, and other pathogens have no effective means of locomotion of their own and that in order for them to get from one host to another, they must take advantage of "free rides." Although man is often not aware of it, it is his own living habits that make the transfer of these organisms possible. Were it not for his ignorance and indifference in the matter of polluting the drinking water of his fellow men with his excrement there would be no typhoid fever; and but for his carelessness in coughing and spitting, tuberculosis would cease to exist.

Chapter XIV

EPIDEMIOLOGY OR THE NATURAL HISTORY OF DISEASE

Epidemiology deals with the natural history of disease. Where it occurs, in whom it occurs, and under what conditions it occurs are its chief concern. These questions can be answered in terms of the life habits of the host and the characteristics of the parasite. The occurrence of plague and pestilences in the past, the presence or absence of certain diseases at present, and the types of disease that are most likely to occur in the future may be explained on the basis of epidemiology. Knowing the epidemiology of a disease, it is possible to predict the type of control measures that will be most effective. It is, of course, not always possible to put them into practice but modern preventive medicine is based largely on epidemiological principles.

Epidemiology differs from the other medical sciences in that it is concerned with infectious disease as a mass phenomenon. It sceks to explain disease not in the individual but in the group. It has been called a collective science because it depends upon the data obtained by other sciences. Each of these approaches the problem from its own special point of view and, utilizing characteristic methods, tools, and techniques, collects information which may be of use to the epidemiologist in interpreting the natural history of disease.

Bacteriology

Thus the bacteriologist who is primarily interested in the specific cause of disease studies the etiologic agent. He tries to find the answers to the following questions:

What does the organism look like and how can it be identified?—information that is used for diagnosis.

How does the infectious agent enter, what tissues does it affect, where does it localize, and how does it leave the body?—information that sheds light on the symptoms and on the method of transmission of the infectious disease. From the practical standpoint, this knowledge is most necessary for control.

Does the infectious agent attack other hosts? If so, is man the natural or common host? If not, what animals are the sources of infection for man? Information on this point is also obviously of great significance in the control of the disease.

Is the infectious agent transmitted from animal to animal, from animal to man, or from man to insect and back to man?

Does the infectious agent grow apart from animals or man? A study of the physiology of the organism can determine whether it multiplies in milk, food, or other non-living material. The materials in which an organism can grow have a direct bearing upon what may be used as a medium of transmission.

How resistant is the organism to drying, heat, cold, or light? Some bacteria produce spores which live for ten to twenty years or even longer and are capable of infecting man and animals years after their escape from the infected host. On the other hand, some bacteria, as for example the gonococcus, the causative agent of gonorrhea, and the treponema of syphilis survive only a short time away from man. These diseases are usually transmitted only by direct and intimate contact.

Does the infectious agent vary with respect to its disease-inciting powers? Many species of bacteria dissociate and virulent strains become relatively non-virulent or *vice versa* as a result of their passage through animals or cultures on artificial media. It is suspected, though not definitely proven, that changes in virulence are involved in the sudden appearance or disappearance of some diseases in their epidemic phases.

Immunology

The immunologist is primarily interested in the defensive mechanisms of the body. When the tissues come in contact with the disease-inciting organisms or their toxic products, certain cells react in a characteristic manner. The phagocytic cells may engulf and destroy the organisms and other cells may produce antitoxin or other types of antibodies which counteract the toxic substances. These reactions are, of course, of tremendous importance in determining the outcome of infection. If the host recovers, he may or may not be immune to subsequent infection by the same organism. If he is, the age distribution of the disease may be quite different than if he is not, for there will be fewer second attacks in later years.

Measles is characteristically a childhood disease. This is not because adults are naturally immune but because nearly all have had an attack during childhood as a result of which they have developed an active immunity. Proof that adults are not naturally immune comes from Panum's classical studies on measles in the Faroe Islands. Measles had been absent for sixty-five years and when reintroduced from Denmark in 1846 occasioned a severe outbreak in which people of all ages were attacked except those who had had measles sixty-five years earlier. A highly contagious disease that has a much greater prevalence in children suggests that it is also a disease that immunizes.

In poliomyelitis or infantile paralysis the younger age groups are most

frequently attacked and the immunologist has shown that there is a progressive increase in the percentage of the population who have neutralizing antibodies for the infective virus. At the age of twenty most people have such antibodies and relatively few contract the disease thereafter. Since most of them have not had recognizable cases of poliomyelitis, and since the only known way in which they could have obtained such antibodies is by contact with the virus, it is assumed that most individuals have unrecognized attacks of poliomyelitis before reaching adulthood. This could be true only if the virus were widely prevalent. In such a way immunologic studies of this sort contribute greatly to a better understanding of diseases.

In some diseases, as for example in jungle yellow fever in South America, evidence points to an animal reservoir of the virus. Immunologic studies on wild animals of the region are valuable in determining which kinds have antibodies and, hence, harbor the virus. The direct detection of the virus in such animals is frequently extremely difficult because the virus may not be obtainable for more than a few days during the disease, whereas the antibodies remain for long periods or even for life.

One further illustration—in diseases of the intestinal tract it is frequently difficult to prove the connection between organisms isolated from the feces and the disease. However, antibodies usually appear in the blood stream against organisms which produce the disease and do not appear against those which do not, even though they may be multiplying in the intestinal tract. It was because of this fact that the Japanese bacteriologist, Shiga, was able to prove the relationship between an organism he had isolated from a case of dysentery and the disease. He took the serum from the individual after recovery and tested it for its ability to agglutinate the various organisms he had isolated. One was agglutinated by this serum and hence assumed and later proven to be the cause of the dysentery. Such indirect immunological studies are extremely useful when working with viruses or when working with organisms that do not produce disease in experimental animals.

Pathology

The pathologist is primarily interested in the effect of infection on the body. Modern medicine is dependent upon the information supplied by the pathologist and could not have developed nor existed without him. While we will not undertake at this time to outline his special contributions, we can indicate how the information he has obtained is utilized by the bacteriologist and immunologist and epidemiologist.

In general, each disease manifests itself by a characteristic pathology In some these changes are in the intestinal tract; in some in the brain, and so on. The nature of these changes is characteristic of the causal agent. In many virus diseases intracellular inclusions are found and the finding of such inclusion bodies suggests a virus etiology and gives the investigator a lead as to how to proceed to isolate or identify the etiologic agent.

The location of the disease process also aids in determining the manner in which the organism can leave the body, a fact of paramount importance, as we have seen, in disease control.

In many diseases the final verdict in diagnosis is left to the pathologist, but in making diagnosis, he, in turn, depends upon immunologic and bacteriologic and clinical findings in addition to his own.

Medicine

The physician's interest lies primarily in the alleviation of suffering and in the cure of disease. He deals with the individual patient and by virtue of his training he alone is qualified to make a clinical diagnosis and prescribe treatment. He leans heavily on supporting sciences for aid in diagnosis and for materials and suggestions as to curative agents. He interprets the findings of the specialists with a view to obtaining a complete picture of disease as it affects the individual. While he is making use of the other sciences, he is also observing and collecting data that are of immeasurable value to them. He is the keystone in the arch upon which our knowledge of disease rests.

Vital Statistics

In order to control disease it is necessary to know where it occurs, how frequently it occurs, under what conditions it occurs, in whom it occurs, how many succumb, and what the cause of death may be.

Vital statistics are records of the vital events of life such as birth, growth, marriage, sickness, and death. They are an inventory and a history of the population. They record facts as to the origin, nationality, race, sex, age, occupation, and place of residence. Demography is a broader term sometimes used for the statistical study of human life.

The collection of data on the population is an ancient custom. Churches recorded marriages, deaths, and births; and the ancient rulers collected information for military and taxing purposes. The colonists recognized the need for accurate data on the number of people, and in 1787 Congress passed a bill providing for the taking of a census for the purpose of taxation and elections.

PERIOD IN HISTORY	AVERAGE LIFE	SOURCE OF INFORMATION
Early Iron and Bronze Age.	Perhaps 18 years	Prehistoric bones
About 2000 years ago.....	About 22 years	Ages at death on Egyptian mummy cases
Middle Ages (Before 1276).	About 35 years	Inheritance of property records in England
1687–1691.....	33.5 years	Records in Germany
Before 1789.....	35.5 years	Death records in New Hampshire and Massachusetts
1838–1854.....	40.9 years	Records in England and Wales
1900–1902.....	49.2 years	Records in United States
1945.....	65.8 years	Records in United States

From a *Statistical Bulletin, Metropolitan Life Insurance Company.*

Our present Federal Bureau of Census conducts a census every ten years. Each census has seen the inclusion of new questions. At present these cover a large variety of topics of interest to the government, business, manufacturing, and professional groups. In 1902 Congress authorized a permanent census act that included authorization to collect birth and death statistics from states or subdivisions which were at that time keeping satisfactory records. Beginning in 1915, the Bureau of Census established registration of births for some of the states. By 1930 all but Texas were included and at present all are. In analyzing data taken at various times in the United States it should be remembered that only recent data are comparable.

States are divided into registration districts varying considerably as to size with a local registrar for each district. He is responsible for reporting births and deaths to the state registrar and to the local or state health officer. Thus there is a direct connection between the registrar and the health department; in fact, in many instances the state registrar appoints a member of the health department staff as deputy registrar.

Reporting of Deaths: In reporting deaths a standard certificate containing data relative to the cause of death is filled out and filed with the local registrar. This is usually done by the physician in charge. There was considerable variation in the classification of disease used in reporting formerly but this has been remedied by the adoption of an International Standard Classification of Cause of Death. Burial permits are necessary before interment can be made and a death certificate properly filled out is required before these can be issued. Since the fact of death is usually known to a number of people, the number of deaths reported is fairly accurate. The cause assigned is less so but is becoming more dependable every year.

Reporting of Illness: Illnesses due to infectious diseases deemed of public health importance are required by law to be reported. The following list

TABLE 5

Crude Death Rate for Selected Causes

	1935	1945
Diseases of the Heart	245.4	321.5
Cancer and other malignant tumors	108.2	134.5
Intracranial lesions of vascular origin	85.7	97.9
Nephritis	81.3	66.7
Pneumonia all forms and influenza	104.2	51.8
Accidental death all forms	78.4	72.7
Tuberculosis (all forms)	55.1	40.1
Diseases peculiar to the first year	40.2	38.5
Diabetes mellitus	22.3	26.1
Senility, ill-defined and unknown causes	24.00	22.0
Syphilis	15.4	10.7
Cerebrospinal (meningococcus) meningitis	2.1	1.3
Whooping cough	3.7	1.3
Diphtheria	3.1	1.2
Dysentery	1.9	1.2
Acute rheumatic fever	1.8	1.0
Poliomyelitis	0.8	0.9
Typhoid and paratyphoid fevers	2.8	0.4
Scarlet fever	2.1	0.2

Rates per 100,000 estimated population.

Data from Vital Statistics: Special reports Vol. 26, No. 1, 1947.

Note: The first ten diseases listed accounted for 78.7 per cent of the total deaths. Diseases of the heart accounted for 30.3 per cent of the deaths. The diseases of older age groups show a considerable increase and reflect changes in the age distribution of our population.

of communicable diseases for which notification is usually required is taken from Reprint No. 1697 of the United States Public Health Reports.

Actinomycosis
Ancylostomiasis (hookworm disease)
Anthrax
Chicken pox (varicella)
Cholera
Conjunctivitis, acute infectious
Dengue
Diphtheria
Dysentery, amebic (amebiasis)
Dysentery, bacillary
Encephalitis, infectious, lethargic and nonlethargic
Favus
German measles (rubella)
Glanders (farcy)
Gonorrhea
Influenza
Leprosy
Malaria
Meningococcus meningitis
Mumps (parotitis)
Paratyphoid fever
Plague, bubonic, septicemic, pneumonic
Pneumonia, acute lobar

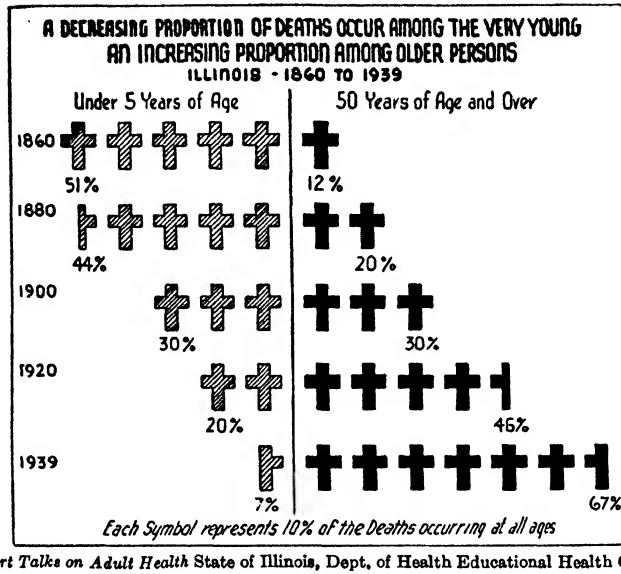
Poliomyelitis
Psittacosis
Puerperal infection (puerperal septicemia)
Rabies
Rocky Mountain spotted (or tick) fever
Scarlet fever (scarlatina)
Septic sore throat (streptococcus throat infection)
Smallpox (variola)
Syphilis
Tetanus
Trachoma
Trichinosis
Tuberculosis, pulmonary
Tuberculosis, other than pulmonary
Tularemia
Typhoid fever
Typhus fever
Undulant fever (brucellosis)
Whooping cough
Yellow fever

The physician is required to notify the health department of such of these cases as he attends. The accuracy of the reports of the number of cases of specific diseases is quite variable. Some are readily diagnosed by the physician, whereas in others, as for example in brucellosis or undulant fever, the symptoms are variable and accurate diagnosis may depend upon laboratory findings. In some diseases there are many mild cases which do not come to the attention of the physicians and hence are not reported. This is illustrated by reports from one city which listed seventy-nine cases and seventy-nine deaths from influenza for the year. If this were true, which it obviously is not, the case fatality of influenza would have been one hundred per cent. The physicians do not report and people do not seek medical attention except in the more severe cases. Reports of infectious diseases which show epidemic tendencies or seasonal distribution are more accurately diagnosed and reported during epidemics or seasons in which the incidence is high.

Bearing in mind that deaths from specific diseases are more accurately reported than cases and that deaths are always reported whereas for one reason or another cases are not, the case fatality usually given for any specific disease is far too high.

The reporting of illness due to the venereal diseases deserves separate attention. For various reasons persons who have or have reason to suspect that they have contracted syphilis or gonorrhea are prone to consult quacks or charlatans and their cases are not reported. Others seek aid and advice from druggists and it is of interest to know that a recent survey showed that well over half the druggists approached made a diagnosis and offered to sell remedies, this in spite of the fact that only licensed physicians are qualified to diagnose and prescribe treatment.

Records from hospitals and clinics are more apt to be accurate from the standpoint of diagnosis than those from private physicians; but there is another type of error in hospital records worthy of mention. In many diseases, as for example, diphtheria, scarlet fever, and whooping cough, the milder cases are not so apt to be hospitalized as are the more severe.



From *Short Talks on Adult Health* State of Illinois, Dept. of Health Educational Health Circular, # 84

FIG. 40

TABLE 7

Age Distribution of Population in United States

AGE	1940	1930	1920	1910	1900	1890	1880
All ages	100.0	100.0	100.0	100.0	100.0	100.0	100.0
Under 5	8.0	9.3	10.9	11.6	12.1	12.2	13.8
5–9	8.1	10.3	10.8	10.6	11.7	12.1	12.9
10–14	8.9	9.8	10.1	9.9	10.6	11.2	11.4
15–19	9.4	9.4	8.9	9.9	9.9	10.5	10.0
20–24	8.8	8.9	8.8	9.8	9.7	9.9	10.1
25–29	8.4	8.0	8.6	8.9	8.6	8.3	8.1
30–34	7.8	7.4	7.6	7.6	7.3	7.3	6.7
35–39	7.2	7.5	7.4	7.0	6.5	6.2	6.0
40–44	6.7	6.5	6.0	5.7	5.6	5.1	4.9
45–49	6.3	5.7	5.5	4.9	4.5	4.4	4.2
50–54	5.5	4.9	4.5	4.2	3.9	3.7	3.7
55–59	4.4	3.8	3.4	3.0	2.9	2.7	2.5
60–64	3.6	3.1	2.8	2.5	2.4	2.3	2.2
65–69	2.9	2.3	2.0	1.8	1.7	1.6	1.4
70–74	2.0	1.6	1.3	1.2	1.2	1.1	1.0
75–79	2.0	0.9	0.8	0.7	0.7	0.6	0.6
80–84		0.4	0.4	0.3	0.3	0.3	0.3
85–over		0.2	0.1	0.1	0.1	0.1	0.1

die; and mortality statistics refer to fatal cases only. It is customary to express these figures as *rates*, or the number of cases or deaths occurring

every year in units of population of 1000 or of 100,000. The base ordinarily used in dealing with cases or deaths from specific causes, such as typhoid fever or tuberculosis, is 100,000 because the rates can then be stated in whole numbers. The base used in expressing the total death rate is usually 1000. Thus, in 1946, the death rate from tuberculosis was said

TABLE 8

Crude Birth and Death Rates, Maternal and Infant Mortality Rates, and Stillbirth Ratios: Registration States,[1] for Specified Years

(Births and deaths exclusive of stillbirths. Birth and death rates per 1,000 estimated population; maternal and infant mortality rates and stillbirth ratios per 1,000 live births)

YEAR	CRUDE BIRTH RATE	CRUDE DEATH RATE	MATERNAL MORTALITY RATE	INFANT MORTALITY[2] RATE
1945	[3]19.6	[4]10.6	2.1	38.3
1944	[3]20.2	[4]10.6	2.3	39.8
1943	[3]21.5	[4]10.9	2.5	40.4
1942	[3]20.9	[4]10.4	2.6	40.4
1941	[3]18.9	[4]10.5	3.2	45.3
1940	[3]17.9	[4]10.7	3.8	47.0
1939	17.3	10.6	4.0	48.0
1938	17.6	10.6	4.4	51.0
1937	17.1	11.3	4.9	54.4
1936	16.7	11.6	5.7	57.1
1935	16.9	10.9	5.8	55.7
1934	17.2	11.1	5.9	60.1
1933	16.6	10.7	6.2	58.1
1932	17.4	10.9	6.3	57.6
1931	18.0	11.1	6.6	61.6
1930	18.9	11.3	6.7	64.6
1925	21.3	11.7	6.5	71.7
1920	23.7	13.0	8.0	85.8
1915	25.0	13.2	6.1	99.9
1910	—	14.7	—	—
1905	—	15.9	—	—
1900	—	17.2	—	—

[1] Includes entire continental United States, beginning with 1933.
[2] Deaths under 1 year of age.
[3] Based on total population including armed forces overseas.
[4] Excludes armed forces overseas.

to be 36.4, that is 36.4 deaths in 100,000 persons; and the total death rate or the deaths from all causes, about 10 in 1000 persons.

Crude, Specific, and Standardized Rates: Rates expressed in terms of all persons in the population unit are called *crude rates.* Now, some diseases, such as measles or whooping cough, are most prevalent in childhood; some,

such as pneumonia, in infancy and old age; some, such as heart disease and cancer, in old age; and some, evenly distributed through all the ages. Populations may also vary in their composition. Wars, epidemics, differences in birth rates, and migrations may considerably alter the age distribution of a population. To give a true disease picture, it is necessary to make corrections for these differences. This is done by the use of either specific or standardized rates.

Specific rates are expressed in terms of the proportion of cases or deaths in a particular age group.

Standardized rates are expressed in terms of a standard population. The standard population used is the population of Sweden in 1890, which is standard for age distribution only, or the population of England and Wales for 1901, which is standard for both age and sex.

In some diseases it is necessary to make corrections for race and occupation before comparing morbidity and mortality rates.

Case Fatality Rate: The term case fatality rate refers to the percentage of deaths, that is, to the number of deaths in every 100 cases of a particular disease.

Prevalence: The prevalence of a disease is a function of its incidence times its duration.

The epidemiologist takes the information obtained from various sources, adds to it his own observations, and attempts to present a complete picture of disease as a group phenomenon. He makes frequent use of statistical analyses and "spot maps" in running down the source of infection and determining the manner of transmission.

The epidemiological approach to disease can be well illustrated in the case of typhoid fever. The two principal facts which serve as a starting point are a knowledge of the mode of transmission and of the incubation period. In typhoid fever, infection results from the ingestion of milk, food, or water contaminated by discharges from cases or carriers. The incubation period is, on the average, seven to fourteen days and only in rare instances as short as three days or as long as four weeks. Therefore, in all probability, infection must take place seven to fourteen days previous to the onset. The epidemiologist must then try to find the source. He may proceed as follows:

Data To Be Obtained: He first obtains a complete list of all known and suspected cases from health officers, hospitals, and physicians. From the records of these cases, he secures the following items: name, address, age, sex, occupation, race, date of onset, and any other information deemed pertinent.

By further inquiry he finds out where the individuals ate, and what foods

they consumed during the period from seven to fourteen days before the onset of typhoid. He pays particular attention to their sources of water and milk. Then, having gathered this information, he may proceed to analyze it with a view to finding a common factor.

Arrangement of Data: As an aid to finding the common factor, a map of the region is secured and the location of the cases spotted. Next, the milk routes of the various companies serving the community and the water supplies are indicated on the map, and the public eating places are located. If there are known carriers in the community, their location is also indicated. Then an age, sex, and occupational distribution of the cases is made.

Analysis and Interpretation: If the cases are grouped in one section of the city, it narrows down the search for the common factor. If most of the cases are in houses served by a particular milk company, that milk might be suspected as the conveyer. If the cases are more common in children and women and if the outbreak is explosive, this is further evidence that milk is probably involved.

If the water supply is wells, or if there are several distribution systems, contamination occurring in one of them would give rise to a localized outbreak.

If the cases are limited to adults taking part of their meals away from home, some restaurant or hotel might be suspected of serving contaminated food, water, or milk.

If the cases are scattered throughout a city and occur in persons eating in many different places, the water supply might be suspected.

Having tentatively incriminated a milk supply, let us say, the source of its contamination is next sought. Was the milk pasteurized? If so, where? Have any new handlers been added recently to the employees? What persons were in a position to contaminate the supply? All persons who could possibly have contaminated the milk supply would be investigated. Bacteriological examinations of stools and urine would be made to determine the presence of a carrier. The health history of the employees would also be checked to see if any had had typhoid fever at some time and might possibly be carriers. The water supply and the equipment of the plant would also be carefully investigated. On the basis of information obtained by such procedures, the epidemiologist would determine whether that milk supply was indeed the source of the infection and, if it were, recommend logical steps for action by the public health officers.

Epidemiological studies are valuable in determining the incubation period, the period of communicability, the value of immunization, the duration of immunity either naturally or artificially acquired, the effectiveness of isolation and quarantine, and a number of such facts concerning

disease. They serve the health department as a guide for administrative action directed toward preventing the spread of infection and, if possible, eliminating its source.

SUMMARY

It is evident then that the kind and incidence of infectious disease in any species reflect the life habits and nutritional requirements of the host and the parasite. The native or natural susceptibility of the host and the way in which it lives will determine the kinds of disease that can prevail. It is the natural susceptibility of any host that determines whether a parasite can increase or multiply in that particular species, a condition necessary for its survival. If it can survive and multiply, its portal of entry, site of localization, mode of exit, and its resistance and longevity will determine its manner and chance of transmission.

The infectious diseases of man are an indication of his habits and customs. It is not happenstance that certain diseases have been more prevalent in one group or in one historical period than in another. As a group changes its cultural pattern, its diseases change. Some are completely eliminated, others increase markedly. Many diseases that formerly raged have all but disappeared, not because of conscious control nor the application of preventive methods, but simply because man has changed his personal habits. Louse-borne typhus fever, for example, vanished where the louse did. Water-borne, milk-borne, and droplet diseases are most prevalent in countries civilized enough to build large cities but not enlightened enough to deal effectively with the resulting pollution of air and water and food supplies.

In a disease such as leprosy which has disappeared from most of the temperate zone, the pathogen seems to have lost its infectivity. And some diseases, as for example, scarlet fever, are far less dangerous than they were a hundred or even fifty years ago for reasons not entirely known but probably because the pathogens have lost much of their virulence.

From the standpoint of an epidemiologist, diseases might be grouped as follows:

1. Droplet-borne diseases in which man is the only important host—tuberculosis, diphtheria, scarlet fever, measles, the common cold, influenza, and, probably, poliomyelitis. The portal of entry is the upper respiratory tract and although food, water, and milk may actually convey the organisms, the infection does not take place through the intestinal tract.

2. Water and food-borne diseases in which man is the important host—typhoid fever, bacillary and amoebic dysentery, cholera, and paratyphoid fever. The portal of entry is the mouth. The mode of exit is the intestinal or urinary tract.

3. Insect-borne diseases in which man is not the natural host—bubonic plague, most rickettsial diseases as scrub typhus and Rocky Mountain spotted fever, murine and endemic typhus, and equine encephalitis in man. The parasite is introduced into the blood stream by the insect vector.

4. Insect-borne diseases in which man is the important host—epidemic or louse-borne typhus fever, urban yellow fever, and malaria. The parasite is introduced into the blood stream by the insect vector.

5. Diseases transmitted to man by contact with animals—brucellosis, tularemia, foot and mouth disease (rarely), anthrax, and rabies.

6. Diseases transmitted by human contact—gonorrhea, syphilis, and lymphogranuloma inguinale.

SECTION III
COMMON INFECTIOUS DISEASES

Chapter XV

SOME INFECTIONS DUE TO THE STAPHYLOCOCCI

It need not surprise us to find that the healthy human skin harbors billions of microorganisms; for, although it appears smooth to us, in relation to the size of the microorganisms it is a veritable series of canyons and mountains in which there is ample moisture, a suitable temperature, and a constant supply of food in the form of dead body cells. The flora of the skin is varied in kind and although bacteria predominate, yeasts and fungi and even protozoa may be present. Many of these microorganisms are there as a result of contamination. The hands, for instance, come in contact with the mouth and become covered with the microbes normally present there. They also come in contact with all manner of foreign objects harboring microorganisms of every sort. As a rule such organisms do not grow on the skin and so need not concern us here. Of the different species of bacteria for which the skin is the normal habitat, by far the greatest proportion are gram-positive cocci of the Family *Micrococcaceae* and of these the staphylococci are of greatest interest.

CHARACTERISTICS OF THE STAPHYLOCOCCI

Ecology: The staphylococci are parasitic. The bodies of men and animals appear to be their normal habitat and they are present almost universally on the skin. They may also be found in the mouth and intestinal contents of man and animal, in the air, in sewage, in water, and in milk. Some are pathogenic.

Morphology: The staphylococci (*staphylo* meaning grape and *coccus* meaning round) are round, nonmotile, nonsporulating cells averaging from 0.7 to 1.0 micron in diameter and arranged in grape-like clusters, as the name indicates, or in pairs. When they are grown in liquid medium, they sometimes form short chains which may be confused with chains of streptococci.

Staining: All true staphylococci are gram-positive when freshly isolated and when in young cultures not more than eighteen to twenty hours old. On aging some strains tend to be rather readily decolorized and some become gram-variable.

Physiology: All species of staphylococci produce pigment and are named according to the color of the colonies on agar: *albus* for white colonies,

aureus for golden, and *citreus* for yellow. The property of pigment production is variable and aureus strains which, in general, are most active physiologically and pathogenically may be dissociated and form pure albus or citreus colonies.

Staphylococci grow readily on most media. Most strains of aureus liquefy gelatin, albus strains do not. Carbohydrate fermentation is also variable and consequently of little use in identification or classification.

They will grow at temperatures ranging from 12° C. to 43° C., their optimum growth temperature being about 37° C. or body temperature. They are facultative anaerobes.

Resistance: The resistance of the staphylococci to heat, cold, and disinfectants is greater than that of most nonsporulating bacteria.

Variability: As may have been gathered, the staphylococci vary greatly with respect to their morphology and physiology. By subjecting cultures to various procedures a number of variations may be observed: smooth cultures may vary to produce rough or viscous colonies, aureus cultures may dissociate to produce colonies ranging from a deep golden to white, and cultures may be induced to vary in respect to carbohydrate fermentation and proteolysis.

Pathogenicity: Staphylococci also vary in regard to pathogenicity. Those isolated from the skin, air, and dust are relatively non-pathogenic. Those isolated from boils, carbuncles, and other lesions are pathogenic. Aureus strains are most virulent. Citreus strains are rarely so. In general, staphylococci are more virulent for man than for animals.

MECHANISM OF DISEASE PRODUCTION

Staphylococci produce a number of toxic substances that unquestionably determine the characteristic features of the infections produced. Although some of the toxins may produce more than one reaction, we shall, for convenience, consider them under separate headings.

Endotoxins: Staphylococci do not possess any very potent endotoxins, although abscesses may be produced experimentally by the injection of large numbers of dead washed cells.

Exotoxins: Filtrates of cultures of the pathogenic staphylococci contain several exotoxins, some of which probably produce more than one reaction. These exotoxins are demonstrable in a number of ways and are named according to the nature of the reactions they produce.

Many strains of staphylococci produce *hemolysins* which lyse or break down red blood cells. When these strains are grown on blood agar, the colonies are surrounded by clear colorless zones and when they are inoculated into tubes of liquid media containing red blood cells, a clearing results. How much lysis of red blood cells occurs in the body during

infections is not known but since hemolytic strains are more virulent than non-hemolytic strains there is reason to believe it occurs. The hemolysin is antigenic and antihemolysins are produced in immunized animals or man.

Leucocidins, the substances that destroy leucocytes, are produced both *in vivo* and *in vitro*. While nearly all varieties of *Staphylococcus aureus* produce them, the amount produced by different strains may vary as much as a hundredfold. The leucocidins are antigenic and antileucocidins may be obtained from immunized animals and man as well as from 'normal' individuals.

Necrotoxins are produced by some staphylococci. When staphylococcal filtrates are injected into the tissues of animals, particularly of rabbits, necrosis occurs. Neutralizing antibodies to the necrotoxins may be produced in animals and are found in the serum of some humans.

Lethal Toxins: The intravenous injection of staphylococcal filtrates into rabbits produces a toxemia which may be fatal. Although there appears to be a definite relation between the hemolyzing and necrotizing power of filtrates and their lethal power, there is evidence that the toxins involved are different.

Enterotoxins: Filtrates from many strains of both albus and aureus produce violent intestinal disturbances, diarrhea, and vomiting in humans. Many outbreaks of "food poisoning" caused by eating such foods as cakes with cream fillings, cream puffs, or chocolate eclairs have been proven due to an enterotoxin produced by the staphylococcus. The conditions necessary for its production are not clear but it has been shown that media containing a high starch and carbon dioxide content favor it. Indirect evidence for this is that a large number of the outbreaks of staphylococcal food poisoning have been traced to foods having a high starch content. The enterotoxin is distinct from the other staphylococcal toxins.

Other Products of Staphylococcal Growth: In addition to the toxic substances mentioned, the staphylococci produce other substances which may influence infection. These are: *staphylocoagulase* which coagulates or clots citrated or oxalated blood, hyaluronidase which alters the permeability of the skin and aids or hastens the spreading of staphylococci, *chemotactic substances* which attract the polymorphonuclear leucocyte to the site of infection, and *fibrinolysin* which breaks down blood clots by destroying the fibrin. Fibrinolysin production is more commonly associated with hemolytic streptococci than with the staphylococci although it has been reported for some strains. The accumulation of the leucocytes gives rise to pus and to the term *pyogenic* or 'pus-producing' which is frequently applied to the staphylococci.

DISEASES DUE TO STAPHYLOCOCCI

The staphylococcus is the common cause of a whole host of infections. These are almost invariably associated with a characteristic pus formation which is of primary value in the diagnosis of the cause of the infection.

Septicemia: While there is a tendency for staphylococci to produce infections that remain localized at the site of the initial lesion, they do get into the blood stream and occasionally multiply there producing septicemia or what is commonly called "blood poisoning." The streptococci are, however, more commonly associated with this condition.

Pyemia: Not infrequently the staphylococci enter the blood stream and are carried to other parts of the body where they produce localized abscesses. The term pyemia is used to designate such a condition. A number of very severe and often fatal infections in man are due to staphylococcal pyemia.

Boils, Pimples, . . . The staphylococcus is the primary cause of pyogenic infections such as pimples, styes, boils, carbuncles, and furuncles. It is by far the most common cause of pus in wounds. Prior to the introduction of antiseptic and, later, aseptic surgery, pus in surgical wounds was considered a good sign and was spoken of as "laudable pus." Experience had shown that if it appeared the patient's chances of surviving were better than if it did not. At that time the chances were that all wounds were contaminated either with staphylococci or streptococci. The streptococci, under such conditions, would probably produce a rapidly fatal septicemia without the formation of pus; whereas the staphylococci would be localized by the body defenses, pus would be produced, but eventually the infection would be overcome. The modern conception of "laudable" surgery is no pus and no infection.

The site of the infection is of considerable importance. Boils on the upper lip or around the nose are particularly dangerous because of the chances of a fatal septicemia should the staphylococcus invade the venous sinuses.

Stitch Abscesses: The skin is difficult to sterilize even by the vigorous methods employed in preparation for surgery, consequently the slightly virulent staphylococci normally present on the skin may not all be destroyed or removed before an operation and may invade at the site of the stitches that close the wound and produce mild inflammations known as stitch abscesses.

Impetigo: The staphylococci are the usual cause of *impetigo contagiosum* that occurs so commonly in children. The small boils or sores are spread over the face and body by scratching. Impetigo often assumes epidemic proportions in nurseries and schools.

Osteomyelitis: In addition to the common and more superficial infections, the staphylococci are the prime cause of osteomyelitis of the bone marrow commonly following blows or trauma. Such infections may be very acute and often develop into a septicemia.

Other pyemias such as kidney and liver abscesses are also the result of blood-borne staphylococci.

Staphylococci also may produce bronchopneumonia, meningitis, puerperal sepsis or child bed fever, mastitis, middle ear infections, peritonsillar abscesses such as quincy sore throat, tonsillar abscesses, and a number of other diseases.

BACTERIOLOGICAL DIAGNOSIS OF STAPHYLOCOCCAL INFECTIONS

This is relatively simple. Microscopic examination of direct smears from pus will reveal gram-positive cocci with their characteristic irregular grouping. Plain agar cultures will show the characteristic aureus or albus colonies. Blood agar plates will differentiate the hemolytic from the non-hemolytic strains. It must be remembered that staphylococci are frequently present in lesions as secondary invaders, and that this fact must be considered in making a diagnosis.

SEROLOGY AND IMMUNITY

Numerous attempts have been made to classify the staphylococci serologically. In general, it can be said that such classification does not parallel the classification based on physiology or pigment production. However, there appears to be a close association between serologic types and pathogenicity. The agglutination reaction is of little value in differentiation but the precipitin reaction employing chemically fractionated antigens is valuable. The *type* specificity is associated with the polysaccharide fractions and the group specificity with the protein. That there is considerable specificity among the staphylococci is indicated by the fact that although animals and man may be readily immunized against staphylococci, autogenous vaccines are far more effective curative agents than vaccines made from stock strains or from strains isolated from other individuals. The use of toxoid prepared from filtrates containing toxic substances has been successful in the treatment of chronic and acute staphylococcal infections.

The resistance to staphylococcal infection depends upon general body resistance as well as upon a specific response to infection. It is a common observation that staphylococcal infections frequently occur during childhood and adolescence and that after having a series of boils, the individual remains comparatively free. This is due to an actively acquired immunity; although if the infection produced a strong immunity, secondary crops of

boils would not be so common. The frequently successful treatments of staphylococcic infections by the use of toxoid or autogenous vaccines also indicate that some degree of active immunity is produced.

Staphylococcal antitoxin, prepared by injecting toxin into horses, has been used in the treatment of a number of staphylococcal infections, including osteomyelitis, septicemia, and meningitis. Although the data available are limited, it would seem that the use of staphylococcal antitoxin is of questionable value.

PREVENTION AND CONTROL

In general, the staphylococci freshly isolated from boils, carbuncles, or abscesses are far more virulent and invasive than those commonly present on the skin. Persons coming in contact with such discharges should be particularly careful to avoid contaminating wounds or skin abrasions. Surgical procedures may be indicated in some cases of boils, but staphylococcus infections are usually self-limiting, a fact which makes it difficult to assess the value of any treatment. Chemotherapeutic agents of the sulfa-group and penicillin are effective for most types of staphylococcus infections.

SUMMARY

The staphylococci are normal inhabitants of the skin and although they present a fairly homogeneous group, there is considerable variation in their characteristics. They are opportunists and tend to invade when the resistance of the body is lowered or the surface of the skin is broken. They probably do not penetrate the intact skin but may enter by way of the hair follicles or sweat glands. Although they may invade any organ or tissue, they usually produce a lesion characterized by infiltration of leucocytes and pus formation. From a local lesion, they may enter the blood stream and be carried to other parts of the body where they may produce secondary lesions.

Staphylococci produce an enterotoxin when they are growing in certain foods. This toxin produces a type of food poisoning when ingested by man.

The staphylococci produce a number of toxic substances which probably account for the type of disease resulting. Immunity to infection may be increased by the use of toxoid or autogenous vaccines. It appears to be short lived, however, and such injections are more often used as curative than as preventive measures.

Treatment with penicillin is, in general, the most effective therapeutic measure.

Chapter XVI

SOME INFECTIONS DUE TO THE STREPTOCOCCI

As a cause of suffering and death the streptococci are probably the most important group of bacteria. They may produce mild or severe disorders in almost every tissue or part of the body. They are the primary cause of a number of specific diseases; as secondary invaders they are responsible for a number of others; and as a terminal cause of death they are probably the most important of all human pathogens. In addition to specific diseases which they are known to cause, they have been implicated in many more maladies.

CHARACTERISTICS OF THE STREPTOCOCCI

Although they had previously been described by Ogsten (1881) and Fehleisen (1893), it was Rosenbach (1884) who first used the term *Streptococcus* to describe the round organism growing in chains and, since it was associated with pus, he gave the name *Streptococcus pyogenes* to the species.

Morphology and Staining: The streptococci are round or ovoid cells. Division takes place on one plane and the cells do not readily separate, hence the characteristic arrangement in pairs or in short or long chains. They are nonmotile, nonsporulating, and gram-positive. Some species produce capsules.

Physiology: Most species of streptococci are facultatively anaerobic, a few are strict anaerobes, and some are microaerophilic. The colonies on artificial media are small and their growth in liquid media slight, although it becomes greater if dextrose or other fermentable sugar is added. Streptococci do not grow readily apart from animal protein or milk and this has a bearing on the way in which many streptococcal diseases are transmitted. The streptococci producing scarlet fever and septic sore throat, for instance, grow readily in milk which, consequently, often acts as the vehicle by means of which they are spread.

The streptococci ferment many sugars with the production of acids. They do not, as a rule, ferment inulin and are insoluble in ox bile or bile salts, a fact which serves to distinguish the *Streptococcus viridans* from the pneumococcus. They grow best at body temperature, but will grow in temperatures ranging from 20° C. to 42° C. Their growth on blood media is characteristic and is used in classification.

Classification: For practical purposes streptococci are first separated according to their hemolytic reactions on blood agar, and then further subdivided according to immunologic and biochemical reactions and

pathogenicity. Brown has made an intensive study of their reactions on blood agar and distinguished three basic groups:

The *Alpha Type* or *Streptococcus viridans* produces a greenish discoloration and partial hemolysis of the blood cells surrounding the colony in twenty-four to forty-eight hours. The green color is due to the action of the liberated hydrogen peroxide which changes the hemoglobin to metahemoglobin and probably to other compounds. The green-producing streptococci are characteristically associated with sub-acute and chronic focal infections such as middle ear infections, dental infections, endocarditis, and possibly with certain types of chronic and acute rheumatism.

The *Beta Type* or *Streptococcus hemolyticus* hemolyzes or lakes red blood cells and the colonies on blood agar are surrounded by clear zones. It is characteristically associated with the more acute infections. There is an almost infinite variety of strains within the hemolytic group which may be further distinguished by serologic, toxogenic, and other tests.

The *Gamma Type* or *gamma streptococci* produce no visible change on blood agar. They are found in milk and dairy products.

Immunologic Reactions: It is only natural that since the *Beta* hemolytic streptococci are most important from the standpoint of disease, they should prove of greatest interest and be studied most intensively. Biochemical reactions alone have not proven of much use in classifying the members of the group so considerable attention has been paid to their serologic relationships. Griffith, using the agglutination reaction, and Lancefield, using refined precipitin tests, have shown that these streptococci fall into a number of groups, the members of which may differ considerably in respect to their fermentation reactions. Lancefield found that *Beta* hemolytic streptococci contain a group-specific partial antigen, a polysaccharide haptene called a "C" substance, and a type-specific protein antigen called an "M" substance.

She distinguished five serologic groups which correlated with natural habitat and host range and designated them by letters.

Group A is pathogenic for man causing scarlet fever, septic sore throat, tonsillitis, and puerperal sepsis. It sometimes causes mastitis in cattle.

Group B is pathogenic for cattle and is the common cause of mastitis. It may be present in milk.

Group C is pathogenic for cattle, horses, and other animals. It produces strangles in horses and is only occasionally found in man.

Groups D and E are found in dairy products and milk and are not pathogenic.

To complicate further the difficult problem of classification that they present, the streptococci tend to vary or dissociate. It may be said that, in general, any classification of the streptococci must necessarily be tenta-

tive until more data are on hand concerning the significance of the differences observed in their physiology and serology.

Resistance: The streptococci die off rather rapidly when exposed to air in objects such as glass slides, telephone mouthpieces, and the like, but when protected from drying, as by sputum, for instance, they may survive for several weeks. They are killed by an exposure of thirty minutes to a temperature of 55° C. Pasteurization of milk destroys the pathogenic types. They are neither highly resistant nor particularly susceptible to disinfectants.

Pathogenicity: The streptococci range from harmless saprophytes and parasites to highly virulent pathogens. They are the opportunists *par excellence*, since they tend to invade whenever the resistance of the body is lowered or the defensive mechanism disrupted. In man and animals they produce pyogenic infections and septicemias and are the primary cause of a number of specific diseases.

Diseases Due to Streptococci

To Streptococcus viridans	*To Streptococcus hemolyticus*
Sub-acute bacterial endocarditis	Scarlet fever
Rheumatic fever (according to some workers)	Puerperal sepsis
Arthritis (according to some workers)	Erysipelas
Middle ear and sinus infections	Septic sore throat
Abscesses of teeth	Tonsillitis
Various focal infections	Middle ear infections
	Pyogenic infections of many tissues
	Bronchopneumonia
	Rheumatic fever

A classification of streptococci based on the specific diseases produced is not satisfactory for, although some workers hold opinions to the contary, there are many who feel that the same streptococci may produce tonsillitis, scarlet fever, puerperal sepsis, or erysipelas. Strains differing in many other respects may produce scarlatinal toxin or erysipelas toxin. The clinical manifestations of infection due to the same hemolytic streptococcus are many and the type of clinical disease may, within limits, be due as much to the reaction of the individual and the location of the lesion as to the organism.

MECHANISM OF DISEASE PRODUCTION

Many features of streptococcal diseases may be related directly to demonstrable toxic products.

Endotoxins: There is little evidence that the streptococcal cell contains

within itself any poisonous or toxic substances that play a part in the direct production of disease symptoms.

Exotoxins: Streptococci produce a number of extracellular substances, some of which, although differing in some respects from the classical bacterial exotoxins, may, nevertheless, be considered as such. The toxic substances are demonstrable in a number of ways and are named according to visible manifestations of their presence.

Hemolysin: As previously mentioned, some varieties of streptococci produce an hemolysin which breaks down red blood cells. It is thermolabile and heating for thirty minutes at 58° C. to 60° C. inactivates or destroys it. The hemolytic strains are the more virulent and invasive but it is not easy to relate the symptoms of streptococcus infection definitely to the streptohemolysin. It seems to be of great importance in septicemia.

Leucocidin: The streptococci, in common with a large number of pathogenic bacteria, produce leucocidins. They are thermolabile, being inactivated by heating for thirty minutes at 60° C. It is probable that the streptoleucocidin enhances the invasiveness of streptococci by preventing phagocytosis.

Fibrinolysin: A number of streptococci produce fibrinolysin, a substance discovered by Tillet and Garner in 1933, which breaks down the clotted fibrin of blood. It also acts on fibrinogen, changing it so that it no longer forms fibrin. It is heat stable and is not inactivated even by heating to 100° C. for fifty minutes. Its species specificity is rather remarkable. Strains of streptococcus isolated from humans break down human fibrin but not the fibrin of animal blood. Strains of streptococci isolated from horses will lyse horse fibrin but not human fibrin. It seems probable that the fibrinolytic substance may be one determining factor in the pathogenicity of certain strains for certain species of animals. During the course of infection the body apparently builds up an antibody against the fibrinolysin. The serum taken from individuals who have recovered from streptococcal infection inhibits fibrinolysis by the streptococcus or culture filtrates. The clotted fibrin of such individuals is highly resistant to lysis. The fibrinolytic substance undoubtedly explains in part the peculiar invasiveness of streptococci.

Hyaluronidase: Hyaluronidase is present in filtrates of some streptococcal cultures and lysed cells. It will be recalled that this factor changes the permeability of tissue in some fashion so that various inanimate substances as well as bacteria and viruses can spread through it more rapidly. Its relation to virulence, however, is not clearly known.

Scarlatinal Toxin: Certain strains of streptococci produce a powerful toxin which will elicit the symptoms of scarlet fever and which will be considered in connection with the disease.

PRINCIPAL DISEASES DUE TO THE HEMOLYTIC STREPTOCOCCUS

SCARLET FEVER

Scarlet fever is a disease usually characterized by a sudden onset with nausea, vomiting, sore throat, head ache, and enlarged glands of the neck. A rash usually appears after twenty-four to forty-eight hours, first on the neck and upper chest and later on the face and the rest of the body. It is characterized by small red spots scattered over a reddened area, sometimes patchy with normal skin between patches. Sometimes bleeding occurs. The skin is itchy. The rash disappears in about eight days leaving the skin dry and rough. The scales peel for a period of ten to twenty days. The streptococci are not present in the scales and consequently the scabs and peeling skin are not infectious. In the early stages red spots appear on the tongue which show through the white furlike coating, producing "strawberry" tongue.

Scarlet fever is essentially a toxemia, the major symptoms being due to a generalized toxin produced by an organism that remains localized in the throat. It varies greatly in severity. Many cases are so mild that they are missed entirely, others may be fatal. One attack usually confers a lasting immunity.

Etiology

The specific cause of scarlet fever is *Streptococcus hemolyticus*. Streptococci were implicated in scarlet fever as early as 1885 and a number of workers presented confirming evidence but final proof was not available until 1923 when Dick and Dick succeeded in producing scarlet fever in human volunteers by the use of pure cultures. There is still some question as to whether there is only one specific scarlatinal streptococcus or whether a variety are capable of producing the disease. Certainly there is a difference in the various strains isolated from cases and it would appear that the disease may be due to a variety of streptococci which have one common characteristic—that of producing a soluble toxin called the scarlatinal toxin. It is doubtful whether there is sufficient difference between the so-called *Streptococcus scarlatina*, sometimes named as the specific cause, and other strains to warrant a strict separation. It should be noted further that the same streptococcus that produces scarlet fever in one individual may produce other diseases such as sore throat, middle ear infections, or septicemias in others.

Scarlatinal Toxin: Scarlet fever is primarily a toxemia due to the exotoxin, Dick toxin, produced by the streptococcus. When this toxin is injected in large doses into susceptible individuals, it produces the characteristic symptoms of scarlet fever. When injected into susceptible indi-

viduals in small amounts, it produces a local swelling and reddening, the extent of which depends upon the dosage and the susceptibility of the individual. The Dick toxin is relatively thermostable, temperatures from 94° to 100° C. for a period of forty-five minutes being necessary to inactivate it. It is antigenic and antitoxin may be produced by injecting the toxin. Toxoid may be prepared by the addition of formalin to toxic filtrates and is antigenic and relatively non-toxic.

Animals are relatively resistant to the scarlatinal toxin, a fact which makes experimentation difficult. However goats and some rabbits react to it and they are sometimes used to test its strength.

Scarlatinal Antitoxin: The serum from recovered cases of scarlet fever will neutralize the scarlatinal toxin *in vitro*. When such serum is injected into the reddened areas of the skin in cases of scarlet fever, a blanching results, indicating a neutralization of the toxin *in vivo*. This is known as the *Schultz-Charlton phenomenon* and is useful in diagnosis, for it indicates that the patient is suffering from scarlet fever. Neutralizing antisera may be produced by immunizing animals against the toxin or toxoid, or by infecting them with toxogenic strains of the streptococcus. The fact that neutralizing antibodies appear in the sera of individuals who have recovered from scarlet fever led to the development of the Dick Test.

The *Dick Test* is a test for susceptibility to scarlet fever and depends upon two observed facts: first, that scarlatinal toxin when injected intradermally produces a reddening at the site of injection, and, second, that antitoxin neutralizes the toxin *in vivo* and thus prevents its action. The test measures the amount of antitoxin present in an individual. A standard unit of toxin called a *skin test dose*, which is the smallest amount of toxin that will produce a reddened area at least one centimeter in diameter when injected intradermally into the arm of a susceptible person, is so injected into the individual to be tested. The volume used is 0.1 cubic centimeter of a suitable diluted toxin and a red spot should appear in twenty-four hours and disappear in a few days if the individual is susceptible. In actual practice, control tests with boiled toxin are sometimes used to determine whether the reaction is an allergic one or is due to lack of antitoxin.

Experience has shown that, in general, a person who has sufficient antitoxin to neutralize a skin test dose is resistant, or *Dick negative*, and will not contract scarlet fever when exposed. Persons who do not have enough antitoxin to neutralize a skin test dose are susceptible, or *Dick positive*, and likely to contract scarlet fever if exposed. The test is the same in principle as the Schick test for susceptibility to diphtheria but is not so reliable. A considerable number of persons who have had scarlet fever remain Dick positive and a considerable number of Dick negative reactors

contract scarlet fever. However, the test is of definite value and is used to determine susceptibility and to standardize antitoxin.

The Value of Antisera: Neutralizing antisera have been prepared commercially by immunizing horses with scarlatinal streptococcus cultures which contain the toxin as well as the whole organisms or with filtrates of such cultures. The potency of the antitoxin thus produced is measured by its ability to neutralize the toxin, the unit of measurement being the least amount of antitoxin which will neutralize fifty skin test doses of toxin. The use of antitoxin in early stages of scarlet fever alters the course of the disease and often leads to marked improvement in a few hours. It also reduces the number of complications.

TABLE 9*

Scarlet Fever: Cases, Deaths, and Deaths per 100 Cases—Ages under 15 Years
State of New Jersey, 1930 to 1934

AGE PERIOD, YEARS	CASES		DEATHS		DEATHS PER 100 CASES
	Number	Percent of total, all ages	Number	Percent of total, all ages	
All Ages	40,738	100.0	338	100.0	0.8
Under 15	34,868	85.6	261	77.2	0.7
Under 1	171	0.4	12	3.5	7.0
1	633	1.6	31	9.2	4.9
2	1,566	3.8	28	8.3	1.8
3	2,477	6.1	37	10.9	1.5
4	2,959	7.3	32	9.5	1.1
5–9	18,942	46.5	85	25.1	0.4
10–14	8,120	19.9	36	10.7	0.4

* Tables taken from *The Mortality from the Principal Communicable Diseases of Childhood*, Metropolitan Life Insurance Company.

Epidemiology

Source of Infection: Although discharges from the nose, ears, and suppurating glands or abscesses are infectious, the main source of infection is discharges from the throat of acute, mild, or missed cases of scarlet fever. Discharges from the throats or noses of healthy carriers may also spread the disease. Scaling skin is never infectious.

Mode of Transmission: Scarlet fever is usually spread by direct contact with cases or carriers or indirectly by contact with articles freshly soiled by their discharges or by contaminated milk or milk products.

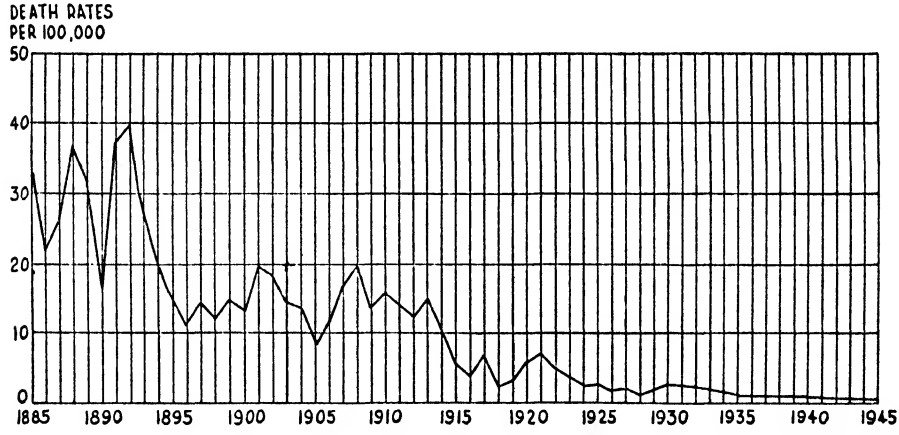
The *incubation period* is from two to seven days, the disease usually occurring between the third and fourth day.

The *period of communicability* is variable. It is usually at least twenty-

one days after onset and always until after abnormal discharges have ceased.

Susceptibility: The majority of young children, particularly the group from one to five years of age, are susceptible. Most adults are not.

Prevalence and Distribution: Although scarlet fever is world wide in distribution, it is not so important in the tropics as in the temperate zones. In the United States it is far more common in the northern states than in the southern, and in urban than in rural districts. There has been a progressive decline in the death rate from scarlet fever in the United States in the last fifty years. A hundred years ago in Baltimore records show a death rate from scarlet fever of 600 per 100,000. In Providence the average mortality from 1875 to 1884 was 40. In 1945 the rate for the



Scarlet fever: Crude annual death rates per 100,000 total persons—all ages. Twenty-one American cities, 1885 to 1945.

United States as a whole was 0.2. In 1935 there were 2718 deaths, in 1947 there were 84,379 cases and only 198 deaths. The case fatality rate, or the number of deaths per hundred cases, is less than one in the milder forms of the disease but may be as high as twenty or more in the severe forms. It varies with age.

Although the reason for this downward trend is not clear, it is generally agreed that the hemolytic streptococcus causing scarlet fever has undergone a change in virulence or type in the past half century. In all parts of the world there is considerable difference in the virulence of the strains producing current outbreaks. It is realized that we are fortunate at present and that a change to the more virulent type may occur at any time. Of course, on the other hand, the decline may be due to some as yet un-

known factor and there may actually be little possibility that the more virulent type will again become prevalent. It should be noted that there has been very little change in the death rate since the introduction of Dick testing.

The *seasonal distribution of cases* of scarlet fever is characteristic and remains unchanged whether the number of cases in any one year is high or low. In the United States the incidence is lowest in July and August and at about the time schools open there is an increase which continues steadily until the peak is reached in March and April.

Age Distribution: Scarlet fever is definitely a disease of childhood although any age may be attacked. The new-born possess an antitoxic immunity as evidenced by the Dick reaction and a low incidence. After the antitoxic immunity wears off sometime during the first year, the incidence increases until it reaches a maximum in the five to nine age group and then rapidly recedes. The case fatality is higher in the younger ages.

Sex Distribution: The incidence is slightly but consistently higher in white males than in females up to the age of fourteen years. After that the order is reversed. In the colored race the situation is the opposite.

Race Distribution: The death rate from scarlet fever is between two to three times as high in the white as in the colored race.

Control

Since the primary source of infection is individuals harboring the streptococcus or articles or milk contaminated by such individuals, control logically resolves itself into preventing direct or indirect contact. Its effectiveness depends upon prompt recognition of cases, isolation until they are no longer infective as determined by cultural methods—which is usually about twenty-one days, and the proper disposal of discharges so as to prevent contamination of milk or food.

Milk, as we have said, affords a handy means for the transmission of scarlet fever. It is easily contaminated by carriers and incipient cases, the streptococci multiply rapidly in it, and it is consumed in quantity by children in the most susceptible age. Consequently, the safeguarding of milk by pasteurization is a particularly effective control measure.

The quarantine of contacts has had little effect on the number of secondary cases but is, nevertheless, recommended. Since scarlet fever is primarily a disease of childhood and adolescence, it is obvious that it occurs most frequently in children in school. Parents, teachers, and health officers are always confronted with the question of whether or not to close schools when a case or two occurs. While local conditions must be taken into account, it is doubtful whether it is advisable to close urban schools. It may be a good thing to close rural schools although prompt

isolation of a case and strict quarantine of contacts appears to be effective in isolated districts.

Serotherapy: The fact that one attack usually confers a permanent immunity, probably antitoxic in nature, suggests the possibility of inducing an artificially acquired immunity by the injection of toxins. Such injections do lead to an immunity and are to be recommended for nurses, medical students, and doctors, who are Dick positive and whose chances of contracting scarlet fever are great.

The preparation used to induce active immunity is unmodified toxin and the usual procedure is to begin with the injection of 500 skin test doses and increase the dosage until 80,000 to 100,000 skin test doses are being given. This is a prolonged process and consequently is difficult to carry out on any large number of people. While the material used is not very toxic for most individuals, cases of miniature scarlet fever are occasionally produced by the vaccination. The protection afforded is not so great as that afforded by immunization to diphtheria. Convalescent serum or antitoxin is effective if given early in the disease and is a means of preventing deaths and reducing complications.

Chemotherapy: Sulfonamides and penicillin are effective in reducing complications.

SEPTIC SORE THROAT

Epidemic sore throat or streptococcic sore throat is an acute inflammation of the throat sometimes accompanied by middle ear infections, pneumonia, or peritonitis, and sometimes ending in a fatal septicemia. The disease usually occurs in epidemic form.

Etiology

The cause of septic sore throat is a hemolytic streptococcus. The term *Streptococcus epidemicus* has been given to the encapsulated streptococcus isolated from epidemics of sore throat. Since some strains show many of the characteristics of strains isolated from scarlet fever and others of strains isolated from erysipelas, many workers are not sure that the organisms producing streptococcic sore throat warrant being designated as a separate species.

Source of Infection: Although organisms may be transmitted from any case of sore throat, tonsillitis, or other streptococcic infection, the most common source of an epidemic is milk from an infected cow.

Mode of Transmission: Septic sore throat may be transmitted by direct or indirect contact with cases. An important means of indirect contact is raw milk that has been contaminated with the hemolytic streptococcus by milkers or handlers with sore throat or, as is most frequently the case,

by the udder of a cow which has been infected by persons harboring the germ. An infected cow will usually show signs of mastitis and during an epidemic all cattle showing signs of mastitis are to be suspected, even though the most common cause of mastitis in cattle is a bovine streptococcus called *Streptococcus agalactiae* which is not pathogenic for man.

The *incubation period* is one to three days.

The *period of communicability* in the cow is for as long as streptococci are being discharged. In man it is until the symptoms disappear and for varying periods afterwards.

Prevalence and Distribution: Septic sore throat is primarily a milk-borne epidemic disease and occurs in every country where raw milk is consumed. In 1911 an epidemic occurred in Chicago in which there were 10,000 cases. It is most prevalent in the spring and early summer but may occur at any time of the year. Single cases also occur.

Control

Control of epidemic septic sore throat is based on the knowledge of its mode of transmission. Because cases give rise to cases, the usual procedures for preventing contact with cases or with their discharges must be employed. Because the epidemic is primarily a milk-borne disease, pasteurization of milk is the most important single control measure. Control of milk handlers and inspection of cows is also important.

Serotherapy: One attack does not appear to confer immunity and artificial active immunization is not practiced.

Chemotherapy: Sulfonamide and penicillin are dramatically effective and their more general use should prevent complications and materially reduce the death rate.

ERYSIPELAS

Erysipelas is an acute infectious disease of the skin, characterized by redness, swelling, and the appearance of large and small vesicles. The rash spreads with the advance of the organism. The disease is due to toxin-producing hemolytic streptococci which are present in huge numbers in the lymph spaces and in the periphery and in advance of the skin lesion, not in its center. It is a clinical condition which is distinguished from other streptococcus infections such as cellulitis and lymphangitis—inflammations of the cells and lymphatics, respectively.

It appears that the streptococci from erysipelas comprise a more or less distinct group although they show characteristics similar to those isolated from scarlet fever and septic sore throat.

Serum which neutralizes the toxin can be produced but its curative value is questionable. Treatment with sulfonamides and penicillin is of great value.

An interesting feature of erysipelas is that one attack does not confer any lasting immunity. Indeed one attack is frequently followed by several more. Workers experimenting on rabbits found that skin areas which had been covered with the lesions were more resistant to subsequent injection of the streptococci than areas hitherto unaffected. This would indicate a local rather than a general immunity to the hemolytic streptococci. It is a quite different phenomenon from that of the antitoxic immunity produced by the hemolytic streptococci in scarlet fever.

PUERPERAL SEPSIS

Puerperal sepsis or childbed fever has long been recognized as one of the greater hazards in child bearing. Before the relation of bacteria to disease was suspected, its contagiousness was recognized. In 1843 Oliver Wendell Holmes read his famous paper, "On the Contagiousness of Puerperal Fever," and Semmelweis in Vienna in 1847 had studied its transmission. But, although these men and many others since have clearly shown that the doctor or attendant is the usual source of the infection and that infections can be reduced by proper attention to sepsis, the death rate from puerperal infections was, until recently, lamentably high in all civilized countries.

Etiology

Although infection from a number of organisms may occur during the period of childbirth, the hemolytic streptococcus is the primary cause of childbed fever. There is probably no specific type involved, for childbed fever is a wound infection and any virulent streptococcus which gains entrance is likely to cause it. Early observers noticed that it frequently followed cases of erysipelas and scarlet fever.

Anaerobic streptococci, not belonging to the *Streptococcus hemolyticus* or *viridans* group, are normal inhabitants of the vaginal tract. In a number of cases of milder puerperal sepsis they have been isolated in pure or mixed cultures. They appear to be able to invade the wounds resulting during childbirth although normally they are of no significance.

Source of Infection and Mode of Transmission: Granting that hemolytic streptococci are the prime cause of puerperal sepsis, the question arises as to whether the source is endogenous or exogenous, that is, whether they come from within the patient or from some outside source. If hemolytic streptococci were present previous to labor, it would be easy to understand how they might be the cause of infection for we know their

tendency to invade injured tissues or wounds. However, experiments show that hemolytic streptococci are not normally present in the vaginal tract or cervix. This fact and epidemiological evidence indicate that the usual source of infection is an outside one, such as the throat or hands of the patient or of the physician or intern in attendance.

Immunity and Serotherapy: Although the same streptococcus may be involved, there is little evidence that immunity to scarlet fever protects against puerperal sepsis. The proportion of cases appears to be as high in Dick negative as in Dick positive reactors. This might be expected, for scarlet fever is a toxemia and immunity is antitoxic not antibacterial. At the present time there is no effective way of increasing the immunity to puerperal fever by the use either of vaccines or serum.

Chemotherapy: As in other acute infections due to hemolytic streptococci, sulfonamide and penicillin are dramatically effective.

RHEUMATIC FEVER

Rheumatic fever is an infection which manifests itself in a number of ways. Acute and chronic rheumatism, chorea, St. Vitus Dance,"growing pains," rheumatic heart disease, and rheumatoid arthritis are some of the many names for various clinical types of rheumatic infection. As a cause of suffering, disability, and death, it is one of the leading infectious diseases. Unfortunately, it does not receive the consideration accorded to far less important though perhaps more dramatic diseases. It is often a chronic disease with acute periods of greater or lesser severity. The clinical symptoms vary according to the age of the patient.

Symptoms

"Rheumatic fever licks the joints and bites the heart," it is said. The onset may be sudden or gradual. It usually follows an infection such as sore throat, tonsillitis, or scarlet fever; but history of previous infection is sometimes lacking. The disease shows itself in a fever, loss of appetite, nose bleed, irritability, jerky and uncoordinated movements, twitching (St. Vitus Dance), painful joints ("growing pains"), and other ways. These are evidence of infection but the greatest danger lies in damage to the heart which develops after the initial symptoms.

Joint involvement is not common in children and adolescents but often occurs in persons over twenty years old. The term, *rheumatoid arthritis*, is given to this type of rheumatic infection and the symptoms are referable to an inflammation of the soft tissue and synovial membranes of the joints. There is some question as to whether it is etiologically related to *osteo-arthritis*, a disease of the joint cartilage and bone.

Rheumatic Heart, which is the chief manifestation of rheumatic fever

accounts for from fifteen to forty per cent of all clinical heart disease according to Hedley. Compared to other infectious diseases, it ranks fourth as a cause of death.

Etiology

The etiology of the diseases characterized by the rheumatic state is perplexing but it seems that rheumatic fever, rheumatoid arthritis, and several others are infectious diseases and that streptococci of the hemolytic type are causally related to them. Viridans and non-hemolytic streptococci have also been implicated by some workers and some have suggested that a virus might be involved. The rheumatic state frequently follows other infections. Gonococcal arthritis is a common complication of gonorrhea.

While the evidence seems to indicate that streptococci are the cause of rheumatic fever, they can be isolated from the joints and synovial fluids in only a small per cent of the cases. The infection is not one in which the organism proliferates in the regions or tissues displaying the prominent symptoms. Streptococci can be isolated from the blood stream in many cases and from focal infections of the tonsils and other tissue. Since the affected tissue is not invaded, it seems likely that the damage is due either to toxic substances liberated by the organisms or to allergic reactions to the bacterial protein, and the bulk of the evidence points to the conclusion that the rheumatic state is a manifestation of allergy. It seems that what probably happens is that the streptococci establish themselves in the throat, tonsils, or other tissue and produce a mild or severe infection, as the case may be. The body responds to the foreign bacterial protein by producing antibodies which sensitize the joints or other tissue. When the antigen gets into the blood stream again, it combines with the antibodies present in the sensitized tissue and shock is produced. The evidence for this is indirect but it is known that the development of the rheumatic state is associated with the appearance of antibodies to hemolytic streptococci, and that unless these appear in high titre following a case of sore throat, tonsillitis, or some other infection, the individual does not subsequently develop rheumatic fever or other manifestations of the rheumatic state.

Mode of Transmission: If rheumatic fever is due to the hemolytic streptococci, then the disease is transmitted by discharges from infected persons, either cases or carriers, and the organism gets into the body and leaves it by way of the mouth and nose.

Susceptibility and Immunity: Rheumatic fever is most common in children from seven to ten years of age. While only one attack may occur, the disease has a tendency to become chronic, probably being reactivated

whenever a focal infection flares up or the individual is reinfected with hemolytic streptococci. Males are less frequently attacked than females.

Prevalence: Rheumatic fever is more frequent in urban than in rural communities, in the poor than in the rich, and in the poorly nourished than in the well-fed. A great deal of the difference may be due to crowding.

The incidence of rheumatic heart disease is greater in the northern than in the southern parts of the United States. It is relatively rare in the tropics. Although it is estimated that the United States has between 30,000 to 50,000 deaths a year attributable to rheumatic fever, less than 2000 deaths are reported each year from acute rheumatic fever.

A study made by Paul and Dixon on Indian children showed an infection rate of 4.5 per cent in Montana and Wyoming, only 0.5 per cent in New Mexico and Arizona, and 2.2 per cent in the New England States. This shows an unusually high rate in regions that are arid though cold which is in contrast to the popular belief that a moist damp climate is a predisposing factor to rheumatism. Epidemiologically rheumatic fever is a crowd disease following closely upon hemolytic streptococcal infection of the upper respiratory tract. In army camps there were a number of cases following streptococcal infection of the throat and scarlet fever. When sulfadiazine was given to all the personnel, the streptococcal infections disappeared rapidly and, after a lag of several weeks, there were no more cases of rheumatic fever. This is corroborative evidence of the causal relationship of hemolytic streptococci to the disease.

Sulfa drugs will prevent recurring attacks in children but neither sulfa nor penicillin is of value in the treatment of acute cases. In fact, there is some evidence that they are harmful. Salicylates given in large doses appear to be very helpful.

Control

In the past few years there has been a reduction of about twenty-five per cent in the death rate from rheumatic heart disease in children under fifteen. Since the death rate from all forms of heart disease has been increasing, this suggests that better care and treatment of cases of rheumatic fever in the younger group is forestalling irreparable damage to the heart.

There is no specific preventive measure. Immunization is not effective. There is some evidence that the sulfonamide compounds prevent recurrence of the infection and so check damage to the heart. The most effective treatment, however, appears to be complete rest until all symptoms of infection have disappeared and the damage already done the heart has been repaired. Clinicians emphasize the importance of early diagnosis

and early treatment. Parents must always be alert for early manifestations.

The rheumatic state appears to be another instance in which a defensive mechanism that operates to the distinct benefit of the individual in many diseases is itself responsible for others.

OTHER DISEASES DUE TO HEMOLYTIC STREPTOCOCCI

There are a number of other diseases in which hemolytic streptococci play a part, either as the primary cause or as secondary invaders. They are particularly important as a cause of bronchopneumonia following measles, influenza, and other diseases. They are a serious cause of infections in wounds producing a local inflammation or a septicemia.

DISEASES DUE TO STREPTOCOCCUS VIRIDANS

In general the diseases due to the green-producing streptococcus are less acute and more chronic than those due to the hemolytic group. *Streptococcus viridans* is the most common cause of vegetative endocarditis and many focal infections.

These infections are not influenced by sulfanilamide nor are vaccines or serum therapy effective. Previous to the introduction of penicillin the fatality rate in subacute endocarditis was about ninety-nine per cent. Penicillin has proven to be effective in many cases if given early. Large doses must be used in order to maintain an adequate concentration in the lesions and treatment must be kept up for a month or more.

Chapter XVII

THE PNEUMOCOCCI AND PNEUMONIA

It was in the eighties that the pneumococcus was first described, isolated, cultured, and accepted as the causative agent of lobar pneumonia. In 1880 Eberth described organisms, undoubtedly the pneumococci, which he had found in exudate from the lungs of pneumonia patients but thought them to be a variety of diphtheria bacillus. Then, in 1881, Pasteur and Sternberg, working independently, isolated, for the first time, from the blood of rabbits injected with saliva, the organisms we now know as *Diplococcus pneumoniae*, and the investigations of Friedländer (1882), Fränkel (1884–86), and Weichselbaum (1886) definitely established its causative association with lobar pneumonia and certain other infections.

Since that time studies on the pneumococcus have resulted in the accumulation of much information regarding its peculiarities and characteristics and have thrown light on the relationship of dissociation to virulence.

The pneumococci or *Diplococcus pneumoniae* is also known by a variety of other names such as *Streptococcus pneumoniae*, *Micrococcus lanceolatus*, etc.

Morphology: The pneumococci are fairly large, elongated, and somewhat lance-shaped organisms. They usually occur in pairs, hence their name, *Diplococci*, but sometimes, especially when grown on artificial media, in short chains. A well-defined polysaccharide capsule surrounds the pneumococcus in animal exudates or tissue but is usually lost on artificial media although in milk cultures it is quite pronounced. The capsular material, sometimes referred to as the *specific soluble substance*, or *SSS*, is serologically type specific and its presence is associated with virulence.

Physiology: The pneumococci are parasites and do not grow readily apart from the host. If growth occurs in ordinary culture media, it is sparse. Fresh veal infusion broth or veal infusion blood agar will give good growth. The colonies, when grown on blood agar, are small, moist, granular, and translucent, with discrete and well-defined edges, and surrounded by a greenish discoloration similar to that produced by *Streptococcus viridans*.

The pneumococci usually ferment dextrose, sucrose, lactose, and inulin. As previously mentioned, the latter sugar is not fermented by the streptococci and consequently is used as an aid in distinguishing the two.

Another curious feature peculiar to the pneumococci is their sensitivity to ox bile or bile salts. The addition of ten per cent ox bile or sodium desoxycholate to a young broth culture or a suspension of pneumococci

dissolves them in a few minutes leaving the liquid clear. The streptococci are resistant to the bile and salts so that this difference may also be used to distinguish the two groups.

The pneumococci are sensitive to acids and grow best at a hydrogen-ion concentration of 7.6 to 7.8. They are readily destroyed by ordinary disinfectants or by heating at a temperature of 50° C. for ten minutes. They are fairly resistant to cold and will grow at temperatures ranging from 25° C. to 42° C., their optimum growth temperature being that of the body, about 37° C.

They may live for months in dried sputum and survive in the dust of the sick room but do not grow away from the body.

Pneumococcus Types and Typing

The pneumococci comprise a large group of closely related organisms. The cells proper or their nucleoproteins are serologically identical, differing only with respect to the chemical nature of the carbohydrate capsule. This may be lost when the pneumococcus is grown on artificial media and regained when it is passed through animals. The carbohydrate capsule interferes with the process of phagocytosis, making it possible for the pneumococcus to invade a host and the severity of the invasion, or the virulence of the organism, is determined by the type of capsule. The effective antibody response is against the capsule, not against the cell, and is consequently type specific.

Another fact of interest and possible significance is that by causing one type of pneumococci to loose its capsules and then proceeding to grow it in the presence of a killed encapsulated pneumococcus of another type, the first type may be induced to produce capsules like the second. Another way of causing a change of type is to grow the organism in the presence of its homologous antiserum. Although there is no evidence that this transmutation of types takes place under any save experimental conditions, there is a possibility that it might occur in the body. An individual might conceivably build up antibodies to a relatively non-virulent type which he was carrying and, in the presence of such antibodies, that strain might change to some other type. This bit of speculation would offer an explanation of some of the sporadic cases of pneumonia.

Early research on the pneumococci distinguished three readily recognizable serological types which were named *Types I, II, III*, and, apart from these, a large variety of strains which were placed in a group called *Group IV*. More recently this fourth group has been shown to consist of at least twenty-nine distinguishable types varying in virulence, pathogenicity, and prevalence.

Prevalence and Pathogenicity of Types: The percentage distribution of

serologic types of pneumococci varies widely and differs with age and country and from year to year. Type I accounts for over 30 per cent of all cases of lobar pneumonia, Type II for about 20 per cent, Type III for 10 to 15 per cent, and the rest of the cases are due to the other twenty-nine types.

In bronchopneumonia the situation is different. Type I and II infections are rare but Type III accounts for about 20 per cent and Type VIII for about 30 per cent of all cases.

The virulence of the various types differs and the case fatality rates, which can be stated in general terms only, are about 25 per cent for Type I, 40 per cent for Type II, and 40 to 60 per cent for Type III.

About fifty to eighty per cent of normal persons are carriers of one or more types, nearly all of which belong to Group IV. The carrier rate for Types I, II, and III is low but about four times as high in persons who are in contact with cases as in non-contacts. Carriers of Type III are reported to be more frequent than carriers of Types I and II.

Pneumococcus Typing: Inasmuch as effective antisera are available for all types of pneumococci, prompt determination of the type is indispensable for serum treatment. Typing also aids in tracing the spread of infection and affords a way of obtaining information concerning the interrelationship of the members of the species.

It has been demonstrated in every disease in which antisera are of value, that the earlier they are given the more effective they are. Pneumococcus infections are no exception and a rapid means of typing is essential. Several serological methods are available, all of which depend upon the use of carefully prepared type-specific antisera. The *Neufeld "quellung" reaction* is the most widely used. Neufeld noticed as early as 1902 that when pneumococci were mixed with homologous antiserum the capsule became swollen while the cells within the capsule remained normal in size. This reaction was rediscovered in 1931 and since then has become popular as a simple and rapid method of typing. The test may be run on cultures from cases, on peritoneal washings from a mouse injected with washed sputum from cases, or directly on sputum. A few drops of sputum are placed on a slide and mixed with undiluted type-specific antisera. Methylene blue is added to stain the organism and increase the contrast. Microscopic examination shows the capsules of homologous organisms swelling noticeably in a few minutes. Capsules of heterologous types do not.

PNEUMONIA

Pneumonia is an inflammation of the lung tissue, more particularly of the walls of the air sacs or alveoli, which become filled with an exudate that interferes with their function. The onset is abrupt with chills, followed by fever and pain in the chest, and often accompanied with coughing or difficulty in breathing.

The pneumonias may be classified as *primary* or *secondary* and as *lobar* or *lobular*, more frequently known as *bronchopneumonia.*

In lobar pneumonia one or more lobes of the lungs are completely involved; in lobular pneumonia the involvement is patchy and scattered throughout the lungs. Lobar pneumonia is usually a primary pneumonia and in about ninety-five per cent of the cases is due to the pneumococcus.

Lobular or bronchopneumonia is usually secondary to other infection and has been called the dreaded ally of all disease. It is a contributory cause of death in half or more of the fatal cases of whooping cough and measles and a terminal bronchopneumonia is the cause of death in the majority of diseases in which the patient has been weakened by long illness.

Of the primary pneumonias, nearly all are lobar and due to the pneumococcus. The secondary pneumonias are bronchopneumonias and may be due to a variety of organisms, the pneumococci and streptococci being most commonly involved.

Etiology of Lobar Pneumonia

Ninety-five per cent of the cases of acute lobar pneumonia are caused by *Diplococcus pneumoniae.* Occasionally cases are due to *Friedländer's bacillus*, *Streptococcus hemolyticus*, the influenza bacillus, and other bacteria.

Mechanism of Disease Production

The nature of the disease produced by the pneumococci suggests the involvement of a toxic substance whose existence has not yet been demonstrated. Neither the carbohydrate capsular material nor the nucleoprotein of the cell is toxic. Leucocidin, a necrotoxin, and a substance which causes capillary hemorrhage are produced and account, in part, for the characteristics of the infection.

The sequence of events in the production of pneumonia is somewhat as follows. At the start of the infection the pneumococci begin to multiply rapidly in the lung causing damage to the cells. Their presence and their toxic products cause the capillaries of the air sacs to distend and fluid leaks into the sacs, filling them and rendering them useless. Phagocytes mobilize in the lung tissue and increase in the blood but in the early stages of the disease they seem unable to engulf the pneumococci with any degree of efficiency. As they continue to multiply without restraint, although most of them remain in the lung, many also get into the blood stream and as a result of toxins absorbed, the patient may become critically ill with high fever and delirium.

After about five to seven days his temperature may fall, the delirium disappear, and he may show an abrupt change for the better within a few hours. This phenomenon is called the crisis. At this time the phagocytes

suddenly begin to engulf and digest the pneumococci with great avidity. The blood stream is soon cleared and the pneumococci in the lung are rapidly destroyed.

There is ample experimental evidence that the crisis is brought about by the appearance of opsonins which enhance phagocytosis and thus play a predominant role in recovery. In the early stages even though ample numbers of phagocytes are present, the pneumococci, protected by their carbohydrate capsules, are not engulfed. However their presence stimulates the production of antibodies and in about six to seven days these reach sufficient concentration to sensitize the organisms so that they can be taken up by the phagocytes. Should the opsonizing antibodies fail to appear, the organisms will continue to multiply and death will ensue.

Source of Infection and Mode of Transmission: Pneumonia is a disease with a low order of communicability. Man is the source of infection, for the pneumococcus does not naturally infect animals and does not live saprophytically. It gets into the body by way of the upper respiratory tract, localizes in the lungs, and leaves in discharges from the mouths and noses of infected persons or carriers. However, unless the body is less resistant than normally, contact resulting in the entrance of pneumococci into the upper respiratory tract does not lead to disease.

A curious feature in the epidemiology of pneumonia is that, although under certain conditions and in certain seasons it may be epidemic, there are so few secondary cases traceable directly to contact with cases. Since both the virulence and the dosage of pneumococci must undoubtedly be greatest from cases this would seem to be evidence that the resistance of the host is the important factor in determining pneumococcal infection.

There is ample evidence to suggest that low economic status with its attendant overcrowding and undernourishment increases susceptibility to pneumonia. Acute alcoholism, prevalence of mild respiratory infections, influenzas, and exposure to dusts of various kinds are also predisposing factors.

The pneumococci are widely distributed and commonly found in the mouths of about forty to eighty per cent of healthy individuals. The types most frequently found, as previously mentioned, belong to the heterogeneous Group IV, although, a small percentage of people carry the more virulent fixed Types I, II, and III. Of these fixed *types*, the most common in carriers is Type III, but the incidence of Type III pneumonia is lower than one would expect in view of its prevalence and, strangely enough, the case fatality rate highest.

The chances of developing lobar pneumonia as compared to the chances of infection seem to depend upon the type of pneumococcus encountered. Group IV pneumococci seem to be able to establish themselves readily in

the nasopharynx but are capable of invasion only when the resistance of the body is lowered by another infection. Few people carry Types I, II, or III pneumococci and yet about eighty per cent of all cases of lobar pneumonia are due to one of these types; so it would seem that if Types I, II, and III succeed in establishing themselves they are more likely to produce pneumonia.

The *incubation period* is difficult to determine accurately but is usually considered to be one to three days.

The *period of communicability* is also difficult to determine definitely but may be considered to be as long as the discharges contain virulent organisms in sufficient number to infect.

Prevalence of Pneumonia: Pneumonia has the highest case fatality of all the acute infectious diseases with a high incidence. It ranks seventh as a cause of death. In 1935 there were 132,625 deaths, a rate of 104.2. In 1945 there were 58,196 deaths, a rate of 44.1. In 1946 there were 53,541 deaths, a rate of 38.3. This decrease is largely due to chemotherapy.

Age, Sex, and Race Distribution: Although pneumonia occurs in all ages, there is a difference in the age distribution. The excess mortality in males over females in both white and colored people is significantly higher in the age group from twenty-five and over. An explanation of the age differential death rate is found in part in Table 10 which shows clearly the relation between occupation and mortality. While there may be physiological differences between the resistance of men and women to *Diplococcus pneumoniae*, the hazards of occupation greatly influence the death rate.

Occupation: Of course occupation and income and standards of living are directly related and the differences that might be expected in the death rates of the various economic classes show up when deaths are listed by occupation. These differences do not, however, account entirely for the differences between the death rates in the various occupations.

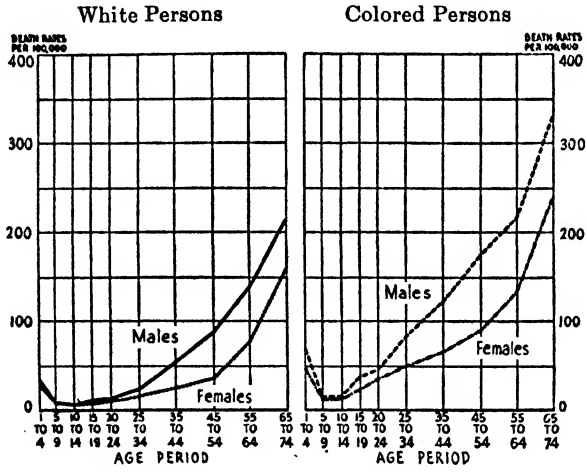
Geographic and Climatic Distribution: Although pneumonia occurs in every country and in every climate, it shows a definite geographic, climatic, and seasonal distribution. It is more prevalent in the United States than in England and more prevalent in the northeastern states than in the Great Plains Region. It appears to be less frequent in the regions where there is a combination of low humidity and high altitude.

