Glandular Physiology and Therapy

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CONTENTS

INTRODUCTION *

MORRIS FISHBEIN, M.D.
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More than fifteen years have passed since there appeared the first collection of articles on "Glandular Physiology and Therapy," published under the auspices of the Council on Pharmacy and Chemistry of the American Medical Association. Our knowledge has advanced more in these fifteen years than in all the previous centuries of the life of man. So rapid has been the advance that it was necessary to revise the first series in 1927 and the second in 1935. With the passing of the third five-year period, the Council on Pharmacy and Chemistry has considered it desirable again to survey the advancement of our knowledge.

Conspicuous among the reasons for publishing a summary of endocrinology in 1924 was the existence of a pseudoscientific therapy based on glandular materials promoted by pharmaceutical manufacturers with but slight evidence as to actual utility. Much of this empirical and unwarranted therapeusis has disappeared. With the establishment of a new Food and Drug Law and with the coming of the new powers given to the Federal Trade Commission under the Wheeler-Lea Bill, it is likely that still further improvement will occur. The sale of extracts of tonsil, kidney, spleen and heart and, indeed, of mixtures of these with innumerable other preparations, is likely to be better controlled in the future than it has been in the past. No doubt obvious charlatanism will be controlled. However, the difficulty of evaluating therapeutic results and the great psychologic factor involved in most glandular disturbances combine to confuse considerably many physicians who, witnessing the marvels of scientific glandular therapy, are ready to accept as established claims for much that is in no way proved.

The entire volume published in 1927 included only 98 pages. The series issued in 1935 included thirty-one contributions and made a book of 528 pages. In the

* This series of articles is published under the auspices of the Council on Pharmacy and Chemistry. The opinions expressed in these articles are those of the authors and do not necessarily represent the views of the Council.

present series the number of manuscripts is not greatly increased but the amount of material is considerably larger. Particularly important are the articles concerned with the endocrinology of the female reproductive mechanism. Interesting also are those articles which discuss new aspects of our knowledge of the adrenal and the pituitary. Extraordinary advances have been made with reference to the antihormones. Of special importance also are those articles which discuss the interrelationships of various portions of the glandular apparatus. The investigators who have contributed to this series of articles are all men of note in the fields about which they write. Appreciation is due the Council's special committee on this symposium, Drs. E. M. K. Geiling, chairman, W. W. Palmer, and Elmer L. Sevringhaus. Dr. S. C. Freed of the headquarters staff of the Council has had general supervision over the series, besides contributing two articles of his own. He has also assisted in editing the articles toward uniformity of expression and avoidance of excessive duplication.

During the process of publication of this series of articles in The Journal of the American Medical Association so many new contributions were made to our knowledge of glandular physiology and therapy that it became necessary to make extensive revision of the text before inclusion of the material in book form. This book may, therefore, be said to bring the accumulation of information up to the date when it was finally sent to the printer, namely, October, 1941.

CHAPTER I

RELATIONSHIP OF ANTERIOR LOBE OF THE HYPOPHYSIS TO OTHER ENDOCRINE GLANDS

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The laboratory and clinical research which has been carried on since the last edition (1935) of Glandular Physiology and Therapy has not revealed any essentially new interrelationships between the hypophysis and the other glands of internal secretion. Nor have investigations revealed that any of the interrelationships of the hypophysis given in the last edition were erroneous. Important investigations have been reported, however, during this period. Those in the field of carbohydrate and fat metabolism have been especially fruitful. Contributions of preceding years, among which those from the laboratory of Houssay are especially noteworthy, laid the basis for this subsequent extensive work, which has revealed the importance of the interrelationship between the hypophysis and the adrenal cortex in the metabolism of fats and carbohydrates. The results from the work in this field have not yet sufficiently matured to enable one to determine what practical value they will have in clinical medicine, but they contribute greatly to an understanding of the physiology of the hypophysis.

Some advance has been made in the purification of preparations containing the pituitary hormones, though the advances, owing to the protein nature of these hormones, have not kept pace with those made in investigations on the chemically more simple hormones (steroids) of the adrenal gland and the gonads.

Aside from the so-called metabolic principles, five hormones or principles continue to be generally recognized as issuing from the anterior lobe of the hypophysis. These are: (1) the growth (somatotropic) principle; (2) the gonadotropic hormone(s) or complex; (3) the thyrotropic hormone; (4) the cortico-

tropic (adrenotropic) hormone, and (5) the lactogenic hormone.[1] The evidence for the secretion of these separate factors is based on data secured from (1) the effects of hypophysectomy and (2) the effects of the injection of hypophysial extracts in (*a*) animals in which an endocrine deficiency has been established by the ablation of one of the endocrine glands, usually the hypophysis, and (*b*) normal animals.

EFFECTS OF HYPOPHYSECTOMY

The syndrome from a total deficiency of the anterior lobe of the hypophysis has been studied in all common laboratory animals, in monkeys and in man (Simmonds's disease). The studies are revealing in regard to the relationship of the hypophysis to the other glands of internal secretion and so merit a brief description.

The picture presented after hypophysectomy is quite uniform throughout all species of animals. The thyroid, the adrenal cortex and the gonads and accessory reproductive organs undergo profound involution, and their functional activity is greatly reduced. Parathyroid changes are less definite. Lactation ceases abruptly. The thymus involutes, although this may be an indirect effect. The capacity for muscular work is decreased, and activity is reduced. The appetite is diminished, hypoglycemia of varying degrees of severity develops, the circulation becomes sluggish, and the blood pressure is lowered. In young animals growth ceases abruptly, although very young rats may grow slightly. The viscera are small. Resistance to trauma and infection is greatly diminished, and the basal metabolism is lowered. The resultant effect, then, is atrophy or involution of most of the endocrine glands and polyglandular deficiency. Survival for extensive periods, however, is possible and is dependent to a considerable extent on maintaining the normal food intake and so preventing hypoglycemic coma or convulsions (especially in rabbits and monkeys) and on maintaining normal body temperature. Less care seems to be necessary with rats, ferrets and dogs in order to secure prolonged survival than with other species, including man. It is not probable that

1. Other hormones from the hypophysis have been described, but their existence and action are not as well established as the existence and action of the five listed. These other tropic hormones are a medullotropic (Collip) and a pancreatropic hormone (Anselmino, Harold and Hoffman).

even with the greatest care any animal with complete ablation of the anterior lobe of the hypophysis will survive for a normal life span.

A small fragment of functioning gland is able, however, to maintain an animal in an apparently normal condition, and even a fragment of microscopic size has some effect.[2] In rats as little as 10 per cent of the gland will prevent the development of the disabilities, and in monkeys certainly not more than one fourth of the gland is necessary. The hypophysis thus has a great margin of safety, as is true for the other endocrine glands. A fragment which remains appears to have little or no capacity to regenerate, although with regard to this data on monkeys and man are not available.

Experimental work has not sufficed to elucidate the condition of hypofunction in a gland of normal size, for such a condition has not been reported to occur in animals and therefore cannot be studied in them. It is essentially a clinical problem, arising in certain cases from a preponderance of nonsecretory (chromophobe) cells at the expense of secretory (chromophil, i. e., acidophil and basophil) cells.

Experimentally it has been found that a genetic deficiency of one of the two secretory cell types of the anterior lobe of the hypophysis may exist. In the particular strain of mice in which this defect appeared, the acidophils were totally absent, but the chromophobes and basophils were present.[3] The disabilities of hypophysectomy were all present except the atrophy of the genitals, thus supplying additional significant evidence that the basophils elaborate the gonadotropic hormone complex. That a similar condition may occasionally exist in the human subject is indicated in a case recently reported by Shorr and co-workers.[4] The opposite condition, overactivity of a cell type, has long been known from studies on acromegaly, in which there is a tumor of the acidophils.

Experimental and clinical work on the anterior lobe of the hypophysis thus shows that this gland influences,

2. The literature is reviewed by Smith, P. E., in Allen, Edgar; Danforth, C. H., and Doisy, E. A.: Sex and Internal Secretions, ed. 2, Baltimore, Williams & Wilkins, 1939, p. 391.

3. Smith, P. E., and MacDowell, E. C.: The Differential Effect of Hereditary Mouse Dwarfism on the Anterior Pituitary Hormones, Anat. Rec. **50**: 85 (July 25) 1931.

4. Reported at the Annual Meeting of the Association for the Study of Internal Secretions in June 1940.

and in most cases directly controls, all of the other glands of internal secretion. These interrelations were all established at the time that the last edition of this book was published, and much of the work since then has been directed toward determining the number of hormones elaborated by the hypophysis and their action and toward the purification of preparations of these hormones.

ACTION OF PITUITARY HORMONES

The Growth Principle.—That the secretion of the hypophysis is essential for general body growth except in the earlier stages of development is unquestionable. The question of the existence of a specific growth-promoting principle would appear to have been raised largely for three reasons: 1. Preparations of the growth-promoting factor have not been free from substances affecting specific organs—thyroid glands, gonads and adrenal glands. 2. Growth is such a complex process, as has been especially pointed out by Stockard,[5] that it is difficult to conceive of its being due to a single hormone. Each of the pituitary tropic hormones is essential for the growth of the specific organ which it affects. 3. The injection of prolactin—a lactogenic factor—causes growth in pigeons and dwarf mice, as shown by Riddle and collaborators.[6] Moreover, in still lower forms (amphibian larvae) the hypophysis can be ablated without inhibiting growth to any great extent.

Specific endocrine stimuli, however, seem to be more necessary for the promotion of growth in mammals than in lower forms, and an interest in comparative physiology should not blind one to the fact that in medical practice the responses of mammals, especially those of man, are of predominant interest. In regard to the contamination of extracts of the growth factor with other anterior pituitary factors, it has been greatly reduced without impairing the growth-stimulating property of the extract.[7] It thus appears, as stated by Evans

5. Stockard, C. R.; in discussion on Evans.[7]

6. Bates, R. W.; Riddle, Oscar; Lahr, E. L., and Schooley, J. P.: Aspects of Splanchnomegaly Associated with the Action of Prolactin, Am. J. Physiol. **119**: 603 (July) 1937. Bates, R. W.; Laanes, Theophil, and Riddle, Oscar: Evidence Against the Individuality of the Growth Hormone, Proc. Soc. Exper. Biol. & Med. **33**: 446 (Dec.) 1935.

7. Evans, H. M.: The Hypophyseal Growth Hormone, A. Research Nerv. & Ment. Dis., Proc. (1936) **17**: 175, 1938. Meamber, D. L.; Fraenkel-Conrat, H. L.; Simpson, Miriam E., and Evans, H. M.: The Preparation of Pituitary Growth Hormone Free from Lactogenic and Thyrotropic Hormones, Science **90**: 19 (July 7) 1939.

nearly twenty years ago, that in mammals a specific principle is secreted by the hypophysis which is essential for general body growth. This principle appears to influence skeletal growth mainly by stimulating the epiphysial cartilages, although the effect extends to the soft tissues and the viscera, also.

The Gonadotropic Complex. — The investigations reported by Cushing and collaborators and by Aschner on dogs some thirty years ago revealed that the gonads atrophied after hypophysectomy. Work in 1927 in which atrophic gonads of hypophysectomized rats were repaired and immature ovaries were stimulated to precocious development by administration of pituitary extract gave conclusive proof that there was a factor (or factors) secreted by the hypophysis which was necessary for the structural and functional maintenance of the gonads and through them of the accessory reproductive organs. It was soon discovered, however, that the injection of extracts of hypophyses from different species did not give equal quantitative responses of the different types of tissue composing the gonads. In fact, Evans and Long showed in 1922 that crude extracts of beef hypophyses caused the formation of large amounts of lutein tissue in the ovaries of normal mature rats but had no follicle-stimulating effect. When it was found that the hypophyses of other species—rats, horses, sheep, man—gave pronounced follicle-stimulating effects, indirect evidence was thus supplied that more than one gonadotropic hormone had been encountered.

Fractionation and purification of gonadotropic extracts were undertaken by several investigators (Fevold, Hisaw and Leonard,[8] Wallen-Lawrence,[9] Evans and associates[10]). The present consensus from the labora-

8. Fevold, H. L.; Hisaw, F. L., and Leonard, S. L.: The Gonad-Stimulating and Luteinizing Hormones of the Anterior Lobe of the Hypophysis, Am. J. Physiol. **97**: 291 (May) 1931. Fevold, H. L.: The Follicle-Stimulating and Luteinizing Hormones of the Anterior Pituitary, in Allen, Edgar; Danforth, C. H., and Doisy, E. A.: Sex and Internal Secretions, Baltimore, Williams & Wilkins Company, 1939, p. 966.

9. Wallen-Lawrence, Zonja: Proof of the Existence of a Follicle-Stimulating and a Luteinizing Hormone in the Anterior Lobe of the Pituitary Body, J. Pharmacol. & Exper. Therap. **51**: 263 (July) 1934.

10. Evans, H. M.; Korpi, Karl; Simpson, Miriam E.; Pencharz, R. I., and Wonder, D. H.: On the Separation of the Interstitial Cell Stimulating, Luteinizing and Follicle Stimulating Fraction in the Anterior Pituitary Gonadotropic Complex, Univ. California Publ. Anat. **1**: 255, 1936.

tories in which these studies are being made is that two gonadotropic principles are elaborated by the anterior lobe of the hypophysis. The determination of the number of gonadotropic hormones is not easy to make, however, and in my opinion has not been definitely made. The length of treatment, the rate of absorption, the dosage and the site of injection (subcutaneous or intraperitoneal) all influence the response. That the solution of the problem is difficult is attested by the fact that within a six year period investigators in one prominent laboratory have stated that there was only one, that there were four and later that there were two pituitary gonadotropic hormones. The two postulated hormones are usually designated as the follicle-stimulating and the luteinizing hormone. The second hormone is sometimes designated as the interstitial cell–stimulating hormone instead of the luteinizer.

The hypophysial luteinizing hormone is not identical with the gonadotropic principle of human pregnancy urine, which is sometimes designated as the luteinizing hormone.[11] Little is known of the chemical composition of the gonadotropic factors, a statement which is also true of the other hypophysial principles.

The Thyrotropic Hormone.—It has been known since the classic work of Rogowitsch in 1889 that the hypophysis is influenced by the thyroid. The determination of an effect of the hypophysis on the thyroid is much more recent. This effect was first demonstrated by hypophysectomy and the injection of hypophysial extracts in amphibia[12] and later in mammals.[13] The threshold of the stimulus of structural response differs greatly in different species, the thyroids of rats being unchanged in structure by high doses of pituitary thyrotropic extract, whereas the thyroids of guinea pigs and rabbits undergo profound changes even with low doses. The injection of the thyrotropic extract in responsive species results in most of the thyroid changes

11. Some commercial descriptive advertisements are misleading in that it is indicated that the hormones from these two sources are identical. All the evidence indicates that the gonadotropic substance in human pregnancy urine is from chorionic tissue, not the hypophysis.

12. The literature is reviewed by P. E. Smith (Relations of the Activity of the Pituitary and Thyroid Glands, in Harvey Lectures, Baltimore, Williams & Wilkins Company, 1930, p. 129).

13. Loeb, L., and Bassett, R. B.: Effect of Hormones of Anterior Pituitary on Thyroid Gland in the Guinea-Pig, Proc. Soc. Exper. Biol. & Med. **26**: 860 (June) 1929. Aron, M.: L'hormone préhypophysaire excito-sécrétrice de la thyroïde, Rev. franç. d'endocrinol. **8**: 472 (Dec.) 1930.

characteristic of exophthalmic goiter, i. e., increase in size of the thyroid, depletion of iodine, loss of colloid, and hyperplasia and hypertrophy of the cells, with the characteristic irregular type of follicles. The infiltration by lymphocytes and the increase in connective tissue characteristically present in hyperplasia of human thyroids are not present in the hyperplasia experimentally induced. The absence of these changes may be due to the shortness of the period of treatment.

The injection of thyrotropic extract causes also a rapid and marked rise in basal metabolism, although a rise in metabolic rate from pituitary extract has also been reported in thyroidectomized animals.[14] The thyrotropic extract induces a hyperplastic response of thyroid tissue in vitro[15] and also of transplanted thyroids;[16] so it can act independently of any innervation. In man massive doses have been shown to be effective in cases in which hypothyroidism is due to underfunction of the pituitary and not to lack of responsiveness of the thyroid. The thyrotropic extract of pituitary may have a limited field of usefulness, in that its administration will give information as to whether or not hypothyroid states are due to lack of responsiveness of the thyroid to stimulation by the hypophysis. It seems improbable, however, that it can have any extensive clinical usefulness.

The Corticotropic Hormone.—A stimulating action of the anterior lobe of the hypophysis on the adrenal cortex was first shown in amphibia. Hypophysectomy caused profound atrophy of the cortical but not of the medullary component of the adrenal, an action which

14. This effect is stated to be due to a specific metabolic principle and not to a tropic hormone (Collip). This principle is remarkably thermostabile and is resistant to boiling in dilute acid and alkali and to peptic digestion. Its injection causes a sharp rise in the metabolic rate for only a few hours. Collip states that it probably has its origin in the pars intermedia but that it is not identical with the melanophore-expanding hormone.

15. Eitel, Hermann; Krebs, H. A., and Loeser, Arnold: Hypophysenvorderlappen und Schilddrüse: Die Wirkung der thyreotropen Substanz des Hypophysenvorderlappens auf die Schilddrüse in vitro, Klin. Wchnschr. **12**: 615 (April 22) 1933.

16. Houssay, B. A.; Biasotti, A., and Magdalena, A.: Hipófisis y tiroides; Acción del extracto del lóbulo anterior de la hipófisis sobre la histologiá de la tiroides del perro, Rev. Soc. argent. de biol. **8**: 130 (May-June) 1932. Marine, David, and Rosen, S. H.: The Effect of the Thyrotropic Hormone on Auto- and Homeotransplants of the Thyroid and Its Bearing on the Question of Secretory Nerves, Am. J. Physiol. **107**: 677 (March) 1934.

was confirmed in mammals some ten years later.[17] The atrophy could be prevented or the involuted glands restored to a normal condition by the administration of anterior lobe. There has been much question as to whether or not this cortex-stimulating action of the hypophysis was due to a distinct hormone, but the evidence supplied by Collip and co-workers,[18] Moon [19] and others shows that it is distinct from the thyrotropic, growth-promoting lactogenic and other hypophysial hormones. It appears to have no action other than that of stimulating the cortex of the adrenal.

The injection of a pituitary extract containing the corticotropic factor restores the adrenals after hypophysectomy. It partly restores the work capacity of hypophysectomized animals, although their work capacity still remains somewhat subnormal, as is the case also when cortical extracts are administered.[20]

The injection of cortical extract has been shown to repress the liberation of corticotropic hormone from the hypophysis.[21] It seems probable that the enlargement of the adrenals in normal animals under conditions of stress is due to liberation of unusual amounts of corticotropic hormone by the hypophysis.

As in the case of the thyrotropic extract, it is not probable that the corticotropic extract will prove of much clinical value.

The Lactogenic Hormone.—The pituitary lactogenic hormone (prolactin; galactin; mammotropin) is purely a secretogogue and does not cause development and growth of the mammary glands. It is the only pituitary hormone that has been secured in crystalline form,[22]

17. Smith, P. E.: The Pigmentary, Growth and Endocrine Disturbances Induced in the Anuran Tadpole by the Early Ablation of the Pars Buccalis of the Hypophysis, in American Anatomical Memoirs, Philadelphia, Wistar Institute of Anatomy and Biology, 1920, No. 11; Hypophysectomy and a Replacement Therapy in the Rat, Am. J. Anat. **45**: 205 (March) 1930.

18. Collip, J. B.; Anderson, E. M., and Thomson, D. L.: Adrenotropic Hormone of Anterior Pituitary Lobe, Lancet **2**: 347 (Aug. 12) 1933.

19. Moon, H. D.: Preparation and Biological Assay of Adrenocorticotropic Hormone, Proc. Soc. Exper. Biol. & Med. **35**: 649 (Jan.) 1937.

20. Ingle, D. J.; Moon, H. D., and Evans, H. M.: Work Performance of Hypophysectomized Rats Treated with Anterior Pituitary Extracts, Am. J. Physiol. **123**: 620 (Sept.) 1938.

21. Ingle, D. J.: The Effects of Administering Large Amounts of Cortin on the Adrenal Cortices of Normal and Hypophysectomized Rats, Am. J. Physiol. **124**: 369 (Nov.) 1938.

22. White, Abraham; Catchpole, H. R., and Long, C. N. H.: Crystalline Protein with High Lactogenic Activity, Science **86**: 82 (July 23) 1937. Shipley, R. A.; Stern, K. G., and White, Abraham: Electrophoresis of Anterior Pituitary Proteins, J. Exper. Med. **69**: 785 (June) 1939.

although the potency of the crystals does not exceed and even may not equal that of the amorphous powder.

The test used for determining lactogenic activity is the response of the crop gland of the pigeon. Riddle has shown that his special preparation, prolactin, has other effects in pigeons, stimulating general body growth and the gastrointestinal tract. These effects are not secured in mammals by the injection of prolactin. Prolactin also stimulates the basal metabolic rate of thyroidectomized pigeons.[23]

The injection of prolactin gives but a temporary increase in milk production in cows,[24] and clinical studies show little or no response following its injection in man.[25]

Relations of the Anterior Lobe of the Hypophysis to Carbohydrate and Fat Metabolism.—The interrelationships of the anterior lobe of the hypophysis and other internal secretory glands in carbohydrate and fat metabolism is a subject which is complex. The reported studies dealing with the injection of pituitary extracts concern themselves largely with "effects" produced by very crude extracts, for the principle or principles producing these effects are in most cases very labile and a degree of purification equal to that achieved with the other pituitary factors has not been obtained. These studies, particularly those of Long and Lukens, have shown an important interrelationship between the hypophysis and the adrenal cortex in carbohydrate metabolism.

Although this complex interrelationship will be discussed more fully in another section, it seems justifiable to discuss briefly some phases of it here. Certain important findings stand out in this work. These are: (1) the greatly increased sensitivity to insulin of hypophysectomized animals, first reported by Houssay and Magenta, in 1924, which was shown later to be due to the loss of the anterior lobe only (Houssay and

23. Riddle, Oscar; Smith, G. C.; Bates, R. W.; Moran, C. S., and Lahr, E. L.: Action of Anterior Pituitary Hormones on Basal Metabolism of Normal and Hypophysectomized Pigeons and on the Paradoxical Influence of Temperature, Endocrinology **20**: 1 (Jan.) 1936.

24. Folley, S. J., and Young, F. G.: The Effect of Continued Treatment with Anterior Pituitary Extracts on Milk Volume and Milk-Fat Production in the Lactating Cow, Biochem. J. **33**: 192 (Feb.) 1939.

25. Stewart, H. L., and Pratt, J. B.: Effect of Prolactin on Mammary Gland Secretion, Endocrinology **25**: 347 (Sept.) 1939.

Potick [26]); (2) the amelioration of experimentally induced diabetes by hypophysectomy, reported by Houssay and Biasotti [27] in 1930 and frequently referred to as "the Houssay phenomenon"; (3) the production of temporary diabetes by injection of anterior pituitary extracts [28] and the production of permanent diabetes by a relatively short course of injections of increasing doses of crude pituitary extracts prepared at low temperatures, reported by Young [29] (diabetogenic effect), in which there is an injury of the islands of Langerhans in the pancreas,[30] with reduction of the pancreatic content of insulin;[31] (4) the amelioration by adrenalectomy of diabetes induced by pancreatectomy, reported by Long and Lukens,[32] and (5) the reinstitution of the diabetic condition by the injection of massive doses of extract of adrenal cortex, reported by Lukens and Dohan.[33] The injection of pituitary extracts causes ketosis in fasting animals.[34] It also will inhibit the action of injected insulin (glycotropic action of Young [35]) and will maintain the levels of muscle glycogen in fasting hypophysectomized animals (glycostatic effect [36]). The rate of absorption of carbohydrates

26. Houssay, B. A., and Potick, D.: Antagonisme entre l'hypophyse et l'insuline chez le crapaud, Compt. rend. Soc. de biol. **101**: 940 (July 17) 1929.

27. Houssay, B. A., and Biasotti, A.: Le diabète pancréatique des chiens hypophysectomisés, Compt. rend. Soc. de biol. **105**: 121 (Oct. 16) 1930.

28. Evans, H. M.; Meyer, Karl; Simpson, Miriam E., and Reichert, F. L.: Disturbances of Carbohydrate Metabolism in Normal Dogs Injected with the Hypophyseal Growth Hormone, Proc. Soc. Exper. Biol. & Med. **28**: 857 (April) 1932. Baumann, E. J., and Marine, David: Glycosuria in Rabbits Following Injections of Saline Extract of Anterior Pituitary, ibid. **29**: 1220 (June) 1932.

29. Young, F. G.: Permanent Experimental Diabetes Produced by Pituitary (Anterior Lobe) Injections, Lancet **2**: 372 (Aug. 14) 1937.

30. Richardson, K. C., and Young, F. G.: Histology of Diabetes Induced in Dogs by Injection of Anterior-Pituitary Extracts, Lancet **1**: 1098 (May 14) 1938.

31. Campbell, James, and Best, C. H.: Production of Diabetes in Dogs by Anterior-Pituitary Extracts, Lancet **1**: 1444 (June 25) 1938.

32. Long, C. N. H., and Lukens, F. D. W.: Effects of Adrenalectomy and Hypophysectomy upon Experimental Diabetes in Cat., J. Exper. Med. **63**: 465 (April) 1936.

33. Lukens, F. D. W., and Dohan, F. C.: Further Observations on the Relation of the Adrenal Cortex to Experimental Diabetes, Endocrinology **22**: 51 (Jan.) 1938.

34. Burn, J. H., and Ling, H. W.: Excretion of Acetone Bodies on Fat Diet as Affected by Injection of Pituitary (Anterior Lobe) Extract and by Pregnancy, Quart. J. Pharm. & Pharmacol. **6**: 31 (Jan.-March) 1933.

35. Young, F. G.: The Identity and Mechanism of Action of the Glycotropic (Anti-Insulin) Substance of the Anterior Pitutiary Gland, Biochem. J. **32**: 1521 (Sept.) 1938.

36. Bennett, L. L.: The Interrelation of Pituitary and Adrenal in the Control of Carbohydrate Levels in the Rat, Endocrinology **22**: 193 (Feb.) 1938.

by the intestine, which is decreased by hypophysectomy, can be restored to normal by the administration of thyroid.[37]

It is tempting to ascribe these various metabolic effects to separate hormones but, as emphasized by Long,[38] this is not justifiable with the evidence available.

For the maintenance of diabetes after pancreatectomy, the presence of the adrenal glands has been shown to be essential by Long and Lukens. The ablation of these glands ameliorates the diabetic condition, and although amounts of cortical extract sufficient to maintain the life and health of the experimental animal do not restore the diabetic condition, massive doses will cause recurrence.[39] It appears that the pituitary stimulation of the adrenals, mediated by the corticotropic hormone, is thus essential for the maintenance of the diabetic condition in experimental animals. The opposite condition, hypoglycemia, characteristic of fasting hypophysectomized animals, appears to be attributable, at least in part, to the absence of the stimulating effect of the hypophysis on the adrenals.

Ketosis from the injection of pituitary extract is also dependent on the presence of the adrenals, for this condition is diminished by adrenalectomy.[38] The inhibiting effect of injected pituitary extract on injected insulin (glycotropic action) and the maintenance of muscle glycogen in fasting hypophysectomized animals by the injection of pituitary extract appear to be direct effects, not involving the adrenals.[38]

The inactivation of the thyroid induced by hypophysectomy apparently explains the decreased rate of carbohydrate absorption of pituitaryless animals. Althausen[40] showed that the injection of thyroxin increases the rate of absorption of sugars by the intestine in normal rats, and Russell,[37] following this lead, was able to show that thyroxin restored the normal

37. Russell, J. A.: The Effect of Thyroxin on the Carbohydrate Metabolism of Hypophysectomized Rats, Am. J. Physiol. **122**: 547 (June) 1938.

38. Long, C. N. H.: Diabetes Mellitus in the Light of Our Present Knowledge of Metabolism, Tr. & Stud., Coll. Physicians, Philadelphia **4**: 21 (April) 1939.

39. Lukens, F. D. W., and Dohan, F. C.: Further Observations on the Relation of the Adrenal Cortex to Experimental Diabetes, Endocrinology **22**: 51 (Jan.) 1938.

40. Althausen, T. L.: Influence of the Thyroid Gland on Intestinal Absorption of Dextrose, Galactose, and Xylose, J. Clin. Investigation **16**: 658 (July) 1937.

rate of carbohydrate absorption in hypophysectomized rats. The dose necessary was less than that required for the restoration of the metabolic rate. It thus seems justified to refer the decrease in the rate of absorption of sugars after hypophysectomy to the loss of the thyrotropic hormone and the consequent inactivation of the thyroid.

The observations made in experimental work on the role of the anterior lobe of the pituitary and the interrelationship to other internal secretory glands in carbohydrate and fat metabolism, although of potential importance in medical practice, are not for the most part clinically applicable at present. An exception can perhaps be made with regard to the results of this work relating to the genesis of diabetes mellitus. Although it is generally recognized that many persons with diabetes do not show the islet lesions and thus differ from dogs made permanently diabetic by the injection of pituitary extracts, nevertheless, in other diabetic persons such lesions are present, and it seems not unreasonable to believe that overactivity of the hypophysis was a factor in causing this injury of the islets. As shown by White [41] and others, the incidence of diabetes is higher in children who are unusually tall for their age than it is in those whose growth has not been so rapid, an observation which suggests that increased activity of the hypophysis may be associated with the juvenile onset of this disease. It is also reasonable to assume that there are variations in the susceptibility of the pancreatic islets of different persons to injury. Permanent diabetes does not develop in all dogs from the injection of diabetogenic pituitary extract, and in other species (rats, cats) no injury to the islets results from the injection of such a pituitary extract unless part of the pancreas has been removed.

RECIPROCAL ACTION OF INTERNAL SECRETIONS ON THE HYPOPHYSIS

The functional activity of the hypophysis, i. e., the rate of formation and release of the various pituitary hormones, is influenced by the secretion of the other endocrine glands, so that a balance exists between the various glands. This balance is also influenced by the

41. White, P.: Diabetes in Childhood and Adolescence, Philadelphia, Lea & Febiger, 1932.

activity of the other tissues and organs of the body and provides a mechanism which is responsive to the needs of the body. Some examples of the response of the endocrine glands to environmental conditions are the hypertrophy of the adrenal cortex under conditions of stress (cold, toxins, and so on), the hyperplasia of the thyroid of the animal kept in a cold environment, and the enlargement and activation of the gonads in some birds and some mammals, which have a seasonal breeding period, with increased light.[42] Some of the responses, at least, are due to increased activity of the hypophysis. Activation and enlargement of the gonads can be induced in the winter season and in some immature animals by increased illumination. After removal of the eyes, gonad activation can be induced in some birds by direct illumination of the hypophysis through a tube leading to that gland.[43]

Adrenal and Hypophysis.—In a series of papers Ingle [21] has shown that the injection of an extract of adrenal cortex causes a regression of the adrenal cortex which is nearly equal to that resulting from hypophysectomy and that this regression is due to a decrease in the output of corticotropic hormone by the pituitary, for the injection of a corticotropic extract of the pituitary prevented the cortical atrophy. It has been mentioned that the adrenal cortex atrophies after hypophysectomy. In intact animals the cortex hypertrophies under conditions of stress, but in hypophysectomized animals this hypertrophy does not take place. If placed in the cold, the animals thus operated on will succumb at temperatures which will be survived by normal animals or by hypophysectomized animals given an extract of adrenal cortex. Although it cannot be assumed that the adrenal glands are alone responsible for survival with noxious stimuli or under conditions of increased stress, nevertheless they are an important factor in the resistance and survival. The immediate stimulus causing the hypertrophy is the corticotropic secretion of the hypophysis.

42. The extensive literature is reviewed by M. A. Bissonnette (The Influence of Light upon Pituitary Activity, A. Research Nerv. & Ment. Dis., Proc. [1936] **17**: 361, 1938).

43. Benoit, J.: Etude du mécanisme de la stimulation par la lumière de l'activité testiculaire chez le canard domestique, Bull. biol. **71**: 393, 1937.

Gonads and Hypophysis.—The mechanism operating between the gonads and the hypophysis is similar to that described for the adrenals and the hypophysis. Both clinical and experimental studies show this interrelationship. The fact that the pituitary gonadotropic substance is excreted in man in the urine has given an unexcelled opportunity to study the factors which influence its secretion. The output of gonadotropin in the urine increases in amount if the gonads are removed or undergo involution, as at the menopause. As has been extensively shown by clinical studies, the administration of gonadal products, especially the estrogens, represses this enhanced output. In experimental animals the removal of the gonads results in increased potency of the pituitary, with an increase in the number of basophils. These structural changes can be prevented or the normal structure restored by the injection of estrogen or androgen.

Thyroid and Hypophysis.—Although a structural alteration of the hypophysis from the extirpation of an endocrine gland was first noted from thyroidectomy, and the administration of thyroid alters the proportion and structure of certain of the cell types of the hypophysis, nevertheless a demonstration of the influence of thyroxine on the functional activity of the hypophysis as complete as that in the case of the gonads and adrenals has not been given. The evidence, however, indicates that the output of thyrotropic hormone is repressed by unusual amounts of thyroid secretion. The administration of thyroid causes an involution of this gland, an effect which by analogy with relationships already discussed is presumably due to a decreased output of thyrotropic hormone. Attempts to assay the urinary excretion of the thyrotropic substance have given conflicting results.

In the preceding discussion it has been shown that excessive amounts of the secretion of the gonads, of the cortex of the adrenal and probably also of the thyroid inhibit the corresponding tropic hormones of the hypophysis, thus supplying an important mechanism for the maintenance of a balance between the hypophysis and each of these glands. There is also some evidence that an excessive amount of secretion of one of these glands influences the rate of secretion of the pituitary tropic hormones affecting other glands or structures. The

administration of excessive amounts of estrogen promptly inhibits the growth of experimental animals and will inhibit the development of the mammary glands. The administration of thyroid increases the gonadotropic potency of the hypophysis, although the opposite condition, thyroid deficiency, does not reduce the pituitary content of gonad-stimulating hormone. The inhibiting effect of thyroidectomy on growth can be prevented by administering a hypophysial extract containing the growth-promoting factor, so that the inhibition probably is due, in part, to decreased secretion of this growth hormone.

RESPONSIVITY OF ENDOCRINE ORGANS AND OTHER TISSUES TO ENDOCRINE PRODUCTS

It is a fundamental tenet of the concept of internal secretion that the endocrine organs secrete chemical hormones ("messengers") which have specific effects, whether their action is on other endocrine organs, on a tissue group or on all the tissues of the body. This concept, however, does not justify the assumption that a hormone will induce its characteristic effect in all cases. In other words, a capacity of the end organ to respond to a hormone is also essential. Of the many examples which show this, two only will be given. Growth depends not only on the presence of the growth factor but on the responsivity of tissue, as is well shown by results obtained from injections of an extract containing the growth-promoting factor in two races of dogs—dachshund and collie. The injections in the former stimulate primarily a growth in the length of the vertebral column; the injections in the latter, an increase in the length of the legs.[44] There seems to be an inherent, hereditary capacity of response in the vertebral and appendicular components of the skeleton, respectively, in these two strains of dogs. The importance of the growth responsivity of various structures of the body has also been shown by breeding experiments in dogs. Experimental work on the gonads shows that at early ages some of the components of these glands are unresponsive to gonadotropic hormones; responsivity develops with aging. Although the

44. Evans, H. M.; Meyer, Karl, and Simpson, Miriam E.: The Growth and Gonad-Stimulating Hormones of the Anterior Hypophysis, Memoirs of the University of California, Berkeley, Calif., University of California Press, 1933, vol. 11.

gonadotropic hormones are essential to sexual maturation, it would not be justifiable to attribute sexual maturity solely to the onset of the secretion of these hormones; the development of responsivity to these hormones is also an essential factor. The responsivity of the end organ thus plays an important part in the action and the interrelations of the endocrine glands.

CHAPTER II

GROWTH HORMONE OF THE ANTERIOR LOBE OF THE PITUITARY GLAND

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INTRODUCTION

Nutritional studies in the domain of the vitamins have long since shown the fallacy of designating any one of these factors as peculiarly growth promoting; as a matter of fact, growth cannot ensue with all of these factors present in a diet which is inadequate for proper nutrition. Just as normal growth is dependent on a multiplicity of these extrinsic factors, so also the same may be said as to intrinsic factors, for more than one of the glands of internal secretion can be shown to have very definite relations with growth. When the thyroid, for example, is removed from a young mammal, growth is always impaired; growth ceases abruptly after hypophysectomy. In the last-mentioned instance, the claim that the cessation of growth has resulted from the removal of a pituitary hormone essential for growth has only gradually gained validation.

As regards the nutritive, or extrinsic, factors influencing growth, the observation that there is, in fact, a lowered intake of necessary food ingredients in hypophysectomized animals may be paralleled with the observation that starvation of normal animals causes regressive changes in the gonads and thyroids resembling those produced by hypophysectomy.[1]

1. Neither quantitative nor qualitative undernutrition has as yet been shown to produce the distinctive picture of hypophysectomy. The conveyal of the hypophysial growth hormone invariably increases food consumption, but no increase in the inflow of nutritive elements has as yet been shown to imitate the distinctive effects of the conveyal of the hypophysial growth hormone. M. O. Lee (Relation of the Anterior Pituitary Growth Hormone to Protein Metabolism, A. Research. Nerv. & Ment. Dis., Proc. (1936) **17**: 193-222, 1938) was the first to show that these effects can be detected in growth hormone–treated animals restricted to the same nutritive intake as untreated controls.

As regards the intrinsic factors essential for growth, it is, of course, conceivable that the stasis of growth after hypophysectomy is only secondarily due to the loss of the hypophysis, that primarily it is due to subnormality of one or more of the endocrine organs—the target organs—known to be directed by the pituitary. The production of excessive growth, or gigantism, by overconveyance of hypophysial hormones could be similarly explained as due to the administration of excessive amounts of the pituitary substances which affect certain target organs.

Fortunately it is possible to utilize crucial tests of the validity or invalidity of these explanations as to how the hypophysis regulates growth.

1. One can attempt to produce a restoration of growth in hypophysectomized animals or an overgrowth of normal animals by direct conveyance of one or more of the different principles secreted by the target organs, e. g., thyroxin, extract of adrenal cortex, sex steroids, insulin and others; or

2. One can attempt to bring about a restoration of growth in hypophysectomized animals or an overgrowth of normal animals by administering one or more of the special anterior pituitary principles affecting the target organs, e. g., extracts containing, respectively, the thyrotropic, lactogenic, adrenocorticotropic and gonadotropic factors; or

3. One can attempt to produce a typical restoration of growth in hypophysectomized animals or an overgrowth of normal animals with anterior pituitary extracts devoid of the four just mentioned factors that influence target organs or containing inconsequential amounts of them.

Much effort has been expended on such experiments.[2] The outcome indicates that growth-stimulating anterior pituitary extracts do not attain their result by virtue of their content of the four well known principles affecting target organs. The growth hormone is hence

2. (*a*) Evans, H. M.: The Hypophyseal Growth Hormone: Its Separation from the Hormones Stimulating the Thyroid, Gonads, Adrenal Cortex and Mammary Glands, A. Research Nerv. & Ment. Dis. Proc. (1936) **17**: 175-192, 1938. (*b*) Evans, H. M.; Simpson, M. E., and Pencharz, R. I.: Relation Between the Growth Promoting Effects of the Pituitary and the Thyroid Hormone, Endocrinology **25**: 175-182, 1939.

clearly separate from the target organ hormones.[3] An equally decisive answer cannot be given as to whether the promotion of growth by anterior pituitary extracts is due to one or more of the incompletely separated metabolic "hormones" of the anterior lobe, i. e., the ketogenic, glycotropic, glycostatic, contrainsular, diabetogenic, pancreatropic, nitrogen-retaining and other factors.

It will be the task of future research to investigate the metabolic effects of highly purified preparations containing the growth factors, but the results of such inquiries—whatever they may be—cannot be expected to invalidate the appropriateness of the term "growth hormone," a designation founded on the most obvious and remarkable effect of this substance—increase in body size. The inquiries in question may be expected, on the other hand, to elucidate the method of action of the growth hormone—a subject on which investigators are totally uninformed—and may hence contribute significantly to understanding of the biochemical mechanism of growth.

BIOASSAY

Efforts to purify extracts of the growth-promoting factor have hitherto employed almost exclusively the simple test of increase in the total body weight of the normal or the hypophysectomized animal (rat) during a stated interval of time. The Laqueur school has recently suggested certain features of skeletal growth as criteria for the potency of such extracts.

The tests employing gains in body weight, though abbreviated by many investigators, are time consuming for high accuracy unless considerable numbers of animals are employed for each dose level of each preparation. These difficulties have been somewhat lessened by the recent establishment of accurate graphs of the growth response.

3. Much confusion was introduced by the Riddle school (Bates, R. W., Laanes, T., and Riddle, O.: Evidence from Dwarf Mice Against the Individuality of Growth Hormone, Proc. Soc. Exper. Biol. & Med. **33**: 446-450, 1935. Schooley, J. P.; Riddle, O., and Bates, R. W.: Analysis of Pituitary Support of Growth of Body and Viscera in Pigeons, Anat. Rec. **72** [supp.]: 90, 1938) by the erroneous claim that this was not the case—a claim based on the promotion of growth in silver dwarf mice by a thyrotropic extract and in hypophysectomized pigeons by a lactogenic extract of the anterior lobe. The last-mentioned accomplishment has been repeatedly confirmed in this laboratory by the employment of a highly purified lactogenic extract, but no significant growth of hypophysectomized mammals is caused by this purified mammotropin at corresponding dose levels, whereas growth factor preparations devoid of lactogenic effects always promote marked growth of the hypophysectomized mammal at very much lower dose levels.

Normal Versus Hypophysectomized Animals.—The young hypophysectomized rat has hitherto and correctly been regarded as the crucial test object for growth hormone research. It may, however, be allowable to point out that hypophysectomized animals suffer from many deficiencies. As was determined in the inception of growth hormone research, normal animals may be used for accurate tests of growth factor potency, provided female rats 5 months of age or older are employed. Such animals exhibit a "plateau of growth" but can be induced to grow rapidly by appropriate growth factor dosage. They are more resistant to the toxic effect of crude extracts and in general are hardier than hypophysectomized animals but are notably less

TABLE 1.—*Comparison of the Body Weight Increase of Normal "Plateaued" and of Hypophysectomized Female Rats Treated with Anterior Pituitary Growth-Promoting Substance ("Globulin" Fraction) for Ten Days*

Test Group	Animals	Daily Dose, Mg.	Weight Increase per Day, Gm.
Normal	63	1.0	2.93
Hypophysectomized	59	0.1	1.36

sensitive than the latter to low dosage with the growth principle, as table 1 shows.

Normal animals have the great advantage of possessing a hypophysis and hence possessing numerous hypophysial hormones—doubtless, some unknown—the absence of which in the hypophysectomized animal may possibly militate against the success of growth factor preparations as purification of these proceeds.

When hypophysectomized rats are given injections of growth-promoting extracts, males and females respond alike.[4] Advantage attaches to the choice of very young animals and to the allowance of a sufficient postoperative interval to establish that the operation has been complete—a fact disclosed by stasis of growth. For example, females 26 to 30 days old are hypophysectomized and an eight to ten day interval is allowed to elapse before injections of the growth-

4. Chou, C.; Chang, C.; Chen, G., and Van Dyke, H. B.: Observations on the Quantitative Assay of Growth-Promoting Extract of the Hypophysis, Endocrinology **22**: 322-334, 1938.

promoting extract are begun. Only those animals are employed for the test in which a gain of less than 6 Gm. in total body weight has ensued.[5] Greater increase in body weight is caused by the same dosage when the administration of the growth factor preparation is begun on the day of hypophysectomy.