There is a pronounced seasonal distribution, pneumonia being most prevalent in late winter and early spring.

Differences in occupation and economic status must always be kept in mind when interpreting the influence of geography or climate.

Immunity: Recovery from an infection by *Diplococcus pneumoniae* leads to the appearance of opsonins in the serum. In fact, it seems to be

related to their appearance in the blood stream for the opsonins appear and the pneumococci disappear at the time of crisis. Such immunity as does occur after recovery is apparently not great and is of short duration,



Pneumonia, lobar and unspecified: Averages of annual death rates per 100,000 by color, sex, and age—ages 1 to 74 years.

TABLE 10

Standardized Death Rates from Pneumonia per 100,000 Gainfully Occupied Males—Ages 15 to 64 in 10 States in 1930—by Occupation Classes

OCCUPATION CLASS	DEATH RATE PER 100,000
All gainfully occupied males in selected occupations	69.3
Professional men	38.8
Agricultural workers	43.4
Clerks and kindred workers	50.5
Proprietors, managers, and officials	53.0
Skilled workers and foremen	59.7
Semiskilled workers	71.6
Unskilled workers	135.9

Table from *The Mortality from Influenza and Pneumonia*, Metropolitan Life insurance.

for one attack is frequently followed by another or by several. There is little evidence that the immunity is other than an enhanced capacity of phagocytosis.

Serotherapy: Effective antisera have been prepared for all types. The antisera for Type I is most effective and its early administration lowers the case fatality from about twenty-five to twelve per cent. Administration of antisera to Type II has resulted in lowering the case fatality from about forty per cent to twenty-five. Favorable results are also reported with antisera prepared against Types V, VII, and VIII, but antisera for Type III, which is highly fatal, does not appear to be effective.

Vaccination has been attempted but the problem is beset with difficulties because of the numerous types of pneumococci. The results have not been uniformly successful although they offer considerable promise.

Chemotherapy: Sulfonamides, penicillin, and streptomycin are very effective in the treatment of pneumococcal and streptococcal pneumonia. They are ineffective in the case of virus pneumonias. However, less than one per cent of the pneumonias reported are due to viruses.

OTHER DISEASES DUE TO THE PNEUMOCOCCI

In addition to producing lobar and bronchopneumonia, the pneumococci produce various other infections, some of which may occur as complications following pneumonia and some of which occur as independent infections. Inasmuch as there is a bacteremia in pneumococcus pneumonia, it is not surprising that organisms get transported to other sites where they may produce local manifestations. The meninges and the middle ear are frequent sites of such secondary localizations. Not infrequently the pneumococci produce a conjunctivitis in one or both eyes. Pneumococcal peritonitis and acute arthritis also occur as a result of pneumococcal infection.

Although under experimental conditions the pneumococci may be pathogenic for animals, they are not the cause of pneumonia in horses, sheep, and other animals.

Animals are readily immunized against pneumococcus infections and the immunity is type specific.

Chapter XVIII

INFLUENZA AND THE COMMON COLD

INFLUENZA

Influenza is an acute infectious disease commonly characterized by a sudden onset, a fever of from one to seven days' duration—usually about three—, severe prostration and exhaustion, aches and pains in the back and limbs, a discharging nose, and bronchial symptoms. Uncomplicated cases are comparatively mild but so weaken the patient that complications are likely to follow. A common secondary infection and cause of death is bronchopneumonia.

The clinical manifestations of influenza are so variable that it is difficult to make a positive diagnosis in many instances. During epidemics or pandemics the symptoms are more clear cut and diagnosis is more certain but many sporadic cases undoubtedly are not recognized. The prevailing symptoms in different epidemics vary, probably with variations in the types of organisms associated with the influenza virus as secondary invaders.

Etiology

Epidemic influenza is caused by a filterable virus. The studies on the etiology of influenza are of particular interest and in order to appreciate our present concept it is necessary to review some of the earlier ones. During the influenza pandemic of 1889 and 1890, investigators attempted to isolate the causative agent and in 1892 the German bacteriologist, Pfeiffer, isolated a small gram-negative rod which he found in cases of influenza. This organism, known as the *influenza bacillus* or *Pfeiffer's bacillus* and, still more recently, as *Hemophilus influenzae*, was accepted as the causative agent until about 1918. During that great pandemic ample opportunity was afforded for a study of influenza, and bacteriologists found that the influenza bacillus was present in throats of healthy persons as well as in the throats of those ill from the disease. This fact alone did not rule out its connection with influenza since the persons might have been immune carriers, but they also found the organism widely prevalent in mild respiratory infections which were not influenza and found it relatively scarce in some localities where influenza was prevalent and abundant in others. From 1918 until 1928, a number of investigators isolated organisms which seemed to be associated with the disease but the theory that influenza was due to a filterable virus was gaining ground as a result of the work of Long,

Bliss, and Carpenter who had produced a disease like influenza in chimpanzees by the use of bacterial free filtrates from cases of influenza.

The difficulties in proving the etiology of influenza were many. There was then no suitable experimental animal and no good criterion for precise diagnosis. When human volunteers were used to test the cultures or filtrates of nasal washings or discharges from cases, it was not possible to say whether their reactions were the result of the inoculations or of previous

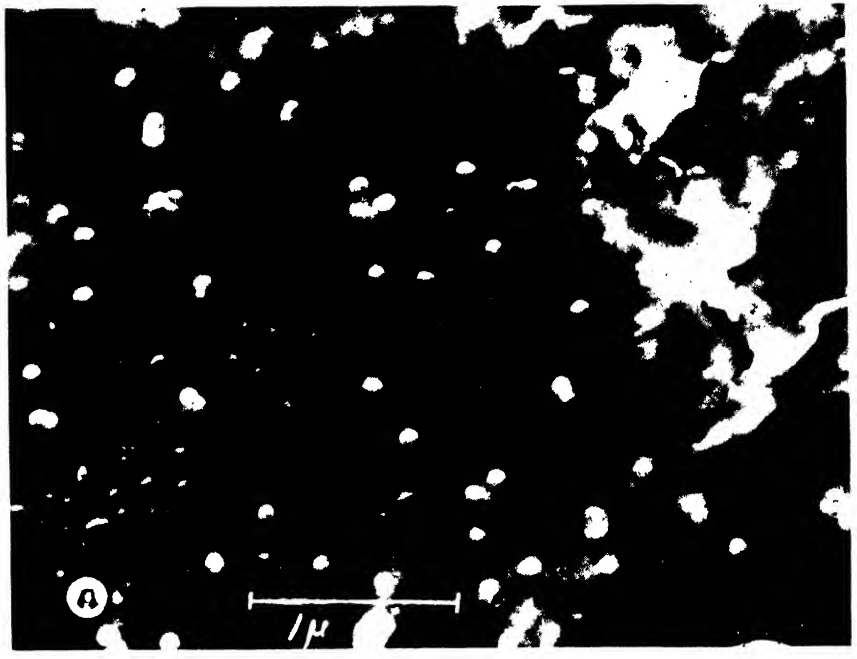


FIG. 41. Electron shadow micrograph of the influenza virus. These virus particles are about 75 millimicra in diameter and are globular in shape.

Courtesy of Dr. R. C. Williams, Dr. R. W. G. Wyckoff, and the Proceedings of the Society for Experimental Biology and Medicine.

exposure to some other infection. During epidemics when cases were more readily recognized and any amount of material for testing was available, the danger of accidental exposure during the tests was especially great.

In Iowa, in 1918, a disease appeared in hogs which was so similar to human influenza that it was promptly named swine influenza or "hog flu." The disease, which has a mortality of from four to twenty per cent or over, strikes a herd with the swiftness of human epidemic influenza and a day or two after the first animals show symptoms the whole herd may be affected.

There is a high fever, extreme prostration and weakness, and a slow uneventful recovery. Because of its simultaneous appearance with human influenza, some workers suspected a connection but attempts to infect hogs with nasal washings from cases of human influenza were negative.

In 1931 Richard Shope of the Rockefeller Institute, in a series of brilliant investigations on "hog flu," showed the disease to be due to a filterable virus and a bacterium very similar to *Pfeiffer's bacillus* which he called *Hemophilus influenzae-suis.* The virus alone produces a mild sort of disease in hogs and the bacterium alone produces no disease, but a combination of the two produces typical swine influenza. The virus infection alone confers an immunity, the bacterium alone does not.

Following closely on Shope's announcement of the "complex" etiology of swine influenza, Smith, Andrewes, and Laidlaw in England succeeded in infecting ferrets with filtrates of nasal washings from typical cases of human influenza and so in isolating the influenza virus. On comparison, Shope's virus of swine influenza and the human influenza virus were found to be very similar but not identical. The human influenza virus in combination with *Hemophilus influenzae* produces the same disease in hogs as does the hog virus in combination with the bacterium. Hogs rendered immune to the human strain as a result of infection with the human influenza virus are also immune to the Shope virus. However, ferrets infected with the human strain are immune to the hog strain but not *vice versa.* The human strain appears to have a broader antigenicity. Two and possibly three serologically distinct types of virus have been obtained from cases of human influenza. In experimental animals they do not cross immunize. Of interest and practical importance is the fact that the virus can be transmitted from ferrets to mice and grown in tissue cultures and chick embryos. Thus the virus supply can be maintained for experimental purposes and economical experimental animals are made available. Even though there may not be so close an association between a specific bacterium and the influenza virus in human influenza as there is in swine influenza, the bacteria normally present in the upper respiratory tract, as well as those not normally present, probably determine some of the characteristic features of epidemic influenza.

Epidemiology

Source and Mode of Infection. Influenza is a droplet infection. The virus is found in the discharges from the mouth and nose of infected persons and transmission is, therefore, by contact with such persons, freshly contaminated articles, and contaminated air. It has been shown that the virus remains alive for several hours in the air of rooms in which it has been sprayed. Thus it would appear that during epidemics the presence of

infected persons breathing, coughing, sneezing, and expectorating in rooms, street cars, and other closed places could and probably does contaminate the air so that others may be infected by it several hours later.

With the exception of swine, no animal is known to harbor the influenza virus or even a closely related one. Of course, since swine are susceptible, there is always the distinct possibility that they may serve as a reservoir.

The *incubation period* is short, usually from twenty-four to seventy-two hours.

The *period of communicability* is probably during the time of incubation as well as during the acute stages and convalescence.

Immunity: In spite of the high contagiousness of influenza, all persons are not susceptible, which is to say that even during severe epidemics about one-quarter of the people escape. Whether this is due to lack of innate susceptibility or whether those who escape may have a mild and unrecognized form of the disease is not known. It may be that relative immunity to the bacteria present plays a part in determining susceptibility. Acquired immunity to the disease as a result of an attack seems to be short-lived but whether this is because there are other strains to which second and third attacks may be due is not known.

Serotherapy: Antisera have been prepared by injecting the virus into horses, but their value in treatment is questionable.

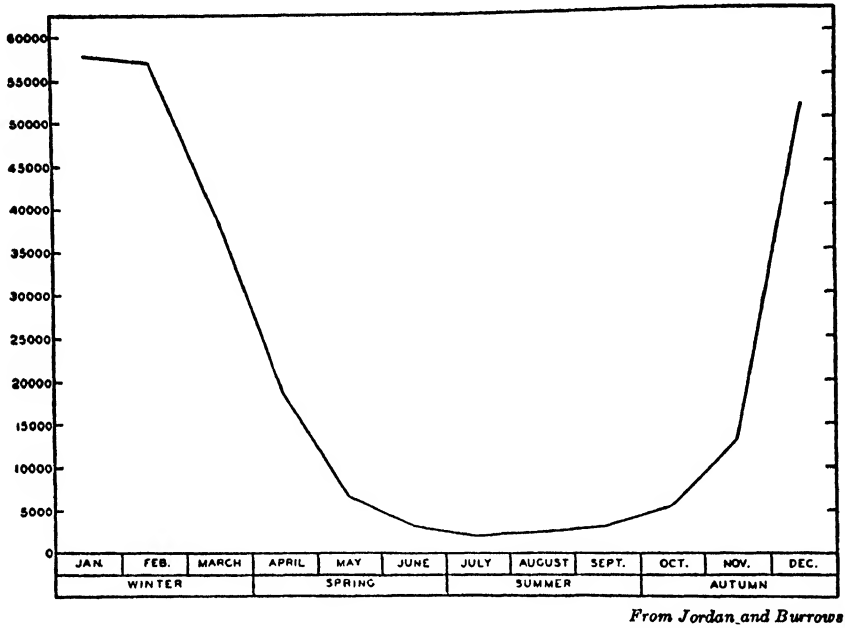
Active Immunization: Types A and B influenza virus have been propagated in the chick embryo and vaccines prepared of killed suspensions. They have been shown to protect experimental animals and to produce antibodies in man. These vaccines have been given to large groups and reduced the incidence of influenza. The immunity is of short duration.

Prevalence and Distribution: Influenza occurs as sporadic cases, local outbreaks, nation-wide epidemics, and pandemics. The pandemics are of particular interest and have occurred in cycles of several decades. The more important were: 1510, 1557, 1580, 1593, 1658, 1675, 1729–32, 1762, 1780, 1788–90, 1830, 1836–37, 1846–47, 1857–58, 1889, and 1918. Lesser epidemics occurred in between. Because of differences in the registration and recording of deaths, it is difficult to get any exact notion of the incidence during any one epidemic but as many as fifty per cent or more of the population may be affected. It disturbs every phase of life. Transportation is crippled, trade is halted, and hospital service is entirely disrupted because of the number of doctors and nurses who are attacked.

The total number of deaths due to influenza in the 1918 pandemic was probably between ten and twelve million. It is estimated that in the United States alone between 450,000 and 600,000 people perished. In contrast to that, less than 50,000 died from war injuries.

Usually influenza travels at the same speed as man but at times it

apparently breaks out simultaneously in widely separated parts of the globe. During the 1918 pandemic, communities that were completely isolated remained free of influenza. The explosiveness of the epidemic is probably due, in part at least, to the short incubation period, the probable spread in the very early stages, the high susceptibility rate, and the number of persons affected with mild cases who do not remain in bed and are mingling with susceptibles.



From Jordan and Burrows

The seasonal incidence of influenza. Averages of reported cases by months for the years 1930 to 1938 inclusive. Data from Supplements to Public Health Reports.

There appear to be differences in virulence between the early and later stages of the epidemic and the recurring waves. At the beginning of the wave the cases are mild. They increase in severity and become attended by complications as the crest is being reached.

Influenza shows a definite seasonal pattern although the great epidemics and pandemics differ somewhat from normal seasonal distribution. There are about 500,000 reported cases and 10,000 deaths a year. Deaths in the winter and spring months are eight to nine times as great as those in summer months. The more severe epidemics show an earlier rise and reach their peaks earlier than do the lesser ones.

Age, Sex, and Race Distribution: The mortality is greatest in the extremes

of life, being highest in the group aged from 65 to 74 and next highest in the 1 to 4 year group. It is lowest in the 10 to 14 year group. The mortality during the 1918 pandemic was not typical of the preceding or following periods but showed a high mortality in the 25 to 34 year group and a relatively low mortality for the older group. As in the case of some of the earlier pandemics, there was an excessively high death rate in pregnant women. A sex distribution of deaths shows no difference up to about thirty-five years. Between 35 and 55, mortality is higher in the males. After 60 years, it is higher in the females. The death rate in colored people is nearly twice as high as in white.

Control

Strict isolation of groups is hardly possible but, from the standpoint of the individual case, it is probably the most effective means of control if for no other reason than to prevent contact with possible carriers of bacteria that might induce complications. Hospitalization is not always to be recommended because the probable overcrowding may bring additional exposures to other organisms. The patient should remain in bed. Quarantine is ineffective but, of course, visiting should be discouraged. Masking as carried out by many individuals and groups is of doubtful value as a protective device. Closing schools did not seem to check the spread of the disease in 1918.

Epidemiology of Swine Influenza

The epidemiological features of an infectious disease may be as constant and as typical as its symptoms. Just as the clinician seeks to account for such symptoms as fever or toxemia, so the epidemiologist tries to account for the factors of seasonal distribution, periodicity, and the like. In some instances the explanation is relatively simple, in others, it is not discovered.

Swine influenza showed a seasonal distribution and periodicity that was difficult to account for. Dr. Shope attacked the problem and his investigations and solution stand as one of the classics in epidemiology.

Swine influenza occurs yearly in the middle western states but is usually limited to the months of October, November, and December after which it disappears until the next October. Shope found that the bacterium, *Hemophilus influenzae suis,* which in connection with the virus produces the disease, was present in swine all year and hence the absence of the disease could not be attributed to its disappearance. The virus, however, did not persist in swine all year. Its whereabouts from January to October were a mystery. Since viruses are not saprophytic and do not persist for any great length of time away from living tissue, an intermediate host was suspected and the final solution involved not one but two, both of them

worms. The hog lung worm was the carrier. During the acute stages of influenza this worm in the respiratory tract of the hog picks up the virus which gets into its eggs. These are coughed up, swallowed, excreted in the feces, and the next stages in their development depend upon the earth worm. After being eaten by the earth worm, the eggs of the lung worm hatch and the larvae develop. These larvae, which are infected and harboring the influenza virus, persist in the earth worm until it is eaten by the swine. Then the larvae are liberated, undergo two further developmental stages, and as adults, reach the respiratory tract of the hogs. At this time the hogs do not develop influenza unless *Hemophilus influenzae suis* is present to provide a stimulus. If they are harboring this organism, they will develop influenza when the infected lung worm reaches the lungs. If the organism is absent, they will not.

The periodicity and seasonal distribution of swine influenza is thus related to the life cycle of the lung worm, a part of which is spent in the earth worm.

THE COMMON COLD

The common cold is the most frequent of all infections and although it is not itself a cause of death, from the economic point of view it is one of the most important diseases or group of diseases. It is responsible for one third of all absenteeism in industry and its cost per year in the United States runs to over two billion dollars. The clinical symptoms are variable, ranging from a slight congestion of the mucous membranes and a soreness of the throat to conditions very difficult to distinguish from mild cases of influenza.

Etiology

The true epidemic infectious cold is due to a filterable virus although a variety of agents may produce symptoms of a cold. Exposure to draughts, sudden changes in temperature, wet feet, and so on may produce a congestion and inflammation of the upper respiratory tract which renders it susceptible to the invasion of the bacteria normally present. Such colds are usually mild and are not transmissible.

The allergic response of many individuals is similar to the symptoms of a cold.

Mechanical and chemical irritants may also produce symptoms of a cold. These may all be predisposing factors to the true infectious cold but they are not the cause. No amount of exposure will produce a true infectious cold in the absence of the virus, a fact well established by observations of explorers in the far north and of others living in isolated communities.

The bacteria commonly associated with colds are the staphylococcus, *Streptococcus viridans* and *hemolyticus*, the pneumococcus, *Neisseria catarrhalis*,

Pfeiffer's bacillus, diphtheroids, and a number of others. It seems likely that the peculiar character and severity of the infectious cold may be due to the different reactions produced by these secondary invaders. Many of them are normally present and anything which reduces bodily resistance may precipitate an invasion with attending cold symptoms. These are the so called autogenous colds.

Although colds are not in themselves essentially dangerous, they do predispose to all manner of secondary infections such as bronchopneumonia, middle ear infections, sinus infections, and, probably, an increase in the tuberculous process.

Epidemiology

Source and Mode of Infection: The cold is transmitted by direct contact. The source of the virus is the discharges of the upper respiratory passage of infected persons. Coughing and sneezing scatter the virus and bacteria into the air where they are readily taken in through the mouth or nose of susceptible individuals.

A cold is most contagious in its early stages, and transmission usually takes place before the infected individual takes the proper precautions. Epidemiologically, colds behave like other highly contagious diseases to which there is little immunity, spreading rapidly through families and groups.

Prevalence and Distribution: Colds are world-wide in distribution and the only places free from them are isolated communities. Records show that the average person has about two colds a year. A recent Gallup poll in the United States showed eighteen million cases in a single week in November.

Control: The effectiveness of hardening measures, irradiation, cold vaccines, and so on is difficult to evaluate. None of them appears to be effective as far as the group is concerned although many individual members insist that they are benefitted. Numerous individuals claim increased resistance to colds from the use of vitamines. As far as curative agents are concerned, the old saying that a treated cold lasts two weeks and an untreated one a fortnight tells the story at present. This does not mean, of course, that a sore throat, a cough, or any other aggravating condition should not be alleviated by the use of various remedies.

Prevention seems to resolve itself under two headings: (1) avoiding infection and (2) building up resistance. Neither of these is easy of accomplishment since it is almost impossible to prevent contact and no one is certain as yet just how to build up specific resistance. General well-being, proper diet and exercise, and protection against undue exposure are probably the best safe-guards against a cold and its consequences.

The autogenous cold may be prevented and the individual benefitted by removing such foci of infection as tonsils; but when groups are considered as such, colds are as common in groups of those who have had their tonsils removed as in groups of those who have not. *Autogenous vaccines* appear to be of some benefit in individual cases in the prevention of autogenous colds.

Bacterial cold vaccines do not appear to afford any protection against the virus cold nor do sulfa drugs or penicillin. However, the latter are of use in preventing complications due to secondary invaders.

Chapter XIX

WHOOPING COUGH, MEASLES, AND MUMPS

WHOOPING COUGH

Whooping cough or pertussis is an acute infectious disease of childhood. As a cause of death in infants and young children it exceeds scarlet fever, measles, and diphtheria. Like measles, whooping cough is widely prevalent, occurs in epidemics and is also endemic, and predisposes to bronchopneumonia.

Symptoms

Whooping cough is characterized primarily by a catarrhal involvement of the trachea and bronchi, a slight fever, and a paroxysmal cough that ends in a whoop. The coughs are so rapid and explosive that there is no chance for inhalation. The whoop, which follows a series of coughs, is really a gasp for air.

The onset of the disease, the catarrhal stage, is often like a cold and lasts about ten days. Examination of the blood during this stage reveals an increase in the number of all white blood cells with a greater increase in the type known as the lymphocytes. This condition is known as *lymphocytosis*. After a period of about ten days the paroxysmal stage sets in and continues for a varying length of time, usually from four to six weeks, after which the coughing becomes less frequent. The cough is due to the effort made to raise the tenacious mucous. It is sometimes followed by vomiting and nosebleed.

The disease varies in severity. It may be very mild and the whoop may be absent. If severe, it is apt to leave the child weak and subject to complications. When death occurs, it is usually due to bronchopneumonia.

Etiology

The infectious agent in whooping cough is *Hemophilus pertussis* or the Bordet and Gengou bacillus. In many cases there may be a mixed infection with pneumococci or streptococci.

Morphology and Staining: Hemophilus pertussis is a small ovoid gram-negative rod about 0.2 micron in width and 0.5 micron long. It is nonmotile and does not form spores. The smooth virulent forms are encapsulated and are known as Phase I. When freshly isolated, all strains are smooth. They become rough and loose their capsules upon cultivation on artificial media. There is a change in antigenicity corresponding to the change from smooth to rough. The practical importance of this

reversion lies in the fact that rough strains are of no value for immunizing against the disease.

Physiology. Hemophilus pertussis is a strict parasite and does not naturally multiply away from man. It is encountered only in cases of the disease. It does not ferment the ordinary sugars, reduce nitrates, nor form indol and yet is more easily cultivated on artificial media than is *Hemophilus influenzae* which does all three.

Bordet and Gengou first isolated *Hemophilus pertussis* on a medium containing a glycerin extract of potato and defibrinated rabbit or human blood. It is most readily isolated by the cough plate method which consists in holding a petri dish containing potato-glycerin-blood medium before the mouth during a paroxysm of coughing. The droplets expelled contain the organism. The colonies which appear in twenty-four to forty-eight hours are small, greenish, and thick, resembling drops of mercury. *Hemophilus pertussis* is a strict aerobe.

Pathogenicity and Mechanism of Disease Production: Hemophilus pertussis is pathogenic for man and for some experimental animals. Only the Phase I smooth organisms are virulent. The characteristic symptoms of the disease are probably due in part to mechanical disturbances and in part to endotoxic substances.

Immunity: There appears to be little natural immunity to *Hemophilus pertussis.* An attack of whooping cough usually confers a definite immunity and second attacks, when they do occur, are mild and not usually followed by complications. Antibodies appear in the serum as a result of an attack and, in a large per cent of the cases, the complement-fixation reaction becomes positive in the third or fourth week after the onset. Antibodies may also be induced by injecting killed organisms.

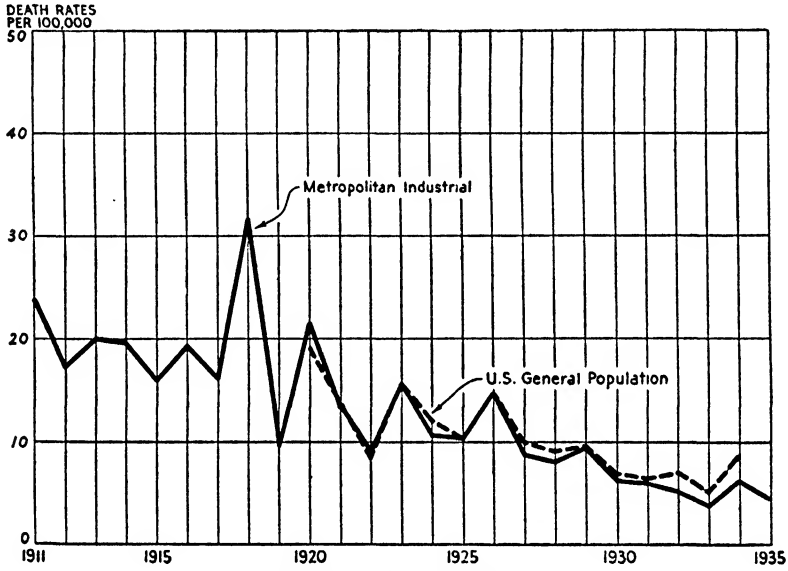
Prophylactic Immunization: Although reports on the value of prophylactic immunization are somewhat conflicting, it is generally agreed that vaccines made from freshly isolated encapsulated Phase I strains are of definite value. The dosage required is large; seven to eight cubic centimeters of vaccine, each containing about ten billion organisms, are given in several doses. The immunity conferred by such vaccines is sufficient to protect the child for a period of at least five to seven years after which whooping cough, if it does occur, is relatively harmless. All children should be vaccinated by the time they are two years old and the earlier the better.

Epidemiology

Source and Mode of Infection: Whooping cough is a droplet infection and the infectious agent is found in discharges from the nose and mouth. It is transmitted by contact. Carriers, if they exist, are rare.

The *incubation period* is usually seven days and probably never exceeds sixteen.

The *period of communicability* is from about seven days after exposure to about three weeks after the onset of the characteristic whooping stage. Whooping cough is most communicable during the early stages before clinical diagnosis is readily made.



WHOOPING COUGH

Standardized Annual Death Rates per 100,000
Total Persons Ages 1–14 years.
Metropolitan Life Insurance Co. Industrial Department 1911–1935
and U. S. Registration-States of 1920–1934.

Whooping cough is world wide in distribution. It occurs in every climate and in every season although it is most prevalent in the spring with another rise in the late summer. It is endemic and occurs in epidemics of variable severity.

The death rate has been declining steadily and in 1946 the crude death rate was 0.9 per 100,000 population. Many factors probably influence the downward trend in childhood diseases. Immunization is, of course, important but better living conditions in general and the smaller size of the average family have also had a significant effect.

Whooping cough is a disease of childhood, about ninety per cent of the cases occurring in children less than ten years old. About sixty per cent

of the deaths occur in infants less than one year old. As the age increases, the case fatality rate decreases.

Whooping cough is the only childhood respiratory disease in which the mortality is higher in females than in males. The difference is smallest in the youngest groups and becomes progressively greater as the age increases. The mortality rate averages about forty per cent higher in girls than in boys.

The mortality is greater in the colored race than in the white, and distinctly higher in rural than in urban districts.

There has been a decided decrease in the number of reported case of whooping cough during the past few decades. Comparison of figures from year to year shows considerable variation with a distinct downward trend.

In comparing death rates, it is essential to use standardized rates because the proportion of children is less now than it was twenty-five or thirty years ago. The greatest decline in numbers has been in the youngest age group which shows the highest mortality.

Prevention and Control. Because of the high case fatality—ten to twenty-five per cent in infants less than one year old—every effort should be made to prevent exposure of infants to whooping cough and to increase their specific resistance. Active immunization is recommended for all infants before the second year, preferably before the age of six months. Some recommend immunization at the age of three months because of the seriousness of the disease in infants. Some even recommend that pregnant women be immunized in order to confer a higher degree of passive immunity to the infant.

MEASLES

Measles is one of the common infectious diseases of childhood. It is also one of the most highly contagious of communicable diseases and consequently one of the hardest to control. Measles is a fairly mild disease. However, it is frequently complicated by pneumonia and the total number of cases is so great that, even with its low mortality, it is one of the leading causes of death in children.

Symptoms

The characteristic features of measles are fairly constant as compared to those of many other acute diseases. In the early stages there are the familiar cold symptoms. There is catarrh of the eyes, nose, and throat, a sensitivity to bright light, and a fever of about 102.5°F. Before the characteristic rash breaks out, small discrete bluish-white spots surrounded by a red zone appear upon the inner part of the cheek opposite the molar teeth. These are known as *Koplik's spots* and, if they can be identified, afford a means of early diagnosis.

The early stages are followed by an eruption of little red spots which

reaches its height in two to three days and disappears in a week. A branny flaking or desquamation follows. The great danger from measles is the secondary infection. Common complications are middle ear involvement and bronchopneumonia.

Etiology

A filterable virus is now generally accepted as the causal agent of measles.

The *source of infection* is secretions from the mouth and nose of cases of measles. There are no proven carriers. Measles is transmitted by direct contact with cases and indirectly by freshly contaminated objects and, probably, contaminated air. Epidemiological evidence suggests that the virus is short-lived and probably does not survive apart from the host for more than twenty-four hours.

The *incubation period* of measles is considerably longer than that of the common cold or influenza. About eight to ten days after exposure, fever occurs, and twelve to fourteen days later the rash appears.

Measles is most contagious during the early stages and before definite diagnosis can be made. It remains contagious for about five days after the eruption. The virus is not present in the scales and the disease is not contagious during the period of scaling.

Susceptibility and Immunity: There appears to be no natural immunity to measles. All persons who have not had the disease are susceptible. Infants born of immune mothers are immune for six to nine months. One attack usually confers immunity although second attacks do occur. The concentration of neutralizing antibodies is high following an attack of measles and antibodies are present in the serum throughout life although they gradually diminish in concentration.

Immune serum has a pronounced effect on the course of the disease if given before the symptoms appear. If it is given before the fourth or fifth day after exposure to the virus, it protects the child completely. Symptoms do not develop and the virus, presumably, is prevented from multiplying and consequently does not serve as an antigen and does not produce an immunity. If the serum is given from the fifth to the seventh day after exposure, infection takes place and the individual is rendered immune but the disease is very mild. Complications do not occur. If serum is given after the onset of symptoms, it has little effect. The recommended procedure is to give it on about the fifth day after exposure.

The dosage depends upon the sources. Four to ten cubic centimeters of convalescent serum or twenty to fifty cubic centimeters of whole blood from immunes is sufficient. Placental extracts are sometimes used in place of convalescent or immune serum. They contain diphtheria and scarlatinal antitoxins and neutralizing antibodies for the measles virus as well as for other viruses.

"Gamma globulin" obtained from pooled human plasma has recently been made available and since it contains antibodies in high titer it is most effective.

Prevalence and Distribution of Measles: Measles is found all over the world and is most frequent in the younger age groups. It presents characteristic epidemiological features which can be explained by the fact that it is extremely contagious and that one attack usually confers immunity. Its periodicity is one such feature. It occurs in cycles of two to four years in thickly populated areas. When the virus is introduced into a susceptible population, it attacks all non-immunes, immunizing them. Then the epidemic wanes until a sufficient concentration of non-immunes is reared when it occurs again.

Measles is commonly and characteristically a disease of childhood, not because adults are naturally resistant but because they are immune as a result of childhood infection.

In discussing the epidemiology of measles, mention must be made of the studies of Panum on an outbreak which occurred in the Faroe Islands. Panum's paper is one of the classics of epidemiology.

In 1846 the Faroe Islands had been free from measles for a period of sixty-five years. Then it was reintroduced by a sailor who had come down with a case during his voyage there. In a few weeks measles was rampant and had attacked every age, old and young alike. None who were exposed and who had not had the disease—that is, who were less than sixty-five years old—escaped.

There have been other instances of persons in isolated communities who escaped infection in childhood only to take the disease in later life. During the World War, measles was prevalent in many camps among troops recruited from rural regions.

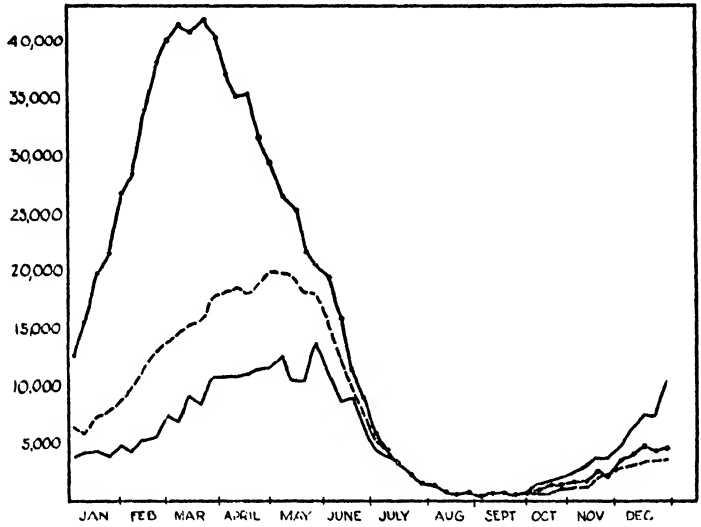
The death rate from measles is highest during the second year of life and practically all deaths from measles occur between the ages of one to four. The case fatality rate, however, is highest in the first year and becomes progressively lower as age increases. It is fortunate that the chance of contracting measles is not so great during that first year.

The death rate, which is slightly higher in males than in females and in white than in colored persons has been dropping steadily and is now 0.2. The case fatality rate among younger children is:

	Fatalities per 100 cases
Group aged 0–1	4
1–2	2.5
2–3	0.8
3–4	0.4
4–5	0.2
5–9	0.1

Measles is usually highest in March and drops to a low in late August and September. In general, it is true that the greater the incidence in any year, the earlier in the year the peak is reached.

Since 1933 there has been an increase in incidence of measles and the first part of 1938 showed the greatest incidence in years. There has been a decline in the mortality during the last fifty years. There are no specific measures for the prevention of measles to account for the decline. The use of serum is not common enough to do so. Whether there has been a change in the virulence of the virus or whether improved living conditions



Seasonal distribution of measles. Number of reported cases plotted by weeks 1938 (uppermost), 1937 (lowermost), and median 1928–1936. Redrawn from U.S.P.H.R.

have increased general resistance to the bacteria which produce fatal complications such as bronchopneumonia is not evident.

Many persons who recover from measles are left with permanent damage which may be an indirect cause of death in subsequent years. Thus the number of deaths attributable to measles is really greater than indicated by the mortality records.

Control

The control of measles is extremely difficult. It is so highly contagious that all those who are exposed and who have not had the disease contract it. As a rule, a child has exposed his contacts before there is a chance of isolating him. There is, as yet, no vaccine by means of which active im-

munization may be produced and there is no chemotherapeutic agent that is effective. To be sure, the severity of the disease may be lessened by the use of convalescent serum or gamma globulin if the date of exposure is known but if it is not, serum treatment is of very little value. If older children in a family contract measles, children less than five should be protected by the use of convalescent or immune serum. When measles is prevalent in a community, great care should be taken to prevent the exposure of children under five.

Insofar as the individual who has contracted measles is concerned, isolation is recommended for two reasons: first, to prevent his giving it to others, and, second, to prevent others from giving him secondary infections, since measles predisposes to bacterial invasion.

Although chemotherapeutic agents have no effect on the virus they do control secondary bacterial infections which are responsible for the fatalities.

The question of whether or not schools should be closed in the face of an epidemic must be considered. It is generally agreed that the closing of schools is of little value in preventing further cases. However it has been suggested that when a case occurs, the school remain open for eight to ten days and the contacts be closely watched for early symptoms. It might then be closed for a week and those who do not show symptoms at the end of that time, be allowed to return.

MUMPS

Mumps or epidemic parotitis is an infectious disease probably due to a virus. It is usually characterized by a swelling of the salivary glands which causes a fullness of the face in front of and under the ears and is responsible for the pain in eating. One or both sides may be affected. The salivary glands, parotid, submaxillary, and sublingual, are most frequently involved but the mammary glands, the testes, and the pancreas may also be affected. The disease is highly contagious and spreads with great rapidity in a susceptible population.

Mumps is caused by a virus which is present in the saliva and is transmitted by droplets. Carriers are unknown and transmission by persons in contact with cases probably does not occur.

The incubation period is from two to three weeks and the disease appears to be infectious in the pre-clinical stage. Because of that, isolation of cases is not effective in controlling epidemics.

Mumps is not a serious disease in children but may be in adult males, should the testes become involved. An encephalitis sometimes follows as a complication of the disease.

Domestic animals such as dogs and cats are said to be susceptible to mumps and may transmit the infection to man.

Chapter XX

DIPHTHERIA

Diphtheria has been and is endemic all over the world. It was probably one of the epidemic diseases among the Romans but until the middle of the nineteenth century was not really serious in any country of Europe except France, where it was first recognized as a clinical entity by Bretonneau of Tours in 1826. George Washington died of it and great epidemics used to spread through the country in five to ten year intervals. From 1850 to 1880 many cities in the United States experienced particularly violent epidemics. Death rates ran as high as 500 and, on occasion, 1000 or over per 100,000 population. At the turn of the century diphtheria was still one of the most dreaded of diseases. Today it is relatively rare. The death rate in 1947 was 0.9.

In 1884 Loeffler discovered the causative agent. The bacillus had been seen and described by Klebs in the previous year but its significance as the cause of diphtheria was first proven by Loeffler. The disease is of particular interest to the bacteriologist and epidemiologist because researches on diphtheria have suggested methods of approach to many other bacterial diseases. There is, perhaps, no other disease about which the bacteriologist knows so much nor in which his laboratory findings have resulted in such immediate and remarkable benefit to the afflicted. The conspicuous success of the practical application of his findings lent an impetus to the development of the whole science of bacteriology, immunology, and preventive medicine.

Diphtheria was extremely prevalent and receiving a great deal of attention during the last half of the nineteenth century when the science of bacteriology was developing. As it happens, it was a happy choice of disease for the new science to work upon for several reasons.

It is a readily recognized clinical entity and accurate diagnosis is possible by bacteriological and clinical findings.

The causative agent is readily grown in pure culture and easily manipulated in the laboratory.

Experimental animals are not difficult to find.

The mode of transmission is well known.

The organism secretes a powerful exotoxin which stimulates an equally powerful antitoxin.

The use of antisera is attended by spectacular clinical improvement.

It was possible to work out methods for producing active immunity which give nearly one hundred per cent protection.

The immunity of individuals can be accurately measured.

Recognition of Diphtheria

Diphtheria is an acute infectious disease generally of the air passages of the throat and tonsils, and, sometimes, of the nose. The site of infection is marked by the formation of a tough dirty-white or greyish membrane called a false or pseudo-membrane. Because of the leathery appearance of this false membrane, Bretonneau gave the disease the name of *diphtheria* from the Greek word meaning leather.

Diphtheria is a localized infection with a generalized toxemia. The principal symptoms are due to the local disturbance and mechanical obstruction of the air passages, in part, and, in part, to the powerful toxins elaborated by the organism. The bacillus grows on the surface and rarely penetrates to any extent. It does not invade but produces a toxin, and tissue in contact with the toxin reacts in a characteristic fashion: many of the epithelial and connective tissue cells become degenerated, the capillaries become distended with blood, and the phagocytes mobilize. The blood plasma leaks out of the distended capillaries to form a fibrinous clot in which phagocytes and necrotic tissue cells become entangled. The false membrane thus formed may cover the entire surface of the throat and larynx effectively closing the air passages and producing a condition known as *membranous croup or laryngeal diphtheria* in which death is caused by suffocation.

The toxin affects the heart, liver, kidneys, glands, and the nervous system, and death occurring as a result of its action is due to heart failure.

At the onset, diphtheria does not display any distinctive symptoms. The child is not very ill, the throat may not be sore, and the temperature may be only slightly above normal, rarely over 101°F. As the disease progresses, the throat may swell, the voice become nasal, and symptoms due to mechanical obstruction become more evident. There is a fetid odor given off by the membrane and a pronounced swelling of the cervical lymph glands. The throat presents a dull red appearance with patches of greyish white membrane.

Etiology

The specific cause of diphtheria is *Corynebacterium diphtheriae* or the *Klebs-Loeffler bacillus*. The prefix *Coryn* means club, and the diphtheria bacillus and other members of this group are slender somewhat club-shaped rods.

Morphology and Staining: The diphtheria bacilli present a distinctive

appearance in smears, partly because of the shape of the organisms and partly because of their typical arrangement. It is one of the few pathogens that can be diagnosed fairly accurately by microscopic examination.

The organisms are characteristically variable in shape. The typical forms from a culture are long, slender, nonsporulating rods ranging from 1 to 6 microns in length and 0.3 to 1 micron in width, with swollen and rounded ends. Some are slightly curved or bent. When stained with Loeffler's methylene blue or other suitable stains, the swollen ends present a characteristic barred, striated, or granular appearance due to uneven staining. Short rods that stain evenly may also be seen. Some strains are more irregular than others and attempts have been made to differentiate types on the basis of morphology but this seems hardly justified because of the variation within the strains and the variations induced by the age of the culture and composition of the medium.

The arrangement of the cells, as we have said, is typical. They tend to lie almost parallel to each other. It seems that when a cell enlarges and divides into two, they both continue to elongate before becoming separated and thus cause a tension on the cell membranes or covering. When the membrane breaks, it does so on one side and the tension on the other side causes the cell to be pulled back, forming characteristic V's, N's, W's, or "palisades."

Corynebacterium diphtheriae is gram-positive and not acid-fast. It is nonmotile, does not produce spores or capsules, and grows best at body temperature.

Resistance: Since the diphtheria bacillus does not form spores, it has no resistant stage and is readily killed by disinfectants or by boiling for one minute or heating at 58° C. for ten minutes. Pasteurization of milk destroys it. Low temperatures have no appreciable effect. When dried in diphtheritic membrane, it lives for several months.

Physiology: All members of the genus *Corynebacterium* are parasites and are found closely associated with the skin of man and animals. They grow readily on a variety of laboratory media, particularly if serum, blood, or meat infusion is added. They also grow readily in milk, a fact of considerable public health significance since milk may serve as a means of transmission.

The most useful medium for the isolation and identification of the diphtheria bacillus is Loeffler's medium, which contains beef blood serum and glucose meat infusion or blood agar. The colonies grow luxuriantly on this medium, making early identification possible. On medium containing potassium tellurite, the organisms reduce the salt and the colonies appear black.

Corynebacterium diphtheriae ferments a number of sugars but the carbo-

hydrate fermentation does not correlate with pathogenicity and is not used for differentiation. It is slightly proteolytic.

The physiological feature of greatest practical interest is the ability of the organism to produce a potent exotoxin. This will be considered in the following paragraphs.

Pathogenicity: The genus *Corynebacterium* has, for convenience, been divided into several species of which only *C. diphtheriae* produces an exotoxin. It is, perhaps, sufficient for our purposes to consider the genus as consisting of two groups: the *true* diphtheria bacillus which produces a powerful exotoxin and the *diphtheroids* which are morphologically similar but do not produce toxin.

The true diphtheria bacillus is pathogenic for man and a number of animals such as guinea pigs, rabbits, cats, dogs, fowls, and horses. Mice and rats are resistant. The guinea pig is most widely used for experimental purposes and the horse for the production of antiserum.

The characteristic feature of the experimental disease in animals is that the organisms nearly always remain localized at the point of inoculation, whereas symptoms are evident in a number of organs and tissues. This is because the organisms do not possess a high invasive power, but, once established, secrete the exotoxin which is readily absorbed.

Diphtheria Toxin: The presence of an exotoxin in filtrates of *C. diphtheriae* was first demonstrated by Roux and Yersin in 1889. Since then it has been the subject of a vast amount of investigation and, although its properties are now rather well known, its exact chemical nature is a mystery. It is protein in nature and behaves like a number of other proteins when treated by physical and chemical agents—it is thermolabile, being rapidly inactivated at a temperature of 60° C. and destroyed in less than five minutes by boiling—it is modified or inactivated by treatment with formalin or precipitation with such agents as aluminum hydroxide. When thus treated, it loses its *toxicity* but retains its *antigenicity* and is then called *toxoid*, or, by some, *anatoxin*.

For the purpose of producing antitoxin it is desirable to have a toxin of high potency as free as possible from other proteins or antigenic substances. The strain of the organism to be used in the production of toxin is obviously of importance since there is considerable difference in the toxogenicity of various strains. It should be remembered, however, that the strains differ only in the amount of toxin produced, not in the kind. And there is no direct relation between invasiveness and toxogenicity. Experiments have shown that some strains readily establish themselves and only a few organisms are necessary to kill a guinea pig but that these strains produce such a small amount of toxin that, if their filtrate is used, a large dose is necessary. There are other strains that produce potent

toxic filtrates in which huge doses of living organisms are necessary to kill guinea pigs because the strains do not readily establish themselves. The strain most commonly used in the production of antitoxin is known as Park No. 8. It is neither particularly virulent nor invasive but is a high producer of toxin.

When diphtheria bacilli are grown on artificial media, they frequently dissociate, undergoing a smooth to rough transformation with a subsequent loss of toxogenicity and virulence.

The medium used for the production of toxin is of great importance. The optimum hydrogen-ion concentration is about 7.8, hence fermentable sugars are not added because the resulting acidity inhibits the formation of the toxin or destroys it as it is formed.

The medium is inoculated with suitable strains of the organisms, the culture incubated at 34° to 37° C. for about seven days, and then filtered to remove all bacteria. The toxin thus produced is unstable and undergoes a rapid loss of potency if exposed to oxidation by air and light. The next step, to determine its potency, is accomplished as follows. Guinea pigs weighing about 250 grams are inoculated with varying dilutions of the filtrate. The highest dilution which will, on the average, kill the guinea pigs in four to five days is the *M.L.D., minimum lethal dose.* The M.L.D. of the toxic filtrate varies but potent strains yield sufficient toxin so that one cubic centimeter of a dilution of 1:500 contains one M.L.D. Many filtrates are much stronger.

Various methods have been developed for concentration and purification and the toxin has recently been isolated in a chemically pure state.

Diphtheria Antitoxin: In 1890 Behring and Kitasoto discovered that the serum from rabbits immunized against the diphtheria bacillus was capable of neutralizing the toxin and of protecting animals injected with the virulent organisms. Further investigation demonstrated that such antitoxin possessed a distinct and specific curative effect when injected into humans. Because of the dramatic results obtained with the use of antitoxin, methods for producing it in large amounts were soon developed and by 1894 it had become generally available to the medical profession.

Diphtheria antitoxin is produced commercially by injecting horses with increasing amounts of toxin. Injections are made every five to seven days and, after a period of two to three months, the horse serum is tested for potency in neutralizing the toxin. The methods used for titrating the serum are somewhat complicated but a number of units have been worked out which make testing and comparison fairly accurate.

Ehrlich, to whom we owe much of our information concerning toxin and antitoxin reactions, defined a unit of antitoxin, *A.U.*, as the smallest amount which would neutralize 100 M.L.D. of toxin. Since the toxin is

unstable and its M.L.D. decreases on standing, antitoxin, which is relatively stable, is used as a standard. Standard antitoxins that may be used to determine the potency of toxin and of other batches of antitoxin are kept in various countries by government institutions. The present unit is an arbitrary standard approximately equal to the amount needed to neutralize at least 100 of the original M.L.D.

The early methods of standardizing toxin or antitoxin were perforce worked out on animals. Later, precipitin or flocculation tests were developed. They are more rapid and probably as accurate.

Antitoxin prepared from horses contains from 300 to 1500 A.U.; that marketed usually contains from 500 to 700 A.U. Many attempts have been made to concentrate and purify the antiserum so as to reduce the amount of liquid and foreign protein.

Epidemiology

Source of Infection: Corynebacterium diphtheriae is found in the discharges from the nose and throat of cases and carriers. However, any diphtheritic lesion in wounds, on the genitalia, or on the conjunctiva may serve as a source of the organism.

Mode of Transmission: Diphtheria is transmitted by direct personal contact with cases and carriers, or indirectly by contact with articles soiled by discharges from cases and carriers, or by milk and other dairy products. Because, except under unusual conditions, the organisms do not live on objects for more than a few weeks, the danger of contracting diphtheria from them depends upon the length of time which has lapsed since they were contaminated.

The *incubation period* of diphtheria is usually two to five days but may occasionally be longer.

The *period of communicability* is for as long as the cases harbor and discharge virulent bacilli and is variable. A large percentage cease to discharge organisms in two weeks or less, some remain carriers for long periods of time.

Susceptibility: The Schick Test. In no other disease does the clinician have as accurate and easily performed a test for susceptibility. In 1913 Schick worked out a method for determining susceptibility. It is based on antitoxic immunity and consists, in brief, of injecting 1/50 M.L.D. of toxin into the skin. If no antitoxin is present, this amount produces a local swelling and reddening at the site of the injection; if antitoxin is present no reaction occurs. Experience has shown that if sufficient antitoxin is there to neutralize a Schick test dose, the individual is immune to diphtheria; if not, he is susceptible.

The reading and interpretation of the reaction to the Schick test requires some experience. Some individuals are sensitive to proteins and give a reaction resembling the Schick positive reaction even though they do have sufficient antitoxin to neutralize the Schick toxin. In actual practice it is, therefore, necessary to control the test. Since the Schick test is usually performed by making an intradermal injection into the flexor surface of the right forearm, the control test consists of making a similar injection into the left forearm, using a portion of the Schick toxin that has been heated to 70° C. for five minutes to detoxify it.

The actual reading of the test may be made four to seven days later. There are four recognized reactions.

1. *Negative Reaction:* If no reaction develops which persists by the end of the fifth day, the test is said to be negative and the individual is *not susceptible* to diphtheria.

2. *Positive Reaction:* If a lesion ranging in size anywhere from a dime to a quarter, and in color from pink to red develops at the site of the test injection, the individual is said to be Schick positive, that is, *susceptible.*

3 and 4. *False or Pseudo Reactions:* If a reaction develops at the site of the control, the individual is sensitive to proteins. Such reactions are usually smaller, not circumscribed, and tend to a bluish color. If the pseudo-reaction appears on both arms, the individual is recorded as *negative-pseudo.*

If the individual is sensitive to the protein as well as to the toxin, the reactions will be different on the two arms, the right arm showing the typical Schick positive reaction and the left arm, the positive pseudo-reaction. Such individuals are recorded as *positive-pseudo.*

It should be noted that this is a test for immunity to the toxin and not for immunity to the organism. A large number of Schick tests have been performed by a number of workers in different cities and countries and the results all point to the same conclusions. The new born are immune as a result of congenital passive immunity if the mother is actively immune. The infant gradually loses this passive immunity until at the age of six months or a year, it becomes susceptible. Then the percentage of susceptibles decreases until at the age of twenty or so, eighty to ninety per cent of the population is immune. The figures in Table 11 give the percentage of susceptibles obtained by Schick testing the population.

The validity of the Schick test as a measure of immunity is amply demonstrated by experience. In a population that has not been artificially immunized, the age distribution of cases parallels the susceptibility distribution. During the early stages of a case of diphtheria, the Schick reaction is positive; it becomes Schick negative following recovery. There

are exceptions. A small per cent of those who recover from a case of diphtheria remain Schick positive and a small per cent of those who contract diphtheria suffer second attacks.

Cases of diphtheria do not occur in contacts who are Schick negative but in those who are Schick positive.

The change in the Schick reaction in various age groups cannot be accounted for on the basis of actual cases resulting in immunity since a large percentage of those who become Schick negative have never had recognizable cases. It is logically assumed that, since the diphtheria bacillus is wide spread because of the large number of carriers in the population, the chances for contact with it are great. Many children who do not develop recognizable cases of diphtheria have enough of an infection so that they develop an immunity. Diphtheria is not unique in

TABLE 11

Susceptibility of Various Ages to Diphtheria (as Indicated by Diphtheria-Toxin Skin Test)

AGE	PER CENT SUSCEPTIBLE
Under three months	15
Three to six months	30
Six months to one year	60
One to two years	70
Two to three years	60
Three to four years	40
Four to ten years	30
Ten to twenty years	20
Over twenty years	12

this respect. The same situation pertains to scarlet fever and probably to a number of other infectious diseases.

The percentage who develop an immunity is apparently proportionate to the percentage of carriers or cases with whom they come in contact, and the chances for such contacts are dependent upon the density of the population. Thus we find that while the susceptibility curves are similar for rural and urban groups, the former show a lag of about five years.

Immunization Against Diphtheria

There are two methods of producing immunity to diphtheria—one produces a passive, the other, an active immunity. The former is largely a curative measure, the latter, a preventive one.

Passive Immunization: Diphtheria antitoxin is an effective curative agent for the treatment of cases of diphtheria and is equally effective for

preventing the development of cases if introduced shortly after the time of exposure. Its effectiveness in preventing deaths depends upon how early in the disease it is administered. If given on the first day of the disease, it reduces the case fatality rate to practically zero; if given on the second day, it is still very effective; if given on the third day, less so; and if given after the fourth day, of little, if any, value. The reason for this is found in the fact that the antitoxin is not really a curative measure. It does not repair damage already done but prevents more.

Since the effectiveness of antiserum depends on its being given on the first or second day of the disease, it follows that early diagnosis and prompt use of antiserum is of utmost importance. Furthermore, the physician is justified in giving antitoxin in all suspected cases and to all susceptible contacts.

The dosage employed is of considerable importance. In mild cases, 3000 to 5000 units are usually given; in moderately severe cases, 10,000 units; and in severe cases, 20,000 to 50,000. The antitoxin is usually injected subcutaneously in the thigh although in very severe cases it appears to be more immediately effective if injected intravenously.

The effectiveness of antiserum as a therapeutic agent in diphtheria is illustrated by a comparison of the case fatality rates before and after the introduction of serum treatment. According to some authorities, at least forty-five per cent of all cases used to end fatally. After the use of antiserum became general, the case fatality dropped to thirteen per cent. There is, for all that, some question as to whether this drop should be attributed entirely to the use of antitoxin since diphtheria was apparently becoming less virulent even before the discovery of the causative agent and several years before the discovery of the value of antitoxin. The discovery of the diphtheria bacillus and the development of accurate diagnosis during this period undoubtedly led to the reporting of far more cases and this would also make an apparent decrease in fatality.

Serum reactions sometimes occur following the use of diphtheria antitoxin. The physician should know whether the patient has hay fever or any other allergy and whether he has had any previous serum treatment. Skin tests for hypersensitivity are usually made by injecting 0.1 cubic centimeter of a 1:10 dilution of the antiserum intracutaneously into the forearm. If no reaction occurs in fifteen minutes, anaphylactic shock is not likely, although the so-called 'serum sickness' may occur even if the immediate reaction is absent.

If a reaction does occur within fifteen minutes, the patient is sensitive to horse serum and liable to anaphylactic shock. The serum may still be administered without danger if the patient is desensitized by giving only 0.5 cubic centimeter subcutaneously and doubling this dose every

half hour until the entire amount has been given. If serum sickness develops after a period of about twelve hours or if the more acute anaphylactic reaction occurs, a half to one cubic centimeter of a 1:1000 adrenalin chloride solution should be given.

A dangerous reaction following the use of any antiserum is extremely rare but is the cause of most of the objections to the use of sera or vaccines by the layman, who often does not distinguish between the two.

Active Immunization Against Diphtheria: It has been amply demonstrated that diphtheria can be prevented by active immunization with toxin-antitoxin or with toxoid. The toxin alone is so poisonous that sufficient amounts to immunize cannot be given. However, if it is treated with antitoxin so that the mixture is only slightly toxic, it can be used safely. The toxin-antitoxin mixture dissociates very slowly in the body, and the toxin liberated stimulates the production of a large amount of antitoxin.

Toxin-antitoxin is usually given in one cubic centimeter injections spaced about a week apart. Infants less than one year are given 0.5 cubic centimeter for the first injection. Three doses are sufficient to establish a negative Schick reaction in about eighty per cent of the persons immunized. Those who remain Schick positive should be given another series.

Toxin-antitoxin as an immunizing agent for diphtheria is being supplanted by detoxified toxin or toxoid. The latter has many advantages and is now used almost universally for immunizing children. Since adults sometimes get severe reactions from the use of toxoid, the toxin-antitoxin is still used for them.

Toxoid is prepared by treating toxin with 0.2 per cent to 0.4 per cent formalin or by precipitating with alum. The mixture is incubated at 37° to 40° C. until it becomes nontoxic. This may take several weeks or months, although the initial drop in toxicity is very rapid.

Alum precipitated toxoid contains enough antigenic material so that in a large percentage of the population one injection is sufficient to immunize. Since toxin-antitoxin requires three injections, the advantage of alum precipitated toxoid is obvious. Then, too, the slight chance that the child will become hypersensitive to the horse serum contained in the toxin-antitoxin mixture is eliminated.

The use of toxin-antitoxin or toxoid has greatly reduced the death rate. In a number of cities where immunization of the younger age groups has been practiced, there has not been a single death from diphtheria for a number of years. Serving unwittingly as a control on these cities, have been other cities where such methods have not been practiced and in which the death rate has been from four to as high as ten per 100,000.

Carriers

From the point of view of control of diphtheria, the carrier is far more difficult to deal with than the case. It has been calculated that the chance of contracting diphtheria from a case is about ten times as great as the chance of contracting it from a carrier; but there are about ten times as many who become infected from carriers as from cases because the number of carriers is so much greater.

Numerous studies have been made to determine the incidence of carriers and although the results vary somewhat, they show that the diphtheria bacillus is prevalent in a good proportion of the population all the time. The incidence of carriers is highest during epidemics and is higher in those in contact with cases than in those in contact with other carriers. During

TABLE 12

Diphtheria: Cases, Deaths, and Deaths per 100 Cases—Ages under 15 Years
(State of New Jersey, 1930 to 1934)

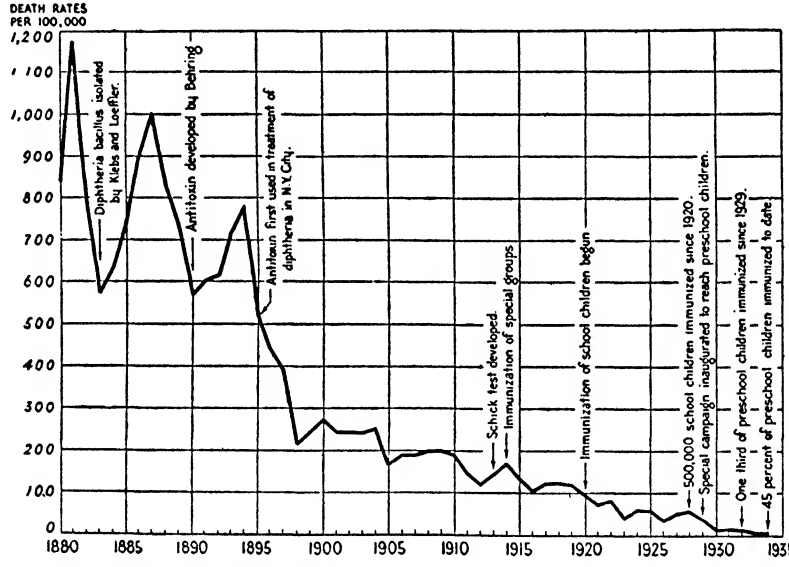
AGE PERIOD, YEARS	CASES		DEATHS		DEATHS PER 100 CASES
	Number	Per cent of total, all ages	Number	Per cent of total, all ages	
All Ages	9,373	100.0	658	100.0	7.0
Under 15	7,526	80.3	602	91.5	8.0
Under 1	180	1.9	40	6.1	22.2
1	446	4.8	78	11.8	17.5
2	693	7.4	95	14.4	13.7
3	810	8.6	88	13.4	10.9
4	825	8.8	71	10.8	8.6
5–9	3,366	35.9	182	27.7	5.4
10–14	1,206	12.9	48	7.3	4.0

epidemics, the carrier rate in contacts may run from ten to fifteen per cent on an average, while in the general population, from a half to one per cent harbor the virulent organism. The rate in schools runs from about one-tenth to five per cent.

It should be pointed out that the carriers harbor the organisms for variable periods of time and that during an entire year perhaps forty to sixty per cent of the population have been carriers. Twenty years ago eighty per cent of the population became Schick negative before reaching the age of twenty-one. Army tests show an increase in the number of Schick positive adults. It seems that with the decrease in the incidence of diphtheria, fewer people are getting a natural immunity and more remain susceptible into adulthood. We have to assume that Schick positives who become immune without developing the disease are carriers during

the period of immunization and since eighty to ninety per cent of the people become Schick negative before reaching the ages of twenty to twenty-five, it follows that nearly everyone is a carrier at least once during the first twenty years of life.

The effect of artificial immunization on the carrier state is of interest. Since the immunity gained by such immunization is antitoxic and not antibacterial, we might not expect to find any resistance to the carrier state. Certainly there seems to be none and rendering the population



*Manhattan and Bronx Boroughs.

DIPHTHERIA

Crude Annual Death Rate per 100,000 children under 10 years of age
New York City 1880–1934.

Schick negative does not decrease the percentage of carriers. It is of interest in this connection to know that the carrier rate is high in the tropics although the disease is rare.

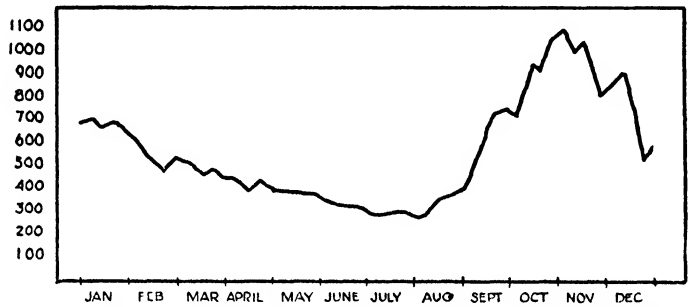
Prevalence and Distribution of Diphtheria: Diphtheria occurs in every age, race, climate, and country and it shows a characteristic distribution. It strikes swiftly, takes its toll, and leaves only to reappear in a few years and repeat its deadly performance.

Age: Diphtheria is characteristically a disease of childhood although any age is susceptible if not immunized as a result of contact with the organism. The age distribution is due not to an inherent "maturation im-

munity" developed as a result of physiological changes that naturally occur during the aging of the individual; but rather to the development of a specific antitoxin which appears as a result of stimulation by the toxin. The incidence is greatest during the early school years but the case fatality is highest during the first year of life, when, fortunately, the disease is rare as a result of congenital immunity.

Sex and Race: The mortality from diphtheria is slightly higher in females than in males and considerably higher in the white than in the colored race. The downward trend has been far more rapid in the white than in the colored race, however, and the difference is becoming less and less.

Geographic, Climatic, and Seasonal Distributions: Diphtheria is more prevalent in the northern than in the southern states. It is rarely present in the tropics, a fact that must be corrected for when comparing the



Seasonal distribution of diphtheria. Median number of reported cases by weeks for a four year period. Redrawn from U.S.P.H.R.

death rate in colored and white populations. However, in the United States, the strides made in prevention have been greater in the northern and eastern states than in the southern and western and, as a consequence, many cities in the southwest show a much higher death rate.

The highest incidence of diphtheria in the United States is in the autumn shortly after the opening of schools and the lowest in late summer. In the southern hemisphere the months of highest and lowest incidence are, of course, reversed.

Trend in Mortality: Although in any community the morbidity and mortality show a wide variation from year to year, the differences are more or less ironed out when the rate for the entire United States is calculated. The rate for the country as a whole varies from year to year with a steady decline in the number of deaths since about 1870, the crude death rate for 1946 being 0.9 per 100,000 population.

In Europe the incidence of diphtheria showed a steady decrease up until about 1936 and then in Germany it began to increase markedly, probably

due to lack of immunization and to mass movements of children to camp. At about the same time the severity of the disease increased and antitoxin treatment frequently failed. By 1941 and 1942 the new more virulent form spread to occupied countries. In Norway the incidence increased over one hundred fold. Diphtheria was one of the leading epidemic diseases of Europe during World War II. This was not true in the United States where the rate continued to decline.

A number of factors must be considered in trying to explain this decline. First, there was the introduction between 1895 and 1900 of the use of antitoxin in the treatment of cases; but, while it reduces the mortality, it does not change the incidence.

Second, there was the development of Schick testing which made active immunization with toxin-antitoxin and, later, with toxoid, practical. The first efforts at active immunization were directed largely to children in the schools. This considerably reduced both the incidence and the death rate. The latest efforts are being directed to the preschool age group, and available evidence suggests that by immunizing thirty to forty-five per cent of this group and sixty to seventy per cent of the school age group, diphtheria can be eliminated.

The pasteurization of milk has prevented milk-borne epidemics or outbreaks and thus has contributed to the general reduction in cases and deaths.

But, in addition to the effect of these control measures, there is perhaps another factor to be taken into account. The death rate had started to decline rapidly before the discovery of the cause of the disease or of specific measures for combatting it. This decline is best explained by assuming a change in the virulence of the organism. Organisms isolated during some outbreaks are more virulent than those isolated during others and it is undoubtedly true that the diphtheria bacillus has become less virulent during the past fifty years.

That the change in death rate is not wholly due to this last factor is shown by the difference in death rates in any community, city, or state where active control measures are being carried out as compared to similar communities, cities, or states, where such efforts are not being made.

Prevention and Control

As is usually the case, the methods of prevention and control of diphtheria are the logical outcome of specific knowledge of its etiology and epidemiology.

Rosenau has outlined these procedures for the control of diphtheria epidemics—

1. Early recognition and isolation of cases.
2. Finding and quarantining carriers of virulent bacilli.
3. Discovery of the susceptibles by means of Schick testing.
4. Immunization with toxoid of all susceptibles.
5. Inspection of all susceptibles twice daily.
6. Use of antitoxin upon the first appearance of suggestive symptoms.
7. Pasteurization of milk.
8. Epidemiologic study to disclose source of infection, methods of spread, etc.
9. Disinfection.
10. Schick testing after three months.

The procedures used to combat an epidemic in schools are not the same as those used to prevent an epidemic in the general population. Diphtheria as a disease could be eliminated by actively immunizing infants between their ninth and twelfth months. Immunization should be directed at this age group because it has the highest case fatality. Schick testing before immunization is not necessary in this group but should be employed afterward to see whether an active immunity has been acquired. Active immunization does not, however, eliminate the organism since a certain proportion of the immunes serve as carriers.

The procedure to be adopted in any isolated instance of diphtheria will depend upon circumstances. The treatment of clinical cases consists in prompt use of antitoxin. It is the only remedy. There are no specific chemicals of therapeutic value.

Chapter XXI

TUBERCULOSIS

"Take of new Milk two Gallons, distil it with Mint, Roman Wormwood, of each two Handfuls, to a Gallon. Then Take of Garden Snails, washed first in common Water, and then Small Beer, half a Peck, of Earth-Worms slit and wash'd a Pint, of Angelica a Handful and half, Agrimony, Betony, Rue, of each a Handful. Put the Herbs in the bottom; upon these lay the Snails and Earth-worms, and upon the top of all lay of Shavings of Hartshorn half a Pound, of Cloves an Ounce, of Saffron three Drams, Infuse them in two Quarts of Syder, and a Quart of the best Malaga Sack, and then distil them in an ordinary Still. These Liquors must be drank plentifully."

Sir Richard Morton; Phthisiologia, or A Treatise of Consumption, 2d Ed., London, W. & J. Innys, 1720.[1]

Throughout the entire history of civilized man the White Plague or tuberculosis has been a familiar and deadly disease. Its antiquity may be judged from the fact that human skeletons dating back some seven thousand years show evidence of infection. Animals, too, suffer from tuberculosis and in both man and animal the disease shows similar epidemiological features. It is a disease of civilized man and of domesticated animals. People living a nomadic existence and animals in the wild escape infection; whereas people in cities and animals in zoos or barnyards are infected. This is undoubtedly due not to any inherent resistance but rather to the fact that the chances of infection increase in direct proportion to crowding and because the more rigorous struggle for existence among primitive people and wild animals leads to the earlier death of the tuberculous and, consequently, reduces the chances of spread. This would not be true if the disease were communicable in the early stages.

The effect of crowding has been shown in civilized societies, for it has long been recognized that tuberculosis is more common among urban than rural dwellers; that it is a disease of soldiers in the barracks but not in the fields; of monks in monasteries and nuns in convents, but not of priests in the parish; and that people living in barren and sparsely populated mountainous regions are less apt to be infected than those living in the fertile and more densely populated valleys.

[1] In an article in The American Review of Tuberculosis, January, 1948, McLeod, Riggins, and Gearhart quoted this recipe with the following comment: "The following concoction is illustrative of the empirical nature of tuberculosis therapy offered as recently as some two hundred years ago by that renowned author and clinician, Sir Richard Morton, "for the cure of consumption in the second degree." From its ingredients one might guess that it not only contained vitamins, but perchance even an antibiotic! It almost certainly was an unpleasant medicament."

Although until quite recently there were no adequate records on the percentage of deaths due to tuberculosis, there is reason to believe that it reached its height as a result of the development of the factory system. The herding of families into cities, the over-work and under-nourishment which tended to reduce resistance, and the crowding, both in the factory and at home, which increased the degree of exposure, made conditions ideal for the spread of tuberculosis. About seventy-five years ago the disease reached its height. It has been declining steadily since. In 1910 tuberculosis ranked first as a cause of death in the United States; by 1948 it had dropped to seventh place. Tuberculosis is a part of the price man pays for imperfect civilization.

The earlier names for the disease are referable to the characteristic wasting of advanced tuberculosis. The Greek word *phthisis* and the Latin *consumptio* and *tabes* all refer to wasting away. The Greeks, Romans, and Arabs recognized and described methods of treatment which often included rest and a change of environment.

During the Dark Ages, when the scientific attitude was in eclipse, many unsound notions were held as to the cause of tuberculosis and many strange ideas developed regarding its cure.

It was generally believed that all disease was an expression of Divine Wrath. Since the kings claimed to rule by Divine Right and to be earthly representatives of God, it was not unreasonable to suppose that they would be endowed with some divine powers, one of which was popularly supposed to be the healing of the sick by touching.

The "Royal Touch" was introduced into England by Edward the Confessor. He and subsequent kings practiced it in the treatment of tuberculosis of the skin and lymph nodes of the neck, and this type, *scrofula*, became known as the *Kings Evil*. Healing by touch later became a church ceremony. Since it was the custom for the king to give the afflicted a gold coin as he touched him, it is little wonder that the treatment was popular. Holmes states that Charles II (1660–85) touched a hundred thousand cases of scrofula, more than any of his predecessors. Although this number may be an exaggeration, it does indicate the high incidence of the disease. The same author says that William of Orange, who assumed the throne in 1688, was more honest than some of his predecessors, and that, on the few occasions when he could be persuaded to touch a scrofulous person, reportedly remarked, "May God give you better health and more sense."

However, there are plenty of writings to show that long before the tubercle bacilli were discovered by Koch in 1882 many persons held the idea that tuberculosis was a contagious disease due to natural causes. The reproduction of tuberculosis by the introduction of tuberculous material was

accomplished by Klencke in 1843 and later by Villemin in 1865. Villemin, working with rabbits, demonstrated conclusively that tuberculosis was transmissible, a view proposed without experimental evidence by William Budd some ten years earlier. With the fact of transmissibility settled, the remaining problem was the discovery of the specific causative agent and it is here that Robert Koch enters the picture.

Etiology

The specific cause of tuberculosis is *Mycobacterium tuberculosis* or the tubercle bacillus. The discovery of the tubercle bacillus, its isolation and cultivation on artificial media, and the production of experimental tuberculosis by the use of the pure cultures was accomplished by Koch in 1882 and is one of the land marks of bacteriology. It is important not only in connection with tuberculosis specifically, but because it laid the frame work for future investigation on the etiology of all disease. It is a classical example of accurate and thorough bacteriological technique. Later workers showed that the tubercle bacillus of Koch was but one of a group of several species and varieties, each capable of producing tuberculosis but differing with respect to physiology, pathogenicity, and host range. In addition, there are several non-pathogenic species.

Morphology and Staining: The tubercle bacillus is a small thin rod measuring about 2 to 4 microns in length and 0.2 to 0.5 micron in width. It is nonsporulating and nonmotile. It appears to be surrounded by a cell membrane containing or consisting primarily of a waxy substance to which the tubercle bacillus owes many of its distinctive properties. Stains, for instance, do not readily penetrate unless left in contact for long periods or heated during the process. Once stained, the bacilli resist decoloration by such agents as acid-alcohol. They are, therefore, said to be acid-fast, and this characteristic is exceedingly useful for identification and diagnosis.

Of a number of staining methods developed for this purpose, the most widely used is known as the Ziehl-Neelson method. It consists of applying a solution of carbolfuchsin to a smear of tuberculous material, heating it for about five minutes, decolorizing by the use of acid-alcohol, and then counterstaining with methylene blue. The tubercle bacilli appear bright red against a blue background of cellular material, other bacteria, and debris. The bacilli in old cultures frequently stain irregularly giving them a blurred appearance. In young cultures and in tissues, many of the cells are not acid-fast and frequently show the presence of gram-positive granules, called *Much granules* after their discoverer. The significance of these granules is debated, many workers asserting that they represent the filterable stage in a postulated life cycle of the tubercle bacilli. Without going into a thorough discussion of the findings of various workers in regard

to this life cycle, it may be said that the rods appear to segment into three or more units, that these units become reduced to fine granules from which very small delicate rods develop, and that these rods later develop into the typical tubercle bacillus. Only the later stage is acid-fast.

Resistance: The resistance of the tubercle bacillus to drying and chemical agents is greater than that of other nonsporulating organisms and appears to be related to the waxy substances of the cell wall. Then, too, the tubercle bacillus is usually discharged in material, such as sputum, which tends to protect it, a fact always to be considered when disinfecting discharges from tuberculous patients. The tubercle bacillus may live for several months in sputum if protected from direct sunlight. Direct sunlight will destroy it in a few hours and diffuse sunlight in a week or less.

The temperature employed in the pasteurization of milk, 142° F. for thirty minutes, is sufficient to destroy the bacillus and to provide a fair margin of safety. Boiling for one minute is sufficient to destroy the organism in milk.

Five percent phenol will destroy it in sputum in a few minutes. Lysol is even more effective. Disinfectants such as bichloride of mercury and many other mercurials that combine with proteins to form insoluble mercury precipitates may protect rather than destroy it.

Classification of the Acid-Fast Bacteria: The tubercle bacillus is now placed in the order *Actinomycetales* and the genus to which it belongs is called *Mycobacterium*. In 1874 Hansen discovered the first member of this group, the leprosy bacillus. The important members of the genus are:

Mycobacterium tuberculosis, var. *hominis*. This variety is the common cause of human tuberculosis and is also pathogenic for the monkey, dog, and parrot. Experimentally, it is highly pathogenic for the guinea pig, but not for cats, goats, cattle, or domestic fowls.

Mycobacterium avium. This is considered a distinct species and causes tuberculosis of chickens and swine but not of cattle. It is doubtful whether it causes tuberculosis in humans although a few cases have been reported.

Mycobacterium paratuberculosis. This organism, also called Johne's bacillus, is the cause of chronic diarrhea in cattle and sheep.

Mycobacterium leprae. Although all experimental attempts to produce leprosy in man have failed, this organism is believed to be the cause of the disease since it is found only in lesions of persons suffering from leprosy. Although claims of successful animal transmission and artificial cultivation have been made, they have not been accepted.

Mycobacterium piscium, marinum, themnopheos, ranae, and several other species cause tuberculosis of cold-blooded animals such as fish, frogs, turtles, lizards, and snakes and are referred to as the cold-blooded types.

Mycobacterium smegmatis. This organism is found growing as a sapro-

phyte in the smegma, which is a soap-like secretion in the folds of the skin of the genitalia.

There are numerous saprophytic acid-fast bacteria widely distributed on soil, water, water-pipes, and on grasses and grains. *Mycobacterium phlei*, the timothy grass bacillus, and *Mycobacterium butyricum*, which is often found in butter, are representatives of this group. All appear to be closely related, hence Bergy has included them all in one species, *lacticola*. Our interest here lies chiefly in the pathogenic types, particularly in *Mycobacterium tuberculosis*.

Physiology: Mycobacterium tuberculosis is a strict parasite in nature. For cultivation special media, usually containing glycerine, egg, potato, and serum or whole blood, must be used. Growth is slow, and visible colonies do not appear until two or three weeks after inoculation. The bacillus is aerobic and grows on the surface of liquid media with characteristic pellicle formation. The optimum temperature for growth is approximately that of the natural host. Human and bovine strains grow best at 37° C. and will not grow above 41° C., whereas the avian type grows best at 40° C. and will grow at temperatures as high as 44° C. The avian strain grows more rapidly than the human or bovine strain and is more easily cultivated on glycerine-free media.

Isolation of Tubercle Bacilli

The ordinary procedures used to isolate most pathogenic bacteria, such as streaking plates of suitable media with material from lesions, sputum, pus, and the like, are not satisfactory for the isolation of tubercle bacilli, chiefly for two reasons. In the first place, since it takes several weeks for tubercle bacilli to produce visible colonies, whereas many other bacteria grow out in twenty-four to forty-eight hours, the latter will overgrow any tubercle bacilli present. Second, during the three to eight weeks incubation period, the media will become too dry to support good growth. Therefore, in order to isolate the tubercle bacillus from material containing other bacteria, the latter have to be destroyed, or a medium must be used which inhibits growth of all save the tubercle bacilli, and suitable precaution taken to prevent drying.

Tubercle bacilli are more resistant to digestion with strong alkali or acid than are the nonsporulating and non-acid-fast types of contaminants which can, therefore, be destroyed by treating the material with equal parts of four per cent sodium hydroxide for thirty minutes at 37° C. After treatment, the mixture is neutralized and inoculated. Such treatment does not destroy all spores; but the spore forms are gram-positive and readily inhibited by dyes such as crystal violet which are incorporated in the medium. To prevent excessive drying, test tubes are stoppered with

cotton plugs impregnated in hot paraffin and then capped with tin foil or other suitable material.

Animal inoculation affords a sensitive method for detecting tubercle bacilli and guinea pigs are the animal of choice. They are inoculated subcutaneously into the region of the groin with untreated tuberculous material or with material which has been concentrated and digested in the manner described in the preceding paragraph. About six weeks after inoculation, the animals may be killed and examined for typical lesions in the lymph nodes. The organisms are readily isolated by culture from guinea pigs.

Variation

The tubercle bacillus undergoes colonial, cellular, and physiological variation. On suitable media, smooth strains give rise to rough and intermediate strains; and, less frequently, rough strains give rise to smooth strains. In general, the smooth strains are virulent, and the rough strains less so, if not entirely avirulent. The virulence of strains diminishes when they are cultivated for long periods. *B.C.G., Bacilli Calmette-Guérin,* is such an attenuated bovine strain developed by Calmette and Guérin for the purpose of prophylactic immunization. Its use will be discussed later.

Chemistry of the Tubercle Bacillus: Extensive chemical analyses have been made on the tubercle bacillus, principally by Anderson and his colleagues at Yale. Lipoids, proteins, carbohydrates, and phosphatides have been extracted and investigated in an effort to find some clue as to the mechanism by which this bacterium incites disease. The several fractions have been injected into animals. The characteristic response of the body cells with the production of the tubercle is clearly due to chemical fractions liberated when the tubercle bacillus disintegrates. The phosphatide fraction stimulates nodule formation, and its activity appears to be due to the phthioic acid it contains. Lipoids stimulate the formation of monocytes, fibroblasts, and giant cells. The insoluble protein fraction stimulates the production of monocytes and giant cells, and the carbohydrate fraction stimulates the formation of neutrophilic leucocytes.

Products of the Tubercle Bacillus: There is no indication that the tubercle bacillus excretes or secretes products such as exotoxins, hemolysins, leucocidins, fibrinolysins, or other soluble substances of the type usually associated with bacterial virulence or pathogenicity. Filtrates from cultures of tubercle bacilli or dead disintegrated bacilli are not particularly toxic when injected into persons or animals who have never been infected. However, if injected into previously infected or sensitized animals, even small amounts may produce a violent reaction. This is an allergic response first noted by Koch and is the basis for the tuberculin test.

Koch's Phenomenon—Tuberculin: Koch observed that tuberculous animals were more resistant to a second inoculation of living tubercle bacilli than normal animals were to the initial inoculation, and that the tubercle bacilli were destroyed at the site of injection. This observation quite naturally suggested to him that it might be possible to produce a degree of resistance by injecting dead bacilli.

He prepared his material for injection as follows: Tubercle bacilli were grown in flasks of five per cent glycerine broth for six to eight weeks. Contents of the flasks were then evaporated to one-tenth their original volume, and the concentrated liquid was filtered. The sterile filtrate contains disintegrated products of tubercle bacilli, growth products, and media, and is known as *O.T.* or *Original Tuberculin.* The formula by which it was prepared was, at first, kept secret.

Koch concluded that tuberculin increased the resistance of healthy animals and that it was also a curative agent. In 1890 he startled the world with the announcement that he had a cure for tuberculosis, and, at his suggestion, physicians injected tuberculin into tuberculous patients. The effect on patients varied but in many instances the results were tragic and fatal. The effects could not be predicted and although it is probable that some were benefitted, the practice proved too dangerous to be recommended.

Tuberculin Tests

The fact that infected persons or animals are hypersensitive to tuberculin makes it possible to use tuberculin as a test for infection. When tuberculin is injected into uninfected animals or persons there is little or no reaction. When introduced into infected persons or animals, the reactions are of three types—local, focal, and general, depending upon the dosage and the reactivity of the body.

The *local* reaction occurs at the site of the injection and consists of an inflammation which may lead to necrosis. This is the typical tuberculin reaction.

The *focal* reaction occurs in the region of the lesion and may conceivably be of such character as to stimulate the defensive mechanism.

The *general* reaction is evidenced by fever, chills, pains in the joints, nausea, and vomiting.