Standard Growth Response Graphs.—It is now generally agreed that if a growth factor extract is given at various dose levels to groups of standardized animals, a straight line relationship exists between the logarithm of the dose level and the response in body weight.[6] The responses of normal "plateaued" female rats to growth factor preparations of differing potencies (A, B, C, D) have been charted in this way, and the resulting lines, representing the relation between the logarithm of the dose and the growth resulting, are approximately parallel (chart). When the dose levels are computed in "units" instead of milligrams of substance, the parallel lines coincide.[7] (The normal rat unit has been arbitrarily defined as the amount of any preparation that confers a gain of 40 Gm. in total body weight in a twenty-day period, seventeen injections, and the hypophysectomized rat unit, as that amount which confers a 10 Gm. gain in ten days, nine injections; the amount of substance required for unit response in the latter case is approximately one tenth that required in the former.) The straight line relationship between the response and the logarithm of the dose is valid only for a limited range of dose levels. This is true for hypophysectomized as well as for normal rats. The optimum range of responsiveness in body weight gain for the hypophysectomized animals is

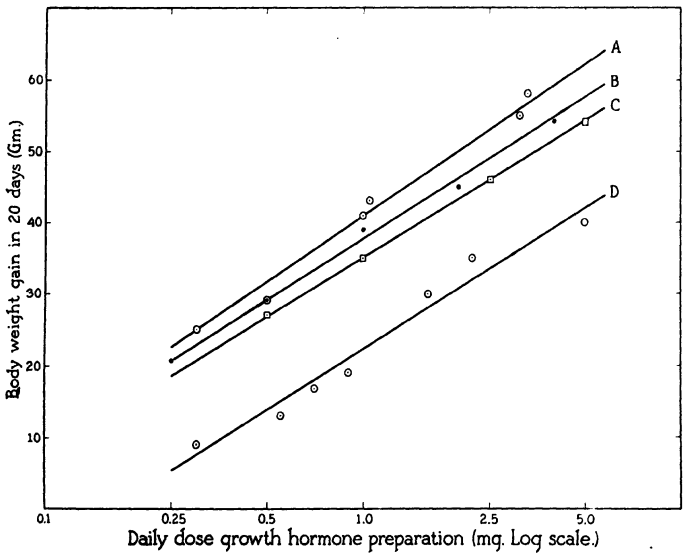
5. Under such circumstances the nose-anus dimension has usually remained constant, although an increase of 1 cm. in the anus–tail tip measurement is regular; the latter measurement may increase by 2 cm. in twenty days in young completely hypophysectomized females but is then at stasis.

6. (*a*) Bülbring, E.: The Estimation of the Growth Hormone of the Anterior Lobe of the Pituitary Body, Quart. J. Pharm. & Pharmacol. **11**: 26-33, 1938. (*b*) Evans, H. M.; Uyei, N.; Bartz, Q. R., and Simpson, M. E.: The Purification of the Anterior Pituitary Growth Hormone by Fractionation with Ammonium Sulfate, Endocrinology **22**: 483-492, 1938. (*c*) Fraenkel-Conrat, H. L.; Meamber, D. L.; Simpson, M. E. and Evans, H. M.: Further Purification of the Growth Hormone of the Anterior Pituitary, ibid. **27**: 605-613, 1940. (*d*) Fevold, H. L.; Lee, M.; Hisaw, F. L., and Cohn, E. J.: Studies in the Physical Chemistry of the Anterior Pituitary Hormones, ibid. **26**: 999-1004, 1940. (*e*) Light, A. E.; de Beer, E. J., and Cook, C. A.: Biological Assay of Anterior Pituitary Growth Hormone, Proc. Soc. Exper. Biol. & Med. **44**: 192-196, 1940. (*f*) Chou and co-workers.[4]

7. Marx, W.; Simpson, M. E., and Evans, H. M.: Endocrinology, to be published.

between 10 and 20 Gm. in ten days; that for normal rats is between 20 and 60 Gm. in twenty days.

Different periods of time have been proposed for these assays by different workers. Daily injections are given for periods ranging from three to twenty days. But when one studies the relationship between the logarithm of the dose and the response for a given preparation for periods ranging from five to twenty days, it becomes evident that the slope of the line increases with the period of time of the injections, at



Standard growth response curves; pituitary preparations of different growth-promoting potencies injected into normal "plateaued" female rats.

least up to fifteen days. Since the accuracy of the method will be the higher the steeper the slope of the curve, the advantage of using a fifteen day test as contrasted with shorter tests is obvious.

It has been suggested that it would be preferable to compute the body weight gain in per cent of body weight and, also, to administer the preparation in strict proportion to body weight.[8] If marked variations in body weight are encountered within experimental

8. Chou and co-workers.[4] Light and co-workers.[6e]

groups of rats of the same age, this might well be advisable. It is more important, however, to standardize both the age and body weight of experimental animals. That standardization of age is of primary importance is shown in table 2, from which it is evident that if rats

TABLE 2.—*Average Responses of Hypophysectomized Rats of Different Ages and Weights to an Anterior Pituitary Growth-Promoting Extract Given in Doses Proportional to Body Weight*

Experiment	Rats	Age, Days		Body Weight at Onset of Experiment	Daily Dose, Mg.	Gain in Body Weight in 20 Days	
		At Operation	At Onset of Experiment			Gm.	Percentage
I	12	28	56	72.8	0.100	29.4	40.4
	10	59-62	85-89	140.5	0.193	34.3	24.4
II	11	29-30	52	78.5	0.090	26.8	34.1
	12	62-63	82-83	143.3	0.164	22.3	15.6

are hypophysectomized at different ages, thus having different body weights, and are given injections, after the same postoperative periods, of doses proportional to their body weights, their gain is not the same even when computed as per cent of body weight, for the younger animals are more responsive.

Seasonal and Unaccountable Variations.—Every laboratory conducting routine growth factor tests has encountered annoying variations in the response of animals to a standard preparation at various times of year. In order to meet this difficulty, Light and co-workers [6e] stressed the importance of a standard reference preparation.

Skeletal Changes Due to the Growth Hormone.—It has been proposed by Freud and co-workers [9] that the increase in tail length rather than in body weight of hypophysectomized rats be used as the test for the effect of the growth extract. These investigators believe this to be a more specific and, thus, more reliable criterion for the effect of the growth-promoting substance. The method may possess advantages due to its specificity,

9. Freud, J., and Levie, L. H.: Hypophyse und Schwanzwachstum der Ratte. Ein Test für Wachstumshormon, Arch. internat. de pharmacodyn. et de thérap. **59**: 232-242, 1938. Freud, J.; Levie, L. H., and Kroon, D. B.: Observations on Growth (Chondrotrophic) Hormone and Localization of Its Point of Attack, J. Endocrinol. **1**: 56-64, 1939.

but these are offset by the following considerations: Young untreated control animals show some continued tail growth for the first few weeks after complete hypophysectomy, and the additional effects produced by injections of the growth-promoting substance in a short test interval are not sufficiently great. Furthermore, the percental error of measurement is larger for the tail length increase than for the body weight gain. There is even some question as to whether the purified extract reported by Freud and collaborators to maintain normal growth in the tail is identical with the growth-promoting factor since it apparently had little or no effect on body weight in the treated animals as compared with untreated controls. The question demands further research.

A more important proposal by the Amsterdam school relates to the proliferation of the cartilage of the epiphysial disk. This effect of the hormone is definite and easily demonstrable.[10] In hypophysectomized animals indubitable effects of the growth substance can be seen after three daily injections.[11] It must be emphasized that while the growth factor is chondrotropic, its effects are registered not solely by the proliferation of epiphysial cartilage cells but by the stimulus to all the normal processes of osteogenesis at the epiphysial-diaphysial junction.[12]

Conditions Necessary for the Continued Growth of Hypophysectomized Animals.—It has been repeatedly observed that hypophysectomized rats will at first respond maximally to partially purified preparations of the growth principle but will subsequently show less response or indeed exhibit growth stasis. Fortunately, the lessened response seldom or never occurs during the typical test period (ten to fifteen days), so that efforts to purify the preparation are not thereby impeded. It is improbable that the lessened response is associated with the development of an antigrowth substance, for potent crude extracts would be more prone to cause the development of antisubstances, and yet they permit continued and eventually gigantic

10. Ray, R. D.; Evans, H. M., and Becks, H.: Effect of the Pituitary Growth Hormone on the Epiphyseal Disc of the Tibia of the Rat, Am. J. Path. **17**: 509-528 (July) 1941.

11. Kibrick, E.; Becks, H., and Evans, H. M.: Article in preparation.

12. Ross, E. S., and McLean, F. C.: The Influence of the Growth Promoting Hormone of the Anterior Lobe of the Pituitary upon Growth Activity in the Long Bones of the Rat, Endocrinology **27**: 329-339, 1940.

growth of hypophysectomized animals. It is probable that hypophysectomized animals eventually need for growth more of their hypophysial hormones than the particular one—the growth hormone. This concept is supported by the fact that normal rats continue to grow for long periods when given injections of partially purified preparations that fail to give continuous growth in hypophysectomized rats. It remains for future research to disclose the identity of these cooperating hypophysial hormones, but the indications of their indispensability cannot invalidate the concept of the growth hormone itself.

PRESENT STATE OF PURITY AND CHARACTERISTICS OF GROWTH PROMOTING PREPARATIONS

Growth promoting preparations are at present generally made from fresh or acetone-desiccated anterior pituitary tissue by preliminary aqueous alkaline extraction followed by fractionation with ammonium sulfate.[6b,d] The precipitate formed at 0.4 saturation with ammonium sulfate is designated the globulin fraction. This fraction may be further purified by isoelectric precipitation. Such a product is still contaminated with the adrenocorticotropic and lactogenic factors and with smaller amounts of the thyrotropic and interstitial cell–stimulating factors. The latter three contaminants can be removed by cysteine treatment of the globulin fraction.[6c] These preparations contain 20 to 100 hypophysectomized rat units per milligram, the daily dose required for a daily gain of 1 Gm. in body weight (1 unit) varying from 10 to 50 micrograms. The Laqueur school [13] has employed different methods—adsorption on norite (a specially activated plant charcoal) and elution with phenol. Their methods have not yet been given complete publication. Although purified, the best growth promoting preparations cannot yet lay claim to being single homogeneous substances. The enumeration of chemical characteristics must be taken as applicable only to impure preparations. The analysis for nitrogen content, together with other analyses, indicates that one is dealing with a protein or a mixture of proteins.

The growth-promoting potency of preparations is greatly reduced or destroyed by boiling, and partial

13. Dingemanse, E., and Freud, J.: Purified Growth Hormone from Beef Anterior Pituitary, Acta brev. Neerland. 5: 39 and 109, 1935.

destruction of potency occurs on subjection to temperatures above room temperature for several hours. Relatively crude preparations are stable in the cold in alkaline solutions up to p_H 11.0 but less stable or destroyed in acid solutions below p_H 4.0. The potency is reduced by the action of strong oxidizing agents, such as hydrogen dioxide (H_2O_2), but is more stable in the presence of reducing agents, such as cysteine, ascorbic acid and glutathione. Ninety-six per cent acetic acid, 90 per cent phenol and 50 per cent urea dissolve the substance and do not very appreciably reduce its potency within an hour or two.

METABOLIC EFFECTS OF GROWTH HORMONE

Among the earliest noted effects of the growth factor on metabolism was the action on protein or nitrogen metabolism, namely, the decrease of the nonprotein nitrogen and amino acid nitrogen of the blood and of urinary total nitrogen.[14] Consequently, the hormone has been considered by some as gaining its effects primarily "by increasing the amount of nitrogenous material available for cell growth and multiplication."[14g]

Reiss, Schwarz and Fleischmann[15] have reported that in dogs and rabbits after injections of "growth hormone" the blood urea was increased while at the same time the blood nonprotein nitrogen and blood arginine dropped from 10 to 50 per cent. They made the interesting but inadequately founded suggestion that the hormone is effective by activating arginase in the tissues. Mirsky and Swadesh[16] and Mirsky[17]

14. (*a*) Teel, H. M., and Watkins, O.: The Effect of Extracts Containing the Growth Principle of the Anterior Hypophysis upon the Blood Chemistry of Dogs, Am. J. Physiol. **89**: 662-685, 1929. (*b*) Teel, H. M., and Cushing, H.: Studies in the Physiological Properties of the Growth-Promoting Extracts of the Anterior Hypophysis, Endocrinology **14**: 157-163, 1930. (*c*) Gaebler, O. H.: Some Effects of Anterior Pituitary Extracts on Nitrogen Metabolism, J. Exper. Med. **57**: 349, 1933. (*d*) Lee, M. O., and Schaffer, N. K.: Anterior Pituitary Growth Hormone and the Composition of Growth, J. Nutrition **7**: 337-363, 1934. (*e*) Gaebler, O. H.: Effects of Thyroparathyroidectomy and Carbohydrate Intake on the Action of Anterior Pituitary Extracts, Am. J. Physiol. **110**: 584-592, 1935. (*f*) Eyres, G. B., and Lee, M.: Determination of the Nitrogen Partition in Tissues, J. Biol. Chem. **115**: 139-148, 1936. (*g*) Howes, N. H.: Anterior Pituitary and Growth in the Axolotl (Amblystoma Tigrinum [Green] Neotenic Form): II. The Effect of Injection of Growth-Promoting Extracts upon the Utilization of Food, J. Exper. Biol. **15**: 447-452, 1938. (*h*) Lee.[3]

15. Reiss, M.; Schwarz, L., and Fleischmann, F.: Beiträge zur Beziehung zwischen Hypophysenvorderlappenwachstumshormon und Eiweissstoffwechsel, Endokrinologie **17**: 167-170, 1936.

16. Mirsky, A., and Swadesh, S.: The Influence of Anterior Pituitary Gland on Protein Metabolism, Endocrinology **25**: 52-56, 1939.

17. Mirsky, A.: The Influence of the Anterior Pituitary Gland on Protein Metabolism, Endocrinology **25**: 52-56, 1939.

suggested two distinct effects of anterior lobe extracts on protein metabolism, a direct one on protein catabolism in the muscles and an indirect one through the pancreas, stimulating protein anabolism, and that the stimulation of growth depends on this pancreatropic effect.

The relations of anterior lobe factors to carbohydrate metabolism (not here discussed) represent a field which is far from complete elucidation, but it may be noted that Shipley and Long [18] have felt it not unlikely that the ketogenic, diabetogenic and growth-promoting hormones are identical. Greaves and co-workers,[19] who partially purified preparations of the respiratory quotient–reducing and ketogenic factors, suggested that these are related to the growth hormone, since in their procedures of purification the potencies of the three principles ran parallel. The complexities of the situation may be further illustrated by the fact that Marks and Young [20] have recently shown that certain anterior lobe extracts increased the insulin content of the pancreas of the rat to almost twice normal values. In ammonium sulfate fractionation of these extracts the insulin-increasing (pancreatropic) substance accompanied the diabetogenic and growth-promoting factors, but the investigators did not consider it to be identical with either of these substances, for extracts could be made from commercial dried anterior lobe powder which had insulin-increasing but not diabetogenic or growth-promoting properties. Nor did they feel that diabetogenic and growth-promoting hormones were identical, for "stale" crude extracts of fresh anterior lobe tissue were not diabetogenic though growth promoting and insulin increasing.

It should be stressed that the studies of the metabolic effects of growth hormone have hitherto been performed with relatively crude preparations. The study of the metabolic effects of highly purified growth hormone preparations is urgently demanded and will occupy the attention of the immediate future.

18. Shipley, R. A., and Long, C. N. H.: Studies on the Ketogenic Activity of the Anterior Pituitary: I. The Relation of Ketonaemia to Ketonuria in the Rat; II. A Method for the Assay of the Ketogenic Activity; III. The Nature of the Ketogenic Principle, Biochem. J. **32**: 2242-2256, 1938.

19. Greaves, J. D.; Freiberg, I. K., and Johns, H. E.: Preparation and Assay of Anterior Pituitary Fractions Rich in Ketogenic and Respiratory Quotient-Reducing Substances, J. Biol. Chem. **133**: 243-259, 1940.

20. Marks, H. P., and Young, F. G.: The Hyophysis and Pancreatic Insulin, Lancet **1**: 493-497, 1940.

RELATION OF ANTERIOR PITUITARY GROWTH HORMONE TO OTHER HORMONES

A functional thyroid gland, or the presence of its products, is clearly important in the regulation of growth. Young thyroidectomized animals cannot undertake normal growth, evidently because of impairment of the hypophysis, which exhibits no eosinophilic elements and presumably does not secrete growth hormone. Normal or better than normal growth is conferred on such animals by the injection of an extract containing the growth factor. Conversely, thyroxin improves the growth secured with standardized preparations of the growth substance.[21]

Inhibition of growth results from administration of estrone (theelin) in high doses,[22] which may be assumed to divert the pituitary gland from the secretion of growth hormone, although the consequent pituitary hypertrophy indicates the assumption of other activities on the part of the gland. Testosterone in high doses also inhibits growth but in low doses may be stimulating to the pituitary and secondarily to growth.[23] The smaller size of the female among mammalia may be related to the inhibition of growth by estrone and the greater size of the male to the stimulation of growth by testosterone.

Both adrenocorticotropic hormone and some of the cortical principles of the adrenal gland when given in high doses inhibit somatic growth in young rats.

Thymus extracts have been claimed to stimulate growth, though this has not been adequately confirmed. Certain it is that thymic hypertrophy is always seen in animals which have responded to growth promoting extracts, which may hence be said to be "thymotropic."[24] Yet as Reinhardt and co-workers[25] have recently shown, the thymus gland is not necessary for the action of the growth hormone.

21. Smith, P. E.: Increased Skeletal Effects in A. P. Growth-Hormone Injections by Administration of Thyroid in Hypophysectomized, Thyro-Parathyroidectomized Rats, Proc. Soc. Exper. Biol. & Med. **30**: 1252-1254, 1933. Evans and co-workers.[26]

22. Zondek, B.: The Inhibitory Effect of Follicular Hormone on the Anterior Lobe of the Pituitary Gland, Lancet **1**: 10-12, 1936; Impairment of Anterior Pituitary Functions by Follicular Hormone, ibid. **2**: 842-847, 1936.

23. McEuen, C. S.; Selye, H., and Collip, J. B.: Effect of Testosterone on Somatic Growth, Proc. Soc. Exper. Biol. & Med. **36**: 390-394, 1937.

24. Uyldert, I. E., and Freud, J.: Thymus Weight of Normal and Hypophysectomized Rats and the Influence of Anterior Pituitary Extracts, Acta brev. Neerland. **8**: 188-190, 1938.

25. Reinhardt, W. O.; Marx, W., and Evans, H. M.: Effect of the Pituitary Growth Hormone on the Thymectomized Rat, Proc. Soc. Exper. Biol. & Med. **46**: 411-415, 1941.

CLINICAL EMPLOYMENT OF EXTRACTS CONTAINING THE GROWTH-PROMOTING SUBSTANCE

The therapy of uncomplicated dwarfism in children will undoubtedly be greatly aided by purification of extracts containing the growth-promoting substance. In some instances crude extracts have been recommended, through belief that long-continued response is more apt to occur with them than with highly purified preparations, but the disadvantages of crude extracts are manifold. The quantities injected must be large, and undesirable consequences may ensue, such as gonadotropic and diabetogenic effects. The extracts already available have demonstrated that dwarfed children can in some cases be made to resume a growth as rapid as that characterizing the earliest epochs of childhood, and a considerable number of such children have now been treated for their dwarfism with partial or complete success.

CHAPTER III

CORTICOTROPIC (ADRENOTROPIC), THYROTROPIC AND PARA-THYROTROPIC FACTORS

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THE CORTICOTROPIC FACTOR

It is to be noted that the word "corticotropin" (or "corticotrophin") is now being widely used as a descriptive term for the principle in the anterior lobe of the pituitary gland hitherto commonly spoken of as the adrenotropic factor. This is in line with the general recommendations of the Third International Conference on the Standardization of Hormones.[1]

Physiologic Significance of Corticotropin.—There is now a mass of evidence that the maintenance of the cortex of the adrenal gland, in the morphologic as well as the functional sense, is dependent on the secretory activity of the normal anterior lobe of the pituitary. The atrophic adrenals of the completely hypophysectomized animal are not entirely functionless, however, since removal of them from rats hypophysectomized some weeks previously is not tolerated, and death ensues quickly. The function of the corticotropic factor is therefore to maintain the normal structure and function of the adrenal cortex.

Nature of the Corticotropic Factor.—Until such time as the hormones secreted by the anterior lobe are obtained in pure form, it will be impossible to know positively whether the substance acting on the adrenal cortex is a single substance having only this specific hormonal activity or a substance having other physiologic effects, tropic or otherwise, in addition to the corticotropic property. Collip[2] reported that an isoelectric protein fraction obtained from an acid acetone

1. Report of the Third International Conference on the Standardisation of Hormones, Bull. Health Organ. League of Nations 7: 887-899 (Oct.) 1938.

2. Collip, J. B.: Properties of Anterior Lobe Extracts, in Cold Spring Harbor Symposia on Quantitative Biology, Cold Spring Harbor, L. I., New York, 1937, vol. 5, pp. 210-217.

extract had the properties of three hormones of the anterior lobe, namely, the growth-promoting factor, corticotropin and prolactin. The corticotropic activity of such extracts was shown to be fairly resistant to heating over a wide p_H range; the greatest stability was shown at p_H 3 to 5. Moon,[3] using the Lyons method [4] for the preparation of prolactin, showed that the material insoluble at p_H 6.5 is rich in corticotropin. This fraction had little prolactin activity, whereas a fraction obtained by isoelectric precipitation at p_H 5.5 was rich in prolactin but had a lower corticotropic content. It can be positively stated that corticotropin is separate and distinct from thyrotropin and from gonadotropin.[5] All available evidence points to corticotropin being of protein nature.