P.P.D.: Many other tuberculins have been prepared, the most important being known as P.P.D., *purified protein derivative.* It was developed by Seibert, consists of the active principle with little or no extraneous matter, and is the one most frequently used in tuberculin testing. The standardization of tuberculin testing is not entirely satisfactory.

It was formerly believed that tuberculin positive individuals remained

positive for life, even though the lesions were completely healed. This has now been proven fallacious. Investigators at the Henry Phipps Institute have shown that ten or more per cent of those positive became negative within a few years. Long concludes that tuberculin sensitivity is often the result not of persistence of an original sensitization, but of recent, and presumably repeated, intake of tubercle bacilli.

Significance of the Tuberculin Test: It is generally agreed that a positive tuberculin reaction—one in which a local inflammation occurs in eighteen to twenty-four hours, followed by necrosis after forty-eight hours or more—is indicative of hypersensitivity or allergy and that this state does not occur in uninfected persons or animals. The test can, therefore, be used to determine infection but it gives little or no indication, by itself, of the degree of infection or the severity of the disease. Although persons having latent tuberculosis or healed lesions react positively, persons with active tuberculosis are more sensitive.

The tuberculin test, then, is not a test for active tuberculosis but merely for infection. Forty years ago when tuberculosis was the chief single cause of death, over ninety per cent of the adult population were tuberculin positive. As the death rate has decreased, there has been a corresponding decrease in the percentage of tuberculin positive adults. A few years ago sixty to seventy per cent of the college students were positive, at present only thirty per cent or less are. It would seem, therefore, that the tuberculin test might become more valuable for the recognition of infection as the percentage of positives becomes less.

A negative tuberculin test does not always mean absence of infection. People may lose their reactivity to tuberculin temporarily while suffering from other diseases and, in advanced tuberculosis, patients are often negative.

There is also a period of desensitization following the tuberculin test during which tuberculin positive individuals or animals will not react. A few unscrupulous persons who knew of this used to desensitize cattle so that infected cows gave a negative reaction when they were tested by government officials. The present methods of eradication of bovine tuberculosis preclude such chicanery.

Methods of Tuberculin Testing: There are several methods by which the tuberculin may be administered in the test.

Ophthalmotuberculin Reaction: This is performed by instilling a suitable preparation of tuberculin into the conjunctival sac of man or cattle. A positive test is indicated by a conjunctivitis which lasts for several days. This test is not used to any extent in man.

Von Pirquet Test: This is a cutaneous test. The forearm is cleaned with alcohol and two scratches not deep enough to cause bleeding are made a

few inches apart. The tuberculin is rubbed into one of the scratches; the other is left as a control. A positive reaction is indicated if, within twenty-four to forty-eight hours, small bright red papules and vesicles appear. These later become dark red and persist for about a week.

Mantoux Test: This is the most widely used test and is preferably made with *P.P.D.* A suitable dilution of tuberculin is injected intradermally and, if no reaction occurs, a much stronger dilution (250 times the first, in the case of *P.P.D.*) is injected.

Patch Test: Recently a new method has been developed in which the tuberculin is dried on a strip of filter paper. After cleansing the skin, the filter paper is taped on to the arm and allowed to remain for twenty-four hours. Then a part of the patch is removed and the underlying area examined. If a positive reaction occurs, the rest of the patch is removed; if not, it is allowed to remain another twenty-four to forty-eight hours and a second examination is made at the end of that time. The Patch Test has many advantages over the other methods and will probably become widely adopted.

TUBERCULOSIS IN MAN

Tuberculosis occurs principally as a disease of the lungs, although practically any part or organ of the body may be infected. In the United States in 1946 there were 50,911 deaths. 46,939, or about ninety-two per cent, were due to pulmonary infection and 3,972, or two and three tenths per cent, to tuberculosis of the meninges and central nervous system. The more common non-pulmonary infections involve the meninges, the intestinal tract, the lymphatics of the head and neck, the bones and joints, the serous membranes, the larynx and the skin (lupus). It appears that many of these represent extensions of infection from the lungs. Others may be primary infections.

Intestinal tuberculosis due to the human strain is almost always the result of swallowing the bacilli coughed up from the lungs, whereas intestinal tuberculosis due to the bovine strain appears to be a primary infection caused by ingesting the bacilli.

The bovine strain shows a greater tendency than the human strain to attack the bones, joints, and other tissues. It rarely produces pulmonary tuberculosis in man. The bacilli are seldom present in the blood stream and it is doubtful whether the blood is important in the transport of the infectious agent from one part of the body to another, except, perhaps, in the case of miliary tuberculosis.

The principal lesion is a characteristic nodule or tubercle. Its formation is the result of cellular activity induced by the presence of the tubercle bacilli and their products.

The constitutional symptoms of frank pulmonary tuberculosis are a dry hacking cough, fever, loss of appetite, loss of weight, weakness, and pallor. In the early stages the symptoms are vague and diagnosis without x-ray is difficult. The disease manifests itself in several forms depending upon the infecting dosages and resistance.

A Childhood Type—Miliary Tuberculosis: This is an acute, rapidly fatal, generalized infection. The term miliary refers to millet seed, and the lungs have numerous small lesions just about that size. Many believe that this type is blood-borne. Miliary tuberculosis occurs principally in primitive people of all ages, and in children of civilized groups. There is little tendency to cavity formation or to the deposition of fibrous tissue, which may be explained by the fact that this type of tuberculosis is usually found in individuals who have never been exposed previously and whose body defenses are not capable of localizing the organism.

Adult Type—Active Pulmonary Tuberculosis, Phthisis, or *Consumption:* This is a more chronic and a more localized infection. The tubercles are larger and appear in the apical region of the lung, not in the hilum glands, as in the childhood type. There is a tendency to cavity formation and a deposition of fibrous tissue. This is the most common form of the disease and in it the body shows a rather high degree of resistance which appears to be related to previous infection and sensitization.

Fibrotic Tuberculosis: The fibrotic form shows a tendency to the production of sclerotic tissue. It is a very chronic form of the disease.

Miscellaneous Forms: Tuberculosis may take on many other forms. In children and young adults, the meninges may become involved, producing a fatal tuberculous meningitis. Another rapidly fatal type is tuberculous pneumonia, often referred to as 'galloping' consumption.

Mechanism of Disease Production

In the main, the symptoms of tuberculosis appear to be due to the peculiar and characteristic tissue response of the body rather than to the damage or destruction of tissue by the tubercle bacillus or its products. The tubercle bacillus does not produce exotoxins of the type elaborated by the diphtheria or tetanus bacillus, nor does it produce hemolysins, leucocidins, fibrinolysins, or any other destructive substance of the type usually associated with disease-producing bacteria. The bacillus cannot be considered as having invasive capacity in the ordinary sense of the word. It does, however, have an extremely high resistance to the destructive agencies of the body and, as a matter of fact, the characteristic features of tuberculosis are dependent upon the resistance of the tubercle bacillus and not on its offensive capacity. Death is due to the manner in which the body reacts.

The wandering phagocytic cells which play such an important role in defense against many bacteria do not digest the tubercle bacillus although they do ingest or engulf it. Its waxy capsule seems to resist digestion by the phagocytic cells.

Tubercle bacilli may even multiply within the phagocytic cell and be liberated in some other region when the phagocyte dies and disintegrates. Thus, the very mechanism which destroys many other types of bacteria is sometimes responsible for the transport of the tubercle bacilli to other regions of the body.

FIG. 42. Experimental tuberculosis of the spleen and liver in monkeys

Most of the signs and symptoms of tuberculosis, including the formation of the tubercle, are referable to the response of the various tissue elements to the chemicals liberated when the tubercle bacillus disintegrates. The first reaction, that is, the ingestion and transportation of the tubercle bacillus, is nonspecific and similar to that which follows the introduction of any foreign substance. The second, that is, the response to the disintegration products of the organism, is specific and characteristic only of infection with the tubercle bacillus or of the introduction of its component parts.

The tubercle is a characteristic feature of tuberculosis, and all tubercles, regardless of their site, are essentially alike. The stimulus that calls forth

the chain of events resulting in tubercle formation comes from the chemical constituents of the disintegrating bacilli. As previously mentioned, the lipoid, protein, phosphatides, and carbohydrate fractions call forth specific responses that lead to the formation of the tubercle. The monocytes of the reticulo-endothelial system are most conspicuous in the development of the tubercle. These form in layers around the organisms. Later the so-called giant cells appear. The polymorphonuclear leucocytes and the lymphocytes from the blood stream are also active at the edge of the focus of infection and it is the combined reactions of these and the other cells that finally result in a tubercle.

The tubercle may then undergo a necrosis beginning at the center. This is followed by caseation and softening and, finally, by the deposition of calcium salts which makes a hard nodule. Since the tubercle bacilli are within the nodule or tubercle they are completely walled off, a process which does not result, however, in their immediate death. Nor is the tubercle connected with surrounding tissue by capillaries. With the exception of certain dyes and oils, drugs or other agents injected into the blood stream do not penetrate it. When the tubercle is close to a blood vessel, it may cause a hemorrhage. In some cases, the bacilli proliferate faster than the body defenses can complete the walling-off process and the infection spreads.

The type of reaction or the nature of the disease varies with the species of animal and species and virulence of the bacillus. In man, the lung is the common site of localization although any tissue or organ may be involved. The organism may reach the lung directly by inhalation or it may be transported from other regions of the body by the blood stream. In either case, it does not penetrate the mucosa but is dragged there, so as to speak, by the wandering phagocytic cells. The chain of events is essentially as follows. After the wandering phagocytes, that is, the polymorphonuclear leucocytes, have ingested the tubercle bacillus, they migrate through the tissues, enter the primary lymphatics of the region, and are carried to the lymph nodes where they may be and usually are arrested. If so, and if the tubercle bacillus multiplies, the lymph nodes will show evidence of infection. The region at which the primary tubercle develops will depend upon where the phagocytes are arrested and whether the organism proliferates to any extent. If the initial entrance is by way of the tonsil, the cervical lymph nodes will be involved; if by way of the intestinal mucosa, the mesenteric lymph nodes; if by way of inhalation, the tracheobronchial lymph nodes. The tubercle bacillus grows slowly, and frequently the primary infectious process is completely walled off, in which case the only evidence of infection will be the primary tubercle, called a *Ghon* tubercle.

In other instances there may be little or no local defensive reaction or the growth rate of the organism may outstrip the walling-off process, and the organism may be transported to and arrested in other lymph nodes or may pass into the circulatory system and finally lodge in the capillaries of the lungs. Thus pulmonary tuberculosis may result from infection through the gastro-intestinal tract or from infection by inhalation. While there is some difference of opinion as to which is the more common, most investigators are of the opinion that pulmonary tuberculosis is usually the result of inhaling droplets containing the organisms rather than of ingestion of the organism.

Immunity to Tuberculosis

There is no absolute immunity to tuberculosis. All persons, regardless of age, sex, race, or occupation, are susceptible to infection, although there is considerable difference in the degree of susceptibility or of resistance. What, if anything, can be done to increase the resistance of the individual is not at all clear. The assertion, often made and as often denied, that the only immunity to tuberculosis is tuberculosis implies that persons who have been infected and have overcome the infection are less apt to develop active tuberculosis when exposed to reinfection than those who have never been infected. There are some workers who feel that the enhanced resistance or infection immunity is offset in part at least by the chance that the primary tubercle may break down and the organism escape and produce tuberculosis in some other region of the body. It has been abundantly demonstrated that infection leads to sensitization or allergy. However, not all workers are agreed as to whether the allergic individual, or the one who is tuberculin positive, is capable of withstanding a larger dose of tubercle bacilli than the non-allergic or non-sensitized person. Such specific immunity as does develop appears to be related to the reactivity of the tissue cells and not to the presence of free-circulating antibodies, although agglutinating, precipitating, complement-fixing, and other antibodies do appear following infection. The time required to produce the allergic state or a positive tuberculin reaction is from four to eight weeks.

Specific Immunization: In spite of the tragic results that attended Koch's efforts to produce a vaccine for tuberculosis, an effective immunizing agent is still being sought. It is doubtful whether dramatic results should be expected of such an agent in view of the fact that one attack of tuberculosis does not confer a high degree of immunity. Then, too, artificial immunization, in general, appears to be most effective against bacteria which produce powerful exotoxins such as the diphtheria and tetanus bacilli and, in acute disease; and less effective or ineffective against pathogenic

bacteria which do not secrete powerful destructive substances and which produce chronic disease.

Attempts to immunize artificially against tuberculosis are based on the idea that sensitivity or allergy to the organism or its components is an asset and not a liability, although, as previously stated, there is no general agreement on this point.

BCG: The most widely used method of prophylactic immunization was developed by Calmette and Guérin. After making over two hundred and fifty successive transfers of a bovine strain of the tubercle bacillus on a medium containing glycerine and ox bile, they found that the strain would no longer produce progressive tuberculosis in the calf, that it persisted in the calf for two years, and that so long as it persisted the calf was immune to tuberculosis. This strain was known as *Bacille Calmette-Guérin* or *BCG*. On the basis of their experiments they attempted human vaccination with it.

BCG is open to criticism of two sorts. First, while it is true that it does sensitize—and the evidence shows that it does because tuberculin negative children become tuberculin positive after vaccination—many workers doubt that this sensitization is of any protective value. And, second, the vaccine consists of a living attenuated organism and there is always the theoretical possibility that the strain may revert to a virulent state.

The problem of evaluating the effectiveness of a vaccine in any chronic disease is a difficult one. Results of the early experiments with BCG vaccination were not convincing but more recent studies, particularly some in Sweden and Norway, show that BCG vaccination does afford a great deal of protection. However, it is not a substitute for other methods of control and should be used, when indicated, only in conjunction with them.

Passive Immunization: Although free-circulating antibodies appear in the blood stream of infected men and animals, their introduction has no effect on resistance to infection nor do they alter the course of the disease.

Diagnosis

The diagnosis of tuberculosis presents a difficult problem. Since it is usually an insidious disease with a slow onset, it may be fairly advanced before the person suffering from it seeks medical attention. In fact, many surveys show that even at present from eighty to ninety per cent of the cases diagnosed are in the advanced stages. The constitutional signs in more advanced cases may be quite evident and readily recognized by the experienced clinician, but diagnosis in the early stages when there may be no visible signs depends upon laboratory findings.

Bacteriological Methods: Since the finding of the tubercle bacillus is positive evidence of infection, bacteriological methods for demonstrating

its presence are of considerable importance. The material usually examined in suspected pulmonary tuberculosis is the sputum, although in children, stomach washings may be more satisfactory. If tuberculosis in other forms is suspected, the urine, the joint fluid, the spinal fluid, or other materials may be examined. The material can be concentrated and treated to destroy non-acid-fast organisms.

Examination may be by one or all of the following methods:

1. *Microscope*—Examination for the presence of acid-fast bacilli by means of the Ziehl-Neelson stain.

2. *Culture*—Inoculation of material on media suitable for the growth of the tubercle bacilli.

3. *Guinea pig*—Inoculation of suspected material into guinea pig and examination of animal after several weeks incubation.

The culture and animal inoculation are the more sensitive but require from three to eight weeks for completion.

Immunological Reactions: The only immunological test commonly used is the tuberculin reaction. Since this does not distinguish between mere infection and active tuberculosis, it is of limited value; although when the results are viewed in the light of other findings, they may be very significant.

X-Ray: The x-ray is of paramount importance in the diagnosis of tuberculosis. By its use it is possible to detect the early stages before clinical symptoms are evident and when bacteriological findings may not be positive. The characteristic tubercular lesion casts a shadow on the x-ray plates. The location of the lesion and the degree of involvement are evident from the x-ray, and by taking a series of pictures over a period of time, the progress of the disease may be followed. As mentioned before, advanced tuberculosis is relatively easy to diagnose but latent and chronic tuberculosis are not. The x-ray is particularly valuable in the diagnosis of the so-called "hidden" cases which physical examination by competent physicians will often fail to detect. Since, from the standpoint of both treatment and prevention, it is essential to get early diagnosis, a more general use of the x-ray is urged by the medical profession.

Epidemiology

It is just as essential to know the epidemiology or the natural history of a disease as it is to know its bacteriology, pathology, or clinical aspects, for epidemiology often gives the clue to its control. Tuberculosis is an infectious disease of man and animals. It attacks both sexes, all ages, races, and nationalities of man, but its prevalence and distribution are by no means equal or uniform, largely because of the manner in which the disease is transmitted.

Source of Infection: Since human infection due to the bovine strain of

the tubercle bacillus is now relatively rare in the United States, thanks to pasteurization of milk and the eradication of tuberculosis in cattle, and since the human strain does not commonly infect animals, it follows that man himself is the chief source of infection. Over ninety per cent of tuberculosis of humans is pulmonary and the principal source of infection is the sputum of active cases. The discharges from lymph nodes, skin, or the intestinal tract contain the organisms but are relatively of little importance as sources of new cases.

Mode of Transmission: Since the sputum is the principal source of the infectious agent and since man acquires infection by inhalation or ingestion of the bacilli, it is obvious that the disease is transmitted by direct or indirect contact with sputum. Whether infection in the case of pulmonary tuberculosis is mainly by the inhalation or ingestion of infective droplets, which may have been expelled by sneezing and coughing, or of organisms floating in dried particles of sputum, is a disputed point. Both probably play a part. The disease may also be contracted through kissing or by contact with articles, utensils, and food contaminated with sputum.

Tuberculosis is not highly contagious as compared to measles or influenza. Frank infection rarely follows casual contact but usually results from long and intimate exposure with active or "open" cases. That is why it is often called a "family" disease. Dosage plays the predominant role. Pathologists working a great deal with tuberculous tissue may acquire a localized tuberculous infection of the skin known as "pathologist's wart."

The incubation period is variable and difficult to determine, as is the case in chronic diseases with a slow onset. It may be a matter of weeks but is usually months before symptoms become evident.

The period of communicability is for as long as the individuals are disseminating the organism. It begins when a lesion becomes open and ends when, and if, the lesion is healed. The severity of the disease is not necessarily a measure of the number of organisms liberated and individuals who are not very ill may produce sputum containing large numbers of organisms.

Susceptibility and Immunity: Susceptibility to infection is general and high as judged from the percentage of persons who develop primary lesions when exposed. Since the vast majority do not develop clinical tuberculosis, it would seem that there is also a high degree of resistance. Resistance to the disease as distinguished from resistance to infection is variable and is conditioned by such factors as age, race, sex, fatigue, undernourishment, and occupation. This will become apparent from what follows.

Prevalence and Distribution of Tuberculosis: Tuberculosis is one of the most common communicable diseases of man. Its incidence rose rapidly

along with industrialization and reached its peak a little after the middle of the nineteenth century. In many industrial towns, the mortality rate ranged from 400 to as high as 700 or more for every 100,000 inhabitants. Had man deliberately designed conditions favorable for the propagation of tuberculosis, he could not have chosen better ones than those which prevailed in the industrial centers in the eighteen hundreds. The factor of crowding alone would insure continual transfer of tubercle bacilli from the

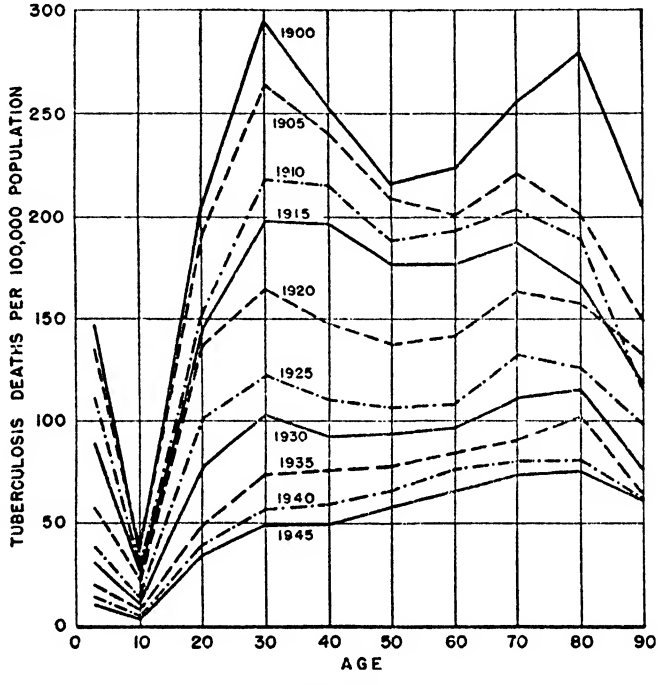


FIG. 43. Tuberculosis Mortality in the United States in 1946
(Evelyn H. Halpern, Public Health Reports)
Death rates for tuberculosis by age: Death-registration States, quinquennial years, 1900–1940

sick to the healthy. Add to that the under-nourishment, exposure, fatigue, and lowered resistance due to other diseases, and it is easy to understand why tuberculosis was the leading cause of death. The mortality rate began to decline from 1850 to 1875 and has continued downward ever since with the exception of the period during and following the first World War of 1914 to 1920. There was a rise then, some of which was probably a reflection of the influenza epidemic. It is a common observation that during a severe outbreak of influenza, deaths from many other causes show an increase. After the war the mortality dropped sharply and in a few

years reached a level somewhat lower than that which had prevailed before. The rate of decline since then has been continuous. In 1946 the death rate had reached a low of 36.4. There has been a sharp increase in tuberculosis in Great Britain and Europe. Concentration and prison camps, malnutrition and crowding, and excessive strain have made their influence felt. Reliable statistics are not available from many of the countries but the death rate is three to five times as high as it was before the war. There was

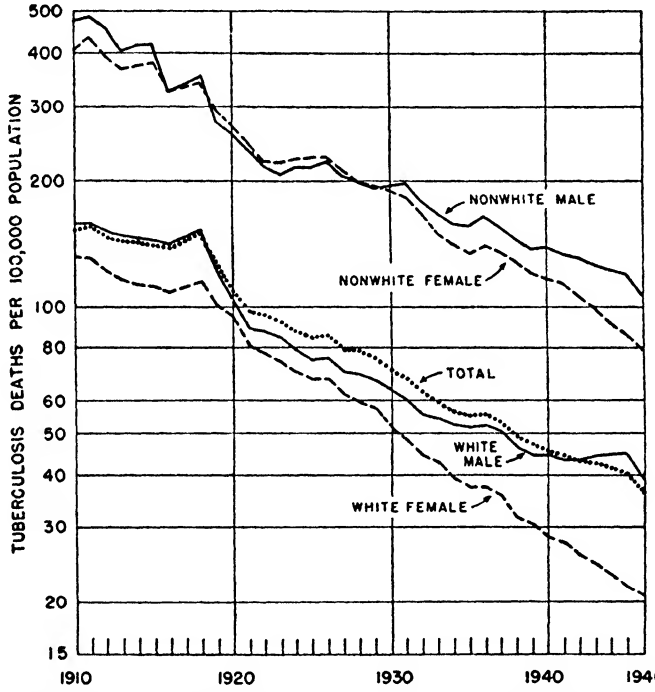


FIG. 44. Tuberculosis Mortality in the United States in 1946
(Evelyn H. Halpern, Public Health Reports)
Death rates for tuberculosis (all forms) by race and sex: Death-registration States, 1910–1946

a slight increase in tuberculosis in some cities in the United States as a result of increased industrial activity in connection with the war.

There are about nine active cases for every death reported.

Sex and Age: There is a definite difference in sex and age distribution in tuberculosis. The sex difference varies with age, there being no significant difference until after the age of ten. Between the ages of ten and thirty, the females show a higher mortality, but after thirty years, the death rate among males exceeds that of females and continues to do so to the end of life. Figures for the United States show that from 1931 to 1935 the num-

ber of deaths was fifty per cent higher in white males and ten per cent higher in colored males.

The maximum excess in male mortality occurs in the group between fifty and sixty. The higher mortality in females under thirty was still more pronounced in the last century, indicating that the reduction in total death rates has been greater in females than in males. There is considerable difference in age sex distribution in the whites and non-white, as shown in Figure 45.

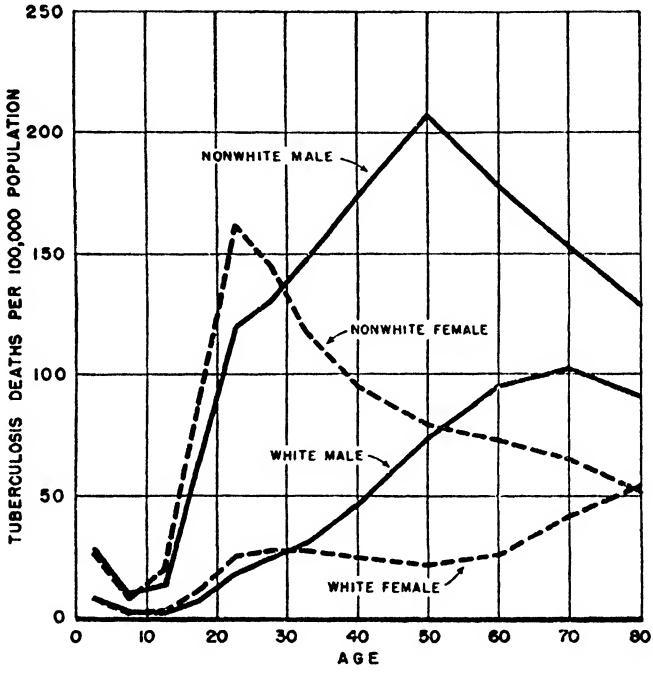


FIG. 45. Tuberculosis Mortality in the United States in 1946
(Evelyn H. Halpern, Public Health Reports)
Death rates for tuberculosis (all forms) by age, race and sex: United States, 1946

Many explanations have been advanced for the age-sex differences in mortality from tuberculosis. Physiological changes that occur in the maturing female and the strain of bearing and rearing children may contribute to the excess in deaths in females in the fifteen to thirty year age group The excess in male over female deaths is related to the degree of industrialization. Certain occupations are known to predispose to the disease. After the age of thirty, women withdraw from industry more or less and tend to lead a more sheltered life at home, whereas males are ex-

posed to excessive physical exertion, unsanitary working conditions, dusts, and other hazards which predispose to tuberculosis, and the trend is reversed.

Race, Nationality, and Family: The average death rate from tuberculosis is two to four times as high in negroes as in white people. In many cities it is ten times as high. This appears to be due in part to a genetic difference, that is, to a greater inherent susceptibility on the part of the colored race. However, much of the difference is undoubtedly due to the economic handicap imposed on the negro which causes a lower standard of living, crowding, lack of nursing or medical care, malnutrition, and the presence of other diseases.

Indians and Eskimoes also show a high susceptibility. The tuberculosis death rate in Alaska was almost ten times as great as that of the average for the forty-eight states.

Mexicans and Porto-Ricans in the United States show a very high mortality rate.

There is considerable difference in the tuberculosis death rate of the various nationalities. The Irish appear to be most suceptible, the Jews most resistant. It is of interest to note that the Jews are just as susceptible to infection, as evidenced by the fact that they become tuberculin positive, but they show a greater resistance in that fewer Jews develop active tuberculosis. For centuries the Jew has lived under conditions that would allow for the more rapid spread of the disease and natural selection may have played its part.

The effect of natural selection on resistance to disease might be expected to play a part only in diseases which kill before the reproductive age. It might determine the survival of resistant types to tuberculosis, but probably would not be a consideration in the case of cancer, for instance, or other diseases which attack persons in later life.

It is sometimes said that tuberculosis runs in a family, and while it is true that families may differ in their resistance to tuberculosis, the question of dosage is probably a more deciding factor. If one member of a family has an active case of tuberculosis, the other members are very apt to be exposed to large numbers of the organisms.

Many attempts have been made to correlate susceptibility with body type and some believe that there is a definite relation and that persons who are thin are more susceptible than those who are stout.

Occupation: It has long been known that tuberculosis is more common in some occupational groups than in others. In general, it can be said that the inhalation of sharp particles of dust predisposes to the disease. The death rate is significantly higher in persons who are exposed to silica dust, as in sand blasting, tool-grinding, quarrying, and some types of

mining. Unless the particles are sharp and insoluble, the fact of dust inhalation as such does not seem to be important, for the inhalation of carbon in sufficient amounts to produce a "black" lung does not appear to predispose to tuberculosis.

The Bureau of Census figures on the death rate from tuberculosis in various occupational groups are of interest.

Group	*Death rate**
Professional men	26.2
Proprietors, managers, and officials	43.2
Agricultural workers	46.5
Clerks and kindred workers	65.8
Skilled workers and foremen	72.1
Semi-skilled workers	102.1
Unskilled workers	184.9

* Tuberculosis death rates per 100,000 employed males.

The association between income as represented by these groups and tuberculosis is quite striking. This is further evidence that the problem of tuberculosis is economic and social as well as medical. Nor may the high incidence in the unskilled and unemployed be the result of their low economic station but rather the cause of it, since tuberculosis so often leads to weakness and fatigue.

Climate: The direct effect of climate on tuberculosis is insignificant. Its apparent effect is more likely due to a number of factors such as occupation, income, standard of living, and the like.

Housing: The evidence on the effect of housing as such is difficult to assess since crowding occurs in groups of low income where lack of proper food, over-work, and industrial hazards are common. In view of the manner in which tuberculosis is spread, inadequate housing might be expected to be of considerable importance.

A Word of Caution: It must be emphasized that while there are many factors that predispose to tuberculosis, it is not easy to assess the significance of each. Since there are sex, age, race, and individual differences, these must be corrected for when we try to say how important such factors as occupation, nutrition, housing, climate, and the like may be. The converse is also true.

Trend in Tuberculosis: There has been a consistent downward trend in the mortality rate in this past seventy-five years in the United States and Europe. In 1900 the death rate was about 200 per 100,000 and in 1946 it was 36.4 per 100,000 in the United States.

In any disease, a decline in the death rate may be due either to a reduction in the case fatality rate, to a decrease in the number of cases, or to both. Of course, the figures may be subject to error due to changes in diagnosis, reporting, and so on. A reduction in the case fatality rate may

be an indication of a loss in the virulence of the organism without a loss of infectivity, to an increased resistance of the host, or to therapeutic agents such as serums or drugs, or to other curative measures. If there is no change in the case fatality rate, then any decline must be due to a reduction in the number of cases. This might be brought about by the use of vaccines, or other preventive measures which reduce the chances of infection, or it might be due to a loss of infectivity of the organism. There is no evidence that the tubercle bacillus has undergone any change in virulence or infectivity and hence the decline in the death rate cannot be attributed to this factor. Pasteurization of milk and eradication of bovine tuberculosis has played only a minor role since only a fraction of human tuberculosis and that mainly in children was ever due to the bovine strain.

Drolet has analyzed the problem and on the basis of a comparison of case fatality rates concludes that there has been little change. That is to say, the ratio of deaths to cases is not over five per cent less than it was twenty-five years ago although the mortality has dropped forty to sixty per cent. This is in spite of the fact that the number of cases isolated in hospitals or sanitariums has increased from four per cent in 1915 to twenty per cent in 1934. It appears, therefore, that such treatment as was given during this period did not appreciably enhance the chances of survival of the infected. A wider use of surgical treatment involving collapse therapy has undoubtedly lowered the case fatality rate in the last fifteen years.

Drolet concludes that the reduction in the death rate has been due to preventive rather than curative measures. The segregation of active cases appears to have been the most important single factor. No doubt a higher standard of living and better food and working conditions have contributed both to increasing the resistance of the individual and to preventing infection.

Control of Tuberculosis

The control of tuberculosis involves treatment and prevention; the two are intimately related.

Treatment: The principles of treatment are primarily based on increasing the power of resistance of the individual by rest and proper nutrition. Fresh air and sunlight are pleasant and contribute to the general well-being but are not curative as such. Climate is of little importance. The best climate is the one where the patient gets the most rest. There are no specific drugs that destroy the tubercle bacillus or its toxic products nor are there antibodies that can be used to bring about a passive immunity.

Rest, complete rest, physical and mental, is the all important curative measure. Its principal value lies in the fact that the nearer the lung approaches complete immobility, the faster the healing will be. Therein lies the value of collapse therapy which consists of immobilizing the lung

by one of a number of methods such as pneumothorax, diaphragmatic hemiparalysis, and thoracoplasty. Collapse therapy is not a cure-all but produces conditions favorable for healing.

Treatment of tuberculosis does two things—it enhances the chances of recovery for the individual and it reduces the spread of infection to others.

Although proper nutrition is important to recovery, there are no special foods that are curative as such.

Sanitoria: Sanitoria serve a two-fold purpose: they provide adequate care of the ill, and they prevent new cases by segregating infective cases. Since sanitarium care is expensive, there is no reason why patients, particularly those who are sputum negative, cannot be taken care of at home, providing facilities are available and the patient is cooperative with respect to rest, eating, and the disposal of discharges.

Early Diagnosis: It has been amply demonstrated that the chances of recovery depend upon the stage in which treatment is begun. It is often said that if taken in the early stages, about eighty-five per cent of the cases recover; if taken in the moderately advanced stages, forty to sixty per cent; and if taken in the far advanced stages, less than fifteen per cent. Early diagnosis is therefore essential in the anti-tuberculosis program. Unfortunately, human nature being what it is, many who might have reason to suspect tuberculosis are afraid to seek medical advice and consequently do not get benefit of early treatment. Then, too, the symptoms associated with tuberculosis in the popular mind: the night sweats, loss of weight, pallor, fatigue, and persistent coughs, are the symptoms of advanced and not early infection. At any rate, in many states eighty-five per cent of those who first seek medical attention or on which a positive diagnosis of tuberculosis is first made, are in the moderate to far-advanced stages. Tests made in Illinois in 1938 on summer school students at three state normal colleges revealed that twenty-six out of twenty-eight hundred persons examined had active tuberculosis. Most of them were teachers and were subjecting children to daily exposure to infection.

The hope for early diagnosis appears to lie in the screening method, which consists of tuberculin testing all contacts of discovered cases and x-raying the positive reactors. By such a method, tuberculosis can be detected in the early or hidden stages. The advantage of such a method both from the standpoint of the individual and of society is obvious. Tuberculin testing of students in schools and colleges periodically and x-raying of the positive reactors would go a long way in further reducing the death rate.

SUMMARY

Tuberculosis is a specific infectious disease of man and animal due to the *Mycobacterium tuberculosis*. It is a disease of civilization and increases in proportion to the degree of crowding. At present over ninety-five per

cent of the cases in the United States are due to the human strain and most cases are pulmonary.

Transmission is by inhalation or ingestion of the tubercle bacilli liberated in the discharges from cases. Since the lung is the common site of infection, the sputum is the common source of the organism. Transmission may be indirect through contact with contaminated utensils and articles such as desks, glasses, handkerchiefs, and bedding, or direct by kissing or inhalation of droplets.

The tubercle bacillus does not produce toxins, hemolysins, or other destructive substances and owes its capacity to incite disease to its high resistance to the body defense mechanism. It is not an invasive organism in the ordinary sense.

The body reacts non-specifically and specifically to the tubercle bacillus. When the bacilli first lodge in the tissue they are attacked by the defensive cells of the body. If the dosage is small and the host resistance high, the infection does not progress; if not, it does. As the tubercle bacilli die and disintegrate, the body cells become sensitized and react specifically. Special cells surround and wall off the organisms. The tubercles thus formed are characteristic of the disease. If the walling-off process be complete, the infection is halted; if not, it may continue and several tubercles may be formed before the host defenses overcome the invader; or the disease may continue to progress, finally producing death.

Infection is evidenced by a positive tuberculin reaction but the degree of invasion is not indicated. Most adults living in cities are tuberculin positive and the percentage increases with age and crowding.

Although the tubercle bacillus is antigenic and sensitizes the infected host and although antibodies are produced, they do not play a demonstrable role in recovery. Vaccines are of little if any value. Specific drugs have not been found. The disease spares no race, age, or sex, although some ages and races are more prone to develop active tuberculosis than others. The mortality is highest in males except in the age group from fifteen to thirty.

Control depends largely upon early diagnosis and segregation of open cases.

The case fatality rate varies with the stage of the disease at which treatment is begun. There has been little change in the case fatality rate since the beginning of the century although the mortality rate has dropped from over two hundred to thirty-six during this period. Most of this decrease is due to prevention of new cases, made possible by the establishment of sanitoria. The incidence and mortality are related to occupation and economic status and the control of the disease involves economic and sociologic problems as well as medical problems.

Chapter XXII

LEPROSY

There is probably no disease held in such dread and horror or about which there are so many popular misconceptions as leprosy. The dramatic allusions to the "unclean" and "outcast" leper in the Bible and the harrowing descriptions of leprosy and the lot of lepers in the Middle Ages as drawn in such well-known books of fiction as *Ben Hur* and *The Cloister and the Hearth* have, in many instances, aroused a sort of morbid interest in the "Disease of Lazarus."

History of Leprosy

Leprosy, probably one of the oldest of the diseases of man, was widespread long before the Christian era and was always held in extreme dread. During the Middle Ages it became very general in Europe. In the eleventh and twelfth centuries leper houses were common and laws were enacted governing the activities of the leprous. By the fourteenth and fifteenth centuries there had been a great decrease in the number of cases and at the end of the sixteenth century, few new ones were appearing in England, France, or Germany. It persisted longer in southern Europe and Russia.

Norway was an exception. Here the disease was on the increase until about 1850 when it began to decline until, at the present time, no new cases are appearing. The prevalence of leprosy in Norway during the past century is of particular interest in two connections. First, it was in Norway that most of the early scientific work on the disease was done. The pathology, gross and microscopic, was studied in great detail before the middle of the nineteenth century by Danielson and Boeck and was followed by an extensive investigation by Hansen on the etiology. In 1874, eight years before Robert Koch published his classical paper on tuberculosis, Hansen described the bacillus which is still considered the cause of leprosy.

The second point of interest in this connection is that leprosy was prevalent in Norway during the period when large numbers of Norwegians were migrating to the United States. The disease was introduced into the north central states, Minnesota, particularly, by these immigrants, and an opportunity was thus provided for studying its communicability in a hitherto untouched region.

Symptoms

Leprosy is usually classified into the *neural* and *cutaneous* types, according to the predominating symptoms. In the neural or anaesthetic type, the

peripheral nerves are primarily involved and the major symptoms are referable to neural damage. Since all feeling may be gone from the affected parts of the body, mechanical insult is unnoticed. Thus a leper may allow a cigarette to burn until his fingers become scorched or he may run a nail through his foot and continue to walk without the slightest sensation of pain. This type of leprosy is rarely in itself fatal. However, the secondary infections which are apt to occur in the affected areas may lead to very serious or fatal complications.

The cutaneous or nodular type of leprosy is characterized by nodules on the skin of the face, legs, arms, and trunk, and is the type that leads to distortion and mutilation. The disease may be benign or malignant and the degree of involvement probably depends upon the resistance of the individual.

Etiology

The specific cause of leprosy is considered to be *Mycobacterium leprae.* This is the organism discovered and described by Hansen in 1874, but although he and many workers since his time have tried to grow it and reproduce the disease experimentally, all such attempts have failed to yield clear-cut results.

In 1932, Soule and McKinley successfully cultivated an organism found constantly associated with leprosy, apparently the same one described by Hansen. There is some doubt as to whether it is the specific cause of the disease because there is no satisfactory experimental animal on which to test its ability to produce leprosy. But in spite of the fact that in this disease it has not been possible to fulfill all of Koch's postulates, the *Mycobacterium leprae* described by Hansen is considered to be the specific cause.

Mycobacterium leprae as it occurs in tissues is a pleomorphic acid-fast nonsporulating rod. The organisms show a characteristic arrangement, appearing in clumps or bundles. Little is known about their physiology except that they appear to be strict parasites limited in host range to man. Chemically and serologically they are so closely related to the tubercle bacillus that the two are classed as different species of the same genus.

Epidemiology

The source of the infectious organism is man. The cutaneous type is, presumably, communicable since the lesions are loaded with the organisms. The neural type is probably not.

A remarkable feature of leprosy is its low order of communicability. As evidence of this, it is only necessary to recall that there were at least two hundred, and probably more, cases of leprosy in the Scandinavian immigrants, most of whom settled in Minnesota. These two hundred or more

cases gave rise to less than a dozen new ones. Had leprosy been the highly contagious disease it is popularly supposed to be, there should have been a vast number. Other evidence points to the same conclusion: under modern conditions, leprosy is transmissible only when contact is long and close and even then there are many who escape. There are numerous instances of husbands who have had leprosy without transmitting it to their wives.

Over one hundred attempts have been made to transmit leprosy experimentally from active cases to "volunteers." There is only one recorded instance in which the volunteer contracted leprosy and since he had been in contact with lepers, it is doubtful whether the infection was due to the experimental inoculation.

Nurses, doctors, and attendants who care for lepers do not contract the disease. The publicity given to the three or four notable exceptions has engendered an entirely erroneous notion of the communicability of leprosy. The disease is, of course, communicable and conditions that favor its communicability seem to be a very low order of personal hygiene, a low standard of living, and a hot humid climate.

Mode of Transmission: Because of the low order of communicability, it is hard to say just how leprosy is transmitted. Probably it is transmitted by contact. The possibility of insect transmission appears to be remote. Hereditary transmission must, of course, be considered, but much of the evidence points in the other direction so it can probably be ruled out. In the tropics, about fifteen per cent of the children living with infected parents develop the disease. If the children are removed from the infected parents soon after birth, very few develop it.

Incubation Period: Leprosy is usually an exceedingly chronic disease and it is very difficult to say what the incubation period may be. It may vary from a few months to twenty-five years or more. The average incubation period is probably from two to seven years.

Susceptibility and Immunity: The average individual is relatively resistant to leprosy. This resistance may be inherited since there are, apparently, differences in the susceptibility of races, groups, and families.

Present Prevalence and Distribution: At the present time there are probably between one to two million cases of leprosy in the world. There are approximately five hundred known cases in the United States and probably about as many unrecognized. Sixteen deaths were reported for 1946.

Geographically, the disease is limited pretty much to the regions in the tropics where there is a high rainfall and a high temperature, although it does occur in Japan, parts of China, northern India, and in parts of South America. In the United States it occurs endemically in a few re-

gions of Texas and Louisiana. In other regions of the United States where it has been known it is practically extinct.

Age, Sex, and Race Distribution: Leprosy is most contagious during childhood and the greatest number of cases develop before the age of fifteen or twenty years. Considering the long incubation period, this means that infection must have taken place some years earlier.

There is little difference in the sex distribution of leprosy in children, but in adults the infected males outnumber the females about three to one.

Negroes are more resistant than white people. Mexicans are less resistant than native Americans.

Control

During Biblical times the method for the control of leprosy was segregation. In the Middle Ages, leper asylums or leprosaria were very common in Europe and the lepers who were not confined were required to observe certain regulations designed to prohibit their mingling with the public in its normal routine and to give warning to any chance wayfarer they might encounter.

At the present time identified lepers in the United States are required to take treatment at the National Leprosarium in Carville, Louisiana.

Segregation, while it has difficulties, appears to be the only specific solution to the problem. Undoubtedly, improved standards of living have played an important part in reducing incidence. Removing infants from their infected parents prevents their infection and, if this could always be accomplished, would soon eliminate the disease.

Conclusion

Leprosy is not of great public health interest in the United States. Although it has been imported on various occasions, it does not maintain itself except in a few areas in Texas and Louisiana. It is a disease of great historical interest and because of the dread in which it was formerly held and the popular misconceptions of its communicability, it has seemed advisable to review some of its main features here.

Chapter XXIII

DYSENTERY, TYPHOID FEVER, AND CHOLERA

A more favorable environment for the growth of microorganisms could scarcely be conceived than the digestive tract filled with warm moist food stuffs in various stages of degradation. At birth it is sterile but shortly after, certainly with the first feeding if not before, microorganisms are introduced into it and from then on a vast and varied number inhabit this enormous culture tube. The predominating kinds are determined by the nature of the individual's diet and the difference in the acidity of the various levels of the digestive tract.

The intestinal flora of the breast-fed infant is relatively simple. In fact, during the first few weeks after birth, as much as ninety-nine per cent of the total number of bacteria in his feces consists of *Lactobacillus bifidus*. The few other organisms present belong largely to the coliform and enterococcus groups.

The intestinal flora of the bottle-fed infant is subject to far greater variation due, in part, to the difference in the character of the food introduced and, in part, to the greater chances of introducing more varied flora. *Lactobacillus acidophilus* replaces *bifidus* in the aciduric group, the coliform organisms, particularly *Escherichia coli*, are present in large numbers, and also some gram-positive aerobic and anaerobic spore-formers.

The normal stomach in both the child and the adult is too acid to provide favorable conditions for bacterial growth so the number and kind of bacteria present depends upon the number and kind introduced. The empty stomach may be sterile and there are not many organisms in the duodenum or upper jejunum. The bacteria introduced with the food tend to be killed off rapidly by the gastric juice, but many which may not be particularly resistant, as, for instance, the typhoid bacillus and the cholera vibrio, are sufficiently protected by the food mass to pass unharmed into the intestine where they encounter more favorable conditions.

In abnormal conditions of the stomach, such as hypoacidity, certain species of bacteria, notably the sarcinae, may actually increase in numbers.

The enterococci seem to predominate in the upper levels of the small intestine. In the lower levels, other groups of bacteria become more evident, and in the large intestine, the flora is abundant and extremely complex. There the reaction is favorable for bacterial growth, the digestive enzymes of the body appear to have little or no inhibiting action, and the bacteria increase in such tremendous numbers that from thirty to fifty per cent of the total dry weight of the feces is comprised of living

and dead microorganisms. Rettiger has calculated that the average man excretes about 128,000,000,000,000 bacteria per day, in kind and number varying with his diet, age, and state of health.

Under normal conditions these organisms neither harm nor benefit their host. If the diet is too heavily protein, the proteolytic bacteria may become too numerous and their toxic products may affect the health; or, if the diet is excessively carbohydrate, the saccharolytic bacteria may become too abundant and produce a diarrhea. Only a comparative few of the many kinds of microorganisms normally present in the intestinal tract are capable of creating intestinal disturbances.

Of course, there are many agents, other than bacteria, that may produce intestinal disturbances. Naturally poisonous foods like some mushrooms; chemical agents, both organic and inorganic; and allergic responses to common foods may produce similar symptoms, but although it may be difficult to distinguish these disturbances from those due to bacteria, we need not consider them here. There are also a number of bacterial and virus diseases, as, for example, scarlet fever, septic sore throat, and poliomyelitis, in which nausea and vomiting occur in the early stages of the disease, but these are not considered as infections of the gastro-intestinal tract.

Enterobacteriaceae

Many of the infections of the gastro-intestinal tract are due to closely related bacteria of the colon-typhoid-dysentery group so we shall consider the bacteriology and serology of the group as a whole before discussing the specific diseases produced by the different members.

The family *Enterobacteriaceae* are short, nonsporulating, gram-negative rods. Some are motile and a few are encapsulated. Except for those species that form capsules, they all look alike under the microscope. The family is subdivided into five tribes which may be briefly described as follows.

Tribe I. Escherichieae. Included in this tribe are three important genera: *Escherichia*, *Aerobacter*, and *Klebsiella*. The *Escherichia* are parasites and are commonly found in the intestinal tract of man and animal. Occasionally they are pathogenic. The *Aerobacter* are saprophytes, normally found on plants, in soil, and in milk, and occasionally in the intestinal tract of man and animal. The *Klebsiella* are parasitic and pathogenic for man and animal. They are also encapsulated which serves to distinguish them from the other two genera. All three are similar in respect to size and shape, measuring about five tenths micron in width and two microns in length. The common species of each is *Escherichia coli*, *Aerobacter aerogenes*, and *Klebsiella pneumoniae*.

Escherichia coli, the colon bacillus, is the predominating bacterium in the intestinal tract of man and animal where it lives as a saprophyte. Under unusual conditions, it may produce a peritonitis, an infection of the urinary tract such as cystitis, a gall bladder infection known as cholecystitis, and rarely, a meningitis in infants. It produces a disease known as *white scours* in new-born calves who have not received colostrum. Because it is always present in the intestinal tract of man, it is used as an indicator of fecal pollution of water.

Aerobacter aerogenes produces unpleasant flavors in milk and dairy products. Since it is normally present on grains, grasses, and in soil, it is commonly found in water supplies too. In examining water for fecal pollution, care must be taken to distinguish it from *Escherichia coli*.

Klebsiella pneumoniae is commonly found in the respiratory tract of man and certain animals. This organism has also been called *B. mucosus capsulatus* because of its mucoid capsule and *Friedländer's bacillus* because Friedländer first described it as the cause of pneumonia. *Klebsiella pneumoniae* or closely related strains are also found associated with many other disease conditions.

Variation is common in all three of these genera and numerous species have been described.

Tribe II. Erwineae. The *Erwineae* is composed of two genera, *Erwinia* and *Phytomonas*. They are plant pathogens producing soft rots, wilts, and other diseases of many common plants. None are pathogenic for animals.

Tribe III. Serrateae. The type species is *Serratea marcescens* or *B. prodigiosus*. Members of this group produce a red or pink pigment. They are saprophytic on decaying plant and animal matter.

Tribe IV. Proteae. This tribe contains highly proteolytic organisms and is associated with putrefaction. The most frequently encountered representative of the group is *Proteus vulgaris*. It is closely allied with the *Eschericheae* but does not ferment lactose. *Proteus vulgaris X 19* and several other strains are of special interest because they are agglutinated by sera from persons who have had typhus fever, Rocky Mountain Spotted Fever, or some other rickettsial infections, a phenomenon named after the workers who first described it and known as the *Weil-Felix reaction*.

Tribe V. Salmonelleae. This tribe is composed of the common pathogenic intestinal bacteria. The two important genera are the *Salmonella* and *Shigella*.

Salmonella. The genus *Salmonella* is composed of a number of species pathogenic for man and animal. To this group belong the typhoid bacillus,

formerly called *Bacterium typhosum* or *Eberthella typhosa*, and the bacteria that produce paratyphoid fever and food poisoning.

Classification of the Salmonella: Due chiefly to the brilliant work of Bruce White and his associates and to Kauffman, the antigenic composition of the members of the *Salmonella* has been charted. Classification of the fifty or more species is now largely on the basis of serology, although physiology is also considered. The human diseases produced by members of the group may be divided into two kinds: a slow and continued fever like a mild typhoid called *paratyphoid*, and an acute gastro-intestinal disturbance or *enteritis*.

Paratyphoid Fever: Salmonella paratyphi (paratyphoid A) and *Salmonella schottmülleri* (paratyphoid B) are the most common causes of paratyphoid fever in the United States. Paratyphoid fevers are transmitted by water and food and are like typhoid in their epidemiology. Carriers exist. The incidence is low, the case fatality rate very low.

Gastroenteritis: Several members of the genus *Salmonella*, chiefly *Salmonella enteritidis*, *Salmonella aertrycke*, *Salmonella cholerae-suis* (*suipestifer*), and *Salmonella typhi-murium* produce acute gastroenteritis. The incubation period is short, ranging from one to twenty-four hours and seldom being more than forty-eight. Because transmission is usually through food, the term "food-poisoning" is applied to these infections.

Salmonella cholerae-suis is of additional interest because it was once thought to be the cause of hog cholera, hence its name. Hog cholera was found to be a virus disease and while this bacterium is present in many cases, it is not the specific cause.

The Salmonellas usually encountered in food poisoning are primarily animal pathogens. Rats and mice may be naturally infected with *Salmonella aertrycke* or *Salmonella typhi-murium* and become carriers contaminating food in vermin-infected premises. Several outbreaks of food poisoning have been definitely traced to droppings from rodents.

Salmonella aertrycke and *Salmonella enteritidis* have been used in preparations designed for the eradication of rodents and called "Rat Virus." Several outbreaks of food-poisoning and a few deaths in humans have been traced to the use of such cultures when the poisoned rats contaminated the food.

The host range of these organisms is wide. Calves, swine, sheep, cats, and fowls may be infected as well as rodents.

Salmonella Infections of Animals: Several species of Salmonella that are not pathogenic for man produce specific diseases of animals. *Salmonella pullorum* is the cause of bacillary white diarrhea of chickens. *Salmonella sanguinarium*, or *Shigella sanguinarium* according to the most

recent classification, is the cause of fowl typhoid. *Salmonella abortus equi* produces contagious abortion of mares. *Salmonella abortus avi* produces a similar condition in sheep.

Shigella: The genus *Shigella* is composed of nonmotile, nonflagellated, gram-negative rods which produce dysentery in man. The *Salmonella* and *Shigella* are closely related and cannot be separated from each other or from the *Escherichia, Aerobacter,* or *Proteus* by microscopic examination but there is a considerable difference in their physiological behavior.

BACILLARY DYSENTERY OR FLUX

Dysentery or flux is an inflammation and ulceration of the mucous membrane of the intestinal tract and is accompanied by severe abdominal pain, bloody diarrhea, and, often, a profound toxemia. The incubation period is from two to seven days and the onset is sudden with fever and frequent stools containing blood and mucus. There are two kinds of dysentery, bacillary and amoebic, and either kind may be acute or chronic. A protozoa, *Endamoeba histolytica* is more commonly associated with chronic dysentery. Bacillary dysentery, which is usually an acute disease, is caused by bacteria belonging to the genus *Shigella.*

History

Shiga, a Japanese bacteriologist, isolated a hitherto undescribed bacterium from cases of dysentery in 1898. In the next few years, Flexner, Strong, Kruse, Park, and Hiss isolated organisms from numerous widely scattered outbreaks of dysentery and studied them by means of fermentation reactions and agglutination tests. These organisms are all closely related but differ in several respects. Park gave the name *paradysentery* to all but the type isolated by Shiga.

Shigella dysenteriae is the type originally isolated by Shiga. It produces a powerful exotoxin and is the only member of the pathogenic gram-negative rods that does.

Shigella paradysenteriae contains a number of varieties, each named after the man who first described it, i.e., Flexner, Strong, Hiss, Sonne, and other types. The paradysentery group ferments mannite and produces indol while the Shiga type does not. The Sonne type is of interest in that it ferments lactose only after ten to fourteen days. Differentiation between the types is largely on the basis of agglutination reactions and physiology.

Resistance of Shigella: The dysentery bacilli are not particularly resistant to heat nor to chemicals. They are destroyed by heating at 55° C. for one hour. Although they may live for a month in a dried state, they usually do not survive more than a few days in stools, nor more than a

week in water. Their resistance to drying is of obvious importance in the spread of the disease.

Pathogenicity. Toxins: Shigella dysenteriae is the most virulent of the group and is the only one to produce a powerful exotoxin. All varieties produce more or less potent endotoxins, as demonstrated by animal inoculation.

Antitoxins: The antitoxin prepared against the Shiga exotoxin is of considerable value in treatment. Antitoxins can also be obtained against the endotoxins but they are not therapeutically effective.

The dysentery bacilli do not produce a generalized infection but remain localized in the deeper tissues of the intestinal mucosa. They are excreted with feces from which they can be readily isolated but they cannot be cultivated from the blood. Proof of the etiological significance of the dysentery bacilli isolated from stools has often come from laboratory accidents or from experiments on volunteers, and, indirectly, from the fact that agglutinins appear in the blood stream following an attack. Shiga had isolated ten strains from cases of dysentery and, since there was no experimental animal, he took serum from his patients and tested its action on each type. One type was agglutinated and thus indirectly implicated as the causative agent. Later experiments confirmed his findings.

Unlike many of the *Salmonellas*, the dysentery bacilli do not normally infect animals, hence there are no animal hosts to consider when outlining control measures.

Case Fatality: The case fatality is highest in the Shiga type. In some outbreaks it may be as high as twenty-five to forty per cent or more, and in others only one to two per cent. It is highest in the group aged one to ten and next high in the oldest group.

Epidemiology

Transmission: Bacillary dysentery is contracted mainly by eating food contaminated by fecal material from persons suffering from the disease or from carriers. Water and milk are less important as vehicles than in typhoid fever. Flies are probably transmitting agents of some importance. Infected flies have been caught in the rooms of patients and it si known that dysentery bacilli can live for at least twenty hours in their intestines. The fly probably also acts as a mechanical carrier, transferring bacilli on its feet from feces to food. The seasonal distribution of dysentery is usually correlated with the prevalence of flies, and the incidence decreases in the late autumn when the flies decrease in number.

Period of Communicability: The stools from persons suffering from acute dysentery may consist of an almost pure culture of dysentery bacilli.

During this period the danger to contacts is especially great but since greater care is usually exercised then, it is likely that the less acute cases and carriers are actually more important in the spread of the disease. Many persons discharge bacilli for a month or longer after recovery. Some become chronic carriers and provide a continual source of infective material. This is particularly important, of course, if they are preparing or handling food.

Immunity: The younger age groups are the most susceptible and resistance increases with age. An attack confers a temporary immunity.

Prevalence: Like plague, typhus, and cholera, dysentery has, in the past, been one of the great and deadly diseases. It has been particularly destructive to armies in the field and to restricted groups in jails, asylums, or on ship board. Bacillary dysentery is world wide in distribution and occurs endemically, in epidemics, as sporadic cases, and in localized outbreaks. Recorded cases give little indication of the true incidence of the disease since many cases are so mild that a physician is not consulted and the disease not reported. The incidence is decreasing in proportion to the improvement in sanitation and higher standards of living.

Bacillary dysentery is most prevalent in the tropics and, in cooler regions, occurs most frequently in the summer months. The higher temperatures undoubtedly allow for a rapid increase in organisms which have come in contact with food or milk either directly from the infected individual or through the agency of flies. The effect of high temperatures on the resistance of the individual is not clear, but some workers believe that they reduce the activity of the secretory glands of the stomach and thus lead to a lowering of body resistance to bacterial infection.

Many outbreaks of dysentery, referred to as "asylum dysentery" have occurred in insane asylums and prisons, where lack of personal cleanliness and a low order of sanitation often prevail. Here the attack rate may approach fifty to sixty per cent of those exposed and the case fatality rate may be as high as twenty per cent or more. Relapses and reinfections are common and carriers appear to play a particularly important part. In summer resort regions in the northern states a number of outbreaks have been occasioned by primitive methods of sewage disposal.

Control

Reporting is essential to the control of dysentery. Epidemiological studies should be carried out whenever possible to determine whether the infection is water-, milk-, or food-borne; and when they are indicated bacteriological examinations should be made for the detection of carriers. Food handlers should be inspected. The control of flies by DDT prevents transmission from that source.

Some of the sulfa drugs and streptomycin seem to be moderately effective in reducing the severity of the disease.

Isolation: Infected individuals should be isolated until they no longer excrete organisms. All bowel discharges must be disinfected and rigid personal cleanliness of the attendants is requisite. There is no need for quarantine of contacts.

Summer Diarrhea

Summer diarrhea, infant diarrhea, summer complaint, cholera infantum, and stomach flu are some of the names applied to a gastroenteritis which occurs epidemically in the summer and autumn, though not absolutely limited to these seasons.

Etiology

There is little doubt that summer diarrhea is a bacterial infection probably caused by a number of bacteria of the dysentery group. Bacteriological findings are conflicting and indicate that this disease is not a bacterial but an epidemiological entity and that several species of bacteria either alone or in combination are capable of producing it. Dysentery bacilli of the Shiga, Flexner, and Sonne types are most frequently isolated. In some outbreaks, organisms belonging to the Salmonella groups are implicated, and in others *Proteus vulgaris* or *Clostridium perfringens*.

The incubation period is variable; the average, as judged from the time between secondary cases in a family, runs from four to eight days but in many instances is only twenty-four to forty-eight hours.

Epidemiology

As everyone knows, summer diarrhea is extremely prevalent and attacks all ages. In rural districts and smaller towns it occurs about the time of harvest. The disease is characterized by a short incubation period and high infectivity and is often referred to as "stomach flu." The source of the infection and the manner of its transmission are varied but associated with food and water. At that time of the year flies are prevalent and have easy access to privies, the food is not properly refrigerated, and the heat may be intense causing everyone to drink a great deal more water than usual and often from a common cup or dipper. Such conditions are ideal for the transfer of an intestinal infection. There is little bacteriological evidence to say how many of these factors are purely coincidental and how many bear a casual relationship.

The mortality is confined almost entirely to the first two years of life. It is an important cause of death during the second summer, although the

case fatality rate is highest in infants less than six months old, where it may reach a figure of twenty to forty per cent.

It has been estimated that in the past the bottle-fed baby's chance of contracting summer diarrhea was ten times as great as the breast-fed baby's. The fatality rate was also higher in bottle-fed babies. A better knowledge of artificial feeding of infants and insistence upon pasteurized milk, or milk which has been brought to a boil, and upon properly sterilized bottles and nipples have served to bring about a great reduction in the number of deaths in this group. Where care is exercised, there is now little difference in the fatality rates of breast and bottle-fed infants. Whether breast feeding may confer an increased resistance to the infection is not clear, but it does serve to delay exposure and attack until the second year when the case fatality is not so great.

In general, the babies of the lower income groups suffer most from summer diarrhea. Ignorance of the gravity of the disease and of the way it is spread coupled with living conditions which make for a ready transmission of infection account for this. During its second year when a baby is almost continually creeping on the floor and putting all manner of things into its mouth, the chances for infection are tremendous, particularly if its surroundings are not too clean.

Flies are instrumental in the spread of summer diarrhea just as they are in the spread of other forms of dysentery. Some studies made in England showed that the reduction in deaths from summer diarrhea paralleled the increase in motor cars. With the increase in motor transportation and hauling there has been a decrease in horse-drawn vehicles, the reduction in the number of horses has eliminated the breeding places for flies, and the reduction of flies has lead to a reduction in summer diarrhea.

Epidemic Diarrhea of the New-Born

The first epidemic of infectious diarrhea of the new-born was reported in 1934 and since then outbreaks have occurred in many hospitals in the United States. The disease is described as a severe intestinal toxemia with an acute onset, yellowish stools without blood or mucus, rapid loss of weight due to dehydration, and shock. The case fatality rate as reported for 750 children in twenty-seven outbreaks which occurred between 1934 and 1937 was 47.5.

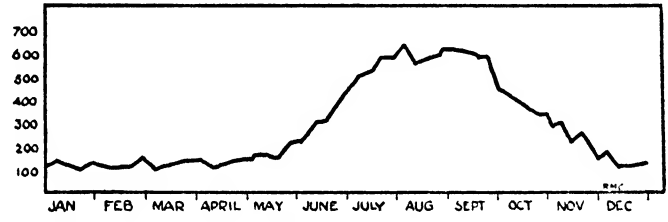
The disease has been studied carefully, but no one has been able to cultivate a bacterium which can be implicated. It has been suggested that it may be a virus disease but there is no proof that a virus is involved.

The infection spreads rapidly involving most of the exposed children who are less than a month old. Ordinary hospital precautions do not

seem to be sufficient to limit its spread. Strict isolation and aseptic handling of infants is effective. There is little or no difference in the attack rate of breast and bottle fed infants.

Typhoid Fever

Typhoid fever is a specific infection due to the typhoid bacillus, *Salmonella typhosa*. The onset is slow, the patient may feel indisposed for five or six days with a little fever, headache, and fatigue. After about a week, the fever rises, diarrhea or, sometimes, constipation sets in, there may be severe abdominal pains, a general and pronounced weakness, loss of weight, and sometimes delirium. There may be perforations of the intestinal tract followed by hemorrhage. A cough may be present. An eruption of small rose-colored spots appears on the abdomen. The fever lasts three to four weeks. There is no evidence that the bacilli multiply in the blood and



Seasonal distribution of typhoid fever. Median number of reported cases by weeks for 1935–1939. Data from P.H.R., May 23, 1941.

the disease is not a septicemia. Recovery is gradual. The disease varies from mild and unrecognizable cases, often referred to as "walking typhoid," to severe and fatal infections. The case fatality rate runs about ten per cent and there has been practically no change in it since the disease was first recognized.

Little is known about the mechanism by which the *Salmonella typhosa* induces illness. It does not produce an exotoxin. There is a toxemia, to be sure, but this is attributed to endotoxic substances.

Typhoid fever is a generalized infection and it is only in the later stages that the infection is active in the intestinal tract. The course of the infection is probably as follows. The typhoid bacilli enter by way of the mouth, usually in water, milk, or food. They pass on through the stomach, enter the upper intestines, and set up an inflammation of the intestinal walls. The lymphoid tissue, particularly *Peyer's patches*, are invaded and the organisms multiply rapidly. From the lymph nodes the organism gets into the blood stream and a bacteremia results. Although there are no antibodies in the blood stream at this stage, the organisms

disappear for a time due to the activity of the reticulo-endothelial system. However, they are not destroyed but proliferate in the spleen, liver, bone marrow, and gall bladder, and from these regions again invade the blood stream where they remain until the appearance of antibodies.

When a sufficient concentration of antibodies occurs, clearing of the blood stream again takes place. Bacteriolysis occurs with the liberation of endotoxins.

If local foci of infection do not clear up, the bacteria may again invade the blood stream and a relapse occur. In rare instances, a typhoidal pneumonia, pleurisy, meningitis, or endocarditis may develop. The so-called "rose spots" represent localized foci of infection and are, in effect, small colonies of the organisms.

In the later stages, secondary infections may occur. The intestines may perforate with a resulting peritonitis. Death may follow from peritonitis, or, in its absence, from a toxemia.

In fatal cases the organisms may remain present in the blood.

It is not always easy to isolate typhoid bacilli from the feces because they are present only in small numbers in the early stages of the disease Many workers interpret this to indicate that the organisms do not multiply freely, if at all, in the intestinal tract. On the other hand, they may be present in great numbers in the urine, as many as 50,000,000 per cubic centimeter having been reported. Since the intestinal tract normally harbors flora rich in coliform bacteria, it would be difficult to isolate typhoid bacilli unless they were present in fairly large numbers. Eosin methylene blue, Wilson and Blair's bismuth sulphate medium, and Leifson's desoxycholate-citrate agar are some of the special media developed to make the detection of *Salmonella typhosa* more certain.

Bacteriological Diagnosis: The bacteriological tests used are blood, stool, and urine cultures, and agglutination tests. Positive findings depend upon the stage of the disease and are correlated with the route of invasion. Stool cultures are usually positive during the later stages. Blood cultures may be temporarily positive following the first invasion, negative following the first clearing, and positive again following the second invasion of the blood stream. This occurs during the first week of the disease. At the end of the first or at the beginning of the second week, the bacteria disappear, antibodies appear, and the agglutination tests become positive.

Stool cultures are usually positive during the disease and the bacteria may be eliminated for long periods after recovery. About five per cent of the cases remain carriers for several months and a smaller per cent become chronic carriers for life. In the chronic carrier state, the focus of infection is usually the gall bladder or urinary tract. Isolation of the typhoid

bacillus from the feces or urine from carriers is not always possible since the organisms may be eliminated intermittently. Several examinations may be necessary.

Typhoid Carriers

The carrier state is of particular importance in the control of typhoid. As we have said, about two to three per cent become chronic carriers. The percentage increases with the age at which the attack was experienced. More women than men become carriers. The carrier state has been detected, that is, typhoid bacilli have been isolated, from persons who gave no evidence of having had a clinical attack of the disease.

Treatment of carriers by chemicals has not been successful. Removal of the gall bladder by surgery eliminates the source of infection from those in whom the gall bladder was the only focus.

The importance of carriers in endemic typhoid and in starting water-food, and milk-borne epidemics has been proven time after time. The classic example is "Typhoid Mary." The following summary of her activities is taken from Rosenau's *Preventive Medicine and Hygiene:*

Mary Mallon was a cook in a family for three years, and in 1901 she developed typhoid fever. About the same time a visitor to the family had the disease. One month later the laundress in this family was taken ill.

In 1902, Mary obtained a new place, and two weeks after her arrival the laundress was taken ill with typhoid fever. In a week, a second case developed, and soon 7 members of the household were sick.

In 1904, the cook went to a home on Long Island. There were 4 in the family, besides 7 servants. Within three weeks after her arrival, 4 servants were attacked.

In 1906, Mary went to another family, and 6 of the 11 members of this family were attacked with typhoid between August 27 and September 3. At this time, the cook was first suspected. She entered another family on September 21, and on October 5 the laundress developed typhoid fever.

In 1907, she entered a home in New York City and two months after her arrival 2 cases developed, one of which proved fatal. During these five years, "Typhoid Mary" is known to have been the cause of 26 cases of typhoid fever.

She was virtually imprisoned by the New York Department of Health in a hospital from March 19, 1907. Cultures taken every few days showed bacilli now and then for three years. Sometimes the stools contained enormous numbers of typhoid bacilli and again for days none could be found.

"Typhoid Mary" then escaped from observation until 1914. In October of that year, she was engaged as cook in the Sloane Hospital for Women in New York. In January and February of 1915, an outbreak of typhoid occurred, principally among the doctors, nurses, and help of the institution, involving 25 cases. The cook was suspected but she left the premises on a few hours' leave, and did not return or leave her address. She was, however, located by the Health Department under an assumed name, and an investigation established her identity as the famous "Typhoid Mary."

A subsequent study of her career showed that she had infected still other individuals beyond those already mentioned, and that she may have given rise to the

well-known water-borne outbreak of typhoid in Ithaca, New York, in 1903, involving over 1300 cases. The fact is that a person by the name of Mary Mallon had been employed as a cook in the vicinity of the place where the first case appeared, and from which contamination of the water supply occurred.

The chronic carrier is only too often not aware of her condition and the consequences of her ignorance are often tragic. The following example was reported in Illinois in 1938. In 1902, the mother of two girls recovered from typhoid fever. In 1924, shortly after visiting at her home, a son-in-law contracted typhoid and died. In 1938 several members of the family had typhoid, including the other son-in-law, and at this time bacteriological examinations showed that the grandmother and one daughter were carriers.

The importance of carriers and the value of protective immunization is nicely illustrated by an outbreak of typhoid in 1946. The source of infection was a carrier ignorant of the fact who prepared orange juice for some three hundred and sixty-one persons. There were seventeen cases of typhoid among the one hundred and forty who were not immunized and only one in the two hundred and eleven who had been immunized.

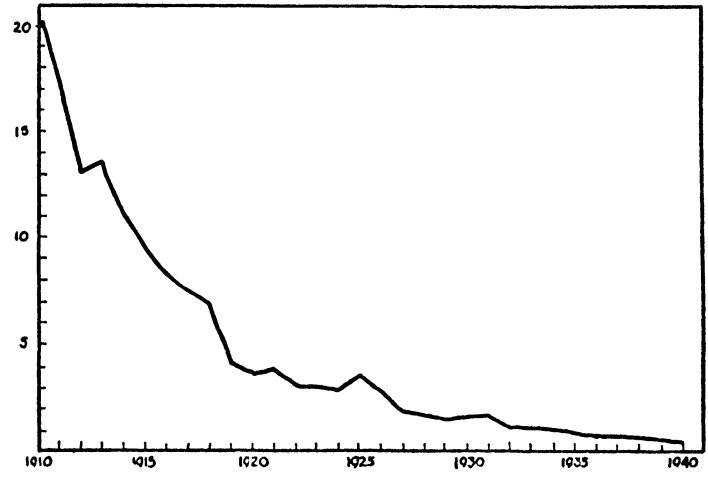
Immunity: It is difficult to gauge the resistance of normal individuals to *Salmonella typhosa*. Undoubtedly we vary in resistance. The fact that carriers have been found in those who have not shown evidence of infection indicates this. Such individuals have probably come in contact with small doses for when the dosage is large, over fifty per cent of those exposed develop the disease.

One attack of typhoid confers a permanent although not an absolute immunity. Second attacks are very rare. Immunity to *Salmonella typhosa* is specific and does not protect against infection with the organisms producing paratyphoid fever.

Typhoid Fever Vaccination: Vaccination using heat-killed cultures of *Salmonella typhosa* has proven remarkably successful in protecting against infection. As early as 1898, Wright successfully inoculated 4000 men in India. Two years later Leishman vaccinated about 100,000 British troops in the Boer War somewhat less successfully. In 1909, Russell introduced typhoid vaccination into the United States army and in 1911 it was made compulsory. The results obtained were so dramatic that there remained no doubt as to the effectiveness of the procedure.

Most of our information on protective vaccination comes from a study of typhoid fever in armies. In the Franco-Prussian War (1870 to 1871) sixty per cent of all deaths in the German army were from typhoid. In the Spanish-American War there were over 4400 cases of typhoid and 248 deaths in one division of 10,759 men. In the Civil War, the rate was nearly as high. In contrast to these high death rates from typhoid fever

are those that prevailed in the German and Allied armies in World War I and which gave ample evidence of the protective value of typhoid vaccination. Of course, sanitation was much improved but does not wholly account for the almost complete absence of typhoid. A total of over 4,000,000 men were in the American army from 1917 to 1918 and in this group there were only 1529 typhoid cases and 227 deaths. Jordan points out that if the rate in World War I had been the same as the rate in the Spanish-American War, there would have been 68,164 deaths. Attention to water supplies and immunization of the troops eliminated typhoid as a problem in World War II.



Total typhoid rate per 100,000 for seventy-eight cities, 1910-1940. Data from J. A. M. A. January 17, 1942.

The duration of immunity following vaccination is fairly long—just how long is not known. A study of an outbreak of a water-borne typhoid occurring in Chamberlain, South Dakota in 1932 sheds some light on the question. In this epidemic there were 282 cases of typhoid and 29 deaths in a population of approximately 1500. Since the infection was water-borne, all ages were exposed. The highest attack rate was in the six to ten age group which had 37.8 per cent of the cases. It decreased with age. There were only three cases among the sixty ex-service men exposed who had all presumably had typhoid vaccination in the army some twelve to fifteen years before and they were so mild that had there been no epidemic they would probably have gone undiagnosed. One patient was not sure whether he had received the full course of injections. The other two were vaccinated with *Lipo vaccine* and received only one injection.

Other studies confirm the belief that immunity, although not absolute, increases resistance for fifteen to twenty years and probably for life.

Until a few years ago the vaccine most commonly used was made up of a mixture of three organisms, *Salmonella typhosa* (typhoid), *Salmonella paratyphi* (paratyphoid A), and *Salmonella schottmülleri* (paratyphoid B). This was known as T.A.B. vaccine and contained 500,000,000 typhoid, 250,000,000 para A, and 250,000,000 para B organisms in each cubic centimeter. It was usually given in three subcutaneous injections spaced a week apart. The first injection consisted of 0.5 cubic centimeter and the others of one cubic centimeter each. Immunity reached its height in about a month.

Because the paratyphoid infections are usually mild and because the constitutional reactions following vaccination with T.A.B. were due to them, they have been omitted from the new vaccine. At present the vaccine commonly used contains only *Salmonella typhosa* strain No. 58, which is highly immunogenic and highly virulent.

Passive Immunization: Vaccination after exposure or during the early stages of typhoid fever has been tried but the results obtained are not clear cut. Serum treatment has not proven successful and is not used to any extent. There is some evidence that effective antiserum may be produced if the vaccine is prepared from strains of *Salmonella* that have *O* and *Vi* antigen. That a curative agent is needed is shown by the fact that the case fatality rate of ten per cent is as high today as it was twenty or even forty years ago.

Prevalence: The death rate from typhoid and paratyphoid in 1900 was in excess of 30 per 100,000. In 1944 it was 0.4, a decline of ninety-nine per cent. The case rate from typhoid and paratyphoid dropped seventy-five per cent in the country as a whole between 1935 and 1945. Ten years ago there were over 20,000 cases a year. At present there are 5,000. This decrease is due to installation of sewer systems, the supervision of typhoid carriers, the inspection of food supplies, the examination of food handlers, antityphoid inoculations, and the adoption of central water supplies with filtration and chlorination of water.

CHOLERA

Asiatic cholera is a disease whose origin and abode is the delta of the Ganges. Although it had been present there for centuries, it was not until 1817 that it invaded other parts of the globe. Since then there have been four periods in which it has spread rapidly in epidemic form. The first epidemic was from 1817 to 1823 and was limited to other parts of Asia. The second was from 1826 to 1837 and reached Europe in 1830 and America in 1832. During this period the disease was especially

virulent in the larger cities of Europe, particularly London and Paris. From 1846 to 1862 a third appeared, and the last great epidemic was between 1864 and 1875.

In the United States the first epidemic entered New York in the early 1830's and spread inland from that point. The second importation through New Orleans in 1848 spread up the Mississippi River and its tributaries. It was carried across country by the 'forty-niners' who left contaminated water holes from the Mississippi to California. The last two invasions were less extensive in the United States.

Cholera has been epidemic in Russia several times since 1900. A few cases have appeared in England and America from time to time but the disease has not gained a foothold because of the rigorous methods of suppression and the generally good sanitary conditions.

Recognition of Cholera

Cholera is a specific infection of the intestinal tract. The onset is sudden with vomiting and a profuse diarrhea which results in the loss of water and salts from the body tissue and leads to collapse. There is great thirst, pain, and cramps. The excreta have a characteristic appearance due to the presence of epithelium from the intestinal walls and mucous and are called "rice water stools." The muscular cramps are due to dehydration and loss of salts and the administration of sodium chloride or sodium bicarbonate is said to be very beneficial. The loss of water from the blood may be great enough to raise the concentration of the red blood cells to seven or eight million.

The organism grows profusely in the intestinal tract and the discharges may contain an almost pure culture of cholera bacilli. They do not penetrate the mucosa deeply and the symptoms are due to the liberation of toxin produced on the autolysis of the organism. Asiatic cholera is not related to the so-called *cholera infantum* or *cholera morbus*.

Etiology

Asiatic cholera is caused by a spirillum, *Vibrio cholerae*, sometimes called the *comma bacillus* or *Spirillum cholerae*. Koch discovered the organism in the intestinal contents of patients in 1883. Von Pettenkofer did not believe that this organism was the sole cause of cholera and to prove his point drank a pure culture. His assistant Emmerick also drank some and developed a severe cholera. Von Pettenkofer, who developed a mild diarrhea only, insisted that the experiment had been inconclusive and had only proved his famous X Y Z theory. This was, in essence, that there were three factors in the production of disease: X, the germ, Y, the host, and Z, the soil, and he insisted that the host and soil or environment

were of particular significance. While we now believe that all these factors play a part in the development of disease in the individual, von Pettenkofer over-emphasized the specific character and the importance of Z, particularly.

Morphology: The vibrio are short curved rods about 1.5 microns in length. Single cells have the appearance of a comma. They are actively motile and give the impression of vibrating, hence the name, vibrio. They may occur in chains or be joined in pairs so as to form a semicircle. They do not form spores, have no capsules, and are gram-negative but do not stain as readily as the gram-negative rods. In old cultures, involution forms are common.

Physiology: Vibrio cholerae grows readily and rapidly in ordinary media. It is aerobic and grows best at 37° C. but will grow at room temperature. Most rapid growth is secured in strongly alkaline media. When a peptone broth at a pH of 8 to 9 is inoculated with feces, *Vibrio cholerae*, if present, grows more rapidly than other organisms and after three to eight hours, pure cultures of it may be obtained by transferring a loopful of the surface growth to fresh media. In Dunham's peptone solution, growth is rapid and indol is produced. The addition of a few drops of concentrated sulfuric acid brings out a bright red color. This is known as the *cholera-red reaction* and is of value because all cholera strains give the reaction and non-cholera strains do not.

Endotoxins are liberated on autolysis but there is some question as to whether true exotoxins are produced.

Bacteriological Diagnosis: The bacteriological diagnosis of cholera depends upon the isolation and the identification of *Vibrio cholerae*. The finding of comma-shaped organisms in the stained preparations of the flecks of mucous from rice water stools is strongly indicative, but separation of the true cholera vibrio from non-pathogenic forms depends upon physiological and serological findings. Agglutination and adsorption tests and Pfeiffer's reaction are specific.

The Pfeiffer Phenomenon: Pfeiffer observed that when normal guinea pigs were inoculated with *Vibrio cholerae* they developed a peritonitis which was usually fatal, but that when immunized animals were so inoculated, the organism was destroyed. He followed the fate of these organisms after injection into the peritoneum by removing a small amount of exudate at intervals of ten to sixty minutes. On microscopic examination he found that the organisms would first loose their motility, then swell up, and finally disintegrate or dissolve. The same reaction took place if the organism and immune serum were injected into normal guinea pigs or if the two were mixed in a test tube. It is a highly specific reaction and is known as the Pfeiffer Phenomenon.

Resistance of Vibrio cholerae: The resistance of *Vibrio cholerae* is not

great. They do not remain viable for more than two or three hours in a dried state. Heating for fifteen minutes at 55° C. kills them. Like the typhoid bacilli, they survive only a few days in highly polluted water but may live a month in pure water.

Epidemiology

The epidemiology of cholera is referable to its bacteriology. Animals are not naturally susceptible and hence man is the only source of infection. The organism leaves the body in the feces and enters it by way of the mouth. Transmission of epidemic cholera is usually by means of water. The short incubation period of one to five days allows for a rapid spread Food is a vehicle of transmission, particularly for the endemic form. Flies are probably of some importance as transmitting agents. Contact infection is rather common in persons associated with the sick, although the disease is not highly contagious and doctors and nurses who use intelligence in the handling of cholera patients and their excreta do not become infected.

Carriers: Chronic carriers of cholera are rare. Convalescents may be carriers for a month or two but usually excrete the organisms for only a week or two. Carriers are important in endemic cholera and in starting epidemics. Persons who never develop recognizable cases of cholera may become carriers.

Immunity and Susceptibility: Susceptibility is general but, as in all other diseases, there are differences in the resistance of individuals. One attack confers an active immunity that lasts for about a year.

Vaccines have been tried since 1884 and the one prepared by Haffkine is effective. Vaccination is used primarily in armies, and the figures show that the incidence and the case fatality rate is much reduced in the inoculated groups. In regions where cholera is prevalent, vaccination is not widespread but is confined pretty much to armies, travellers, physicians, and nurses.

Chemotherapy: Sulfonamides and streptomycin are of value in treatment.

Control

The control of cholera depends upon the fact that infection takes place when germs from the feces of a case or carrier are transferred to the mouth of healthy persons by means of drinking water, food, fingers, and, less commonly, milk.

Water purification and adequate sewage disposal are prime factors in the prevention of epidemics. The proper disposal of excreta from cases prevents secondary cases. Carriers are important in keeping the infection alive.

To prevent the spread of cholera from infected to non-infected parts

of the world an efficient quarantine must be maintained. To the United States Public Health Service must go the credit for preventing its entrance into the United States on innumerable occasions. Ships which arrive from infected ports are detained in quarantine until the passengers are given a clean bill of health, and bacteriological examinations are conducted on persons showing suspicious symptoms. The incubation period of a disease has a direct bearing upon the effectiveness of ship quarantine. In cholera the incubation period ranges from one to five days and since the time for ships to come from infected ports is in excess of this, the chances are that persons who may have contracted the disease will show recognizable symptoms before their arrival or shortly after so that they need not be detained long. Airplane travel has altered that situation and offers a real hazard in spite of the vigilance of all concerned.

Chapter XXIV

PLAGUE AND TULAREMIA

"The harp that once through Tara's halls
The soul of music shed,
Now hangs as mute on Tara's walls
As if that soul were fled.
So sleeps the pride of former days,
So glory's thrill is o'er,
And hearts that once beat high for praise
Now feel that pulse no more!

No more to chiefs and ladies bright
The harp of Tara swells;
The chord alone that breaks at night
Its tale of ruin tells."

—*Thomas Moore*

History

The ancient writers spoke of plagues whenever they meant to portray any disease of man or beast with a high mortality. It might be typhoid fever, dysentery, typhus, smallpox, or the disease later known as the bubonic plague or the Black Death. Hence, when he encounters the term plague (from the Latin *plaga* meaning blow), the medical historian looks for a more detailed description of the disease in order to tell which particular one is being discussed. From these descriptions, it is probable that the bubonic plague occurred in great epidemics centuries before the Christian era.

The first well-authenticated epidemic occurred in Egypt in 542 B. C. and lasted to the end of the century. Plague was prevalent in Syria and Libya as well, and, probably, in Ethiopia. The Mediterranean seaports were the commercial centers of that day and plague has usually been distributed from some such focal point. In the succeeding years the plague spread over Central Europe, France, and Germany, and laid waste to Ireland between 543 and 548. It was at that time that Tara, the residence of the king, was abandoned. Nearly everyone there died and the place came to be held in such dread that it was never again inhabited.

About a hundred years later the plague again ravaged Ireland and this time also reached England. Then, for several hundred years, no great epidemics were reported in Europe although plague continued to exist in Syria and the Valley of the Euphrates. It was not until 1094, two years before the Crusades, that Europe again experienced the devastation of the plague and with the Crusades of the twelfth and thirteenth centuries,

epidemics became more frequent and virulent, reaching a peak between 1347 and 1349.

This great pandemic from 1347 to 1349 apparently entered Europe by way of Constantinople. In three years it swept over the continent, killing about twenty-six million people, almost a quarter of the population. Many of the stricken were covered with small black pustules and scarcely any of them lived. It might have been because of these black spots but was more likely because of the dark dread aroused by its extremely high fatality that the plague became known at that time as the Black Death.

Plague reached England in the later part of 1348 and is said to have killed at least one in every ten persons. Many country villages were entirely wiped out. The Scots, according to a contemporary account by Le Baker de Swynebroke, indulged in a little premature rejoicing, believing that they could obtain power over the stricken English. "But sorrow following on the heels of joy, the sword of the anger of God departing from the English drove the Scots to frenzy through leprosy no less than it had done the English through abscesses and pustules."

In the fifteenth, sixteenth, and seventeenth centuries, the plague attacked frequently but the outbreaks were, in general, limited. London was visited every few years. In 1625 there were 35,417 deaths, the next year only 134, and two years later 4. The plague broke out several times during the next thirty-five years but caused only a few deaths each year. In 1664 there were only six plague deaths recorded in London. Then the Great Plague occurred and in 1665, there were 68,596 deaths. After that the plague subsided and after 1680 there are no plague deaths recorded.

During this period, plague was also rampant on the continent. In 1437, Cairo was almost depopulated. In 1576, Venice lost 70,000 and Moscow 200,000 of its inhabitants. In 1656 Naples lost 300,000 by plague, Rome, 145,000; and Genoa, 60,000. It is reported that in the fourteenth century outbreaks, from a third to a half of the entire population of Norway and Sweden died from plague.

Following the great epidemics plague remained endemic in only a few of the countries. Succeeding epidemics were due to new importations.

The Venetians were the first to introduce rational measures of control. Between 900 and 1500 they experienced sixty-three epidemics. Eventually they concluded that there was a connection between the merchants and merchandise from plague-infested countries and the appearance of the disease. They instituted the first quarantine measures on the ships and the crew and thus secured some measure of protection for themselves.

A more important factor in the disappearance of the plague from Europe was the discovery and settlement of America and the subsequent shift in the center of trade from the Mediterranean to Amsterdam and London. Their

connections with the endemic plague countries were made by sea and not by land. Quarantine was easier to apply and more effective.

Another factor which will be discussed later has to do with a change in the species of rat that predominated.

Changes in housing also played a part, particularly in London. After the Great Fire in 1665, the authorities decreed, as a fire preventive measure, that partitions of houses must be made of brick or stone. They thus unwittingly but nevertheless effectively closed one of the favorite breeding places of the black house rat.

We need not try to draw a word picture here of a panic-stricken populace fleeing before the plague nor of a decimated community trying to carry on. The plague was the greatest calamity that has befallen man during the period of recorded history and its influence cannot be measured.

It would, however, be most unfortunate to think that our interest in plague is purely historical. During the seventeen and eighteen hundreds, plague was endemic and epidemic in the Near East, in Russia, and in the Orient. It was, however, receding and finally disappeared from many countries, remaining endemic in India and parts of China. In 1894 plague broke out in Canton and later in Hongkong. It was during this epidemic that the etiological agent was discovered independently by two bacteriologists, Kitasato and Yersin. The plague spread to Bombay and thence to large parts of India. From India it spread to nearly every port in the world including some in North and South America. It appeared in several parts of the United States but gained a foothold only in San Francisco where, following the fire, sanitary conditions were disturbed.

Our present concern with plague is due to the fact that the plague bacillus, *Pasteurella pestis*, has become established in ground squirrels which serve as a reservoir for the infection and thus create a real public health problem.

Plague is a definite threat to us at all times and it is only the vigilance of the United States Public Health service and our rather high order of sanitation that have kept the disease from gaining epidemic proportions in this country. Thousands still die of it in various plague spots of the world—countries of the Near East and Far East for the most part where multitudes are crowded together in miserable quarters.

Recognition of the Disease

Plague may be bubonic or pneumonic. The more common in man is the bubonic form, so-called because of the enlargement of the glands of the groin. This is characteristic and diagnostic. The swollen glands are called "buboes" and hence the name bubonic. The organisms are present in the lymphatics and may be cultured from them. In severe cases there

is a septicemia as a result of the passing of the organism from the buboes into the blood stream where, in fatal cases, it multiplies rapidly. Hemorrhage may occur. From the early accounts, it probably did occur more frequently in the great epidemics of the past. Ordinarily the bubonic form is not contagious.

Should the lungs be invaded, the pneumonic form develops and it is always fatal. Numerous organisms are present in the lungs and sputum and the disease is highly contagious. It is very different from the bubonic form in epidemiology.

Etiology

The specific cause of plague is *Pasteurella pestis*, a short, thick, gram-negative rod with well-rounded ends and a tendency to bipolar staining. It is nonmotile, nonsporulating, aerobic, and can be cultivated readily on ordinary media. It is destroyed by heating to 50° C. for one hour. When dried it does not survive more than a few days at ordinary temperatures but may live for months at a temperature of 0° C.

The *Pasteurella* as a group are responsible for a disease called hemorrhagic septicemia of animals. All members of the genus are animal pathogens but two members, *pestis* and *tularense*, are also pathogenic for man.

Epidemiology

Source of Organism: The rat is the main source of infection although other rodents, such as the ground squirrel in the western states, also carry *Pasteurella pestis*. Experimentally, mice and cats are very susceptible, and cattle, horses, swine, and dogs can be infected. The blood of infected animals and the sputum of human cases of pneumonic plague contain the organisms.

Mode of Transmission: The pneumonic form is obviously directly communicable since vast numbers of organisms are present in the sputum. Judging from the number of cases following contact, it is highly contagious.

The bubonic form is not directly communicable from person to person. Man contracts the disease from rats through the agency of two species of rat fleas: *Xenopsylla cheopis* and *Ceratophyllus faciatus*. Since their host, the rat, plays such an important part in the spread of plague, a few words about his habits as well as theirs will not be amiss.

RAT PLAGUE

The rat becomes infected from the bite of the infected flea. The organism produces a septicemia and is present in tremendous numbers in the blood stream before the death of the animal, hence the chance of other

fleas also becoming infected is exceedingly great. When the rat dies, the fleas leave the body and seek new hosts. In the absence of sufficient other rats, they readily attack and infect man and in areas where plague is endemic, the danger is proportional to the concentration of rats. If the number of rats increases, an epizootic may suddenly occur which will destroy a large proportion of them. The death of so many rats means a scarcity of rat hosts for the fleas and they turn to man.

There are several principal species of rat concerned in plague transmission: *Rattus norvegicus*, the grey rat, *Rattus rattus*, the black rat, and *Rattus alexandricus*, the ship rat. The grey rat is usually found living in sewers, stables, and warehouses. It is fiercer than the black rat which lives in or close to houses and tends to drive it out and destroy it. It is believed that the black rat was introduced into Europe by the returning Crusaders and that it became widely established there because of the character of the housing. Epidemics of plague followed the extension of this species, and it was not until the grey rat was introduced that the plague began to decline. Many students of the problem feel that the grey rat was responsible to a great extent for the decline, since, as previously stated, it destroys the black rat, and, at the same time, does not live in such close contact with man.

The relationship of *Pasteurella pestis* to the flea is of considerable interest. The flea becomes infected during the act of feeding. The organism multiplies in the proventriculus or foregut of the flea, finally attaining sufficient numbers to plug the entrance to the stomach. The flea, thus starved, attempts to feed and sucks up blood which cannot pass into the stomach because of the plugged foregut. This becomes greatly distended and, during the act of sucking, organisms are regurgitated into the puncture-wound, thus very effectively inoculating the host. Should the blood succeed in getting past the foregut and into the stomach, the flea may be even more dangerous since the blood containing the organisms may then get into its pharyngeal pump and be introduced in greater numbers into the host.

Fleas remain infected for a varying length of time. Low temperatures and high humidity are favorable for the survival of the organisms in the flea, high temperatures and low humidity are not. The former conditions, by the way, are not the most favorable for the propagation of the flea or rat.

There is evidence that the survival of *Pasteurella pestis* in a rat is inversely related to the number and activity of the rat's phagocytes taken in by the flea. After the infection has reached its height in the rat, the phagocytes are more numerous in its blood stream and fleas fed during this period do

not harbor the infection so long as those fed during the early stages of the disease. In fact, fleas fed on immune rats are said to harbor the infection for a short period only, which has suggested to some investigators that recovered rats may play a part in terminating an epizootic.

The distribution of pneumonic plague depends upon crowding. It occurs in winter and in colder countries.

The distribution of bubonic plague is determined by the species of rat, by the factors that determine the rat population—such as breeding places, natural enemies, and food supply, and by climatic factors that affect the breeding of fleas.

As previously mentioned, *Pasteurella pestis* is present in rodents other than the rat. In some regions in the western states, the ground squirrel is heavily infected. Plague infection has also been reported in the following animals or their ecto-parasites: jack rabbits, marmots, chipmunks, mice, wood rats, pack rats, cottontail rabbits, bush rabbits, badgers, prairie dogs, and gophers. Plague in rodents is called *sylvatic plague* and has been extensively studied by K. F. Meyer who has focused attention on its dangers to the country. Although the number of human cases contracted from ground squirrels is not great, the danger is a real one. Any organism pathogenic for man and present in wild animals is difficult to control. A small number of human cases may be expected every year in hunters, particularly in boys, since they find it easy to capture the diseased animals. One fatal human case was reported from California: that of a twelve year old boy. Infected wood rats were found in the vicinity and he presumably acquired the infection from them.

The possibility that rats may be infected by wild rodents and may start an outbreak is not to be overlooked. Sylvatic plague is working eastward and has been reported as far east as North Dakota and Kansas.

Scavenger birds have also been studied in relation to the epidemiology of sylvatic plague. Although they do not become infected, they may spread the infection. They may serve as accidental hosts to infected fleas and transport them over wide areas. If they have fed on infected rodents, they may disseminate the plague bacilli in their regurgitated casts. These consist of such indigestible material as bones and have been shown experimentally to be highly infectious.

Susceptibility and Immunity: All persons are naturally susceptible to infection by *Pasteurella pestis* and one attack confers a lasting immunity in a high proportion of cases. An active immunity which lasts for six to twelve months may be induced by the use of a vaccine made from the organism. Antisera does not appear to be of much value.

Therapy: Sulfadiazine and streptomycin are effective according to the present scanty reports.

Control

Control of plague consists in getting rid of the rats and this is best done by eliminating their breeding places, keeping food supplies out of their reach, careful poisoning and trapping, and rat-proofing buildings. If plague is endemic in a community, it may be necessary to do some wholesale razing of rat-infested buildings in poor districts. In a recent outbreak in Peru it was found that DDT effectively controlled plague in man as well as in rats by destroying the rat flea which transmits the infectious agent.

Ships coming from plague ports are held in quarantine and fumigated routinely. Metal shields are used to try to keep rats from leaving by way of the hawsers.

There should also be periodic examinations of rats and other rodents for signs of infection by *Pasteurella pestis.*

TULAREMIA

Tularemia is a disease primarily of wild life and secondarily of man. In 1910, McCoy of the United States Public Health Service investigated a plague-like disease of rodents in California and two years later succeeded in cultivating the causative agent. In 1920, Francis, to whom we owe much of our information on tularemia, found, in the course of some investigations on a disease called Deer Fly Fever, that it was due to the same organism. He named it *Bacterium tularense* after the county in California where McCoy had first described the rodent disease.

The disease is not a new one and it is evident from records that cases of tularemia had previously occurred following the skinning of infected rabbits. It was known as "market" or "rabbit" fever.

Etiology

The specific cause of tularemia is *Bacterium* or *Pasteurella tularensis.* The placing of this organism in the genus *Pasteurella,* as the Americans do, has some basis for justification, although the British prefer to place it in the genus *Brucella.* The organism shows some characteristics of both genera, some of neither.

Morphologically, *Pasteurella tularensis* is a very small nonmotile, nonsporulating rod measuring about 0.2 micron in width and 0.3 to 0.6 micron in length, and frequently rather coccoid in appearance. It is gram-negative and while it stains poorly with methylene blue it stains readily with gentian violet. In animals, the organism appears to have a capsule. Bipolar staining is not so common as in the other pasteurellas. Antigenically it is related to and cross-agglutinates with the brucellas, a fact of considerable practical importance when a diagnosis of either of these diseases is to be made.

Pasteurella tularensis is naturally a parasite. Some strains can be grown on ordinary laboratory media but one containing blood serum, egg, or the amino acid cystine is usually used. *Pasteurella tularensis* is aerobic. Its physiological requirements appear to be readily met in animals since it has an extremely wide host range.

Pasteurella tularensis, like many pathogens, is not particularly sensitive nor particularly resistant to drying, heat, or chemicals. Exposure to moist heat at 55° to 60° C. for ten minutes destroys it. Cooking renders contaminated meat safe for consumption although the organism may live for four or five months or more in refrigerated rabbits.

Recognition of Tularemia

The symptoms of tularemia in man are characteristic of the way in which the infection is contracted. There are four clinical types:

Ulceroglandular Type: This type often follows the bite of deer flies or ticks, or contact with infected rabbits and is common in hunters and butchers. The principal primary lesion appears on the skin in the form of a raised area or papule which later develops into an ulcer. This ulcer shows a stubborn resistance to healing. Later the regional lymph glands become enlarged and ulcerate.

Oculoglandular Type: This infection results from rubbing the eyes after the hands have become contaminated by crushing ticks or other insects or, more commonly, from skinning and dressing infected rabbits. The principal primary symptoms are a conjunctivitis followed by a swelling of the regional lymph glands.

Glandular Type: Most cases of this type have followed skinning and dressing wild rabbits. Although there is no apparent primary lesion, there is an enlargement of the glands of the axilla.

Typhoidal Type: The typhoid type has been recorded as following tick bite or the eating of improperly cooked rabbit and is the type most common in laboratory workers. Tularemia is a hazardous disease to experiment with and most investigators who have handled *Pasteurella tularensis* extensively have become infected. There is no external sign such as a primary lesion nor any enlargement of the glands.

In all types, the onset of the disease is sudden with a headache, general pains, chills, vomiting, and a fever which may reach 105° F. but usually lasts only two or three days. When pneumonia develops, the case fatality is high, otherwise it is from four to five per cent. Convalescence is slow and drawn out. Weakness may persist for months.

The incubation period is from twenty-four hours to ten days, with an average of about three days.

Infection in Rabbits: The infection is generalized and the organism

grows in all parts of the body. The spleen, liver, bone marrow, and lungs show innumerable small lesions plainly visible to the naked eye.

Diagnosis: Laboratory diagnosis depends upon finding tularemia bacilli in discharges from ulcerating lesions, or obtaining specific agglutination of a tularense antigen with the patient's serum. Cross-agglutination also occurs with the Brucellas so adsorption tests should be run.

Epidemiology

Source of Infection: The source of infection is usually diseased wild rabbits, although woodchucks, skunks, muskrats, tree squirrels, cats, deer, fox, sage hens, geese, and quail have been shown to be infected. This list does not purport to be complete but is given to indicate the wide variety of wild animals known to be infective. Experimentally nearly all animals are susceptible to a greater or lesser degree.

Domestic animals appear to be more resistant, although tularemia has been reported in sheep and dogs. The chicken, pigeon, turkey, hog, cow, and horse appear to be resistant.

Mode of Transmission: Pasteurella tularensis is a very highly invasive organism and may infect through any one of the common portals or routes of entry to the body. It may be contracted by the bites of infected ticks or flies, by the handling of infected rabbits or other animals, by eating insufficiently cooked infected rabbit meat, or, rarely, by the bite of infected animals or by the drinking of water contaminated by the urine of infected water rats, muskrats, and beaver.

Insect Vectors

Ticks: The wood tick, *Dermacentor andersoni*, and the dog tick, *Dermacentor variabilis*, become infected from feeding on infected animals, rodents usually, and transmit the disease to other animals and man. *Pasteurella tularensis* is transmitted from the adult tick to the young through the egg, a rare occurrence with a bacterial agent but common with the rickettsiae. The organism is present in the gut of the tick and is eliminated in the excreta. Man is infected through the tick puncture wound when it becomes contaminated with the infectious excreta, or through the intact skin when infected ticks are crushed on it.

The tick-borne infection of man shows a distribution corresponding to the seasonal and geographic distribution of the ticks, as would be expected. Wood tick-borne tularemia is common in the spring in the Middle and Northwest states, and dog tick-borne tularemia is more common a bit later in the Eastern and Southern states and is not restricted to a few months.

Flies: The deer fly, *Chrysops discalis*, is responsible for a number of cases

of tularemia every year. This fly is not particularly restricted in its feeding habits but can turn to a number of animals. It does not become infected itself but serves as a mechanical carrier. In feeding on infected rodents, its mouth parts become contaminated so that when it bites man, infection follows. Dogs, coyotes, and other animals may likewise transmit the infection by biting a human after having been in contact with infected rabbits. Instances of this are rare but have been reported. Since the fly bites the exposed parts of the body, such as the face, neck, and hands, whereas the wood tick prefers the parts covered by hair or clothing, the distribution of the primary lesions and enlarged lymph glands usually differs.

In general, spring infections are wood tick-borne; summer infections are dog tick- and fly-borne; and autumn and winter infections, the result of contact with infected rabbits or the ingestion of infected rabbit meat. The incidence of tularemia is directly correlated with the opening of the rabbit hunting season.

When considering the possibility of contracting tularemia, it must be remembered that infection can take place through intact skin.

Susceptibility and Immunity: All individuals appear to be susceptible. One attack leads to a permanent immunity which, although not absolute, confers a high degree of resistance.

Distribution and Prevalence: It is very likely that tularemia is present in every state of the United States. It is present in Canada, Mexico, Russia, Norway, Sweden, Japan, and probably other countries. The incidence and case fatality rate are low. About 1000 cases and 125 deaths are reported every year. However, since the case fatality rate is less than 5, obviously many milder cases are not reported. Tularemia appears to be increasing in the United States. In the past few years, Illinois, for example, has shown a great increase in cases due primarily to infection following the killing of diseased rabbits. The incidence of the disease in rabbits appears to be increasing, so it seems likely that there will be more cases in humans in the next few years. The Northeastern states seem to have a very low incidence of tularemia in rabbits and few cases in humans.

Control

Control of tularemia depends upon a number of factors. Prompt and careful removal of wood ticks and avoidance of tick-infested areas during the tick season will reduce the hazard from this source.

Careful handling of wild rabbits and wearing of rubber gloves during their skinning and preparation will help reduce infection from this source. The blood is infectious and must be avoided. Thorough washing of hands and the use of disinfectants should be practiced on general principles. It

may be effective in some instances although it cannot be depended upon. The thorough cooking of rabbits destroys the organism and renders the meat safe.

Most cases of tularemia occur in December and about ninety per cent are contracted from rabbits, most of these from the cottontail. The observations that cottontails died within seven days after infection and that the insect vectors were immobilized by frost suggested that the number of cases of tularemia could be reduced by moving the hunting season back several weeks until after cold weather set in. The number of infected rabbits killed by hunters is then greatly reduced and also, consequently, the number of human cases.

It is difficult to control the disease in the wild rabbit for it is transmitted by the wood and dog tick, the rabbit louse, *Haemaphysalis ventricosus*, and the rabbit tick, *Haemaphysalis leporis-palustris*, and, since there areso many efficient vectors, can spread rapidly.

Tame rabbits appear to be free from the disease, not because they are not susceptible but because they do not harbor the insect vectors. Control of the wild rabbit population seems to be indicated but involves a number of factors since, in many states, rabbits are protected by law for the benefit of sportsmen.

Streptomycin is used in treatment and with outstanding success.

Chapter XXV

BRUCELLOSIS (UNDULANT FEVER, BANG'S DISEASE)

History

The genus *Brucella* is responsible for the disease of man known variously as Malta Fever, Mediterranean Fever, or undulant fever; the disease of cattle known as Bang's disease or contagious abortion, and localized and generalized infections in goats, swine, horses, and other animals. Inasmuch as the same organism produces contagious abortion in cattle and undulant fever in man, the term *Brucellosis*, which refers to diseases due to the genus *Brucella*, is to be preferred.

While diseases caused by this group of organisms have their origin in antiquity, it was not until the middle of the last century that attention was drawn to obscure fevers in the British troops on the Isle of Malta during the Crimean War. David Bruce, in 1887, performed a series of experiments on material taken from patients and discovered an organism which was later proven to be the cause of the disease. This organism he described as a coccus and named *Micrococcus melitensis*. A commission headed by Bruce made a thorough study of Malta Fever and found that the disease was contracted by drinking raw goat's milk and that it was not transmitted from person to person.

In 1897 a Danish bacteriologist, Bang, discovered and isolated the specific cause of contagious abortion in cattle. He described it as a small rod and named it *Bacillus abortus*.

While undulant fever in man and contagious abortion of cattle both received considerable attention for the next twenty years, no one appears to have suspected any relationship between them until Alice Evans published the results of her studies in 1918 and drew attention to their close relationship antigenically, morphologically, and culturally.

Classification of Brucella: There are a number of closely related organisms belonging to the genus *Brucella*. Whether they should be considered separate species or varieties of the same species need not concern us here, but it does explain the names different workers have used to designate them.

Brucella melitensis, also written *B. melitensis var. melitensis*, refers to the caprine or goat strain.

Brucella abortus, also written *B. melitensis var. abortus*, refers to the bovine strain. It is also known as Bang's bacillus.

Brucella suis, also written *B. melitensis var. suis*, refers to the porcine or hog strain.

Characteristics of Brucella

The three species can be separated on the basis of pathogenicity, agglutination, sensitivity to dyes, and biochemical reactions. Table 11 shows the essential differences.

Morphology and Staining: The brucellas are small nonsporulating gram-negative rods, ranging in size from 0.4 to 0.8 micron in width, and from 0.4 to 2.5 microns in length. *Brucella suis* is slightly larger than *melitensis* or *abortus*. Coccoid forms are very numerous in smears made from cul-

TABLE 13

SPECIES	BRUCELLA MELITENSIS	BRUCELLA ABORTUS	BRUCELLA SUIS
Infectivity for guinea pigs	++	+	++
H_2S formation	−	++	++++
Tolerance 10% CO_2	+	++	+
Nitrate reduction	+	−	+++
Growth on media containing:			
Thionin 1:50,000	+++	−	+++
Basic fuchsin 1:25,000	+++	+++	−
Pyronin 1:100,000	+	+++	−

tures of some strains of *melitensis*, less numerous in smears of *Brucella abortus*.

Physiology: Brucellas are aerobes and grow best on media containing liver infusions. *Brucella abortus* is peculiar in that it requires a concentration of about ten per cent carbon dioxide for its initial isolation. After a series of transfers on laboratory media, it becomes adapted to and grows readily under ordinary atmospheric conditions.

Some strains produce a brown pigment on aging.

Resistance: Heating at 142° to 145° F. for twenty minutes is sufficient to destroy the brucellas. Some workers report that these temperatures will destroy them in less than five minutes.

The longevity of the brucellas in dairy products such as ice cream and cheese, and in soil and stable dust, water, and discharges from the uterus of infected cows is obviously of considerable interest from the standpoint of the control of the disease in animals and man. Huddleson cites reports from several workers to the effect that *Brucella melitensis* will live for twenty to sixty days in soil, and forty-two days in sterile tap water; that *Brucella abortus* will live in the infected bovine uterus, stored in an ice chest, for at least seven months, that it can be recovered after thirty days from ice cream made from naturally infected milk stored at 32° F., and that it remains alive in butter stored at 46.4° F. for at least 142 days. In bovine

tory infections and epidemiological evidence, *melitensis* is the most invasive strain, *abortus* the least.

The fact that brucellosis is common in veterinarians, butchers, and handlers of meats, suggests that milk is only one source of infection. The organism may be carried to the mouth on the hands or infection may take place through the skin. In cases of brucellosis of man, the organisms may appear in the urine, though infection from this source seems a remote possibility.

Susceptibility: Inasmuch as the infection is contracted from drinking milk, more cases than actually appear might be expected among children and women if they were highly susceptible to brucellosis. Immunity following attack is uncertain.

Incubation Period: The incubation period is always difficult to determine in a disease which has such a gradual onset. It probably varies from about six days to eleven or more weeks.

Prevalence and Distribution: Brucellosis is present all over the world. It occurs in every part of the United States, but because of the uncertainty of diagnosis and the number of mild cases there are no satisfactory figures upon which to base an estimate of its incidence. It is more common in rural than in urban populations because the former drink more raw milk and handle live stock. Many of the urban group who contract undulant fever do so during vacation when they drink raw milk. The disease shows an occupational distribution, being particularly high in veterinarians and in packing house employees who handle the carcasses of hogs.

In 1946, sixty-nine deaths were reported. There has been a progressive increase in reported cases every year, indicating either that the disease is spreading or that the medical profession is becoming more aware of its existence. The latter is certainly true, and the former may be.

The case fatality rate is said to be two or three per cent. Better diagnosis would undoubtedly lower this estimate, since it is doubtful whether even ten per cent of the cases are ever recognized. Accuracy of reporting in the different states is variable. Although the case fatality rate is low, the disease is important because of the long period of intermittent illness.

Diagnosis: Laboratory diagnosis is the only satisfactory method. There are a number of tests which have been suggested and, when several are used, diagnosis will be accurate.

Agglutination Test: Agglutinins appear in the blood of infected persons. These may be identified by using a suspension of *Brucella* antigen. There is cross-agglutination with *Pasteurella tularense*, the causative agent of tularemia, and the serum should be adsorbed with this antigen before adding the *Brucella* antigen.

Skin Tests: Huddleson has developed a skin test made by injecting one

tenth cubic centimeter of the proper dilution of *Brucellergen*, the nucleoprotein of *Brucella*, intradermally into the forearm. Individuals who have been infected show a local reaction at the site of injection and many also show rather severe systemic reactions resembling brucellosis.

Opsono-Cytophagic Test: The opsono-cytophagic test is a modification of the Wright opsonic index test. It is based on the fact that the blood from persons who have had contact with *Brucella* shows an enhanced phagocytic ability. In brief, the test is carried out by adding one tenth of a cubic centimeter of citrated blood to an equal amount of *Brucella* suspension, mixing thoroughly, and incubating in a water bath at 37° C. for thirty minutes. After incubation, a large drop of the sedimented cells is removed, spread evenly on a clean glass slide, dried, treated with acetic acid and formalin to dissolve the red blood cells, rinsed, and stained with Bordet-Gengou stain, carboltoluidin blue, or Wright's stain. The slides are then examined microscopically for the presence of phagocytic activity by the polymorphonuclear phagocytes. The first twenty-five to fifty phagocytes are examined for engulfed bacterial cells. The reaction is relative, and its significance lies in the fact that in persons immune to *Brucella* as a result of contact, there is a higher percentage of cells engulfing bacteria and a greater number of bacteria within the cells. The interpretation of this test is open to some question, but it yields significant information on the immunity of the individual. By use of the three tests, agglutination, skin, and opsono-cytophagic, in addition to blood cultures, diagnosis of brucellosis may be made certain.

Control of Brucellosis of Man

Control of human brucellosis involves the control of the disease in animals, particularly in cattle, but also in swine, and, in small areas, in goats, and probably in sheep. Eradication of the disease from dairy cattle is difficult but is being accomplished.

Control of occupational brucellosis in veterinarians and others coming in contact with infected animals or animal tissue is a problem.

Most cases, however, are contracted from drinking raw milk. Even certified milk is not safe. Pasteurization is the only sure guarantee of safety. It kills the organisms and there are no records of brucellosis ever having been contracted from drinking properly pasteurized milk.

Vaccination has not been extensively tried although some favorable results have been reported.

There are no specific curative agents. Serum, sulfonamides, penicillin, and streptomycin have been tried but the results have not been encouraging.

Patients should be instructed to wash their hands thoroughly. Some states prohibit infected persons from handling milk or milk products, utensils, or foods.

Cooking destroys the organism.

BRUCELLOSIS IN CATTLE

Brucellosis, contagious abortion, or Bang's disease in cattle used to rank second only to bovine tuberculosis in economic importance. Inasmuch as bovine tuberculosis has been practically eradicated, brucellosis now ranks first. About ten per cent of the cattle are infected. The incidence is highest in dairy cattle, many herds showing from seventy to eighty per cent infection, but the disease is also found in range cattle and in bison and deer.

Some of the economic loss is due to a decrease in the milk yield which may be reduced as much as twenty per cent, even in the absence of apparent infection. The greater part of the loss is due to the loss of calves through abortion. It has been estimated that five to ten per cent of dairy cows abort annually.

The *Brucella* show a tendency to localize in the mucous membranes of the uterus of the cow, setting up an inflammation and necrosis which interferes with the nutrition of the fetus and leads to its death and expulsion. Infected cows nearly always abort once, sometimes twice, but seldom more, because infection leads to sterility. If an infected cow delivers a living calf, it may or may not be infected. Uninfected calves may be raised free from infection if kept properly isolated. Since the organisms are present in the milk it is essential that healthy calves do not nurse.

There is a tendency for infected cows to retain the placenta, and farmers and veterinarians very often become infected in assisting in its removal.

The *Brucella* are present in the vaginal discharges, the urine, and the milk. Cattle contract the infection from eating food or drinking water soiled by contaminated discharges.

Infected bulls also transmit the disease to uninfected cows.

BRUCELLOSIS OF GOATS

The problem of brucellosis in goats is important in the United States chiefly in the region adjoining Mexico. Here the goat industry is of relatively greater importance and infected goats have been present for a long time. The disease may cause abortion in goats but is usually chronic. The goat milk may contain tremendous numbers of organisms. Control depends upon elimination of infected herds and pasteurization of milk.

Since *Br. melitensis* or the goat strain also infects cattle, goats alone are not the only source of danger. Infection of man with the goat or *melitensis* strain has been reported from many states where the possibility of contact with goat milk can be ruled out.

BRUCELLOSIS IN SWINE

Brucellosis in swine is more or less limited to the "hog belt," that is, to the central middle western states. In hogs, the disease is usually self-limited. It may cause abortion and undoubtedly reduces the vigor of the hog. The *suis* organism is pathogenic for cattle and more virulent for man than *abortus* but less virulent than *melitensis*. Its high invasiveness for man accounts for the prevalence of undulant fever in hog handlers and packing plant workers.

BRUCELLOSIS OF OTHER ANIMALS

Brucellosis of sheep has been reported but is not common. The horse is susceptible to infection. When the organism localizes it produces fistulous 'withers' and 'poll evil.' Abortion of mares, however, is due to an entirely different organism.

Small animals are varyingly susceptible experimentally.

Control of Brucellosis in Cattle

The control of brucellosis or contagious abortion or Bang's disease in cattle is dependent upon accurate diagnosis. Agglutinins which appear in the blood of infected animals may be detected by the use of *Brucella* antigen. Huddleson, to whom we owe much of our information about this disease, has developed a rapid agglutination test which is accurate, easy to perform, and easy to interpret.

Three methods are used in the control of Bang's disease: Testing and segregation of reactors, Testing and slaughtering of reactors, and Calfhood vaccination.

The first method involves the maintenance of two herds and may be inconvenient and expensive. Its success depends upon individual circumstances.

The test-and-slaughter method did not become widespread until 1934 when it was adopted in conjunction with the program for the reduction of surplus cattle. The testing was carried out under the direction of the Bureau of Animal Industry and farmers were indemnified for the animals condemned and slaughtered. This program has resulted in a great reduction of the disease in many areas.

The third method, called calfhood vaccination, gives promise of being effective. As early as 1910, attempts were made to produce an active

immunity to Bang's disease by injecting a killed organism as a curative measure. Later it was tried as a preventive measure; but, at that time, was not successful. Vaccines made of living brucella cultures were also tried and were shown to increase resistance to subsequent infection but neither did this method prove successful in practice. One reason was that only virulent strains were effective and that these had a tendency to localize in the udder so that vaccinated animals became chronic carriers.

In 1925, Buck tested the virulence of a large number of *Brucella abortus* strains and found that one, known as *B 19*, did not localize in the udder but did produce an immunity, as evidenced by the fact that vaccinated animals did not abort. He then vaccinated calves five to six months of age and exposed them to infection during their first pregnancy. They did not abort but similarly exposed controls did. Later work has substantiated his findings and the present indications are that calfhood vaccination with *Brucella abortus*, strain 19, will prove an effective preventive measure.

It should be noted that vaccinated calves develop agglutinins for *Brucella abortus* and consequently show a positive Bang test. Many cities require Bang testing of cows and will not allow milk from positive reactors to be sold. Many states will not allow their entrance. It seems that these regulations may have to be modified so as to take into account the fact that a positive Bang test in vaccinated cattle does not indicate infection.

The expenditure of money and effort in the eradication of Bang's disease is justified for reasons of herd and human health. The loss of milk yield and the loss of the calf crop is so great that every effort should be made to prevent infection in the herd; and the fact that man contracts the disease from infected animals and that it is chronic and incapacitating makes it an important public health problem.

An interesting fact and one that may lead to misunderstanding of the factors involved is the flaring up of contagious abortion in a herd when known healthy animals are introduced. Owners of herds which have been free of abortion for some time and hence have been judged to be no longer infected, may find that, when healthy animals are introduced, abortion flares up. It may occur first in the new animals or it may occur first in the old. The explanation offered is that the herd has gained an infection immunity but that it still harbors the organism. Healthy animals, being more susceptible, become infected and disseminate a large enough dosage of an organism of enhanced virulence to overcome the resistance of the older residents of the herd and thus these may develop abortion.

CHAPTER XXVI

RICKETTSIAL DISEASES: TYPHUS AND ROCKY MOUNTAIN SPOTTED FEVER

RICKETTSIAE[1]

The *Rickettsiae* are very small pleomorphic rods, variable in size but averaging about 0.3 to 0.5 micron in length and 0.5 micron in width. The shorter ones are almost coccoid in appearance, the longer ones bacillary. They are gram-negative and stain poorly with ordinary dyes but special stains such as Giemsa, Castaneda's, or Machiavello's are very satisfactory.

The *Rickettsiae* are obligate parasites whose normal habitat appears to be insects, where they accumulate, usually, inside of the cells lining the intestinal tract. They show a degree of specificity for insects, but some species are able to parasitize a wide variety of mammals. A few species are pathogenic for man and animals and it is in these that we are interested here. All rickettsial diseases of man with the exception of Q fever are insect transmitted.

The *Rickettsiae* differ from ordinary bacteria in that the pathogenic forms cannot be grown apart from living cells. However, they grow readily in tissue culture and in the yolk sac of the developing chick embryo. This last method was developed by Cox for the production of vaccine against Rocky Mountain spotted fever and typhus.

The pathogenic rickettsiae may be divided into a number of species and strains depending upon the type of disease produced in man and animals, the insect host, and serological and cross-immunity tests.

Weil-Felix Reaction: Weil and Felix found that certain strains of *Proteus* were agglutinated by the sera from typhus fever patients. The strain called *Proteus X 19* gave the highest titre and the ability of sera from typhus patients to agglutinate the strain was a fairly constant feature. Since sera from such patients do not agglutinate the typhoid bacilli or organisms producing other diseases which might be confused with typhus, the Weil-Felix reaction has considerable diagnostic value.

We are here confronted with a peculiar phenomenon. There is no phylogenetic relationship between *Proteus* and *Rickettsia.* Although it was suggested at one time that *Proteus* might play a part in the production of the disease either as a primary cause or as a secondary invader, it has been shown that *Proteus X 19* does not produce typhus and is not present in all of the cases. Immunizing animals against *Proteus X 19* does not protect

[1] For an authoratative discussion of the biology of the rickettsiae, see *Insect Microbiology* by Edward A. Steinhaus—Comstock Pub. Co.

them against subsequent inoculation with the rickettsiae, nor does recovery from the rickettsia of typhus protect guinea pigs against virulent strains of *Proteus*.

Some workers have postulated a very close relationship between *Proteus* and *Rickettsia* and suggested that they are different phases of the same organism. Perhaps the most logical explanation lies in the fact that *Proteus X 19* and *Rickettsiae* have a common antigenic factor in no way related to the immunity produced by either of the organisms and not necessarily indicating a phylogenetic relationship.

With the development of the chick embryo technique for culturing rickettsia, it has become possible to use the rickettsiae themselves in complement fixation tests. These are highly specific and have now largely supplanted the Weil-Felix Reaction in research on and diagnosis of rickettsial infections.

TYPHUS

History

The disease now known as typhus fever appears in earlier literature under such names as brig fever, ship fever, gaol fever, crowd fever, and spotted fever—this last name being descriptive of the accompanying rash, a symptom also present in spinal meningitis and typhoid fever and one of the reasons the three have been confused. In Spain and Mexico the disease is called *tabardillo*, a name derived from the Spanish word *tabardo* signifying a spotted or vividly colored cloak. The term typhus (from the Greek word *typhos* meaning smoke) refers to the coma or stupor resulting from the fever and was not used until the middle of the eighteenth century.

Typhus appears in two forms, epidemic and endemic. The epidemic form, sometimes called European or Old World typhus, raged from 1500 to about 1850 in Britain and Europe. It was often associated with the plague although it seems not to have been prevalent until about two hundred years later and to have remained for about two hundred years longer. The names: crowd, ship, brig, or gaol fever, and the fact that it follows famine, fire, flood, and wars suggest the presence of factor common to such conditions—that factor is the louse.

Epidemic typhus is a louse-borne disease and its appearance in epidemic form previous to and its disappearance with the advent of the Saturday night bath is thereby explained.

Endemic typhus is very different in its epidemiology, being transmitted from rat to rat and from rat to man by the rat flea. The rat serves as a reservoir of the infectious agent and if the population is lousy, the epidemic type follows the introduction of the endemic disease into the human population.

Epidemic Typhus:

That louse-borne typhus should have been so prevalent during the sixteenth, seventeenth, and eighteenth centuries reveals a good deal about the ecto-parasitic condition of our forebears. They were lousy, a condition they regarded as undesirable but also as practically unavoidable. The word, louse, was then, as it is today, a term of reproach and the insect was the subject of many a ribald jest. Thus, when Theodore Hook was turned out of her house by the Third Countess of Holland, he wrote,

> "My Ladyship said when I called at her house,
> She didn't esteem me three skips of a louse;
> But I freely forgave what the dear creature said,
> For women will talk of what runs in their head."

Among the ladies of the court, it was the fashion to carry long curry combs for scratching their backs. The gentlemen shaved their heads and wore wigs because of the difficulty they had in keeping their own hair free from lice. Samuel Pepys records his indignation at finding his new periwig already infected with nits.

McArthur has given us the following vivid description of the migration of lice from the body of the murdered Thomas à Beckett.

"... and next day, after some debate, it was decided to remove the clothing in preparation for burial. The dead Archbishop was clad in an extraordinary accumulation of garments. Outermost there was a large brown mantle; next, a white surplice; underneath this, a fur coat of lamb's wool; then, a woollen pelisse; then, another woollen pelisse; below this, the black cowled robe of the Benedictine order; then, a shirt; and finally, next to the body, a tightly-fitting suit of coarse haircloth covered on the outside with linen, the first of its kind seen in England. The innumerable vermin which had infested the dead prelate were stimulated to such activity by the cold, that his hair-cloth garment, in the words of a chronicler, "boiled over with them like water in a simmering cauldron," and the onlookers 'burst into alternate fits of weeping and laughter, between the sorrow of having lost such a head, and the joy of having found such a saint.' "

Typhus fever has been one of the great pestilences of man, ranking with smallpox and bubonic plague. It has always been associated with wars and military campaigns and has exercised no little influence on their outcomes. It appeared in Maximillian's army in 1552 and his soldiers spread it all over Europe. It was rampant all through the Thirty Years War and it played havoc with Napoleon's army on its disastrous retreat from Moscow. In fact, it had a significant part in every major war in Europe until 1914. Even then it appeared on the Eastern Front and only the

rigorous delousing measures taken by both German and Allied forces kept it from spreading.

Although typhus was probably prevalent earlier, the first clearly described outbreaks in Britain were in 1522, 1577, and 1586 and were associated with the Black Assizes, so-called because it was during the trials of the lice-infested prisoners that the gaol fever, or typhus, spread to the others and the epidemics began. In the next two hundred years many outbreaks occurred in army hospitals, universities, and in prisons. These last are of particular interest.

At that time persons were thrown into prison for debt, for failing to subscribe to current religious practices, or for incurring the wrath of officers of the King, as well as for more serious offenses. Many of the prisons were privately or semiprivately owned and operated for profit. Food was inadequate and sanitary conditions appalling. The floors were of mud covered with accumulated filth and topped with straw. There were often no toilets, no water for bathing, and crowding was beyond belief. In 1696 when a tax was placed on windows, the jailors promptly bricked them up to avoid paying and conditions were still worse. When a windmill ventilator was installed on Old Bailey, it is recorded that two of the workers died from the stench issuing from the flues. As a matter of fact they undoubtedly contracted typhus during the process of installation.

Ireland was long an endemic focus for typhus and the Irish immigrants brought it into England on numerous occasions. Perhaps the greatest epidemic in Ireland occurred in 1847 during the potato famine when it is said that over 300,000 people died; about 20,000 of starvation and the rest from dysentery, typhus, and typhoid.

An extensive outbreak in Europe occurred in Poland, Russia, Serbia, and Bulgaria during and after the first World War.

As World War II progressed, conditions, particularly in Naples, were ideal for a rapid spread of typhus and in the winter of 1943–44 a severe outbreak threatened. Naples had a population of about one million. The city had been bombed. Food, fuel, and soap were scarce and undernourishment, privation, and exposure were the lot of the inhabitants. Many of them were living in the crowded caves used for air raid shelters. Under such conditions filth and lice were inevitable and when typhus was introduced, probably by Italian troops returning from Russia or North Africa, it kindled rapidly. But for the prompt action of the United States of America Typhus Commission the outbreak would, in all probability, have reached gigantic proportions. The Commission set up a program of case finding, contact delousing, mass delousing, and immunization. Their success is shown by the trend in the incidence. The first known cases occurred in February, 1943, and during the summer of 1943 typhus was

smoldering. As colder weather set in and crowding in the shelters increased, the number of new cases increased. In October there were 29 cases, in November there were 61, in mid-December, 83, and by the end of the month, 288.

Dusting with DDT was begun on December 15th, 1943. In less than a month a sharp decrease in cases was noted and by the end of February the outbreak was under control. Since the incubation period of typhus is from five to twenty days, a lag of several weeks is to be expected before the decline since cases occuring during this period were infected before control of lice was begun.

The Naples outbreak illustrates two points nicely. First, where conditions are such that filth, poverty, and crowding prevail, populations become heavily louse-infected and provide perfect conditions for an outbreak of typhus. Second, properly organized control measures and the use of DDT can eliminate the infection from the population.

Typhus was introduced into the New World by the immigrants, and epidemics have occurred in nearly all ports, the latest being in Philadelphia in 1880. In the United States the disease has never spread over wide areas. A small outbreak occurred in the Navajo Indians in New Mexico in 1920.

Recognition of the Disease

The symptoms of classical epidemic typhus are variable. The onset may be sudden, resembling influenza, and marked by headache, general pain with weakness of the limbs, chills, and a fever that usually lasts about fourteen days and may reach 104° F. Four to six days after the onset an eruption appears, beginning usually on the lower chest and upper abdomen and rarely occurring on the soles, palms, or face. This rash is quite different in appearance and distribution from that of spotted fever. It persists from two to nine days and then disappears rapidly. Bronchitis and sore throat often occur in the later stages, an unproductive cough is usually present, and the patient may be sensitive to light. Delirium is common. The Weil-Felix reaction is positive after the first week. The disease may be confused with a number of other infections although its epidemiology will help to differentiate it.

Etiology

The specific cause of typhus fever is *Rickettsia prowazeki*. It has never been cultivated apart from living cells and little is known about its physiology.

Epidemiology

Source of Infection: The source of the infection is the blood of infected man and rats.

Mode of Transmission: The etiological agent is transmitted from man to man by the body louse, *Pediculus vestimenti* or *corporis*. The infection is not contracted except through the intervention of the vector and hence is not contagious in the ordinary sense.

A word about the habits of the louse might not be out of order at this point. Three species of lice are parasitic on man: *Pediculus capitis*, the head louse, *Pediculus vestimenti*, the body louse, and *Pediculus pubis*, the crab louse. The last is not involved in the transmission of typhus.

These lice can feed only on human blood and remain on man or in his clothing during their whole life span. Lice prefer a temperature only slightly lower than that of the body and if the body temperature goes up, as in fever, or when the body cools, as in death, the lice migrate and seek another host. Hence the high infectivity of typhus.

The female louse lays five or more eggs a day. These are attached to the hair or clothing by an insoluble cement. They hatch in about eight days and the lice are full-grown in about a month. The young louse literally blows itself out of the egg case by swallowing air from the air hole in one end of the egg cap, expelling it from its intestine, and thus developing pressure sufficient to propel itself forward and break out. When oily preparations such as kerosene are applied, the oil covers the air hole; the young louse cannot get enough air to force its way out and dies inside.

The body louse thrives best when it inhabits persons who wear heavy or many clothes and consequently is found among groups who depend upon much clothing rather than artificial heat for warmth.

Period of Communicability: The communicability of epidemic typhus depends upon the presence and number of lice. The patient is capable of infecting lice and, hence, of serving as a source of infection for about thirty-six hours after his temperature has reached normal.

Lice feed on typhus fever patients and develop the infection. The rickettsiae increase to tremendous numbers within the insects and appear in their excreta which is highly infectious. The infected lice do not introduce the organisms during the feeding process but deposit their excreta, in which the rickettsiae may survive for long periods, near the puncture wound. This becomes thoroughly inoculated when the bite is scratched. The infected lice die of typhus in about twelve days.

Susceptibility: All persons are susceptible and the sex, race, or age distribution is determined by chances of exposure to infected rats or lice.

Prevalence: For centuries typhus fever was one of the most dreaded of

all diseases in Europe. It was constantly present and took its greatest toll during times of distress. It was associated with crowding and filth. Epidemic typhus is now absent from countries with sanitary living conditions and high standards of personal cleanliness although it still exists in parts of eastern Europe, Africa, South America, and the Orient. In 1941 epidemics occurred in the Mediterranean region, particularly in Spain.

Its communicability was so high and the mortality so great that it often was a determining factor in the destiny of nations. The reader is urged to read "Rats, Lice, and History" by Hans Zinsser for an instructive and entertaining account of typhus fever.

Louse-borne typhus has a mortality of twenty to forty per cent.

It occurs in the temperate and colder zones in winter and spring when crowding and, consequently, lousiness is greatest.

Control

The control of typhus is, as usual, determined by the way the infectious agent is transferred. The link that connects typhus with man is broken when people develop a desire for personal cleanliness and get rid of their lice. The control of rats is, of course, essential to an elimination of the disease.

There is no specific therapeutic of accepted value although para-aminobenzoic acid may be of some benefit and aureomycin looks promising. The fact that epidemic typhus is not present in the United States is due to standards of living high enough to demand housing free from rats and lice.

Methods of immunization, using cultures of rickettsiae as a vaccine, have been worked out but have not been sufficiently tested to say just how effective they may be; nor is the chance of exposure to typhus great enough to justify their wide use by the civilian population in the United States.

ENDEMIC TYPHUS

While the classical epidemic typhus is still present in parts of the world, it has lost its hold on western Europe and is not a problem in the United States. There are, however, several variant forms which are of importance. There is present in the United States a mild form of endemic typhus, also referred to as the *murine* type, since it is primarily a disease of rats. It is transferred from rat to man and from rat to rat by the rat flea, *Xenopsylla cheopis*.

Endemic typhus, in contrast to epidemic typhus, has a low case fatality rate, one or two per cent, as compared to twenty to forty. It occurs most frequently in the late summer or autumn. The highest attack rate is in adult males, particularly those engaged in handling food. Since endemic typhus is borne by the rat flea and since rats are found where there is food,

it might be expected that workers in the food industry would frequently be exposed to infection.

Endemic typhus occurs as sporadic cases and does not show a tendency to become epidemic. Where several cases occur in the same area, they usually originate from a common source.

What animals other than rats may serve as a source for human infection cannot be answered definitely. It has been shown that the rickettsia have, experimentally at least, a wide host range and that the woodchuck, the opossum, several species of mice, squirrels, rabbits, and the skunk are susceptible. The grey fox is not, apparently. Since endemic typhus is primarily an urban disease, however, even though these animals are susceptible, they are not important as sources of infection.

Endemic typhus is resident in several sections of the United States, particularly along the eastern and southeastern coasts where it has been introduced by immigrants from Europe. It has been gradually working northwest and in the past ten years 71 cases and 27 deaths have been reported as far north as Detroit. In 1945 there were over 5000 cases and 377 deaths reported from typhus in the United States.

The problem of control centers around the control of the rodents. Rat proofing of grain storage houses and the elimination of rat breeding places is indicated. An interesting approach to the control of endemic typhus that has proven very successful is to use DDT to control the rat fleas. Although not a substitute for rat eradication, it may be more immediately applicable in some circumstances. Vaccination has proven successful. The disease is often confused with typhoid, black measles, scarlet fever, meningitis, and other diseases; but can be distinguished from them by several means, the Weil-Felix reaction being positive in most cases of endemic typhus.

TABARDILLO

In Mexico there is a form of typhus called tabardillo. It was probably present before the white men came and is considered native to America. It is louse-borne and has been introduced into several parts of the United States on numerous occasions, and where conditions were favorable, there have been localized outbreaks.

RICKETTSIAL POX

In 1946 there occurred in New York City a hitherto undescribed disease at first thought to be an atypical form of chickenpox. It was limited to one housing development where a total of 124 cases were described in a population of 2000.

The initial lesion at what is probably the site of infection left a scar on

healing. Regional lymph glands became enlarged and about a week after the appearance of the initial lesion the acute stage, consisting of fever, sweats, chills, headache, and backache began. Several days later a rash appeared. The total duration of the disease was about eighteen days and there were no deaths.

Etiology: The cause of rickettsial pox is *Rickettsia akari.* In this outbreak the natural host appeared to be the mouse. Mice trapped in the vicinity were shown to have antibodies against the causative agent and rickettsia were isolated from one mouse. What other rodents may be infected in nature is not known.

Transmission: Present evidence suggests that the transmitting agent is a rodent mite, *Allodermomanyssus sanguineus.* The disease, like most other rickettsial diseases, is not transmitted by contact and control measures resolve into control of mice.

Q FEVER

This rickettsial disease was first described in 1937 in slaughterhouse workers in Australia and the name of Q fever was given to it because it occurred in Queensland. Since then it has been described on several occasions in the United States.

Unlike other known rickettsial diseases Q fever does not display a characteristic skin rash. The onset is acute with fever, chills, headache, and body aches and pains. Coughing and chest pains are common and the sputum may be blood tinged. Q fever is primarily a respiratory infection and is often diagnosed as atypical pneumonia. The illness lasts about one to three weeks with a long convalescence, especially in older persons. The fatality rate is very low, only one death having reported.

The cause of Q fever is *Rickettsia burneti.*

Epidemiology: Interest in Q fever in the United States was aroused by an outbreak in the National Institute of Health in Washington in 1940 with the appearance of fifteen cases with one death among employees. In 1947 another outbreak involving forty-seven cases occurred in the National Institute of Health at Bethesda, Maryland. It was due to a laboratory infection. Several outbreaks occurred in troops in the Mediterranean area and in Panama. In 1946 two outbreaks occurred, one in Texas and one in Chicago. Persons contracting the disease were engaged in handling cattle or were slaughterhouse workers handling cattle and sheep. Transmission was by way of contact with infected tissue or fluids.

In California in 1947 an outbreak occurred among persons who lived near or visited dairies. Studies showed that sixteen per cent of the cattle tested in the dairy herds in question had agglutination antibodies for *R. burneti.*

Many persons who did not give a history of Q fever also had antibodies and presumably had had the disease in a mild form.

Rickettsia were isolated from raw milk by guinea pig inoculation which suggests that milk may serve as a transmitting agent. Pasteurization renders the milk non-infective for guinea pigs.

In Australia the bandicoot is probably the most important natural reservoir of Q fever although rodents and marsupials are susceptible. The infection is mild. The rickettsia are transmitted by several species of ticks. The scrub tick *Ixodes holycyclus* transmits infection from the bandicoot to cattle, and the cattle tick, *Boophilus annulatus*.

Little is known about the primary host in the United States but calves are readily susceptible and the natural outbreaks in man have implicated cattle. Because of the mildness of the disease and the unlikelihood of its being properly diagnosed, it seems likely that many more human cases have occurred than have been reported.

TSUTSUGAMUSHI DISEASE

Tsutsugamushi, known also as scrub typhus, mite-borne typhus, Japanese river fever, flood fever, kedani, tropical typhus, and rural typhus occurs in many parts of the Orient, in the Malay States, and appears to be widely distributed in the islands of the southwest Pacific.

The etiological agent is *Rickettsia tsutsugamushi*.

Epidemiology: Tsutsugamushi disease in Japan is transmitted by the mite much like our chiggers or red bugs. The name of the vector is *Trombicula akamushi*. This mite in its larval stage is parasitic on the vole which has been found naturally infected with *R. tsutsugamushi*. The larval mite form lives in the surface soil and crawls about on the grass a few inches from the ground. When it finds a warm-blooded animal it attaches itself and feeds for two to five days after which it drops off and subsequently develops into a nymph and then an adult. Both nymph and adult are free-living vegetative feeders. Since only the larval stage feeds on animals, it follows that it must retain the rickettsiae acquired by feeding on the infected animal through the complete developmental cycle from larva to larva of the second generation.

In the Federated Malay States a wild rat appears to be the natural host and the vector is a mite belonging to the genus *Trombidium*.

Control: Vaccines offer promise and a recently developed antibiotic, aureomycin, is said to give encouraging results. Cutting the grass, the use of repellants, and such preventive measures directed against the mite are also of value.

ROCKY MOUNTAIN SPOTTED FEVER

Among the early settlers in the Bitter Root Valley of Montana there occurred, in the early spring, a disease characterized by chills, headache, pains, soreness of the muscles, and, sometimes, vomiting and nosebleed. Delirium was common, indicating that the brain and spinal cord had become involved. The outstanding feature, however, was the appearance, two to five days after the onset of the symptoms, of a rash, first on the

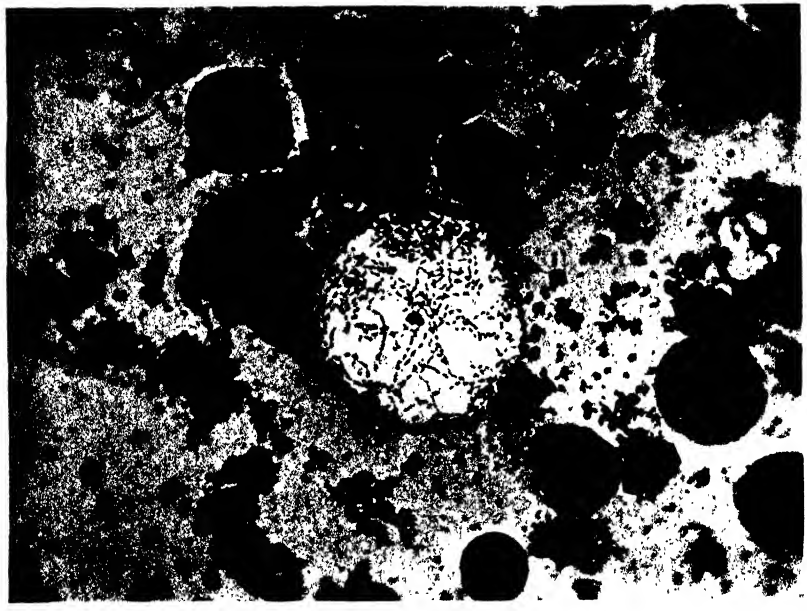


FIG. 46. Rickettsia in skin exudate of a guinea pig

extremities, the wrists and ankles, then on the back, and later all over the body. Because of the mottled or spotted appearance due to the rash, the disease was given the name *spotted* fever and because it was thought to occur only in a restricted area of the northwest, Rocky Mountain spotted fever. It has since been found that the disease occurs in most parts of the United States and that it is increasing in incidence.

Etiology

The specific cause of Rocky Mountain spotted fever is a rickettsia known by any one of three names, *Rickettsia rickettsi*, *Rickettsia dermacentroxenus*, or *Dermacentroxenus rickettsi*.

Epidemiology

Ticks are the primary source of infection. Although man becomes

infected and could serve as a source of the infectious agent, the manner of transmission is such that he does not.

Mode of Transmission: Rocky Mountain spotted fever is contracted primarily from the ticks. The Western type is transmitted by the wood tick, *Dermacentor andersoni*, and the Eastern type by the dog tick, *Dermacentor variabilis*. *Amblyomma americanum* is responsible for numerous cases in the southwestern states. Man contracts the infection as a result of being bitten or of crushing infected ticks while removing them from him-

FIG. 47. The female tick, *Dermacentor andersoni*, the vector of spotted fever, Q fever, tularemia, tick paralysis, and, probably, Colorado tick fever.

Courtesy of Dr. R. A. Cooley

self, dogs, or livestock. The transmission and distribution of Rocky Mountain spotted fever, Western and Eastern types, can best be interpreted in terms of the life cycles and feeding habits of the tick.

Life Cycle of Dermacentor: The normal adult female tick attaches itself to an animal and begins to engorge and, consequently, to increase greatly in size. After feeding for about a week, it drops off, lays between two to six thousand eggs, and dies.

The eggs hatch into six-legged larvae which feed on rodents until engorged and then drop to the ground where they may remain over the winter

if they do not chance to find a host. When the engorged larvae molt, they emerge as eight-legged nymphs which engorge again, molt, and are then adult ticks. The cycle requires two to three years.

The tick may become infected in any stage and, once infected, is capable of transferring the rickettsia to its offspring. Even the infected males produce infected offspring, for the rickettsia are present in the spermatozoa. This hereditary transfer complicates the matter of control. In any stage, the tick is also capable of infecting its animal host and the host may then serve to infect non-infected ticks.

There is a curious incubation period necessary before the adult tick carrying rickettsiae can infect. It seems that it has to digest a blood meal before it is capable of transmitting the infectious agent and, hence, prompt removal of a tick often will serve to prevent infection. Ticks that have had such a blood meal are infectious after a period of ten hours and capable of infecting a new host immediately. This required period of incubation after a blood meal may be why only a small percentage of tick bites in an infected area leads to infection.

In contrast to the *Rickettsia prowazeki* which invariably kills the louse, the *Rickettsia rickettsi* does not seem to have any harmful effect on the ticks.

The incubation period of Rocky Mountain spotted fever is from three to ten days.

Weil-Felix Reaction: The *Proteus X* reaction is not always positive and the titre is low. The complement fixation reaction is a valuable diagnostic test.

Case Fatality Rate: The case fatality rate of Rocky Mountain spotted fever is variable and depends upon the virulence of the strain of *Rickettsia*. The crude case fatality rate reported for the Eastern type was 18.4 per hundred, of the Western type 28. There are small districts in Montana and Idaho where the rickettsiae appear to be so virulent that the disease is fatal in over ninety per cent of the cases. In adjacent areas the fatality is much lower.

A relatively higher proportion of children contract the disease in the Eastern states. The case fatality rate in both types is higher in adults than in children and, when this is corrected for, there appears to be little difference in the case fatality of the two types.

In comparing the figures for the Eastern and Western types, it must be remembered that the Public Health Service has developed a vaccine which has been used extensively, particularly in the Bitter Root Valley of Montana where the disease was most highly fatal.

Distribution: Rocky Mountain spotted fever has been reported from every geographic district of the United States. A few years ago it was thought to be limited to a few of the Northwestern states but every year

sees it reported from new areas. This may be due in part to a better recognition of the disease and, in part, to an actual extension.

The Western type occurs from March to June with the highest incidence in May when the wood ticks are active. It is particularly prevalent in sheep herders, campers, and foresters, who are naturally more apt to pick up ticks.

The Eastern type is most prevalent during the late spring and early summer, the highest incidence being in July. Again it is related to the life cycle and habit of its vector, the dog tick. The types are mutually exclusive since there is little overlapping of the ticks. Maryland, Virginia, and adjoining states show the highest incidence of the Eastern type, and Montana, Idaho, Wyoming, and Oregon, the highest incidence of the Western type. In 1945 there were 475 cases and 128 deaths reported for this disease. In some states more deaths were reported than cases.

Control

The control of Rocky Mountain spotted fever presents a number of difficult problems. Control of the wood tick by introducing a species of wasp which parasitizes the egg of the tick was attempted unsuccessfully.

Control of rodents and the dipping of live stock is probably of some importance.

The individual may best protect himself by avoiding infected areas during the wood tick season, by wearing boots or leggings which make it difficult for the ticks to attach themselves without crawling up to his neck, by prompt removal of ticks, and by vaccination. It should be emphasized again that care must be taken not to crush the tick.

One attack of the disease leads to a permanent immunity; at least records of second attacks are few or doubtful.

Vaccination: Spencer and Parker, and Cox of the United States Public Health Service have developed very effective vaccines which afford a high degree of protection for at least one season and probably for longer. They give almost complete protection against the more prevalent and milder form of the infectious agent and greatly reduce the seriousness of the less prevalent but highly virulent strain. Vaccination after the tick has bitten may be of some value in reducing the gravity of the disease.

Treatment. Antisera prepared in the rabbit is effective if given early. Sulfonamides are definitely not recommended. Penicillin is useful to control the pneumonia that may follow. Para-aminobenzoic acid is beneficial but the dosage required is rather great. It has been suggested that para-aminobenzoic be given prophylactically in cases of suspected exposure.

OTHER RICKETTSIAL DISEASES

There are a number of other rickettsial diseases which may be mentioned in passing. Some belong to the murine or endemic typhus group,

others are probably identical with or, at least, very similar to Rocky Mountain spotted fever.

Shop typhus is probably spread by rat fleas.

The *Fievre Boutonneuse*, which occurs in Tunis and other parts of the Mediterranean, is transmitted by the dog tick, *Rhipicephalus sanguineus*, and ticks serve as the reservoir.

Trench fever: A disease, subsequently called trench fever or five-day fever, broke out in the troops during the World War in 1915. It was shown to be transmitted from man to man by lice. Although the rickettsia have not been seen in man, they have been seen in lice which have fed on infected persons and it is generally assumed that they are the cause of the disease. The strain has been called *Rickettsia quintana* and is extracellular in the louse. Trench fever is said to be present in Russia and Poland as well as in parts of the Near East. The case fatality rate is extremely low.

Sao Paulo fever of Brazil is probably identical with or at least very similar to Rocky Mountain spotted fever. It has a high case fatality, about sixty to eighty per cent, and is transmitted by a tick, *Amblyomma cajennense.*

SUMMARY

The rickettsia are like bacteria in that they can be seen and stained. However, they are intracellular parasites and do not grow apart from living cells and in these respects resemble the viruses. They appear to be naturally associated with insects, are obligate parasites, and some forms are pathogenic. They are the specific cause of epidemic typhus, endemic typhus, Mountain spotted fever, and other similar fevers. Some rickettsial diseases have an extremely high case fatality; others do not. They are all transmitted by insects and their geographic, climatic, seasonal, and occupational distribution is explained by the distribution of their vectors. Vaccination is effective in some cases; in others it has not been adequately tested. Control centers in the control of the vector.

In the United States, endemic typhus and Rocky Mountain spotted fever do not produce a great number of deaths but do, nevertheless, represent an important public health problem.

Chapter XXVII

VENEREAL DISEASES: SYPHILIS AND GONORRHEA

It is, perhaps, unfortunate that the name venereal (from Venus, the Goddess of Love) should have been applied to this group of diseases, not always nor necessarily contracted in venery. The name and its associations have tended to over-emphasize the moral and social issues often involved in transmission and are in part responsible for these infections being treated as disgraces rather than as diseases. The tendency has been to keep them secret and hidden rather than to admit that they are serious communicable diseases whose presence in our society is a constant menace to every one, the innocent as well as the culpable, and whose rational control is a public health problem, as well as a moral one.

The frank and honest approach to this problem instituted a few years ago by Dr. Parran of the United States Public Health Service has convinced the public that venereal diseases must be treated as objectively as any other infectious and highly communicable disease. This change in attitude has had much to do with the progress made in their control.

Great Britain and the European countries, too, are treating the problem with more candor than in the past and meeting with a considerable degree of success. The Scandinavian countries have gone a long way toward eliminating gonorrhea and syphilis.

The venereal diseases are the great plagues of our times and the principal causes of physical and mental disability. It is a sad commentary on our acknowledgment of social responsibility to realize that present medical knowledge is sufficient to keep these diseases under control, did we but have the courage and sense to make use of it.

There are at least five separate and distinct venereal diseases: gonorrhea, soft chancre or chancroid, syphilis, lymphogranuloma inguinale, and granuloma inguinale. Although these diseases are not clinically nor etiologically related, they are alike epidemiologically and, hence, may best be treated in the same chapter. Gonorrhea and syphilis are by far the most important and should receive the greatest attention.

SYPHILIS

There are few diseases about which the average person shows as much curiosity, both salutary and morbid, as he does about syphilis. There are few diseases that have had a greater influence on modern history and there is probably no infectious disease of greater concern to civilized man. Yet, in spite of all this, and in spite of accurate methods of diagnosis, rather complete knowledge of the manner of transmission, and satisfactory

methods of treatment, the average person remains both misinformed and uninformed.

History

The origin of syphilis in Europe has been investigated by medical historians and paleopathologists. Some believe that it has been present in Europe since ancient times and some that it was introduced by the sailors who returned with Columbus from his voyage to the West Indies. There is a great deal of evidence to support this latter view and, if it be true, then syphilis is one disease that can be dated to the exact day of its introduction into an uninfected country.

In the autumn of 1494, Charles VIII of France gathered together a great army of mercenaries from every part of western Europe, including many from Spain, and invaded Italy. He met with little resistance, captured Naples in February of 1495, and prepared to settle down for a permanent occupation. However, his army was attacked by a new and violent disease; the Pope, the Emperor, the Doge of Venice, and the King of Spain united to force him out; and in a few weeks' time, Charles and his army were in disorganized retreat. The spread of the new disease over Europe followed the disbanding of his mercenaries and their return to their own countries.

There was no name for the disease, and, as it was recognized, each country attributed it to the one from which it had gotten it. The Neapolitans and Italians, who acquired it in 1495, called it the Spanish or French disease and the writers in Latin referred to it as *Gallicus morbus*. The French mercenaries called it the Neapolitan Disease. In 1497 it was recognized in England and called the French Disease, and the Russians, who were farther removed and did not meet with it until 1499, called it the Polish Disease. In a contemporary report by Oviedo, who knew Columbus and the members of his crew, we find this—"Many times in Italy I did laugh, hearing the Italians say the French disease and the French calling it the disease of Naples; and in truth both would have hit on the right name if they had called it the disease from the Indies." (Taken from "The Origin of Syphilis" by William Pusey.)

Other parts of the world acquired the disease when infected Europeans made contacts with them. The Portugese carried it to Africa in a short time; it was first recognized in India after the arrival of Vasco de Gama in 1498; and in Canton, China in 1505 after European contacts.

As we have said, syphilis was immediately recognized as a new disease. It was extremely virulent. The fever was high, the bone and joint pains extreme, and the rash so severe that the disease was often called the Great Pox to distinguish it from the smallpox.

The rapid spread of syphilis over the Old World was an indication of the moral laxity and sexual promiscuity of the times, as well as of the virulence of the organism in a hitherto unexposed population. However, within fifty years, the disease had diminished in severity and taken on its present character.

In 1530 Fracastor published a clever poem, *Syphilis sive Morbus Gallicus* (Syphilis or the French Disease), in which he told how the shepherd, Syphilis, was stricken with the new and dreadful disease for worshiping his own king instead of the Sun God. From then on the disease of the shepherd was known as syphilis, the medical name it bears now.

Etiology

The specific cause of syphilis is *Treponema pallidum*, discovered and described by Schaudinn and Hoffmann in 1905.

Morphology: Treponema pallidum (*Treponema* meaning threadworm, and *pallidum* meaning pale) is a delicate, slender, thread-like, spiral organism with from six to twelve or more coils and tapered ends. It measures on the average about six to fourteen microns in length and about 0.25 micron in thickness. There are flagella and the organism is able to move back and forth and from side to side with a spiral twisting, resembling a corkscrew in action. Reproduction is by transverse fission and no nucleus is demonstrable.

The treponema are like the protozoa rather than the bacteria in that they cannot be stained by the ordinary stains. A special stain such as Giemsa's solution or Fontana's silver impregnation method is required.

Physiology: The *Treponema pallidum* is an obligate parasite. It cannot be cultivated by ordinary methods; in fact, it is exceedingly difficult to culture on any medium. Noguchi, using ascitic or hydrocele fluid and sterile rabbit kidneys or testicular tissue placed in deep tubes covered with vaseline, reported that he was able to cultivate it, but there is some question as to whether the spirochete obtained was *Treponema pallidum*. At any rate, the organism is exceedingly fastidious in its growth requirements and does not, in nature, multiply away from its host—a fact which has a direct bearing on its transmission. Not only does the *Treponema* fail to grow away from its host, but it is very delicate and dies rapidly when subjected to drying. It is killed by a temperature of 39° C. in a period of five hours, which may account for the success of fever therapy and the use of malarial infections in the treatment of general paresis, a late stage of syphilitic infection. Weak disinfectants and soap and water quickly destroy the organisms.

Man is the only natural host of *Treponema pallidum*. Anthropoid apes are susceptible experimentally but are not infected in nature. Rab-

bits may also be infected experimentally by inoculating material from a chancre into the anterior chamber of the eye or into the testicle where it produces localized infections.

Recognition of the Disease

Syphilis has been called the "great imitator" because of the variety of signs and symptoms which follow infection. There are, however, three rather constant and typical clinical stages of the disease.

Primary Stage: Usually two to four weeks after exposure, although it is sometimes six weeks or longer, a sore or chancre appears at the site of inoculation. This sore is small and not painful, easily escaping notice, especially in the female. Sores on the genitalia are more apt to arouse suspicion than those on other parts of the body, as, for example, on the lips, mouth, or skin of the face; and non-venereal syphilis is very apt to go undiagnosed. Since the *Treponemas* begin to invade the tissue shortly after inoculation, they are pretty well established before the local lesion or chancre appears and infection is suspected. Of particular significance from the standpoint of transmission is the fact that the primary chancre is alive with spirocheates.

The primary lesion heals spontaneously and disappears in a short time, regardless of treatment. This is unfortunate because the individual who does not seek medical advice and treatment or who places his confidence in quacks or salves feels that he is cured when his lesion disappears. Nothing could be further from the truth. Regardless of the severity or duration of the primary lesion, the spirocheates invade from the time of exposure and after five to eight weeks, or, perhaps, after a year or more, the second stage will manifest itself.

Secondary Stage: The secondary stage of syphilis is characterized by a number of signs and symptoms. An eruption or rash, which does not itch, may appear over the body. Small lesions or sores, much like fever blisters, may appear on the mucous membranes of the mouth or on the tongue or tonsils. Wart-like lesions may develop on the genitalia or on other parts of the body. Swelling of the lymph glands, a slight fever, undefined pains in the joints, a headache, and sore throat are not infrequently present. Loss of hair or alopecia is a common sign. While all of these manifestations may be present, many may be absent or so slight as to go unnoticed. The sores contain innumerable *Treponemas* and are highly infectious. The secondary stage passes in a short time, but the spirocheates continue to invade and sooner or later—it may be in a few months or it may be as long after as thirty years—the third stage of syphilis becomes evident.

Tertiary Stage: Because of the transient character or apparent absence of the first two stages of syphilis, many persons may not know they are

infected until the tertiary stage, which usually begins about four to six years after the initial infection. Even then syphilis may not be suspected, because it is in this stage that the disease may simulate a number of conditions.

During the latent period following the first and second stage, the organisms have invaded the brain, spinal cord, liver, spleen, heart, and blood vessels, and the symptoms that now appear are referable to an involvement of these tissues and organs. Heart disease, heart failure, apoplexy, locomotor ataxia, insanity, and blindness are some of the conditions that may result. The neural involvements, or neurosyphilis, may occur at early or late stages in the disease.

CONGENITAL SYPHILIS

The symptoms previously described refer to contact infection. Another tragic type of syphilis is the congenital form which is transmitted from a diseased mother to the foetus. Infection takes place through the placenta.

Several possibilities may follow pregnancy in an infected mother. The child may be born entirely free from infection. The foetus may be infected early in pregnancy and be destroyed, in which case a miscarriage results, or at a later stage causing abortion—a large percentage of miscarriages or abortions are due to prenatal syphilis. The child may be delivered at term, showing sores and other manifestations of syphilis. The child may show no signs of disease at birth but develop them later.

Control of Congenital Syphilis

Congenital syphilis can be prevented by early and adequate treatment of the pregnant syphilitics. Numerous studies show that although fetal infection may occasionally occur earlier than the fourth month of pregnancy, it usually takes place after this period, and that, for practical purposes, congenital syphilis can be prevented if treatment is begun by the fourth month and continued until term. Unfortunately a large number of these expectant mothers are never treated before the fifth month or later.

Epidemiology

Source and Mode of Infection: From what has been said previously, it might be inferred, and correctly, that discharges from lesions or sores on the skin and mucous membranes of infected persons, blood from infected persons, and freshly soiled articles are all sources of infectious material.

Since the lesions are teeming with *Treponemas*, contact with them is particularly dangerous and since they are commonly present on the genitalia, it is obvious that syphilis is contracted primarily by sexual intercourse. Direct contact with lesions on the mucous membranes of the mouth, as in kissing, is responsible for a number of infections. The most

dangerous syphilitic is the one in the first year of his disease when the lesions are open. After the fourth year there is little danger of his transmitting the infection.

Although the blood stream harbors the organisms at times, the chance of contracting syphilis from this source is not great, except in cases where blood transfusions are made from infected persons. Instances of this are, of course, extremely rare, since, except in great emergencies, blood donors are tested for evidence of syphilis before their blood is used.

The danger of contracting syphilis from articles soiled by discharges is greatly reduced because the *Treponemas* are so sensitive to drying that they perish rapidly away from the body. Probably not more than five per cent of the cases may be traced to indirect transmission. Razors, drinking glasses, and other articles coming in contact with the chancres may be heavily contaminated and present a source of real danger.

Susceptibility and Immunity: All persons, regardless of age, sex, or race, are susceptible to infection. There is no natural immunity. The infected individual acquires an immunity to superinfection and remains resistant for as long as he remains infected. If cured, he becomes susceptible to subsequent infection.

Prevalence and Distribution: The exact prevalence of syphilis is unknown and its approximate prevalence can only be estimated. Such data as are available show that it is one of the leading infectious diseases and is present in every country, regardless of race, climate, or age of population.

In the World War II reports from selective service boards showed an infection rate of about twenty per thousand for whites and from a hundred and eighty to three hundred and twenty per thousand for negroes.

It is more frequent in males than in females and is most often contracted between twenty and thirty-five years of age. In college students, the attack rate is between one and two per thousand, in other groups of the same age, it may run as high as one hundred to one hundred and eighty.

In 1945, 350,000 cases were reported in the United States but estimates indicate that there are nearly again as many unreported.

Control

Because of its high incidence, its destructive effect on those infected, and the social and economic consequences of infection, syphilis ranks as the outstanding public health problem of the day. The difficulty encountered in its control is not that the medical profession lacks sufficient information concerning its spread or effective curative drugs. It is the attitude of society toward a disease whose transmission is so intimately

linked with the sexual urge. It has been extremely difficult to break through the taboos and persuade the people to think of syphilis objectively.

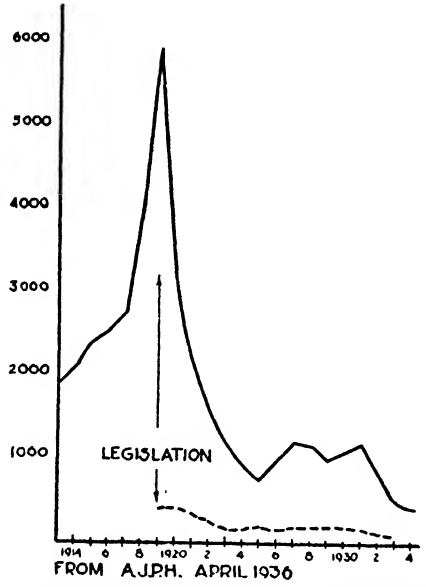
Venereal disease is so intimately connected with prostitution, and prostitution with crime and crooked politics, that many self-respecting people have brushed the situation aside as of no concern to decent folk. Nothing could be further from the truth. The fact that syphilis and gonorrhea are primarily transmitted by sexual intercourse and that most prostitutes are infected and play a large part in transmission is apt to lead to the erroneous conclusion that syphilis is never contracted by the innocent, but that it is punishment for sin and serves the guilty right. Syphilis is a menace to all. Prostitution and immorality are major factors in its spread, but the innocent also suffer. On the basis of chance, the probabilities are that the syphilitic will marry a non-infected individual, who will, in all likelihood, contract the disease. It has been estimated that about forty per cent of the cases of syphilis are acquired innocently in this way. Because of this situation, many states have laws requiring blood tests before the issuance of marriage licenses.

In the control of any infectious disease, it is important to trace the sources of infection. In the case of venereal diseases it is exceedingly difficult to carry out this sort of study. However, a number of epidemiologists have developed the technique to a fine point and have trained workers who are capable of getting information as to where and from whom the disease was acquired. By dint of skillful and honest detection, these investigators have not only found the contacts, but have often succeeded in getting treatment instituted and so in preventing further cases. From some such case-finding studies in Washington, it was found that the transmission rate was 1 to 1.5, that is, that every infected person was responsible for an average of 1.5 new cases. When infected persons are treated, the transmission rates approach 1 to 0. Such studies are rare but indicate that with properly trained workers and adequate funds, syphilis can be traced from case to case, and that, when cases are known, treatment can be instituted and a large number of new cases prevented.

The control of venereal disease requires adequate facilities for laboratory tests, trained personnel for case-finding and contact-tracing, funds for treatment, and an organized effort at educating the public in regard to the transmission, danger, and treatment of the disease. In addition to the educational approach, some countries have passed laws making the transmission of syphilis a punishable offense, hoping in this way to force infected persons to take the necessary treatment. It might be remarked here that treatment to render the infected non-infectious does not necessarily cure the individual.

Several European countries, Sweden in particular, have achieved remarkable success in the control of syphilis and gonorrhea. This table shows the incidence and trend in Sweden.

The control of venereal disease, it can be seen, is a social, economic, and medical problem. It requires an honest and objective approach on the part of everyone. A beginning has been made and it is probably safe to predict that the next generation will see a saner, more scientific, and more effective handling of this great plague.



Syphilis in Sweden. Number of new cases ——. Cases infected abroad -----

Diagnosis: The laboratory occupies such an important place in the diagnosis of syphilis that its value cannot be over-emphasized. A great many tests to determine infection have been developed and the public is familiar with the names, at least, of some of them.

Wassermann Test: This test was developed by Wassermann in 1906. It was accurate in about ninety-five per cent of the cases, when properly carried out, and soon came to be depended upon for the diagnosis of syphilis.

In studying the bacteriolytic power of antibodies, Bordet observed that if the serum containing them were heated, it did not bring about lysis of the bacteria; and also that its lytic power could be restored by the addition of a few drops of serum taken from a normal animal, which, consequently, did not contain antibodies. He concluded that bacteriol-

ysis or cytolysis depended upon two factors: the specific antibody and a nonspecific substance present in normal blood. He called this nonspecific substance *alexin*: it is more commonly known as *complement* because it completes the reaction.

Complement is a component of all animal blood, is thermolabile, being inactivated by heating at 56° C. for ten minutes, and is nonspecific.

If bacterial antigen and its homologous antibody are mixed in the presence of complement, the complement is bound or fixed; but if antigen and heterologous antibody are mixed together, they do not combine, and the complement is not bound but remains free in the mixture. Its presence in the free state can be detected by adding red blood cells plus hemolysin. Since the lysis of red blood cells also depends upon complement, if it is present, they will lyse; if it is not, they will remain intact. If lysis does not occur, it means that the complement was fixed or bound and thus indicates that the antigen and antibody combined and bound the complement. If lysis does occur, it means that the complement was available and, hence, was not used up or bound.

In the Wassermann Test the patient's serum is mixed with antigen plus complement. If the serum contains syphilitic antibodies, they will combine with the antigen and the antigen-antibody complex will bind or fix the complement. If no syphilitic antibodies are present, the complement will remain free. The next step is to determine whether there is free or unbound complement. This is accomplished by adding to the first system, an indicator system, consisting of the red blood cells of sheep and anti-sheep hemolysin. If complement is free, lysis of the red blood cells will occur and the mixture will become clear. If it is not, the cells will remain intact and the mixture will appear cloudy. Since only persons who are infected with syphilis develop antibodies, the presence of antibodies is proof of infection.

The Wassermann Test is quantitative and recorded as 1+, 2++, 3+++, or 4+++++. In a 4 plus test, all the complement is bound by the syphilitic antigen and antibodies and there is no lysis of the red blood cells of the indicator system. In a negative test, all of the complement is free to act in the indicator system and complete lysis occurs.

To perform the test, guinea pigs are needed as a source of complement, sheep as a source of red blood cells, and rabbits to produce antibodies for the sheep red blood cells. The test is, therefore, expensive and complicated.