Preparation of Potent Extracts of Corticotropin.—Although it has been my experience that most protein fractions obtained from extracts of anterior lobes have more or less corticotropic activity, I have found the following method satisfactory for the preparation of an extract rich in corticotropin and having a minimum of other principles of the anterior lobe.

Prime fresh tissue which has been largely defatted and dehydrated by extraction with alcohol is extracted twice with 5 volumes of 0.25 per cent acetic acid for each volume of original gland tissue used. The residues are then extracted a third time with 5 volumes of 0.25 per cent acetic acid, the mixture being heated to 75 C. for fifteen minutes, then cooled and filtered. The tissue residues are then suspended in 5 volumes of 0.25 per cent acetic acid and placed in a boiling water bath for four to six hours, the mixture being frequently stirred. The mixture is then filtered, and the filtrate is concentrated at low temperature and pressure until the volume is equal to the original volume of gland used. Sufficient concentrated hydrochloric acid is then added to make a 0.25 per cent concentration of this reagent, and the acidified mixture is placed in a boiling water bath for twelve hours. The extract at this stage is adjusted to p_H 4 and is ready for assay on the hypophysectomized rat. The salt content can be reduced by a short dialysis in cellophane. Fractionations of such extracts with a view to obtaining the corticotropic principle in purer form are being undertaken. An earlier observation that extracts obtained from simple hydrolysates of pituitary tissue (treatment on a

3. Moon, H. D.: Preparation and Biological Assay of Adrenocorticotropic Hormone, Proc. Soc. Exper. Biol. & Med. 35: 649-652 (Jan.) 1937.
4. Lyons, W. R.: Preparation and Assay of Mammotropic Hormones, Proc. Soc. Exper. Biol. & Med. 35: 645-648 (Jan.) 1937.
5. Collip, J. B.; Anderson, E. M., and Thomson, D. L.: The Adrenotropic Hormone of the Anterior Pituitary Lobe, Lancet 2: 347 (Aug. 12) 1933.

boiling water bath for twelve to twenty-four hours with 10 volumes of 0.25 per cent hydrochloric acid) produced a number of physiologic effects when tested in the appropriate manner suggested the process described here for the preparation of extracts of corticotropin. In addition to retaining corticotropic properties, these simple hydrolysates were found to contain appreciable amounts of the metabolic, glycotropic and ketogenic substances. Feeding of hypophysectomized rats with such potent preparations has given no evidence that the corticotropic substance is active when taken orally.

Bioassay for the Corticotropic Substance.—In my opinion the most satisfactory method of assay for corticotropin is by the use of hypophysectomized rats. These animals should not be used until an adequate time has elapsed after hypophysectomy to insure that cortical atrophy has progressed to a marked degree. One month is a safe interval. In the method of assay originally described,[6] one adrenal was removed from the test animal prior to beginning injections of the extract to be assayed. This was weighed and sectioned, and served as the control. The injections were made twice a day for six days, and then the remaining adrenal was removed, weighed and sectioned for histologic study. It was suggested that a 50 per cent increase in weight together with positive histologic evidence of cortical repair should be considered a unit effect. A more extensive study of the response of hypophysectomized rats to a single corticotropic preparation administered at increasing levels of dosage showed that the accurate assay of such extracts for corticotropin is a very difficult matter.[7] It is now believed that much greater uniformity can be obtained if restoration to normal or nearly normal size of the adrenals is accepted as a unit effect. It is suggested also that the period of injections be increased to ten days, that several animals be used and that biopsy is unnecessary.

The method of Moon [8] for the assay of corticotropin consists in the treatment of normal 21 day old male rats for three days with the extract to be tested. Moon defined a unit as that amount of extract tested in this manner which caused a fifty per cent increase in the weight of the adrenals. An inert protein solution is used as a control in the Moon test. The Moon method of assay has much to commend it, and in the case of purified extracts there should be little danger of nonspecific effects being obtained, for it is well known that adrenal enlargement due to cortical hypertrophy is readily obtained by treatment of normal rats with injections of a foreign protein.

General Considerations.—The ability of the normal anterior lobe to secrete corticotropin quickly in increased amounts in response to a great variety of

6. Collip, J. B.: Some Recent Advances in the Physiology of the Anterior Pituitary, J. Mt. Sinai Hosp. 1: 28-71 (May-June) 1934. Collip.[8]
7. Collip, J. B.: Results of Recent Studies on Anterior Pituitary Hormones, Edinburgh M. J. 45: 782-804, 1938.

so-called nonspecific agents would seem to constitute one of the chief arms of defense on the part of the body to injurious stimuli.[8] As yet no success has been had in the demonstration of corticotropin in the blood. It would be of clinical value if more sensitive methods for the detection of this substance were available, so that actual assays of blood and urine in cases of Cushing's disease could be made.

No specific clinical value for preparations containing corticotropin has been demonstrated. There is the possibility that they might be of definite prophylactic value in cases of anticipated shock. There is likewise the possibility that in certain cases of Addison's disease there might be benefit from this form of therapy. Presumably, although there is no direct proof of it, the corticotropic substance causes the adrenal cortex to function to the full measure of its capacity. I have observed that the activity of hypophysectomized rats as measured in the running wheel cage is greatly increased after prolonged treatment with corticotropic extract. In view of the important, though as yet none too well defined, role which the adrenal glands play in carbohydrate and protein metabolism, as well as other phases of metabolism, a very important place must be assigned to the corticotropic hormone among the family of active principles of the anterior lobe.

THE THYROTROPIC HORMONE

Since the publication of the last edition of "Glandular Physiology and Therapy," in which knowledge of the thyrotropic hormone was reviewed, numerous papers have appeared dealing with this subject, but, as Van Dyke remarked in his excellent review of the physiology and pharmacology of the pituitary gland,[9] recent investigations of the thyrotropic hormone from various biologic standpoints have yielded a disappointingly small crop of facts. Much of the effort has been expended in consolidating or extending slightly knowledge which was already available.

Heyl and Laqueur[10] expressed the view that the principle responsible for thyroid enlargement differs

8. Selye, Hans: Thymus and Adrenals in the Response of the Organism to Injuries and Intoxications, Brit. J. Exper. Path. 17: 234-248 (June) 1936.

9. Van Dyke, H. B.: The Physiology and Pharmacology of the Pituitary Body, Chicago, University of Chicago Press, 1939, vol. 2.

10. Heyl, J. G., and Laqueur, Ernst: Zur quantitativen Bestimmung der thyreotropen Wirkung von Hypophysenvorderlappenpräparaten und die Einheit des thyreotropen Hormons, Arch. internat. de pharmacodyn. et de thérap. 49: 338-354 (Jan. 15) 1935.

from that which causes the histologic signs of hyperplasia. Heyl [11] emphasized that certain pituitary extracts cause obvious histologic signs of thyroid stimulation without increasing the weight of the gland, while other extracts elicit a pronounced increase in weight. He isolated a protein-free acetone-soluble pituitary fraction which had no effect on the thyroid by itself but caused marked enlargement of the gland when given in combination with a highly purified thyrotropic preparation that in itself caused only histologic signs of hyperplasia. He concluded that, in addition to the thyrotropic hormone, the hypophysis produces an activator responsible for the increase in weight. More recently, Billingsley,[12] studying this question in my laboratory, summarized his results as follows:

A number of thyrotropic extracts have been simultaneously assayed by the weight and histology of the guinea pig thyroid, and by the metabolic rate. No correlation can yet be expressed between these, except that increase in weight and hyperplasia apparently are associated more often than are either of these responses with an increased metabolism. The metabolic rate was found to respond most readily to thyrotropic extracts, and the guinea pig was more sensitive than the hypophysectomized rat. The anterior pituitary appears to have a two-fold action on the thyroid: one which influences the secretion of the thyroid hormone, the other to change the morphology of the gland. It is doubtful whether an identical mechanism is involved in each case, as indicated by comparative studies of the three assay methods.

Relations of the Thyrotropic Hormone to Other Hypophysial Principles.—Riddle and his co-workers [13] showed that active gonadotropic pituitary preparations tested in pigeons do not always exert a thyrotropic effect. This indicates that the two hormones are not identical. Yet more recently, Tolksdorf and Jensen [14] and Jensen and Tolksdorf [15] came to the conclusion that the thyrotropic factor is identical with the inter-

11. Heyl, J. G.: Thyreotropes Hormon und Wachstum der Schilddrüse, Acta brev. Neerland. **4**: 102, 1934.

12. Billingsley, L. W.: Factors Affecting the Metabolism of Small Animals, Thesis, McGill University Graduate School, 1937.

13. Riddle, Oscar; Bates, R. W., and Dykshorn, S. W.: Thyroid Hypertrophy as a Response to the Gonad-Stimulating Hormone of the Pituitary, Proc. Soc. Exper. Biol. & Med. **30**: 794-797 (March) 1933.

14. Tolksdorf, Sybylle, and Jensen, H.: Mechanism of Pituitary Gonadotropic Antagonism, Proc. Soc. Exper. Biol. & Med. **42**: 466-469 (Nov.) 1939.

15. Jensen, H., and Tolksdorf, Sybylle: The Relation Between the Interstitial-Cell-Stimulating and Thyrotropic Effects of the Anterior Pituitary, J. Biol. Chem. **133**: xlix-l (May) 1940.

stitial cell–stimulating, or luteinizing, factor, since all pituitary fractions containing thyrotropin proved also to be active luteinizers and vice versa. Since purified thyrotropic preparations do not inhibit the action of insulin in the mouse, Jensen and Grattan [16] concluded that the glycotropic action is not due to the thyrotropic principle, thus confirming the view of Young.[17]

Mechanism of the Thyrotropic Action.—Schneider [18] and Schneider and Widmann [19] observed that in the dog an extract containing thyrotropin increases the iodine content of the blood without a period of latency, thus differing from the action of thyroxin. Similarly in the guinea pig it leads to a rapid appearance of signs of thyrotoxicosis. Although these signs are not evident before the thyroid undergoes hyperplasia, they develop much sooner than after treatment with thyroxin. These experiments suggest that the actions of the thyrotropic principle cannot be regarded as due merely to a liberation of thyroxine.

The fact that the thyrotropic hormone acts directly on the thyroid without any intermediary has been demonstrated by Eitel and his associates,[20] who found that an extract containing this factor when added to slices of dog thyroid increased the consumption of oxygen by the thyroid tissue and caused histologic changes similar to those usually seen in vivo.

Bioassay for the Thyrotropic Substance.—The Third International Conference on the Standardization of Hormones [1] agreed that extracts of the thyrotropic factor should be assayed in comparison with an international standard preparation which is distributed by the National Institute for Medical Research, Hampstead, London, England; 250 micrograms of this preparation are equivalent to 1 unit. It also agreed that only those tests can be considered safe which are based on actual observation of a stimulation of the thyroid, since other effects may be due to impurities in the extract.

16. Jensen, H., and Grattan, J. F.: The Identity of the Glycotropic (Anti-Insulin) Substance of the Anterior Pituitary Gland, Am. J. Physiol. **128**: 270-275 (Jan.) 1940.

17. Young, F. G.: The Identity and Mechanism of Action of the Glycotropic (Anti-Insulin) Substance of the Anterior Pituitary Gland, Biochem. J. **32**: 1521-1539 (Sept.) 1938.

18. Schneider, E.: Morbus Basedow und künstliche Steigerung der Schilddrüsentätigkeit, Arch. f. klin. Chir. **173**: 421-428, 1932.

19. Schneider, E., and Widmann, E.: Klinische und experimentelle Untersuchungen zum Problem des Kropfes und der Basedowschen Krankheit: III. Mitteilung, Deutsche Ztschr. f. Chir. **238**: 206-215, 1932.

20. Eitel, Hermann; Krebs, H. A., and Loeser, Arnold: Hypophysenvorderlappen und Schilddrüse: Die Wirkung der thyreotropen Substanz des Hypophysenvorderlappens auf die Schilddrüse in vitro, Klin. Wchnschr. **12**: 615-617 (April 22) 1933.

In the axolotl, preparations of the thyrotropic substance induce metamorphosis, and this effect has been suggested as a basis for the assay of extracts for the thyrotropic principle.[21]

The frog tadpole has been used for the assay of preparations for the thyrotropic substance by Cuyler and co-workers,[22] who standardized such preparations on the basis of an accelerating effect on metamorphosis.

The grass snake (Tropidonotus natrix) is a particularly good test object, according to Mason,[23] because its thyroid, unlike those of laboratory rodents, shows surprisingly little variation from the normal resting condition unless stimulated by pituitary extracts. The disadvantage of this method is that few laboratories are equipped with this experimental material.

The thyroid of the pigeon or of the dove likewise undergoes pronounced enlargement and shows histologic signs of hyperplasia after administration of an extract containing thyrotropin and is a suitable test object for assay purposes. It is important, however, to use either immature[24] or hypophysectomized[25] birds in order to make sure that the untreated controls have resting thyroids.

Immature chicks were first employed for such bioassays by Stimmel and associates,[26] who considered the decrease in the iodine content of the thyroid caused by the thyrotropin in an extract as an indicator of activity. Later Smelser[27] and Cope[28] used the increase in the weight and the histologic signs of hyperactivity elicited by the thyrotropic factor in the 1 day old chick as a basis for bioassay. Kabac and Liapin[29] made an accurate detailed study of this test object, using 5 to 6 day old chicks and estimating thyrotropic potency merely by the increase in thyroid weight. They stated: "As an arbitrary unit of thyrotropic preparation we designate the amount which brings

21. Spaul, E. A.: Experiments on the Injection of Pituitary Body (Anterior Lobe) Extracts to Axolotls, Brit. J. Exper. Biol. **2**: 1, 1924.

22. Cuyler, W. K.; Stimmel, B. F., and McCullagh, D. R.: Quantitative Studies with Thyrotropic Hormone, J. Pharmacol. & Exper. Therap. **58**: 286-293 (Nov.) 1936.

23. Mason, E. M.: Assay of Thyrotropic Hormone, Nature, London **142**: 480-481 (Sept. 10) 1938.

24. Riddle, Oscar: Studies on Pituitary Functions, Endocrinology **15**: 307-314 (July-Aug.) 1931.

25. Miller, R. A., and Riddle, Oscar: Stimulation of Adrenal Cortex of Pigeons by Anterior Pituitary Hormones and by Their Secondary Products, Proc. Soc. Exper. Biol. & Med. **41**: 518-522 (June) 1939.

26. Stimmel, B. F.; McCullagh, D. R., and Picha, Valerian: The Thyrotropic Hormone of the Pituitary Gland and Iodine Metabolism, J. Pharmacol. & Exper. Therap. **57**: 49-55 (May) 1936.

27. Smelser, G. K.: Assay of Thyrotropic Hormone on Day-Old Chicks, Proc. Soc. Exper. Biol. & Med. **37**: 388-390 (Nov.) 1937; Chick Thyroid Responses as a Basis for Thyrotropic Hormone Assay, Endocrinology **23**: 429-438 (Oct.) 1938.

28. Cope, C. L.: The Young Chick as Test for the Thyrotropic Hormone, J. Physiol. **94**: 358-364 (Dec.) 1938.

29. Kabac, J. M., and Liapin, N. I.: A Quantitative Study of the Thyrotropic Action of Extracts of Anterior Pituitary on Chicks, Bull. de biol. et de méd. expér. de l'U. R. S. S. **5**: 334-338 (April) 1938.

the weight of both thyroids of the chick to 8 mg. (on the average) after daily subcutaneous injections during five days. The experiments were carried out on 5 day old chicks, and autopsy was performed one day after the last injection." Bergman and Turner,[30] who employed the 1 day old white leghorn chick, emphasized that males are more sensitive than females. They defined the unit of thyrotropic activity as the total amount of extract administered subcutaneously once daily during four days which will cause a mean increase of 50 per cent (to about 5.4 $\pm$ 0.26 mg.) in thyroid weight in 20 chicks whose body weights average 55 $\pm$ 10 Gm. This unit corresponds to about one quarter of the guinea pig unit employed by the same investigators.

The guinea pig is the most commonly employed test object for thyrotropin. It represents an excellent test object, but a relatively young animal must be used in order to insure that the thyroid is in the resting stage before the thyrotropic preparation is administered. Aron [31] and Aron and Klein [32] stated that this is always the case in animals weighing less than 250 Gm. Loeser [33] used guinea pigs weighing 180 to 220 Gm. However, several subsequent investigators found that even in the small animal the thyroid may show signs of hyperplasia and of absorption of colloid under apparently normal conditions.[34] This variability of the normal thyroid structure induced Benazzi [35] and Junkman and Schoeller [36] to employ only animals weighing between 100 and 150 Gm. Aron [37] insisted, however, that the 200 Gm. guinea pig is suitable

30. Bergman, A. J., and Turner, C. W.: A Comparison of the Guinea Pig and Chick Thyroid in the Assay of the Thyrotropic Hormone, Endocrinology **24**: 656-664 (May) 1939.

31. Aron, Max: (*a*) Action de la préhypophyse sur la thyroïde chez le cobaye, Compt. rend Soc. de biol. **102**: 682-684 (Nov. 29) 1929; (*b*) Particularités histologiques de la réaction de la thyroïde aux extraits de lobe antérieur d'hypophyse, ibid. **103**: 145-147 (Jan. 24) 1930; (*c*) L'hormone préhypophysaire excito-sécrétrice de la thyroïde, Contribution à l'étude du fonctionnement thyroïden, Rev. franç. d'endocrinol. **8**: 472-520 (Dec.) 1930; (*d*) Note de technique sur la mise en évidence et évaluation quantitative des faibles taux de "thyréo-stimuline" préhypophysaire présents dans le sang ou l'urine, Compt. rend. Soc. de biol. **109**: 218-220 (Jan. 29) 1932.

32. Aaron, Max, and Klein, M.: Sur la présence, dans l'urine humaine, d'une substance douée de la même action sur la thyroïde que l'extrait préhypophysaire, et sur l'interprétation de la réaction de diagnostic de la grossesse, Compt. rend. Soc. de biol. **103**: 702-704 (March 7) 1930.

33. Loeser, Arnold: Die Darstellung thyreotrop wirksamer Extrakte aus Hypophysenvorderlappen, Arch. f. exper. Path. u. Pharmakol. **166**: 693-702, 1932.

34. del Castillo, E. B., and Magdalena, A.: Hypophyse et thyroïde. Pouvoir excito-thyroïdien du sérum sanguin, Compt. rend. Soc. de biol. **108**: 917-918 (Dec. 4) 1931. del Castillo, E. B.: Hypophyse et thyroïde: La thyroïde les jeunes cobayes comme réactif pour déceler l'activité thyréostimulatrice du sérum, ibid. **111**: 461-464 (Nov. 4) 1932.

35. Benazzi, M.: Sul test di Aron per la tireostimolina preipofisaria, Boll. Soc. ital. di biol. sper. **8**: 1212, 1933.

36. Junkman, Karl, and Schoeller, Walter: Ueber das thyreotrope Hormon des Hypophysenvorderlappens, Klin. Wchnschr. **11**: 1176-1177 (July 9) 1932.

37. Aron, Max: Sur le titrage biologique de la thyréo-stimuline préhypophysaire: Le "seuil des mitoses" dans la thyroïde des cobayes traités, Compt. rend. Soc. de biol. **123**: 250-253, 1936.

for assay purposes. He defined the unit as $\frac{1}{100}$ of the amount necessary to produce a 50 to 100 per cent weight increase in the thyroid of a 200 Gm. guinea pig twenty-four hours after this amount is administered in a single injection. Histologically, 100 units causes absorption of colloid and secretory phenomena in the enlarged acinous cells throughout the glandular parenchyma. Ten units exerts such an effect only in the central portion of the gland; 40 to 45 units represents the amount which is most easily gaged in a single test since it causes absorption of colloid, hyperplasia and the appearance of mitotic figures, but none of these signs is sufficiently marked to make it impossible to differentiate the effect from that of still higher doses. Well aware of the variability of the thyroid even among young normal guinea pigs, Paal and Kleine [38] described a "resting diet" which causes the thyroid to become entirely free of any signs of activity and thus makes it particularly suitable for bioassay purposes. De Fremery [39] advocated pretreatment with the thyroid depressing diiodotyrosine for the same reason. Krogh and associates [40] emphasized that a few vacuoles in the colloid of the thyroid gland cannot be considered as significant, and this is in agreement with my own observations. However, if this is kept in mind, 200 Gm. guinea pigs are quite suitable for assay purposes, unless extremely small doses have to be detected. Such guinea pigs have also been used by Rowlands and Parkes,[41] who adopted as a unit "the thyrotropic activity contained in an amount of extract which given daily for 5 days will cause the thyroids of the 200 Gm. guinea pigs to attain a weight of 60 mg., i. e., about double the normal." This method of assay has been accepted by Junkman and Loeser.[42] Starr and Rawson,[43] Rawson and Starr [44] and Starr and others [45] measured the height of the thyroid epithelium and found it to be about 3.75 microns in normal females weighing 180 to 225 Gm. Using this "microhistometric" method, they

38. Paal, Hermann, and Kleine H. O.: Ueber die Abhängigkeit der Schilddrüsen-funktion von alimentären und hormonalen Faktoren, Beitr. z. path. Anat. u. z. allg. Path. **91**:322-342, 1933.

39. de Fremery, P.: Die Wirkung des Dijodtyrosins auf die Rattenschilddrüse, Acta brev. Neerland. **5**: 35-36, 1935.

40. Krogh, Marie; Lindberg, Anna-Louise, and Okkels, Harald: Studies on the Thyroid Gland: III. Experimental Hyperactivity of the Thyroid Gland, Acta path. et microbiol. Scandinav. **9**: 37-54, 1932.

41. Rowlands, I. W., and Parkes, A. S.: Quantitative Study of the Thyrotropic Activity of Anterior Pituitary Extracts, Biochem. J. **28**: 1829-1843 (May) 1934.

42. Junkman, Karl, and Loeser, Arnold: Die Wertbestimmung des thyreotropen Hormons der Hypophyse, Arch. f. exper. Path. u. Pharmakol. **188**: 474-488, 1938.

43. Starr, Paul, and Rawson, R. W.: Graphic Representation of Thyroid Response to Stimulation by Thyrotropic Hormone, Proc. Soc. Exper. Biol. & Med. **35**: 603-605 (Jan.) 1937.

44. Rawson, R. W., and Starr, Paul: Direct Measurement of Height of Thyroid Epithelium: A Method of Assay of Thyrotropic Substance; Clinical Application, Arch. Int. Med. **61**: 726-738 (May) 1938.

45. Starr, Paul; Rawson, R. W.; Smalley, R. E.; Doty, Ellis, and Patton, Helen: The Microhistometric Method Applied to Thyrotropic Hormone Assay, West. J. Surg. **47**: 65-75 (Feb.) 1939.

assayed urine for its thyrotropic activity in various diseases by the increase in epithelial height which it produces. Heyl and Laqueur [10] insisted that the histologic changes, namely, the increase in the epithelial height and the vacuolation of the colloid, are the only satisfactory indexes of thyrotropic activity. They worked out a scale of six different stages of histologic signs of activity which they designated by the letters p to u. They termed "borderline dose" the amount which when given in two intraperitoneal injections on two consecutive days will cause in two thirds of the treated 150-200 Gm. guinea pigs a reaction "s" in the middle part of the thyroid within forty-eight hours. This reaction "s" is defined as a thickening of the cells in which the nucleus becomes round and the protoplasm develops on the distal pole of the cell to about the width of the diameter of the nucleus. In order to keep their unit as close as possible to those generally in use, they defined 1 guinea pig unit as one quarter of the "borderline dose." Wilcke [46] pointed out that it is important always to use the same fixative, since Susa's—which causes practically no shrinkage—makes the cells appear higher than does ordinary solution of formaldehyde U. S. P. diluted 1:10. He claimed that for the Heyl and Laqueur test the latter fixative is preferable. More recently Bergman and Turner [80] defined 1 guinea pig unit as the total amount of an extract containing the thyrotropic factor which when administered subcutaneously once daily on five successive days causes a mean increase of 50 per cent (to about 26.4 $\pm$ 1.63 mg.) in the thyroids of 10 male guinea pigs whose weights average 155 $\pm$ 15 Gm. According to Bastenie and Zylberszac,[47] 0.025 mg. of colchicine given for every 30 Gm. of body weight to 220-250 Gm. guinea pigs makes the mitogenetic action of the thyrotropic extract readily detectable, since colchicine arrests mitotic division in the metaphase. This drug should be administered about nine hours before killing the animals.

The decrease in the iodine content of the thyroid furnishes another test of thyrotropic activity in guinea pigs.[48]

The thyroid of the mouse is generally not considered to be a suitable test object for bioassay purposes, although Paal and Kleine [38] claimed that by using a special diet one may sufficiently repress the thyroid even in this species to make the effect of thyrotropic evident.

46. Wilcke, J.: Einfluss der Fixierung auf das histologische Bild der Schilddrüse bei Meerschweinchen, Acta brev. Neerland. **5**: 99, 1935.

47. Bastenie, P., and Zylberszac, S.: Mise en évidence de stimulations hormonales par la colchicine. Détection de stimulation thyroïdienne par l'extrait antéhypophysaire, Compt. rend. Soc. de biol. **126**: 446-448, 1937.

48. McCullagh, D. R., and Stimmel, B. F.: A Biochemical Method for the Assay of the Thyrotropic Hormone of the Pituitary Gland, J. Biol. Chem. **109**: lxii-lxiii (May) 1935. Cuyler and others.[22] Stimmel and others.[20]

The thyroid of the rat is normally too variable to be useful for bioassay purposes. In hypophysectomized animals, on the other hand, the restoration of the atrophic epithelium gives a good index for thyrotropic activity.[49] The low basal metabolism of the rat is restored to normal by the thyrotropic factor, a change which has been advocated for bioassay purposes.[50] However, in view of the more recent discoveries showing that pituitary extracts other than those containing the thyrotropic principle may raise the basal metabolic rate, this method is not strictly specific.

Among other assay methods in which the rat is used should be mentioned that of Cuyler and co-workers,[22] which is based on the action of the thyrotropic hormone in decreasing the iodine of the thyroid, and that of Karnofsky and Cronkite,[51] based on the observation that this principle accelerates the eruption of the incisor teeth in the newborn rat.

Thyrotropic Content of Blood.—Van Caulaert and associates [52] found that dog serum has the ability to stimulate the thyroid of the immature guinea pig. Fellinger [53] prepared an extract of dog blood which was definitely thyrotropic in the guinea pig.

Horse serum likewise shows thyrotropic activity.[52]

Guinea pig blood, unlike that of most other species so far examined, does not contain any thyrotropic principle demonstrable by the guinea pig thyroid test.[54]

Rabbit and rat blood are definitely thyrotropic in the guinea pig thyroid test.[52] Aron [55] claimed that rat blood is particularly active in stimulating the thyroid of the immature rabbit.

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49. (*a*) Collip, J. B.: The Standardization of Anterior Pituitary Hormones, Am. J. Obst. & Gynec. **33**: 1010-1016 (June) 1937. (*b*) Hertz, Saul, and Oastler, E. G.: Assay of Blood and Urine for Thyreotropic Hormone in Thyrotoxicosis and Myxedema, Endocrinology **20**: 520-525 (July) 1936.

50. Anderson, E. M., and Collip, J. B.: Studies on the Physiology of the Thyreotropic Hormone of the Anterior Pituitary, J. Physiol. **82**: 11-25 (Jan.) 1934.

51. Karnofsky, D., and Cronkite, E. P.: Effect of Thyroxine on Eruption of Teeth in Newborn Rats, Proc. Soc. Exper. Biol. & Med. **40**: 568-570 (April) 1939.

52. van Caulaert, C.; Aron, Max, and Stahl, J.: Sur la présence de l'hormone préhypophysaire excito-sécrétrice de la thyroïde dans le sang et le liquide céphalo-rachidien, et sur sa repartition dans ces milieux et dans l'urine, Compt. rend. Soc. de biol. **106**: 607-609 (Feb. 13) 1931.

53. Fellinger, Karl: Klinische und experimentelle Untersuchungen über das Verhalten und die Bedeutung des thyreotropen Hormons im Blute, Wien. Arch. f. inn. Med. **29**: 375-406, 1936.

54. Aron, Max: Recherches sur les indices d'activité de la préhypophyse selon l'âge et l'espèce par la méthode du titrage physiologique de l'hormone dans le milieu intérieur, et sur leur correspondance avec les manifestations morphologiques de l'activité de la thyroïde, Comp. rend. Soc. de biol. **106**: 609-611 (Feb. 27) 1931. van Caulaert and others.[52]

55. Aron, Max: Distinction entre l'hormone préhypophysaire excito-sécrétrice de la thyroïde et le principe stimulant de l'ovaire renfermé dans les extraits préhypophysaires, Compt. rend. Soc. de biol. **106**: 1044-1046 (April 16) 1931.

Human blood is active in producing thyroid hyperplasia in immature guinea pigs.[56] Fellinger [53] showed that the active substance is readily extracted from the serum by 40 per cent acetone, from which it precipitates after the concentration is raised to 85 per cent. Aron [55] claimed that the thyrotropic activity may also be demonstrated by the effect of normal human blood on the thyroid of the immature rabbit.

Thyrotropic Content of Urine.—Emerson and Cutting [57] were unable to demonstrate any thyrotropic substance in dog urine using the guinea pig thyroid test. Similar negative results have been obtained with guinea pig urine by Aron,[54] who used the same test. Rat urine, on the other hand, is very active in stimulating the thyroid of the immature guinea pig.[58]

Aron and collaborators [59] and Starr and associates [45] were able to demonstrate in the urine of man the presence of thyrotropic substance, using the thyroid of either the guinea pig or the rabbit as an indicator. Katzman and Doisy [60] stated that a modification of their tungstic acid process for the extraction of the gonadotropic factor from pregnancy urine is suitable for the extraction of a thyrotropic substance from urine. Nielsen [61] claimed that the urine of some men stimulates, while that of others depresses, thyroid development in the rabbit. Antognetti and Geriola [62] and Emerson and Cutting [57] found normal urine quite inactive in the guinea pig thyroid test. Similarly, Jones [63] was unable to extract the thyrotropic principle from normal urine with a method which gave good recoveries when a pituitary extract containing this principle was simply added to urine. He concluded that either the hormone is not excreted in an active form or its chemical properties are

56. (*a*) Aron, Max; van Caulaert, C., and Stahl, J.: Recherches sur le diagnostic des troubles fonctionnels du lobe antérieur de l'hypophyse (préhypophyse) et sur certains déséquilibres endocriniens auxquels ils participent, Presse méd. **40**: 1981-1984 (Dec. 31) 1932. (*b*) Bodart, F., and Fellinger K.: Ueber die thyreotrope Wirkung des Serums bei endokrinen Erkrankungen, Wien. klin. Wchnschr. **49**: 1286-1287 (Oct.) 16) 1936. (*c*) Aron.[21d] (*d*) Caulaert and others.[52]

57. Emerson, Kendall, Jr., and Cutting, W. C.: Urinary Thyrotropic Hormone, Endocrinology **23**: 439-445 (Oct.) 1938.

58. Aron, Max; van Caulaert, C., and Stahl, J.: L'équilibre entre l'hormone préhypophysaire et l'hormone thyroïdienne dans le milieu intérieur à l'état normal et à l'état pathologique, Compt. rend. Soc. de biol. **107**: 64-66 (May 8) 1931.

59. Aron, Max: L'hormone thyréo-stimulante de la préhypophyse est-elle éliminée par le rein et présente dans l'urine? Compt. rend. Soc. de biol. **114**: 20-23, 1933; L'hormone thyreo-stimulante de la préhypophyse est-elle présente dans l'urine? ibid. **116**: 272-273, 1934. Aron.[21d] Aron.[55] Aron and others.[56] Aron and Klein.[52]

60. Katzman, P. A., and Doisy, E. A.: A Quantitative Procedure for Determining Normal Excretion of Prolan, Proc. Soc. Exper. Biol. & Med. **30**: 1188-1191 (June) 1933.

61. Nielsen, Herman: Ein "Thyreotest" durch Harninjektion in Kaninchen, Klin. Wchnschr. **12**: 508 (April 1) 1933.

62. Antognetti, L., and Geriola, F.: Studi sui "tests" ormonici: Nota tredicesima. L'hormone tireotropo preipofisario, Endocrinol. e. pat. costit. **11**: 395-410 (July) 1936.

63. Jones, M. S.: A Study of Thyrotropic Hormone in Clinical States, Endocrinology **24**: 665-671 (May) 1939.

so altered that it is not extractable with his method. Giedosz,[64] on the other hand, observed marked stimulation of the rabbit thyroid following administration of normal human urine. Sendrail and Tamalet[65] claimed that 15 cc. of normal urine (the amount employed for one 3 day assay) contains from 4 to 30 Aron guinea pig units.

Thyrotropic Content of Blood and Urine in Various Diseases.—In Simmonds's disease the thyrotropic activity of the urine is below normal as judged by the guinea pig thyroid test.[66]

In acromegaly Fellinger [53] was able to recover thyrotropic substance in unusually large quantities from the blood, using his acetone extraction method. The urinary excretion of thyrotropic substance is increased in this disease, according to Nitzescu and Timus [67] and Sendrail and Tamalet,[65] who used the guinea pig thyroid test. The latter investigators obtained similar results in Cushing's disease. On the other hand, Jones,[63] who used the chick for assay, obtained negative results.

In myxedema and other types of hypothyroidism Aron,[68] Aron and Klein [32] and Antognetti and Geriola [62] obtained variable results, while Hertz and Oastler,[49b] who followed restoration of the thyroid in hypophysectomized rats, and Rawson and Starr [44] and Sendrail and Tamalet,[65] who employed the guinea pig thyroid test, observed increased elimination of thyrotropic substance. Emerson and Cutting,[57] using the latter test, found that following thyroidectomy the thyrotropic activity of the blood increases regularly, but such increase is only occasionally observed in spontaneous hypothyroidism. Jones,[63] who with his chick test could not detect any thyrotropic substance in the urine of normal persons or patients suffering from a variety of diseases, found a positive result in a case of myxedema.

The blood of patients with hypothyroidism is rich in thyrotropic substance, according to Fellinger,[58] Hertz

64. Giedosz, B.: Ueber thyreotrope Substanzen im menschlichen Harn, Klin. Wchnschr. **13**: 1507 (Oct. 20) 1934.

65. Sendrail, M., and Tamalet, L. J.: Le test hypophysaire d'Aron en clinique, Toulouse-méd. **40**: 1-15 (Jan.) 1939.

66. Anderson and Collip.[50] Sendrail and Tamalet.[65]

67. Nitzescu, I. I., and Timus, D.: Die Ausscheidung des thyreotropen Hormons aus dem Hypophysenvorderlappen durch den Harn bei Akromegalie, Spitalul **58**: 5, 1938.

68. Aron, Max: (*a*) Méthode biologique de diagnostic des états d'hyperactivité et d'hypoactivité de la préhypophyse chez l'homme, Compt. rend. Soc. de biol. **105**: 585-586 (Nov. 28) 1930; (*b*) Le titrage des hormones préhypophysaires dans l'urine humaine; son intérêt dans l'exploration fonctionelle des diverses glandes endocrines, Bull. Acad. de méd. **111**: 273-275 (Feb. 20) 1934.

and Oastler [49b] and Bodart and Fellinger,[56b] who also noted that following thyroidectomy the usually low thyrotropic activity of the blood of patients suffering from exophthalmic goiter undergoes a rapid and pronounced increase. It appears that the production of thyrotropic hormone is increased in thyroid insufficiency just as the output of gonadotropic hormone rises after the gonads are removed or have undergone involution.

In hyperthyroidism Aron [68a] obtained variable results, while Krogh and Okkels [69] claimed that no thyrotropic activity can be demonstrated in the urine by the rabbit thyroid test. Smith and Moore,[70] Antognetti and Geriola,[62] Emerson and Cutting,[57] Rawson and Starr,[44] Starr and other co-workers [45] and Sendrail and Tamalet,[65] all of whom used the guinea pig thyroid test, usually found subnormal quantities of thyrotropic substance in the urine, and in most cases this principle appeared to be entirely absent. Jones,[63] who used the chick test, likewise failed to detect excretion of thyrotropic substance. Drouet [71] emphasized, however, that increased excretion of thyroxine, which may antagonize the effect of the thyrotropic hormone in the intact animal, may be the reason why most investigators were unable to detect the latter factor in the urine of hyperthyroid patients. He pointed out that negative results from urinary assays do not prove that thyrotropic substance is not excreted unless it is made certain that no substances antagonistic to the thyrotropic hormone are eliminated. It should be emphasized, however, that Hertz and Oastler,[49b] who used hypophysectomized rats for their assays, were also unable to demonstrate thyrotropic activity in the urine of hyperthyroid patients, although in the absence of the pituitary the thyroid hormone would not have exerted an inhibitory effect. It is of interest, however, that, according to Nielsen,[61] in exophthalmic goiter the urine actually causes atrophy of the epithelium in the rabbit thyroid, which suggests some antithyrotropic effect.

69. Krogh, Marie, and Okkels, Harald: L'hormone thyréostimulante préhypophysaire est-elle présente dans l'urine? Compt. rend. Soc. de biol. **113**: 635-638, 1933; L'hormone thyréostimulante préhypophysaire est-elle eliminée par le rein? ibid. **113**: 638-641, 1933.

70. Smith, M. G., and Moore, E.: Is Anterior Pituitary Hormone Demonstrable in Urine of Graves' Disease, in Urine of Guinea Pigs Injected with Anterior Pituitary Extract, Proc. Soc. Exper. Biol. & Med. **30**: 735-739 (March) 1933.

71. Drouet, P. L.: Le rôle de l'hypophyse dans l'hyperthyroïdie et le syndrome para-basedowien. Contribution à l'étude de l'hyperpituitarisme, Rev. franç. d'endocrinol. **12**: 101-136 (April) 1934.

The blood of patients with hyperthyroidism likewise contains subnormal amounts of thyrotropic substance, according to the guinea pig assays of Fellinger [53] and Bodart and Fellinger.[56b]

In obesity Merklen and co-workers [72] invariably observed an increase in the elimination of thyrotropic substance, using the guinea pig test, while de Prat [73] obtained more variable results.

In various other diseases the results so far reported are too contradictory to justify detailed comment.[74] It is noteworthy, however, that, according to Aron,[74a] in most cases the elimination curves of the thyrotropic and gonadotropic substances tend to run parallel.

Preparation of Active Extracts.—Greep [75] showed that a fairly complete separation of thyrotropic from gonadotropic substance could be obtained by treatment of extracts with benzoic acid. The thyrotropic factor was precipitated with the benzoic acid, and the gonadotropic factor remained in the filtrate.

Lambie and Trikojus [76] have recently described a method of extraction and purification of the thyrotropic principle. Collip [77] has found that, in addition to the thyrotropic substance which is obtained in primary dilute acetic acid extracts, an isoelectric protein fraction can be prepared from the gland residues which is highly potent in thyrotropic properties. Moreover, guinea pigs treated daily with the latter fraction may not show resistance to it for many months. This is confirmatory of the results of Werner,[78] who found that thyrotropic

72. Merklen, P.; Aron, Max; Israel, L., and Jacob, A.: Tests histologiques de l'hyperfonctionnement préhypophysaire chez certains obèses, Bull. et mém. Soc. méd. d. hôp. de Paris **51**: 1402-1406 (Nov. 4) 1935.

73. de Prat, J.: Thesis, Paris, 1937.

74. (*a*) Aron, Max: Parallélisme des taux respectifs d'excrétion de la thyréo-stimuline et de la gonado-stimuline préhypophysaires dans le milieu intérieur chez l'homme en des conditions normales ou pathologiques, Compt. rend. Soc. de biol. **113**: 443-445, 1933. (*b*) Sendrail and Tamalet.[65]

75. Greep, R. O.: Separation of a Thyrotropic from the Gonadotropic Substances of the Pituitary, Am. J. Physiol. **110**: 692-699 (Jan.) 1935.

76. Lambie, C. G., and Trikojus, V. M.: The Preparation of a Purified Thyrotropic Hormone by Chemical Precipitation, Biochem. J. **31**: 843-847 (June) 1937.

77. Collip, J. B.: Anterior Pituitary Hormones, in Piersol, G. M., and Bortz, E. L.: Cyclopedia of Medicine, Surgery and Specialties, Philadelphia, F. A. Davis Company, 1939, pp. 637-656.

78. Werner, S. C.: Prolonged Injection of a Thyrotropic Extract Without Development of Refractoriness, Proc. Soc. Exper. Biol. & Med. **34**: 390 (April) 1936; Antibody Nature of Refractoriness to Injections of Hypophyseal Extracts Containing Thyrotropic Hormone, ibid. **34**: 392 (April) 1936.

extracts prepared by different methods differed widely in their ability to cause development of the refractory state in guinea pigs.

Effect of Iodine on Action of Thyrotropic Hormone.—Friedgood [79] has reported that the administration of sodium iodide to guinea pigs receiving an extract containing the thyrotropic factor caused a remission of the symptoms of hyperthyroidism. Anderson and Evans [80] observed that in guinea pigs potassium iodide may prevent the metabolic action of thyrotropic extracts (perhaps because it prevents the discharge of thyroxine) without interfering with the effect on the thyroid itself. Franck [81] found in guinea pigs treated with an extract of the anterior lobe that the basal metabolism decreased after an initial increase. During the period of decreased basal metabolic rate the thyroid showed storage of colloid. This storage appeared simultaneously with an enlargement of the Golgi apparatus and was considered to be due to a special action of the thyrotropic principle. Iodine administered to such animals prevented both the initial rise and the eventual fall in the basal metabolic rate. According to Franck, extracts of the anterior lobe cause degranulation of the eosinophils and an increase in the number of basophils in the anterior lobe of the guinea pig. These effects are inhibited by iodine. He concluded that the basophils of the pituitary secrete a thyrotropic hormone which stimulates storage of colloid in the thyroid follicles. The secretion of this hormone is prevented by the administration of iodine.

THE PARATHYROTROPIC HORMONE

Although the existence of a parathyrotropic hormone has not been proved as yet and may be regarded as rather doubtful, certain clinical and experimental evidence now available concerning this question will be discussed.

79. Friedgood, H. B.: Similarity of the Iodin Remission in Experimental Anterior Hypophyseal Hyperthyroidism, the Hyperthyroidism of Acromegaly and That of Exophthalmic Goiter, Endocrinology **20**: 526-536 (July) 1936.

80. Anderson, E. M., and Evans, H. M.: The Effect of Thyreotropic Hormone Combined with Small Amounts of Iodine upon the Function of the Thyroid Gland, Am. J. Physiol. **120**: 597-603 (Nov.) 1937.

81. Franck, S.: Histophysiologie de la préhypophyse. Prehypophyse et glande thyroïde soumises à l'action de l'iode, Compt. rend. Soc. de biol. **125**: 569-573 (Feb. 26) 1937.

Parathyroid Tumors.—Adenoma of the parathyroids has repeatedly been observed in association with hypophysial tumors,[82] a fact which suggests that a close relationship exists between these two glands.

Hypophysectomy.—In the dog Aschner [83] was unable to detect any change in the parathyroids following ablation of the hypophysis, while Koster [84] claimed that the parathyroids were invariably subnormal in size. Houssay and Sammartino [85] stated that degenerative lesions and necrosis of the parathyroids may be observed in 66 per cent of all hypophysectomized dogs, while they are seen in only 10 per cent of the normal controls. After lesions of the tuber cinereum they were noted in about 41 per cent of the cases.

In the rat Smith [86] claimed to have noted atrophy of the parathyroids following hypophysectomy.