Kahn Test: Shortly after the Wassermann test was developed, it was shown that the syphilitic antigen would flocculate in the presence of syphilitic sera and a number of workers developed tests based on this fact. The Kahn test is probably the most accurate and is the most widely used. In this test the antigen is a fine lipoidal extract of beef heart. When the

syphilitic blood serum is added to the antigen in a 0.85 per cent solution of sodium chloride, the lipoid particles flocculate and form a coarse emulsion.

The *Kline Test* can be performed in a few minutes. It is a microscopic precipitation reaction and the results can be read on the microscope.

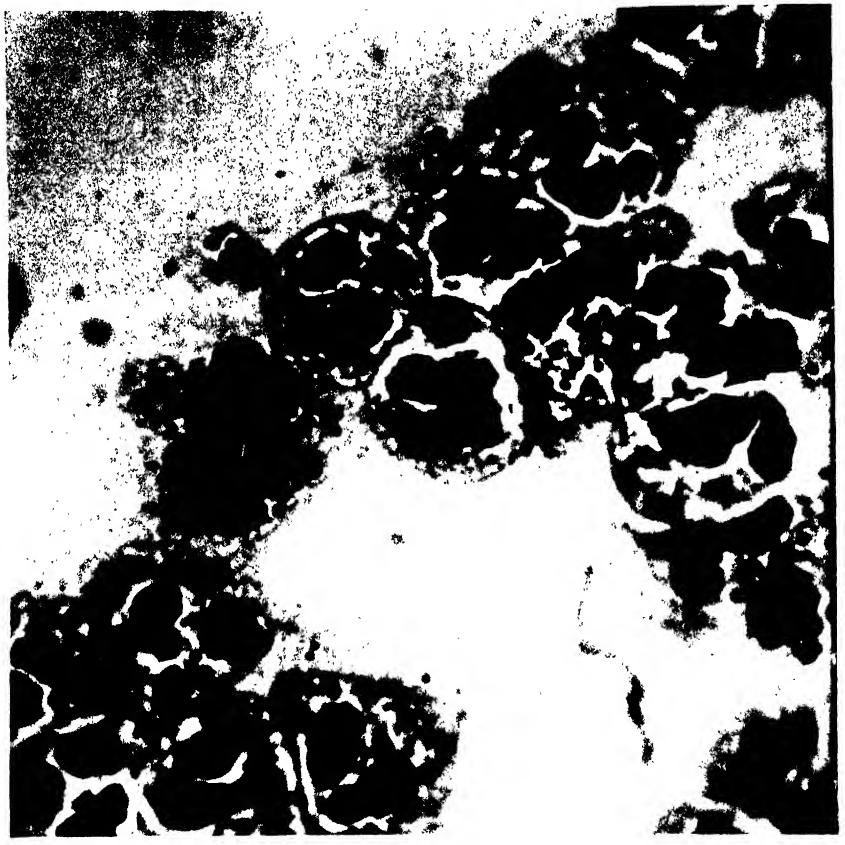


FIG. 48. *Neisseria gonorrhea* in pus

Treatment of Syphilis: Mercury has been used in the treatment of syphilis ever since the disease was introduced into Europe. Various mercurials have been employed in the treatment of the sores or skin lesions, but, because of their toxicity and tendency to cause bleeding of the gums, their use has been largely discontinued.

The use of arsenicals dates to the work of the German chemist, Paul Ehrlich. After years of intensive research and after preparing and testing hundreds of compounds, he finally chose one, the 606th one prepared, as having the most promise. This compound, known as *606*, *salvarsan*, or

arsphenamine, was the first successful chemotherapeutic agent in the treatment of syphilis and of other spirocheatal infections.

The exact mechanism by which arsphenamine, neosalvarsan, or any of the other arsenicals destroy the spirocheates is not known. They are not highly destructive to spirocheates *in vitro* and their effect is probably an indirect one.

Bismuth is used in conjunction with arsphenamine.

The most important single advance in the treatment of syphilis in recent years has been penicillin therapy. Penicillin, relatively nontoxic and rapidly effective, brings about a high percentage of cures. Its use in pregnant women prevents congenital syphilis.

GONORRHEA AND GONOCOCCUS INFECTIONS

The term gonorrhea refers to a form of gonococcus infection in which the genital tract is involved. There are other gonococcus infections known as ophthalmia neonatorum, vulvovaginitis, and, according to recent evidence, perhaps rarely, a kind of meningitis.

Gonorrhea is an infection of the mucous membranes of the genital tract. In the initial stage, the vagina and uterine mucosa of the female and the urethra of the male are involved. After the initial acute stage, the infection tends to become chronic and spreads to the surrounding tissues and to remote parts of the body. Arthritis and endocarditis are common manifestations of the chronic stages and there may be a chronic discharge at the original site of infection.

Etiology

The specific cause of gonorrhea is *Neisseria gonorrheae*, or the gonococcus. In 1879 Neisser discovered the coccus in pus from a number of cases of gonorrheal infection and in 1885, Bumm grew it in pure cultures and produced typical cases in inoculated humans.

The gonococcus is a small nonmotile, nonsporulating, gram-negative coccus from 0.6 to 0.8 micron in diameter. The individual coccus is flattened on one side, giving it a kidney bean shape. The cocci usually occur in pairs, however, and the pair looks somewhat like a biscuit. This appearance is characteristic, especially in smears from body discharges. There are several different strains, but all produce gonorrhea.

The pathogenic *Neisseria* are extremely sensitive to drying, to heat and cold, to aging, and to disinfectants. Drying for a few minutes destroys large numbers; none will survive more than a few hours. Ordinarily the organisms are discharged in pus which protects them until it is completely dried. Moist heat at a temperature of 55° C. destroys them in less than five minutes.

Cultures of gonococci have to be transferred every twenty-four to forty-eight hours to be kept alive. They will remain viable longer at 37° C. than at room temperature but may be stored for long periods at room temperature after rapidly freezing and drying *in vacuo*.

Disinfectants, such as silver nitrate, potassium permanganate, and tincture of iodine, are very destructive. The gonococcus is also sensitive to ordinary soap.

The physiology of the gonococcus is of considerable interest since it determines to a large extent the epidemiology of gonorrhea. The gonococcus is a strict parasite and very fastidious as to growth requirements. Its growth temperature range is narrow, from 30° to 38.5° C., with an optimum at 37° C. Although it is aerobic, slightly increasing the carbon dioxide and lowering the oxygen pressure will increase the rate of growth. The gonococcus will not, as a rule, grow on plain agar, but can be cultivated on a variety of media enriched with blood serum, hydrocele, or ascitic fluid. Since it is exacting in its physiological requirements and since it is so sensitive to drying, it does not grow naturally apart from the body and dies rapidly when discharged. This accounts for the fact that gonococcus infections are, as a rule, contracted only by means of direct and close contact with infected persons.

Pathogenicity and Host Range: The gonococcus is not only exacting as to its growth requirements on artificial media, but is also limited in its ability to parasitize animals. Man is the natural host and the only animal in which the organism is capable of maintaining itself. Mice, rabbits, and guinea pigs are resistant to infection.

The gonococcus produces a substance in the nature of an endotoxin. Anti-gonatoxins have been developed and used with some success.

Symptoms

In adults, the pathogenesis, or course of events following infection with the gonococcus is somewhat as follows. The gonococcus gains entrance to the mucous membranes of the genital tract and in about a week sets up an acute inflammation in the urethra of the male or in the vaginal or uterine mucosa of the female. This is accompanied by a discharge of pus containing huge numbers of gonococci. Many cases, whether treated or not, tend to recover spontaneously after this acute stage.

But the organism frequently invades the deeper tissues, the inflammation spreads, the acute stage subsides, and the infection becomes chronic. The organism may enter the blood stream and set up an endocarditis, one of the more common complications; or it may produce a gonococcus arthritis. It is the chronic stage that is most destructive and most difficult to treat.

Diagnosis: The diagnosis of gonococcus infection is based on finding the

causative organism. Two methods are commonly used, microscopic and cultural, but skin tests and serological tests have also been suggested.

Direct Microscopic Method: The finding of gram-negative intracellular diplococci in smears made from the discharges of the genital tract is pretty conclusive evidence of gonococcus infection. The source of the pus and the history of the patient are, of course, important considerations.

Diagnosis is made more readily in the male than in the female because the gonococci are more numerous in discharges from the male.

The chronic cases are more difficult to diagnose. Pus may be absent. If it is present, the number of organisms may be too few to detect by direct microscopic methods. Cultural methods are more accurate in such cases

Cultural Methods: The isolation of the gonococcus is readily accomplished by inoculating any one of a number of special media. North's gelatin agar fortified with ten per cent sheep blood is very satisfactory. The blood is added while the gelatin agar is hot thus producing a "chocolate" agar. The identification of the gonococci has been greatly simplified by McLeod who noted that they produced an oxidase which could be detected by dyes. Plates of the media are streaked with swabs from the urogenital tract, incubated for forty-eight hours at 36° to 37° C., and then flooded with a one per cent aqueous solution of tetra-methyl or dimethyl paraphenyline diamine hydrochloride. Oxidase-positive colonies turn bright purple when treated with the tetra-methyl compound; and a bright pink which changes to a deep red and finally to black when treated with the dimethyl compound. Other members of the *Neisseria* also show this reaction but they are not commonly encountered in urogenital smears. Smears from the oxidase-positive colonies should be gram stained to check the morphology and staining reactions.

Complement-fixation and skin tests are of value in chronic cases where material for culture is not available.

Source of Infection: The infectious agent is in the discharges from infected persons, usually from the lesions of inflamed mucous membranes of the urogenital organs.

Mode of Transmission: Gonorrhea is a venereal disease and usually contracted through sexual intercourse. The danger of contracting it in any other way is practically negligible since the organism is extremely sensitive to drying and does not grow away from the human body. In order to transmit the disease, contact must be direct and intimate.

The incubation period of gonorrhea is usually from three to five days, but may be shorter or a trifle longer.

Period of Communicability: It is obvious that an individual remains infectious as long as the organisms are being discharged. This is true regardless of whether the infection is acute or chronic, recent or of long

standing. Since relapses frequently occur, the individual may be non-infective following the acute stage and become infectious later when a relapse occurs.

Susceptibility and Immunity: All persons are susceptible. One attack followed by recovery does not lead to immunity. There is probably some sort of infection immunity that confers a resistance to reinfection for as long as an individual is infected.

An interesting point in this connection is the suggestion that a person who has apparently recovered from an attack of gonorrhea and believes himself to be cured, may, unknowingly, transmit the infection to another who will develop acute gonorrhea and, in turn, reinfect him. The explanation may be that the organism loses its virulence during its sojourn in the original donor but is virulent for a person who has never had an infection. Such an organism might set up an acute infection in a susceptible person and acquire a degree of virulence sufficient to overcome the acquired immunity the donor possessed as a result of his original infection.

Prevalence: All the available evidence suggests that gonorrhea is one of the most widespread and prevalent of all infections in adults. It is more common in males than in females and has its highest incidence in the eighteen to forty-five age group. Over 300,000 cases were reported in 1945 but reporting is very incomplete so there were probably many more.

It is not possible to give more than an estimate of the total number of new cases that occur every year because only a small per cent are ever reported. There are a number of reasons for this. Since there is a common but mistaken belief that gonorrhea is not a serious disease—'no worse than a bad cold'—, infected persons do not seek medical advice or, if they do, tend to patronize quacks who promise "cures." Even reputable physicians are often lax in reporting the disease. The recent efforts on the part of the United States Public Health Service to educate the public and medical profession have resulted in better reporting, and, consequently, in an apparent increase of gonorrhea cases. It is still probable that not more than five to fifteen per cent of the new cases are recorded.

It has been estimated that from five to twenty per cent of the males have gonorrhea at some time or other, some investigators put the figure as high as fifty per cent. This incidence is highest in negroes in the lower income groups, and in urban areas.

Control

Theoretically, the control of gonorrhea should be simple. Actually it is very difficult. The public health aspects of control are similar to those involved in the control of syphilis and have already been discussed in connection with that disease.

Treatment: When sulfanilamide was first introduced clinicians reported over eighty per cent of the cases cured but very soon afterward the percentage of cures dropped to seventy per cent, to fifty per cent, and, in some groups, to twenty per cent. As other sulfa drugs were introduced the situation was repeated. The reason is the rapid appearance of sulfa resistant strains for, in an extensively treated population, only those strain are propagated and it does not take long before most the cases are due to such strains.

Penicillin is so dramatically effective that the army uses it almost exclusively. Ironically enough, it is such an effective cure that many persons have lost their dread of contracting gonorrhea.

Artificial fever therapy or pyrotherapy has been suggested, and favorable results have been reported by some workers. The idea is that since the gonococcus is very sensitive to temperatures slightly above normal body temperature, it might be possible to raise the patient's temperature somewhere between 41° and 42° C., which would be high enough to destroy the organism,—a sort of *in vivo* pasteurization.

VULVOVAGINITIS

Vulvovaginitis is frequently caused by the gonococcus, although it may be caused by other organisms. It is most common in young girls about ten years of age. It is difficult to treat but not a particularly serious disease since recovery eventually takes place and there appears to be no permanent damage.

It is interesting to note that recovery takes place at puberty with the maturing of the tissue of the genital tract. This has suggested that the injection of hormones to mature the tissue might have an effect on the gonococcus infection. The sex hormone, Antuitrin S, has been given in numerous instances, the tissue has matured, and the infection cleared up. This is an interesting case of tissue susceptibility to infection.

Vulvovaginitis is characteristically a disease of asylums, hospitals, and institutions and appears to be highly contagious. Soiled bedding, towels, and clothing play a large part in its spread. It is not a venereal disease. Penicillin is effective in treatment.

OPHTHALMIA NEONATORUM

This is a very serious gonococcus infection of the eyes of the newly born, incurred during the infant's passage through an infected birth canal. Since the gonococci are on the surface, disinfectants are effective if applied immediately. The method suggested by Credé is commonly used and, in many states, is mandatory and routinely applied whether gonorrhea of the mother is suspected or not. It consists of dropping a few drops of one

percent silver nitrate into the infant's eyes immediately after birth. Since this method has become common practice, the number of cases of blindness from gonococcus infection has decreased very markedly. Diagnosis of ophthalmia neonatorum may be made by microscopic examination of a smear.

CHANCROID OR SOFT CHANCRE

Soft chancre or chancroid is a specific infection characterized by multiple ulcers which may resemble the primary sore or hard chancre of syphilis, hence the term, chancroid. These ulcers are different, however, in that they do not become hardened or indurated but remain soft, are filled with pus, and are very painful. Unless complications involving an extension of the infection to the inguinal lymph glands follow, the ulcers heal spontaneously. In many cases, however, there is such an inflammation of the inguinal glands, which, may in turn, ulcerate.

The specific cause of chancroid is a small gram-negative bacillus belonging to the hemophilic group and called *Ducrey's bacillus* or *Hemophilus ducreyii*, in honor of Ducrey who first isolated the organism and showed its connection with the disease.

The disease is spread almost entirely by sexual contact. It does not represent a particularly serious public health problem.

Diagnosis involves a direct microscopic examination of smears made from the ulcerating sores, cultures, and skin tests. It is very important that this infection be distinguished from syphilis, hence, a dark field examination of the pus should be made to determine the absence of spirocheates.

There is little reliable data on the prevalence of chancroid. It was said to have been very prevalent in the soldiers during and following the war and this has been interpreted to mean that little importance was attached to the infection.

Ordinary soap and water is said to be a highly effective preventive if applied immediately after exposure. Treatment is local and the arsenicals used in the treatment of syphilis are not effective. Sulfadiazine is effective.

LYMPHOGRANULOMA VENEREUM

This is the only venereal disease that has been shown to be caused by a virus. Following exposure there may be a small lesion on the genitalia, but the principal evidence of infection is the swelling of the inguinale lymph gland and the formation of buboes. In the female, the regional lymph glands drain into the nodes around the rectum which then becomes involved.

The incubation period varies from one to three weeks.

This is the only virus disease that can be diagnosed by a skin test. Frei found that infected persons developed an allergic response to material taken from the buboes. The *Frei Test* is performed as follows: material from the buboes is heated and injected into the skin and, if the individual is infected, a characteristic skin reaction occurs.

Lymphogranuloma venereum is prevalent in the southern states and in the tropics where it is called climatic bubo. It is a common disease of the colored population.

GRANULOMA INGUINALE

Granuloma inguinale is an endemic venereal disease in the southern states and most prevalent in negroes and poorer whites. The disease is presumably transmitted by sexual intercourse and remains localized in the region of the genitalia. The characteristic lesion is an ulcer. It is caused by a pleomorphic, non-motile rod, *Donovania granulomatis*, that cannot be grown on artificial media. It can be grown in the developing chick embryo.

Chapter XXVIII

SOME INFECTIONS DUE TO THE SPORE FORMING BACILLI

Several diseases of men and animals are due to the sporeforming bacilli. Many of these are wound infections and present a somewhat different picture than do the diseases due to non-sporeforming bacteria or viruses. To appreciate these differences and to understand the unique problem of control, it is important to have an understanding of the morphological and physiological characteristics of the sporeformers.

The family *Bacillaceae* is divided into two genera: the *Bacilli* and the *Clostridia*. Both form spores and both are gram-positive. The natural habitat of the *Bacilli* is the soil and many are very active decomposers of proteins and carbohydrates. They vary widely in their temperature requirements, some growing at low temperatures and some in hot springs. The spores are heat resistant and survive temperatures of boiling water for hours. The *Bacilli* are a common cause of food spoilage. The genus *Bacillus* comprises well over a hundred species, only one of which is of especial interest in connection with disease. This is *Bacillus anthracis*, the cause of anthrax.

ANTHRAX

Anthrax, splenic fever (*Milzbrand*, Gr., *Charbon*, Fr.) is primarily a disease of animals but it also occurs in man. It is of interest historically because it was the first disease proven experimentally to be caused by a living organism. In 1850 Davaine and Rayer saw the bacillus in the blood of animals dying of anthrax. Pollender saw it in 1855. In 1877 Koch isolated the organism in pure culture, reproduced the disease by inoculation with the culture, and not only established the organism as the specific cause of anthrax but laid down the pattern which has since served as the method of approach to the study of the causes of all infectious diseases.

Etiology

Bacillus anthracis is a large sporeforming gram-positive aerobic rod. Capsules may be produced when it is growing in animals but are not present in organisms growing on artificial media. Spores, too, form readily in cultures but are not found in the animal although when the blood or tissues of a diseased animal are exposed to the air, the bacilli sporulate rapidly, a fact of considerable practical importance. Spores of different strains all show a relatively high resistance to heat. It requires three hours at 140° C dry heat to destroy them and at least five to ten minutes of live steam at 100° C. Boiling will destroy them in about ten to fifteen minutes. Vege-

tative cells are not particularly resistant to heat but are destroyed at a temperature of 54° C in thirty minutes or less.

Although *Bacillus anthracis* is pathogenic for man and for a wide variety of animals, the disease is most important in cattle and sheep. Horses, swine, deer, rabbits, guinea pigs, and mice are also susceptible. Rats and dogs are relatively resistant.

Little is known about the mechanism of disease production and although the symptoms may suggest the presence of an exotoxin, such a toxin has not been demonstrated.

The ease of infection and the symptoms are related to the portal of entry. Infection is usually through cuts or scratches, by way of mouth, or by inhalation. Experimental animals are readily infected by subcutaneous inoculation. Feeding spores to susceptible animals leads to infection though the more resistant animals are infected by this route with difficulty. Feeding vegetative cells to susceptible species does not lead to infection which suggests that such cells may be destroyed in the stomach.

Anthrax is a septicemia in cattle and sheep and is rapidly fatal in about seventy-five per cent or more of the cases. In no other bacterial infection does there appear to be such extensive multiplication of the organism. The blood and capillaries of all tissues are found to be teeming with *Bacillus anthracis* and the capillaries of the liver and spleen are packed with them. The spleen becomes enlarged and is deep red in color, hence the name "splenic fever" and "Milzbrand."

Three forms of anthrax are recognized in cattle, the periacute, the acute, and the subacute. In the periacute the time between the appearance of the first symptoms and death may be only a few hours or a matter even of minutes. In the acute form, the disease may last a day or two, and in the subacute the disease may last several days.

There are few signs of disease in the periacute stage but there may be bloody discharges from the mouth, nose, or anus. In the acute form the discharges are more common. The spleen is usually greatly enlarged and dark red in color. Other organs and tissues show characteristic changes of diagnostic value.

In the more resistant animals the infection is not generalized but is localized forming a carbuncle or pustule, hence the names anthrax and charbon meaning coal or carbuncle.

Under natural conditions cattle and sheep usually contract anthrax by swallowing the spores, and the fact that spores may live in soil for twenty to thirty years makes control difficult. Infection in farm animals may occasionally be through abrasions.

In man, anthrax exists in three forms and each form is related to the route of infection.

Malignant Pustule: Infection through the skin gives rise to a boil or abscess called a malignant pustule. This may heal or may spread and be followed by a septicemia which is highly fatal. If the abscess is opened and allowed to drain, healing may be fairly prompt. Man is rather more resistant to anthrax than are cattle or sheep as evidenced by the tendency for the process to localize. The localized form also reflects the nature of man's contact with the spores and has occurred following the use of shaving brushes made from bristles harboring the spores and as a result of contact with infected hides and other infected animal products.

Pulmonary Anthrax: Inhalation of spores leads to infection of the lungs, a form of anthrax called "woolsorters" disease because it is associated with the handling of wool. While this form of the disease is less frequent, it is the most highly fatal.

Intestinal Anthrax: This form has been reported in man following the eating of the flesh of diseased animals. The intestinal route of infection is rare in man but is common in cattle, sheep, and other animals.

Control

The control of anthrax calls for the close cooperation of the Bureau of Animal Industry, the United States Public Health Service, and the medical and veterinary professions. It is difficult because of the high resistance of the anthrax spores.

Control in animals: The following procedures are indicated.

Prompt disposal of carcasses. This is accomplished by complete incineration of the carcass along with the bedding and other materials soiled by discharges, or by deep burial. The carcass should be buried to a depth of at least six feet and covered with quicklime to prevent dogs and other animals from scattering the bones and flesh. Carcasses should never be buried in swampy ground or where water might carry the spores to the surface. Water is often a factor in the spread of anthrax from one farm to another. Crows and buzzards who prey on diseased carcasses may also play a part in spreading the spores.

It is also necessary to have control and disinfection of effluents and wastes from factories where spore-infected hides, wool, or hair have been encountered and bacteriological examination of shipments before such products are processed if they come from areas not known to be free of anthrax.

Control in Man: Antisera, sulfa drugs, penicillin, and streptomycin are effective but must be given early. Therefore rapid and early diagnosis is essential. The prevention of industrial anthrax by sterilization of hides and hair is difficult because no satisfactory method has yet been found for destroying the spores without damaging the hides.

Vaccination: Several types of animal vaccine are available and where

anthrax occurs more or less regularly, vaccination of cattle is indicated. There is no method of immunization in man.

In cattle the disease may be too rapidly fatal to allow effective treatment with antisera but in the less acute cases, antisera may be effective.

Prevalence

Anthrax reporting is not very accurate but it does indicate that although both agricultural and tannery anthrax have shown decreases, the total number of cases of human anthrax has increased by sixteen per cent due to the five fold increase in anthrax due to imported wool or hair. The case fatality rate has dropped eighty per cent due to promptness and rigorousness of treatment. About eighty cases a year are reported.

The bulk of the cases occurs in employees in tanneries and woolen mills with agricultural workers ranking second. Sporadic cases traced to shaving, hair, and tooth brushes have been reported and one death occurred due to infection contracted cleaning laboratory test tubes. Three cases were attributed to fertilizer or soil because they were acquired while gardening.

About ninety per cent of the cases in tannery workers were contracted from imported goat skins. Baled skins stored in warm, leaky, and damp warehouses may become heavily contaminated with organisms from the skin of one infected animal.

Not only hides but also bone meal from infected animals may harbor the anthrax bacillus and it has been suggested that some of the sporadic outbreaks in livestock may have been due to this source. Outbreaks of anthrax have occurred in mink farms where infected meat, usually horse, had been used for feed.

Some Infections Due to the Clostridia

The clostridia are anaerobic, sporeforming, gram-positive rods. The natural habitat of most clostridia is the soil although the spores of some are found in the intestinal tract of man and animals.

Physiologically the clostridia are a heterogeneous group. Most of the species are free-living saprophytes. Those that grow in the intestinal tract of animals are also essentially saprophytes and there is some question as to whether the disease-producing forms depend entirely upon multiplication in the animal for survival.

Some of the clostridia, for example, *Clostridium butyricum*, fix nitrogen. Some, such as *Clostridium acetobutylicum*, *Cl. butylicum*, and *Cl. pasteurianum*, are rather active fermentors of carbohydrates and produce butyric, acetic, lactic, and formic acids, and butyl, ethyl, amyl, and propyl alcohols.

The clostridia commonly associated with disease are *Cl. botulinum*, the

cause of botulism (see page 379), *Cl. chauvoei*, which causes blackleg in sheep and horses, *Cl. tetani*, the cause of tetanus or lockjaw, and *Cl. septicum*, *Cl. perfringins* (*welchii*), *Cl. histolyticum*, *Cl. oedematiens*, and several others which are associated with gas gangrene.

BLACKLEG

Blackleg is an acute infectious disease, primarily of young cattle. It is also known as symptomatic anthrax, emphysematous anthrax, black quarter, and quarter ill. Blackleg occurs in nearly all countries of the world, and in nearly all of the United States with the possible exception of the southern Atlantic and Eastern Gulf states. The greatest losses occur in the cattle feeding and grazing states. It is one of the important bacterial diseases of cattle and should not be confused with anthrax although before the work of Pasteur the two diseases were regarded as the same.

Young cattle, six to eighteen months old, appear to be most susceptible and cattlemen report that high grade cattle are more susceptible than scrubs. In this country there appears to have been an increase of the disease paralleling the improvement in cattle breeds. Cattle that are gaining weight and in good condition appear to be more susceptible than those in poor condition. Sheep and goats are susceptible, a few cases have been reported in swine, while horses, dogs, and cats as well as man are immune.

Blackleg occurs in all seasons of the year but is most prevalent in the spring and fall.

The spores of *Clostridium chauvoei* survive for several years in soil and the animal becomes infected when the spores gain entrance through puncture wounds and abrasions of the skin. It is thought that infection may also occur through the mucous membranes of the mouth, the tongue, and the intestinal tract. Since the spores do not germinate in the air, large open wounds are not apt to become infected. The symptoms consist of localized swellings. The thigh or shoulder are most commonly involved and because of the extensive discoloration observed after skinning, the disease is named blackleg, black quarter, or quarter ill. The swellings contain considerable gas formed by the organism and produce a crackling sound upon slight pressure.

Cl. chauvoei produces a weak exotoxin but the disease is primarily a bacteremia. Death usually occurs in twelve to thirty-six hours and only a few animals recover. There is no successful treatment.

Control: Vaccination is the only reliable means of controlling blackleg and several types of vaccine are available.

To reduce the hazards of infection, animal carcasses should be either incinerated or buried as in anthrax. Burning of pastures is also recommended. The grass should be allowed to grow high and burned off when it is dry. This must be done for several years in succession.

TETANUS

Although tetanus or lockjaw is an ancient disease, its sporadic occurrence does not readily suggest infection. However, in 1884, Carle and Rattone showed that it was transmissible and in the same year Nicolaier described the tetanus bacillus. He observed the organism in material taken from animals that had developed tetanus after being inoculated with soil. Kitasato, in 1889, isolated the organism in pure culture from pus which he had heated so as to destroy the non-sporulating organisms. He then produced tetanus by animal inoculation and thus proved the causal relationship of the organism to the disease. His studies showed that the bacilli did not invade the blood stream and that the symptoms were due to a toxin. Von Behring and Kitasato, in a series of brilliant researches, then demonstrated that the injection of the tetanus toxin into animals stimulated the production of a substance which appeared in the blood stream and neutralized the toxin. Their studies laid the framework for subsequent studies on the nature of immunity and served as a basis for the development of the whole field of serum therapy.

Tetanus is an acute disease of the central nervous system and is characteristically associated with muscular spasm. The spasms usually begin with the muscles of the jaw and the neck and, in severe cases, involve the voluntary muscles of the body. The frequency with which the muscles of the jaw are involved is responsible for the common name, lockjaw.

Morphology: The specific cause of tetanus, *Clostridium tetani*, is a long, slender, gram-positive, motile, spore-bearing rod. The rods vary considerably in length but average about 3 to 5μ in length by 0.3 to 0.5μ in width. The spherical spores are at the tip end and are usually wider than the diameter of the rod, giving a "drumstick" appearance to the sporulating cell.

Physiology: The tetanus bacillus grows in a variety of laboratory media but requires anaerobic conditions, or, at least, a low oxidation-reduction potential for spore germination. This fact appears to account for its rather low infectivity when introduced into healthy tissue. It grows best on dead tissue in deep wounds to which the air does not reach. The bacillus is found in soil, particularly cultivated soils, but there is some question as to whether it multiplies there. Its natural habitat is the intestinal tract of animals. It has frequently been isolated from horse manure and human feces and, in one investigation, was found in nine out of sixty-three samples of street dust in Baltimore.

Resistance: Tetanus spores resist boiling at 100° C for over one hour but are readily destroyed at temperatures of 105° to 110° C.

Tetanus Toxin: Our principal interest in the tetanus bacillus is in the extremely potent toxin it produces. Tetanus toxin is readily destroyed by heat and is filterable. It is extremely toxic when injected. When given

by mouth it is harmless and is apparently digested by the digestive enzymes. It appears to be a protein and does not pass through semipermeable membranes nor is it absorbed by the mucous membranes.

The toxin consists of at least two fractions, one called *tetanolysin* which hemolyses red blood cells, the other called *tetanospasmin* which shows an affinity for nerve cells. Only the tetanospasmin fraction appears to be of any significance in the disease picture.

Animals vary greatly in their sensitivity to the toxin. The horse is highly sensitive, whereas chickens are said to be over two hundred thousand times as resistant.

Epidemiology: Tetanus is an infectious but not a contagious disease. It occurs sporadically—there are about 450 cases a year in the United States—and cases do not give rise to other cases.

Tetanus is a wound infection and the number of cases in a population is related to this fact. It did occur most frequently, as would be expected, in times of war and was particularly important in World War I because of the deep penetrating nature of wounds suffered in trench warfare and the chance of contamination of such wounds with soil heavily seeded with tetanus spores. Due to the effectiveness of immunization, tetanus was a rare disease in the Allied Forces in World War II. There were only sixteen cases reported in the entire United States army and navy from 1942 to 1945 and in fourteen of these the individuals had not received complete immunization.

The incidence of tetanus is much lower than might be expected in view of the wide distribution of spores in cultivated and manured soil and this is because the spores do not germinate in fresh wounds or in healthy tissue. When introduced into experimental animals, washed spores do not readily produce tetanus, but if soil, calcium salts, or other agents that destroy tissue are introduced along with tetanus spores, tetanus will develop. The presence of other bacteria, relatively harmless in themselves, may provide suitable conditions in a wound for the germination of tetanus spores. The presence of *Clostridium welchi* or of other members of the gas gangrene group appears to promote the development of tetanus, particularly in war wounds. In short, tetanus spores do not germinate in well-aerated tissue, but when any agent is present that destroys tissue and produces a necrosis, it leaves the tissue without sufficient oxygen and, when the oxidation-reduction potential is lowered to a certain point, the tetanus spores germinate, the organism begins its vegetative multiplication, and produces its toxin. It is the anaerobic nature of the organism that accounts for the fact that tetanus is, in civil life, associated with powder burns and deep puncture wounds. The rusty nail as such is not dangerous but it is more apt to be contaminated with tetanus spores because it has been exposed longer and

it may produce more destruction of tissue and hence more necrosis, thus providing better conditions for the germination of the spores. Unless other bacteria are present, the wound may be so small and there may be so little local disturbance that it may go unnoticed or may be healed at the time generalized tetanus sets in. Tetanus spores can survive for long periods in healthy tissue, germinate when conditions become favorable, and give rise to the so-called ideopathic tetanus. In such cases no wound is evident. It has been suggested that such cases are due to spores that have been inhaled, transported by the lymphatics, and, later, given rise to infection.

Tetanus spores have been found in the war wounds of many soldiers who never developed tetanus.

Sources of Infection: The direct source of tetanus spores is usually the soil. However the spores are in the intestinal tract of animals and man. The spores do not normally escape from wounds and hence the patient is not a dangerous source of infection. Tetanus has been traced to the use of nonsterile catgut sutures and has, in the past, occasionally followed vaccination. However modern methods of controlling vaccine production and the rigorous tests now made for the presence of harmful bacteria have now eliminated that hazard. If tetanus should happen to follow vaccination, it is most likely the result of spores from the soil having gotten into the vaccination wound.

Incubation Period: The incubation period in untreated human cases runs from less than three days to eight weeks, with an average of about seven days. The use of antitoxin prophylactically greatly lengthens the incubation period and in treated cases the average time of onset of symptoms may be forty to fifty days. This suggests that spores persist for long periods in the wound and that, since passive immunization lasts for only about three to four weeks, repeated injections of antitoxin should be given until all danger of the disease is past.

Case Fatality Rates: The case fatality rate in untreated cases may run as high as 85, in fact, it was this high before antitoxin therapy was used prophylactically. The shorter the incubation period, the higher the case fatality and, in general, the deeper and more heavily soiled the wound, the higher the percentage of deaths. Wounds on the upper part of the body are more dangerous than those on the legs or trunk.

Tetanus following infection of the umbilical cord of the newly born is highly fatal. This is called *tetanus neonatorum* and is prevalent in the Negro in our southern states. Among the natives of some of the Caribbean islands it ranks third as cause of death in the new-born.

Immunity: Natural Immunity: There is a wide difference in the natural resistance of different species, a resistance not dependent upon the presence of substances that neutralize the toxin. Although there is a greater

tendency of the horse to injure himself and to suffer cuts and bruises which become infected, horses and mules, for instance, are more susceptible to tetanus than are cattle.

Acquired Immunity: As stated earlier, Von Behring and Kitasato laid the ground work for studies on acquired immunity by showing that the injection of tetanus toxin into animals stimulated the production of antitoxin which neutralized the toxin *in vivo* and which when injected into animals protected them from large doses of toxin. They also showed that the antitoxin protected against active infection with virulent tetanus bacilli. The nature of this protection consists in the neutralization of the toxin. Antitoxin has no effect on the capacity of the bacilli to grow in tissue.

The horse is an excellent animal for use in the production of antitoxin. Tetanus toxin-antitoxin mixtures or tetanus toxoid is first injected and when sufficient antitoxin has developed, toxin alone is injected. When the titre of antitoxin in the serum of the horse has reached a high level, the horse is bled, and the antitoxin is purified and standardized. Tetanus antitoxin has been used extensively in animals, particularly horses, with great success. Its use is indicated following accidental wounds, surgery, and castration.

Tetanus antitoxin is likewise effective in preventing tetanus in humans. The experience of the armies in World War I showed that the case fatality rate after the prophylactic use of tetanus antitoxin became general dropped from about eighty to about fifteen.

The value of antitoxin depends upon the time at which it is given. The toxin combines with nervous tissue and damage to the nerves is responsible for the symptoms. Once the symptoms appear, the damage has been done and antitoxin does not repair tissue damage, it merely prevents it. It follows that to be effective, antitoxin must be given before the toxin has combined with the nervous tissue. While the use of antitoxin is frequently said to have curative value, it is really a preventive measure.

Tetanus toxoid is a highly effective agent for producing active immunity and has been used extensively in horses and humans. It is usual in army practice to give the troops three weekly injections of one cubic centimeter each and to give a booster injection each year. Tetanus toxoid combined with diphtheria toxoid is an effective method for immunizing against both diseases simultaneously.

The reported incidence of tetanus in the unimmunized Japanese army was 10 per 100,000 and there were over 400 cases and 300 deaths in the civilian population during the Manilian operation. There were no deaths reported from tetanus in the immunized American troops in World War II. British experience likewise indicated that in time of war tetanus is a negligible cause of illness or death in immunized persons.

Chapter XXIX

POLIOMYELITIS, ENCEPHALITIS, RABIES, AND PSITTACOSIS

Many infectious diseases produced by bacteria and by viruses show a marked affinity for the central nervous system. Some are old; others are either new to man or newly recognized. Some may be primary, others may be secondary and follow as complications or extensions of infection from some other focus.

Although the diseases produced by the viruses are quite different, it is convenient to treat them as a group. The more important of the primary virus diseases are poliomyelitis or infantile paralysis, various types of encephalitis, and rabies.

POLIOMYELITIS

(Infantile Paralysis or Acute Anterior Poliomyelitis)

Poliomyelitis (from *polio* meaning gray, *myelos* meaning marrow or pith, and *itis* meaning inflammation) is an infection of the central nervous system, particularly of the anterior horns of the grey matter of the spinal cord and of the motor nuclei of the medulla. The *paralytic* type is rather readily diagnosed but there are many mild cases that never show recognizable features. Although it is not so fatal as a number of other diseases, it leaves in its wake a larger number of cripples and handicapped individuals.

Typically, the acute type of poliomyelitis begins with a moderate fever, headache, vomiting, constipation, and periods of sleeplessness, alternating with periods of irritability which may last three to four days. Later there is a stiffness of the neck and spine, tremors, tenderness, and muscular weakness, followed by a flaccid paralysis. The paralysis is due to the destruction of the nerve cells located in the front or anterior portion of the cord. They are the ones that send out the motor impulses which stimulate motion in muscles. When they are destroyed and can no longer send out the impulses, the muscles no longer contract and are paralyzed. The severity of the paralysis depends upon the number of motor cells destroyed. If only a few are damaged, paralysis may be merely temporary.

Not all cases show the gastro-intestinal symptoms. In a small proportion, the first indication of illness is paralysis.

The stage preceding paralysis is called the *preparalytic* stage, and attacks which do not progress to paralysis are described as *abortive*. Such attacks are not readily diagnosed as poliomyelitis and must often go unrecognized. Hence the incidence of the disease is far greater than reported.

Etiology

The specific cause of poliomyelitis is a neurotropic filterable virus. The disease was first reproduced in animals by Landsteiner and Popper, in 1908, who inoculated monkeys with bacterial-free filtrates of emulsions of the spinal cord taken from two fatal cases in humans. In 1910, Flexner and Lewis were able to produce the disease by inoculating monkeys intraperitoneally, subcutaneously, intravenously, and intraneurally with bacterial-free filtrates. At that time they were not able to produce infection by feeding the virus to the monkey.

So great was the interest this disease aroused that many extensive studies followed. Much was learned about the properties of the virus and about the nature of the disease, but the investigators have always been handicapped by the lack of an inexpensive experimental animal. It was not until 1939 that Armstrong found it was possible to infect the cotton rat with one strain of the virus and, shortly thereafter, that mice could be infected with the strain adapted to the cotton rat. These inexpensive rodents have proven very satisfactory for some types of research on poliomyelitis.

Properties of the Virus: The virus of poliomyelitis is extremely small, being between ten to fifteen millimicrons in diameter. It is resistant to drying and cold and will live for at least eight years in fifty per cent glycerol, if kept cold. It will live for at least three months in sterile water, and over a month in milk. Its resistance to heat is not great since it is inactivated in thirty minutes at 42° C. Pasteurization of milk, consequently, will destroy it.

The poliomyelitis virus appears to be considerably more resistant to chlorine than are the intestinal bacteria, a fact of particular interest since the virus has been found in stools of acute and abortive cases and of healthy carriers and has been recovered from sewage. A concentration of 0.5 parts per million of chlorine—more than twice the concentration maintained in drinking water—will not inactivate it in one and a half hours, although it will in four.

Epidemiology

We have mentioned upon several occasions the fact that answers obtained in experimental work always come out in terms of the techniques used, and that the "climate of opinion" influences the deductions drawn from observations. This is nicely illustrated in the case of poliomyelitis, for the concepts of its epidemiology have been modified from time to time as information has accumulated and as newer tools and techniques have been introduced.

In 1840, Heine described individual cases of flaccid paralysis of the lower

extremities of children. The first extensive studies on the epidemiology of poliomyelitis were carried out in Sweden between 1905 and 1911 by Wickman and followed a series of outbreaks that began in that country in about 1880. From its epidemiological features, he concluded that poliomyelitis was a contagious disease, transmitted from case to case or carrier to case by contact, and that it could be transmitted by milk. At this time the infectious agent was unknown.

In 1908, Landsteiner and Popper transmitted the disease to monkeys and isolated the virus. Following this, there were extensive studies on the experimental disease, but they were largely limited to one species of monkey, the *Macacus rhesus*. Features which determine poliomyelitis transmission, such as the route of entry and mode of exit of the infectious agent, were studied intensively in this animal and an attempt made to explain the natural disease in man on the basis of the results obtained from this monkey in the laboratory.

It was shown experimentally that the *rhesus* monkey could be readily infected by rubbing the virus on its nasopharynx; that the virus was present in the nasal secretions of the infected monkey, of human cases, and of carriers; that it was found in highest concentration in the nervous system although it was also found in the glands and stools; and that it was not present in the blood. The disease could not be easily transmitted by intravenous injection but was readily induced by injection into the brain or nerves. Attempts to infect the *rhesus* monkey by introducing the virus directly into the intestinal tract were negative, although if the virus were introduced by way of the mouth, infection occasionally followed.

Since the virus was present in the nasal secretions and since the *rhesus* monkey could be readily inoculated by introducing the virus into the nasopharynx, the idea developed that poliomyelitis was probably a droplet infection. The presence of the virus in stools was not then considered significant and the failure to find it in the blood seemed to eliminate transmission by blood-sucking insects, although the seasonal distribution had suggested this possibility, for poliomyelitis is most common in late summer and early autumn.

Later work showed that the virus was not only present in the stools of of cases and healthy carriers, but that a carrier might harbor it for long periods. During an epidemic, Paul, to whom we owe much of this discussion, and his coworkers, isolated the virus from urban sewage. It was not so readily demonstrable during inter-epidemic times. Perhaps poliomyelitis will have to be put in with the water-borne diseases although epidemiological evidence scarcely supports this point of view. It should be noted, however, that finding the etiological agent of a disease in stools may mean merely that it is excreted, not that the disease is intestinal, nor

that infection takes place through the intestinal mucosa. The causative agents of scarlet fever, tuberculosis, and other non-intestinal diseases can be isolated from the feces.

Since it has been found, more recently, that the *cynomolgous* monkey could be infected readily by feeding, the gastro-intestinal tract has been suspected of being a most likely portal of entry.

The possibility that poliomyelitis might be insect-borne was, as we have said before, suggested by the summer and early autumn prevalence of the disease. In 1912, Rosenau and Brues succeeded in transmitting the disease from monkey to monkey by the stable fly, *Stomoxys calcitrans* and the work was confirmed shortly afterwards, although later attempts were unsuccessful.

In 1941, a group of investigators, Paul, Trask, Bishop, Melnick, and Casey, reported two instances in which the virus was isolated from collections of flies. The first was from flies caught out of doors around the kitchen of a camp in Connecticut where three frank cases of poliomyelitis had occurred and where there were two convalescent intestinal carriers of the virus. The second was from Alabama from flies trapped near privies used by three households where cases of poliomyelitis had occurred. The collection of flies represented many species with green bottle flies, blow flies, and house flies predominating and the various species were not examined separately.

While the finding of the virus in flies does not prove that they transmit poliomyelitis, it is suggestive and indicates that they were able to pick it up and carry it. Obviously it would be of interest to know how and where they got it and what they do with it.

The transmission of poliomyelitis is generally believed to be primarily by droplets and occasionally by milk. Sabin and Ward regard poliomyelitis as epidemiologically similar to typhoid and dysentery in which the chief source of infection is the stools from cases and carriers and transmission is direct or indirect. Since both the house fly and blow fly have been shown to harbor the virus, they may play a part, presumably by contaminating food. However suppression of flies with DDT has not seemed to have any effect upon the incidence of poliomyelitis.

Another observation of interest is that over the world in general there is a positive correlation between the degree of sanitation in regard to water purification and sewage disposal and the number of cases of poliomyelitis.

Incubation Period: The incubation period may be from four to thirty-four days. Twelve days is the average.

Period of Communicability: There is evidence that the infected person is capable of infecting others during the incubation period and for some time

afterward. Multiple cases in families are more frequent than was previously suspected.

Immunity: The nature of immunity to poliomyelitis has been studied extensively and much pertinent information gleaned although we are still in almost complete ignorance about many phases.

All races are susceptible to poliomyelitis and one attack confers an immunity although second attacks do occasionally occur. Monkeys that recover are, in general, immune but the immunity is not absolute.

When a suspension of virulent virus is mixed with the serum from normal monkeys, incubated, and inoculated into other monkeys, they contract poliomyelitis. If the serum from those that recover is mixed with a suspension of virus, incubated, and inoculated into other monkeys, they show no evidence of disease. It is obvious, therefore, that the serum of monkeys recovered from an attack of poliomyelitis possesses the power to neutralize the virus, and that the serum from normal monkeys does not.

By using neutralization tests, that is, by combining human serum with virus and incubating the mixture, it has been possible to get information on the percentage of individuals who have neutralizing antibodies, a percentage much greater than those who have had clinical poliomyelitis. In general, it may be said that infants born of mothers who have neutralizing antibodies also possess the antibodies and are immune to natural infection until they are about a year old, by which time their sera have lost protective power. Few children between one and five have neutralizing antibodies, but the percentage increases progressively with age. About eighty per cent of the adult population possess them.

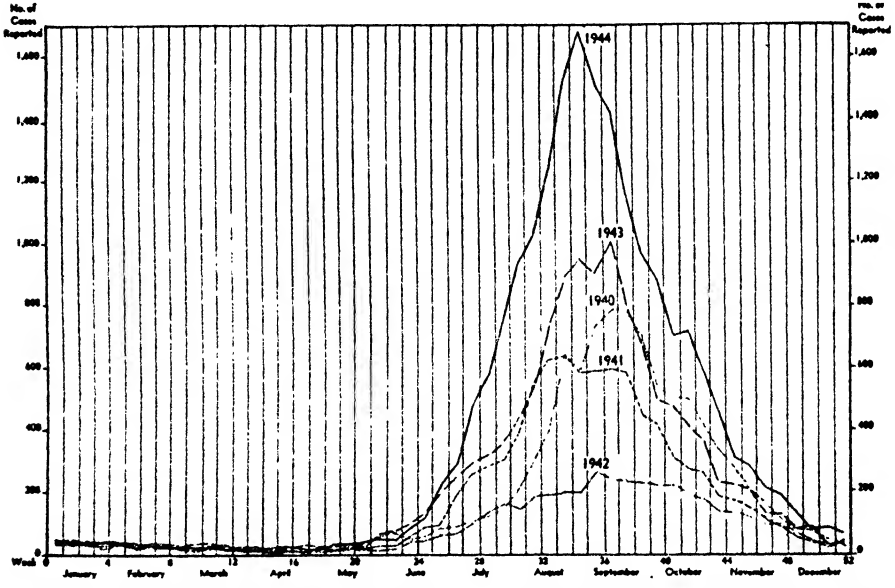
In isolated rural communities there is a lag in the rate at which these antibodies appear.

The presence of neutralizing antibodies to poliomyelitis virus in the population presents a picture very similar to that of scarlet fever and diphtheria. Studies of these diseases have shown a large number of carriers and a large number of Dick and Schick negatives who are immune because they have antibodies in their blood. This indicates that a great many persons who have never had clinical attacks of scarlet fever or diphtheria have become immune as a result of subclinical attacks. Since eighty per cent of the adult population possess antibodies for poliomyelitis and since these do not appear insofar as we know except as a response to infection, it is generally assumed that the poliomyelitis virus is wide spread, that there are many carriers in the population, and that a large proportion of the population has had subclinical attacks of this disease before reaching maturity.

Although finding antibodies following an attack would seem to suggest specific immunity, certain discordant findings make it wiser to observe considerable caution before drawing conclusions. Persons who have a high titre of antibodies in their blood have been known to develop the disease.

The use of immune sera does not appear to have any beneficial effect and attempts to produce an active immunity have not been successful.

Prevalence and Distribution of Poliomyelitis: The early records of poliomyelitis are very incomplete. Sporadic cases appeared from time to time and in 1845 Heine published descriptions of some twenty-seven cases.



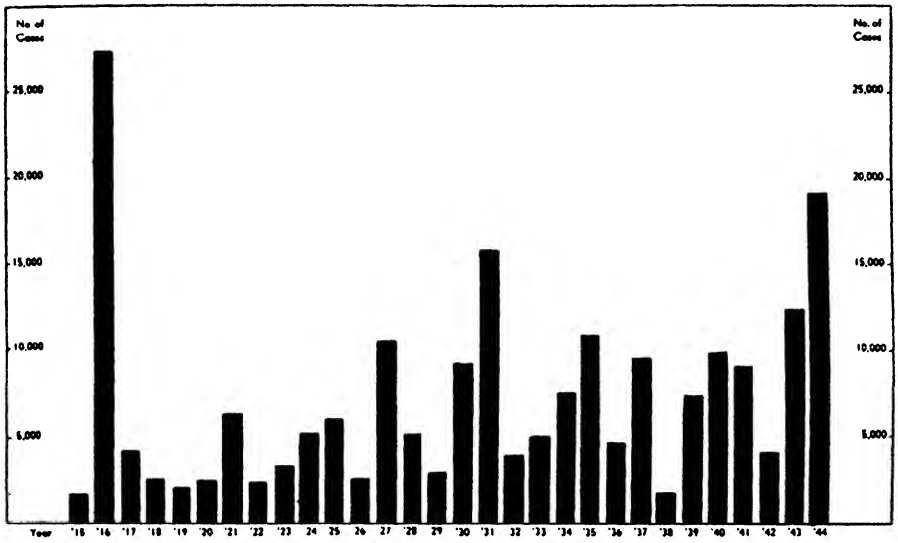
Weekly incidence of infantile paralysis in the United States—1940–1944. Publication #59, National Foundation for Infantile Paralysis.

The first epidemics were those beginning about 1880 in Sweden. In the years that followed, they increased in size and number. The first one recorded in the United States occurred in 1894. There was a progressive increase in the number of outbreaks until 1916 when poliomyelitis swept the entire country. In that year there were nearly 28,000 cases and 6000 deaths. The yearly incidence shows rather wide variation as indicated in the accompanying chart.

The geographic and seasonal distribution of poliomyelitis is of considerable interest. It is a disease of temperate climates and is most common in Scandinavia, Canada and northern United States, Australia, and New Zealand. It is most prevalent in late summer and early autumn.

In recent years severe outbreaks have occurred in nearly all of the states. In general the epidemics have a cyclical tendency. Every four to six years there is an outbreak followed by a period in which there are relatively few cases. In years when the disease has reached epidemic proportions there has been a tendency for the cases to appear early. In a general sort of way, the earlier the cases appear in the spring, the greater the number at peak. In 1946 poliomyelitis reached a high of over 25,000 cases with 1845 deaths. In 1947 there were less than half that number.

In regions such as China and Japan where poliomyelitis is rare the cases that occur are nearly all in children less than five years of age. Although



Infantile paralysis cases reported in the United States 1915–1944. Publication #59, National Foundation for Infantile Paralysis.

there were a good many cases in American troops stationed in Japan, for some unknown reason the disease did not spread to the native population.

There are more cases in males than in females. The ratio is about 56 to 44 and is relatively constant regardless of the severity or size of the outbreak.

Although adults do contract poliomyelitis, most cases occur in children and over sixty per cent in children less than five years old. In recent years however, the trend has been towards a higher attack rate in older persons.

In rural areas, the disease occurs at a somewhat later age, and is correlated with the lag in the appearance of neutralizing antibodies. There is undoubtedly a causal relationship between the appearance of these antibodies and resistance.

Control

The control of poliomyelitis cannot be rational until more is known about its manner of spread and about the factors that influence its transmission. Until such information is available, the expedient thing to do is to follow methods known to be effective in the control of other infectious diseases.

Isolation should be practised for the protection of the patient as well as of the contacts. There is no evidence as to how long the patients should be isolated since it is not known how long they may harbor the virus but it is usually assumed that the period should be for at least two weeks after the onset.

The question of whether exposed children should be quarantined is a pertinent one. Since the outbreaks occur at about the time of the opening of school, the question frequently arises as to whether exposed children should be allowed to attend. In view of our ignorance, it is probably safest to keep such contacts from school for a period of at least two weeks after exposure.

There are, as yet, no effective methods for actively immunizing against poliomyelitis. All attempts at producing effective vaccines have failed. Passive immunization using convalescent or pooled normal serum might possibly be of some value as a curative agent, although the results following its use have not been encouraging.

There are no chemotherapeutic agents of any value. It has been shown that if the nasal passages from monkeys are thoroughly sprayed with alum-picric acid mixtures or zinc sulphate, the monkeys are protected from intranasal infection. This naturally has suggested the use of such sprays for the protection of humans but the results have not been conclusive nor particularly satisfactory.

Another point might be mentioned in passing, and that is the possibility that the normal flora of the upper respiratory tract exercises an antagonistic action against the virus. Armstrong has shown this to be the case with the St. Louis encephalitis virus. If it is also true of the poliomyelitis virus, it may have a bearing upon its seasonal distribution.

Because of our lack of information it is particularly desirable to have accurate reporting of all cases and to check all possible sources of the infection. It seems likely that epidemiological studies of outbreaks will, in time, suggest leads for experimental work and so aid in solving the problem of transmission.

Treatment: When paralysis of the respiratory muscles occurs, artificial respiration using the 'iron lung' is of benefit and has saved many lives.

A great deal of progress has been made in the treatment and after care of paralytic cases. Skillful massage and special exercises have restored

the use of their limbs to many who, in previous years, would have remained hopelessly crippled.

ENCEPHALITIS

Encephalitis, from the word *encephalon* meaning brain and the suffix *itis* meaning inflammation, refers to a characteristic body reaction to many harmful agents: bacterial, virus, protozoan, physical, or chemical. In all of these cases the symptoms are referable to inflammation of the brain and damaged nerve tissue and are characterized by sleepiness or sleeplessness, restlessness, twitching of muscles, paralysis of muscles—particularly those of the eye, mental confusion, alteration of facial expression, severe headaches, and stiffness of the neck. Recovery is usually complete, although some cases show permanent impairment.

There are several encephalitides due to viruses. Of greatest interest to us, perhaps, are St. Louis encephalitis, equine encephalomyelitis, and lymphocytic choriomeningitis.

ST. LOUIS ENCEPHALITIS

In 1933, over one thousand cases of encephalitis occurred at St. Louis, Missouri, about half in the city and the other half in the surrounding districts. This outbreak was shown by Muchenfuss, Armstrong, and McCordock to be due to a virus, now called the St. Louis encephalitis virus. A smaller outbreak had occurred the previous year at Paris, Illinois, but had not been recognized as a new disease. Several have occurred since, particularly in the section around St. Louis. The case fatality rate varies with age, being about four per cent in persons between twenty and twenty-nine and eighty per cent in persons over eighty.

The epidemiological features are of interest. The St. Louis outbreak began in July and ended in October, the attack rate was highest in the older age groups, and the disease occurred along streams, refuse dumps, and open sewers. Water, food, and milk transmission seemed to be ruled out; case to case transmission was not demonstrated; but the agency of an insect, presumably the mosquito, was rather clearly suggested although it was not proven. Since the disease did not seem to be spread by contact, infection by droplets could not have been common.

One attack leads to the appearance of neutralizing antibodies in the serum. Experimentally the mouse is susceptible and it has been suggested that it might be the animal reservoir. It has also been shown that this virus produces an encephalitis in the horse similar to that produced by the equine encephalomyelitis virus and antibodies to it have been found in horses in many states. Birds and rodents have also been found to harbor antibodies.

EQUINE ENCEPHALOMYELITIS

The name "equine" implies that this disease is primarily a disease of horses and mules, and, to be sure, the first recognized outbreaks were in these animals. Subsequent events have shown that the virus produced an encephalitis in man and is widespread in nature. In the light of our present knowledge, it is reasonable to assume that the disease is accidental in both man and horse, and that the natural host is probably wild fowl.

Equine encephalomyelitis has been the most destructive disease of horses and mules in America in recent years. In 1930, an outbreak occurred in the San Joaquin Valley in California, and Meyer, Haring, and Howitt showed that it was caused by a virus. In 1933 and 1934 a more severe encephalomyelitis occurred along the eastern seaboard, and Ten Broeck and Merrill isolated a virus, immunologically distinct from the one first found in California. The viruses are now known as the Eastern and Western type of equine encephalomyelitis virus. The Eastern type appears to be restricted to the region east of the Applachian Mountains, and the Western type is widespread and responsible for outbreaks in states west of the Mississippi and in midwestern Canada.

In 1931, there were 4000 cases of the disease reported in horses. In 1937, there were 170,000 and Minnesota, North and South Dakota, and adjoining states were particularly hard hit. In 1939, only 8000 cases were reported.

Equine encephalomyelitis occurs only in the summer and early autumn, terminating with the first frost. The distribution is spotted and horse to horse transmission has not been demonstrated. In many instances one horse on a farm has been attacked and even though it has been stabled with other horses, they do not contract the disease. This is particularly interesting in view of the fact that the virus is present in the nasal secretions and that horses can be infected experimentally by instilling the virus into the nostrils.

Epizootiology

Evidence points to mosquito transmission and a number of species of mosquitoes has been shown to be capable of transmitting both the Eastern and Western type of disease. To be mosquito transmitted, the virus must be present in the blood stream. In the horse, it is there a short time only so it seems unlikely that the horse is a common source.

As early as 1933, Giltner and Shahan showed that pigeons were susceptible and suggested that they might serve as a source of the infectious agent. In 1939, the Eastern type of virus was isolated from naturally infected ring neck pheasants, thus indicating that these birds might also be

involved. In 1941, Cox isolated the virus from a prairie chicken shot in the field, and so the evidence incriminating birds as a reservoir mounts.

Hammon and his coworkers approached the problem of possible reservoirs from a slightly different angle. Knowing that infected animals develop neutralizing antibodies which are retained in the serum for long periods, and that it is difficult to isolate the virus except in certain and often brief stages of the infection, the workers examined the blood of domestic and wild fowls and animals, particularly rodents, in the region around Yakima, Washington, where an outbreak of encephalomyelitis had occurred in 1940.

They looked for neutralizing antibodies for the St. Louis encephalitis virus and for the encephalomyelitis virus. Fifty per cent of the domestic fowls and fifteen per cent of the wild fowl contained such antibodies for the St. Louis virus; forty-eight per cent of the domestic fowl and only twenty per cent of the wild, for the equine virus. Sixty-nine per cent of the domestic animals and thirty-eight per cent of the wild animals showed neutralizing antibodies for the St. Louis virus. When tested against equine virus, thirty-two per cent of the domestic animals and five per cent of the wild were positive. It would seem that both St. Louis encephalitis and equine encephalomyelitis are very widely distributed in both domestic and wild birds and animals. In 1941, these same workers isolated both viruses from mosquitoes in the same region. All evidence points to the conclusion that the equine encephalomyelitis virus is widespread in birds and is found in mosquitoes, but that the disease in horses is probably incidental and that the horse does not play a part in keeping the infection alive.

ENCEPHALITIS IN MAN DUE TO THE EQUINE ENCEPHALOMYELITIS VIRUS

Although in 1932 and 1933, Meyer had observed three cases of encephalitis in persons in contact with infected animals and had suggested that they might be due to the equine virus, and although in 1937, several cases of human encephalitis had occurred in Minnesota during the height of the outbreak in horses; the first proven cases of this disease in man occurred in 1938 in Massachusetts during an outbreak in horses. In 1940 there was a number of such cases in Washington. The greatest outbreak to date occurred in the summer of 1941 and was centered in North Dakota. According to a preliminary report by Leach, there were 1080 cases in North Dakota, 815 in Minnesota, 434 in Manitoba, 180 in South Dakota, and only 64 reported from Montana.

The cases were mainly in persons living on farms or in small towns. The disease arose in all parts of the state and at about the same time, indicating that it did not spread from a single focus. Cases did not appear to give rise to other cases.

The age and sex distribution of the disease is of interest and is probably explained on the basis of exposure.

	Number of Cases	
Age in years	*Male*	*Female*
Under 1	27	23
1 to 14	82	64
15 to 24	125	29
25 to 44	170	51
45 to 64	176	77
65+	96	43

The epidemiological features of the outbreak point to insect transmission. The season was unusually wet and mosquitoes were extremely numerous.

It is of interest to note that the disease was not so widespread nor so severe in horses as it was in 1937 when no human cases were reported.

In North Dakota, the case fatality rate, as calculated from the number of reported cases, was 8.9; but it seems likely that in reality it was much lower since many cases were not reported.

Higbie and Howitt have developed a vaccine prepared by inoculating the virus into the developing chick embryo. It produces an immunity which protects the horse for at least one season and its usefulness as a practical procedure has been definitely established. Second attacks of equine encephalomyelitis and attacks in vaccinated horses are probably due to the St. Louis encephalitis virus for the two do not cross-immunize.

The control of the disease in man can probably be accomplished by using the chick embryo vaccine but information on its use is, as yet, limited. Treatment is largely symptomatic. Antisera and chemotherapeutic agents are not effective.

LYMPHOCYTIC CHORIOMENINGITIS

While working on the St. Louis encephalitis epidemic, Armstrong and Lillie injected monkeys with material from the brain of a patient who had died of encephalitis in the 1933 epidemic, and recovered a virus not previously described. It was different from the St. Louis virus immunologically and pathogenically. It produced a choriomeningitis in monkeys and their spinal fluid showed an increase in lymphocytic cells and not in the polymorphonuclear or pus cells associated with bacterial meningitis.

In 1935 Rivers and Scott isolated the lymphocytic choriomeningitis virus from two cases of acute meningitis in humans and the etiology of a mysterious disease, sometimes called aseptic meningitis and sometimes known by other names, was cleared up.

The disease, which has been reported from all parts of the world, runs a mild course and resembles mild cases of poliomyelitis and other diseases of the central nervous system. In the acute type, the onset is sudden and

the patient shows such signs of meningitis as severe headache, drowsiness, stiffness of the neck and back, vomiting, and photophobia. There may be retention of the urine and diarrhea. The acute stage is often preceded by the symptoms of a cold or mild influenza. There is a fever of 100° to 103° F. in the early stages but it falls during the second week. The disease runs its course in three to six weeks and complete recovery usually follows. The case fatality rate is extremely low.

A few mild epidemics have been reported, but the cases are usually sporadic and cannot be traced to previous cases.

The disease appears to be endemic in mice and outbreaks have been reported in stocks used for experimental purposes. Armstrong and Sweet isolated the virus from mice caught in a house in which there were two human cases.

The mode of infection of man is unknown. The virus is excreted in the urine of mice but transmission from mouse to man has not been definitely proven. Droplet transmission has been suggested since the early symptoms are upper respiratory and cases have been reported following tonsillectomy.

Of particular interest is the finding that twelve per cent of a population giving no history of attack had neutralizing antibodies, indicating that the disease is rather wide spread in man and that only the acute cases are recognized. Only 1.2 per cent of persons less than seventeen years old showed antibodies; so the infection does not, apparently, usually occur in children.

The virus can be cultivated in the chick embryo. It lives for at least seven months in fifty per cent glycerine, for three months in the dried and frozen state, and is destroyed in twenty minutes at 56° C.

No specific therapeutic measures are available. Isolation of cases is not suggested, for case to case transmission does not seem to occur.

RABIES

Rabies or hydrophobia is another disease present since ancient times. It was accurately described by the Hindus a thousand years before the Christian era. The bacteriologist is particularly interested in rabies because it was while working with this disease that Pasteur laid down some of the foundations of the science of immunology.

Recognition of Rabies

Rabies is an acute infectious disease, primarily of animals, and particularly of dogs, but is readily transmissible to man. The virus is neurotropic and the symptoms are referable to an involvement of the nervous tissue.

Symptoms in Dogs

The symptoms of rabies in dogs have been divided into three stages: prodromal, furious, and dumb or paralytic.

Prodromal: It has often been said that the first sign of rabies in dogs is a change in disposition; the ugly dog may become very friendly and the friendly, playful dog may suddenly become vicious or dejected and want to hide. There is usually a restlessness which later develops to great excitability.

Furious: It is the second stage of great irritability and restlessness that has given rise to the term "mad dog." The animal may suddenly start on what has been termed the "rabid run." He will run about or run long distances, snapping and biting, particularly at any moving object. This characteristic is responsible to a large extent for the spread of the disease. The dog may bite live stock, other dogs, or humans. Children playing and running about are particularly apt to be bitten. Because of the nerve involvement there is a paralysis of certain throat muscles and the dog has a difficulty in swallowing. This is responsible for the impression that he hates water and, hence, for the descriptive term, hydrophobia (*hydro* meaning water, and *phebomai* meaning fear) so often applied to the disease. Frothing of the mouth is also attributable to his inability to swallow. Sometimes it seems that the dog has a foreign object, such as a bone, in his throat, and the owner may reach in to attempt to remove it. The bark of the dog may become throaty or change to a peculiar howl. It is during this period that the dog is most dangerous.

Paralytic or Dumb: The dog may run until exhausted or until paralysis sets in. Then he stumbles and falls, the froth at his mouth becomes tinged with blood, and death follows shortly.

In many dogs the symptoms vary from the typical course described. The first and second stages may be unnoticed or absent and the animal may present only the paralytic or dumb stage. This has led to the statement that there are two kinds of rabies, furious and dumb. All dogs become paralyzed before death. Verification of the diagnosis depends upon laboratory methods.

Etiology

The specific cause of rabies is a filterable neurotropic virus. Like all viruses, it is an obligate parasite and cannot be cultivated on artificial media. Several features of the virus should be borne in mind. It parasitizes the central nervous system and the symptoms of rabies are referable to the damage resulting to nerve cells. It is found in the nervous tissue and in the saliva, where some workers believe it comes from the infected nerve cells of the salivary glands. The virus has also been found in

other tissues. There is some question as to whether it may be present in the milk of infected cows as has been reported.

Animal experimentation shows that the virus travels along the nerves, not through the blood system, from the point at which it was introduced to the central nervous system; and that the symptoms do not appear until the latter has become involved. This explains the long incubation period. The closer to the brain the virus is introduced, the shorter the period and the more dangerous the bite since there may not be time for protective immunization to be established.

The rabies virus produces a characteristic change in the nerve cells. In the cytoplasm mostly, and sometimes in the dentritic prolongation of the nerve cells, there appear bodies of varying shape, ranging in size from one to twenty-five or thirty microns, with an average of about three to ten. These bodies, first described by Negri and now called *Negri bodies*, consist of a more or less homogeneous mass containing corpuscles within which there are smaller bodies consisting of short rods or granules. They are most commonly in the part of the brain known as Ammons horn. They are characteristic of rabies and are of diagnostic importance. These Negri bodies probably represent a sort of colony of virus, but the visible aggregates are not a single organism.

The virus of rabies undergoes a change in virulence when it is passed in series in rabbits. When the virus is isolated from rabid dogs it is called "street" virus. When a rabbit is inoculated subdurally with "street" virus, the incubation period is about two weeks. When virus is taken from this animal and used to inoculate another rabbit, the incubation period is shortened and, after successive passage through forty to fifty rabbits, it is down to about seven days. When further passage does not materially reduce the incubation period, the virus is said to be "fixed."

The chief difference between "street" and "fixed" virus lies in the greater affinity of the "fixed" virus for the tissues of the central nervous system of the rabbit. "Fixed" virus, being constant in its virulence, is used as an immunizing agent.

Incubation Period: Rabies is unique among acute infectious diseases in respect to its incubation period which is long and dependent upon the site of inoculation. The incubation period in man is rarely as short as sixteen days and often as long as ninety, with an average of about forty-two. In dogs, it varies from about fourteen to ninety days, with an average of about twenty-one. In other animals it likewise varies from about fourteen to ninety days.

Because of the long incubation period and because the time of exposure is usually known, since most cases result from dog bites, it is possible in practice to inoculate an individual after exposure and induce an active

immunity which will be effective before natural infection reaches the central nervous system. The procedure used is either that developed by Pasteur or a modification of his method.

Pasteur Treatment for Rabies: The Pasteur treatment for rabies is not a treatment in the usual sense, since it is not a clinical treatment of the disease after the symptoms have appeared; but is rather a preventive treatment, effective only if started early enough to produce active immunity before the symptoms appear.

The Pasteur treatment is based on Pasteur's findings that the virus could be inactivated by drying in air over such agents as potassium hydroxide. When the cord from rabbits that have died from rabies is dried for five days, it is usually no longer infective. By eight days, it is completely inactivated. Pasteur's original method consists, in brief, of giving a series of about fourteen injections over a period of about that many days. The first injection consists of an emulsion of cord dried fourteen days, the second of cord dried thirteen days, and so on, until on the fourteenth day when cord dried one day only is used. By the end of two or three weeks, dogs so treated can tolerate the injection of material so virulent that it would kill non-treated dogs. There have been many modifications of the method of preparing the virus for immunization, some of which are inferior and some superior to Pasteur's original method. The Semple method is most widely used.

Case Fatality Rate: The case fatality rate of rabies in humans is one hundred per cent. Although by no means all persons bitten by rabid dogs develop rabies, once the symptoms have appeared, the outcome is invariably death. There is no record of a human being ever recovering from rabies.

There is some question as to whether dogs may survive infection; but if they ever do, it is so seldom that the canine fatality may also be considered about one hundred per cent.

Source and Mode of Transmission: The source of the virus is chiefly the saliva of rabid dogs, although other rabid animals also carry the infectious agent. The disease is not transmitted from man to man. The virus enters the wound made by the bite of the rabid animal and the nature and severity of the wound is of considerable importance in determining the possibility of infection and the length of the incubation period. Wolf bites are most dangerous because they are apt to be deeper and there is greater chance for the virus to become established. The virus, too, is said to be more virulent. The bite from horses or cattle is far less dangerous because their teeth are such that they do not pierce the skin. Bites through heavy stockings or trousers are less apt to lead to infection because there is a good chance that the virus will be wiped off the teeth or absorbed by the cloth-

ing. The more severe the bite and the closer to the head, the more dangerous. Bites on the face are, of course, particularly bad.

Period of Communicability: The virus is present in the saliva of dogs for three to eight days before the onset of symptoms. This makes it difficult to decide what course to follow when a dog which is a child's pet and has probably been licking the child's face and hands more or less regularly, becomes rabid. Although the virus does not presumably penetrate intact skin, it is not always certain that the skin is intact just because obvious wounds are absent. Scratches and inapparent breaks might allow the entrance of the virus. As a matter of fact, there are no records of infection incurred in this manner.

The dog remains infective until death and the virus survives for some days after, so that the carcasses of rabid dogs should be handled with care. Of course, the greatest danger is during the furious stage of the disease when the dog becomes mad and bites without provocation.

Susceptibility and Immunity: All mammals are susceptible to rabies. Wild animals, such as the wolf, fox, skunk, rabbit, rat, and squirrel are easily infected. Even birds are susceptible although they do not become readily infected in nature. Domestic animals, such as the horse, mule, and cow, are readily infected, indeed rabies in animals is an economic problem in some southern states. There was an interesting outbreak of rabies in cattle in Brazil and another in Trinidad in which the disease was spread by blood-sucking bats.

Prevalence and Distribution: Rabies is world wide in distribution although a few countries are free from the infection. It has been introduced into England, eliminated, reintroduced, and again eliminated by prompt action. The Scandinavian countries, Iceland, and Australia are free from the disease.

In the United States, it has been eliminated from a number of states but the danger of reintroduction is extremely great. The modern tendency to travel by trailer presents a distinct hazard in regard to rabies because persons travelling in that fashion are more apt to take their dogs with them.

Rabies, of course, occurs most frequently in dogs. There is a common superstition that the season known as "dog days" drives dogs mad. This, of course, is not true. "Dog days" have absolutely no connection with rabies, which is more common in colder months.

The disease is rare in humans but is more prevalent in men than in women and occurs most frequently in persons less than twenty years old. Forty to fifty deaths a year are reported in the United States.

Control of Rabies

The factors which determine the transmission of rabies are known and

methods for its control are readily available. However, in actual practice, control is difficult, not because the procedures are complicated or expensive but because of the attitude of a great many people. Control of disease is often largely a problem in human psychology even when it concerns dogs.

The control or elimination of rabies depends primarily upon the control of the dog population. The measures employed are humane and logical and dog lovers should support them.

License: All dogs should be licensed, and the unlicensed, ownerless, and homeless dog, destroyed. The wandering dog is obviously particularly dangerous since he is likely to infect other dogs over a wide area.

Muzzling: Since rabies is transmitted from dog to dog and from dog to man by bites, it is obvious that a muzzled dog cannot transmit the infection. Since there are no carriers of the virus and since all infections terminate fatally, consistent muzzling of all dogs for a period as long as the incubation period of the disease—in practice, for about two years—would eliminate the virus except for what is resident in wild animals.

Quarantine: In regions where rabies is known to exist and where dogs have possibly been exposed, quarantine should be in effect for at least six months. This length of time is necessary because of the long incubation period of the disease.

Vaccination: Vaccines have been developed which are said to protect dogs against rabies. The preparation usually employed is a phenol or chloroform inactivated virus. In one method, only one injection is given. Immunity lasts for about a year and dogs must be revaccinated every year.

The effectiveness of the one-injection method of vaccination is open to question and cannot be entirely relied upon. Another method requiring a number of injections is more effective but is also much more expensive.

In order to be effective, muzzling and quarantine must include all dogs. Halfway measures or ineffective enforcement do not control the infection and serve only to shake the confidence of the public in the measures. When muzzling and quarantine measures have been enforced consistently, they have proven effective.

The prevention of rabies in exposed persons consists of two steps: first, local treatment of the wound, and, second, active immunization by use of the Pasteur treatment.

The wound produced by a rabid animal should be cauterized with fuming nitric acid introduced drop by drop. Such treatment is apparently effective if cauterization is done even twenty hours after the patient is bitten, but it is advisable to treat the wound as soon as possible. The common disinfectants such as iodine, mercurochrome, and such, do not cauterize and are not effective.

The Pasteur treatment of immunization should be begun immediately.

When to Vaccinate: One of the most difficult questions to answer is what to do after being bitten by a dog who may be rabid or may be just angry. The following procedure has been suggested for instances of this kind.

If there is any reason to believe that the dog might be rabid, or if rabies is known to be present in the area, call a physician, have the wound cauterized, and begin the Pasteur treatment.

Do not kill the dog, but have him kept tied up and under observation by a competent veterinarian for a period of two weeks.

The natural reaction after being bitten by a dog or after seeing a child bitten is to get a shot gun and kill the dog. This should not be done unless the dog cannot be captured and it is absolutely necessary for the safety of others. Then the dog should never be shot in the head, for the brain is necessary for diagnosis. Even then diagnosis of rabies cannot be made readily by laboratory examination unless the disease has progressed far enough for characteristic changes to have taken place. Negri bodies are not present in the early stages of the disease. If the dog can be kept chained or caged and is rabid, the disease will usually be recognized within a few days. After death of the dog, its head should be sent to the State Health Department where confirmation of the diagnosis will be made by microscopic examination and animal inoculation. If the dog was not rabid, the Pasteur treatment should be discontinued.

In case the bite is due to a stray dog which cannot be identified or captured, the only safe course is to take the entire course of injections.

In the last analysis, the control of rabies depends upon educating dog owners to the dangers of the disease in the hope that they will cooperate in enforcing muzzling and quarantine measures, if not for the protection of their fellow men, for the protection of their dogs.

Summary: Rabies is an infectious disease due to a specific virus which parasitizes the nervous system. The outstanding features of rabies are its long incubation period and its high case fatality rate. Rabies is contracted from the bite of an animal, usually a dog. Prevention is based on control of the dog population, licensing and fixing of owner responsibility for the dog, muzzling, and quarantine when rabies is present. Muzzling and quarantine are effective control measures.

Active immunity can be produced by use of the Pasteur treatment.

There are no curative measures, drugs, or sera of any value and once symptoms appear the animal or person invariably dies.

PSITTACOSIS OR PARROT FEVER

Psittacosis is an acute infectious virus disease, primarily of parrots, parrokeets, and love birds, hence its name which comes from the Greek word, *psittakos* meaning parrot. The disease is not confined to members of the parrot family but occurs in other birds, in animals, and in man.

Recognition of the Disease in Parrots

In parrots the disease is characterized by diarrhea, ruffling of feathers, shivering, sneezing, weakness, and loss of appetite. Recovery often occurs and then the bird is immune for some time. Characteristic lesions appear in the spleen, liver, and, sometimes, in the lungs. The virus can be recovered from these tissues, as well as from the blood and nasal discharges.

Many animals are susceptible experimentally. The white mouse is a reliable diagnostic animal. Characteristic lesions appear in it and *elementary bodies* may be demonstrated by suitable methods.

Recognition of the Disease in Humans

The onset of the disease is characterized by chills, fever, headache, and weakness. After a short time the lungs may become involved and a cough may or may not be present. The sputum may be yellowish and thick, the tongue heavily coated. Other symptoms are loss of appetite, constipation, delirium, and pains in the chest and abdomen. Phlebitis is said to be a common complication and convalescence is slow.

Psittacosis may be confused with a number of diseases, as for example, influenza and typhoid fever. The incubation period is from five to fifteen days. The case fatality is from twenty to forty per cent, increasing with the age of the patient.

Laboratory findings are albumin in the urine and a low white blood cell count after the first few days. Diagnosis may be made by inoculating white mice with sputum or blood and demonstrating the characteristic elementary bodies or the Levanthal-Coles-Lillie (L.C.L.) bodies.

Etiology

The specific cause of psittacosis is a virus. Not much is known about its properties but it is infectious for a number of birds and animals and can be grown in tissue culture though not in the absence of living cells.

Source and Transmission: Common sources of the infection are parrots, love birds, and canaries. Man contracts infection from contact with the infected birds, both active cases and carriers. Since the virus is present in the nasal discharges and droppings, these probably play an important part in spreading the infection. The disease is highly contagious among birds but does not appear to be so from person to person. The habit of kissing birds or feeding them from the mouth is very dangerous.

The infection is an extremely dangerous one to investigate in the laboratory and is very often contracted in spite of great care. This is accounted for by the fact that the virus is air-borne and the infected birds flap their wings and so disseminate the dried matter containing the virus.

Prevalence and Distribution: Psittacosis is a rare disease in man. It is more common in females than in males, not because they are more susceptible but because of their closer association with pet birds. It is more common in the age group twenty to fifty than in the younger group. Cases in younger persons are usually milder and the case fatality rate lower. The disease usually occurs as localized outbreaks among persons handling birds. Several cases may occur in the same household, but they are the result of contact with sick birds and not of person to person transmission.

In the Faroe Islands, psittacosis occurs in persons who handle the Arctic Fulmars, birds which are caught and used as food. The disease is confined principally to the women who pick the feathers off the birds and occurs during August to September when the birds are caught.

Control

The prevention and control of psittacosis demands a recognition of the dangers of this disease. Government regulations govern the importation and shipping of birds belonging to the parrot family and keep infected birds out of the country. However, the disease is endemic in parts of California and in spite of efforts made to prevent its spread, there is a danger from this source. Another point to be kept in mind is the possibility that other birds not now known to carry the disease may become infected and transmit the infection to man.

The individual can prevent infection by observing a few practical precautions:

Never purchase pet birds until being assured that the breeder, or distributor does not have psittacosis in his stock.

Exercise extreme caution in handling sick birds and never kiss or feed birds from the mouth.

Chapter XXX

MENINGITIS

The term meningitis refers to an inflammation of the meninges, the three membranes that envelop the brain and spinal cord. It is a clinical and not an etiological concept for it may be due to a large variety of organisms and may be a primary disease or a secondary localization or extension of an infection of some other part of the body.

Meningitis may be chronic or acute. When it comes as a secondary manifestation it is usually acute and the case fatality rate in untreated cases is extremely high. Tuberculous meningitis, secondary to tuberculosis of some other part of the body, is about one hundred per cent fatal. Untreated cases of meningitis due to staphylococci, streptococci, or pneumococci are likewise highly fatal. Meningitis may follow typhoid, plague, and a host of other infections, and bacteria not considered pathogenic ordinarily, such as *Escherichia coli*, occasionally produce meningitis. The fact that a meningitis may be produced by so many different bacteria, most of which show little virulence under ordinary conditions, is evidence that the meninges are highly susceptible to infection. Once bacteria have effected an entrance, they find conditions there very favorable for growth.

The most important primary meningitis is due to a gram-negative coccus which causes a cerebrospinal meningitis.

MENINGOCOCCUS MENINGITIS

Meningococcus meningitis, cerebrospinal meningitis, or cerebrospinal fever is an acute infection characterized by an inflammation, usually at the base of the brain, and the symptoms are referrable to damage to this region. The onset may be so sudden and the disease so acute that death occurs within twenty-four to forty-eight hours after the first evidence of illness. There is an intense headache, nausea, often vomiting, and frequently, a rash. In epidemics the subcutaneous hemorrhages give rise to the so-called purpuric or purple spots. They are a common occurrence and hence the name "spotted fever" is sometimes given to this disease.

In some instances the disease is more chronic and recurrences or relapses are then common.

Etiology

The cause of cerebrospinal meningitis is *Neisseria meningitides*, a gram-negative coccus. Because it is frequently found intracellularly in phagocytic cells in the spinal fluid it is also sometimes called *N. intracellularis* but it is more commonly known as the *meningococcus*.

The cocci usually appear in pairs with the adjacent sides flattened resembling coffee beans but they may appear singly, in tetrads, or in larger aggregates.

There are eleven species of Neisseria. All but two are aerobic. All are parasitic and typically associated with the mouth and upper respiratory tract. *N. gonorrhea* and *N. meningitis* are frankly pathogenic. *N. catarrhalis* and several others have been associated with mild upper respiratory infections.

There are a number of varieties or serological types of the meningococcus and these differ in virulence. Cultures readily lose their virulence upon subculture.

The meningococcus does not grow on ordinary laboratory media and a medium containing blood and known as "chocolate" agar is usually used for abundant growth. It will grow on synthetic media containing glutamic acid. Physiologically the *Neisseria* behave more like the gram-positive than like gram-negative bacteria.

The meningococcus is a very delicate organism, readily destroyed by drying, direct sunlight, or heating. In subcultures it dies rapidly after three or four days and transfers must be made frequently to keep the culture alive.

Epidemiology

Meningococcus meningitis occurs as sporadic cases, chiefly in young children, and is endemic in cities in the late winter and early spring. There seems to be no direct connection between cases. Every five or six years epidemics occur in which the most severe cases are in young adults. Such epidemics are frequent in camps and were very severe in World War I. The epidemics are peculiarly erratic in their behavior. A camp epidemic may spread to other camps and to civilians or it may remain localized. There is no good explanation for its spread or localization but it seems quite probable that the virulence of specific strains, the effect of crowding on transmission, and the effect of exposure and fatigue on resistance of the individuals are critical factors in determining epidemic tendencies.