Hypophysectomy and Pancreatectomy.—The degenerative changes which occasionally occur following hypophysectomy are much more commonly observed in the dog in which the pancreas is also removed. Mere removal of the pancreas, however, does not elicit such changes except in rare cases.[87]

Hypophysial Extracts.—In the cat Anselmino and co-workers [88] claimed that the so-called parathyrotropic extracts of the pituitary cause hyperemia and histologic signs of increased activity in the parathyroids without actually increasing their size.

In the dog, following treatment with hypophyseal extract, enlargement of the epithelial cells, follicle

82. Hadfield, G., and Rogers, H.: Two Parathyroid Tumors Without Osteitis Fibrosa: One Associated with Acromegaly. J. Path. & Bact. **35**: 259-263 (March) 1932. Gerstel, G.: Ueber multiple Tumoren der Drüsen mit innerer Sekretion bei einem Akromegalen, Frankfurt. Ztschr. f. Path. **52**: 485-499, 1938. Husslein, J.: Zur Frage der Pagetschen und Recklinghausenschen Knochenerkrankung, Beitr. z. klin. Chir. **169**: 276-298, 1939.

83. Aschner, B.: Ueber die Funktion der Hypophyse, Arch. f. d. ges. Physiol. **146**: 1-146, 1912.

84. Koster, S.: Étude expérimentale de la fonction de l'hypophyse chez le chien, Arch. néerl. de physiol. **13**: 601, 1928; Experimentelle Untersuchungen der Hypophysenfunktion der Hunde: II. Arch. f. d. ges. Physiol. **224**: 212-216, 1930.

85. Houssay, B. A., and Sammartino, R.: Les parathyroïdes dans l'insuffisance hypophysaire et pancréatique, Compt. rend. Soc. de biol. **114**: 729-732, 1933; Die Epithelkörperchen bei den Hypophysen- und Pankreasinsuffizienzen des Hundes, Beitr. z. path. Anat. u. z. allg. Path. **93**: 405-416 (July) 1934.

86. Smith, P. E.: The Disabilities Caused by Hypophysectomy and Their Repair, J. A. M. A. **88**: 158-161 (Jan. 15) 1927.

87. Houssay, B. A., and Biasotti, A.: La diabetes pancreática de los perros hipofisoprivos, Rev. Soc. argent. de biol. **6**: 251-296, 1930. Houssay, B. A., and Sammartino, R.: Altérations des parathyroïdes des chiens pancréatoprivés, Compt. rend. Soc. de biol. **120**: 735-736, 1935. Houssay and Sammartino.[85] Collip.[6]

formation and hyperemia have been observed without enlargement of the total volume of the gland,[88] and the structural changes were often accompanied by hypercalcemia.[89] Recently Ham[90] reported changes supposedly characteristic of the parathyrotropic hormone in the parathyroids of dogs treated with pituitary extract.

In the guinea pig Anselmino and associates[88] also claimed to have seen parathyroid stimulation after the administration of pituitary extract.

In the dwarf mouse Kemp and Marx[91] claimed to have seen parathyroid enlargement following administration of a preparation containing the growth-promoting factor of the anterior lobe.

In the rabbit Hertz and Kranes,[92] Anselmino and associates[88] and Cattaneo[93] observed enlargement and changes after treatment with various pituitary extracts, which they interpreted as characteristic of the parathyrotropic hormone.

In the rat Anselmino and co-workers[94] claimed to have obtained particularly obvious parathyroid enlargement accompanied by hypercalcemia following treatment with their extract of the parathyrotropic factor, and Hoffmann and Anselmino[89] emphasized that the hypercalcemia is prevented by parathyroidectomy.

It should be emphasized, however, that the existence of the parathyrotropic hormone has by no means been proved.[95]

88. Anselmino, K. J.; Herold, L., and Hoffmann, F.: Ueber die Wirkung des parathyreotropen Hormons des Hypophysenvorderlappens bei verschiedenen Tierarten, Ztschr. f. exper. Med. **97**: 51-59, 1935.

89. Hoffmann, F., and Anselmino, K. J.: Ueber die Wirkung von Hypophysenvorderlappenextrakten auf den Blutkalkspiegel, Klin. Wchnschr. **13**: 44-45 (Jan. 13) 1934.

90. Ham, A. W., and Haist, R. E.: Histological Effects of Diabetogenic Anterior Pituitary Extracts, Anat. Rec. **76** (supp. 2): 89 (Feb.) 1940.

91. Kemp, Tage, and Marx, Lore: Beeinflussung von erblichem hypophysärem Zwergwuchs bei Mäusen durch verschiedenen Hypophysenauszüge und Thyroxin: II. Endokrine Organe, Acta path. et microbiol. Scandinav. **14**: 197-227, 1937.

92. Hertz, Saul, and Kranes, Alfred: Parathyreotropic Action of the Anterior Pituitary: Histologic Evidence in the Rabbit, Endocrinology **18**: 350-360 (May-June) 1934.

93. Cattaneo, M.: Richerche sperimentali sull'ormone paratireotropo del lobo anteriore dell'ipofisi, Riv. di pat. sper. **9**: 361-370, 1938.

94. Anselmino, K. J.; Hoffmann, F., and Herold, L.: Ueber die parathyreotrope Wirkung von Hypophysenvorderlappenextrakten, Klin. Wchnschr. **12**: 1944 (Dec. 16) 1933.

95. Bomskov, Christian: Methodik der Hormonforschung., Leipzig, Georg Thieme, 1939, vol. 2.

CHAPTER IV

THE ANTERIOR LOBE OF THE HYPOPHYSIS IN INTERMEDIARY METABOLISM

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The role of the anterior lobe of the pituitary in intermediary metabolism, especially in carbohydrate metabolism, has been reviewed by Houssay,[1] Russell,[2] Long,[3] Van Dyke,[4] Young,[5] Thomson and Collip[6] and others in recent years, in greater detail and with fuller bibliography than is possible in these pages. The subject is one of the greatest difficulty and complexity, since it is seldom possible to tell how widely the repercussions of any experimental interference may spread through the endocrine system. Great variations in behavior between species and between individuals in any one species are the rule rather than the exception. None of the anterior pituitary principles has yet been obtained in a state of indubitable purity, and any extract of the gland may or may not, depending on the source and the method of preparation, contain not only the thyrotropic and the corticotropic (adrenotropic) hormone

1. Houssay, B. A.: Certain Relationships Between Parathyroids, Hypophysis and Pancreas, in Harvey Lectures, Baltimore, Williams & Wilkins Company, 1936, vol. 31, pp. 116-134; New England J. Med. **214**: 1128-1136 (June 4) 1936; Diabetes as a Disturbance of Endocrine Regulation, Am. J. M. Sc. **193**: 581-606 (May) 1937.

2. Russell, Jane A.: Relation of the Anterior Pituitary to Carbohydrate Metabolism, Physiol. Rev. **18**: 1-27 (Jan.) 1938.

3. Long, C. N. H.: Influence of Pituitary and Adrenal Glands on Diabetes Mellitus, in Harvey Lectures, Baltimore, Williams & Wilkins Company 1937, vol. 32, pp. 194-228; Medicine **16**: 215-247, (Sept.) 1937; Diabetes Mellitus in Light of Our Present Knowledge of Metabolism, Tr. & Stud., Coll. Physicians, Philadelphia **7**: 21-46 (April) 1939. Long, C. N. H., and White, A.: Intermediary Carbohydrate Metabolism, Ergebn. d. Physiol. **40**: 164-203, 1938.

4. Van Dyke, H. B.: Physiology and Pharmacology of the Pituitary Body, Chicago, University of Chicago Press, 1939, vol. 2.

5. Young, F. G.: Experimental Investigations on Relationship of Anterior Hypophysis to Diabetes Mellitus, Proc. Roy. Soc. Med. **31**: 1305-1316 (Sept.) 1938; Anterior Pituitary Gland and Diabetes Mellitus, New England J. Med. **221**: 635-646 (Oct. 26) 1939; Pituitary Gland and Carbohydrate Metabolism, Endocrinology **26**: 345-351 (Feb.) 1940.

6. Thomson, D. L., and Collip, J. B.: Endocrine Glands, Ann. Rev. Physiol. **2**: 309-346, 1940.

(which must secondarily influence metabolic processes in many ways), the growth-promoting hormone (which presumably promotes anabolic processes even if its primary influence is on skeletal growth) and prolactin (which may directly or indirectly control metabolic adaptations to lactation) but also an uncertain number of principles active in metabolism but less clearly defined at present. For example, O'Donovan and Collip [7] demonstrated the existence of a factor which rapidly elevates the metabolic rate and depresses the respiratory quotient in normal and in thyroidectomized rabbits and guinea pigs and which is apparently derived from the pars intermedia, being closely associated with the melanophore-expanding hormone. Extracts which contain this factor may be largely freed from the "accepted" pituitary substances by exposure to high temperatures and still display manifold effects on intermediary metabolism. Even true posterior lobe principles (known and unknown) may be present in so-called anterior pituitary extracts, and may be overlooked.

A comparatively simple example of the apparent contradictions which abound in this field of study may be given. The hypophysectomized animal, with its atrophic thyroid, has a low metabolic rate, and its peripheral tissues consume abnormally small amounts of dextrose at all blood sugar levels [8]; in other words, its tolerance for injected dextrose is low,[9] but since the rate of absorption from the intestine is also greatly reduced, the tolerance for orally administered sugar is high.[10]

7. (*a*) O'Donovan, D. K., and Collip, J. B.: Specific Metabolic Principle of Pituitary, and Its Relation to Melanophore Hormone, Endocrinology **23**: 718-734 (Dec.) 1938. (*b*) Neufeld, A. H., and Collip, J. B.: Studies of the Effects of Pituitary Extracts on Carbohydrate and Fat Metabolism, ibid. **23**: 735-746 (Dec.) 1938. (*c*) Billingsley, L. W.; O'Donovan, D. K., and Collip, J. B.: Specific Metabolic Principle of Pituitary, ibid. **24**: 63-68 (Jan.) 1939.

8. Soskin, Samuel; Levine, R., and Lehmann, W.: Influence of Hypophysis on Carbohydrate Metabolism, Am. J. Physiol. **127**: 463-469 (Oct.) 1939. Soskin, Samuel: The Blood Sugar, Its Origin, Regulation and Utilization, Physiol. Rev. **21**: 140-193 (Jan.) 1941.

9. Biasotti, A.: Tolerancia de los perros hipofisoprivos a la glucosa, Rev. Soc. argent. de biol. **10**: 124-130 (June) 1934. Russell, Jane A., and Cori, G. T.: Comparison of Metabolic Effects of Subcutaneous and Intravenous Epinephrine Injections in Normal and Hypophysectomized Rats, Am. J. Physiol. **119**: 167-174 (May) 1937.

10. Corkill, A. B.; Marks, H. P., and White, W. E.: Relation of Pituitary Gland to Action of Insulin and Adrenaline, J. Physiol. **80**: 193-205 (Dec. 5) 1933. Phillips, R. A., and Robb, Phoebe: Carbohydrate Metabolism Studies in Hypophysectomized Albino Rats, Am. J. Physiol. **109**: 82-83 (July) 1934; Metabolism Studies in Albino Rat, Carbohydrate Studies After Hypophysectomy, Endocrinology **25**: 187-192 (Aug.) 1939.

FASTING HYPOGLYCEMIA AND THE GLYCOSTATIC PHENOMENON

Many observers have noted that when hypophysectomized animals of any species are caused to fast, the blood sugar falls rapidly to levels dangerously low. In hypophysectomized monkeys, for example, after eighteen hours' fast the blood sugar averaged 59 mg. per hundred cubic centimeters, while in normal animals it was still 110 mg.;[11] and in rats, whose metabolism is more intense, the blood sugar concentration may be halved in eight hours' fasting after hypophysectomy, whereas controls show only a 20 per cent decrease.[12] Many hypophysectomized animals have died in hypoglycemia, and many have been dramatically resuscitated from a cold and deathlike torpor by injections of dextrose.[13] The phenomenon is not ascribable to a breakdown of the mechanism of glycogenolysis, since the stores of liver glycogen usually show similar rapid exhaustion. Nor can it be said to be primarily due to exaggerated sensitivity to the insulin produced by the animal's own pancreas, since fasting hypoglycemia is observed in the depancreatized-hypophysectomized animals described on a subsequent page. It has been claimed[14] that injected thyroxin will maintain the blood sugar of hypophysectomized animals during fasting, but it is more widely believed that the tendency to hypoglycemia is a consequence of the atrophy of the adrenal cortex which follows hypophysectomy, since adrenalectomized animals also show this tendency, especially if their mineral metabolism is controlled, and since injected cortical steroid substances will protect both hypophysectomized and adrenalectomized animals against it.[15]

11. Smith, P. E.; Dotti, Louis; Tyndale, H. H., and Engle, E. T.: Effect of Hypophysectomy on Blood Sugar of Rhesus Monkeys, Proc. Soc. Exper. Biol. & Med. **34**: 247-249 (March) 1936.

12. Russell, Jane A.: Carbohydrate Levels in Fasted and Fed Hypophysectomized Rats, Proc. Soc. Exper. Biol. & Med. **34**: 279-281 (March) 1936.

13. Mahoney, William: Hypoglycemia Hypophysiopriva, Am. J. Physiol. **109**: 475-482 (Sept.) 1934.

14. Soskin, Samuel; Levine, R., and Heller, R. E.: Role of Thyroid in Carbohydrate Disturbance Which Follows Hypophysectomy, Am. J. Physiol. **125**: 220-226 (Feb.) 1939.

15. Russell, Jane A., and Craig, J. M.: Adrenal Cortical Hormone and Anterior Pituitary Extract on Carbohydrate Levels in Fasted Hypophysectomized Rats, Proc. Soc. Exper. Biol. & Med. **39**: 59-62 (Oct.) 1938. Corey, E. L., and Britton, S. W.: Hypophysial and Adrenal Interrelationships and Carbohydrate Metabolism, Am. J. Physiol. **126**: 148-154 (May) 1939. Fitzgerald, O., and Verzar F.: Die Wirkung von Nebennierenrindenhormon auf den Glykogengehalt der Leber von hypophysektomierten Ratten, Arch. f. d. ges. Physiol. **242**: 30-34, 1939.

Two rival interpretations of the hypoglycemic tendency have been put forward. On one hand, it is urged that the hypophysectomized (or adrenalectomized) animal prefers carbohydrate as a fuel and is spendthrift of its stores thereof. Evidence for this is seen in experiments in which dextrose is administered orally to rats which have fasted for twenty-four hours; the normal rat will oxidize about half the dextrose administered (in the following four hours) and will store about one third of it as liver and muscle glycogen in roughly equal shares, but the adrenalectomized or hypophysectomized rat will oxidize more and store less, whereas administration of adrenal cortex extract increases storage.[16a] Moreover, the amount of dextrose which must be supplied to prevent hypoglycemia developing after evisceration is almost twice as great in hypophysectomized as in normal rats.[16b] On the other hand, hypoglycemia may be an indication that the supply of new carbohydrate by glyconeogenesis is lagging behind the demands of the peripheral tissues. That the supply is, in fact, reduced after hypophysectomy is indicated by an analysis of blood leaving the liver;[17] furthermore, if one makes the orthodox assumption that a large and fairly constant proportion of the amino acids liberated in the breakdown of tissue protein constitutes the main source of new carbohydrate for the fasting animal, the excretion of nitrogen becomes a measure of the intensity of glyconeogenesis and shows the latter to be restricted after hypophysectomy. During fasting the nitrogen excretion tends to be low,[18] and is raised by the hormones of the adrenal cortex when they maintain the blood sugar level;[16a] the increase in nitrogen excretion and in stored carbohydrate which occurs when rats are exposed to low atmospheric pressures is not seen after extirpation of the hypophysis or of the adrenal cortex.[19] There is, then, fair if not con-

16. (*a*) Long, C. N. H., and Katzin, B.: Effect of Adrenal Cortical Hormone on Carbohydrate Stores of Fasted Hypophysectomized Rats, Proc. Soc. Exper. Biol. & Med. **38**: 516-518 (May) 1938. Katzin, B., and Long, C. N. H.: Effect of Adrenal Cortical Extract on Carbohydrate and Protein Metabolism of the Rat, Am. J. Physiol. **126**: P551 (July) 1939. (*b*) Russell, Jane A.: Carbohydrate Metabolism in the Eviscerated Rat, ibid. **133**: P 434 (June 1) 1941.

17. Crandall, L. A., and Cherry, I. S.: Effects of Insulin and Glycine on Hepatic Glucose Output in Normal, Hypophysectomized, Adrenal-Denervated and Adrenalectomized Dogs, Am. J. Physiol. **125**: 658-673 (April) 1939.

18. Braier, B.: Metabolismo nitrogenado de los perros hipofisoprivos en el ayuno, Rev. Soc. argent. de biol. **7**: 140-157 (1931).

19. Evans, Gerald: Adrenal Cortex and Endogenous Carbohydrate Formation, Am. J. Physiol. **114**: 297-308 (Jan.) 1936.

clusive evidence that the fall in blood sugar and liver glycogen in fasting hypophysectomized animals indicates that a decrease in the production of hormones in the adrenal cortex has made impossible acceleration of the protein breakdown to a point at which glyconeogenesis would be adequate; and in any case the protective action of anterior pituitary extracts may be ascribed tentatively to the corticotropic factor which they contain.[20]

But the fasting hypophysectomized rat or mouse (other species have hardly been examined) shows a decrease in muscle glycogen which is more striking and appears more rapidly after extirpation of the gland than that in adrenalectomized animals; moreover, anterior pituitary extracts may display a "glycostatic" power to retard this decrease even when they contain little corticotropic substance and even in rats which have been adrenalectomized as well as hypophysectomized.[21] During an eight hour fast the concentration of muscle glycogen may fall from 0.55 to 0.50 per cent in a normal rat and from 0.50 down to 0.30 per cent in a hypophysectomized rat; the higher respiratory quotient of the latter shows that the vanished carbohydrate has been oxidized, while the nitrogen excretion affords no evidence that glyconeogenesis diminished.[22] It is tempting to speculate that this using-up of muscle glycogen is due to a deficiency in the supply to the peripheral tissues not merely of dextrose, as in the adrenalectomized animal, but also of some other fuel (acetoacetic acid, for example; in which case this might be the inverse of the ketogenic phenomenon discussed later), but there is no supporting evidence at present for any such interpretation. It must be noted also, as a type of synergism yet unexplained, that the glycostatic phenomenon is most easily obtained when adrenal cortical principles are present in quantity, though it does not require that the gland itself should be intact.[20]

20. Russell, Jane A.: Relationship of the Anterior Pituitary and Adrenal Cortex in Metabolism of Carbohydrate, Am. J. Physiol. **128**: 552-561 (Feb.) 1940.

21. Russell, Jane A., and Bennett, L. L.: Maintenance of Carbohydrate Levels in Fasted Hypophysectomized Rats Treated with Anterior Pituitary Extracts, Proc. Soc. Exper. Biol. & Med. **34**: 406-409 (May) 1936. Bennett, L. L.: Interrelation of Pituitary and Adrenal in the Control of Carbohydrate Levels in the Rat, Endocrinology **22**: 193-196 (Feb.) 1938.

22. Fisher, R. E.; Russell, Jane A., and Cori, C. F.: Glycogen Disappearance and Carbohydrate Oxidation in Hypophysectomized Rats, J. Biol. Chem. **115**: 627-634 (Oct.) 1936.

SENSITIVITY TO INSULIN AND THE GLYCOTROPIC PHENOMENON

The extreme sensitivity of the hypophysectomized animal to insulin, observed by Houssay and Magenta [23] in the dog, has been confirmed in many other species; it is from ten to thirty times greater than that of intact animals, the hypoglycemia being both intense and prolonged. Some increase in sensitivity is also observed in animals deprived of their adrenal medullae or of the sympathetic nervous system or even of the thyroid gland; but these observations seem irrelevant in face of the fact that pretreatment with suitable anterior pituitary extracts may decrease the exaggerated response to insulin of the hypophysectomized animal [24] and completely prevent insulin hypoglycemia in the normal animal; [25] this is called the "glycotropic" effect. In attempting an interpretation one must bear in mind that extracts showing glycotropic activity do not raise the blood sugar level or affect carbohydrate stores in normal animals, do not prevent the development of hypoglycemia in fasting hypophysectomized animals [26] and do not retard the fall in blood sugar that follows hepatectomy in the rabbit (though they prevent further acceleration of this fall by insulin [27]). Hence the factor is not a general inhibitor of carbohydrate consumption but a rather specific "anti-insulin"; yet any chemical interaction between these agents is unlikely, since the glycotropic factor must be administered some hours

23. Houssay, B. A., and Magenta, M. A.: Sensibilidad en los perros hipofisoprivos a la insulina, Rev. Asoc. méd. argent. (Soc. de biol.) **37**: 389-406, 1924.

24. Houssay, B. A., and Potick, D.: Antagonisme entre l'hypophyse et l'insuline chez le crapaud, Compt. rend. Soc. de biol. **101**: 940-942 (July 17) 1929. di Benedetto, E.: Extrait antéro-hypophysaire et résistance à l'insuline, Compt. rend. Soc. de biol. **112**: 499-501 (Feb 10) 1933. Newton, W. H., and Young, F. G.: Influence of the Glycotropic (Anti-Insulin) Factor of the Anterior Hypophysis on the Insulin Sensitivity of the Hypophysectomized Rabbit, J. Physiol. **94**: 40-46 (Oct. 14) 1938.

25. (*a*) di Benedetto, E. C.: Extrait antéro-hypophysaire et résistance à l'insuline, Compt. rend. Soc. de biol. **112**: 499-501 (Feb. 10) 1933. (*b*) Cope, O., and Marks, H. P.: Further Experiments on Relation of Pituitary Gland to Action of Insulin and Adrenaline, J. Physiol. **83**: 157-176 (Dec. 31) 1934. (*c*) Young, F. G.: Identity and Mechanism of Action of the Glycotropic (Anti-Insulin) Substance of the Anterior Pituitary Gland, Biochem. J. **32**: 1521-1539 (Sept.) 1938. (*d*) Jensen, H., and Grattan, J. F.: Identity of the Glycotropic (Anti-Insulin) Substance of the Anterior Pituitary Gland, Am. J. Physiol. **128**: 270-275 (Jan.) 1940.

26. Russell, Jane A.: Action of Insulin and of Anterior Pituitary Extract in Normal and Hypophysectomized Rats, Am. J. Physiol. **124**: 774-790 (Dec.) 1938.

27. Himsworth, H. P., and Scott, D. B. McN.: Action of Young's Glycotropic Factor of Anterior Pituitary Gland, J. Physiol. **92**: 183-207 (March 14) 1938.

before the insulin to be effective. Part of the sugar which disappears from the blood under the influence of insulin is laid down in the muscles as glycogen, and it is possible that the glycotropic factor prevents this.[26] Beyond this the attempt at interpretation becomes frankly speculative; one could, for instance, work out a hypothesis equating the glycotropic and ketogenic effects, while, on the other hand, Jensen and Grattan [25d] produced evidence that the glycotropic and corticotropic factors are identical.

It was formerly thought that hypophysectomized animals were resistant to the hyperglycemic action of epinephrine and that this might explain their sensitivity to insulin, but it is chiefly when the solution of epinephrine hydrochloride is injected subcutaneously that the sluggish circulation of the hypophysectomized animal, with its atrophic thyroid, prevents epinephrine from acting with its usual vigor.[28] Pretreatment with certain pituitary extracts diminishes the response to epinephrine; this is due to the presence of an unidentified factor probably originating in the posterior lobe.[29]

EFFECT OF HYPOPHYSECTOMY ON PANCREATIC DIABETES

No discovery has more sharply focused attention on the role of the anterior lobe of the hypophysis in carbohydrate metabolism than that of Houssay and Biasotti,[30] who showed, first in toads and then in dogs, that hypophysectomy profoundly modifies and moderates the intense diabetes usually produced by complete pancreatectomy; this discovery has been repeatedly confirmed and extended. Survival without insulin is greatly prolonged, and the animals are as likely to die in a hypoglycemic crisis as from diabetes; glycosuria and hyperglycemia do not become extreme, and are

28. Braier, B.: Influence de l'adrénaline sur le métabolisme azoté et la glycémie des chiens hypophysoprives, Compt. rend. Soc. de biol. **108**: 491-493 (Oct. 30) 1931. Heinbecker, P. T., and Weichselbaum, T. E.: Blood Sugar Response to Intraperitoneal Epinephrine Injections in Normal and Hypophysectomized Dogs, Proc. Soc. Exper. Biol. & Med. **37**: 527-529 (Dec.) 1937. Russell and Cori.[9] De Bodo, R. C.: Sweet, J. E., and Bloch, H. I.: Role of the Anterior Pituitary in Adrenaline Hyperglycemia and Liver Glycogenolysis, Am. J. Physiol. **133**: P 218 (June 1) 1941.

29. Stenström, T.: Das Pituitrin und die Adrenalinhyperglykämie, Biochem. Ztschr. **58**: 472-482, 1914. Neufeld, A. H., and Collip, J. B.: Further Studies on the Antagonist to Adrenalin Hyperglycemia in Pituitary Extracts, Endocrinology **25**: 775-781 (Nov.) 1939.

30. Houssay, B. A., and Biasotti, A.: Hypophysectomie et diabète pancréatique chez le crapaud, Compt. rend. Soc. de biol. **104**: 407-410 (May 30) 1930; Le diabète pancréatique des chiens hypophysectomisés, ibid. **105**: 121-123 (Oct. 16) 1930.

controlled by small amounts of insulin; ketosis is slight or absent; metabolic rate and nitrogen excretion do not show the usual increase, and weight is lost slowly if at all; liver glycogen stores are quite well maintained, and although the tolerance to administered dextrose is quite low,[31] excretion is not quantitative, and the respiratory quotient is not pegged at the fat level, while during fasting the low values of the urinary dextrose-nitrogen ratio indicate that much of the carbohydrate produced by glyconeogenesis is being consumed.

Long and Lukens [32a] showed in cats and dogs that adrenalectomy affected pancreatic diabetes in much the same way as hypophysectomy did in Houssay's experiments, and they proved that it was the cortex rather than the medulla of the adrenal which was of importance. Their animals received cortical extract in quantities sufficient to prevent serious disturbance of water and salt metabolism but apparently insufficient to replace the function of the glands in carbohydrate metabolism; in later experiments they found that adrenalectomy abolished glycosuria in partially depancreatized rats, maintained after the second operation with saline solution.[3] The effect of thyroidectomy on pancreatic diabetes is slight and inconstant.

Discussion of these adrenal-pituitary relations will be deferred until the action of pituitary extracts on animals doubly operated on has been described, but it may be well to point out here that while in the dog and still more in the cat pancreatectomy alone produces exceedingly severe and rapidly fatal diabetes, there are other species (including pig, sheep and monkey) in which the diabetes is of a relatively mild type, with low dextrose-nitrogen ratios, little ketosis and great sensitivity to insulin—resembling the condition observed in the hypophysectomized-depancreatized, or "Houssay," dog or cat, or clinical diabetes of moderate severity. It is especially interesting to note that cases have been encountered in the clinic in which fibrotic or other

31. Chambers, W. H.; Sweet, J. E., and Chandler, J. P.: Carbohydrate Metabolism in the Hypophysectomized Depancreatized Dog, Am. J. Physiol. **119**: 286-287 (June) 1937. Lichtman, A. L.: Fatty Acids and Glucose in Blood of Depancreatized Dogs, J. Biol. Chem. **120**: 35-40 (Aug.) 1937.

32. (*a*) Long, C. N. H., and Lukens, F. D. W.: The Effects of Adrenalectomy and Hypophysectomy upon Experimental Diabetes in the Cat, J. Exper. Med. **63**: 465-490 (April) 1936. (*b*) Long, C. N. H.; Lukens, F. D. W., and Dohan, F. C.: Adrenalectomized-Depancreatized Dogs, Proc. Soc. Exper. Biol. & Med. **36**: 553-554 (May) 1937.

change had almost completely obliterated the islet tissue of the pancreas, yet the diabetes resembled that produced by pancreatectomy in the monkey [33] rather than that in the dog, and was less severe than in many other cases of clinical diabetes in which there was little or no pathologic alteration of the islets.

DIABETOGENIC ACTION OF ANTERIOR PITUITARY EXTRACTS

The injection of anterior pituitary extracts of many kinds is not infrequently followed by slight transient hyperglycemia; this has been ascribed to contamination with posterior-pituitary principles or to nonspecific stimulation of epinephrine discharge, and does not in any way merit the name "diabetogenic." But if fresh simple extracts that have been cautiously prepared are administered in large doses day after day to well nourished dogs, cats or rabbits, one may observe in many individual animals after some days a gradual but maintained rise of the blood sugar beyond glycosuric levels, moderate ketonuria, increased excretion of nitrogen and other symptoms of diabetes.[34] At its height the condition may simulate in severity that produced by pancreatectomy, from which it differs chiefly in maintenance of body weight, maintenance of liver glycogen and resistance to insulin treatment.[5] It is interesting to compare this with the resistance to insulin exhibited in those patients with diabetes in whom acromegaly and hyperthyroidism point to hyperfunction of the anterior lobe of the pituitary as the primary disturbance. Yet it must not be supposed that the secretion of insulin continues unimpaired during the temporary diabetes produced by anterior pituitary extracts, for degranulation and hydropic degeneration

33. Collip, J. B.; Selye, Hans, and Neufeld, A. H.: Experimental Pancreatic Diabetes in the Monkey, Am. J. Physiol. **119**: 332 (June) 1937; Canad. M. A. J. **37**: 287-288 (Sept.) 1937.

34. Johns, W. S.; O'Mulvenny, T. O.; Potts, E. B., and Laughton, N. B.: Studies on the Anterior Lobe of the Pituitary Body, Am. J. Physiol. **80**: 100-106 (March) 1927. Evans, H. M.; Meyer, Karl; Simpson, Miriam E., and Reichert, F. L.: Disturbance of Carbohydrate Metabolism of Normal Dogs Injected with the Hypophysial Growth Hormone, Proc. Soc. Exper. Biol. & Med. **29**: 857-858 (April) 1932. Baumann, E. J., and Marine, David: Glycosuria in Rabbits Following Injections of Saline Extract of Anterior Pituitary, ibid. **29**: 1220-1223, (June) 1932. Houssay, B. A.; Biasotti, A., and Rietti, C. T.: Acción diabetogena del extracto antero-hipofisario, Rev. Soc. argent. de biol. **8**: 469-481 (Aug.-Sept.) 1932. Evans, E. I.: Diabetogenic Principle of Anterior Pituitary, Proc. Soc. Exper. Biol. & Med. **30**: 1370-1371 (June) 1933.

of the beta cells of the islets of Langerhans occur,[35] the amount of insulin extractable from the pancreas falls to a low level,[36] and when such a pancreas is transplanted into a diabetic animal it seems to produce little insulin.[37] It seems unlikely that the anterior pituitary extract should have a directly harmful influence on the pancreas and more probable that the islets become exhausted because of the tendency of substances in the pituitary extract to elevate blood sugar and neutralize insulin action; similar changes in the islets are observed when blood sugar levels are kept high by injections of dextrose,[38] and a supply of extra insulin may protect the islets against the anterior pituitary extract.[39]

Extracts which are diabetogenic in the dog have no such action in the guinea pig, mouse or rat; in the last, indeed, they cause a considerable increase in the quantity of islet tissue found in the pancreas and in the amount of insulin extractable therefrom;[40] this "pancreotropic" effect is not now obtainable in the circumstances in which it was first described.[41] One concludes that in some species the islet tissue is sufficiently adaptable to bear any strain that has yet been thrown upon it.

The type of experimental diabetes described in the foregoing paragraphs is well called temporary. Not merely does it soon subside after the treatment has ceased, but it does not persist for more than a few days even when the injections are continued at a constant level; the diabetic symptoms vanish, though resistance to insulin (glycotropic effect) persists. But an increase in the amount of anterior pituitary extract given will reestablish the diabetic state for a few days more, until

35. Richardson, K. C., and Young, F. G.: Histology of Diabetes Induced in Dogs by Injection of Anterior-Pituitary Extracts, Lancet **1**: 1098-1101 (May 14) 1938. Richardson, K. C.: Influence of Diabetogenic Anterior Pituitary Extracts on the Islets of Langerhans in Dogs, Proc. Roy. Soc., London, s. B **128**: 153-169 (Jan. 4) 1940.

36. Best, C. H.; Campbell, James, and Haist, R. E.: Effect of Anterior Pituitary Extracts on the Insulin Content of the Pancreas, J. Physiol. **97**: 200-206 (Dec. 14) 1939.

37. Houssay, B. A., and Foglia, V. G.: Diabetes antero-hipofisaria y funcion endocrina pancreatica, Rev. Soc. argent. de biol. **12**: 237-252 (Aug.) 1936.

38. Woerner, C. A.: Studies of Islets of Langerhans After Continuous Intravenous Injection of Dextrose, Anat. Rec. **71**: 33-57 (May 25) 1938.

39. Campbell, James; Haist, R. E.; Ham, A. W., and Best, C. H.: Insulin Content of the Pancreas as Influenced by Anterior Pituitary Extract and Insulin, Am. J. Physiol. **129**: P328-P329 (May) 1940.

40. Richardson, K. C., and Young, F. G.: Pancreotropic Action of Anterior Pituitary Extracts, J. Physiol. **91**: 352-364 (Dec. 14) 1937. Marks, H. P., and Young, F. G.: Pancreotropic Action of Anterior Pituitary Extracts, Chem. & Industry **58**: 652, 1939.

41. Anselmino, K. J.; Herold, L., and Hoffmann, F.: Ueber die pankreatrope Wirkung von Hypophysenvorderlappenextrakten, Klin. Wchnschr. **12**: 1245-1247 (Aug. 12) 1933.

finally a dose is reached (say, the equivalent of 25 Gm. of fresh gland daily) at which the animal can no longer escape: the diabetes not only persists as long as treatment continues but reaches a point (often marked by transient exacerbation of ketonuria) at which the degeneration of the islets becomes irreversible, so that the diabetes continues indefinitely and unabated even when the extract is withdrawn.[42]

Such permanently diabetic dogs vary in the intensity of diabetes exhibited, but in general their insulin requirements, sugar excretion and dextrose-nitrogen ratios are even higher than those usually recorded for depancreatized dogs on the same diet; ketonuria, however, may be low in the early stages, weight is better maintained, and survival without insulin is much longer; [43a] the islet tissue is altered to a variable extent, and hardly any insulin can be extracted from it; surgical removal of the pancreas may have little influence, once the permanent diabetes is established. One feels that if there are real differences between dogs that are diabetic because their islet tissue collapsed under treatment with an anterior pituitary extract weeks or months previously and dogs that are diabetic because the whole pancreas has been removed, such differences must be due to the loss of the pancreatic acinar tissue in the latter rather than to persistence of any extrapancreatic effect of the anterior pituitary extract in the former. Lukens and Dohan [43b] have found that when partially depancreatized, nondiabetic cats are treated with crude extracts of the anterior lobe, diabetes appears and

42. Young, F. G.: Permanent Experimental Diabetes Produced by Pituitary (Anterior Lobe) Injections, Lancet **2**: 372-374 (Aug. 14) 1937; Preparation and Properties of Diabetogenic Extracts, J. Endocrinol. **1**: 339-355 (Nov.) 1939. Campbell, James and Best, C. H.: Production of Diabetes in Dogs by Anterior-Pituitary Extracts, Lancet **1**: 1444-1445 (June) 1938. Houssay, B. A., and Biasotti, A.: Acción diabetogena de diversas hormonas hipofisarias, Rev. Soc. argent. de biol. **14**: 297-307 (Aug.) 1938. Dohan, F. C., and Lukens, F. D. W.: Persistent Diabetes Following Injection of Anterior Pituitary Extract, Am. J. Physiol. **125**: 188-195 (Jan.) 1939. Loubatières, A.: Récherches sur le diabète sucré permanent consécutif aux injections d'extrait de lobe antérieur d'hypophyse chez le chien normal, Compt. rend. Acad. d. sc. **208**: 1933-1935 (June 12) 1939.

43. (*a*) Marks, H. P., and Young, F. G.: Observations on the Metabolism of Dogs Made Permanently Diabetic by Treatment with Anterior Pituitary Extract, J. Endocrinol. **1**: 470-510 (Dec.) 1939. Dohan, F. C.; Fish, C. A., and Lukens, F. D. W.: Induction and Course of Permanent Diabetes Produced by Anterior Pituitary Extract, Endocrinology **28**: 341-357 (March) 1941. Dohan, F. C.; Chambers, A. H., and Fish, C. A.: Metabolism of Dogs with Permanent Diabetes Produced by Anterior Pituitary Extract, ibid. **28**: 566-579 (April) 1941. (*b*) Lukens, F. D. W., and Dohan, F. C.: Morphological and Functional Recovery of the Pancreatic Islands in Diabetic Cats Treated with Insulin, Science **92**: 222-223 (Sept. 6) 1940; Pituitary Diabetes in the Cat Treated by Low Diet, Insulin, Phlorhizin and Adrenalectomy, J. Clin. Investigation (Proc.) **20**: 444 (July) 1941.

persists after cessation of injections, and the remaining islet tissue shows hydropic degeneration; this change, however, is not irreversible at first; for some weeks it is possible to obtain both structural and functional recovery by treating the cats with insulin or phlorhizin or by adrenalectomy, or even—if the diabetes happens to be mild—by restriction of diet.

ANTERIOR PITUITARY EXTRACTS IN DEPANCREATIZED ANIMALS

It is probably desirable to restrict the term "diabetogenic" to extracts capable of producing temporary if not permanent diabetes in normal dogs,[5] but it has sometimes been applied in a rather different way. To produce diabetes in normal animals, even in the susceptible individuals of relatively susceptible species, one requires large doses of extracts which have been carefully protected from the destructive action of heat or powerful reagents; but in subtotally depancreatized animals (even of resistant species such as the rat) or hypophysectomized-depancreatized ("Houssay") animals, diabetic symptoms can be elicited easily, or intensified if already present, with moderate doses of extracts of many kinds. The conclusion is inescapable that true diabetogenic potency involves the action of one or more very labile factors as well as of relatively stable factors that may be effective by themselves in animals deprived of all or most of their insulin-producing tissue.

Among these relatively stable factors one may certainly include the corticotropic principle, for extracts which evoke intense glycosuria and ketonuria in hypophysectomized-depancreatized cats have little or no effect on adrenalectomized-depancreatized animals;[32a] adrenal cortical steroids cause glycosuria in subtotally depancreatized, and sometimes even in normal, rats; it is surprising to find that natural and synthetic estrogens have a similar action.[44] But in the latter

44. Fry, E. G.; Long, C. N. H., and Ritter, H. B.: Aggravation of Pancreatic Diabetes by Adrenal Cortical Extract, Am. J. Physiol. **126**: 497 (July) 1939. Ingle, D. J., and Thorn, G. W.: Comparison of the Effects of 11-Desoxycorticosterone Acetate and 17-Hydroxy-11-Dehydro-Corticosterone in Partially Depancreatized Rats, ibid. **132**: 670-678 (April 1) 1941. Ingle, D. J.: Production of Glycosuria in the Normal Rat by Stilboestrol and by 17-Hydroxy-11-Dehydro-Corticosterone, ibid. **133**: P 337 (June 1) 1941. Dolin, G.; Joseph, S., and Gaunt, R.: Effect of Steroid and Pituitary Hormones on Experimental Diabetes Mellitus of Ferrets, Endocrinology **28**: 840-845 (May) 1941. Young, F. G.: Influence of Oestrogens on Experimental Canine Diabetes Mellitus, Lancet **1**: 600 601 (May 10) 1941.

test the action of the cortical steroids is not as great when they are administered by themselves as when they are administered simultaneously with anterior pituitary extracts (a further example of the type of synergism already discussed[20]); moreover, anterior pituitary extracts may evoke glycosuria in partially depancreatized adrenalectomized dogs;[45] hence the corticotropic factor is certainly not the only one involved. In considering the identity of the labile factor or factors, one naturally thinks of the "growth hormone," which is closely associated with diabetogenic activity;[46] and it has been shown recently that even brief heat treatment largely destroys the striking power of fresh anterior pituitary extracts to increase the body glycogen of normal fasting mice.[47] In viewing the diabetogenic phenomenon as a whole one may cite the evidence (quoted in the discussion of the hypoglycemia of fasting animals) that anterior pituitary principles often tend to increase glyconeogenesis; one may be inclined also to credit them with the power to diminish the utilization of carbohydrate by the peripheral tissues, even if this seems to elude direct demonstration,[14] and one has, unfortunately, several different unsupported interpretations of the glycotropic phenomenon to draw on as required.

THE KETOGENIC PHENOMENON

The urinary excretion of acetone bodies by the fasting or fat-fed rat, normally quite small, may be greatly increased by injecting anterior pituitary extracts;[48]

45. Houssay, B. A., and Biasotti, A.: Acción diabetogena anterohipofisaria en perros sin suprarenales, Rev. Soc. argent. de biol. **14**: 308-314 (Aug.) 1938.

46. Shipley, R. A., and Long C. N. H.: Studies on the Ketogenic Activity of the Anterior Pituitary, Biochem. J. **32**: 2242-2256 (Dec.) 1938.

47. Neufeld, A. H.; Scoggan, S. M., and Stewart, G. S.: Effect of Pituitary Preparations on the Total Body, Glycogen, Water, Nitrogen, and Fat of Fasted Mice, Endocrinology **27**: 132-136 (July) 1940. Neufeld, A. H., and Collip, J. B.: Effect of Pituitary Preparations on Glycogen Stores of Fasted Mice, ibid. **28**: 926-932 (June) 1941.

48. Burn, J. H., and Ling, H. W.: Ketonuria in Rats on a Fat Diet After Injection of Pituitary (Anterior Lobe) Extract, J. Physiol. **69**: xix (March 15) 1930; Excretion of Acetone Bodies on a Fat Diet as Affected by the Injection of Pituitary (Anterior Lobe) Extract and by Pregnancy, Quart. J. Pharm. & Pharmacol **6**: 31-38 (Oct.-Dec.) 1933. Hoffmann, F., and Anselmino, K. J.: Das Fettstoffwechselhormon des Hypophysenvorderlappens, Klin. Wchnschr. **10**: 2383-2386 (Dec. 26) 1931. Magistris, H.: Das Fettstoffwechselhormon des Hypophysenvorderlappens, Endokrinologie **11**: 176-191, 1932. Butts, J. S.; Cutler, C. H., and Deuel, H. J.: Sexual Variation in Carbohydrate Metabolism: Role of Anterior Pituitary in Metabolism of Diacetic Acid, J. Biol. Chem. **105**: 45-58 (April) 1934. Black, P. T.; Collip, J. B., and Thomson, D. L.: Effect of Anterior Pituitary Extracts on Acetone Body Excretion in the Rat, J. Physiol. **82**: 385-391 (Oct. 17) 1934. Shipley, R. A.: Effect of Adrenalectomy on Ketosis Produced in Rats by Anterior Pituitary Extract, Endocrinology **26**: 900-905 (May) 1940.

there is a simultaneous rise in blood ketones, which is a more sensitive and dependable index of the phenomenon, since acetoacetic and hydroxybutyric acids are to some extent threshold substances. Parallel observations have been made in other species, including the mouse, guinea pig, rabbit, dog and man. The effect is lessened but not abolished after adrenalectomy.

A general discussion of this phenomenon has been published elsewhere;[49] there is good evidence that it indicates an increased outpouring of ketone bodies by the liver rather than a diminished utilization of them;[50] and in general the ketosis is accompanied by a transfer of fat from the depots to the liver, in which large quantities may accumulate.[51] There is accordingly a good deal of support for the view that the phenomenon represents a general stimulation of fat catabolism beyond the capacity of the body to oxidize the ketonic final products. Yet other possibilities exist; fatty acids are not the only conceivable source of acetone bodies, and there may well be different types of ketonemia; if stimulation of fat metabolism does occur, it may be secondary rather than direct.