Source and Mode of Infection: Meningococci are inhabitants of the nasopharynx and are transmitted by droplets. The carrier rate is variable and proportional to the degree of crowding. It may reach fifty to seventy per cent during epidemics and may be as high as twenty-five per cent in the absence of cases. The normal carrier rate is usually said to be from two to five per cent. While the case is more dangerous than the carrier, the latter keeps the infection alive.

The period of incubation is usually about seven days but varies from two to ten. Probably many persons carry the organisms for longer periods before becoming ill.

The period of communicability is variable and for as long as the individual harbors the infectious agent. The use of penicillin and sulfa drugs eliminates the carrier state in about twenty-four hours.

The prevalence of meningococcus meningitis varies widely in any region and from year to year. Epidemics last two to three years and then subside.

Control: The control of meningococcus meningitis consists of the isolation of patients and treatment with sulfa drugs, penicillin, and streptomycin. These agents not only reduce the case fatality rate but limit the period of communicability. (For a discussion of the streptomycin resistant and requiring strains of the meningococcus, see page 141).

Since meningococcus meningitis is frequently epidemic in military camps and since a high carrier rate is usually associated with epidemics, attempts to control epidemics by treating the population at risk with sulfa drugs were tried early in World War II. In one camp the carrier rate in the untreated controls rose from thirty-eight per cent to fifty-five per cent during the period of the experiment while the rate in the treated group dropped from thirty-six to three and one tenth per cent. Twenty-three cases appeared in the control group and none in the treated group. Other experiments confirmed these results.

Vaccination to produce a permanent immunity has been tried but the results are not encouraging. Serum treatment has been used fairly successfully but chemotherapy is far more effective.

INFLUENZAL MENINGITIS

This appears to be a primary disease and is caused by the influenza bacillus, *Hemophilus influenzae*. It is a gram-negative rod. This organism was originally described as the cause of the 1889 influenza epidemic. Later research, however, proved influenza to be a virus disease. About eighty per cent of the cases of influenza meningitis occur in infants and children less than three years of age. The case fatality rate was over ninety-five.

Streptomycin appears to be very effective, particularly if given early.

SECONDARY MENINGITIS

Meningitis may also be produced by the pyogenic cocci: the staphylococci, streptococci, and pneumococci. In fact, it is a not uncommon complication of pneumonia and the middle ear infections due to these organisms. In untreated cases the fatality rate approaches one hundred per cent but the sulfa drugs and antibiotics have been dramatically effective, not only in treating the secondary meningitis but in preventing it when they are used in the treatment of the primary infection.

TUBERCULOUS MENINGITIS

Tuberculous meningitis, as previously noted, is secondary to tuberculosis of some other part of the body. It occurs most frequently in young children and is responsible for from two to three per cent of all deaths from tuberculosis. It has been considered fatal. Streptomycin, however, shows considerable promise in the treatment of this infection.

Chapter XXXI

FOOT-AND-MOUTH DISEASE

Disease is a major problem in production of livestock. In 1942 and in the United States alone the economic loss suffered from the more important animal diseases was conservatively estimated at over $400,000,000. In many countries the percentage loss is much higher. Although the milder and more wide-spread diseases are more serious in the aggregate, in a severe outbreak a single farmer can lose eighty to ninety per cent of his output. With food the number one problem confronting the human race, cutting the percentage losses in the animal industry becomes the concern of everyone. The factors that determine the manner of transmission of infectious diseases of animals and man are essentially alike. The problems of control are also in many respects the same but the measures used to control may be somewhat different because of the difference in the value placed on individual animals as compared with that placed on individual humans.

The control of animal disease requires a great deal of technical information and highly enlightened husbandry. In general the methods used are: the control of insect vectors, sanitation, immunization, and slaughter. In some diseases caused by parasites harbored in the soil, rotation of pasture can be used to break the infective chain. In some, as for example, in hog cholera, immunization is widely practiced. In some, providing the infection rate is relatively low, it may be most practical to slaughter the diseased animals. The practicability of slaughtering as a control measure differs with the epidemiology of a disease and with the different species of farm animals.

An example of an animal disease of great importance and one which clearly demonstrates rather special control measures in foot-and-mouth disease.

Foot-and-mouth disease is a highly communicable disease of cloven-footed animals. Although regarded as a disease of cattle, other cloven-footed animals such as goats, sheep, deer, antelope, bison, and camels are susceptible. Man is not readily infected although a few cases have been reported and the milk from cows showing involvement of the udder produces a serious illness in humans.

Symptoms: As the name implies, the foot and the mouth are characteristically affected. The typical lesions are blisters or vesicles which appear on the mucous membranes covering the tongue, cheeks, palate, and lips, and also on the skin at the top of the hoof and between the toes. The intestinal tract is also involved and in cattle, the udder and teats may be

affected. In swine, the snout shows characteristic lesions, while in deer, sheep, and goats, lesions on the feet are most common. Only one or all four feet may be affected.

The vesicles usually rupture in twenty-four hours leaving a red, raw, eroded surface. Pustules, that is, lesions, filled with lymph or pus, are not formed. The lesions may heal rapidly but are prone to secondary bacterial invasion. In the more severe cases one or even all of the claws may drop off. The sores are very painful and the animals become so lame that they lie down and refuse to get up. In cattle the blisters in the mouth cause a great increase in the flow of saliva which frequently hangs in strings. Because the tongue and mouth and feet are sore, the animals do not forage nor eat and hence, even in the milder cases, lose weight rapidly.

In the mild form it takes ten to twenty days before the animal recovers and feeds normally. In the severe cases it may take months or a year. In cows the milk flow is greatly reduced and it may be a year before it becomes normal. The mortality is low, only about three to five per cent of the animals dying. However, it is much higher in young animals. Calves that have been nursing infected cows may show a mortality of sixty to seventy per cent. Pregnant cows usually abort. When death occurs it may be due to paralysis of the throat resulting in choking, to paralysis of the heart muscles, or to suffocation resulting from the action of the poisons on the tissues of the lungs.

While the direct mortality is low, the loss due to abortion, to decrease in milk yield, and to loss of weight is of such magnitude as to make foot-and-mouth disease one of the most dreaded in the animal industry.

Distribution of foot-and-mouth disease: Foot-and-mouth disease occurs in Europe, South America, China, India, and several other regions of the world. It does not occur in Canada, the United States, New Zealand, Australia, and parts of Africa. In Italy, France, Germany, and the Netherlands, it is endemic and is so firmly established that it is not economical to try to eradicate it. Norway and Sweden are free from the disease. Great Britain has succeeded in eradicating it on the many occasions when it has been introduced.

Foot-and-mouth disease has been introduced into the United States upon at least nine occasions. In 1870, 1880, and 1884 there were separate outbreaks traced to imported animals. Since then there have been no outbreaks from this source because of the strict quarantine and inspection required for imported livestock.

In 1902 foot-and-mouth disease, probably caused by imported vaccine virus, broke out in the New England States. It was eradicated in six months. In 1908 an outbreak involving Pennsylvania, Maryland, New

FIG. 49. Foot and Mouth Disease Reprinted from, Special Report on Diseases of Cattle, "Foot and Mouth Disease", published by the U. S. dept. of Agriculture, Bureau of Animal Industry, 1942.

York, and Michigan occurred and was traced to calves used to produce vaccine virus for smallpox vaccination. The original vaccine had come from Japan. This outbreak was brought under control in five months.

In 1914 an outbreak which began in Michigan spread to twenty-two states before it could be completely eradicated. Hog cholera vaccine virus prepared from hogs infected with foot-and-mouth disease was responsible for the rapid extension of this outbreak.

In 1924 foot-and-mouth disease broke out in California. This time the source was garbage from meat originating in the Orient. Over twenty-two thousand wild deer, nearly sixty thousand cattle, twenty-one thousand swine, and twenty-six thousand sheep were slaughtered before the infection could be eradicated. The same year a small outbreak occurred in Texas and in 1929 there was another in California. This was traced to hogs fed on meat scraps which had been imported contrary to regulations. It spread to cattle but was soon eradicated. In all then, there have been nine separate importations of foot-and-mouth disease in the United States and each time it has been eradicated.

Etiology: Foot-and-mouth disease was the first animal disease shown to be due to a filterable virus. Loeffler and Frosch in 1897 passed the vesicular fluid through a bacteria-tight filter and produced the disease with the bacteria-free filtrate. The virus is one of the smallest known, measuring from eight to twelve millimicra in diameter. There are several immunologically distinct types. It is present in high concentration in the vesicular fluid, as evidenced by the fact that in dilutions of one to a million the fluid retains its infectivity for guinea pigs.

In the acute stage of the disease the virus is also present in the blood, urine, milk, and saliva, and it is of considerable interest to note that they lose their infectivity three to six days after the lesions appear. Inasmuch as these discharges become noninfective in such a short time, it is very important to determine how long the virus persists away from the animal body and whether it is present in the tissues of the animal.

Experiments show that the virus dies rapidly at 37°C. However, the tongue or other tissues from infected animals and the bone marrow of carcasses of infected cattle and hogs stored for three months at freezing temperatures have been shown to be infective. Contaminated hay and grain have been shown to be infective for four months. The survival of foot-and-mouth disease virus in meat scraps, hay and grain, and in refrigerated meat indicates the danger of importing such products from areas not known to be free from the disease.

Foot-and-mouth disease is most commonly spread from infected to healthy animals by way of discharges from lesions on the feet and tongue. The incubation period is from two to seven days and the disease is com-

municable once lesions appear. The disease is spread indirectly from animal to animal and from farm to farm by virus carried on the shoes and clothing or on the hands of milkers. It is probably occasionally carried on the feet of birds, by dogs, cats, and rodents. Feed, utensils, drinking troughs, grass in pastures, stockyards, trucks, and railway stock cars may also be involved. Infected cattle do not commonly appear to remain infectious after recovery but evidence suggests that there probably are recovered carriers and that these may start fresh outbreaks.

Immunity and vaccination: Foot-and-mouth disease is produced by several different immunologic types of virus. In Europe the types are designated by the letters *O*, *A*, and *C* and there are a number of strains of each type. The diseases produced by the different types are alike but there is no cross immunity. Animals that recover from type *O* resist reinfection with type *O* but can be infected with types *A* and *C*. The fact that different immunologic types exist makes it necessary to determine the prevalent type before vaccination and to take the different types into consideration when assessing the value of vaccination. Bivalent vaccines containing types *A* and *O* have been developed.

There are two satisfactory vaccines: the Waldmann which has been widely used in Europe, and the Argentine which has been used successfully in South America. However the duration of immunity following vaccination is very short.

Control: Two general methods are used to control foot-and-mouth disease, the slaughter method and the quarantine method. Great Britain, Norway, and several other countries have completely eradicated foot-and-mouth disease by slaughtering all infected and exposed animals. In the United States this method has been used to eradicate the disease on nine separate occasions. The eradication program has been undertaken by the Bureau of Animal Industry of the United States Department of Agriculture in cooperation with the states in which the outbreaks have occurred. The expense is shared by the State and Federal governments.

In the slaughter method, premises where the outbreak occurs are quarantined, infected and exposed animals are slaughtered and disposed of by burning or burial, and the premises are disinfected. Sodium hydroxide in a one and one half per cent solution has been used successfully as a disinfectant. As a measure of the effectiveness of the procedure, test animals, preferably hogs, are placed on the premises and carefully observed to see if they develop the disease. If they do not, the quarantine is lifted.

The problem of the slaughter and disposal of thousands of cattle, sheep, and hogs is a tremendous task. Road building equipment has been used to dig trenches in which to bury great numbers of the animals. Even then

it has been necessary to slash the hides and cover them with quick lime to keep unprincipled people from skinning the carcasses and selling the hides.

It may be necessary to construct miles of fence to prevent the spread of the disease on ranges. In all cases, the effectiveness of this method depends upon prompt diagnosis and rigorous action. Where the disease has been established for long periods as in Europe, or where it cannot be promptly eradicated, the quarantine method may be the more practical.

The quarantine method implies living with the disease. Animals are vaccinated to reduce losses. Some slaughter may be involved. A belt around the focus of infection is under strict quarantine and animals in this belt are vaccinated.

Treatment with drugs is of no value.

FOOT-AND-MOUTH DISEASE IN MEXICO

The Mexican outbreak of foot-and-mouth disease deserves special mention because it illustrates the need for constant vigilance if countries free from the disease are to remain free. It shows the necessity for international cooperation, the difficulty of eradicating the disease once it has become established, and the need for intelligent and effective cooperation between farmers, veterinarians, and governmental agencies. It also shows how costly the disease is to control and how infinitely greater the cost if it is not controlled.

The trouble in Mexico began when two shipments of Zebu cattle were brought in from Brazil. One shipment consisting of one hundred and thirty bulls arrived in October 1945 and the other of three hundred and twenty-seven head in May 1946. Both shipments were held in quarantine on an island but were later transferred to ranches in the State of Vera Cruz. At this time Mexico was free of infection but a short time later foot-and-mouth disease broke out in that district and, before it was recognized, had gained a foothold over a large area. Mexico mobilized its resources, including the army, to enforce a quarantine and the Bureau of Animal Industry of the United States Department of Agriculture provided about forty million dollars for expenses, and dispatched trained personnel and equipment to the area. It was agreed to try the slaughter method and attempt to eradicate the disease. However, before sufficient forces could be mobilized to effect control, the disease had spread over such a wide area that the program was doomed to failure. There was a lack of understanding of the seriousness of the disease, a great deal of resentment against the slaughter of animals, particularly of the oxen upon which some farmers depended for power, and a general failure to cooperate in the program. Quarantine lines were crossed and new foci of infection continued to develop, many in rather

inaccessible regions, and the areas under quarantine had to be continually enlarged.

The vaccination-quarantine method was then attempted. A protective zone varying from a few miles to forty or fifty in depth was established around the area of infection and the animals in this region were vaccinated. Restrictions on shipments from the infected area were set up and have been continued and it is hoped that the infection can be stamped out eventually. However, as long as foot-and-mouth disease remains on this continent it is a threat to our entire live stock industry and a very serious disaster to Mexican agriculture.

The importation of ruminants and swine is, of course, prohibited but the virus can be transferred by clothing, automobile tires, and in a variety of other ways and, in spite of such precautions as driving cars through vats containing disinfectants to destroy the virus on the tires, the possibility that the infection may get into the United States is real.

When one considers how foot-and-mouth disease can disrupt the agricultural economy of a nation and threaten its food supply when the disease has been transmitted unwittingly and through ignorance, one shudders to think of what might happen if attempts should be made to use the disease as a weapon against a nation during an all out global war.

Chapter XXXII

SMALLPOX OR VARIOLA

The story of smallpox is of special interest because the disease was formerly one of the great scourges of mankind, because the principle of protective inoculation was first worked out to combat it, and because the wide spread practice of vaccination is all that keeps it from resuming its old place as a leading cause of death and disfigurement.

Smallpox or variola is another disease with its beginnings in antiquity. It was present in China long before the birth of Greek civilization. It appeared in Rome in the first century A.D., swept through medieval Europe in devastating epidemics, and, until about a hundred and fifty years ago, was one of the most dreaded pestilences. It is hard for us to realize that from twenty-five to forty per cent died when a population was stricken with smallpox, and to visualize the unsightly scars of the survivors and the many blinded in one or both eyes as a result of the infection. Smallpox was introduced into the Indian population in Mexico in 1500 by a negro slave in service with the Spanish. It spread like wild fire from tribe to tribe and so extreme was its virulence for this susceptible group that it is recorded over three million Indians died from it.

The word pockles or pox refers to the skin eruption, and the term smallpox is used for variola to distinguish it from the Great Pox, or syphilis, which raged over western Europe in the sixteenth century.

Recognition of Smallpox

Smallpox is an acute infectious disease characterized by a fever and a rash and frequently by vomiting, aches, and pains. The eruption appears three or four days after the onset of the fever, first on the forehead and face, then spreading rapidly to the forearms, wrists, hands, and rest of the body. The rash is more abundant on the shoulders and chest than on the abdomen. The small red spots which appear on the face and hands are called the *initial* or *macular* rash. In about twenty-four hours these spots become elevated and are known as *papules*. When this happens the temperature may fall and the patient may feel much better. After about four or five days, the papules turn to watery blisters or vesicles with a central depression. They become filled with foul-smelling pus and are then known as *pustules*. At this stage the temperature may again rise. As the pustule dries, it becomes covered with a scab or crust which eventually drops off leaving a pit or pockmark. There is a most offensive odor during the height of the eruption and an intense itching when the pustules are drying.

In the early stages the rash may be as vivid as in scarlet fever. It is usually discrete, in which case it disappears in about ten or eleven days; but it may be confluent, in which case the disease is more deadly. In the severe cases there may be bleeding into the skin of the face and, as a result, the face may turn purple. This is the type known as the Black Smallpox. Most of these cases end fatally.

There are two types of smallpox. One is the malignant classical disease, characterized by severe pitting and disfigurement and by a case fatality as high as thirty per cent; the other is a mild form known by many names; alastrim, cottonpox, parasmallpox, parva variola, Cuban itch, and others, and has a case fatality in the order of less than one per cent. This is the type prevailing in the United States at present. It is highly contagious and can be prevented by vaccination.

Mild smallpox and chicken pox may show a marked similarity and be confused. The following table, taken from a bulletin published by the Wisconsin State Board of Health shows the chief differences.

Mild Smallpox	*Chicken Pox*
Initial fever drops with appearance of rash.	If initial fever is present, it may be continuous.
Eruption on face, arms, and legs more than on the body.	Eruption more pronounced on body.
Eruption of one crop.	Eruption of successive crops.
Eruption may occur on hands, feet and in mouth.	Eruption rare on hands, feet and in mouth.
Occurs at any age.	Generally in children.
Vaccination scar, old or wanting.	May be vaccinated.
Exposure to smallpox.	Exposure to chicken pox.

Etiological Agent

The specific cause of smallpox is the variola virus. It can be grown in tissue culture and on the chorioallantoic membrane of the chick embryo. Symptoms of smallpox cannot be reproduced in the lower animals but characteristic local lesions may be produced in monkeys, calves, and rabbits. The virus is not so resistant as the pathogenic bacteria to germicides nor heat, but is more resistant to glycerol and phenol.

Mutation of Virus: The variola or smallpox virus is converted into *vaccinia* or cowpox virus on passage through the calf. The cowpox virus produces a mild infection in man which leaves him immune to smallpox. Strains converted from variola to vaccinia appear to be identical with the naturally occurring vaccinia virus taken from cows. Insofar as is known, vaccinia virus cannot be converted to variola.

Guarnieri Bodies. A characteristic feature of vaccinia or variola infection, discovered by Guarnieri in 1892, is the appearance of intracellular

inclusion bodies, known as Guarnieri bodies, within the epithelial cells of lesions. Paschen afterward showed that the inclusion bodies were composed of minute elements which are now called Paschen bodies or elementary bodies and are considered to be the virus.

Laboratory Diagnosis: Diagnosis of smallpox is usually made on clinical grounds, but in case of doubt several laboratory methods are available.

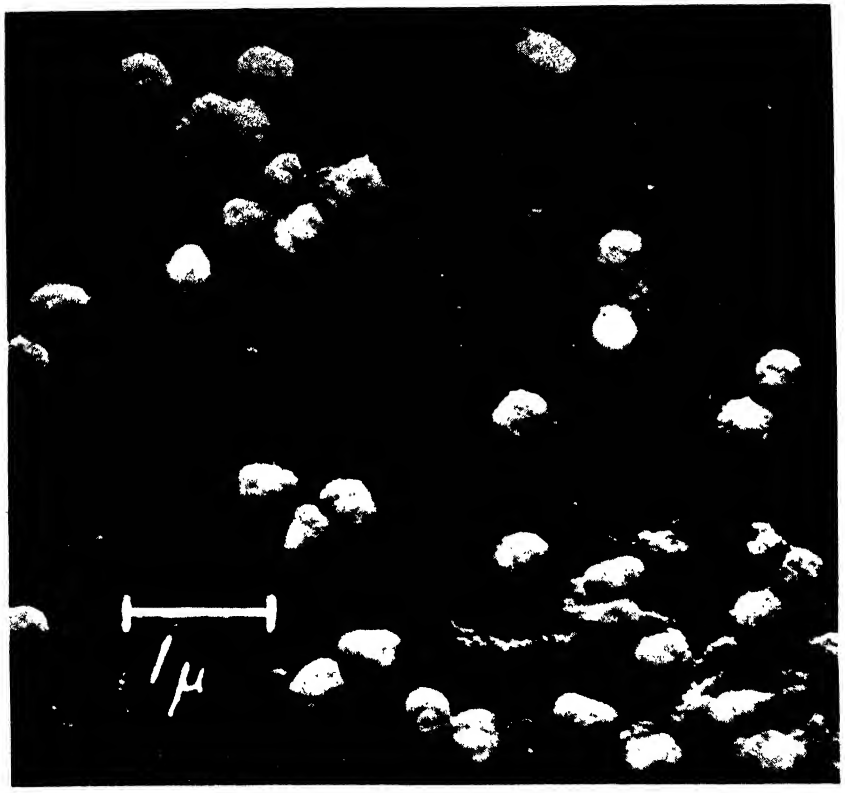


FIG. 50. This electron micrograph shows the small-pox virus. Courtesy of Dr. D. Gordon Sharp

Paul's test consists of introducing suspected material into the scarified cornea of the rabbit's eye. A positive reaction is indicated by the appearance, in about forty-eight hours, of small dewdrop vesicles. Microscopic examination shows the Guarnieri bodies.

Serological tests are perhaps the most practical. Material taken from the lesions is ground up and mixed with serum taken from an immunized rabbit. If the test is positive, a precipitate appears. The serum from patients also shows antibodies and when it is mixed with a suspension of Paschen bodies, agglutination and precipitation occurs.

Rabbit Skin Test: The smallpox virus produces a local reaction when inoculated intradermally into normal susceptible rabbits. It does not produce one in immunized rabbits. Therefore, when material from lesions is inoculated into susceptible and into immune rabbits, a reaction in the susceptibles and not in the immunes is a positive test.

The serological and animal inoculation tests, of course, do not differentiate between vaccinia, variola, or alastrim.

Source and Mode of Infection. Smallpox occurs exclusively in man. The virus is present in the secretions of the mouth and nose, in the vesicles, pustules, and crusts, and in the blood, feces, and urine. It is generally conceded that the nasal secretions are the most important in the spread of smallpox which is, therefore, usually considered a droplet infection. Contact need not be intimate and contaminated articles may play a part. There are no known carriers.

The period of incubation is usually about twelve days, although it may be as short as eight or as long as twenty.

Period of Communicability: Smallpox is communicable from the beginning of the fever until the crusts disappear. It is most contagious in the early stages when the virus is present in secretions of the nose and throat. In the later stages, the contents of the pustules and crusts are infective.

Susceptibility and Immunity: All ages and races are susceptible to smallpox. When the disease is introduced into people who have never been previously exposed from a quarter to a half succumb. It has been estimated that of the twelve million Indians in the United States when the white men landed, about six million died of smallpox.

In unvaccinated populations, smallpox shows an age distribution similar to that of measles. It is primarily a childhood disease, appearing in cycles whenever a large enough proportion of susceptibles accumulates. In populations which are partially artificially immunized, it appears in all ages.

The case fatality rate is variable and depends upon the virulence of the infecting strain. In epidemics due to the malignant strain, the case fatality rate is from five to thirty per cent or even higher. With the alastrim strain, it is less than one per cent.

Prevalence and Distribution: Smallpox occurs endemically and in sporadic outbreaks in countries where vaccination is practised but is not general. In countries where vaccination is not widely practised, it is endemic or epidemic, and in countries where vaccination is universal, it is practically extinct.

In the United States, smallpox occurs chiefly where vaccination is not compulsory. The northwestern states have shown the highest incidence

in the last few years. The disease occurs most frequently in the winter months.

In the ten year period from 1913 through 1922 there were nearly 600,000 cases. During the period from 1921 through 1930 there were nearly 500,000 cases. Since then there has been a rapid and steady decline and in the five year period from 1942 through 1946 the yearly average was 384 cases. In 1946 there were 356 cases and in 1947 only 173 were reported. Most of the cases are mild and it is likely that many are not reported.

The regional or geographic differences in the distribution of smallpox are a reflection of the degree of vaccination. Climate as such has no influence.

Control

Variolation: During the centuries when smallpox was the leading cause of death and disfigurement, it was common knowledge that one attack of the disease conferred an immunity and it is recorded that for this reason mothers would not hire nurses who had not had the pox. The Chinese recognized smallpox as something inevitable and practiced the art of inoculation or 'variolation' which consisted of introducing material from the pustule or scab into the nose or abraided skin. This practice was common in the Near East and was popularized in England in 1716 by Lady Montague, an energetic and highly intelligent woman, who had become familiar with the practice while living in Constantinople. She had her own son inoculated. The practice spread rapidly and was introduced into the colonies by Boylston at Boston in 1721. He inoculated his six year old son and many others. Cotton Mather was soon convinced of its efficacy and used his influence to popularize the practice.

Variolation was not without dangers, although the percentage of deaths numbered one in a hundred or less. The disease produced by variolation was smallpox but was usually mild. However, it was just as contagious as the naturally occurring smallpox, just as severe to those who contracted it, and served to keep the disease prevalent in the population.

Vaccination: Vaccination (from the Latin word *vacca* meaning cow) owes its beginning to the observation of the English farmers that those who milked cows infected with cowpox developed lesions on their hands and did not thereafter develop smallpox when exposed. In fact, it is recorded that several farmers vaccinated their children by this method. Either these homely observations were not given credence by the physicians of the time or they were unaware of them, for when, William Jenner heard a milk maid remark that she would not take smallpox because she had had cowpox, he went to John Hunter, a leading London physician, and asked

him what he thought, to which Hunter replied, "Don't think. Try it." And Jenner did. The story of how Jenner went back to his country practice and on May 14, 1796, used a thorn to transfer material from cowpox lesions on the hand of one Sarah Nelms, a milk maid, to the arm of eight year old James Phipps, obtained a local lesion or 'take' at the site of inoculation, and of how in July he inoculated the boy with material from a case of smallpox and found him to be immune, is one of the classics of medicine. Jenner retested the boy later and tested a number of persons who had previously had cowpox. None developed smallpox. In 1798 he published his observations which encountered considerable opposition, and, although the harmlessness and the efficacy of smallpox vaccination has been proven beyond doubt, that opposition by the ignorant and misguided still continues and because of it thousands have died needlessly.

Vaccination was introduced into the United States in 1800 by Benjamin Waterhouse, the first professor of the 'Theory and Practice of Physic' at the Harvard Medical School. He first vaccinated his five year old son and then two slaves. Shortly after, nineteen children were vaccinated and later exposed to smallpox. None contracted the disease. These demonstrations were so convincing that in 1809, Massachusetts passed a law compelling towns to set up committees in charge of vaccination. The cases and deaths of smallpox dropped immediately and, as so often happens, the next generation, being free of the disease, decided to change the law so as to make the formation of vaccination committees voluntary. This was done in 1837. In the next decade vaccination was not generally practiced and smallpox began to increase. In 1855 vaccination of school children was made compulsory, but it took a wide spread epidemic in 1872 to bring about satisfactory enforcement.

The Vaccine: Successful vaccination depends upon the use of a vaccine which is potent and free from other disease-producing agents. When vaccination became general, it was common practice to inoculate with material from pustules or crusts taken from persons who had been recently vaccinated. While this method produced a satisfactory immunity, it was not practical nor safe, because occasionally other diseases, particularly syphilis, were transmitted along with the vaccine virus.

At present, most of the commercial vaccine is produced in the calf, although it can be produced in tissue cultures and in the chorioallantoic membrane of the chick embryo.

In the commercial production of vaccine, the calf is inoculated with virus taken from other calves or with 'seed virus' taken from the vesicles or crusts of vaccinated children. The virulence of the virus, which is lost on continuous passage through the calf, can be maintained by passage through the rabbit.

For the production of virus, calves are used which have been carefully observed and are free from disease. The abdomen is clipped, shaved, cleaned, and the virus introduced into the scarified skin. In about five or six days, the typical vaccinia vesicles have developed and their contents are harvested. The animal is sacrificed, autopsied, examined for evidence of disease, and if there is any, the lymph is discarded. If not, glycerine and phenol are added to the lymph from the vesicles to preserve it and to destroy contaminating bacteria. Before it is ready for use, sterility and potency tests are run. The yield from a calf is sufficient to vaccinate about fifteen hundred persons.

Although there were formerly a few instances in which diseases were transmitted by the vaccine, the rigorous precautions now taken under the supervision of the United States Public Health Service preclude this possibility.

Vaccination confers an immunity against the malignant and mild forms of smallpox as well as against cowpox. The immunity lasts from five to twenty years, and appears to be as strong and durable as that acquired by an attack of the disease. It is usually recommended that children be vaccinated during their first year. Many suggest before the age of three months, and some suggest doing it during the first three or four weeks. There are many advantages to vaccination in the first few months and no disadvantages.

Methods of Vaccination: Of the many ways of introducing the vaccine, the one commonly recommended is the "multiple pressure" method. The skin is first cleansed with soap and water, then with alcohol or acetone, and then a drop of vaccine is applied. A new sterile needle is held parallel to the skin and the side of the needle point pressed through the drop and against the skin firmly about thirty times. The entire area covered is not over one-eighth inch and care is taken not to draw blood. This method leaves very little mark, is less apt to lead to infection by contaminating bacteria than the old methods of scarification, and is effective. Since there is a scarcely noticeable scar, the site preferred for vaccination is the upper arm because it is not apt to become contaminated or bruised.

Types of Reactions to Vaccination: The type of reaction that follows the introduction of vaccine depends upon the individual's state of immunity. There are four general types.

Primary Vaccinia: If the individual is susceptible; that is, if he has never had smallpox, has never been vaccinated, or has lost his immunity over a period of years, a papule appears on about the fourth day. It rapidly develops into a vesicle surrounded by a narrow zone of redness which increases in size and redness, reaching its maximum on the tenth or fourteenth day. The redness then recedes, the vesicle dries, and a crust is formed which falls off, leaving a typical scar.

Immune Reaction: If the individual is immune as a result of an attack or previous vaccination, the only reaction noted will be a redness and slight swelling in twenty-four to forty-eight hours, usually, seldom as late as seventy-two hours. The site of the reaction is itchy, but papule and vesicle formation are absent and, consequently, no scab appears.

Vaccinoid or Accelerated Reaction: This is an intermediate reaction and indicates partial immunity. Its height is reached in six to eight days and a vesicle and scab are formed.

Negative Reaction: If there is no reaction it may mean that the vaccine was not potent, that the inoculation was not properly performed, or that the individual was already immune. If such a negative reaction is encountered, and it is not definitely known that the individual is immune, another attempt at vaccination should be made.

Postvaccinal Encephalitis

In 1922 a disease was recognized in children which followed vaccination and was called postvaccinal encephalitis. Its occurrence was sporadic and not associated with any particular batch of vaccine. It was first described in England but has also been reported in Holland, Germany, and the United States. Its cause is unknown and less than five hundred cases have been reported altogether. The mortality is from thirty to forty per cent. It has never been reported in children less than one year and is most frequent between the ages of five to fifteen. Considering the infrequency with which it has appeared, it does not present any argument against vaccination.

This disease, also called postinfection encephalitis, follows anti-rabies vaccination and many diseases such as measles and chicken pox.

The history of smallpox vaccination has been a stormy one. There is no other disease in which protective inoculation is so effective and free from danger and discomfort. Its efficacy has been proven time after time during epidemics, but in spite of this, the suggestion that vaccination be made compulsory arouses certain groups to a wild frenzy and the opposition to vaccination during periods when there are no outbreaks is often sufficient to bring about a repeal of vaccination laws. The inevitable result is the development of a susceptible population and whenever a case is introduced into such a community, an epidemic occurs. By promptly vaccinating the population, the disease is again brought under control, but memory is short, and after a few years, opposition develops again.

The effectiveness of vaccination in the control of smallpox and the need to maintain a continuously vaccinated population was amply demonstrated by two events in recent years. In 1945 a soldier returning to Seattle from Japan developed symptoms of smallpox on shipboard. His case started

an epidemic in the Puget Sound Region in which sixty-five cases and twenty deaths occurred before it could be checked by isolation and vaccination.

In 1947 a Mexico City merchant travelling by bus to New York City became mildly ill en route. A few days after arriving in New York he entered one hospital but was shortly after transferred to another where he died. Because he had no history of exposure, because the symptoms were not considered typical, and because he had been vaccinated a year earlier (apparently unsuccessfully), his case was not diagnosed as smallpox until about nine days afterward when two patients, a man and a child, who had been in the hospital with him but who had recovered from their illnesses and been discharged, developed symptoms which again hospitalized them. Their cases were diagnosed as smallpox then. Although attendants and contacts were vaccinated immediately, the man's wife developed the disease and died. This first case, then, led to three cases, and those, before adequate measures could be taken, had led to eight more. This characteristic geometric type of progression accounts for the rapid increase and epidemic character of highly infectious diseases. By prompt isolation and an heroic effort by the New York health authorities who promptly mobilized all their forces and in less than one month vaccinated over six million persons in New York City, what would undoubtedly have been a sizable outbreak was prevented.

These two instances show the continual threat that smallpox is to unvaccinated populations.

Chapter XXXIII

YELLOW FEVER

The stories of plague, smallpox, typhus, and many other pestilences are a part of the history of the Old World. The story of yellow fever is a part of the history of the New World. No other disease took such a heavy toll of its investigators but the drama-laden story of its conquest is one of the brightest chapters in the history of science. Physicians, bacteriologists, entomologists, and engineers played brilliant roles.

It is generally believed that West Africa is the home of yellow fever and that its appearance in other parts of the world is the result of importation. Its introduction into the Western Hemisphere is linked with colonization and the slave trade. The island of Saint Thomas, some two hundred miles off the coast of West Africa, was a center of the slave trade and a port of call for many traders. Although not recognized as such, it was also a center from which yellow fever was widely disseminated, for the disease, probably introduced from Africa, remained endemic there for years and was carried all over the world by vessels touching the port.

Following the Spanish conquest and the colonization of the Western Hemisphere, yellow fever was introduced into the northern part of South America, and into Central America and the southern part of the United States. The islands of the Carribbean were very heavily infected. At times the disease would die out from the various regions and when it was reintroduced, fresh epidemics would occur. Yellow fever has been known as far north as Quebec, but the disease has never gained a foothold except in the tropics and adjoining regions.

According to Bauer, yellow fever first appeared in North America in 1668 when there were severe epidemics in New York and Philadelphia. For over two hundred years the disease struck again and again, particularly in the southern coastal region and the gulf ports, following the Mississippi up as far as St. Louis. Perhaps the most severe epidemic in the United States occurred in 1793 in Philadelphia. There were four thousand deaths and terror of contagion was so great that the inhabitants fled, businesses closed, and about the only persons to be seen abroad were the burial parties. In the next ten years there were several other outbreaks and about ten thousand more deaths.

As time went on the epidemics became less frequent, although the disease remained endemic in the Gulf Region, particularly in New Orleans where the last outbreak occurred as late as 1905.

During all this time, there accumulated many pertinent observations whose significance was not realized. It was noted that yellow fever fre-

quently broke out on board vessels that had called in ports where the disease was prevalent; that crews of vessels lying in harbors were attacked even though no one went on shore; that the disease might be absent on ships from infected ports for weeks and then break out when the hatches were opened, either in mid-ocean or when unloading in distant parts of the world; that the disease was transported from island to island and country to country and that in some regions it gained a foothold and remained endemic, whereas in others the outbreaks were limited to a few weeks. The geographic and seasonal distribution and the fact that the disease died out after the first frost were also noted early. The disease always appeared in ports, along the seaboard and waterways, and never spread inland nor appeared in regions of higher altitude. It always appeared first along the water front and was frequently confined to this district. These significant facts were all explained when it was learned that the disease is transmitted by a mosquito which is limited in distribution, restricted in habits, and sensitive to temperature.

Although there were many contributions by individuals, our present knowledge of yellow fever is largely the result of the work of two groups: a Commission of the United States Army consisting of Reed, Carroll, Agramonte, and Lazear, who went to Havana, Cuba, in 1900; and The International Health Division of the Rockefeller Foundation who studied the disease in West Africa about twenty-five years later.

Recognition of the Disease

Yellow fever is an acute disease characterized by a sudden onset, with pains in the head and back, fever, jaundice, and, in severe cases, of vomiting discolored and blackened blood—hence the descriptive name of "Black Vomit." The symptoms are referable to a necrosis of the liver which produces the jaundice or yellow coloring of the skin and mucous membranes, and to hemorrhages in various organs and tissues, such as the skin, meninges, intestine, covering of the heart, and mucosa of the stomach.

The pathological changes in the liver are characteristic, and diagnosis can be made by examining small portions of it. It is often impossible to secure permission to perform autopsies on the bodies of persons suspected of having died from yellow fever, whereas there is little or no objection to removing a portion of the liver by the use of the viscerotome, an instrument somewhat similar to an apple corer. The development and use of this specific instrument has proven of inestimable value in postmortem diagnosis of yellow fever.

Etiology

Previous to 1900, many careful observations had been made on the epidemiology of yellow fever and although insects were suspected of trans-

mitting the disease, the etiological agent was unknown. The Yellow Fever Commission of the United States Army, working in Cuba in 1902, first established the fact that the organism causing the disease could be passed through filters.

Many investigators did not accept this virus etiology and Noguchi, who was working in South America in 1918, announced that the cause of yellow fever was a Leptospira which he called *Leptospira icteroides*. It seems that he had been working on material from fatal cases of Weil's Disease or yellow jaundice, confused with yellow fever by the clinicians.

However, the virus etiology was confirmed by Stokes, Bauer, and Hudson in 1928 and in 1933, Findlay and Broom, measuring the virus by means of collodion membranes, established its size as 17 to 28 microns. It is killed in five minutes at 55° C. and is also sensitive to drying. It produces a solid immunity, antibodies appearing in the patient's blood on the sixth or seventh day, and lasting for life. Strains of the virus isolated from cases of yellow fever in different parts of the world are immunologically identical

The virus is pathogenic for the Indian monkey, *Macacus rhesus*, and on passage from monkey to monkey still maintains its virulence for man. It is said to be *viscerotropic* and has an affinity for certain tissues such as the liver.

In an attempt to attenuate the virus, Theiler passed it serially through the brains of mice and finally obtained a strain which proved pathogenic for the mouse. This strain is used in serum neutralization tests and enables workers to carry on more extensive investigations than were possible with strains virulent for the monkey only.

Another attenuated strain called 17 D has been propagated successfully in tissue culture and chick embryo and is being widely used as a vaccine.

Epidemiology

Transmission: There are two epidemiological types of yellow fever, both due to the same virus. One, the classical disease, is transmitted by mosquitoes, principally by *Aedes aegypti*. The other, called jungle yellow fever, was discovered in 1932 in Brazil and its manner of transmission is not entirely known.

As early as 1881, Dr. Carlos Finlay of Havana suggested that yellow fever was mosquito borne. However he presented little convincing evidence and his theory was not accepted. Reed, Carroll, Agramonte, and Lazear began a study of the problem and in a series of courageous and carefully planned experiments involving the use of human volunteers from the army camps, as well as members of the Commission, showed that the disease was transmitted by the mosquito, *Aedes aegypti*, and that it was not

transmitted by contact. Volunteers lived in the same room with patients, slept in their soiled bedding, and still did not contract the disease.

They found too that a patient is infective for a mosquito only during the first three days of his fever, and that mosquitoes fed on yellow fever patients after the fourth or fifth day did not transmit the disease.

The virus of yellow fever can penetrate the intact skin, which is probably why a considerable number of investigators have contracted laboratory infections.

Incubation Period: In 1900, Carter studied an outbreak of yellow fever in a small community of Orwood, Mississippi, and demonstrated what is now called the 'extrinsic' incubation period. He showed that when a susceptible individual went into an epidemic region, the incubation period was not more than seven nor less than three days; but that when a case of yellow fever was introduced into a region free from the disease, a period of sixteen to twenty-one days lapsed before the appearance of secondary cases.

To Carter this difference suggested that the organism was multiplying away from man, perhaps in a mosquito, hence the term, 'extrinsic' incubation. When Reed and his group went to Cuba, they had the benefit of Carter's and Finlay's observations and were no doubt influenced by them when planning their experiments.

The 'extrinsic' incubation period represents the time required for a mosquito, fed on a yellow fever patient, to become infective or capable of transmitting the virus. It is generally believed that this represents the time required for the virus to multiply and become distributed in the saliva of the mosquito. It is not, however, necessary to postulate any multiplication in the mosquito, because it has been shown by Davis, Frobisher, and Lloyd that immediately after feeding on an infected monkey, the mosquito contains one hundred and sixty-seven million lethal doses of virus.

The period required for a mosquito to become infective depends, within limits, on the temperature. At ordinary summer temperatures, it is twelve days; and at 37° C., it is only four.

The virus is not transmitted from infected mosquitoes to their progeny, nor is there any evidence that it is pathogenic for the mosquito.

The Mosquito: Aedes aegypti is a domestic mosquito, living around dwellings and not out in the swamps. The larvae are poor swimmers, unable to escape the cannibalistic larvae of other insects. The adults lay their eggs in clean water: in rain barrels, drains, tin cans, vases, and the like where their natural enemies are absent. Even though the food supply is scarce in this type of place, they are better able to survive. It is through a knowledge of the habits of the vector that yellow fever has been so readily brought under control.

Experimental Animals: The early experimental work on yellow fever was limited to human volunteers and consequently after the etiology and manner of transmission were established, little was added to our knowledge. The discovery by Stokes, Bauer, and Hudson that the Indian monkey, *Macacus rhesus*, was susceptible and would develop infection similar to that in man, paved the way for further advances. In this, as in many other diseases, the finding of an experimental animal yielded knowledge which saved many lives.

Jungle Yellow Fever: The epidemiology of the classical or urban yellow fever is referable to the manner of its transmission and the habits of the mosquito vector, *Aedes aegypti*. There is no animal reservoir and when the *aedes* mosquito is brought under control, the disease dies out. In parts of South America there is another epidemiological type of yellow fever known as *jungle yellow fever* because it occurs in or at the edges of jungles or forests. Jungle yellow fever is endemic and occurs as small outbreaks and isolated cases in regions and during seasons when the *aedes* mosquito is absent. Monkeys probably serve as a source of infection since they have been found to harbor antibodies to the yellow fever virus, and two genera of mosquito: *Haemogogus capricorni* and *Aedes leucocelaenus* have been found to carry the virus. However, cases of jungle yellow fever occurred in both animals and man during dry seasons when neither of the mosquitoes was to be found by collectors working on the ground. The manner of its transmission remained a mystery until they discovered that during the dry season *Haemogogus capricorni* lived in the tree tops. This explained the prevalence of yellow fever in workers felling trees, the carry-over of the disease from one rainy season to another, and the appearance of scattered cases during the dry season.

Since the virus responsible for urban and jungle yellow fever is the same, cases of jungle yellow fever can serve as a source of infection for epidemics of urban yellow fever in regions where the *Aedes aegypti* mosquito is present.

Prevalence and Distribution: Yellow fever is now confined to West and Central Africa and parts of South America. In Africa the disease is primarily urban. In South America it is primarily rural and is known as jungle yellow fever.

Why yellow fever does not become more wide spread in the tropics and why it disappears after having been introduced are interesting problems. Carter suggested that in addition to the presence of the virus, the vector, and man, two other conditions must be met. The temperature must be 72° F. or higher or the mosquitoes will be inactive, and the concentration of susceptible individuals must be sufficient to keep enough active cases so that the mosquitoes will become infective. In a small and isolated community, yellow fever dies out. When an outbreak occurs, those that

recover are immune so that after a time there are no susceptibles to develop the disease and to infect other mosquitoes. Carter believed that it was the high birth rate and the large number of susceptibles flowing into Havana every year that kept the disease alive in that city.

In the case of jungle yellow fever, there appears to be no tendency for the disease to die out. This suggests an animal reservoir in which it maintains itself. However it may be carried over in the *haemagogus* mosquito.

In India there has never been any yellow fever although the temperature, the vector, and the susceptible population are present. The Indian monkey is susceptible and would furnish an animal reservoir from which it would be almost impossible to eradicate the disease. It seems reasonable to suppose that an outbreak there would be fatal to millions. Fortunately the virus has never been introduced. In the olden days, India was too distant. With airplane travel, the probability that an infected mosquito may be transported and start an outbreak, or that a person may be bitten by an infected mosquito in Africa, develop the disease after arriving in India, and infect mosquitoes there is a real one and the health authorities are greatly concerned. A similar threat hangs over the southern United States and every effort is made to prevent the introduction of the disease from South America.

Control

The control of yellow fever depends upon control of the vector and vaccination. Mosquito eradication measures based on a knowledge of the habits of *Aedes aegypti* have been sufficient to eliminate yellow fever from the regions of the world where it was formerly the most important disease. The control of jungle yellow fever cannot be accomplished until more is known about its transmission and the animal reservoir.

Vaccination with a living attenuated virus propagated in the chick embryo has been proven safe and effective. It has been given to large numbers of missionaries and employees of governmental agencies and commercial firms who are stationed in regions where yellow fever occurs and to laboratory workers. Troops on duty in yellow fever districts are also being vaccinated. At present, vaccination appears to be the only hope in the control of jungle yellow fever in South America.

SECTION IV

MICROBIOLOGY OF AIR, FOOD, MILK, WATER, SEWAGE, AND SOILS

CHAPTER XXXIV

AIR POLLUTION AND SANITATION

The idea that diseases are spread by air or are due to bad air is ancient. Miasmas and offensive effluvia were commonly associated with epidemics. Miasmas were believed to arise from decaying animal and vegetable matter and to float in the air. The miasmas from swamps were held to be especially dangerous, particularly at night, and the observation that a certain sickness was highly prevalent in those exposed to such miasmas led to naming it *mal' aria* or bad air.

In the seventeenth century typhus, or, as it was more descriptively called, "gaol fever" or "ship distemper", was commonly believed to be due to foul air, and the great and versatile rector, physiologist, and inventor, Stephen Hales (1677–1761) invented an "engine" for ventilating ships and prisons. Newgate and Old Bailey prisons were equipped with his ventilators which consisted of huge bellows operated by windmills. It is recorded that while the ventilators were being installed at Newgate seven of the eleven workmen took ill and died of typhus, contracting it, presumably from the stench. Hales reported excellent success with his ventilators but it might be noted that the prisons were also thoroughly cleaned, the walls washed with vinegar, and the prisoners bathed in vinegar, so it seems likely that the resultant destruction of lice might have been the important factor.

Henle (1809–85), according to Bullock, referred to miasma as "a quiddity unrecognizable by the senses but in some way related to or identical with other deleterious potencies like contagia." He regarded contagia as a kind of second generation of miasm which had passed through its first development in the human body.

In this country as late as 1832 when cholera raged along the Erie Canal, hogsheads of tar were unloaded and set afire to purify the atmosphere.

However, the demonstration that many epidemic diseases, such as typhoid and cholera, were water-borne, and that others, such as typhus and malaria, were insect-borne, seemed to have directed the attention of sanitarians away from the air as a vehicle of infection. When it was found that surgical instruments were the most important source of operative wound infection in hospitals and that such infections could be greatly reduced by sterilization of instruments, the possibility of additional air-

borne infections was largely ignored. However, it is becoming abundantly clear that air-borne infection in homes, nurseries, schools, army camps, factories, stores, transportation systems, in fact, in every place where peoples come together is, indeed, a major problem.

An atmosphere polluted by smoke, dust, gases, vapors, and fumes seems to be one of the by-products of our industrial civilization. Just how important such pollution is from the standpoint of health and well-being is not clear. Although smoke is disagreeable and constitutes an economic nuisance, it has never been shown to predispose to infection. This does not mean that it may not play a role in the total health picture but merely that evidence that does is lacking. Disagreeable and irritating odors emanating from sewage disposal plants, glue factories, fertilizer plants, refineries, and other industrial establishments produce aesthetic discomfort and are undesirable from that point of view, but, again, it has never been conclusively shown that such fumes play any major role in the production of infections.

Dusts, particularly those associated with silica, are injurious and produce a disease known as silicosis. Workers in close contact with such dusts are predisposed to tuberculosis. There is, however, little evidence that pollution of the outside atmosphere with such dusts is an important factor in the transmission of infectious diseases. The effect of dusts, fumes, gases, and vapors within factories is dealt with in industrial hygiene. Our principal concern here is with infections that may be air-borne. Recent studies all point to the same conclusion; namely, that the air within buildings plays a very significant part in infections, particularly in those of the upper respiratory tract.

Air is not a natural habitat for microorganisms and those that are present come from soil, water, vegetation, and man and animals. They are contaminants and the number present in the atmosphere depends largely upon temperature, humidity, and wind or air currents. Aeroplane surveys show that spore-forming bacteria and the spores of fungi are present in the higher altitudes but that the ordinary human pathogens are not. Air-borne fungi, such as those causing black stem rust of wheat, travel for hundreds and perhaps thousands of miles and while there doesn't seem to be any reason that spore-bearing bacteria such as tetanus and anthrax bacilli could not be transported around the world, there is no evidence that infection with these diseases does result from spores carried from afar. Virus transport by air over long distances may be possible, too, but there is no reason to believe that it does take place.

From the sanitary point of view outdoor air does not seem to be an important source of infection. This might be expected because most bacteria associated with disease do not survive in the air for long periods of

time in sufficient numbers to produce infection. The very air currents that disseminate the organisms are conducive to rapid drying, which is very destructive to most bacteria, and to dilution to a point where the dosage is too small to produce infection.

In contrast to outdoor air, the air within buildings is a constant hazard and serves as a vehicle for the transmission of diseases of poultry, livestock, and humans. The source of the infectious agents is animals and man. In man, droplets expelled in coughing, sneezing, singing, and even in ordinary conversation constitute a steady source of air contaminants. Discharges from the intestinal tract and from sores and abcesses, vomit, and scales from the skin are of far less importance. Healthy persons as well as those who are frankly ill are a constant source of microorganisms many of which are pathogens.

Droplets are by far the most important means by which pathogens of the upper respiratory tract leave the body. The fate of the bacteria in droplets depends upon humidity, temperature, and the size of the droplet. Large droplets ranging up to 10 millimicra are projected for some distance during sneezing but fall rapidly to the floor. The smaller droplets, those from 0.1 to 1 millimicra or less, evaporate very rapidly and the bacteria present remain suspended in the air. The normal air currents are sufficient to keep them suspended indefinitely and they will circulate freely through ventilating ducts. Wells and Wells, who have contributed so much to the study of droplet infection, call the particles left after evaporation "droplet nuclei." Bacteria, viruses, and dust particles act as such. It is also interesting to know that particles of this size penetrate to the lung upon inhalation whereas larger dust particles are usually trapped in the nose. The larger droplets settle to the floor and when they evaporate the bacteria are left free in the dust or on the dust particles. Hemolytic streptococci, tubercle bacilli, and diphtheria bacilli have frequently been isolated in the dust swept up from the rooms of patients. The best evidence for the importance of air-borne infection is that disease transmission and cross infection in hospitals is not an uncommon occurrence.

Control of Air-Borne Infection: There are two methods for the control of infectious diseases. One is to increase the resistance of the host by immunization. The other is to reduce the dosage. The control of air-borne infections must follow these two methods.

In the case of a disease like diphtheria, which is chiefly droplet transmitted, active immunization offers the most practical method of control. However, in many respiratory diseases one attack does not confer a lasting immunity or there is, at present, no satisfactory immunizing agent. In these cases air sanitation seems to offer possibilities could it be accomplished

satisfactorily. Ventilation, dust control, irradiation, and disinfection are the methods that have been tried.

Ventilation: Ventilation involves replacing contaminated air with fresh air, the chief effect being dilution. The amount of replacement necessary to accomplish the desired result depends upon the concentration of susceptible persons occupying a given amount of space. Actually, of course, the amount of replacement of air necessary to control infection varies with different disease agents. Under ordinary conditions in the home or in schools, ventilating alone is not an effective means of preventing infection, as witness the contagiousness of such diseases as influenza, the common cold, and measles.

Dust Control: Dust has been shown to harbor pathogenic organisms for relatively long periods. In a hospital or sick room dust from blankets is a particularly important source of pathogens. When patients' beds are made such organisms as hemolytic streptococci float in the air and eventually settle out on beds, desks, or instruments, on the skin and clothing, or are inhaled. In hospitals and occasionally in laboratories dust-borne bacteria are a source of infection. Studies of infections of wounds suffered by bombed civilians in England proved the seriousness of air or dust-borne infections.

The studies on dust control also showed that by oiling floors and applying oil-in-water emulsions to bedclothes bacterial counts could be reduced by about ninety per cent. Oiling of floors and bedding was an effective means of controlling upper respiratory diseases in army camps according to English workers. They found that the average weekly infection rate per 1000 was 38 in the controls as compared to 7 in the test unit. Tests conducted in American camps also showed the effectiveness of oiling in reducing complications due to cross-infection in wards. The action of the oil is, of course, purely mechanical and merely keeps the dust-borne bacteria from flying around.

Irradiation: The effectiveness of germicidal radiation has been studied by many workers and the results are rather difficult to interpret. There is little question but that ultra-violet light in the wave band of 2537 Angstrom units is highly germicidal. But killing bacteria by radiation is a function of time and intensity and the practical problem is to insure sufficient intensity of light for a long enough time to destroy the air-borne pathogens. Reports are at variance as to how much respiratory infections in schools can be reduced by irradiating the upper air in class rooms. Since the ultra-violet light in the intensity necessary to disinfect air is harmful to the eyes, it is not feasible to irradiate occupied rooms and some method other than direct continuous irradiation must be used. Air conditioning that would

remove dust and irradiate the air in the ducts before circulating it into occupied rooms offers a possible solution.

Disinfection: Disinfection of the air by chemicals has attracted the attention of many workers and it has been shown that effective germicidal mists can be produced under experimental conditions where the various factors such as humidity and air currents can be controlled. Triethylene glycol vapor is the most promising chemical disinfectant.

The nature of the problem of air sanitation and the prevention of air-borne infection as contrasted with water purification should be recognized. When a city water supply is chlorinated, all of the water in public places, schools, and homes is made safe. Air sanitation on a corresponding scale would mean that all the air breathed was free from pathogens. While this might possibly be accomplished with isolated groups, such as hospital patients, and possibly in school rooms, it remains to be seen whether persons so protected will become more susceptible to infection upon leaving such institutions. The rapid spread of upper respiratory infections among crowds suggests that if the air in the more crowded places could be properly purified the incidence of many of the upper respiratory infections would be greatly reduced. Since air sanitation is a relatively new field it seems likely that much progress will be made in it in the near future.

CHAPTER XXXV

MICROBIOLOGY AND FOOD

Civilization depends upon an adequate supply of food. It cannot develop nor be maintained in its absence for man as an individual or in a group will not concern himself with the advance of culture when there is a question mark between his meals. Supplying a nation with a diet ample in amount and varied in kind is no small undertaking. It involves production, preservation, storage, and transportation, and it involves a certain amount of control by public health officials to safeguard the consumer against harmful or poisonous agents that may be present in the food itself and against contamination of the food during its handling.

We have already described in some detail how microorganisms are essential to the process of decay in which dead and surplus matter is reduced to simpler forms and then built up again into new living things. The part they play in soil fertility and food production is basic.

Preservation, storage, and transportation problems arise for several reasons. Plants and animals that are used for food by man may also be used by microorganisms. As long as the plant or animal is alive and healthy, it is able to resist the action of the microorganisms of decay always present on its surface; but as soon as the plant is harvested or the animal is killed, the microorganisms invade and decomposition begins. Some products are decomposed readily; while others, such as the cereals or ripe seeds, resist decomposition for long periods.

Most crops are seasonal and in any region the period during which they can be harvested is short, extending from a few weeks to a few months. This means that crops must be preserved unless consumption is to be limited to the season of their harvest.

Because of varying soil and climatic conditions, the different regions of the world appear to be particularly adapted to the production of certain plants or animals. In the United States, we have wheat belts, corn and hog belts, and fruit belts. But, obviously, the regions where crops can be produced most economically and abundantly are not necessarily the regions where the population who consumes them is concentrated. The areas of greatest population density are, of course, the industrial regions. One-tenth of the total population of the United States, for instance, lives within a radius of one hundred miles from New York and the food for this multitude must be brought from the agricultural hinterlands.

The development and improvement of agricultural machinery has greatly increased the amount of food produced per farmer and, as a consequence, fewer and fewer people supply the food for a larger and larger

proportion of the population. In 1870 more than fifty per cent of the people lived on farms and raised most of their own food. This number has declined steadily, until now less than a third live where they can grow their own food. The other two-thirds expect to buy it in small quantity, good quality, and unlimited variety at the nearest market.

So, because crops are seasonal, because no one region grows all the foods demanded by considerations of diet and taste, and because comparatively few people produce all or even most of their own food, the processing, preserving, transporting, and selling of food has become a major industry and one in which the microbiologist has a special interest, for microorganisms make foods, spoil foods, and preserve foods. They bring about changes which are desirable and they bring about changes which are undesirable.

Of course, microorganisms are not the only cause of deterioration in foods. The natural enzymes of the plant and animal cells continue to function for long periods after harvesting or storage and eventually bring about decomposition. A certain amount of such activity improves the flavor of meats, makes them more tender, and hence is considered desirable. Enzyme activity ripens fruit that has been picked green. But, after a certain stage is reached, any more activity results in decay. In general, the methods used to retard enzyme activity are the same as those used to reduce the activity of microorganisms.

Loss of water and oxidative changes due to exposure to air are additional problems encountered in the storage of foodstuffs.

From the public health point of view, the food supply today is potentially far more dangerous than it was when each family grew most of its own. Food that has been handled by a number of people is apt to pick up and harbor pathogenic organisms and so become a vehicle in the spread of some diseases.

MICROORGANISMS AND FOOD SPOILAGE

Bacteria, yeasts, and molds attack and spoil enormous quantities of food every year. We have already seen that these organisms are ubiquitous in nature, that they are capable of utilizing a great variety of materials for food if a small amount of moisture is present, and that they can live and grow in a fairly wide temperature range.

Although microbes use food only in solution, they secrete extracellular enzymes which bring about the initial cleavage, after which they absorb the soluble constituents into the cell and use them to build up cellular material and as a source of energy.

When microorganisms grow in food they act on the fats, carbohydrates, and proteins and bring about a series of chemical changes which lead to the

formation of fractions with entirely different tastes and flavors. They often reduce the nutritive value and affect the palatability. The characteristic changes brought about depend upon the composition of the food and upon the nature of the predominating microorganism. In a few cases, as we have said, these changes may be desirable; ordinarily they are not.

Bacteria are, in general, rather exacting as to moisture, osmotic pressure, and pH requirements and hence are less apt than the molds to find suitable conditions for growth. However, they multiply more rapidly and, if conditions are favorable, tend to overrun the other forms.

The yeasts are more tolerant of a high osmotic pressure and of high acid conditions.

The molds are the most tolerant of high osmotic pressure and high acid concentrations and can grow in relatively dry and fairly acid foods. They are, consequently, a very important cause of food spoilage.

Fruits: The molds and yeasts are largely responsible for the rotting of acid fruits. The green mold commonly found on lemons and oranges belongs to the genus *Penicillium*. The non-acid fruits, such as bananas, are more frequently attacked by members of the genus *Rhizopus*. Bacteria are of secondary importance.

The microorganisms break down the pectin and carbohydrates making the fruits soft and unpalatable.

Since the growth of these microorganisms may be retarded by lowering the temperature, refrigeration and cold storage are commonly employed means of control. Waxy coatings, and fungicidal coatings and wrappings have also been used on some fruits to prevent decomposition and dehydration.

Fruit Juices: Fruit juices readily undergo fermentation. The surface becomes covered with a greyish-white scum of *Mycoderma* which destroys the acids and sugars and creates an undesirable musty flavor.

Yeasts grow rapidly and convert the sugars to alcohol and carbon dioxide. The availability of oxygen determines the character of the fermentation. In open containers, a relatively high concentration of acetic acid is formed; in closed containers, a high concentration of alcohol. Yeasts are of special importance in the production of wine from fruit juices; and bacteria, in the production of vinegar.

Fruit juices may be preserved by pasteurization, by freezing, or by the addition of sugar. Large numbers of bacteria may be removed by filtration.

Cereals: Ordinary cereals do not undergo rapid decomposition unless the moisture content is high. Wheat flour, for example, will keep if the moisture content is fifteen per cent or less, but not if it is seventeen per cent.

Flour: There are certain oxidative changes that take place in flour during storage. Since they appear to improve its quality, it is customary

to 'age' white flour. The nature of the changes is not clearly understood but there appears to be a destruction or inactivation of the protein-digesting enzymes which produce a sticky dough. Bleaching agents probably improve flour in a similar manner.

According to results obtained by Holtman, the bacterial count of wheat flour ranges from about three thousand to seven thousand per gram, and the mold content is from one hundred to seven hundred. The more prevalent types of bacteria found in flour are *Aerobacter aerogenes* and *Bacillus mesentericus*, although other genera are frequently present.

Bread, which contains more moisture, is subject to spoilage by molds primarily, and, to a lesser extent, by bacteria.

Vegetables: Vegetables such as carrots, potatoes, cabbage, and the like are non-acid and subject to attack by bacteria and, to some extent, by molds. The softness accompanying decomposition is due to the breakdown of the pectin material. As a result the adjoining cells lose water and the surrounding tissue becomes soft and watery. Some bacteria convert the soluble sugars to lactic acid, creating a sour taste and smell and making the vegetable unfit for consumption.

A low storage temperature with free circulation of air and proper humidity is important in the control of the attacking microorganisms. Fresh vegetables should be handled carefully and should never be placed in very large piles.

Eggs: Eggs present a difficult storage problem. Most of them are sterile when freshly laid but about ten per cent contain bacteria even then. The surface, of course, is never sterile, but, although the pores in the shell are large enough to permit the entrance of bacteria and of the hyphae of molds, the fresh egg is covered with a mucilaginous film which affords additional protection. If the eggs are kept clean and dry, there is little danger that microorganisms will penetrate. If the eggs are soiled in the nest, or washed, the protective coating is destroyed and the moisture aids penetration of bacteria and molds.

Fresh market eggs have been shown to have from 0 to 100 bacteria per gram, whereas eggs in storage for several weeks may show counts of several thousand. Counts reported on Grade C eggs in the process of being preserved by fast freezing run from 20,000 to over 500,000 bacteria per gram. About a tenth of the bacteria are of the coliform types and a goodly percentage of the remainder is proteolytic or hydrogen sulfide producing forms. There are fewest bacteria in eggs in the winter and early spring. The number tends to increase as the weather becomes warmer and is highest in the hot summer months.

Eggs also deteriorate because of chemical changes involving the release of carbon dioxide. By increasing the concentration of carbon dioxide

within the egg, the rate of these reactions and hence the rate of deterioration, can be retarded. Coating the egg with a mineral oil or with water glass effectively plugs the pores so that the carbon dioxide cannot escape. It also prevents water loss and the entrance of bacteria.

Eggs should be cooled as rapidly as possible and kept at low temperatures which retard the enzyme activity within the egg, slow down bacterial growth, and thus help to preserve them.

Meats: That meats of all kinds are excellent food for numerous species of microorganisms might be deduced from the common knowledge that as soon as the natural defensive mechanisms cease to function, that is, that as soon as an animal dies or is killed, rapid decomposition occurs. Meat from freshly killed healthy animals is seldom sterile but the numbers of bacteria in it are small. The surface, of course, is well seeded with bacteria but if the meat is handled under sanitary conditions and put in cold storage immediately, there should be little spoilage.

Bruises and other injuries allow bacterial invasion and may account for infections next to bones which lead to a condition known as "bone souring."

Ground meats are particularly likely to spoil because the surface bacteria become distributed throughout the meat during the process of grinding. Hamburger may contain tremendous numbers without showing obvious evidence of spoilage. Plate counts run from a few thousand to several hundred million per gram and represent many species of gram-negative bacteria, gram-positive cocci, and spore-forming aerobes and anaerobes.

Although modern methods for meat preservation are a bit more refined than the ancient ones, the principles involved are essentially the same and depend upon preventing or retarding the growth of microorganisms by drying, salting, smoking, or lowering temperatures. Drying is the method most frequently used by primitive people. The American Indians made pemmican by cutting the flesh of buffalo and other game into long narrow strips and hanging them to dry. This prevents activity by microbes, for it increases the osmotic pressure to a point beyond their tolerance. Salting does the same thing. Freezing and refrigeration at low temperatures are, of course, the most commonly used methods of preservation.

In general the methods employed to destroy microorganisms or to retard their growth and so to prevent food spoilage are:

Sterilizing: This is usually accomplished by the use of heat, as in canning; but liquids such as fruit juices may be sterilized by filtration. The first process actually destroys the microorganisms, the second removes them.

Drying, Salting, Pickling, and Preserving: Increasing the osmotic pressure beyond a certain point prevents the growth of the microorganisms associated with spoilage. It may be accomplished by the addition of salts or sugar or by drying. A concentration of eight to ten per cent salt will prevent the growth of most microorganisms. Only a few will grow in a concentration of fifteen per cent.

In making preserves, a large amount of sugar is added. A concentration of fifty per cent is needed to prevent the growth of bacteria, but even this will not prevent the growth of molds. However, molds are aerobic and their growth can be prevented by sealing and excluding air.

Drying increases the concentration of sugar and salts and thus prevents microbial growth.

The vinegars or brines used in pickling are somewhat germicidal.

Smoking and Chemical Preservatives: The preservatives used in foods may be natural products of microbial decomposition, such as alcohol and acetic acid, or artificial compounds such as sodium benzoate. Fish and meats are often salted and then smoked. The process dries the foods and also impregnates them with various germicidal substances present in the smoke.

The use of artificial preservatives is governed by law.

Refrigerating: Lowering the temperature retards the rate of microbial growth. If the temperature is low enough, it prevents decomposition. The ordinary home refrigerator which maintains a temperature from 0° to 10° C., depending upon its adjustment, satisfactorily retards microbial growth for a short length of time.

The temperatures recommended for keeping various foodstuffs are different and each depends upon the nature of the food to be preserved.

Some foods can be frozen without damage and will then keep indefinitely. Most foods are altered in taste or texture by freezing. This change is referable to the destruction of cells as the ice crystals form and depends upon the rate at which the foods are frozen. The more rapid the freezing process, the smaller the ice crystals and, hence, the less the destruction of cells and the less the alteration in taste or texture. This is the advantage of the 'fast-freezing' process which has gained such tremendous popularity in the last few years.

The choice of a method of food preservation depends upon the nature of the food, the time it must be kept in the preserved state, and the expense of processing.

Foods of various kinds: meat, fish, fowl, and dairy products may undergo a certain amount of bacterial decomposition and still not be dangerous nor produce ill effects if consumed. As a matter of fact, partially decomposed food is considered a delicacy by the connoisseur. The British prefer

their game 'high,' the Eskimos consider fish which has undergone considerable bacterial decomposition far more tasty than fresh fish, and the gourmet relishes the distinctive tang of old cheeses in which the microorganisms have had ample time to create characteristic flavors. Whether food which has undergone some microbial decomposition is spoiled or not seems to depend, sometimes, upon our point of view.

MICROORGANISMS AND FOOD PRESERVATION AND PROCESSING

It would be fostering an entirely erroneous impression of microbial activity in foods and beverages, were we to feel that it always is undesirable. The same organisms that produce undesirable changes in some foods are responsible for desirable changes in others and, as a matter of fact, the food and beverage industry depend upon microbial activity for preserving and processing.

Beverages: The use of fermented beverages dates to antiquity and most nations have characteristic drinks which have played a part in their rituals and customs and are celebrated in story and song. In Old England, it was mead, a fermented honey drink; in Germany, it is beer, made from barley or other grains; in Japan, it is saki, a rice wine; in the Mediterranean countries, it is wine, usually made from grapes; in Mexico, it is pulque, a fermented drink made from the juice of the agave; in Russia, it is Kvass, made from barley and rye with peppermint added for flavoring; and in the Scandinavian countries, it is taette, a fermented milk drink.

A more recent innovation is the distillation of the fermented liquors and the production of whiskey, brandy, gin, cordial, liqueur, tequila, and the like.

Beers: Beer is one of several malt beverages made from cereals, usually barley, by a process known as brewing. The essential difference between the processes of wine and beer making is occasioned by the fact that the carbohydrates in fruits are largely sugars, whereas in grains, they are starches. Sugars are readily acted upon by yeasts; starches are not, and the cereal starch must be converted to sugar before fermentation can occur. This conversion is brought about by the enzymes contained in the living seed. The first step in brewing consists of soaking barley in water and allowing it to germinate, a process known as malting. The malt is then dried and heated slowly to a temperature of 75° to 100° C., the degree determining the color and flavor of the final product. This malt contains proteins, carbohydrates, and proteolytic and saccharolytic enzymes.

American barley is high in protein and produces an inferior beer unless twenty to thirty per cent of carbohydrates is added. Corn products such as grits, flakes, or corn sugar; or rice or wheat flakes are commonly used as adjuncts to the malt.

Next the malt is mixed with water, hops are added for flavor and because they contain bacterial inhibiting substances, and the resulting mash is incubated. A temperature of 40° to 50° C. is maintained for about an hour during which the proteolytic enzymes are active; the temperature is then raised to about 70° C. at which the starch-splitting enzymes are active; and then all enzymes are inactivated by raising the temperature to about 75° C. The mash is filtered and the *wort*, or sugary liquid portion, sterilized and inoculated with brewer's yeast, *Saccharomyces cerevisiae*. The grain residue is used as food for live stock.

The addition of yeast to the wort is called *pitching* and the strain used determines to a considerable extent the alcoholic content of the final product.

Fermentation is carried out at temperatures of 6° to 20° C., depending upon the type of yeast used.

As fermentation proceeds, a scum consisting of yeast cells, resins, and proteinaceous materials is carried to the top and is removed. After five to ten days, depending upon the type of yeast and the temperature, the yeasts may flocculate and settle out and at this point the fermentation is complete. The sugars have been converted to alcohols, ethyl mainly, glycerol, acetic and carbonic acid; and esters have been produced by combination. Higher alcohols and acids derived from proteins and fats are also present in small quantities.

The beer is now clarified by settling, and is bottled and carbonated. Packaged beer is usually pasteurized.

The flavor of beers depends upon a number of factors and the actual production of beer is a highly refined and carefully controlled process, much more intricate than indicated in the foregoing discussion.

Wines: When grapes are crushed and the juice expressed, the yeasts normally present on the skin and stems multiply rapidly in the juice, converting the sugar to alcohol, carbon dioxide, and water. Bacteria and molds are also present and if they find conditions favorable will grow and produce acids, carbon dioxide, and water.

There are several general types of wine: sweet, dry, sparkling, and fortified. Sweet wine contains enough sugar to be tasted and is produced by arresting the fermentation at an early stage. Dry wine contains little sugar and is the result of a more nearly complete fermentation. A sparkling wine contains considerable carbon dioxide and is produced by removing the sediment after the fermentation has progressed to a certain point, adding more sugar, and allowing further fermentation. A fortified wine is one to which distilled liquor or spirits have been added.

The color of the wine is due to the pigments of the grape skin extracted during the process of manufacture. In the production of white wine, the

expressed juice is used; in red wines, the skin and seeds are usually left in during the fermentation process.

The flavor or bouquet of wine depends upon the variety of grape and the process of manufacture. In general, wines are named according to the districts in which they are produced or the variety of grape used. However, it is common practice to use names such as *burgundy* or *port* for wines similar in body and flavor, regardless of where they are produced.

It is customary to add sulfur dioxide or potassium metabisulfate to the crushed grapes to inhibit the growth of the bacteria which produce the so-called 'diseases' of wine. While he was working on this problem, Pasteur found that heating the juice to 122° to 140° F. for a few moments would prevent abnormal fermentations and souring, a process which became known as pasteurization and is still used in wines and other fruit juices and, of course, in milk.

Since the yeasts present on the skins may be used to ferment the sugar but cannot always be depended upon to give the proper flavor, it is common practice to inoculate the juice using pure cultures of selected strains of *Saccharomyces ellipsoideus.*

During the fermentation process, air is excluded, otherwise a more complete oxidation would occur and vinegar would be produced. Within limits, the higher the sugar content, the higher the concentration of alcohol. However, in general, yeasts are inhibited by concentrations of thirteen to fifteen per cent alcohol, so wine usually contains not more than twelve per cent.

The temperature at which the fermentation is carried out is of importance and temperatures of 70° to 75° F. appear to give the best flavors and aromas which is why wines are so frequently fermented in caves or cellars. If the temperature is too high, wine yeasts will be retarded and many bacteria will outgrow them; if too low, the rate of fermentation will be too slow to be practicable.

After an initial fermentation period of three to five days, during which sufficient color and tannin are extracted from the skins and pulp, the juice is drained off into casks and allowed to undergo further fermentation. Carbon dioxide collects over the surface inhibiting the aerobic bacteria. After eight to twelve days in casks, the wine is racked, that is, the juice is drawn off from the sediment or *lees*, to prevent the extraction of undesirable flavor from the old yeasts, and to facilitate clearing. It is then stored, usually in white oak barrels, and aged.

As wine ages, a process which takes from three to five years in the better wines, proteins, tartrates, and other substances which give new wine a cloudiness and raw flavor settle out. Heating hastens the precipitation so some modern wines are pasteurized at this stage, and then cooled. Of

equal importance is the development of desirable flavors due, primarily, to the formation of esters from a combination of alcohols and acids. The aging process does not take place in the glass bottle and consequently the age of wine after bottling is of little importance.

Distilled Spirits: Whiskeys are usually made from fermented grains: wheat, rye, corn, and barley. The initial preparation and fermentation are somewhat similar to that used in the production of beer. After the fermentation is complete, the liquid is distilled. Brandies are distilled from fermented fruit juice, tequila from pulque, and rum from fermented blackstrap molasses.

Vinegar: The term 'vinegar' means literally 'sour wine,' and when wine or beer or other alcoholic solutions are further fermented by certain bacteria, a souring occurs, for acetic acid is formed from the alcohol. The first step in the production of vinegar is the production of ethyl alcohol. As raw material for this process, fruits, such as apples, peaches, or cherries, or cereals or syrups, or any other substance capable of undergoing alcoholic fermentation is used. The production of alcohols is an anaerobic fermentation due to yeasts. The conversion of alcohol to acetic acid is an oxidative process and is brought about by aerobic bacteria belonging to the genus, *Acetobacter*. A number of species, particularly *A. aceti*, *A. orleanense*, and *A. pasteurianum*, are commonly found in vinegar.

The *Acetobacter* produce a floating zoogleal mass or membrane which will sink if disturbed. Since the production of acetic acid from alcohol is, as we have said, an oxidative process, the methods used are designed to provide a large surface and an ample supply of air and a support for the zoogleal mass of bacteria.

On the farm vinegar may be made in barrels or casks; and rafts, excelsior, or shavings are often added for support of the *Acetobacter*. In commercial production, the alcoholic solution is commonly allowed to trickle down through towers filled with shavings, rattan, coke, corncobs, or other material which presents a large surface for the growth of *Acetobacter*. The temperature usually employed ranges from 80° to 85°F.

Vinegar made from fruit juices, such as cider, contains many substances besides acetic acid and is improved by aging. Vinegar made from pure alcohol is little more than a four to eight per cent solution of acetic acid.

There are several causes of vinegar spoilage. The more common are "vinegar eels," a nematode worm whose scientific name is *Anguillula aceti*, and vinegar flies, *Drosophylla cellaris*. Both are offensive and may be the cause of unpleasant odors and flavors. In addition, bacteria and yeasts may grow and oxidize the acetic acid to carbon dioxide and water.

Pasteurization and filtration are used to prevent spoilage.

Sauerkraut: The characteristic flavor of sauerkraut is due chiefly to

lactic and other acids which are produced by bacterial fermentation and act as preservatives. In the manufacture of kraut, the cabbage is shredded or crushed and two to three per cent of salt is added. The salt serves several purposes: it adds flavor, it draws the juices out from the leaves by osmosis, and it inhibits the growth of certain types of organisms while allowing the lactic acid producers to grow. Since the cells of the shredded leaves are not immediately killed when they are crushed, they continue to respire and use up oxygen, thus creating anaerobic conditions. Bacteria present on the leaves begin to grow in the brine and convert the sugar to lactic and acetic acids and alcohols. The acids and alcohols react to produce esters which are partially responsible for the characteristic flavor.

There are three types of bacteria in a normal kraut fermentation. *Leuconostoc mesenteroides*, a gas-producing coccus, predominates in the early stages and produces lactic and acetic acids, alcohols, and carbon dioxide. These cocci are relatively sensitive to acid. When the acids accumulate, their rate of growth is retarded and, as the concentration reaches a half to one per cent, they are destroyed.

During the later stages of this period, a non-gas-producing rod, *Lactobacillus plantarum*, begins to multiply freely and soon predominates. It produces a large amount of lactic acid. However, *Lactobacillus plantarum* is also inhibited by the acids produced and a more acid resistant gas-producing rod, *Lactobacillus pentoaceticus* or *brevis* begins to multiply freely and completes the fermentation.

Many other organisms are present, some of which may produce undesirable changes. A common form of spoilage in kraut is due to yeasts that produce a pink or reddish pigment and belong to the torula. Such kraut, which has a pinkish appearance, is sometimes referred to as 'bloody' kraut. The yeasts responsible for this change often appear when the salt is not evenly distributed and will grow where the salt concentration is too high for the desirable bacteria. Too high temperatures and aerobic conditions also favor the growth of these yeasts.

Some types of bacteria produce slimy kraut, and some produce proteolytic changes or rotting.

Lactic acid fermentation may occur in many types of vegetables if conditions are favorable. The Russians use red beets which have undergone lactic acid fermentation in making their famous borsch.

Pickling: The process known as pickling usually refers to the preservation of foods by the use of brine or vinegar or both. In some instances, the vinegar or acid is produced by fermentation. Spices are added for flavoring and not for preservation.

In pickling cucumbers, a salt concentration of about fifteen to sixteen per cent is commonly used. The juices which serve as food for the bacteria

are thus drawn out by a process of osmosis. The fermentation is brought about by a number of bacteria, of which lactobacilli are the more important, and lactic acid is produced. The salt in the brine preserves the green coloring matter, which, as the process continues, becomes distributed throughout the pickle.

If the salt concentration is too low, proteolytic and pectinolytic bacteria will grow and produce a soft pickle. If it is too high, the pickle will shrivel.

Silage: The changes whereby corn or other plants are converted into silage are brought about by enzymes. The initial changes are due to the enzymes of the plant tissue. For some time after the plant is cut, the cells continue to respire and to produce carbon dioxide. Since air is excluded in the silo, the oxygen is soon depleted and anaerobic conditions prevail. The plant enzymes act on the carbohydrates, particularly on the sugar, producing some organic acids and alcohol. The acids have an inhibiting action on yeasts and proteolytic bacteria, but lactobacilli grow and attack the carbohydrates, producing lactic and acetic acid, which also act as preservatives. Since the molds are aerobic, little mold spoilage occurs except on the surface or portion exposed to air. However, if the silage is not properly packed, aerobic bacteria and molds will grow and cause spoilage.

Bread or Panary Fermentation: The use of leavened bread began in the distant past. Leavening is a biological process depending upon yeasts or bacteria. Flour and water constitute a suitable medium for their growth and if left standing, undergo spontaneous fermentation, the nature of which depends upon the predominating microorganisms or leavens. This fermentation results in the production of alcohol or acids, carbon dioxide, and water. The bubbles of carbon dioxide cause the dough to rise and become light. The protein in the dough also undergoes changes which contribute to a better flavor and texture in the loaf.

Bread may be made by several processes. Usually a yeast is added, but, if not, the dough will undergo spontaneous fermentation as in the so-called 'self-rising' or 'salt-rising' breads. In the 'self-rising' bread, bacteria which produce acids predominate, and hence such bread is often called 'sour-dough' bread. The fermentation in the 'self-rising' dough depends upon chance contamination and the predominating organisms are not always the same. Most bread is leavened with yeasts. It is then more uniform and, because of the higher production of carbon dioxide, lighter.

It used to be customary to propagate yeast at home or at small bakeries, but now most of the yeast is prepared commercially. The brewers yeast, sometimes used, is a by-product of the brewing industry and is skimmed from the top of the vats. Bakers yeast is a relatively pure culture grown in vats in a suitable wort made from grains or potatoes. The cells are

washed, mixed with meal or starch, and compressed. Yeasts mixed with meal will remain viable for long periods when dried.

FOOD ADULTERATION

The food industry is regulated under the terms of the Pure Food and Drugs Act passed by Congress in 1906 and revised and passed in 1938. Previous to the passage of this act the food industry was in a deplorable state and adulteration with cheap substances, the use of preservatives injurious to health, and mislabeling were common practices.

To various agricultural experimental stations and particularly to E. F. Ladd of the North Dakota Experimental Station must go a great deal of credit for calling attention to these practices. It was found that, curiously enough, the total production of maple syrup was only about fifteen per cent of the amount sold as 'pure maple' syrup; that pickles and peas were colored with copper sulfate; that samples of "pure" cocoas and chocolates contained as much as ten per cent of cereal; that potted chicken and potted turkey contained no detectable amount of either chicken or turkey; that meats contained borax, sodium sulfate, and other preservatives; that a large proportion of the jams and jellies were colored by coal tar dyes, sweetened with saccharine, and made from inferior substitutes for the fruit named on the label. One sample labeled "Strawberry Jam" was colored with artificial dye, sweetened with saccharine, and abounding with 'strawberry' seeds which, when planted, produced timothy!

The artificial preservatives formerly used were not necessarily harmful; but their promiscuous use often concealed the inferiority of the product.

Another common practice was the use of fictitious names and addresses for the manufacturing companies of the inferior or adulterated food, thus making it difficult to prosecute or affix blame.

The passage of the Pure Food and Drugs Act and the establishment of State Food Regulatory Departments have corrected most of these nefarious and unscrupulous practices.

Chapter XXXVI

FOOD TOXEMIAS AND FOOD-BORNE INFECTIONS

There are many disorders and acute illnesses associated with the ingestion of food and since they are due to different causes and require different methods of control, it is convenient to distinguish types.

There is, of course, the allergic response, which is an individual idiosyncrasy and may be occasioned by the ingestion of any food to which the individual is hypersensitive. Since it is not due to the innate toxicity of the food nor to its contamination, it will not be discussed here.

There are only two types of food illnesses: toxemias and infections.

Food toxemias may be caused by food that is naturally poisonous, food into which poisons have been accidentally introduced, or food containing poisons due to microbial activity. In the first group are such poisons as the solanin of nightshade; in the second, arsenicals used in spraying fruit or vegetables; and in the third, botulinum toxin produced by *Clostridium botulinum*.

Food-borne infections may be due to bacterial or animal parasites. In some cases the food becomes contaminated and serves merely as a vehicle for the infectious agent; in others, as in the case of infected meats, the food is infected because it comes from a diseased animal. These two possibilities call for quite different methods of control.

Ptomaines, which were at one time believed to result from putrefaction, have never been satisfactorily shown to be a cause of food poisoning. Acute illnesses or injuries due to foods which harbor bacteria are, as we have said, either toxemias or specific infections and the term "ptomaine poisoning" is inaccurate and misleading and should not be used.

In the toxemias, the incubation period is short since the toxic products are already present in the food and do not need to be produced by bacteria after they have invaded the body. In fact, ingesting the organisms, even in large numbers, does not give rise to symptoms, for the toxins are not formed within the body.

In the food-borne infections, the organisms multiply in the intestinal tract and symptoms do not appear until sufficient time has lapsed for multiplication and invasion to take place. In some instances this may be as little as twenty-four to forty-eight hours, in others more than a week.

BOTULISM

Botulism is a specific disease caused by eating food containing a powerful poison, an exotoxin produced by a spore-forming anaerobe, *Clostridium botulinum*.

Recognition of Botulism

The symptoms of botulism are quite different from those usually associated with food poisoning or food-borne infections. They are due to a neurotoxin which produces a paralysis of the motor nerves, and the gastrointestinal tract is merely the route by which the toxin gets into the body. First evidence of botulism is great muscular weakness, fatigue, and dizziness. There is no fever. Nausea and vomiting may occur but are not pronounced nor lasting. Constipation and urine retention, not diarrhea, are the rule. One of the most characteristic symptoms is visual disturbance, due to the failure of the eyes to adapt to light. Double vision or diplopia is common and there may also be a drooping of the eyelids known as ptosis. The throat may be dry or there may be excessive salivation. There may be aphonia, a complete or partial loss of voice. There is no damage to the brain and the mind remains clear. If death occurs, it is in from four to eight days, as a rule, and is due to respiratory failure.

Etiology

Botulism is a toxemia, not an infection. The toxin is produced by *Clostridium botulinum* when the organism is growing in foods, not when it is growing in the body. Spores of the organism can be swallowed with impunity and probably never multiply within the body.

Clostridium botulinum was named from the Latin word *botulin* or sausage because the first outbreak which attracted attention followed the eating of sausages. In 1896, van Ermengem first implicated this organism in this disease. He isolated the spores from the organs of persons who had died after eating raw ham and also from remains of the ham.

There are at least five different types of *Clostridium botulinum*. Types *A* and *B* are the cause of botulism in man. *C* is the cause of limberneck or Western duck sickness, and a subtype has been associated with forage poisoning in horses in the United States. *D* and *E* have been found to cause botulism in cattle and horses in South Africa.

Morphology: Clostridium botulinum is a gram-positive spore-forming anaerobe about 4 to 6 microns in length and 0.9 micron in width. The spores are wider in diameter than the vegetative cell and are located near the end. Flagella are present and give the organism motility.

Clostridium botulinum is widely distributed in soils, and the spores may be found on the roots, leaves, and fruits of plants and in the digestive tract of man and animals. Its natural habitat is soil, and it probably does not multiply elsewhere in nature.

The vegetative cells are not particularly resistant to heat but the spores withstand boiling for several hours. The acidity of the medium in which they are suspended greatly influences their thermal resistance, for they are

more easily destroyed in an acid medium than in a neutral one. Esty and Meyer made some studies on the thermal resistance of spores and showed how the temperature necessary to kill them varied with the time of exposure. Under controlled conditions, with a pH of 7, they found that spores were killed in the following length of time at the various temperatures:

In 4 minutes at 120° C. (248° F.)
In 10 minutes at 115° C. (239° F.)
In 32 minutes at 110° C. (230° F.)
In 100 minutes at 105° C. (221° F.)
In 330 minutes at 100° C. (212° F.)

Boiling for over five hours is necessary to kill the spore forms and at higher altitudes where boiling occurs at a lower temperature, even longer.

In the past fifteen years, no outbreak of botulism has been traced to commercially canned foods, although a number have been traced to home processed, usually 'cold-packed', foods. This is understandable because commercial canners process under pressure and obtain a sufficiently high temperature to destroy the spores in an hour or less; whereas, in the 'cold-pack' method, the temperature does not get higher than that of boiling water. The spores may remain dormant for months before germinating—just how long they will survive is not known.

Clostridium botulinum is a fairly strict anaerobe. It grows most rapidly at about 35° C. but will also grow well at room temperature. It is proteolytic and saccharolytic but some strains are more active than others. It is not a fastidious organism and grows well in most common laboratory media and in a wide variety of foods, meats, vegetables, and, even fruits.

Clostridium botulinum is a saprophyte and does not produce infection in man or animal. Although it can be found in the intestinal contents of man and animal, there is little evidence that it multiplies within the body or that it invades under ordinary conditions. Botulism is caused by the ingestion of the toxin.

Toxins: The botulinum toxin is one of the most potent if not the most potent toxin known. This is evident when we find that persons have died from botulism after biting into a single olive and spitting out the flesh because it did not taste right, or after biting into a pod of a string bean and spitting it out. That death results, even though the material is not swallowed, suggests that the toxin is adsorbed through the mucosa of the mouth, a unique characteristic. Diphtheria and scarlet fever toxin and snake venoms are not toxic when taken by way of the mouth for they are not adsorbed through the intact mucosa and are destroyed by the digestive juices.

The botulinum toxin is thermolabile and readily destroyed by heat.

Results obtained by various workers on different strains show some variation but heating for thirty minutes at 65° C. or for two minutes at 80° C. inactivates the toxin under experimental conditions, and boiling for three to ten minutes appears to render food perfectly safe for consumption.

As evidence that heating completely destroys the toxin and makes food safe, Tanner cites an instance in which a housewife tasted some home-canned string beans, then boiled them for ten minutes and served her husband and six children. Three days later she developed botulism and died whereas the rest of the family, who had consumed the boiled beans, showed no evidence of illness.

The fact that boiling destroys the toxin is of great practical significance. If there is any question as to whether food, particularly home-canned non-acid food, is spoiled; it should never be tasted until after it has been boiled for ten minutes.

The conditions favorable for the growth of the *Clostridium botulinum* and for toxin production are of considerable interest but are not well known. Brine, an eight per cent solution of salt, prevents toxin formation and lowers the thermal resistance of the spores. The composition of the food is of importance. In Europe botulism is associated with meats and meat products. In the United States, most outbreaks are associated with spoiled string beans, peas, corn, and spinach. Olives have been responsible for several outbreaks. Fruits, such as apples, peaches, and pears are less likely to be involved. The presence of other organisms undoubtedly plays a part in determining the growth of *Clostridium botulinum* and the production of its toxin. In fact, some bacteria break down the toxin.

Types of Toxins: The toxins produced by the types A, B, and C of *Clostridium botulinum* are immunologically different and the antitoxin produced against one type is not effective against the others.

Antitoxin: Clostridium botulinum toxin stimulates the production of an equally potent antitoxin, which is type specific. In experimental animals, the antitoxin is effective if used before or shortly after the toxin is introduced, but, if given after the symptoms appear, it is not. In man, its use has not been encouraging; no doubt because it is seldom given before the symptoms appear and by this time, the damage to the nerve endings has already been done. In several outbreaks, it was noticed that chickens, that were fed the remains of a meal, developed limberneck before any of the people showed signs of poisoning. Under such circumstances, the administration of antitoxin is certainly indicated. When several persons are attacked, the individual incubation periods may vary considerably, so if a diagnosis is made on the first to show symptoms, there may be time to protect the others by the prompt administration of antitoxin. It is available from the commercial biological houses and a combination of types A

and B should be used without waiting to determine which type of organism is responsible.

The largest outbreak of botulism ever to be reported in the United States occurred in 1931 at Grafton, North Dakota. The following account is condensed from a report of the outbreak by Robert W. Allen and Walter Ecklund in the Journal of the American Medical Association, 1932, Volume 99, pages 557 to 559.

Thirteen persons developed botulism and died following a midnight lunch of beans, hot boiled weiners, vegetable salad, light spice cake, and coffee. The vegetable salad consisted of diced carrots, peas, and cut string beans, served on a lettuce leaf, with a whipped cream dressing.

Of the seventeen persons present, sixteen consumed helpings of all of the food; one did not eat vegetables in any form and his wife consumed his salad in addition to her own. This man did not become ill, but his wife developed symptoms in sixteen hours and her death followed forty-eight hours after the meal. The other three who escaped death, had eaten their salad but, being intoxicated at the time, had also become nauseated and vomited both before and immediately after the lunch. Investigators were able to show by a process of elimination that the salad only could have been responsible for the poisoning. The weiners were ruled out because they were a part of a large batch made up by a local market two days before and caused no other cases of botulism among persons who had bought and consumed them. The lettuce was purchased on the market and was fresh. The drinks served were home made beer, home brew, and wine. They were ruled out because five of those who did not consume any of the intoxicating beverages died of botulism, and three of those who drank them, did not become ill. In this connection it has been said that alcohol detoxifies *Clostridium botulinum* toxin and some have even recommended that small and beneficent drinks of brandy be given. However, it seems likely that in this instance, it was the vomiting immediately after the consumption of the meal that prevented the adsorption of the toxin.

Epidemiology

Botulism occurs in scattered outbreaks. There are no secondary cases, of course, as the disease is not infectious. It never results from the eating of fresh or uncooked fruits or vegetables, but occurs after eating processed, usually home-canned, foods. This is explained by the physiology of the organisms and the high thermal resistance of the spores. As a matter of fact, the methods used in home canning are ideal for the growth of the organism. The heating is sufficient to kill the non-spore formers, some of which would produce a souring that would inhibit toxin production; it drives out the oxygen and thus creates anaerobic conditions, but it does not kill the spores.

The prevalence of botulism is not so high as might be expected since the spores are very widely distributed in nature. Spores have been found in virgin soils and, less frequently, in cultivated soils from all parts of the world. K. F. Meyer and his co-workers, to whom we owe much of our

knowledge of the distribution of botulinum spores, found that over twenty-five per cent of the soil samples from various parts of the United States contained spores. They also obtained positive cultures from vegetables, roots, and fruits in from eight to thirty per cent of the samples tested. Type A spores were obtained twice as often as Type B. In Europe, Type B is most prevalent.

Although *Clostridium botulinum* is widely distributed, the number of cases of botulism is few. The case fatality rate is high, running an average of about sixty. In some outbreaks, it has run as high as one hundred, and in others as low as thirty. This is a quite different situation than pertains in staphylococcus food poisoning where the attack rate is high—in some outbreaks five hundred to over a thousand persons have been attacked—but in which there are no deaths.

Control

The control of botulism is based on the known facts concerning its cause, the conditions necessary for the growth of the organism and toxin production, and its ready destruction by heat. Thorough washing of vegetables to remove spores is to be recommended, since it is easier to sterilize materials that have few spores. Boiling for ten minutes will destroy the toxin. If there is any question as to whether the contents of cans are spoiled, the material should be boiled before tasting. Although it is not always possible to detect obvious spoilage in food containing *Clostridium botulinum* toxin, the history of outbreaks shows that the food in question usually showed signs of spoilage.

STAPHYLOCOCCUS FOOD POISONING

Some, but not all, strains of staphylococci produce enterotoxic substances which give rise to gastro-intestinal disturbances, manifested chiefly by nausea, vomiting, diarrhea, and a moderate rise in temperature. The onset is sudden, the symptoms appearing a few hours after the ingestion of contaminated food and subsiding in a day or two. This type of food poisoning or toxemia is seldom, if ever, severe enough to cause death.

The foods most commonly associated with staphylococcus food poisoning are cream-filled pastries, chocolate eclairs, cream puffs, cakes with cream fillings, salads, and, less commonly, meats. The poisoning is often acquired at picnics, church suppers, and banquets where food, particularly desserts, for a number of people is prepared in advance and in such large quantities that refrigeration may not be possible nor convenient. If staphylococci are present in the food, they multiply at a tremendous rate and produce toxic substances. Many outbreaks occur in institutions, schools, prisons, old folks homes, and the like.

How prevalent staphylococcus food poisoning may be is not known. There is every reason to believe that a great many outbreaks are never reported and that the incidence is probably high. Outbreaks occur in all seasons of the year but are more common in the warmer months.

The control of staphylococcus food poisoning depends upon proper care in preparing foods and in refrigeration. The source of infection is man. One outbreak was traced to a cook who had had a boil on his arm and sliced a ham which was shown later to be highly seeded with staphylococci and was responsible for a number of cases of food poisoning.

Other types of bacteria, namely, *Proteus*, *Streptococcus*, and *Escherichia*, have been suspected of producing similar food poisonings, but the evidence that they do is not entirely convincing.

BACTERIAL FOOD POISONING

Food poisoning of the infectious type is primarily due to organisms belonging to the *Salmonella: Salmonella enteritidis* (Gaertner's bacillus), *Salmonella aertryche* (*typhimurium*), and *Salmonella suipestifer* (*Cholera suis*).

Salmonella enteritidis was first isolated by Gaertner from the meat of a diseased cow that had been responsible for a number of cases of food poisoning, one of which was fatal. *Salmonella enteritidis* is pathogenic for cattle and produces a dysentery in calves. Most outbreaks occur as a result of eating the meat of infected cattle. This type occurs primarily in Germany.

Salmonella aertryche or *typhimurium* is a natural parasite of rodents and is also pathogenic for cattle. It is usually associated with meats that have been contaminated by droppings of rodents. The name *aertryche* comes from a village in Belgium in which an outbreak due to the organism was first described.

Salmonella suipestifer, or *cholerae suis*, as it is sometimes called, was thought to be the cause of hog cholera at one time and is often found associated with the disease although it is not the cause. It is wide spread in hogs and associated with food poisoning following the consumption of pork.

While these are the more important species of salmonellas involved in food poisoning, a great many other species or subspecies have been shown to cause poisoning. In addition to the salmonellas of the food poisoning group, the salmonellas responsible for paratyphoid may also produce food poisoning.

Salmonella food poisoning is usually associated with meats, less commonly with milk, fish, and eggs, and only occasionally with fruits and vegetables.

Eggs, and particularly duck eggs, have been incriminated recently. Ducks, apparently, are susceptible to infection by *Salmonella enteritidis*

and *Salmonella typhimurium*. Some studies made in Germany showed that about one per cent of their eggs was infected.

Salmonella infections are usually associated with meats that have been processed, that is, made into jellies, patties, meat balls, and the like. The extra handling allows for contamination and for a distribution of the organism throughout the mass of material. Improper refrigeration allows for its rapid multiplication. The infections are most common in the summer months.

Food may serve as a vehicle for the transfer of many other infectious agents, *Salmonella typhosa* and *Vibrio cholerae* being the outstanding examples.

Control

The control of *Salmonella* poisoning is rational and depends upon the fact that the source may be infected animals or food contamination in processing. The infected animals consist chiefly of rodents and insects usually carriers. Unless the food is properly refrigerated, the organisms once introduced multiply rapidly. They do not form spores and are destroyed by boiling. In this connection it should be mentioned that it takes considerable time to raise the temperature of the entire contents of a pot or kettle to boiling. Baking in an oven takes even longer.

Acid foods such as fruits are far more readily freed of microorganisms than are non-acid foods.

To safe-guard the consumer, the government has established an inspection service which, although not perfect, goes a long way toward insuring the safety and high quality of the food reaching him.

United States Meat Inspection: As a result of abuses which had arisen and to satisfy nations that imported meats, Congress in 1890 authorized meat inspection by the Federal Government. Later the service was extended to meats that entered interstate commerce, except those slaughtered on the farm by the farmer. The objectives of the present inspection are "to search out and destroy diseased and otherwise unfit meat; to see that meat and meat products are kept clean during the stages of preparation into articles of food; to guard against the use of harmful preservatives and other deleterious substances; to cause sound and wholesome meat to be marked "Inspected and passed"; and to prevent the use of false or deceptive labels and statements on meat foods offered for sale." (E. C. Jars, Chief, Meat Inspection Division of the B. A. I., U. S. D. A.).

At the present time about two-thirds of the meat consumed in the United States and all of that offered for exportation are inspected. During the period from 1930 to 1938 an average of over seventy-two million animals was slaughtered annually in establishments where inspection was carried

on. During that period, 230,741 carcasses were condemned annually, in addition to the animals condemned after inspection in pens.

The cost of maintaining the Federal Meat Inspection service is very low and amounts to 7.6 cents per animal or about one thirty-ninth of a cent per pound on the basis of meats and meat products. The meat which is processed by smoking, salting, cooking, canning, and so on, or converted into bacons, corned beef, sausage, and such, is subject to inspection during the whole course of treatment. Pork products which are eaten without further cooking by the consumer must be so processed as to destroy the trichina worm which causes trichinosis. This can be accomplished by heating to 137°F., freezing to 5°F. or lower, or holding for not less than twenty days by curing methods.

There is no practical method for detecting the presence of this parasite in pork by inspection, so the only safeguard is to treat all pork as if it were from infected hogs and process it in such a way as to destroy the organism if it is present.

FOOD-BORNE PARASITIC INFECTIONS

In addition to the bacterial food-borne infections, there are a number of parasites of animals that may attack man and that must be guarded against. While these are more properly considered in parasitology, a few of the more important will be mentioned here.

TRICHINOSIS

Trichinosis is a serious health problem which involves the swine producers and packers as well as the consumers of pork.

Etiology

The cause of trichinosis is a small round worm called *Trichinella spiralis*. To understand the disease in its various aspects, it is necessary to consider the life cycle of this worm in its several hosts.

The infective stages of the parasite are encysted in the muscle and enter a fresh host when the flesh of infected animals is eaten, for then the digestive juices of the stomach dissolve the capsules inclosing the larvae and thus free them. From the stomach they pass on to the intestinal tract where they develop into mature worms. Mating occurs and the females produce about several hundred eggs.

Recognition of Trichinosis

The clinical manifestations of trichinosis are so varied that it has been confused with over fifty other disease conditions. Maurice Hall of the United States Public Health Service has made a critical study of the problem of diagnosis and the clinical manifestations of the disease and says

that the disease depends upon the presence of the parasite *Trichinella spiralis* in a number of forms: "as infected larvae entering the digestive tract; as older larvae and adults in the lumen of the intestine; as adults among and partly within the villi; as young larvae circulating through the lymphatic, systemic, and pulmonary circulation and entering such tissues as the lymph nodes and glands, the brain, the heart muscles, the striated, voluntary, somatic, and skeletal muscles, and, to some extent, other tissues and various cavities, being reported from the lungs, liver, bile, peritoneal cavity, pleural cavity, pericardial cavity, pancreas, kidney, bone marrow, placenta, human milk, and the pus from the external ear, and from a furuncle; and as larvae degenerating and disintegrating in various sites such as the heart, and encysting and ultimately calcifying in the skeletal muscles."

The severity of the reaction depends upon the dosage, that is, upon the number of larvae ingested. This may vary from one, in which case there will be no increase in the intestinal tract, to tens of thousands. For example, the Iowa State Health Department reports that a sausage disclosed 27,000 trichina larvae in five ounces of meat. Experimentally infected guinea pigs show as many as 10,000 larvae per gram of muscle, or about 280,000 per ounce.

The symptoms associated with larvae and adults in the intestinal tract are gastro-enteritis, diarrhea, constipation, vomiting, pain, intestinal hemorrhage, and fever. Because of the similarity of these symptoms to those induced by other causes, a faulty diagnosis of typhoid or paratyphoid fever, typhus fever, cholera, intestinal influenza, malaria, food poisoning, gastro-enteritis, appendicitis, or many other diseases, even acute alcoholism, has been made.

When the larvae are in the blood stream, there is a change in the blood picture, a characteristic eosinophilia and leucocytosis occurring, which has been considered of diagnostic value. In addition there may be a laryngitis, loss of voice, diaphragmatic breathing, pleurisy, cough, hiccough, asthma, skin eruptions, interference with vision such as diplopia (double vision), photophobia, sweating, weakness, loss of appetite, and a number of other symptoms. Faulty diagnosis of this stage includes undulant fever, pneumonia, scarlet fever, measles, mumps, influenza, and tetanus, to mention only a few.

When the larvae are in the heart, an erroneous diagnosis may be made of heart disease, such as myocarditis, rheumatic myocarditis, and so on.

When the larvae are in the brain, the symptoms include encephalitis, meningitis, delirium, and coma, and have been diagnosed as encephalitis, meningitis, tuberculosis, and poliomyelitis.

It is obvious that trichinosis may present a complicated clinical picture

and that an accurate diagnosis on the basis of symptoms is not easy. Many attempts have been made to develop readily applicable laboratory tests and a number have been suggested.

Laboratory Diagnosis: Since the trichinae encyst in the muscle, it is possible to excise a bit of tissue and examine it. This method is rapid and, if infested muscle is obtained, accurate. However, it is negative in the early stages of the disease and does not become positive until the larvae have encysted. Furthermore, since the larvae may be found long after the initial attack, their finding does not necessarily mean that the clinical symptoms at the time of biopsy are due to the trichinae.

Examining the stools for larvae has not proven of any value.

Neither is the examination of the blood or cerebral fluid for larvae dependable. The larvae are difficult to find and are not present in the early or late stages of the disease.

Serology: Skin tests and precipitin reactions have been developed using the protein of the trichina as antigen. These are being refined and give some promise, although positive tests must be interpreted with caution. Tests are not positive until about eleven to fourteen days after the attack and may remain so for as long as seven years after recovery.

The history of the patient is of great importance in the diagnosis of trichinosis. Evidence that pork or foods containing pork, such as pork loaf, sausages, and such, have been consumed raw or improperly cooked may be a valuable clue to diagnosis.

Immunity: It is rather difficult to say how much if any immunity is gained as a result of infection. One attack does not confer a high degree of immunity although it seems probable that it is followed by a slightly increased resistance.

Epidemiology

Prevalence. The incidence of trichinella infection in the United States is at least twelve per cent. Evidence for this comes largely from the studies of Hall and his associates on the presence of trichinae in the diaphragms of persons dying from various causes in different parts of the United States. According to the data available the United States has a higher incidence than any other country. It has been suggested that this may be related to our higher standard of living for with a greater income there is a greater consumption of pork and more scraps and trimmings find their way to the garbage pail. These uncooked scraps serve to infect hogs on the farm and in the large garbage feeding plants.

There appears to be a considerable difference in the incidence among various groups, a difference correlated with exposure. Men show a higher incidence than women; and men who travel about, such as those in the

army, navy, or merchant marine, show an infection rate of over twenty-five per cent.

It should be noted that the figures obtained from the examination of diaphragms are from persons who had never been diagnosed as having a case of trichinosis. The presence of a few larvae does not mean that persons show clinical trichinosis. Just how heavy a dosage is necessary to produce symptoms is not evident, but it will vary with the size of the person, the condition of his health, and so on.

There is a distinct seasonal distribution of reported cases. December, January, and February are the peak months, and the summer months are the lowest.

The distribution of trichinosis is not uniform in the United States. The highest incidence is along the Northern sea-board and along the West Coast, the lowest in New Orleans, a distribution correlated with methods employed in feeding swine. There are no accurate figures on the total cases of trichinosis and the case fatality rate as calculated from reported cases is much too high because only the most severe or readily diagnosed cases are reported.

Source of Trichinella: Since there is little question but that the chief source of trichinella infection is swine, it is important to determine the incidence and manner of transmission of swine trichinosis. Bears, cats, and dogs may contract the disease and cases in man have been traced to the eating of bears and dogs, although, such meat, obviously does not account for very many cases.

Hogs become infected from eating uncooked scraps of pork containing *Trichinella*. It was formerly believed that rats had an important part in swine trichinosis; but is now apparent that, although they may be heavily infected themselves, they play only a minor role in infecting hogs.

The incidence of trichinosis depends upon the conditions under which the hogs are raised. Many pasture-raised swine are not infected. Grain fed swine show about one to two per cent infection. Garbage fed swine show five per cent infection, and offal fed swine, according to figures obtained during the period from 1885 to 1890, showed an infection rate from six to eighteen per cent.

Hall and Collins believe that there is a correlation between the incidence of trichinosis in man and in hogs and that the regional distribution can be explained by the fact that along the Eastern sea-board and on the West Coast there are a number of large garbage-feeding establishments supplying pork to large cities. Then, too, the practice of feeding swill to hogs is common in New England and hogs thus fed are exposed to infection. In the South, the hogs are raised in an entirely different manner. Many are allowed to run at large in the forests and are never exposed to *Trichinella*. The incidence of trichinosis is low both in hogs and in man there.

Throughout the Midwest, hogs are raised under conditions ranging from the best to the worst and the incidence of trichinosis is intermediate.

Control

The control of trichinosis is very simple in theory but difficult and complex in practice. Since the life cycle, the host range, the manner of transmission, and the longevity and resistance of the trichinae larvae are pretty well known; it is not difficult to outline preventive measures which, if adopted, would soon eliminate or at least reduce to a negligible figure the amount of trichinosis in hogs and man.

In brief, control of trichinosis in swine resolves itself into keeping all uncooked pork out of the food used to feed swine. The danger of garbage fed hogs becoming infected is evident from the fact that one to five out of every hundred pieces of discarded pork contains trichinae and will cause trichinosis if consumed uncooked. The amount of meat scraps in garbage varies, that from hotels being highest, but all garbage contains enough so that there is a demand for it by swine feeders. Garbage disposal presents a problem in all cities. It is either fed to hogs, burned, or disposed of by some other means. Some cities sell garbage for as much as a dollar a ton. Since it would cost a dollar and a half to burn it, this means a saving of two and a half dollars per ton to the city. And, there is no objection to feeding garbage to swine, providing it is cooked. A uniform law covering this point would go a long way toward solving the trichinosis problem.

Trichinosis in humans can be prevented by cooking or otherwise processing pork so as to destroy the trichinae larvae. Pork products that enter interstate commerce are controlled by Federal regulation and are safe, but about thirty per cent of all pork does not come under this inspection and it is largely to this pork that we owe our high incidence of trichinosis in the United States. This includes hogs killed on the farm. It is recognized that there is a dilution factor operative in pork products from packing plants which does not operate in the case of home made sausage. When one to two per cent of the hogs are infected and sausage is made from a large number, the number of trichinae per pound is much fewer than when sausage or other pork products are made from a single infected hog. This is why a higher percentage of acute cases is reported from home-butchered hogs. Proper storage, refrigeration at 5° C. or less for twenty days, destroys trichinae larvae. A proper approach to the problem of trichinosis control calls for intelligent action and for the cooperation of the consumer, the swine grower, the packer, and the processer, as well as the medical and public health profession.

TAPE WORMS

A discussion of tape worms does not properly come in a book on microbiology. It will not be out of place, however, to mention them in connection with diseases contracted from infected pork and beef.

THE PORK TAPE WORM

The pork tape worm, *Taenia solium*, is world wide in distribution but is common only in countries where pork is consumed raw or improperly cooked and where hogs have access to human feces. It is relatively uncommon in the United States, but fairly common in Germany and some of the other parts of central Europe.

The adult worm is from three to eight yards long and made up of anywhere from several hundred to as many as a thousand segments. The individual tape worm has been described as a series of individuals united in a chain-like colony. There is a single head and a mother segment from which daughter segments called *proglottides* are produced. These proglottides are sexually complete and produce large numbers of eggs. The adult stage is present only in man and the segments or proglottides containing the eggs are expelled, usually in groups of five, in the feces. The proglottides or the eggs become distributed and are ingested by the hogs. The action of the digestive juices in the small intestine frees the embryo which, by the aid of hooks, penetrates the walls of the intestines and enters the portal vessels and lymphatics, and, eventually, the peripheral circulation. From there the worm enters the muscles and encysts in the tongue, neck, shoulders, or, less commonly, in other tissues or organs, including the brain and eye.

This stage of the pork tape worm is called *Cysticercus cellulosae*. There is no multiplication in this stage and unless the pork is ingested by man, no further development takes place. However, if man eats raw pork infected with *Cysticerci*, the adult stage develops in his small intestines. Hogs are the normal host for the intermediate stage, although it will develop in dogs and also in man. Man becomes infected as a consequence of ingesting the eggs expelled by another person harboring the adult form or as a result of ingesting eggs expelled in his own feces (auto-infection), in which case the larval forms will encyst. This form of the disease is particularly dangerous as the *Cysticerci* frequently encyst in the eye or nervous system as well as in the muscle tissues and other organs. The seriousness of the infection depends upon the number of *Cysticerci* and upon the tissue or organ infected. The adult stage does not produce serious disorders unless the infection is great.

Prevention

Prevention of pork tape worm infection centers on the control of the disease in hogs. Since man is the only host to the adult stage and since only the adult stage produces eggs, it follows that swine, the intermediate host, must get their infection from man, which they do from eating food or drinking water contaminated with human feces. Proper disposal of such material prevents their infection. It must be remembered that man may serve as the intermediate host and that autoinfection as well as infection of others does take place. Man, infected with the intermediate stage, is not infectious to others.

The adult stage is readily expelled by antihelmintic drugs such as oleoresin of aspidium. The treatment used to expel hook-worm is effective against the tape worm.

Measly pork, that is, pork infected with the larval stage of the tape worm, can be detected by gross examination of the carcass and Government meat inspection includes such an examination. Cooking destroys the larvae but smoking, pickling, and refrigeration are not dependable.

BEEF TAPE WORM

The beef tape worm, *Taenia saginata*, is very similar to the pork tape worm but differs in several anatomical features. The head of the *Taenia saginata* does not have hooks, whereas that of *Taenia solium* is armed with twenty-six to twenty-eight hooklets. It is somewhat longer, measuring from four to twelve yards, and the number of segments is greater, numbering as many as two thousand. The segments or proglattides are expelled singly and may force their way out of the anus, frequently appearing on the clothing or bedding. Fortunately, the larval form does not develop in man, otherwise the danger of autoinfection and infection of other persons would be greater than with the pork tape worm.

When the eggs are swallowed by cattle; the larvae develop, migrate to various parts of the body, particularly to the tongue, heart, and diaphragm, and encyst. The encysted stage is known as *Cysticercus bovis*.

There is no further development unless the beef is eaten raw or improperly cooked. The incidence of beef tape worm is increasing, probably because more rare beef is consumed.

Prevention

Proper disposal of human feces to prevent infection of cattle and thorough cooking of beef is indicated. It will be noted that multiplication of the pork and beef tape worm occurs only in man.

Chapter XXXVII

MICROBIOLOGY OF MILK AND OTHER DAIRY PRODUCTS

It is hardly necessary to dwell here upon the fact that milk is nature's most nearly perfect food, intended for the nourishment of the mammalian young and constituting the chief part of the diet of infants and young children. It might, however, be well to recall that it is potentially a most dangerous food. It is an animal secretion and hence likely to contain bacteria associated with the animal; it is liquid and therefore readily dissolves dirt from utensils and the milker's hands and hence is easily contaminated; and, a factor of particular significance, it need not be cooked to be palatable.

The bacteriologist's interest in milk is due principally to two facts: milk, which we recognize as an excellent food for humans, is also an excellent medium for the growth of microorganisms and consequently may serve as a vehicle by which agents of disease can be transported readily from animal to man or from man to man; and it is so highly perishable that the problem of delivering it to the consumer fresh and clean and safe is accomplished only by rigid adherence to practical bacteriological precepts. The prevention of milk-borne infections is one of the most serious public health problems to which we have found the answer.

Composition of Milk: Milk varies slightly in the relative concentration of its constituents but the following figures may be taken as representative:

Dry matter		12.8%
Minerals	0.7%	
Proteins	8.5	
Carbohydrates	4.9	
Fat	3.7	
Water		87.2%

The proteins, chiefly casein with lesser amounts of lactalbumin and lactoglobulin, can serve as a source of nitrogen for many species of bacteria and can also be used as a source of energy.

The disaccharide lactose is the only sugar normally present in milk. It is split or hydrolyzed by many microorganisms to form two monosaccharides: glucose and galactose. The galactose is of little importance from the standpoint of milk fermentation for it cannot be used as a source of energy and hence is not broken down by many common organisms. The glucose is readily utilized by a great variety of microorganisms.

The fats, too, can be hydrolyzed by some species and not by others.

The minerals present appear to supply some growth requirements of bacteria.

Not only does milk have the necessary food elements, but it is highly buffered and its pH of 6.4 to 6.8 is satisfactory for the growth of most bacteria.

Milk, as we know, is a good source of vitamine A and also contains vitamine G in high concentration, B, C, and D in lesser amounts, and traces of E. How important these may be in influencing the growth of the bacteria present is not known.

Sources, Kinds, and Numbers of Bacteria in Milk: The microorganisms found in milk come from three sources: the cow, the surroundings, and the handlers. Milk, as secreted by the tissues of a disease-free animal, is probably sterile; but, as it enters the teat canal, becomes contaminated and consequently, even when freshly drawn, contains bacteria varying in number from a few to as many as several hundred thousand, the average being a few thousand per cubic centimeter. The first drawn milk contains large numbers of bacteria for those which have been multiplying in the milk ducts and in the teats are washed down. The numbers decrease as more milk is drawn.

The bacteria present in the normal udder usually enter through the opening in the teat and grow in the milk cistern and canals. There appears to be a restraining or inhibiting action on all types of bacterial growth here, for even those that find the udder a suitable environment are not present in as great numbers as might be expected.

Rogers states that the bacteria found in aseptically drawn milk belong to four characteristic groups: staphylococci, streptococci, gram-negative rods, and diphtheroids.

The staphylococci may be present in large numbers and occasionally forms which are virulent for man are found. The first reported case of staphylococcus food poisoning was traced to milk. They may be a cause of mastitis but are not the common one.

The normal udder streptococci are probably not pathogenic for the cow, although it is believed that some cases of mastitis are due to this group. *Streptococcus lactis*, strangely enough, does not grow in the udder to any extent although it is the predominating organism in milk and the chief cause of its souring.

Neither do the gram-negative rods of the coliform group appear to be able to multiply appreciably in the udder for they are not commonly found in milk drawn aseptically.

Diphtheroids are apparently present in large numbers in aseptically drawn milk, but what significance they may have is not known.

Two common bovine infections due to bacteria also pathogenic for man

are Bang's disease and tuberculosis. The organisms causing these diseases are found in the milk from infected animals. There are some other bovine disease conditions in which the pathogens may be found in milk, but usually such conditions are so obvious in the cow that the milk is not likely to be used for human consumption. In anthrax, for example, the anthrax bacillus appears in the milk only just before the death of the cow.

But, while milk, as drawn, is never sterile, the number of bacteria present is not great and the types of organisms are limited. From the moment it is drawn, however, it may become contaminated with a great variety of bacteria from the surface of the teats, from the skin and hair of the cow, from particles of soil, bedding, and manure, from the air, from the utensils, and from the milker and handlers. The number and types will vary depending upon the cleanliness of the surroundings, the animal, and the milker. From the public health point of view, it is the number of certain types that is important.

The soil may be looked upon as the natural habitat for many bacteria that get into milk. Most of these forms are not important but some few species grow and produce undesirable fermentations. Both aerobic and anaerobic spore-formers survive pasteurization.

The more common bacteria normally found on the plants used for fodder are the aerobacter, lactobacilli, and streptococci. *Aerobacter aerogenes* is a gram-negative rod similar in many respects to the colon bacillus, and mucoid variants produce a fermentation characterized by "ropiness."

Since the lactobacilli are found in many natural fermentations of plants, it is assumed that plants are their natural habitat. Several species are of economic importance, among them, *Lactobacillus casei*, found on corn and important in cheese production, *Lactobacillus pentoaceticus*, found in silage, and *Lactobacillus delbruckii*, found in grain mashes.

The natural habitat of the streptococci is not definitely known and their classification is somewhat tentative. However, plants appear to be the natural habitat of *Streptococcus lactis*, the common milk-souring organism.

Cow manure is a common source of contamination and its importance, of course, depends upon the amount of it which gets into the milk. Cow manure contains roughly from five to fifty million bacteria per gram, the count of fresh manure being, according to Rogers, somewhat lower than that of manure which has been dried at body temperature for two days. A tenth of a gram of manure per cubic centimeter of milk will increase the bacterial count by about 10,000, or, perhaps, by as much as 200,000.

The kinds of bacteria introduced with the manure are of considerable importance since the coliform group, fecal cocci, and the anaerobic rods, give rise to undesirable fermentations. Their presence in milk is evidence of manurial contamination.

Unless special care is taken in their washing and handling, the utensils, milking machines, coolers, pails, and such are likely to be mainly responsible for high bacterial counts in milk. There is sufficient food in the form of traces of milk in apparently clean utensils to allow for the growth of tremendous numbers of bacteria, enough to increase the count of milk by several hundred thousands per cubic centimeter. It is because of this that so much attention is given to scalding with hot water, sterilizing with steam or chemicals, and drying. Exposure to sunlight destroys most of the nonsporulating bacteria, such as the *Streptococcus lactis*, but does not destroy the spores of aerobes or anaerobes. When milk is introduced into cans treated in this manner, it may become heavily inoculated with the spores which can germinate and produce undesirable fermentation even after pasteurization. In the production of cheese or butter such spore formers may be particularly annoying.

From the public health standpoint, man, himself, is the most dangerous source of contamination of milk. The numbers of bacteria added from human sources are rarely sufficient to raise the bacterial plate count, but a variety of diseases may be transmitted from person to person through milk. Any organism present in human feces, in the nose or mouth, or in sores is likely to get into the milk by way of the hands of the milker or handler or by sneezing or coughing. The danger of disease transmission depends upon the number of organisms introduced, their ability to survive or grow, and their ability to produce disease when taken into the body by way of the mouth.

The most common diseases transmitted by milk are typhoid and paratyphoid fevers, dysentery, summer complaints of children, diseases of the food poisoning type, diphtheria, scarlet fever, septic sore throat, tuberculosis, infantile paralysis, and several others. When considering milk sanitation, we must add to this list diseases of the animal which may infect humans such as streptococcic mastitis due to human strains, bovine tuberculosis, brucellosis, and, more rarely, foot and mouth disease, [illegible] sibly, rabies and anthrax.

Pasteurization

The term 'pasteurization' means heating milk to a temperature sufficiently high and holding it there for a period long enough to destroy all the disease-producing bacteria commonly present. This process was originally developed by Pasteur for preventing abnormal fermentations in wine and souring in beer, but the term is now applied to the application of the process to a number of materials.

There are two common methods of pasteurization, the *holding* and the *flash* process. In the holding process, the milk is heated to 143° to 145° F.,

kept at this temperature for thirty minutes, and then cooled rapidly. At a temperature of 140° F., *Mycobacterium tuberculosis*, *Corynebacterium diphtheriae*, and the less resistant bacteria are destroyed in a period of twenty minutes or less, although, as an added factor of safety, the time is extended to thirty minutes. If the temperature in the holding process is raised to 148° F. or more, the flavor of the milk is changed.

The time-temperature relationship in the killing of bacteria is nicely illustrated in the pasteurization process. For every degree increase over 140° F., the time necessary to destroy the pathogens can be reduced by one minute. In the flash method of pasteurization, the temperature is determined by the time used. In some instances, the temperature selected is [illegible]° or 160° F. for five to two minutes. A common practice is to heat to 170° F. for about twenty seconds.

The term pasteurization really implies the safe-guarding of milk and covers not only heating but also rapid cooling and bottling or handling in such a way as to prevent contamination. Either the flash or holding method is satisfactory from the safety point of view, for all pathogens and about ninety-nine per cent of all the bacteria are destroyed by pasteurization, and the keeping quality of the milk is greatly improved.

MASTITIS

Mastitis or garget is, perhaps, the most serious disease of dairy cattle, chiefly because it lowers the yield and reduces the quality of milk. It may also be of public health importance because some cases are due to bacteria pathogenic for man.

Mastitis is an inflammation of the udder. It may be acute or chronic, noninfectious or infectious. Acute mastitis of the noninfectious type may follow bruises, injuries, or chilling, and predisposes to infection. This type frequently occurs in cows shortly after freshening, particularly if the animals are producing heavily. Recovery may be complete with little or no permanent damage.

The infectious type of mastitis is usually chronic, followed by acute attacks. The degree of infection is variable but appears to be greater in large herds than in small ones of ten cows or less. In many herds nearly a hundred per cent of the cows may become infected. Thirty to fifty per cent infection is not uncommon.

Etiology

Infectious mastitis may be due to any one of several different species of bacteria. In about ninety-nine per cent of the cases streptococci are involved. Staphylococci and diphtheroids rank next, and many other species have also been shown to produce outbreaks but are not at all common.

Streptococcus agalactiae (*mastitides*) is the cause of nearly all of the streptococcic mastitis. This species or group is not pathogenic for man, so most cases of mastitis are not dangerous to those who consume milk.

Streptococcus agalactiae is a term applied to a distinct serological group of organisms, Lancefield Group B. Most of them are hemolytic but some strains are not. Cows become infected through the opening of the teats but the factors that determine whether or not infection takes place are not exactly clear. Some workers have found that true infection does not take place when the streptococci are first introduced but does when a second inoculation is made shortly after the initial exposure. This suggests that it is necessary for the tissues to become sensitized before the organisms can establish themselves and produce a true infection. The disease is usually chronic for some time, as indicated by an increase in *Streptococcus agalactiae* in the milk, before the symptoms of mastitis or the more acute stage appear. After the acute stage subsides, the disease again becomes chronic.

The streptococcus invades and destroys the milk-secreting tissues thus lowering the yield. Scar tissue is evidenced by an unevenness of the udder and experienced dairy men can determine the degree of involvement fairly accurately by palpation. One or more quarters may be affected.

The chronic cases and carriers, that is, the cows showing little direct evidence of disease, make control by elimination of infected animals difficult.

Diagnosis: The acute form of mastitis is readily recognized by the intense swelling of one or more quarters.

The early and chronic cases in which there is little swelling are not readily diagnosed by examination of the animals but may be discovered by the following tests based on observed changes in the milk.

The Strip Cup: The strip cup equipped with a 120 mesh wire screen is a valuable aid in the detection of mastitis. Normal milk passes through the screen, whereas the milk from infected cows contains clots and flakes which are retained. By drawing the first milk through such a cup an early diagnosis can be made.

Microscopic Examination: For this test the milk should be drawn into sterile test tubes from teats previously wiped clean with a moist cloth dipped in mild antiseptic. The milk is then incubated so that the streptococci will have a chance to increase, centrifuged, and the sediment examined microscopically for the presence of organisms and pus cells.

Blood Agar Plate: A loopful of milk or the sediment from milk which has been incubated and centrifuged may be streaked out on blood agar plates to detect the presence of hemolytic streptococci or staphylococci. Most *agalactiae* strains are hemolytic.

Colorimetric Tests: The reaction of normal milk varies somewhat, but lies between a pH of 6.4 to 6.9, whereas that from infected cows is more alkaline, that is, is above 6.9. Changes in pH can be detected by the use of suitable indicators such as bromthymol blue or bromcresol purple. The indicators may be used in solution or paper may be dipped in the indicator and dried. With bromthymol blue, normal milk gives a yellowish color; mastitis milk, a greenish color. With bromcresol purple, normal milk gives a yellow or yellowish blue color, and milk from infected cows, a range of color from light blue to purple, depending upon the degree of alkalinity. Sometimes a pH of less than 6.2 may be encountered in infected animals.

Tests for Chlorides: There is an increase in the concentration of chlorides in milk from infected animals. Silver nitrate with potassium chromate is used to precipitate the chlorides and the test is considered positive if chlorides are present in a concentration of more than 0.14 per cent.

Tests of Coagulability: Hadley developed a rennet coagulability test which appears to be accurate and practical. A solution is prepared of one part of fresh cheesemaker rennet extract with fifty parts of distilled water. Ten cubic centimeters of milk, the first-drawn milk from each quarter, is collected in test tubes which may be marked to indicate how far they should be filled. To each ten cubic centimeter sample, two tenths of a cubic centimeter of rennet extract is added, the tube is shaken, and allowed to stand for an hour. Normal milk will coagulate but abnormal will not in this period. Readings may be made every fifteen minutes to detect degrees of difference in coagulation time. This test is of particular importance in selecting milk for the making of cheese.

Although the tests described are valuable aids in the diagnosis of mastitis, no single one can be relied upon. It is customary to run several.

Control

Vaccination has not proven successful in preventing mastitis. Chemotherapy may be of value but there is doubt as to whether the animals are ever cured. The bacterial count of the milk is reduced by treating the cow with such compounds as sulfanilamide, but when the drug is discontinued the organisms again increase. A practical procedure that tends to prevent the spread of infection is to milk the infected animals last. It is the carrier, the animal harboring the *Streptococcus agalactiae* but showing little evidence of disease, that tends to keep the infection alive in herds.

Public Health Aspects: Although most cases of mastitis are due to streptococci which are not pathogenic for man, the milk from cows showing evidences of mastitis must be viewed with suspicion. Many large and severe outbreaks of septic sore throat and scarlet fever have been traced

to cows suffering from mastitis due to *Streptococcus epidemicus* and *scarlatina*. Indeed, many outbreaks have been traced to certified milk. The only way a milk supply can be safe-guarded is by pasteurization.

BUTTER

Butter may be manufactured from sweet or sour cream. The separation of the butterfat during the churning process is hastened and is more complete in sour than in sweet cream. Souring of cream is due primarily to *Streptococcus lactis*, but other lactic acid bacteria, which may change the flavor, are also present through chance contamination so that butters made at home from raw cream have varying tastes and flavors. Consequently, in the large scale manufacture of butter, it is customary to pasteurize the cream. This destroys the pathogens and most of the other bacteria present and the cream is then 'ripened' with butter starters or cultures. Butter starters are usually about nine-tenths *Streptococcus lactis* and about one-tenth *Streptococcus citrovorus*, also known as *Leuconostoc citrovorum*, and *Streptococcus paracitrovorus*, also known as *Leuconostoc dextranicum*. *Streptococcus citrovoros* and *Streptococcus paracitrovorus* are often called *associants* and the resulting fermentation is a good example of associated action. *Streptococcus lactis* produces lactic acid mainly. The associants produce some lactic acid but also break down lactic acid to produce volatile acids and other compounds which give butter its characteristic flavor and aroma. In the course of the fermentation acetyl-methyl-carbinol is produced and this, in turn, is oxidized to diacetyl, a volatile compound which is responsible for the flavor and aroma of butter made from sour cream.

The use of pasteurized cream and butter starters not only improves the flavor and aroma but also safeguards the consumer against disease-producing organisms such as those that cause undulant fever, tuberculosis, typhoid fever, septic sore throat, scarlet fever, and diphtheria.

The microbiology of butter is an entirely different problem from that of milk, largely because butter is not a suitable medium for the growth of microorganisms. It is mostly fat, the percentage varying from about seventy-eight to eighty-six per cent, with an average around eighty-two; thirteen to sixteen per cent is water; one to four per cent salt; and one to four per cent milk protein and solids with a small amount of milk sugar. However, there are certain types of microorganisms which do find it a suitable medium for growth and those that attack fats and cause rancidity are of particular importance. Molds and certain yeasts will also grow in butter.

The cream, the utensils, the salt, the water used to wash the butter, and the wrapper contain microorganisms that get into the butter and

cause deterioration. Butter should be wrapped in such a way as to prevent loss of water, contamination, and the growth of molds. It should be kept at low temperatures.

With the general improvement in milk sanitation, there has been a corresponding improvement in the quality of butter although from the public health point of view butter must be considered of some potential danger—far less, however, than milk.

ICE CREAM

The popularity of ice cream as a food for children, particularly, and the fact that it has been implicated in a number of outbreaks of disease makes it imperative that special attention be paid to its sanitary quality. The chief sources of microorganisms in ice cream are the milk, the utensils, and the handlers, although some bacteria may be present in the gelatin or other stabilizers employed.

Freezing, as we have seen, is not an effective means of destroying bacteria and this is borne out by the fact that ice cream made from unpasteurized milk contains on an average of about thirty-seven million bacteria per cubic centimeter—as many as sewage. After freezing, the bacterial count decreases rapidly, although if the holding temperature is above 0° C., certain forms may show an increase. The pathogens show a gradual decrease in numbers but survive for much longer periods than they do in milk or in water. Typhoid bacilli will live for several years and outbreaks of typhoid fever, dysentery, and scarlet fever, have been traced to contaminated ice cream.

The only way to safeguard ice cream is to pasteurize the cream and milk and to exercise proper care in handling.

CHEESE

Cheese as an article of diet dates back farther than any other prepared food with the possible exception of butter. Every nation or group that has domesticated animals and used their milk for food has developed a characteristic cheese and, like its favorite fermented beverage, cheese is part of the history of the people. Cheese is encountered in rituals, rites, and folklore. It has even been used as a basis for reckoning wealth.

Of course, there are many legends about the discovery of the various cheeses. Thus it is related that an Arabian traveller by the name of Kanana, in preparing for a journey, filled his water bag, made from the stomach of a sheep, with milk. After travelling all day, he stopped for his evening meal and was astounded to see a thin watery liquid flowing from his canteen instead of the milk he had expected. Being curious, he took his knife, cut open the bag, and found a tasty and palatable white curd—

the first rennet-curd cheese. His bag, as we have mentioned, was the stomach of a sheep—of one so recently slaughtered that the rennin was still active.

Of the several hundred varieties of cheese, all can be divided roughly into two groups: the acid-curd and the rennet-curd. In the former, the initial coagulation or curdling of the milk protein is brought about by

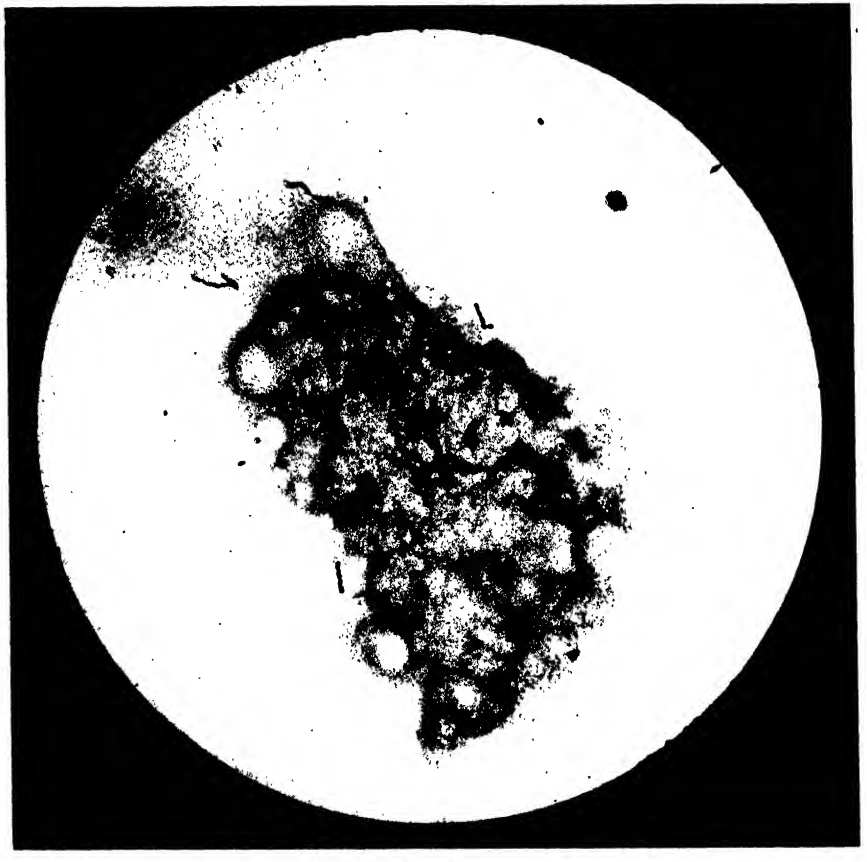


FIG. 51. Streptococci in cheese

acids produced by the fermentation of lactose or milk sugar; and in the latter, the coagulation is brought about by rennet.

Acid-Curd Cheese: The acid-curd cheeses, of which cream cheese is a familiar example, are consumed fresh and do not undergo a prolonged ripening process. They are usually produced by inoculating pasteurized milk with starters of lactic acid-producing bacteria. As the acid in-

creases, the casein precipitates and can be readily separated from the whey.

Acid-curd cheeses may harbor pathogenic bacteria unless they are made from pasteurized milk. The tubercle bacillus has been isolated from such cheeses bought on the market and typhoid epidemics have been traced to them. Again, the only safeguard is to pasteurize the milk.

Rennet-Curd Cheese: Practically all of the commercially important cheeses are produced by the rennet method.

Rennet, rennin, or 'lab' ferment, as it is sometimes called, is one of the digestive enzymes of the gastric juice of young mammals and is also produced by many microorganisms. The rennet used in the cheese industry is obtained from the calf stomach. It coagulates milk by converting the caseinogen to casein. The coagulation may occur at neutrality or in a basic reaction and is, therefore, referred to as 'sweet' curdling.

The flavor and texture of cheeses depends upon the kind of milk, the amount of whey in the curd, the amount of salt, the spices, the size, the temperature, and the kinds of microorganisms present. Most cheese is made from the milk of cows or goats but some is made from the milk of mares or buffaloes. Moisture, salt, and temperature play an important part in determining the nature of the changes that take place during the ripening process.

In Europe, the making of cheese has been an empirical process and the art has been passed from father to son, a highly guarded secret surrounded by mystery. Local climatic conditions have been important in the development of particular flavors and various well-known cheeses such as Roquefort, Limburger, and Stilton, have been named for the districts in which they were first produced.

With the discovery that cheese was dependent upon microbial activity, there has been a great deal of research directed toward a better understanding of the factors involved and cheese-making has become a scientific manufacturing process, carefully controlled through all stages.

The changes that take place depend upon an associated action of many microorganisms. In the early stages, bacteria predominate, first the saccharolytic types, and later, the proteolytic. In some cheeses, such as Swiss cheese, there are a number of kinds of bacteria involved; some are responsible for flavor, and others produce a large amount of carbon dioxide which causes the formation of the characteristic holes or eyes.

Roquefort and Camembert owe their characteristic flavors primarily to the activity of *Penicillium roquefortii* and *Penicillium camembertii*, molds which develop in the later stages of the ripening process.

In the hard cheeses, the predominating microorganisms are bacteria of the lactic acid group and are distributed through the curd. In the

ripened soft cheeses, proteolytic bacteria are active. They gain entrance at the surface and as they grow inward, ripening progresses toward the center. Such cheeses have to be made in small sizes.

Microorganisms may also produce undesirable flavors or textures in cheeses and the process of cheese production resolves itself into the problem of maintaining proper conditions for the organisms that produce the desired changes and of excluding or retarding the growth of those that produce undesirable changes.

As a food for man, cheese ranks very high. In Switzerland, about twenty-five pounds per capita per year is consumed, in the United States only a little more than five.

In some countries cheese is relished for its flavor and the more highly flavored cheeses are the more popular. In other countries, cheese is one of the principal articles of food and the milder cheeses are preferred.

Chapter XXXVIII

MICROBIOLOGY AND WATER

". . . And let no one suppose that this is a matter in which he has no personal interest. The duty itself we may evade, but we can never be sure of evading the penalties of its neglect. This disease[1] not seldom attacks the rich, but it thrives most among the poor. But by reason of our common humanity we are all, whether rich or poor, more nearly related here than we are apt to think. The members of the great human family are, in fact, bound together by a thousand secret ties, of whose existence the world in general little dreams.

And he that was never yet connected with his poorer neighbour, by deeds of charity or love, may one day find, when it is too late, that he is connected with him by a bond which may bring them both, at once, to a common grave."

—*From William Budd.*

"You never miss the water until the well goes dry," go the words of an old song; and anyone who has lived through a drought or a broken water main or far from a pump finds little difficulty in appreciating the importance and remarkable properties of plain, common, ordinary water.

Water is the principal substance in all living things, be they sizable plants and animals or microorganisms. The chemical reactions by virtue of which they digest food, grow, and reproduce are dependent upon water and the enzymes which aid in these processes act only in aqueous solutions. Living things are constructed in such a way that water is absolutely essential to their existence and there is no substitute for it.

Water is a clear, colorless, tasteless, odorless liquid with a boiling point of 100° C. and a freezing point of 0° C. It has one peculiar characteristic—its greatest density is not at its freezing point. It is at 4° C. Were it not for this, all lakes, rivers, and streams, and even the ocean in regions where ice forms, would be frozen solid during the entire year.

It is the best solvent known and the fact that it dissolves so many substances, soil particles, dirt, grease, salts, acids, alkalies, gases, and such, is of paramount importance in water purification.

The value of water, ample in amount and pure in quality, has always been recognized and people of all times have tended to settle where water was plentiful. An adequate supply has often been a problem, but like so many of today's problems, it has taken on special characteristics peculiar to our twentieth century civilization.

[1] Typhoid Fever—Budd (published by the American Public Health Association, printed by George Grady Press, New York; reprint of original essay published in London in 1874.

The Problem

Primitive people used water mostly for drinking and for preparing food. Their per capita consumption was low. Very little extra was used for washing and very little was needed for their simple handicrafts. They did, however, recognize the necessity or, at least, the desirability of having relatively uncontaminated water and threw their refuses down stream from where they got their drinking water. As people developed permanent homes, as population increased, and as civilization developed with its cities and industries, the need for water sanitation became more of a problem and we find some of the greatest engineering feats of the ancient Romans directed to the end of providing enough good water for their city. It is a rather sad commentary on the march of science to know that although the need was recognized thousands of years ago, we still find communities where water sanitation is so inadequate that people actually consume their own or their neighbors' sewage.

The *daily per capita consumption* of water in American cities is about one hundred gallons, in many cities it runs as high as two hundred and in a few, over two hundred and fifty. In contrast, the European cities consume from twenty-five to seventy gallons per capita daily, with only a few using as much as one hundred gallons.

The more obvious factors that determine the amount of water used are the number and kinds of industries, the temperature of the weather, and the quality and cost of the water. When water is not metered or where the cost is low, much is utterly wasted. Installation of meters in some cities has reduced the per capita consumption of water as much as twenty to forty gallons a day. The major causes of waste are faulty plumbing such as leaky pipes and dripping faucets, and carelessness in leaving faucets turned on. This waste is doubly expensive. It increases the cost of water used and increases the cost of sewage disposal.

The amount of water used for different purposes varies a great deal. Hoover has estimated the number of gallons for different classes of users in cities where nearly all the water is metered.

Users	*Minimum*	*Maximum*	*Average*
Domestic	15	50	35
Commercial	10	55	40
Public	5	15	10
Loss	10	40	20

The daily variation is great depending upon such factors as holidays, the work days for the households, the outbreak of fires, the temperature of the weather, the amount used for watering lawns, and so on. Because of the wide hourly, daily, and seasonal variation in demand, a large factor of safety must be maintained in calculating the amount of equipment necessary to maintain adequate pressure on the water mains.

Sources of Water

Water comes to the earth in the form of rain or snow. Some is used by plants, some evaporates, and the remainder collects on the earth's surface in ponds, lakes, streams, rivers, and seas, or penetrates into the subsurface of the earth. For practical purposes the sources of water can be considered as either surface or ground. The surface water: lakes, rivers, reservoirs, and so on, usually serves as a supply for large cities, the ground water as a supply for villages and individual homes. The amount available depends upon several factors, the chief of which is rainfall.

What Is Good Water? There are a number of factors to consider in answering this question and it may not be possible to draw a definite line between good and bad water. The terms ordinarily used in describing water are: pure, contaminated, polluted, and potable.

Pure Water: Pure water, that is chemically pure water, does not exist in nature. It can be prepared only by distillation and even then is not easy to obtain. In nature, all water is impure. As the vapor of the air condenses, the water absorbs gases and when it strikes the ground, it picks up all manner of materials depending upon the character of the surface at that particular point. It is not the fact of chemical impurities but rather the kind and amount that is important from the sanitary standpoint. Hence the word "pure" as applied to water usually refers to water free from substances harmful or detracting from appearance and taste.

Contaminated Water: From the sanitary standpoint, contaminated water is water that contains substances harmful to health; pathogenic microorganisms, and organic or inorganic poisons, for instance.

Polluted Water: We think of polluted water as containing substances, not necessarily harmful but of such character as to offend the senses of smell, taste, and sight. It may have an odor, an unpleasant taste, or turbidity. Pollution usually refers to the physical characteristics of water.

Potable Water: This term, potable, refers to water which is safe from the standpoint of health and is pleasant and palatable to taste. In other words, it's fit to drink!

Hoover has suggested the following standards for judging the fitness of a municipal water supply.

1. That it shall contain no organisms which cause disease.
2. That it be sparkling, clear, and colorless.
3. That it be good tasting, free from odors, and preferably cool.
4. That it be reasonably soft.
5. That it be neither scale-forming nor corrosive.
6. That it be free from objectionable gas, such as hydrogen sulfide, and objectionable minerals, such as iron and manganese.
7. That it be plentiful and low in cost.

Sources of Pollution

Water picks up and carries along in suspension or solution all manner of materials which may spoil its taste and appearance or render it unfit for domestic or industrial uses. The kinds of pollution will depend upon its history. If it flows through fertile land, it is apt to carry a great deal of silt; if it flows over limestone, it will contain salts which make it hard; and if it comes in contact with decaying vegetable matter it will pick up materials which impart unpleasant tastes and colors. It may become mingled with man-made pollution such as industrial wastes, the seepage from mines, or domestic sewage. The amount and kind of pollution will also vary with rainfall, soil types, the type of agriculture, of industry, and the density of the population. And it will vary with the intelligence and farsightedness of the inhabitants.

Sources of Bacterial Contamination

The moment water precipitates in the form of rain or snow, it becomes contaminated with bacteria, some of which may be pathogenic for man but most of which are not. Water serves as a natural habitat for microorganisms, both bacteria and protozoa, whose numbers depend upon the nature of the surrounding soil, the amount of organic matter present in the water, the hydrogen-ion concentration, the temperature, and so on. The so-called normal water bacteria, none of which is pathogenic for man, belong to the spherical, rod, and spiral forms. Many of them are chromogenic and produce red, yellow, orange, or violet pigments.

There is, of course, considerable similarity between the character of the bacterial flora of water and of the surrounding soil. As water runs over, seeps, or percolates through the soil, it picks up microorganisms and unless these are screened out by passage through sand, they find their way into the larger bodies of water.

Since the air does not support the growth of bacteria, the organisms in it must come from the surrounding soil and its inhabitants, plant, animal, and human. But although the numbers of air-borne organisms in a cubic centimeter of rain water may be as high as five to six thousand at the beginning of a rain, after it has rained for a few hours, the number will have dropped to about fifty or less. These organisms do not play an important part in water sanitation.

The number of bacteria in deep wells is usually low. In surface waters, it may run from a few hundred per cubic centimeter for lakes and rivers having a low organic matter content to over a million for water heavily polluted with sewage. Surface waters show a rapid increase in numbers after a heavy rainfall because of the bacteria washed in from the surround-

ing banks. If the dilution of the rain is great enough, this first increase in numbers may be followed by a decrease. The actual plate counts in themselves have little significance but serve merely as indicators of pollution.

The decaying bodies of plants and animals in and on the soil and in water are an important source of the bacteria found in the water. Except during calamities, human bodies do not remain in the water and so do not serve as a source of contamination.

From the sanitary standpoint, it is the excrement of man that plays the greatest part in the contamination of water.

Self-Purification of Water

There is a popular notion that running water purifies itself and, while this is perfectly true, it should be added that still water or stagnant water purifies itself even more rapidly. But, while a certain degree of self-purification of water by physical, chemical, and biological forces does take place, it cannot be depended upon to safeguard drinking water and when it has been, serious outbreaks of disease have invariably followed. Disease-producing bacteria die off in water for several possible reasons.

Unless the amount of organic matter is extremely great, far greater than would be tolerated in drinking water, there is not sufficient food nor is it of the right kind for the growth of the common disease-producing bacteria or protozoa. The question, then, is not one of their multiplication but of their survival and is determined by several factors.

Temperature—In general, the higher the temperature, the more rapidly the pathogens die off.

Light—While sunlight is somewhat destructive to bacteria, it lacks penetrating power and its germicidal effect is limited to a few feet in clear water and a much shorter distance in turbid water.

Dilution—Obviously this is a very important factor in reducing the concentration of organic matter and bacteria. However, it cannot be depended upon to safeguard consumers against disease. It has been observed that the excrement of one typhoid patient was sufficient to pollute a large river so that consumers living miles below contracted typhoid fever.

Oxidation—The changes that take place in purification are in part oxidative and are biological in character. Chemical oxidation is probably of little importance in purification.

Antagonistic Effect of Other Organisms:—The struggle for existence goes on everywhere and water is no exception. Bacteria in water are consumed by protozoa and, perhaps, by some of the smaller metazoa. A species which cannot replenish itself by reproduction soon disappears. It seems

likely that one reason pathogenic bacteria die off more rapidly in stagnant water than in clear is because they are devoured by the greater numbers of protozoa present.

Sedimentation—Sedimentation, in the opinion of Jordan and numerous other workers who have studied the problem, is far the most important factor in the self-purification of water. Anything that promotes sedimentation hastens purification. The specific gravity of bacteria is such that they would not settle rapidly enough to account for self-purification if they did not become entangled in particles of silt and other suspended matter which is heavy enough to settle and to carry them down at the same time. Anything which reduces the flow of a stream will reduce its carrying power for suspended matter and will, as a consequence, hasten purification.

Bacteria, of course, are not destroyed by the process of settling but are merely removed from the upper water. Many are devoured by the protozoa on the bottom and it seems likely that, in the final analysis, it is the protozoa who are responsible for the rapid disappearance of pathogens. Many species of saprophytes find favorable conditions for growth on the bottom of streams and may be found there in large numbers.

It is obvious that no rule can be laid down as to how many miles it takes for running water to purify itself. The distance will depend primarily upon the rate of flow because this will determine the rate of settling and the distance the bacteria will travel in any given time.

One other factor probably plays an important part in the destruction of bacteria in certain highly polluted streams such as the Ganges, and that is *bacteriophage*. Bacteriophage for the organisms responsible for the common intestinal diseases, such as typhoid fever and dysentery, can be readily isolated from any domestic sewage, but it is doubtful whether in ordinary water these phages destroy enough bacteria to be of sanitary significance.

WATER PURIFICATION METHODS

There are a number of methods commonly used in the purification of water. There is filtration through sand filters, with or without previous coagulation induced by the addition of chemicals; water softening by one of a number of processes; and disinfection by the use of chemicals, usually chlorine. The kind of method or combination of methods used depends upon the quality of the water. If it contains little suspended or dissolved matter, disinfection may be sufficient; if it is soft but contains suspended matter, filtration plus chlorination may be sufficient. If it contains dissolved salts, such as those of calcium or magnesium, it will be necessary to soften it before it is satisfactory.

Filters

Two common types of sand filters are used for the removal of pollution from water—the slow sand filter and the rapid sand filter.

Slow sand filters, first used in England about 1830, were used extensively in Europe but never became popular in the United States. They are constructed of concrete, cover about an acre each, and are filled with sand to a depth of one to four feet. The criticisms levelled at this type of filter are: (1) that it is not successful if the water contains large amounts of suspended matter, for the surface soon becomes coated and they can no longer deliver sufficient water, (2) that it covers large areas so that if land is expensive the original cost is prohibitive, and (3) that even when operat-

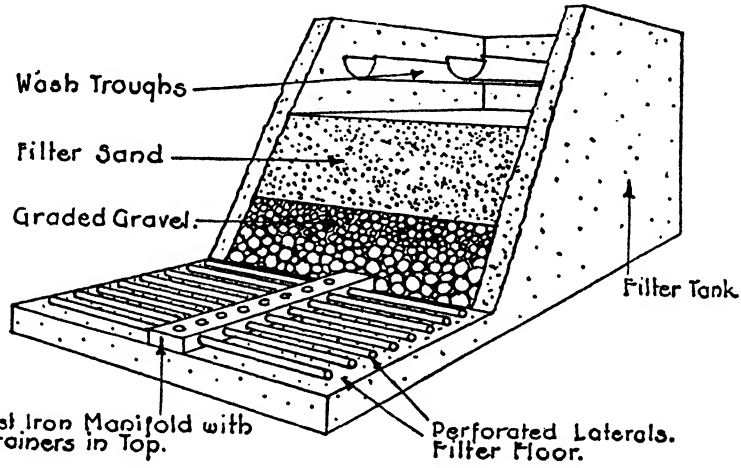


FIG. 52. Cut-away view of gravity filter showing underdrains and filter-bed. Courtesy of National Lime Association.

ing to full capacity, the rate of delivery is only about two to three million gallons per acre.

The mechanism by which bacteria are removed is not purely mechanical but is associated with the gelatinous film or zoogleal mass which covers the surface after the filter has been in operation for a few days.

Rapid sand filters were introduced in the United States about 1890 and 1900 and are now used extensively in modern water purification plants, usually in combination with chemical flocculation and settling basins. Briefly the process is as follows: The raw water is screened to remove large floating objects such as sticks, leaves, or fish. It is then pumped or run by gravity into large basins where it is mixed with flocculating agents, such as aluminum or iron sulfate. After a thorough mixing the water flows to

settling tanks where the suspended floc settles out. The supernatant is then run over rapid sand filters and the effluent pumped into the water mains. At some point between the filters and the city mains, chlorine is usually added.

Flocculation: The formation of a suitable floc by means of chemicals such as aluminum sulfate, ferrous sulfate, ferric chloride, and lime is dependent upon several factors. The aluminum sulfate, iron sulfate, or chlorides are soluble in water. In the presence of natural alkaline substances or the lime usually added, the reactions involved in the formation of insoluble precipitates are essentially these:

$$\begin{array}{rl} Al_2(SO_4)_3 = & 2\ Al^{+++} + 3\ SO_4^{-} \\ 6\ HOH = & 6\ OH + 6\ H^{+} \\ \hline & 2\ Al_2(OH_3) + 3\ H_2SO_4 \end{array}$$

The sulfuric acid (H_2SO_4) reacts with the lime to form calcium sulfate (Ca_2SO_4) which is insoluble. The aluminum hydroxide ($Al_2(OH)_3$) forms a gelatinous precipitate which absorbs and enmeshes bacteria, clay particles, and the like, leaving a clear water with only a fraction of the original number of bacteria in the supernatant. The size and character of the floc are influenced by pH, agitation, and temperature.

In water purification plants, the floc is allowed to settle out in settling basins which can be drained to facilitate removal of the precipitate. After the first settling, the water is passed through rapid sand filters which remove the finer flocs leaving a clear effluent. The choice of coagulant will depend upon the cost of chemicals, equipment available, and the character of the water. Muddy water requires relatively less coagulant than comparatively clear water.

Disinfection of Water: Unless water comes from wells which are properly cased and protected from sewage pollution, it is safe to assume that pathogenic organisms will be present at some time or other. Cities usually get their water from rivers, streams, lakes, or reservoirs, and since these sources are likely to be contaminated, it is customary to disinfect with chemicals. Chlorine is used almost exclusively although other agents have been tried. Chlorine may be obtained in several forms but the least expensive and most satisfactory is the liquified gas which is handled in steel cylinders. These are connected to a special dispensing and measuring device which automatically regulates the amount fed into the water.

The somewhat complicated chemical reactions resulting when chlorine enters water may be summarized as follows:

$$Cl_2 + H_2O = H{\cdot}Cl + H{\cdot}O{\cdot}Cl$$
$$H{\cdot}O{\cdot}Cl = H{\cdot}Cl + O$$

The nascent oxygen is a powerful oxidizing agent and chlorine owes part of its effectiveness to the oxygen liberated. Some of its effect is due to the fact that when chlorine comes in contact with such compounds as amino acids, amines, or ammonia, N-chloro compounds are formed which are also toxic to bacteria. How much of the killing power of chlorine is due to the direct oxidation by the nascent oxygen liberated and how much to the formation of N-chloro compounds in the cells is not known. There is evidence that the latter is the more important and that the effectiveness of chlorine compounds is due to a disturbance of oxidation-reduction systems necessary to the life of the bacteria.

Water containing algae, industrial wastes, and other impurities may acquire a distinct medicinal taste when it is chlorinated. This is due to chlorophenals, chloramines, or other chlorine compounds, and not to the chlorine itself. In regions where the ground is covered with snow during the winter, such tastes usually appear following thaws or spring floods. The organic matter washed in from the fields cannot all be removed by coagulation and filtration and the chlorination of these dissolved substances gives rise to offensive tastes. Such water is not harmful, however.

The amount of chlorine necessary to disinfect water depends upon the amount of organic matter present. Enough must be added to leave a residue of about 0.2 p.p.m. free chlorine. This amount can be determined colorimetrically by means of the orthotolidine test.

Lime may also be used to disinfect water. Its use is not widespread but many purification plants soften water with lime and find that it also destroys most of the bacteria. The time of contact is, of course, important. Many of the smaller water treatment plants use chlorine only as an extra precaution, the lime treatment being considered satisfactory for disinfection.

Water Softening: Hardness is a relative term and the degree of hardness is usually expressed in terms of parts of calcium carbonate per million (p.p.m.). Hard water is undesirable for many reasons. The salts to which it owes its characteristics interfere with the action of soaps, form scale in pipes, foams in boilers, and may, particularly if the salts of magnesium are present in excessive amount, give rise to enteric disturbances.

Hardness is due chiefly to the presence of bicarbonates or sulfates of calcium and magnesium, although the nitrates and chlorides may also contribute. Hardness due to bicarbonates is called temporary or carbonate hardness, and that due to sulfates is called permanent or noncarbonate hardness. The calcium salts come from lime stone, gypsum, and calcium chloride. Calcium carbonate is relatively insoluble in water, about 13 p.p.m., but when carbon dioxide is present the solubility is increased to as much as 1000 p.p.m. Calcium sulfate is more soluble and the

chloride is highly soluble. The presence of carbon dioxide greatly increases the solubility of magnesium salts too. If salts are held in solution by carbon dioxide they can be precipitated by boiling and hence contribute a temporary hardness only.

Iron salts are present in sufficient amounts in some water to cause staining of fabrics and plumbing fixtures.

In addition to these salts, associated with hardness, there are other salts present in the water of certain regions in sufficient concentration to be of public health significance. Fluorides are present in the water of some of the midwest states in sufficient amounts to cause mottling of the teeth. Selenium, iodine, and numerous other elements may also be present in small amounts.

Methods of Water Softening: A discussion of methods of softening water is pertinent to water purification because in the process, the numbers of bacteria are reduced. Softening of water can be accomplished by several methods all of which remove the salts to which water owes its hardness. The addition of soap, a washing powder such as trisodium phosphate, and the use of zeolite base exchange filters are commonly used methods in the home. In water treatment plants, lime and zeolite are most commonly used. Lime treatment converts the soluble salts into insoluble salts readily removed by sedimentation.

Zeolites are complex compounds of aluminum and iron or both. They contain a sodium ion which is exchanged for the calcium or magnesium ion of the hard water. The sodium salts do not confer the property of hardness and hence hard water passed through a zeolite softener becomes soft. The zeolite can be regenerated by adding a concentrated solution of sodium chloride or common salt.

The first large scale softening plant employing lime and soda ash was constructed at Columbus, Ohio in 1908. Since that time many cities have constructed similar plants, although the Columbus plant is still the largest of its kind.

The chemistry of softening hard water by the use of lime and sodium carbonate is expressed by Gainey as follows:

Soluble			*Insoluble*
$Ca(H\cdot CO_3)_2$	+ $Ca(OH)_2$	=	2 $CA\cdot CO_3$ + 2 H_2O
$Mg(H\cdot CO_3)_2$	+ $Ca(OH)_2$	=	$Ca\cdot CO_3$ + $Mg\cdot CO_3$ + 2 H_2O
$Mg\cdot CO_3$	+ $Ca(OH)_2$	=	$Mg(OH)_2$ + $Ca\cdot CO_3$
$Mg\cdot SO_4$	+ $Ca(OH)_2$	=	$Mg(OH)_2$ + $Ca\cdot SO_4$
$Ca\cdot SO_4$	+ $Na_2\cdot CO_3$	=	$Ca\cdot CO_3$ + $Na_2\cdot SO_4$

The calcium carbonates and magnesium hydroxide formed are readily removed by sedimentation. A difficulty encountered in this process is

that the water is supersaturated with calcium carbonate and this precipitates out in the pipes. The addition of carbon dioxide which increases the solubility corrects this difficulty.

Of particular interest to the consumer is the fact that adding chemicals to precipitate the compounds responsible for hardness reduces the total amount of chemicals in the water. The prejudice against the addition of chemicals or the statement that the water is doped with chemicals is not based on facts.

But, even though chemicals can be used to soften water and the bacterial count be greatly reduced thereby, the question of the cost of such treatment must be raised before deciding whether it is practical. All studies that have been made point to the same conclusion—water softening plants soon pay for themselves by reducing expenditures for soap and for replacements of pipes, boilers, and other equipment subject to scaling. Table 12 taken

TABLE 14

CITY	TOTAL HARDNESS OF WATER SUPPLY	ANNUAL PER CAPITA SOAP CONSUMPTION	ANNUAL PER CAPITA COST OF SOAP
	p.p.m.	*pounds*	
Superior, Wisconsin	45	29.23	$3.75
Bloomington, Illinois	70	32.13	4.48
Champaign-Urbana, Illinois	298	38.89	5.93
Chicago Heights, Illinois	555	45.78	7.50

from Hoover's *Water Supply and Treatment* nicely illustrates how the per capita consumption of soap varies with the hardness of the water.

EARLY DESCRIPTIONS OF SOME WATER-BORNE DISEASES

Three bacterial diseases of man transmitted primarily by water contaminated by fresh sewage are cholera, typhoid fever, and dysentery. These diseases occur as scattered cases, endemically, and as devastating epidemics. In times of peace they are a cause of fear and apprehension, in times of war they have been far more destructive than swords or bullets.

Our interest in water-borne diseases is two-fold: first, because unless they are continually guarded against they will assume epidemic proportions, and, second, because it is in connection with them that the epidemiological approach to disease developed.

Two names are preeminently associated with the study of water-borne epidemics: John Snow (1813–1857) and William Budd (1811–1880).

John Snow was an English physician who practiced medicine in London from 1836 until the time of his death. His interests were varied and his

principal contributions had to do with the development of anesthesia and the doctrine of water-borne disease. His name is associated with the use of chloroform in child birth and it was he who delivered Queen Victoria in 1853 and again in 1857 using chloroform to ease her labor.

The first recorded epidemics of cholera in England occurred in 1831 and 1832. London suffered two severe outbreaks, one in 1848–49 and another in 1853–54. One phase of the latter is known as the Broad Street Pump epidemic because it was traced to a contaminated well on Broad Street. Snow's observations and analyses of the London outbreaks had led him to the conclusion that the cause of cholera was a specific microorganism—this was over twenty years before Koch performed his experiments on anthrax.

In his significant work "On the Mode of Communication of Cholera" published first in 1849 and expanded for a second publication in 1855, Snow tells how the pathology of the disease indicated the manner in which it spread and how he had traced cases to cases proving its communication from person to person through the medium of polluted water. His expanded edition includes a description of the Broad Street epidemic—his first recommendation in this outbreak was to remove the handle of the pump!—and contains a map showing the deaths from cholera in Broad Street and the neighborhood and indicating the position of the Broad Street Pump.

He describes the outbreak as follows:

"The most terrible outbreak of cholera which ever occurred in this kingdom, is probably that which took place in Broad Street, Golden Square, and the adjoining streets, a few weeks ago. Within two hundred and fifty yards of the spot where Cambridge Street joins Broad Street, there were upwards of five hundred fatal attacks of cholera in ten days. The mortality in this limited area probably equals any that was ever caused in this country, even by the plague; and it was much more sudden, as the greater number of cases terminated in a few hours. The mortality would undoubtedly have been much greater had it not been for the flight of the population. Persons in furnished lodgings left first, then other lodgers went away, leaving their furniture to be sent for when they could meet with a place to put it in. Many houses were closed altogether, owing to the death of the proprietors; and, in a great number of instances, the tradesmen who remained had sent away their families: so that in less than six days from the commencement of the outbreak, the most afflicted streets were deserted by more than three-quarters of their inhabitants.

. . . As soon as I became acquainted with the situation and extent of this irruption of cholera, I suspected some contamination of the water of the much-frequented street-pump in Broad Street, near the end of Cambridge Street; but on examining the water, on the evening of the 3rd September, I found so little impurity in it of an organic nature, that I hesitated to come to a conclusion.

. . . On proceeding to the spot, I found that nearly all the deaths had taken place within a short distance of the pump. There were only ten deaths in houses situated decidedly nearer to another street pump. In five of these cases the families of the

deceased persons informed me that they always sent to the pump in Broad Street, as they preferred the water to that of the pump which was nearer. In three other cases, the deceased were children who went to school near the pump in Broad Street. Two of them were known to drink the water; and the parents of the third think it probable that it did so. The other two deaths, beyond the district which this pump supplies, represent only the amount of mortality from cholera that was occurring before the iruption took place.

With regard to the deaths occurring in the locality belonging to the pump, there were sixty-one instances in which I was informed that the deceased persons used to drink the pump-water from Broad Street, either constantly or occasionally. In six instances I could get no information, owing to the death or departure of every one connected with the deceased individuals; and in six cases I was informed that the deceased persons did not drink the pump-water before their illness.

The result of the inquiry then was, that there had been no particular outbreak or increase of cholera, in this part of London, except among the persons who were in the habit of drinking the water of the above-mentioned pump-well.

I had an interview with the Board of Guardians of St. James's parish, on the evening of Thursday, 7th September, and represented the above circumstances to them. In consequence of what I said, the handle of the pump was removed on the following day.

. . . There is a Brewery in Broad Street, near to the pump, and on perceiving that no brewer's men were registered as having died of cholera, I called on Mr. Huggins, the proprietor. He informed me that . . . the men are allowed a certain quantity of malt liquor, and Mr. Huggins believes they do not drink water at all. . . .

. . . Dr. Fraser also called my attention to the following circumstances, which are perhaps the most conclusive of all in proving the connexion between the Broad Street pump and the outbreak of cholera. In the "Weekly Return of Births and Deaths" of September 9th, the following death is recorded as occurring in the Hampstead district: "At West End, on 2nd September, the widow of a percussion-cap maker, aged 59 years, diarrhoea two hours, cholera epidemica sixteen hours."

I was informed by this lady's son that she had not been in the neighborhood of Broad Street for many months. A cart went from Broad Street to West End every day, and it was the custom to take out a large bottle of the water from the pump in Broad Street, as she preferred it. The water was taken on Thursday, 31st August, and she drank of it in the evening, and also on Friday. She was seized with cholera on the evening of the latter day, and died on Saturday, as the above quotation from the register shows. A niece who was on a visit to this lady, also drank of the water; she returned to her residence, in a high and healthy part of Islington, was attacked with cholera, and died also. There was no cholera at the time, either at West End or in the neighbourhood where the niece died.

. . . In some of the instances, where the deaths are scattered a little further from the rest on the map, the malady was probably contracted at a nearer point to the pump. A cabinet-maker, who was removed from Philip's Court, Noel Street, to Middlesex Hospital, worked in Broad Street. A boy also who died in Noel Street, went to the National school at the end of Broad Street, and having to pass the pump, probably drank of the water. A tailor, who died at 6, Heddon Court, Regent Street, spent most of his time in Broad Street. A woman, removed to the hospital from 10, Heddon Court, had been nursing a person who died of cholera in Marshall Street. A little girl, who died in Ham Yard, and another who died in Angel Court, Great Windmill Street, went to the school in Dufour's Place, Broad Street, and were in the habit of drinking the pump-water, as were also a child from Naylor's Yard, and sev-

eral others, who went to this and other schools near the pump in Broad Street. A woman who died at 2, Great Chapel Street, Oxford Street, had been occupied for two days preceding her illness at the public washhouses near the pump, and used to drink a good deal of water whilst at her work; the water drank there being sometimes from the pump and sometimes from the cistern.

. . . As there had been deaths from cholera just before the great outbreak not far from this pump-well, and in a situation elevated a few feet above it, the evacuations from the patients might be amongst the impurities finding their way into the water, and judging the matter by the light derived from other facts and considerations previously detailed, we must conclude that such was the case.

. . . All the instances of communication of cholera through the medium of water, above related, have resulted from the contamination of a pump-well, or some other limited supply of water; and the outbreaks of cholera connected with the contamination, though sudden and intense, have been limited also; but when the water of a river becomes infected with the cholera evacuations emptied from on board ship, or passing down drains and sewers, the communication of the disease, though generally less sudden and violent, is much more widely extended; more especially when the river water is distributed by the steam engine and pipes connected with waterworks."

William Budd was also a London physician. He studied the pathology and transmission of typhoid which was then widely prevalent in England and concluded that it was a water-borne disease due to a specific poison. His essay on "Typhoid Fever, Its Nature, Mode of Spreading, and Prevention" was published in 1874 but he states that he had taught the doctrines therein presented previous to 1857.

In an excerpt from the Introductory, his sympathetic appreciation of the "consummation of misery" known as typhoid is evidenced—

". . . Even in the highest class of society, the introduction of this fever into the household is an event that generally long stands prominently out in the record of family afflictions. But if this be true of the mansions of the rich, who have every means of alleviation which wealth can command, how much more true must it be of the cottages of the poor, who have scant provision even for the necessaries of life, and none for its great emergencies! Here, when Fever once enters, Want soon follows, and Contagion is not slow to add its peculiar bitterness to the trial.

As the disease is, by far, most fatal to persons in middle life, the mother or father, or both, are often the first to succumb, and the young survivors being left without support, their home is broken up and their destitution becomes complete.

How often have I seen in past days, in the single narrow chamber of the day labourer's cottage, the father in the coffin, the mother in the sick bed in muttering delirium, and nothing to relieve the desolation of the children but the devotion of some poor neighbour who in too many cases paid the penalty of her kindness in becoming, herself, the victim of the same disorder!"

In referring to an outbreak of typhoid at Cowbridge, Wales, in 1853, which occurred among "persons who had never been in one another's company except in the Cowbridge ball-room" on the occasion of two balls held during the Race Week, he says,

" . . . From this and other considerations I was led to infer that drinking-water was the most probable vehicle of it.

A visit to the courtyard of the hotel left in my mind no doubt that this was the true view of the case. The cesspool and drain, which I was informed had received the bulk of the diarrhoeal discharges from the fever patient, was at the time of the outbreak so near to the well, that, under the conditions of soil and locality, percolation from one to the other was almost inevitable. I further learnt, from persons who were present at the balls, that, as is usual on such occasions, many drinks—lemonade among others—were largely supplied there, and freely drunk.

This much, then, was sure—that a considerable number of the persons who attended the balls drank freely water from a well in close proximity to a receptacle which, for a considerable time, had received the specific excreta from the diseased intestine of a fever patient.

. . . there are one or two collateral points almost equally deserving attention, which it may be well to note at once.

The first is the very large proportion in which the guests were infected. Of the persons who attended the balls, there is reason to believe that from forty to fifty suffered—a truly remarkable proportion when it is borne in mind that many, probably, drank no water at all, or only water that had been boiled.

The second point which this outbreak illustrates, in a striking way, is one to which I have already referred, viz., the very prolific nature of the typhoid poison.

The water which gave fever to all these people could not have amounted to more than a few gallons, at most. The exact cubic contents of the total well-water I do not know . . . but, when we consider, in addition, that the great bulk of the poison cast off by the fever patient must have remained, after all, in the cesspool, the number of contagious units contained in the whole quantity is more easily imagined than calculated."

In another outbreak the fever was propagated by a "fever-tainted brook." His observations follow.

"The outbreak began in the person of the father of the family living in No. 1. There were two circumstances attached to this man which made his case different from that of any other member of his own or his neighbour's household.

1st. He was the only one of the group whose way of life took him away to the neighbouring city; and 2nd, he was the only one who was known to have been exposed to the infection of typhoid fever.

Having a horse and cart, he plied a small trade with Bristol, partly as hawker and partly as huckster. His chief business in the city lay in the filthy back-slums of St. Philip's, where, for some time immediately before his illness, typhoid fever—as I can affirm from my own observation—was epidemic. Whether he got his fever here, it is, of course, impossible to say with absolute certainty, but that in the course of his business he must have been exposed to its specific infection there was no doubt.

That his disease was contracted away from home was further indicated by the fact that when he was stricken all the other inmates of the two cottages were, and, indeed, continued for some time after, to be, in their usual health.

His attack proved to be severe and protracted, and for a considerable time was attended by profuse diarrhoea.

As a matter of course, all the discharges were thrown into the common privy. In

this way, for more than a fortnight, the stream which passed through it continued to be daily and largely fed with the specific excreta from the diseased intestine of the patient.

Some weeks passed away thus, without any fresh incident; but, in the latter end of the third, or beginning of the fourth week . . . several persons were simultaneously attacked with the same fever in all the four cottages.

Not, be it observed, in Nos. 1 and 2 merely, whose inmates might be described as living in more or less contiguity to the already infected man, but in Nos. 3 and 4 also, nearly a quarter of a mile away. . . . before long the majority of the persons living in them were in bed with the fever.

. . . The significance of these circumstances will be appreciated at once when it is added, that those who were attacked in this particular outbreak had not only held no intercourse of any kind with the inmates of Nos. 1 and 2, but had not the remotest suspicion of the origin of the deadly pest which had appeared thus silently in their midst.

The little stream laden with the fever-poison cast off by the intestinal disease of the man who had been stricken with the same fever some weeks before, was the only bond between them."

Bacteria in Ice: Laboratory experiments show that typhoid bacilli may survive for months in ice, but that about ninety per cent are killed during the process of freezing and that after a week or two only about one per cent are alive. Although typhoid and dysentery have been traced to ice made from contaminated water, it seems doubtful whether contaminated ice is of much sanitary importance.

The danger from infection due to frozen excreta from cases of typhoid is far greater. This is well illustrated by an outbreak which occurred in Plymouth, Pennsylvania in 1885. When the source of the infection was finally traced, it was found that in January the excreta of a typhoid case had been thrown on the banks of a brook. They had remained frozen until the March thaws at which time they had been washed into the brook and eventually carried into the municipal water mains to produce about 1000 cases of typhoid in this town of 8000!

Bacteriological Analysis of Water: The bacteria of public health importance are not natural inhabitants of water nor do they multiply there. The water-borne pathogens must come from man, with one exception—*Pasteurella tularensis.* This organism has been isolated from water in which beavers were living, and it seems highly probable that man might acquire tularemia from drinking it. In Russia, water-borne outbreaks of tularemia have been traced to water contaminated by the urine of infected water rats. In general, however, it is man himself who contaminates the water and the bacteriological analysis of water is based on this fact.

The organisms of fecal origin most commonly found in water are the *coli-aerogenes* group, *Streptococcus fecalis,* and *Clostridium welchii,* an anaerobic spore former. Any of these may be used as an indicator of pollution. In the United States, *Escherichia coli* has been chosen as an

indicator because it is the predominating organism in human excreta and is always present. It does not multiply in water but survives for longer periods than the typhoid or dysentery bacillus, and it is readily isolated. It is obvious that if the organism used as an indicator grew in water, its presence or numbers would tell nothing about how recently it had been introduced. If it did not live as long as the pathogen, its absence would give no assurance that the pathogen was absent.

It is often asked why the typhoid bacillus is not used as an indicator. Its use is not feasible because it is difficult to detect unless it is present in massive doses. Then too, the absence of the typhoid bacillus on one day would give no assurance that it might not be present on the next if the water happened to contain any fecal discharges.

In practice, then, the organism used is *Escherichia coli* and it is presumed that if this organism is found in the water there is fecal pollution and the pathogens may be, and in all probability sooner or later will be, present. If it is not found, there is little chance that the pathogens will be.

The method of water analysis recommended by the American Public Health Association and the American Water Works Association is outlined in the Standard Method of Water Analysis. It consists of tests run in three parts: the "Presumptive," the "Confirmed," and the "Complete" tests.

Presumptive Test: This is based on the fact that the *coli-aerogenes* group ferments lactose. Lactose broth tubes are inoculated with 10 cubic centimeters, 1 cubic centimeter, and 0.1 cubic centimeter portions of the water and incubated for twenty-four hours at 37° C. If no gas is present then, the tubes are incubated for another twenty-four hours. The presence of gas is presumptive evidence that the *coli-aerogenes* group is present and, hence, is evidence of fecal pollution. The dilution in which the fermentation of lactose occurs is an index of the number of organisms present.

Confirmed Test: The confirmed test consists of inoculating suitable differential media, such as Endo's or eosin-methylene blue plates (E.M.B. plates) with material from the tubes showing gas. *Escherichia coli* displays a characteristic sheen and can be separated from other lactose fermenters on such media.

Completed Test: To complete the test, transfers are made from isolated colonies, showing characteristic features of *Escherichia* coli, on the E.M.B. plates or Endo plates, to nutrient agar slants and to lactose broth. If, after incubation, microscopic examination of the growth on the agar slants reveals small gram-negative organisms and if the lactose fermentation is positive, the identification of *E. coli* is said to be completed.

Plate Counts: Plate counts are made when it is desirable to determine the total number of bacteria present in water. Suitable dilutions of the

water are plated out on nutrient agar and gelatin. The agar plates are incubated at 37° C. and the gelatin plates at 20° C. The presence of a high bacterial count on the plates incubated at 37° C. is interpreted to indicate human or animal pollution for that is the temperature at which organisms of animal origin grow best. A high count on the gelatin plates, incubated at 20° C. suggests bacteria from water or soil and, hence, non-pathogenic bacteria for they grow best at 20° C.

Chemical Analysis of Water: Chemical analyses are made to determine acidity, hardness, amount of inorganic salts, and amount of organic matter. While of considerable importance in determining the fitness of water, the tests shed little light on its safety from the standpoint of health. Tests are usually made for ammonia, nitrites, and nitrates. It should be observed that the relative proportion of these is also a measure of the time since organic nitrogen was introduced. In protein decomposition, ammonia is given off first; this is then oxidized to nitrites; and later to nitrates. It follows, therefore, that the finding of relatively large amounts of ammonia suggests recent pollution; the finding of large amounts of nitrates, that sufficient time has elapsed for a rather complete decomposition to take place. The presence of large amounts of chlorides may suggest urine pollution.

Standards: The National Institute of Health's Bacteriological Standards for Water[1] apply to common carriers such as busses, railroads, and vessels. They are based on the average density of *E. coli* in the water.

The sanitary quality of drinking water cannot be determined by bacteriological analysis alone. There are many factors to consider besides the total count or the number of *E. coli* present at the time tests were made

Swimming Pools

The extensive development of recreational centers with swimming pools as a center of attraction for children, adolescents, and adults, has created a popular interest in keeping them safe, clean, and free from harmful bacteria. A swimming pool, from the standpoint of sanitation, is a large communal bath tub and any kind of bacteria to be found on the bodies of its users may also be found in the water. It has been shown that such diseases as conjunctivitis, middle ear infections, skin infections, upper respiratory infections, and typhoid fever can be contracted in swimming pools. The transmission of venereal diseases has also been reported and the possibility suggested that infantile paralysis might be spread by this means. The commonest form of infection incurred is ringworm or "ath-

[1] The Public Health Service has published standards governing the requirements for water supplies. These cover chemical and bacteriological tests and are found in the Public Health Reports, vol. 61, no. 11, 1946.

lete's foot." This is not contracted from the water in the pool but from the walks surrounding the pool, the floors of the dressing rooms, and, perhaps, swimming suits and towels.

It is obvious that swimming pools, be they outdoor or indoor, school or municipal, are a hazard to health unless proper sanitary precautions are taken. These resolve themselves into preventing persons harboring transmissible infections from contaminating the water, walks, or floors, and into destroying the organisms that are, nevertheless, introduced. In addition, sanitation includes the removal of any materials, such as dirt or the lint from bathing suits, that interfere with the aesthetic properties of the water.

Contamination can be greatly reduced by requiring everyone to take shower baths with soap before entering the pool and by excluding all persons who show signs of infection such as sores, abscesses, running noses, and so on. But even under such rigid precautions, pathogenic bacteria will get into the water and it will be necessary to employ disinfection as a final safeguard. For this purpose chlorine in the liquid state or in the form of hypochlorites is most widely used. Ultraviolet light or ozone is satisfactory under restricted conditions.

Since chlorine combines with the organic matter present in the water, the more organic matter there is, the more chlorine will be required to maintain a concentration sufficient to destroy the bacteria. This is usually 0.2 to 0.4 parts per million of residual chlorine. Too high a concentration is irritating to the eyes and imparts an objectionable taste to the water.

The lint from suits, hairs, and other miscellaneous fine and coarse solids that accumulate in the water and on the bottom of the pool may be removed by emptying and scrubbing the floor before refilling. More commonly, however, the water is treated with an alum coagulant, filtered, and then passed back into the pool. The floors may be cleaned with suction cleaners.

One of the problems encountered in the operation of swimming pools is the growth of algae. It is of no importance from the standpoint of disease but increases the organic content of the water and makes it necessary to increase the amount of chlorine. It also interferes with the filters and makes the water turbid. Algae can be controlled by the addition of copper sulfate in a dosage of two parts per million.

The sanitary condition of a swimming pool is indicated by its bacterial count, and although it is not possible to say just how many bacteria should be tolerated in a pool, it is usually agreed that the standard should be as high as for drinking water.

Chapter XXXIX

SEWAGE

The purpose of sanitation is to prevent disease and to provide healthful and pleasant surroundings. The proper disposal of sewage is important because sewage serves as a source of disease-inciting agents and gives rise to unsightly conditions and unpleasant odors.

The problem of how to dispose of sewage is not a new one but its complexity and magnitude have vastly increased with industrialization and the growth of cities. Primitive and nomadic people solved the problem by moving away when their refuse and excrement had accumulated to an intolerable degree. But with permanent habitations, denser populations, and the development of industries, the problem has expanded until it now demands the careful and intelligent planning of a group of specialists known as 'sanitary engineers.' Primitive man removed himself from the neighborhood of his wastes: modern man removes his wastes from the neighborhood of himself.

Sewage may be defined as the used water supply of a community to which has been added the wastes incident to the life processes and the industrial activities of the population. It consists of:

1. Human excrement, feces and urine, water used for washing and bathing, for washing dishes, clothes, etc.
2. Industrial waste from tanneries, slaughter houses, sugar and starch refineries, creameries, laundries, breweries, chemical plants, and the like.
3. Storm water, rain and snow water from streets, lawns, etc. In some cities there are separate storm sewers, not connected with sanitary sewers.

The first sewers or sewerage systems were designed to carry off storm water only. They were merely drainage ditches, some open and some closed, which carried the storm water to the nearest stream or field. In some cases these ditches were used also as sanitary sewers, for the inhabitants threw slops and other wastes directly into them. In other cities such practices were unlawful. As a matter of fact, some of the earliest laws relating to sewage were designed to prevent its contamination by human excrement and industrial wastes. A commission on sewers established in the fifteenth century in England laid down strict laws covering sewage disposal. They are of particular interest because they represent a thoroughly modern point of view in that they provided a severe penalty for the pollution of water. They required tanneries and breweries to drain their wastes into cesspools, and stipulated that pig sties should be so located that there would be no drainage into streams.

At that time human excrement was disposed of by a system of collection or by the use of privies. In the former method, the slops were placed in buckets which scavengers would call for nightly and dispose of in a number of ways. The fertilizer value of fecal material was recognized and "night soil" was commonly spread over fields.

In this country and in many European countries, the privy was common. It still is in rural regions. As crowding increased, the number of privies increased until the soil and subsoil became highly saturated with fecal material. The significance of this lies in the fact that water for household purposes was obtained from shallow wells, and in the cities it was impossible to separate these more than a few feet from the privies. The contamination of wells was appalling, and diseases such as typhoid, dysentery, and cholera which were transmitted by water were the leading causes of death.

Then the people went to streams, lakes, and rivers, or sunk deep wells in a search for supplies of ample and good water. At first the water was hauled in tanks or barrels and distributed in buckets, later it was piped directly into the houses. The development of the pressure water system lead to a great increase in the amount of water used and thus to an increase in the amount of household wastes.

However, it was not until after the development of the flush toilet or water closet around 1850 that human excrement became the chief constituent of sewage. This use of water as a vehicle for transporting excrement from the premises did not solve the problem but it did alter it. The sewage was increased in volume and decreased in concentration and the problem now became one of how to handle a large volume of highly polluted water. The natural and easy solution was to conduct it to the drainage system and pipe it to the nearest stream or lake. Thus our modern sewage system developed.

Industrial Wastes: A direct consequence of the industrial revolution is industrial wastes. Their character, composition, and volume vary with the kind of industry. The wastes are constantly changing as new industries are developed, as new processes are introduced, and as old ones are discarded. The problem is further complicated by the fact that some industries, such as canning, for instance, are seasonal and cause the volume of a particular kind of waste to be very great at some times and very small at others.

The following classification of industrial wastes,[1] on the basis of composition, will illustrate the variety of materials from industries which find their way into sewage.

[1] Weston, Modern Sewage Disposal, 1938.

1. Ligneous and resinous wastes, both acid and alkaline, from digestion of wood, paper stock, cotton cloth, and vegetable fibers.
2. The greasy wastes from wool, silk, and woolen cloth, washing, tanning, and meat packing.
3. The highly nitrogenous wastes from glue and gelatine factories, tanneries, and slaughter houses.
4. The highly carbonaceous wastes from starch and glucose factories, creameries, sugar factories, tanneries, pulp mills, and paper mills.
5. The soapy wastes from laundries and cloth washing mills.
6. The acid wastes from mines and metal industries. These may contain iron, copper, zinc, nickel, chromium, or other metals.
7. The phenolic, creosolic, ammoniacal, and tarry wastes from gas and coke works.
8. Wastes containing large amounts of suspended matter, both organic and inorganic, like those from the washing of animal fibers and from tanneries, packing houses, cement mills, some chemical industries, canneries, beet sugar factories, rubber reclaiming mills, silk mills, and pulp and paper mills.
9. Wastes containing mineral oils, like those from oil refineries, garages, and machine works.
10. Wastes containing poisons like arsenic and cyanide.
11. Highly colored wastes from dyeing processes.

Methods of Sewage Disposal

Sewage may be disposed of by irrigation, by dilution, or by treatment in any one or a combination of ways.

Irrigation Method: Disposal by irrigation, that is, by running raw sewage over land which may or may not be used for the growth of crops is not common practice in the United States. If this method is to be considered, the character of the soil, the rate of evaporation, the price of land, the size of the city, and the volume and character of the sewage are factors that must be taken into account.

Dilution: Sewage disposal by dilution, that is, simply by running the sewage into a river, lake, or other convenient body of water is the oldest method developed. It is also the least expensive, and if the dilution factor is sufficient and if the water into which the sewage is dumped is not used for any other purpose, it is reasonably satisfactory. Cities located on the seaboard can use it to best advantage.

If the city is running its sewage into a river, however, the seasonal flow may vary enough to prevent adequate dilution and so present a problem, usually in the hottest months at the end of the summer.

Then, too, as cities increase in size and sewage increases in volume, the

pollution in the river or lake will often reach a point where the dispersion is not adequate and sludge banks and scum develop.

Since many cities are dependent upon streams or lakes for their water supply, to use these same waters for diluting sewage obviously increases the burden of water purification. This was well illustrated by the experience of the city of Chicago. Cribs some distance out in Lake Michigan serve as a source of water for the city. The city's raw sewage was discharged into a river which emptied into the lake. So much of it found its way back into the water system that the typhoid rate in Chicago reached 200 per 100,000. Forty years later, after this practice had been stopped and the drinking water chlorinated, the rate had dropped to 0.5 per 100,000. Part of this reduction, of course, is due to the pasteurization of milk which had become general during this period.

When raw sewage was discharged into rivers, cities below often raised objections and legal action sometimes followed. In addition to the hazards to health, they complained that the introduction of sewage into the water created offensive odors and interfered with recreational activities, swimming, boating, etc.

These unsatisfactory conditions led to a search for ways of treating sewage, and during the past fifty years there has been a growing tendency for cities to treat sewage before allowing it to enter lakes or streams. By getting the sanitary engineer, chemist, biochemist, and bacteriologist together to work on the problem, it has been possible to construct sewerage systems and treatment plants which, though by no means perfect, are highly efficient.

Methods of Sewage Treatment and Microbiology

The methods used for the treatment and disposal of sewage are based upon the fact that sewage is an excellent food for bacteria. Raw sewage consists of inorganic and organic substances. The organic substances, such as fats, carbohydrates, and proteins, both in their native state and in various stages of decomposition contain a vast amount of energy and can serve as nutriment for microorganisms. When the bacteria use these compounds for energy and growth, they oxidize them more or less completely, leaving new compounds of lower molecular weight and less energy. The principal reactions are either hydrolytic, resulting in more soluble compounds, or oxidative, resulting in more stable compounds. When most of the energy in the compounds has been consumed, bacteria will no longer grow readily and the sewage is said to be stabilized; that is to say, it is no longer putresible and does not give rise to vile smelling compounds.

Sewage contains millions of bacteria from the intestines of man and from soil. Some are aerobic, some are anaerobic. When the aerobic bacteria

act on sewage in the presence of an ample supply of oxygen, they produce by-products which are more or less completely oxidized and, hence, stable. The anaerobic bacteria, on the other hand, do not require free oxygen and when they act on sewage, they tend to produce compounds such as the mercaptans, and the compounds: indol and skatol, which are foul smelling and offensive. The type of change that will take place in sewage depends upon the oxygen supply. If sufficient oxygen is present, the aerobic organisms will digest, transform, and stabilize sewage without creating a nuisance. If an insufficient supply is present, the anaerobes and the facultative anaerobes will predominate and the changes will be of such character as to give rise to putrid conditions.

The stabilization of sewage is essentially an oxidation process, and the amount of oxygen consumed will depend upon the nature and amount of organic matter present. Conversely, a measurement of the oxygen needed is a good index of the amount of organic matter present in the sewage.

Biochemical Oxygen Demand: The common method employed to determine the biochemical oxygen demand, also known as the *B.O.D.*, is to measure the amount of oxygen consumed by bacteria growing in sewage. When an active sludge or inoculum of sewage-digesting bacteria is placed in a known amount of sewage and allowed to act, the oxygen present in the water is used up in proportion to the amount of organic matter oxidized. Not all of the organic matter is readily oxidized, but that which is resistant to bacterial oxidation will not create a disturbance if allowed to flow into a stream.

The more rapidly oxidized compounds are used first and the rate of oxidation is most rapid in the early stages of digestion. Although the process is not complete for a period of about twenty days, for practical purposes the test need not be run for more than five, at which time about seventy to eighty per cent of the oxygen demand is satisfied.

The type of process or combination of processes selected for the treatment of sewage will depend upon a number of factors. If it is to be discharged into a stream, the primary consideration is to prevent polluting the stream beyond its capacity to purify itself; that is, the amount of organic matter turned into the stream should not be so great as to consume all of the oxygen present in the water. The load a stream is capable of carrying depends upon its flow, which is a quite variable factor because it depends upon seasonal rainfall and snow. In many instances the flow may be sufficient to take care of the untreated sewage during the greater part of the year and treatment may be limited to the period of reduced flow, usually the summer months.

The practical methods of sewage treatment have been classified by Gainey as follows:

A. Without treatment
   1. By dilution
   2. By irrigation
B. With treatment by one or more methods or combination of methods
   1. Separation of solids and liquids
      a. Floating and coarse solids
         1. By screens
         2. In skimming tanks
      b. Heavy solids
         1. By grit chambers
      c. Coarse and fine suspended solids
         1. By sedimentation
            a. Plain sedimentation
            b. Chemical flocculation followed by sedimentation
            c. In cesspool
            d. In septic tank
            e. In two-story tanks
            f. Flocculation by activation, followed by sedimentation
   2. Treatment of liquid
      a. By oxidation through aerobic bacterial action
         1. Through dilution
         2. Through irrigation
         3. Through intermittent sand filtration
         4. Through contact beds
         5. Through trickling filters
         6. Through activators
      b. By disinfection
   3. Disposal of effluent
      a. By dilution
      b. By irrigation
   4. Treatment of solids
      a. By digestion through anaerobic bacterial action
         1. In septic tanks
         2. In Imhoff tank
         3. In separate digestion tank
      b. By dewatering without digestion
         1. Natural drying
         2. Artificial drying
   5. Disposal of undigested solids
      a. By burning
      b. As fertilizer

Regardless of the method of treatment or combinations of treatments, it is bacterial action that is eventually responsible for the stabilization of the sewage. A sewage treatment plant really does nothing more nor less than allow bacterial decomposition of sewage to take place under controlled conditions. The sewage is confined and is allowed to digest under such conditions as to prevent the creation of offensive smells and odors.

Types of Digestion Tanks:

Cesspools: A cesspool may be described as a covered pool with walls of open construction which allow for rapid seepage into the surrounding soil. Cesspools were of some little importance in the past but today are used only for small volumes of sewage. They are not used where there is a sewerage system.

Septic Tanks: Septic tanks may be small, serving only one residence, or they may be large enough to serve an entire city. The earlier septic tanks

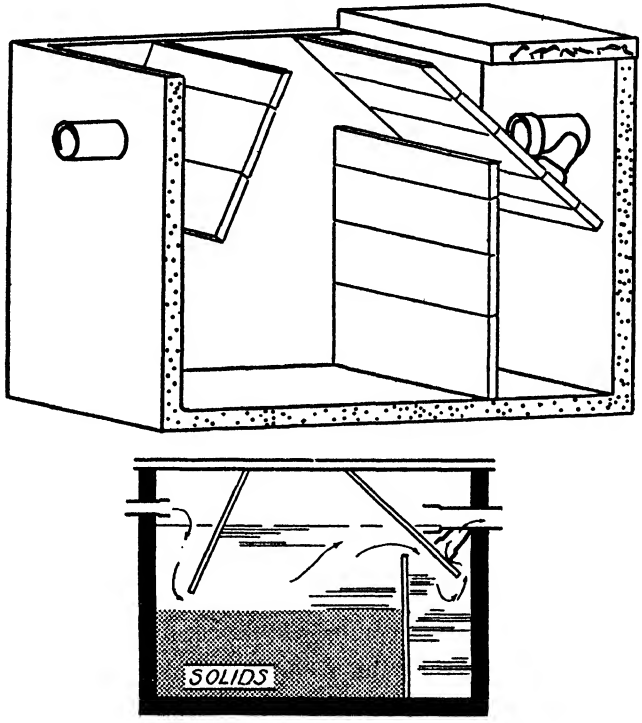


FIG. 54. Sectional view of a septic tank. Redrawn from California Extension Circular No. 82.

were single story, but the two-story tanks, called *Imhoff tanks* after the man who designed them, are a decided improvement.

Septic tanks are usually constructed of concrete or brick although some of the smaller units for the use of single families are made of iron. A system of baffles prevents the floating solids from passing through. These settle rapidly because of the reduced rate of flow and, in about two hours, from forty to sixty per cent will have settled out forming a sludge. The liquid effluent will contain dissolved organic material but the concentra-

tion will be reduced by the amount of the sludge and there will be a twenty-five to sixty per cent reduction in the biochemical oxygen demand of the effluent.

Sedimentation may be hastened by the addition of chemicals and the B.O.D. of the effluent reduced to twenty-five to fifteen per cent of that of raw sewage. Whether chemical sedimentation should be used will be determined by economic factors.

The sludge is reduced in volume by the hydrolytic activities of bacteria. The rate of bacterial growth is rapid and the oxygen is soon depleted. Conditions then become favorable for the strict and facultative anaerobes which bring about an incomplete digestion of fats, carbohydrates, and proteins. This results in a production of many vile smelling partially oxidized compounds. Gas, chiefly methane, is liberated at a rate sufficient to cause a bubbling which tends to stir up the solids. In the single story tanks this is undesirable because some of the solids will flow out in the effluent. To overcome this and to get a better separation of solids from the liquid, the Imhoff or two-story tank was designed.

In the Imhoff tanks the raw sewage flows into a V-shaped trough, so arranged that the solids can slide through but the gas cannot readily escape upwards. The solids settle to the lower digestion chambers and the liquid sewage passes directly into the outlet. The sludge undergoes anaerobic digestion and the escaping methane gas may be collected and used for power for operating the treatment plant, for heat, or light. A properly designed plant can produce about twenty-five to thirty-five per cent of the power it uses. The gas has a B. T. U. of from 400 to 800. In Germany it is compressed and used in automobiles.

As the sludge from septic tanks becomes digested it must be removed.

Levine, in discussing the exact mechanism by which methane is produced, suggests two possibilities: (1) that the substrate is the immediate and direct cause and (2) that methane is produced by a biological reduction of carbon dioxide by hydrogen according to the equation:

$$CO_2 + 4\ H_2 = CH_4 + 2\ H_2O$$

Of the several groups of bacteria capable of producing methane, species of *methanosarcena*, *methanococcus*, and *methano-bacterium* are said to be very active.

The rate of digestion of a sludge depends upon a number of factors. Temperature, the composition of the sludge, and the type of bacterial flora are important.

When sewage is first introduced into a new septic tank, the digestion is slow but after the tank has been operating for several weeks or a few months, the process of digestion becomes much more rapid. During this

time the character of the flora has changed and certain species particularly effective in digesting sewage have become predominant. The digestion of fresh sewage may be speeded up by inoculation with a small amount of "ripened" sewage.

Disposal of Effluent from Septic Tanks: The effluent from septic tanks contains about fifty per cent of the organic matter and has about fifty per cent of the B.O.D. of raw sewage which can be reduced to about ten per cent by aerobic decomposition. The two principal means of disposing of the effluent are irrigation and the use of filters. If suitable land is available, the effluent may be used as a source of water and fertilizer for plant growth, in which case it is conducted over the land by a system of tile pipe. This method is open to many objections and is not commonly employed in the United States. The type of farming, the rate of evaporation, and the character of the soil are factors that restrict its use.

Effluents from domestic septic tanks are usually disposed of by subsurface irrigation if the soil is porous and the ground water not too near the surface. The final decomposition of the sewage takes place in the soil.

Filters: There are several kinds of filters in use for purification of the effluent from septic tanks. They are all constructed so as to provide optimum conditions for the growth of aerobic bacteria and for removing the liquid by some system of filter drainage.

Contact Filter Beds: The contact filter beds are water-tight tanks three to six feet deep and filled with cinders, crushed rock, or rubble. The tank is filled with sewage which is allowed to remain there for a few hours. Then it is emptied by means of a drainage system. When the bed is new, digestion is slow and may require ten to fourteen hours for completion. However, as the bed is used, the surface of the rock or cinder becomes coated with a zoogleal mass consisting chiefly of bacteria and protozoa, although metazoa and the larvae of insects may also be present. The outstanding physical property of this mass is its high absorptive capacity for the oxygen and organic matter of sewage.

The sequence of events appears to be essentially as follows: When the bed is empty the zoogleal mass becomes saturated with oxygen from the air. When sewage is run over the bed, the larger particles are filtered out mechanically and dissolved organic matter is absorbed by the zoogleal film. Aerobic bacteria begin their activities and by a series of hydrolytic and oxidative reactions transform the organic matter into bacterial protoplasms. The material that remains is oxidized and, hence, stabilized. During the process, oxygen is consumed so the tank must be drained and the zoogleal mass permitted to absorb more oxygen. The number of fillings and emptyings per day will vary with many factors, but if two hours

is allowed for contact with sewage and four hours for microbial action and reoxygenation, several runs may be made each day.

If the B.O.D. is not reduced sufficiently by passing through one bed, another, or a series of beds hooked up in tandem, may be used. How complete the stabilization need be will depend upon the amount of water available as a diluent for the effluent.

The role of the protozoa is of particular interest. While they do not digest sewage, they have an important part in the process. When bacteria are growing in any medium, they multiply very rapidly for a few hours and then, as their numbers increase, the rate of growth slows down until a point called the M concentration is reached, where there is no further increase. It is while bacteria are actively multiplying that they use large amounts of food and, hence, it is during this period that purification takes place most rapidly. If the bacteria growing in sewage were allowed to multiply without restraint, they would soon reach such a high concentration that little or no increase in numbers would be possible and there would be no further purification. The protozoa eat the bacteria and in so doing keep the numbers down and prevent the culture from reaching the M concentration. In effect, the protozoa keep the culture in the logarithmic phase. The metazoa probably exert a similar influence on the protozoan population.

Trickling Filters: The trickling filter serves the same function as does the contact filter, it reduces the B.O.D. of sewage. The essential difference lies in the manner in which the sewage is applied. In the trickling filter the sewage is sprayed over the surface of the filter bed by means of stationary or rotating nozzles. There is some difference in opinion among those qualified to judge as to whether the sewage should be applied continuously or intermittently, but it is generally agreed that the trickling filter is extremely efficient. The mechanism of purification is microbial and the process is one similar to that of the contact filter. The selection of material for the filter bed is of paramount importance. Cracked stone of uniform size is preferred. Cinders are not satisfactory because of their tendency to disintegrate, particularly when subjected to freezing and thawing. The efficiency of the trickling filter is due in large measure to the fact that the sewage becomes saturated with oxygen when sprayed through the air, and that, by virtue of this oxygen, the aerobic bacteria are able to oxidize large amounts of organic matter rapidly. The rate of oxidation depends upon temperature. Trickling filters undergo what has been termed spring disgorgement during which large amounts of organic matter, consisting of microbial cells and more or less completely oxidized sewage, break loose from the filter and appear in the effluent. The trickling filter effluent may

be passed into a sedimentation tank and the sediment then returned to a digestion tank. However, if the filter is operating effectively, there will be little sediment.

One drawback to the trickling filter is that it may serve as a breeding place for flies, in which case it is difficult to control them.

Activated Sludge: The discovery of activated sludge was one of the great contributions to sewage disposal. A number of men in the United States and England were working on the problem and Lockett, in 1913, was the first to work out the principles. If air enough to provide ample oxygen is forced through a bottle of raw sewage, the bacteria begin to multiply rapidly but it takes from two to three weeks to bring about complete nitrification and stabilization. Towards the end of this period the finely suspended matter flocculates and the floc settles rapidly, thus allowing for a separation of the liquid from the sludge. If a portion of this sludge is used to inoculate a bottle of fresh sewage, the period required to bring about flocculation, nitrification, and, hence, stabilization is reduced. And, if this process is repeated a number of times, a sludge is obtained which brings about flocculation in an hour or less and complete nitrification in about four to six hours. Such a sludge is said to be "activated."

In its simplified form the activated sludge sewage treatment consists of (1) an aeration tank where the sewage is subjected to vigorous aeration by mechanical stirring or by forcing air through porous plates, (2) a sedimentation tank where the digested sewage is allowed to settle and (3) pumps for forcing a portion of the settled activated sludge back into the aeration tank where it serves to inoculate the incoming sewage. In actual practice, there may be several other stages, such as pre-sedimentation of the incoming sewage. The essential feature is the aeration tank, for the process depends upon efficient and controlled aeration.

The principal drawback to sewage disposal by the activated sludge process is the disposal of excess sludge. This sludge contains much more water than that from the septic tanks and is consequently more bulky. It may be dried by vacuum filtration and used as fertilizer. Many plants sell the sludge. Because of its high water-holding capacity, it serves as an excellent soil conditioner, but, since mineralization takes place during the process of digestion and the nitrates and phosphates are in solution, much of the fertilizer value is lost by way of the effluent. The sludge may also be burned, used to fill in low areas, or hauled away to dumps.

The mechanism of purification in the activated sludge process is analogous to that of the trickling filter. In the former, air is sprayed through the sewage: in the latter, sewage is sprayed through the air. In either case the sewage is well aerated and conditions are favorable for the growth of aerobic bacteria. The activated sludge floc consists of a

matrix of a jelly-like substance produced by slime-forming bacteria in which bacterial cells and inert particles are imbedded. This floc presents a vast surface area upon which the colloidal and dissolved sewage is absorbed. The mechanical agitation or the agitation due to the bubbling of air through the sewage keeps the floc in motion, thus insuring contact with the sewage.

Bacteria Present in Activated Sludge: There are numerous species of bacteria present in activated sludge but the present evidence indicates that most of these are purely incidental to the process of purification and that one group consisting of a single genus is responsible for the process. Butterfield has isolated a short gram-negative rod, *Zooglea ramigera*, which, in pure cultures is capable of producing the characteristic flocculation and reduction in B.O.D. associated with activated sludge. Various species or strains of this organism differ in activity on different substrates.

Chemical Treatment of Sewage: In general, chemicals are added to sewage for two purposes: (1) to hasten sedimentation, and (2) to destroy pathogenic microorganisms.

Chemical Sedimentation: Ferric chloride, chlorinated copperas, alum, and ferric sulfate are the most commonly used chemicals for bringing about a more rapid and more complete separation of sludge and, hence, a greater reduction of the B.O.D. of the effluent. Chemical sedimentation is usually a supplementary process used during certain seasons to increase the efficiency of the plant. The objections to the chemical treatment are the cost of the chemicals, and the cost of disposing of the large amounts of sludge.

Disinfection of Sewage: Disinfection of sewage by the use of chlorine gas has been and is being used in a number of places. The dosage necessary to destroy pathogenic bacteria in raw sewage or in the effluent from septic tanks or from activated sludge plants depends upon the amount of organic matter. It is usually a supplemental process and, in many instances, seasonal.

Utilization of Sewage: The value of sewage as fertilizer is well recognized but there are practical considerations that limit its use in agriculture. In some countries, particularly in Germany, fats and oils are being reclaimed. There are two reasons for doing this: they are of economic value and they interfere with the digestion of sludge. In many instances, industries have found it profitable to recover various materials from the effluents of their plants.

Disease-Producing Agents Transmitted by Sewage

The diseases due to organisms found in sewage are considered elsewhere, but, by way of recapitulation, they may be mentioned again here. The

pathogenic organisms found in human excrement belong to the bacteria, protozoa, viruses, and metazoa.

Disease	*Pathogen*
Bacterial Diseases	
Typhoid fever	Eberthella typhi
Paratyphoid fever	Salmonella paratyphi
	Salmonella schötmulleri
Cholera	Spirillum cholerae
Bacillary dysentery	Shigella dysenteriae
	Shigella paradysenteriae
Protozoan Diseases	
Amoebic dysentery	Entamoeba histolytica
Metazoan Diseases	
Hookworm—Old world	Ankylostoma
New world	Necator americanus
Virus Diseases	
Poliomyelitis (?)	Poliomyelitis virus

There are other pathogenic organisms, as for example, the tubercle bacillus, that may be eliminated in the feces, but sewage seldom, if ever, plays a part in the transmission of this disease.

Chapter XL

MICROBES AND THE SOIL

"The Earth is the Mother of us all—plants, animals, and man . . . "
—Henry Wallace from *Soils and Man*

The importance of the soil to national welfare cannot be overemphasized. Our prosperity and well-being depend upon its intelligent use and that depends upon an understanding and appreciation of the factors that determine its ability to produce crops. A great many have to be considered for the soil is a very complex material.

What we know as the 'soil' is the upper surface of the earth's crust. It varies in thickness, running from as little as six to eighteen inches in humid areas to as much as twelve to twenty feet in arid regions. It may be distinguished from the underlying layers by its properties and composition.

In the formation of soil, physical, chemical, and biological factors interact to produce innumerable changes in the parent rock. The physical force we term 'weathering' disintegrates it. The expansion and contraction caused by freezing and thawing play a most important part in the initial breakdown, and rainfall and wind erosion tend to divide the particles still further.

Chemical changes due to the solvent action of water and the reactions between the minerals and the acids and gases produced by microorganisms in the breakdown of plant and animal residue bring about more changes.

The composition of soil varies with a number of factors such as the character of the rocks from which the mineral elements arise, the climatic conditions of the region, the rainfall and temperature, and the kind of plant life.

Soil consists of solid, liquid, and gaseous matter. It may also be divided into inorganic or mineral elements arising from the disintegration of rocks, and organic matter consisting of plant and animal residues, microscopic organisms, and a certain amount of animal life such as earth worms, insects, and burrowing animals. All these influence the character of the soil.

Inorganic or Mineral Elements of Soil: The mineral elements of the soil, as we have said, vary with the kind of rock from which the soil arises. Although almost all the chemical elements may be present, those in greatest abundance are used either not at all or in very small amounts by the plants.

The following data, taken from *Soils and Man*, Yearbook of Agriculture

for 1938, gives the relative abundance of the various elements present in the upper layers of soil:

Silicon oxide	61.28%
Aluminum oxide	15.34
Iron oxide	6.26
Calcium oxide	4.96
Magnesium oxide	3.90
Sodium oxide	3.44
Potassium oxide	3.06
Titanium oxide	.78
Phosphorus oxide	.29
Sulfur oxide	.25
Manganese oxide	.10

Oxygen, silicon, aluminum, and iron comprise the bulk of the material obtained from the decay of rocks, yet the elements found in greatest abundance in plants are carbon, oxygen, hydrogen, nitrogen, and, in lesser amounts, phosphorus, potassium, calcium, manganese, and sulfur. Many other elements essential for plant growth are needed in very small amounts only.

It is because the elements obtained from the breakdown of rocks are not sufficient and because the "free" nitrogen in the air cannot be utilized, that bacteria are absolutely essential for the maintenance of plant and animal life.

Organic Matter in Soil: Although many of the characteristics of soils are due to their inorganic matter, it is the organic matter which, by and large, determines their productivity. Plants and animals and microorganisms are the sources of the organic matter which consists essentially of proteins, fats, and carbohydrates. These are broken down by microbial action into simpler and simpler compounds, the end result being carbon dioxide, water, ammonia, methane, "free" nitrogen, and simple mineral salts. The process of reducing complex organic matter to simple salts is spoken of as "mineralization."

The rate of decomposition of the various materials differs. Sugars and simpler carbohydrates are hydrolyzed first and proteins are rather readily broken down; while lignins, waxes, resins, and fats are less rapidly decomposed. Because of this difference in the rate of decomposition, the less readily decomposed compounds tend to accumulate and are known collectively as *humus.*

Humus: However, it is more than decomposed plant and animal residues. Since these are acted upon by microorganisms which utilize them for energy and growth materials, it follows that during the process microbial cells are being produced. Thus there are synthetic as well as analytic reactions involved in the formation of humus. As a matter of fact, the

protein in humus is largely in microbial cells and were it not for the proteolytic activity of microorganisms on microbial protein and the autolytic activity of dead cells, the soil nitrogen would soon become locked up.

The formation of humus is conditioned by a number of factors such as temperature, moisture, pH, and aeration; and several of these, in turn, are determined by the amount of humus present. For humus has a beneficial effect on the physical properties of the soil. It gives it a granular structure which aids in aeration and it greatly increases its water-holding capacity.

Humus is unstable and subject to further attack by microorganisms. The more complex compounds are broken down to simpler ones by hydrolytic changes which result in mineralization when they are carried to completion. Thus humus serves as a storehouse for the elements essential for plant growth.

Mineralization is purely incidental as far as the microorganisms are concerned since they are composed of the same elements as the plants and are often competitors for the same substances. Under many conditions this competition may be serious and may result in a loss of soil productivity for a time. When a large amount of material high in carbohydrate is added to the soil, for instance, the nitrogen will be used by the microorganisms for structural processes. If the carbohydrate is greatly in excess, that is, if the carbon-nitrogen ratio is high, there will not be enough nitrogen to supply their needs and the decomposition of carbohydrates will be very slow. If nitrogen is added, it will be used first to supply the needs of the microorganisms. These will decompose the organic material and then, as they die and decompose, the nitrogen will be freed from their bodies and again be available for the plants.

Good husbandry demands that the humus content of the soil be maintained at a high level. It has been said, and with ample justification, that our most important national resource is the organic matter in the soil. The value of such decayed organic matter as stable manure has been known for ages and manure has been spread over the soil to increase its fertility. In spite of this knowledge, the pioneers, finding the virgin soils wonderfully fertile and productive, only too often engaged in agricultural practices that robbed the soil of its organic matter and left it comparatively barren.

The biologic processes in a virgin soil are pretty much in equilibrium. Rocks are disintegrated, liberating minerals; plants are growing, dying, decaying, and thus adding to the organic matter; and the microorganisms are fixing and releasing nitrogen and bringing about numerous other changes which enable plants to grow. The net result is that a large amount of organic matter is stored up. The breaking of the soil imme-

diately upsets this equilibrium. More air is introduced, the bacteria are able to oxidize the organic matter more rapidly, and a large amount of food is made available for plants. If the plants are returned to the soil, it will maintain its fertility; but, since the object of cultivating soils is to obtain food for animals and man, the plants are removed. Thus the stock of organic matter is decreased. The rate of decay and mineralization is at first increased, the food for plants is increased, and the first crops taken from a virgin soil are most luxuriant. But as the cropping continues and as the plants are removed, the organic matter is removed and in a short time the stock which had taken ages to accumulate is depleted. The breaking of the sod also allows for erosion and some of the organic matter has been lost in this way. The rapid decay led to an accumulation of more soluble compounds which were leached out by water. Loss of fertility was the inevitable result.

The amount of organic matter in virgin soils varies from less than two to nearly five per cent, or from about eighteen to forty-five tons per acre. Soils which have been cropped for fifty to sixty years have lost from thirty to forty per cent of their original organic matter. The problem of restoring fertility to such soils is far more complex than the mere addition of organic matter equivalent to that which has been removed.

An old Indian, watching a farmer breaking ground in western North Dakota, kicked the sod back in place and broke his silence with a laconic "Wrong side up." We now know that for large regions of the United States his words were prophetic.

Microorganisms in Soil: The numbers and kinds of microorganisms in soils depend upon the amount of organic matter, the pH, moisture, and temperature, as well as its physical and chemical properties. Most of the microorganisms are in the upper six to eight inches, the numbers decreasing rapidly below this depth. The organisms in the same soils show considerable variation not only in total number but in relative abundance of various groups.

Bacteria predominate in the soil population but there are numerous protozoa, fungi, actinomycetes, and algae. In a representative gram of soil, the following kinds and numbers of bacteria may be found:

Cocci	800,000,000
Bacilli	370,000,000
Azotobacter	1,000,000
Anaerobes	5,000,000
Aerobic nitrogen fixers	5,000 to 0
Anaerobic nitrogen fixers	5,000 to 0
Nitrifying bacteria	3,000
Anaerobic cellulose decomposing bacteria	400

The number of bacteria is usually determined by plating samples of soil on artificial media. There are many sources of error in such determinations because the organisms may be clumped or because many do not grow. Such counts are always too low. Counting by direct examination with the microscope yields a much higher number. However, in spite of the faults of the plating technique, the results are of value for the purposes of comparison.

The number of organisms other than bacteria in the soil is tremendous, as shown by the following data taken from Waksman and Starkey:

Flagellates.....	35,000 per gram,	70 to 170 pounds per acre
Amoeba.....	150,000 per gram,	150 pounds per acre
Bacteria.....	22,000,000,000 per gram,	24 pounds per acre
Nematods:		
In the upper six inches of a Missouri corn field.....		648,000,000 per acre
In the upper two feet of a Utah sugar beet field.....		12,000,000,000 per acre

The various microorganisms are important because of their peculiar physiological activities. These are discussed in some detail in the section on physiology. It is not the fact of numbers that is important. When conditions are such that any one group reaches its maximum number or M concentration, the rate of multiplication is slow and there is little conversion of materials. Consequently it is the factors that determine multiplication that are important. The microflora and fauna react on one another, limit the numbers of any one group, and thus keep the soil population in a dynamic equilibrium.

Soil may be regarded from various points of view. The microbiologist's interest lies chiefly in soil as a medium for microbial growth and in the microbial processes which bring about changes in its constituents. From this broad point of view, the soil may be looked upon as a huge digestive system. Into it go all manner of materials. Plants, animals, and man are eventually returned to it. The digestive enzymes of the microorganisms break down these bodies and convert them to simpler compounds which may be utilized by growing plants. Were it not for their action, there would be no decomposition, the surface of the earth would soon become covered with dead but not decomposing plant and animal bodies, and the process of life would be at stalemate.

The exploitation of our national forests was dramatic and readily appreciated; that of our soils, while far more important, has a humdrum quality and has received only limited attention. Yet soil fertility must be maintained and the problem challenges the economist, the agronomist, and the microbiologist as well as the farmer.

Chapter XLI

BACTERIOLOGICAL WARFARE

"For nation shall rise against nation and kingdom against kingdom and there shall be famines and pestilences and earthquakes in divers places."—*Matt. 24–7*

The concept of disease as a weapon in warfare is not a recent one for it is recorded that Apollo used darts to inflict epidemic sickness on the army before Troy and that in the reign of Commodus many persons were killed by "wicked wretches" who, for a stipulated reward, dipped small needles into pestilent poison and communicated disease. Chemical warfare is foreshadowed by the ancient practice of poisoning enemy wells, thus striking both at man and at the beasts upon whom he depends. Disease and famine have always been associated with war and have, in fact, been more devastating than war itself.

The scriptures are replete with references to plagues and pestilences and both ancient and modern history tell of disease, chiefly infectious disease, that has affected not only the soldiers and civilians in the countries where the wars were waged, but has been carried about to other lands by soldiers, prisoners, and travelers. There was the famous Plague of Athens (430–425 B.C.) so vividly described by Thucydides and the Plague of Galen brought about in Italy (168–166 B.C.), by soldiers returning from Syria, and spreading from Italy to Gaul and to the banks of the Rhine. The history of the Crusades illustrates very clearly the ravages of disease. The crowding of people into besieged cities and the attendant lack of sanitation made conditions ideal for epidemics. The Crusaders, recruited from farm and village, had little "herd immunity" and presented a highly susceptible group that succumbed rapidly when exposed to infections. In 1098 when a Christian army of 300,000 besieged Antioch, disease and famine killed so many that the dead could not be buried. There were 300,000 at the beginning of the siege of Jerusalem in 1098 and only 60,000 alive when the city was taken a year later. The story was the same in the crusade against the heretics in Egypt as indicated from the following account:[1]

"A horrible sight greeted the pilgrims when they took possession of Damietta. Not only the houses, but even the streets were filled with unburied corpses; in the beds dead bodies lay beside helpless and dying invalids, and the infection of the air was intolerable. Of 8,000 inhabitants which the city had had at the beginning of the siege only 3,000 were left, while only 100 of these were healthy."

In the Napoleonic wars we are told that the French army in the Spanish peninsula lost 300,000 from disease and only 100,000 in battle. Napoleon's

[1] *Epidemics Resulting from Wars*, Friedrick Prinzing, Oxford Press, 1916.

Russian campaign was even more disastrous. He set out for battle with over 500,000 troops and came back with less than 8,000. The Russian winter, famine, and battle took their toll but most of his loss were due to typhus fever although typhoid, dysentery, and other infections were also present.

In Haiti it was yellow fever that destroyed the French army killing 22,000 of the 25,000 troops and thus enabling Dessalines to gain control.

In the Civil War, in the northern armies there were 44,000 killed in battle, 49,000 who died of wounds received in battle, and 186,000 dead from disease.

While disease which was not the direct result of battle injury has until quite recently been far more important than bullets as a cause of death in troops, the total number of deaths in the troops from disease has been relatively small, of course, as compared to the total number of deaths from disease in the much larger civilian population. The influenza epidemic of 1918–19 took a toll of about 500,000 lives in the United States. The number of deaths due to battle injury in the United States troops in World War I was about 50,000.

Even so mild a disease as measles usually is can, under certain circumstances, be deadly. When it was introduced into the Fiji Islands in 1875 it caused the death of 40,000 people in a population of 150,000.

Since epidemic diseases such as smallpox, bubonic plague, typhus fever, typhoid, dysentery, cholera, and influenza have so overshadowed war as a cause of death and destruction, it is no more than realistic to consider the possibility of the deliberate use of disease-producing agents in a total, global war. It is merely a recognition of the obvious fact that infectious disease without conscious or planned intervention by man has found fertile soil under the conditions of war and a realization that an attempt might be made to take deliberate advantage of this fact.

Microbiology and allied sciences have provided the information and suggested measures for the control of microorganisms associated with food, health, and disease. They have discovered the principles of infection and resistance, and of disease transmission and control and have thus made it possible to eliminate many diseases and to reduce the threat of others to a point where they are not a major problem in war or in peace.

It should be recognized that it is only because we have determined the nature of most plagues and pestilences and have learned to control them that they have not been important in the two World Wars. But the same knowledge that makes control possible also makes it possible to determine the kinds of diseases and the conditions under which these diseases could be used deliberately.

The diseases that have been so devastating in the past were transmitted

unknowingly and unintentionally. The problem of bacteriological warfare has to do with diseases introduced intentionally and deliberately.

In a general way, defense against or preparation for bacteriological warfare is an exercise in epidemiology, for the natural history or epidemiology of disease gives the clues as to why certain diseases were so wide spread and deadly in certain countries and under certain circumstances and provides much of the information that makes control possible. It is, of course, admitted that we do not know just why some of the epidemic diseases of the past disappeared but in most cases a pretty shrewd guess can be made and in other instances the answers are clear.

It is also evident that for a variety of reasons the most virulent diseases of the past may not be most suitable in waging bacteriological warfare. Nor can it be assumed that the diseases most to be feared in a highly industrialized country would be equally serious in a country with a less highly integrated economy.

We might, as a starting point in our exercise in epidemiological reasoning, consider some of the diseases that have shown a special tendency to become epidemic and see what peculiar conditions have prevailed that made these diseases behave as they did. The diseases are of different epidemiological types and, by and large, these have determined what diseases have prevailed. On the basis of the mode of transmission they may be grouped as follows:

1. Droplet and contact transmitted diseases, such as influenza, smallpox, and tuberculosis.
2. Water-borne diseases, such as typhoid fever, dysentery, and cholera.
3. Insect-borne diseases in which man is the source of infection, such as yellow fever, dengue, malaria, and epidemic typhus fever.
4. Insect-borne diseases in which lower animals are the source of infection, such as bubonic plague.

A number of questions come readily to mind when trying to anticipate just what diseases might be used effectively in bacteriological warfare. How vulnerable are the people of the country to a given disease at the moment? When a Cuban with the Spanish forces brought smallpox into Mexico in 1520, within a short time half the population of Mexico is said to have died of the disease. The population had never experienced the infection and all persons were highly susceptible. Today vaccination is general in most parts of the world and where it is not, smallpox is usually present so that a large proportion of the population is immune as a result of infection. The deliberate introduction of this virus might be scarcely noticed in most countries.

Control of the louse has eliminated typhus fever from most of Europe and

North America. If lice are not present, an epidemic of louse-borne typhus cannot be started.

Control of rats has eliminated the threat of bubonic plague.

Any city depending upon a central water supply may be vulnerable to water-borne diseases. Water purification and chlorination have practically eliminated this type of infection and are a protection against sewage contamination but the possibility that water supplies may be tampered with and intestinal pathogens introduced deliberately could be a real threat. It is ironic that the standard bacteriological tests for water purity would not detect the pathogens, for these tests are based on the assumption that any pathogens will have been introduced in the discharges of cases or carriers and *E. coli* is used as the indicator of fecal pollution.

We may also ask why a certain disease is not epidemic in a population. Is it because the pathogen is absent, because the population is actively immunized, because the insect vector is absent, or because the customs make contact with the disease-producing agent unlikely?

Can the disease be transmitted in some other way than the one in which it is naturally transmitted? Laboratory accidents suggest, in part, answers to this question. Typhus fever, brucellosis, tularemia, and several other diseases are highly dangerous to investigate as indicated by the fact that so many workers contract the disease. Air-borne rickettsial infections have occurred in laboratories, although in nature these diseases are transmitted by insects. Could a city, then, be exposed to clouds or mists containing these organisms?

Pasteurella pestis, the cause of bubonic plague, also produces a pneumonic form of the disease which is droplet transmitted. Could this organism be used to infect a population? *Pasteurella tularense* is another organism that is infective by way of the respiratory tract although it does not produce a communicable disease and person to person transmission does not occur naturally.

Plant and animal diseases could play a very decisive role in any conflict. We have but to recall how rapidly foot-and-mouth disease spreads and what its effect is on our meat and milk supply. True, it has been eradicated on many occasions but successful eradication depends upon prompt mobilization of control measures and while this can be accomplished if the focus of infection is limited, it would be extremely difficult under the exigencies of war if cattle in many areas were infected simultaneously.

A number of practical questions arise of which the following will serve as illustrations:

Can the infectious agent be produced in large quantities? How long will the material remain infectious? How can it be distributed? Will the

disease be contagious and spread after being introduced or will exposure to the original inoculation be necessary? Will carriers develop from recovered cases? If so, will they constitute a hazard after the country is defeated? How long is the incubation period? Does the insect vector already exist in the country? Are there susceptible animals that may be infected and serve as a reservoir? Can the troops using the bacterial or viral agent be immunized? Are effective methods of treatment available?

Off-hand it would seem that bacteriological warfare would have to be tailored very precisely to the conditions that prevail in any one country. Before any nation could use microbial agents offensively, that nation would have to have methods available for their control, otherwise the disease might boomerang.

Unseen dangers are always difficult to face rationally. Perhaps the psychological threat of plagues and pestilences will be as great or greater than the real thing; but, if, because of man's misdirected ingenuity, bacteriological warfare becomes an actuality, the principles used to carry it out or to control it will be essentially those of epidemiology. We will need to know the natural history of a disease, the characteristics of the etiological agent, and the methods of prevention and control to deal with this tragic misuse of science.

GLOSSARY

Abberrant: Deviating from the normal.

Abiogenesis: Spontaneous generation; the production of living from non-living matter.

Abscess: A circumscribed cavity containing pus.

Acid-fast: A term used to designate bacteria that are not decolorized by acids after being stained with aniline dyes.

Acquired Immunity: A specific resistance which is not inherited.

Active Immunity: A specific resistance due to the activity of body cells in response to infectious agents.

Acute: Severe, sharp.

Aerate: To charge or mix with air.

Aerobe: Organism requiring free oxygen for growth.

Agar: A gelatinous carbohydrate prepared from sea weed and used as a base for solid media.

Agglutination: A clumping or bringing together of bacteria or other cells under the influence of specific agglutinins.

Agglutinin: A substance in the blood of immunized animals which agglutinates an antigen.

Algae: Microorganisms, mostly aquatic cryptograms.

Algicide: A chemical that destroys algae.

Allergen: A substance capable of producing an allergic state.

Allergy: A hypersensitive or altered reaction shown by animal or man against a substance to which it is sensitized.

Amboceptor: A substance in blood serum of immunized animals which unites complement with cells.

Amphitrichous: Having flagella at both ends of the cell.

Anabolism: Constructive processes of living cells.

Anaerobe: An organism which grows only or best in the absence of free oxygen.

Anaphylaxis: Hypersensitivity to a foreign protein manifested only by sensitized animals.

Anti-: Prefix meaning against.

Antibiotic: Pertaining to antibacterial substances of biologic origin.

Antibody: A specific substance produced by an animal in response to the introduction of antigen.

Antigen: A substance which, when introduced parenterally into animals, stimulates the appearance of antibodies or immune bodies.

Antiseptic: A substance which inhibits the development of microorganisms causing infection.

Antiserum: A serum which contains specific antibodies.

Antitoxin: An antibody which counteracts or neutralizes a toxin.

Asexual: Having no sexual forms.

Ascomycetes: A group of fungi in which the sexual spores are borne within a cell called an ascus (sac).

Ascospore: A spore formed in an ascus or sac.

Ascus: A sac-like structure.

Aspergillosis: An infection caused by a fungus called aspergillus.

Assimilation: The conversion of food into cellular material.

Attenuate: To weaken or reduce in virulence or infectivity.
Autogenous: Self-produced. As applied to vaccines, the term refers to a vaccine prepared from the organism isolated from the infectious process.
Autolysis: Self-digestion or destruction of the cell by enzymes produced by the cell itself.
Autotrophic: Bacteria which can utilize carbon dioxide and inorganic salts as food.

Bacillus: Technically a genus of Schizomycetes, rod-shaped and forming endospores.
Bacteremia: The presence of bacteria in the blood.
Bactericide: A substance destructive to bacteria.
Bacterin: A suspension of dead bacterial cells used as a vaccine.
Bacteriolysin: An antibody which dissolves bacterial cells.
Bacteriophage: A submicroscopic agent which multiplies within the bacterial cell and destroys it.
Biogenesis: Production of living things from living things.
Brownian Movement: Oscillatory movement of particles due to molecular bombardment.

Capsule: A mucilaginous envelope produced by certain bacteria.
Carrier: A person who shows no symptoms of an infectious disease but harbors and disseminates the specific microorganism.
Catabolism: Destructive processes of living cells.
Catalase: An enzyme found in tissue and some microbial cells which breaks down hydrogen peroxide.
Catalyst: An agent which alters the speed of a chemical reaction usually by increasing it.
Cell: The unit of structure and function.
Certified Milk: Milk produced according to the requirements of the American Association of Medical Milk Commissions.
Chemotaxis: Positive or negative reaction of an organism to chemicals.
Chemotherapy: Treatment of disease with chemicals that destroy or inhibit the causative microorganisms.
Chromogenic: Pigment producing.
Coagulation: Clotting.
Coccus: A round bacterium.
Communicable Disease: A disease which may be transmitted naturally from one to another. One which is "catching."
Complement: A heat-labile enzyme-like substance found in normal blood which is necessary in the lytic reaction brought about by the antibodies.
Conidiophore: A stalk bearing conidia.
Conidium: An asexual spore formed by fungi and borne exogenously.
Contagious Disease: A disease transmitted by personal contact.
Contamination: Soiling with infectious matter.
Cyst: A sac-like resistant structure.
Cytology: Study of cells.
Cytoplasm: The protoplasm of cells as distinct from the nucleus.

Denitrification: Reduction of nitrates with liberation of free nitrogen.
Desensitize: To render insusceptible to anaphylactic shock or related phenomena.
Dick Test: A test to determine susceptibility to scarlet fever.

Diplococcus: Cocci which occur in pairs.
Disaccharide: A sugar consisting of two molecules of monosaccharides.
Disease: A pathologic or abnormal condition of any part of the body or mind.
Disinfect: To destroy infectious microorganisms.
Dissimilation: The breaking down processes involved in metabolism.
Droplet Infection: Transmission of infectious agents by the spray thrown off from the mouth or nose while talking, coughing, or sneezing.
Drug fast: A term applied to organisms that are resistant to the action of drugs.
Dysentery: Inflammation of the intestinal mucosa with bloody evacuations.

Effluent: That which flows out.
Endemic: Disease which is always present.
Endo-: Prefix meaning within.
Endospore: A spore formed within a cell.
Enteric: Pertaining to the intestines.
Enteritis: Inflammation of the intestines.
Epidemic: An unusual number of cases of communicable human disease within a community in a short time.
Epizootic: An unusual number of cases of communicable animal disease within a restricted area in a short time.
Etiology: Science of the causes of disease.
Extra-: Prefix meaning outside.
Extracellular: Outside the cell.
Exudate: A substance which oozes out.

Facultative: Not obligatory, optional.
Fauna: The animal life of a region.
Ferment: An enzyme.
Fermentation: Decomposition of carbohydrates by enzymes.
Fertilization: Impregnation.
Filamentous: Composed of long threads or filaments.
Fission: Reproduction by division.
Flagellum: A whip-like structure appended to a cell.
Flat sour: Formation of acid without gas. Occurs in certain canned foods.
Flora: The plant life of a region.
Fomite: An inanimate agent such as a pencil or doorknob which may serve to spread infection.
Fumigation: Destruction of infectious agents or vermin by gaseous fumes.
Fungus: A multicellular thallophyte which does not have chlorophyll.

Gangrene: Death of soft tissues.
Garget: Inflammation of tissues in cow's udder (mastitis).
Germ: A microorganism.
Germicide: An agent destructive to germs.
Gram-negative: A term applied to bacteria that are decolorized when stained by Gram's method and which take the counter stain.
Gram-positive: A term applied to bacteria that retain the purple stain.

Hapten: A substance such as a polysaccharide which although not antigenic itself will confer specific antigenic properties if linked to a protein.

Hemolysin: An antibody which, in the presence of complement, destroys red blood cells. Also a substance produced by bacteria which destroys red blood cells.
Hemolysis: Destruction or lysis of red blood cells.
Heterotrophic: A term applied to bacteria which cannot use inorganic nitrogen and carbon for growth.
Homogeneous: Of the same composition.
Homologous: Of the same form or function.
Host: A living animal or plant on which another lives as a parasite.
Humor: Any natural body fluid.
Hyaluronidase: A mucolytic enzyme found in some bacteria, seminal fluid, testicular extract, and other tissues, which depolymerizes and hydrolyses hyaluronic acid.
Hydrolysis: Decomposition of or by water.
Hypha: A single filament or thread of a mold.

Idiosyncrasy: An individual peculiarity, usually applied to hypersensitivity to drugs or foods.
Immune Bodies: Antibodies found in the blood of animals and responsible for immunity.
Immunity: A natural or acquired resistance to a disease.
Immunize: To bring about immunity.
Inactivate: To render inactive.
Incubation Period: That period between the time of exposure to an infectious agent and the first symptoms of disease.
Infect: To communicate disease-producing organisms.
Infection: Invasion of the body by a pathogenic agent with disease resulting.
Infectious Disease: A disease due to a living microorganism.
Inflammation: Response of tissues to irritation. Manifested by heat, redness, swelling, and pain.
Inoculation: The introduction of material into an animal or other medium.
Intracellular: Within the cell.
Intramuscular: Within the muscle.
Intravenous: Within the vein.
Invertase: An enzyme which breaks down sucrose of cane sugar.
Involution Forms: Retrograde and abnormal forms assumed by microorganisms grown under unfavorable conditions.
Itis: Suffix meaning inflammation of.

Lactase: An enzyme which acts on lactose or milk sugar.
Laryngeal: Pertaining to the larynx.
Lesion: A structural tissue change caused by injury or disease.
Lethal: Deadly, fatal.
Leucocyte: A white blood cell.
Leucocytosis: An increase in the number of white cells in the blood.
Leucopenia: A reduction in the number of white cells in the blood.
Lipase: Fat-splitting enzyme.
Lophotrichous: Having a tuft of flagella at one end of cell.
Lymphocyte: A white blood cell of lymphoid origin.
Lysin: A cell dissolving substance.
Lysozyne: An enzyme present in saliva, tears, egg white, and many animal fluids, which destroys bacteria by hydrolysing a mucopolysaccharide present in susceptible organisms.

Macro-: Prefix meaning large.
Macrophage: A large mononuclear wandering phagocyte.
Malt: Partially fermented barley seed.
Medium: Materials used for the culture of microorganisms.
Metabolism: The physical and chemical processes involved in the growth and maintenance of living organisms.
Metachromatic Granules: Internal granules that stain a different tint from that of the stain used.
Micro-: Prefix meaning small.
Microbe: A microorganism or germ.
Micron: One millionth of a meter, one one-thousandth of a millimeter or approximately one twenty-five thousandth of an inch.
Monosaccharide: A simple sugar which cannot be split into simpler sugars.
Monotrichous: Having one flagellum at one end of the cell.
Morphology: Science of the form and structure.
Mucopolysaccharide: A group of polysaccharides containing hexosamine that may or may not be combined with protein, and which forms many of the mucins.
Murine: Pertaining to mice or rats.
Must: Unfermented grape juice.
Mutation: Changein form or property.
Mycelium: The cottony structure consisting of a meshwork of hyphae of fungi.
Mycology: The science dealing with fungi.

Nitrification: Oxidation of ammonium compounds to nitrates.

Obligate: Necessary.
Opsonin: An antibody which enhances phagocytosis.
Osmosis: Passage of liquids through semi-permeable membranes.
Oxidase: An enzyme which brings about oxidation-reduction reactions.

Pandemic: A very wide spread epidemic.
Parasite: An organism which lives in, on, and at the expense of another.
Pasteurization: The heating of liquids at temperatures below boiling to destroy organisms causing undesirable fermentations or disease.
Pathogen: A disease-producing microorganism.
Pathology: The science of disease or morbid processes.
Peptonization: The process of converting proteins into peptones.
Peritrichous: Having flagella all around the cell.
Phagocyte: A cell capable of ingesting bacteria or other cells or particles.
Phagocytosis: The ingestion of bacteria or other substances by phagocytes.
Photosynthesis: The construction of complex compounds from carbon dioxide and water by sunlight in the presence of chlorophyll.
Plasma: Fluid portion of the circulating blood.
Plasmolysis: The removal of water from a cell.
Pleomorphism: Different forms of the same species.
Postulate: A self-evident assumption, too obvious to require proof.
Precipitin: An antibody which precipitates or flocculates homologous antigen.
Proliferation: Multiplication or reproduction of cells.
Prophylaxis: Prevention of disease.
Proteolysis: The hydrolysis or decomposition of proteins.
Proteolytic: Having the property of hydrolyzing or decomposing proteins.

Pseudo: Prefix meaning false.
Psychrophilic: Cold-loving.
Ptomaine: A basic nitrogenous substance resembling alkaloids formed in putrefaction.
Purulent: Containing pus.
Pus: An accumulation of phagocytes, bacteria, and cellular debris.
Pustule: A small elevation on the skin containing pus.
Putrefaction: Decomposition of animal or vegetable proteins by microorganisms.
Pyemia: A general septicemia with secondary foci.
Pyogenic: Pus-producing.

Quarantine: Period of isolation for persons, ships, or goods arriving from places infected with contagious disease, or of persons who have been exposed to such infection.
Resistance: The ability of the body to ward off disease.
Reticular: Network construction.
Rhizoid: Root-like structure.

Sanitary: Health-promoting.
Saprophyte: An organism living on dead organic matter.
Schick Test: A test for determining susceptibility to diphtheria.
Sensitize: To render more reactive.
Septate: Divided by a septum or cross-wall.
Septicemia: A diseased condition in which the pathogenic bacteria are multiplying in the blood stream. Often referred to as 'blood-poisoning.'
Septic Tank: A tank in which sewage is decomposed under anaerobic conditions.
Septum: A wall or dividing membrane.
Serology: The branch of science that deal with serums, particularly immune serums.
Serum: The fluid portion of the coagulated blood.
Sewage: The waste matter in sewers.
Sewerage: The system of pipes for carrying sewage.
Smear: A very thin layer of material spread on a glass slide.
Soluble Specific Substance: A complex carbohydrate which is highly type specific and which serves as a hapten.
Sporangiophore: The mycelial thread which bears a sporangium.
Sporangium: A sac at the tip of a fertile hypha containing asexual spores.
Spore: A highly resistant stage of an organism.
Sporulation: The production of spores.
Sterile: Free from living organisms.
Sterilization: Destruction of all living matter.
Suppuration: Pus formation.
Susceptibility: A disposition to yield readily to disease.
Symbiosis: The mutual advantageous association of two or more organisms.
Systemic: Relating to an entire organism rather than to a part.

Terminal Disinfection: Disinfection of the premises after the recovery or death of a patient suffering from an infectious disease.
Therapeutic: Having healing qualities.
Therapy: Treatment of disease.
Thermal: Having to do with heat.

Thermolabile: Easily altered or destroyed by heat.
Thermophile: Heat-loving.
Thermostabile: Heat resistant.
Toxemia: Poisoned condition of the blood.
Toxin: Poison formed by bacteria and other living thing
Toxoid: Bacterial toxin treated so that its toxic properties are destroyed and its antigenic properties retained.
Tubercle: A nodule or small eminence. The characteristic lesion of tuberculosis.

Ulcer: An open sore.

Vaccine: Originally lymph from cowpox vesicles. Now applied to any material used in prophylactic inoculation.
Vacuole: A clear space in the protoplasm of a cell.
Vector: An animal, usually an insect, that carries bacteria, protozoa, or virus from one host to another.
Vesicle: A small blister.
Virulence: Disease-producing capacity.
Virus: A sub-microscopic agent producing disease.

REFERENCES

Section I. Fundamentals of Microbiology

Anderson, C. G.: *An Introduction to Bacteriological Chemistry.* William Wood & Co., Baltimore, 1938.

Buchanan, R. E., and Fulmer, E. I.: *Physiology and Biochemistry of Bacteria*, Vols. I, II, and III, 1928–30. Williams & Wilkins Co., Baltimore.

Burdon, K. L.: *Medical Microbiology.* Macmillan Co., New York, 1939.

Clark, M. W.: *Determination of Hydrogen Ions.* Williams & Wilkins Co., Baltimore, 1928.

De Kruif, P.: *Microbe Hunters.* Harcourt, Brace & Co., New York, 1926.

Dobell, C.: *Antony van Leewenhoek and His Little Animals*, Harcourt, Brace and Co., 1932.

Dubos, Rene J., *The Bacterial Cell*, Harvard University Press.

Fred, E. B.: *The Root Nodule Bacteria of Leguminous Plants.* Chicago University Press, 1928.

Greaves, J. E., and Greaves, E. O.: *Elementary Bacteriology*, 4th Ed. W. B. Saunders Co., Philadelphia, 1940.

Hadley, P.: *Further Advances in the Study of Microbic Dissociation.* Jour. Inf. Dis., 60: 129, 1937.

Henrici, A. T.: *The Biology of Bacteria*, 2nd Ed. D. C. Heath & Co., New York, 1939.

Henrici, A. T.: *Molds, Yeasts, and Actinomycetes.* John Wiley & Sons, New York, 1930.

Henrici, A. T.: *Morphologic Variation and the Rate of Growth of Bacteria.* Carles C. Thomas, Springfield, Ill., 1928.

Henrici, A. T.: *The Yeasts.* Bacteriological Reviews, June, 1941.

Hewitt, L. F.: *Oxidation and Reduction Potentials in Bacteriology and Biochemistry.* P. S. King & Son, Westminster, S. W. 1, England, 1936.

Jordan, E. O. and Burrows, W.: *Textbook of Bacteriology*, W. B. Saunders Company, Philadelphia, 1946.

Koser, S. A., and Saunders, F.: *Accessory Growth Factors for Bacteria.* Bacteriological Rev., 3: 187, 1939.

McCulloch, E. C.: *Disinfection and Sterilization*, Lea and Febiger, Philadelphia, 1936.

Marton, L.: *The Electronic Microscope.* Journal of Bact., 41. 397–413, 1941.

Northrup, John H.: *Crystalline Enzymes.* Columbia Press, New York, 1939.

Porter, John R., *Bacterial Chemistry and Physiology*, John Wiley & Sons, Inc., New York, 1946.

Salle, A. J.: *Fundamental Principles of Bacteriology.* McGraw Hill Book Co., New York, 1939.

Smith, K.: *Plant Virus Diseases.* Methuen & C., London, 1935.

Stephenson, M.: *Bacterial Metabolism*, 2nd Ed. Longmans, Green & Co., New York, 1939.

Waksman, S. A.: *Principles of Soil Microbiology*, 2nd Ed., Williams & Wilkins Co., Baltimore, 1932.

WERKMAN, C. H.: *Bacterial Dissimilation of Carbohydrates.* Bacteriological Rev., 3: 187, 1939.

ZINSSER, H., AND BAYNE-JONES, S.: *Textbook of Bacteriology*, 8th Ed. D. Appleton-Century Co., New York, 1939.

SECTION II. INFECTION AND RESISTANCE

BURDON, K. L.: *Medical Microbiology.* Macmillan Co., New York, 1939.

DUBOS, RENE J., *The Bacterial Cell*, Harvard University Press, 1945.

DUBOS, RENE J., (Ed.): *Bacterial and Mycotic Infections of Man*, J. B. Lippincott Co., 1948.

GAY, F. P., *et al.*: *Agents of Disease and Host Resistance.* Charles C. Thomas, Springfield, Ill., 1935.

GREENWOOD, MAJOR: *Epidemics and Crowd Disease.* Williams and Norgate Ltd., London, 1935.

LANDSTEINER, K.: *The Specificity of Serological Reactions.* Charles C. Thomas, Springfield, Ill., 1936.

MAJOR, RALPH H.: *Disease and Destiny.* Appleton-Century Co., New York, 1939.

PRINZING, FREDERICK: *Epidemics Resulting from Wars.* Oxford University Press, 1916.

RIVERS, THOMAS M., (Ed.): *Viral and Rickettsial Infections of Man*, J. B. Lippincott Co., 1948.

ROSENAU, M. J.: *Preventive Medicine and Hygiene*, 6 Ed. D. Appleton & Co., New York, 1935.

WELLS, G. M.: *Chemical Aspects of Immunity.* Reinhold Publishing Co., New York, 1929.

ZINSSER, H., ENDERS, J. F., AND FOTHERGILL, LeR.: *Immunity Principles and Application.* Macmillan Co., New York, 1939.

SECTION III. COMMON INFECTIOUS DISEASES

American Association for the Advancement of Science (Moulton, Ed.): *The Gonococcus and Gonococcal Infection.* Washington, D. C., 1939.

ANDREWES, C. W., *et al.*: *Diphtheria.* His Majesty's Stationery Office, London, 1923.

BUDD, WILLIAM: *Typhoid Fever.* Published for Delta Omega by the American Public Health Association, New York.

BURDON, K. L.: *Medical Microbiology.* Macmillan Co., New York, 1939.

CARTER, H. R., CARTER, L. A., AND FROST, W. H.: *The Early History of Yellow Fever* Williams & Wilkins Co., Baltimore, 1931.

DUBOS, RENE J., (Ed.): *Bacterial and Mycotic Infections of Man*, J. B. Lippincott Co., 1948.

GAY, F. P., *et al.*: *Agents of Disease and Host Resistance*, Charles C. Thomas, Springfield, Ill., 1936.

GREENWOOD, MAJOR: *Epidemics and Crowd Disease.* Williams and Norgate Ltd., London, 1935.

HAVENS, L. C.: *The Bacteriology of Dysentery, Salmonella and Typhoid Infection and Carrier States.* Oxford Univ. Press. 1935.

HUDDLESON, I. F.: *Brucellosis in Man and Animals.* Oxford Univ. Press, 1939.

LAIDLAW, P. P.: *Virus Diseases and Viruses.* Macmillan Co., New York, 1939.

MacArthur, W. P.: *Old Time Plague in Britain.* Trans., Royal Society of Tropical Medicine.

MacArthur, W. P.: *Old Time Typhus in Britain,* Trans., Royal Society of Tropical Medicine. 20: 487, 1926–27.

Major, Ralph H.: *Disease and Destiny.* Appleton-Century, New York, 1939.

Prinzing, Frederick: *Epidemics Resulting from Wars.* Oxford University Press. 1916.

Rivers, T. M.: *Viruses and Virus Diseases.* Stanford University Press, 1939.

Rivers, Thomas M., (Ed.): *Viral and Rickettsial Infections of Man,* J. B. Lippincott Co., 1948.

Rosenau, M. J.: *Preventive Medicine and Hygiene,* 6th Ed. D. Appleton & Co., New York, 1935.

Simpson, W. J.: *A Treatise on Plague.* Cambridge Press, 1905.

Snow, John: *On Cholera.* Published for Delta Omega by the Commonwealth Fund 1931. Oxford Press, 1936.

Symposium on Virus and Rickettsial Diseases. Harvard University Press, 1940.

Vital Statistics. Special Reports of the Bureau of Census.

Vital Statistics. Sir Arthur Newsholme, Appleton and Co. New York, 1923.

White, B.: *The Biology of the Pneumococcus.* Commonwealth Fund, New York, 1938.

Zinsser, H.: *Rats, Lice, and History.* Little Brown and Co. Boston, 1935.

Section IV. Microbiology of Food, Milk, Water, Sewage, and Soils

American Public Health Association: *Standard Methods of Water Analysis,* 8th Ed., New York, 1936.

Budd, William: *Typhoid Fever.* Published for Delta Omega by the American Public Health Association, New York.

Burdon, K. L.: *Medical Microbiology.* Macmillan Co., New York, 1939.

Gainey, P. L.: *Microbiology of Water and Sewage for Engineering Students.* Burgers Publishing Company, Minneapolis, 1939.

Hammer, B. W.: *Dairy Bacteriology,* 2nd Ed. John Wiley & Sons, New York, 1938.

Hoover, Charles P.: *Water Supply and Treatment.* Bulletin 211, National Lime Association, Washington, 1936.

Pearse, Langdon (Ed.): Modern Sewage Disposal, Federation of Sewage Works Associations, 1938.

Prescott, S. C., and Dunn, C. G.: *Industrial Microbiology.* McGraw-Hill Book Co., New York, 1940.

Rogers, L. A. (Associates): *Fundamentals of Dairy Science,* 2nd Ed. Chemical Catalog Co., New York, 1939.

Snow, John: *On Cholera.* Published for Delta Omega by the Commonwealth Fund, Oxford Press, 1936.

Tanner, F. W.: *Microbiology of Foods.* Twin City Publishing Company, Champaign, Ill., 1932.

United States Department of Agriculture: *Food and Life.* Washington, D. C., 1939.

United States Depar tment of Agriculture: *Soils and Men.* Washington, D. C., 1938

United States Public Health Service. Public Health Engineering Abstracts.

Waksman, S. A.: *Principles of Soil Microbiology,* 2nd Ed. Williams & Wilkins Co., Baltimore, 1932.

Wilson, G. S., *et al.*: *The Bacteriological Grading of Milk.* Medical Research Council, Special Report Series 206, London, 1935.

JOURNALS

American Journal of Hygiene
American Journal of Public Health
American Journal of Tuberculosis

Biological Abstracts

Epidemiological Reports of the League of Nations

Food Research

Journal of Agricultural Research
Journal of Bacteriology
Journal of Experimental Medicine
Journal of Immunology
Journal of Infectious Diseases

Phytopathology

Science
United States Public Health Report
British Journal of Hygiene

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