Shipley and Long,[46] for instance, have suggested that the primary effect of crude extracts of the anterior lobe may be to *retard* the breakdown of tissue protein so that the energy requirements of the body are met by drawing on preformed carbohydrate stores (which are rapidly exhausted to the point of hypoglycemia[52]) and on the fat depots, whose overactive response may produce ketonemia. Some retardation of protein catabolism is, as already pointed out, required of a "growth hormone,"

49. Thomson, D. L.: Anterior Pituitary and Metabolism of Acetone Bodies, A. Research Nerv. & Ment. Dis., Proc. (1936) **17**: 257-267, 1938.

50. Mirsky, I. A.: Source of Blood Acetone Resulting from Administration of Ketogenic Principle of Anterior Hypophysis, Am. J. Physiol. **115**: 424-428 (April) 1936.

51. Best, C. H., and Campbell, James: Anterior Pituitary Extracts and Liver Fat, J. Physiol. **86**: 190-203 (Feb. 8) 1936; Effect of Anterior Pituitary Extract on Liver Fat of Various Animals, ibid. **92**: 91-110 (Feb. 16) 1938. Barrett, H. M.; Best, C. H., and Ridout, J. H.: Study of Source of Liver Fat Using Deuterium as Indicator, ibid. **93**: 367-381 (Sept. 16) 1938. Neufeld, A. H., and Collip, J. B.: Effect of Pituitary Extracts on Ketonuria, Fat Content and Fat Distribution in the Liver and Tissues of Mice, Endocrinology **25**: 768-774 (Nov.) 1939. Campbell, J., and Kennan, H. C.: The Substance of the Anterior Pituitary Gland Which Increases Liver Fat, Am. J. Physiol. **131**: 27-35 (Nov. 1) 1940.

52. Harrison, H. C., and Long, C. N. H.: Effect of Anterior Pituitary Extract on the Metabolism of Fasting Normal and Adrenalectomized Rats, Am. J. Physiol. **126**: 526-527 (July) 1938. Paschkis, K. E.: Influence of Anterior Pituitary Extract on Protein and Carbohydrate Metabolism, ibid. **133**: P 409 (June 1) 1941.

and it has in fact been observed in many experiments.[53] At first sight this appears to be a bewildering contradiction of the thesis already developed, that anterior pituitary extracts (especially those containing the corticotropic principle) stimulate protein breakdown and glyconeogenesis, and certainly it is probable that one is here dealing with another factor. It is quite possible that the balance is held by the islets of Langerhans; nitrogen retention will be favored as long as they continue to produce sufficient insulin, nitrogen loss when they break down under the stimulus. Thus Mirsky,[54] using the rate of increase of nonprotein nitrogen in the blood of nephrectomized dogs to measure the catabolism of protein, finds it to be accelerated by crude extracts of the anterior lobe in depancreatized (diabetic) animals but slightly diminished in normal ones. However, increased nitrogen retention and increased glycosuria may at times be observed simultaneously.[55]

A great deal of effort has been expended in the study of the chemical and physical properties of the factors described and in attempts to separate them by chemical means; the results are of great importance to those actually working in the field but are unfortunately so full of apparent contradictions that they can rarely be used to support general statements and have accordingly been given little attention in this review.

53. Teel, H. M., and Cushing, Harvey: Studies in the Physiological Properties of Growth-Promoting Extracts of the Anterior Hypophysis, Endocrinology **14**: 157-163 (May-June) 1930. Gaebler, O. H.: Some Effects of Anterior Pituitary Extracts on Nitrogen Metabolism, Water Balance, and Energy Metabolism, J. Exper. med. **57**: 349-363 (March) 1933. Lee, M. O., and Schaffer, N. K.: Anterior Pituitary Growth Hormone and the Composition of Growth, J. Nutrition **7**: 337-363 (March 10) 1934. Schaffer, N. K., and Lee, M. O.: Effect of Anterior Pituitary Growth Hormone on Protein Metabolism, J. Biol. Chem. **108**: 355-371 (Feb.) 1935.

54. Mirsky, I. A.: The Influence of the Anterior Pituitary Gland on Protein Metabolism, Endocrinology **25**: 52-56 (July) 1939.

55. Gaebler, O. H., and Galbraith, H. W.: Effects of Anterior Pituitary Preparations in Experimental Pancreatic Diabetes, Endocrinology **28**: 171-178 (Feb.) 1941.

CHAPTER V

LACTOGENIC AND MAMMOGENIC HORMONES

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Investigations of the past twelve years provide much new information concerning the regulation of growth and of secretion in the mammary glands. Both growth and secretion are controlled mainly by hormones, but quite different hormones are involved in these two wholly distinct sets of processes. The anterior lobe of the pituitary exercises a direct control (through its lactogenic hormone, prolactin) over milk secretion; in addition it exercises an indirect control (through estrogen, progesterone and possibly other sterols) and perhaps also a direct control (through a mammogenic pituitary hormone) over the development and growth of the mammary glands.

THE LACTOGENIC HORMONE

The Lactogenic Response.—Stricker and Grüter demonstrated in 1928-1929 that a factor or combination of factors directly essential to lactation is produced by the anterior lobe of the pituitary. Their results were confirmed first by Corner in 1930 and thereafter extended to various species by Nelson and Pfiffner, Turner and Gardner, Asdell and others. Indeed both mammary proliferation and lactation were observed to follow the administration of crude pituitary extracts in females with intact ovaries by Parkes in 1929 (rabbits), by Putnam, Benedict and Teel (bitches) and by Evans and Simpson (rats), but this effect either was or could then be attributed to pituitary influence on hormone production in the ovary. A related response, the enlargement of the crop sacs of pigeons with formation of "crop milk," was reported in 1931 by Riddle and Braucher. All these results were obtained with simple glandular extracts—mixtures of various pituitary products—and provided no indication of the identity of the hormone or hormones that excited this response.

Isolation and Identification of the Hormone.—The individuality of the hormone that excites lactation was established in 1932-1933 through its isolation and study by Riddle, Bates and Dykshorn,[1] who definitely associated it with lactogenic and crop-stimulating functions and called it prolactin. Confirmation of its individuality and lactogenic function was soon supplied by Catchpole and Lyons[2] and Lyons and other associates,[3] who called the hormone mammotropin, and by Gardner and Turner,[4] who also fully described the mammary changes accompanying its lactogenic action in the rabbit. Gardner and Turner called this hormone galactin.

Occurrence.—The hormone is probably present in the hypophyses of all vertebrates but is more plentiful in some species and at certain stages of the life cycle than others. Beef and sheep glands usually yield 30 to 40 international units per gram of fresh tissue, while pork glands contain much less.[5] Pituitaries of fetal calves were once reported[6] to contain much more prolactin (78 + units) than the glands of adult or pregnant cows (38-44 units), but a later report[7] stated these values in essentially the reverse order. In the study represented in the latter report a higher concentration of hormone was found in glands from dairy cattle than in those from beef cattle, and in both studies a somewhat higher concentration was found in glands from pregnant (or lactating) cows than in those of heifers. There is evidence that

1. (*a*) Riddle, Oscar; Bates, R. W., and Dykshorn, S. W.: A New Hormone of the Anterior Pituitary, Proc. Soc. Exper. Biol. & Med. **29**: 1211 (June) 1932; (*b*) Prolactin, a New and Third Hormone of the Anterior Pituitary, Anat. Rec. (supp.) **54**: 25 (Nov.) 1932; (*c*) The Preparation, Identification and Assay of Prolactin—a Hormone of the Anterior Pituitary, Am. J. Physiol. **105**: 191 (July) 1933.

2. Catchpole, H. R., and Lyons, W. R.: The Lactation Hormone of the Hypophysis, Anat. Rec. (supp.) **55**: 49 (March) 1933.

3. Lyons, W. R., and Catchpole, H. R.: Assay with the Guinea Pig of the Lactogenic Hypophysial Hormone, Proc. Soc. Exper. Biol. & Med. **31**: 299 (Nov.) 1933. Catchpole, H. R.; Lyons, W. R., and Regan, W. M.: Induction of Lactation in Heifers with the Hypophysial Lactogenic Hormone, ibid. p. 301. Lyons, W. R.; Chaikoff, I. L., and Reichert, F. L.: Experiments with Hypophysial Lactogenic Hormone on Normal, Ovariectomized and Hypophysectomized Dogs, ibid., p. 303. Lyons, W. R., and Catchpole, H. R.: Availability of the Rabbit for Assay of the Hypophysial Lactogenic Hormone, ibid., p. 305.

4. Gardner, W. U., and Turner, C. W.: The Function, Assay and Preparation of Galactin, a Lactation Stimulating Hormone of the Anterior Pituitary and an Investigation of the Factors Responsible for the Control of Normal Lactation, research bulletin 196, University of Missouri, College of Agriculture, Agricultural Experiment Station, 1933, pp. 1-60.

5. Bates, R. W., and Riddle, Oscar: The Preparation of Prolactin, J. Pharmacol. & Exper. Therap. **55**: 365 (Nov.) 1935.

6. Bates, R. W.; Riddle, Oscar, and Lahr, E. L.: An Assay of Three Hormones Present in Anterior Pituitaries of Seven Types of Cattle Classified for Age, Sex and State of Reproduction, Am. J. Physiol. **113**: 259 (Oct.) 1935.

7. Reece, R. P., and Turner, C. W.: The Lactogenic and Thyrotropic Hormone Content of the Anterior Lobe of the Pituitary Gland, research bulletin *266*, University of Missouri College of Agriculture, Agricultural Experiment Station, 1937, pp. 1-104.

prolactin is formed in the eosinophilic cells[8] and that administration of estrogen increases the amount of it (stored?) in the rat gland.[9] Small amounts have been found repeatedly in postpartum human urine (also in urine of normal men and in that of infants during the first week) since this was first reported by Lyons and Page,[10] who stated that the amount excreted daily is equal to that found in one beef pituitary. It is alleged that two peaks of excretion occur within the normal cycle of the female sex: during menstruation and during ovulation.[11] Positive tests were reported for human serum[12] and for young (not for mature) placentas[13] and notable quantities from cystic human breasts.[14] Hoffmann[15] found less than the expected amounts of prolactin in the urine of seven of eight parturient women whose secretion of milk was subnormal. Rabald and Voss[16] reported recovery of a prolactin-like substance from the livers of healthy cattle and hogs but not from horse liver.

Preparation and Assay.—Currently used methods of preparing[17] prolactin from pituitary tissue involve its initial extraction in acid or alkaline medium (aqueous or 60 per cent alcohol [ethyl alcohol], or acid acetone), its isoelectric precipitation in an aqueous medium and repeated washing of this precipitate. Comparisons of the various methods are available.[18] Current methods of assaying prolactin were surveyed by McShan and Turner,[19] Bergman and Turner,[20] and Bates;[21] crop sacs of pigeons (macro and micro tests available) or of lactation in

8. Schooley, J. P., and Riddle, Oscar: The Morphological Basis of Pituitary Function in Pigeons, Am. J. Anat. **62**: 313 (March) 1938.

9. Reece, R. P., and Turner, C. W.: Experimentally Altering Galactin Content of the Rat Pituitary, Proc. Soc. Exper. Biol. & Med. **36**: 283 (April) 1937.

10. Lyons, W. R., and Page, Emery: Detection of Mammotropin in the Urine of Lactating Women, Proc. Soc. Exper. Biol. & Med. **32**: 1049 (April) 1935.

11. Ehrhardt, Karl, and Voller, H. F.: Untersuchungen über das Laktations-hormon des Hypophysenvorderlappens, Endokrinologie **22**: 19, 1939.

12. Tesauro, G.: Contributo allo studio dell'ormone galattogeno, Pediatria **44**: 665 (Aug.) 1936.

13. Ehrhardt, Karl: Ueber das Laktationshormon des Hypophysenvorderlappen, München. med. Wchnschr. **29**: 1163 (July 17) 1936.

14. Geschickter, C. F., and Lewis, Dean: Lactogenic Substance in the Human Breast, Arch. Surg. **32**: 598 (April) 1936.

15. Hoffmann, Friedrich: Ueber die hypophysäre Hypogalaktie, Zentralbl. f. Gynäk. **61**: 35 (Jan. 2) 1937.

16. Rabald, E., and Voss, H. E.: Ueber Vorkommen und Eigenschaften des Laktationshormons, Ztschr. f. physiol. Chem. **261**: 71, 1939.

17. Bates and Riddle.[8] McShan and Turner.[19] Lyons.[22]

18. Riddle, Oscar, and Bates, R. W.: The Preparation, Assay and Actions of Lactogenic Hormone, in Allen, Edgar; Danforth, C. H., and Doisy, E. A.: Sex and Internal Secretions, ed. 2, Baltimore, Williams & Wilkins Company, 1939, chap. 20, p. 1088. Bergman and Turner.[20]

19. McShan, W. H., and Turner, C. W.: Further Purification of Galactin, the Lactogenic Hormone, Proc. Soc. Exper. Biol. & Med. **32**: 1655 (June) 1935.

20. Bergman, A. J., and Turner, C. W.: Comparison of Methods of Extraction of the Lactogenic Hormone, J. Biol. Chem. **118**: 247 (March) 1937.

21. Bates, R. W.: Methods for the Assay of Prolactin, in Cold Spring Harbor Symposia on Quantitative Biology, Cold Spring Harbor, L. I., New York, The Biological Laboratory, 1937, vol. 5, p. 191.

pseudopregnant rabbits. The micro test in pigeons is said to be sensitive to 0.01 microgram of lactogenic hormone.[22]

For assay of this hormone in urine (or serum) Lyons[22] now injects unconcentrated urine intradermally (micro test) over the crop sacs of pigeons 1 month old. Four daily injections (0.05 to 0.5 cc.) are made at five sites on each crop gland (feathers removed), with necropsy twenty-four hours after the last injection. After stripping away the muscle and the inflammatory exudate which always results from the toxic substances in the urine and which masks minimal reactions, the crop wall is inspected against good light for stimulation at the sites of injection. By using varying quantities on a few birds, a standard or minimal effective dose of the urine (preserved with hexylresorcinol) is determined.

Four quite different macro units ("pigeon units") now confuse the literature, but a practical solution is at hand. A standard preparation of the lactogenic (crop gland-stimulating) substance—to be distributed on behalf of the League of Nations Health Organization—is available from the National Institute for Medical Research, Hampstead, London, England. Samples made in different laboratories were combined to form this standard preparation, and it was decided that "agreement shall be reached among the members of the Conference as to the activity of the standard preparation in 'Riddle-Bates units' per milligramme, and that the international unit shall be thus defined in terms of the standard preparation."[23]

Properties.—Prolactin is insoluble in all fat solvents. It is soluble in water except in the isoelectric region p_H 5-6 and in strongly acid solution, < 0.5. When made acid to the isoelectric point, the extract containing prolactin is precipitated by the usual protein precipitants—e. g., by tannic, phosphotungstic, flavianic and trichloroacetic acids and by trinitrophenol. It may be salted out with sodium (or ammonium) sulfate or sodium chloride, the amount required depending on the p_H. It is also precipitated by basic salts of heavy metals at or near p_H 7.[24] It is rapidly inactivated by trypsin[25] and pepsin.[26] Salt-free solutions at p_H 8 withstand boiling for one hour with little loss;

22. Lyons, W. R.: Preparation and Assay of Mammotropin, in Cold Spring Harbor Symposia on Quantitative Biology, Cold Spring Harbor, L. I., New York, The Biological Laboratory, 1937, vol. 5, p. 198.

23. Report of the Third International Conference on the Standardization of Hormone: IV. Consideration of the Lactogenic (Crop-Gland-Stimulating) Substance of the Anterior Lobe of the Pituitary Gland ("Prolactin," "Galactin," Mammotropin), Bull. Health Org. League of Nations **7**: 892 (Oct.) 1938.

24. Bates, R. W., and Riddle, Oscar: Preparation of Prolactin Free from Other Pituitary Hormones and Preparation of a Mixture of Other Pituitary Hormones Free from Prolactin, J. Biol. Chem. **123**: v (May) 1938.

25. Bates, R. W.; Riddle, Oscar, and Lahr, E. L.: On the Protein Nature of Prolactin and of Follicle-Stimulating Hormones, Proc. Soc. Exper. Biol. & Med. **31**: 1223 (June) 1934.

26. McShan, W. H., and French, H. E.: The Chemistry of the Lactogenic Hormone Extracts, J. Biol. Chem. **117**: 111 (Jan.) 1937.

under other conditions the prolactin may be rapidly destroyed.[27] A crystalline protein with fairly high lactogenic activity was extensively studied by White, Catchpole and Long [28] and found to have the following elemental composition in percentage: carbon 51.11, hydrogen 6.76, nitrogen 14.38, sulfur 1.77, phosphorus 0. In their better preparations of prolactin Bates and Riddle [29] found roughly 2 per cent each of tyrosine, cystine and tryptophan. Tests indicated that free amino groups in prolactin are essential to its activity.[30] More advance has been made in the chemical study of prolactin than in that of any other anterior pituitary hormone.

Applications and Actions.—For increasing the milk yield of domestic animals after lactation has been established or during declining lactation, prolactin has sometimes [31] but not always proved efficacious; likewise, from whole pituitary extracts both success and failure are recorded; [32] still other glandular products (notably thyroxin) [33] have sometimes proved of equal or of greater effectiveness for these unusual requirements. Again, the adrenal cortex—perhaps incident to its regulation of fluid and salts or to the production of sterols supporting mammary growth—seems to contribute something essential to the maintenance of milk flow or even to the initiation of this function in some species,[34] though apparently not in the dog.[35] Similarly, hypophysectomy, thyroidectomy or hysterectomy may markedly

27. Riddle, Bates and Dykshorn.[1c] McShan and French.[26]

28. White, Abraham; Catchpole, H. R., and Long, C. N. H.: A Crystalline Protein with High Lactogenic Activity, Science **86**: 82 (July 23) 1937.

29. Riddle and Bates,[18] p. 1093.

30. Li, C. H.; Simpson, M. E., and Evans, H. M.: Action of Ketene on the Pituitary Lactogenic Hormone, Science **90**: 140 (Aug. 11) 1939.

31. Asdell, S. A.; Brooks, H. J.; Salisbury, G. W., and Seidenstein, H. R.: Experiments in the Physiology of Mammary Development and Lactation, Memoir 198, Cornell Agricultural Experiment Station, 1936. Folley, S. J., and Young, F. G.: The Effect of Anterior Pituitary Extracts on Established Lactation in the Cow, Proc. Roy. Soc., London, s. B **126**: 45 (Sept. 23) 1938. Azimoff.[32]

32. Azimoff, G. I.: Probleme der Laktationsphysiologie, in Proceedings of the Fifteenth International Physiological Congress, Leningrad-Moscow, Aug. 9-16, 1935, Moscow, State Biological and Medical Press, 1938, pp. 132-133.

33. Graham, W. R., Jr.: The Action of Thyroxine on the Milk and Milk Fat Production of Cows, Biochem. J. **28**: 1368, 1934. Folley, S. J., and White, Paul: The Effect of Thyroxine on Milk Secretion and on the Phosphatase of the Blood and Milk of the Lactating Cow, Proc. Roy. Soc., London, s. B **120**: 346 (June 2) 1936.

34. Brownell, W. H.; Lockwood, J. E., and Hartman, F. A.: Lactation Hormone of Adrenal Cortex, Proc. Soc. Exper. Biol. & Med. **30**: 783 (March) 1933. Gaunt, Robert, and Tobin, C. E.: Lactation in Adrenalectomized Rats, Am. J. Physiol. **115**: 588 (May) 1936. Nelson, W. O., and Gaunt, Robert: Initiation of Lactation in the Hypophysectomized Guinea Pig, Proc. Soc. Exper. Biol. & Med. **34**: 671 (June) 1936.

35. Kendall, E. C.; Mason, H. L.; Myers, C. S., and Allers, W. D.: Physiological and Chemical Investigation of the Suprarenal Cortex, J. Biol. Chem. **114**: lvii (May) 1936; personal communication to the author.

affect lactation. Finally, estrogen, though certainly helpful in the preparation of mammary tissue, becomes for one true action of prolactin an agent decidedly adverse to milk yield after lactation has been established.[36]

Since it is thus obvious that so much of the organism —assimilative, endocrine and neural—becomes involved in the initiation, augmentation and maintenance of lactation, the term "lactogenic hormone" is equivocal and its use often a serious error; others (not ourselves) have rather generally ventured to employ this term as an alternative designation for that hormone which was found to initiate milk secretion in prepared mammary tissue and which after its isolation and establishment as an entity was called prolactin. Lactogenesis involves several hormones and many other factors; prolactin is a specific substance. Only full recognition of this distinction, along with awareness of other actions of prolactin, is likely to lead to satisfactory clinical use of this substance.

Clinical use of prolactin hitherto has been restricted largely to postpartum women with deficient lactation. In the earliest tests[37] twenty-nine women whose lactation had failed to develop adequately by the sixth to the ninth day were then given one or two intramuscular injections of prolactin, totaling 75 to 400 units. This dosage (highest, 200 units per injection) was well tolerated. In twenty-five of these women the daily milk yield increased by from 50 to 400 Gm. within three to nine days; there were four or five definite failures, in all of which the total dose used was only 100 to 150 units. In eight other women, whose milk secretion was or presumably would become normal, dosage for a single day with 100 to 400 units at one to fifteen days post partum probably induced no change. Werner[38] noted that eight castrates whose

36. Parkes, A. S., and Bellerby, C. W.: The Effects of Injection of Estrin During Lactation, J. Physiol. **62**: 301 (Jan.) 1927. De Jongh, S. E.: Laktationshemmung durch Menformon, Acta brev. Neerland. **3**: 52, 1933. Nelson, W. O.: The Reciprocal Hypophysial Ovarian Relationship as a Factor in the Control of Lactation, Endocrinology **18**: 33 (Jan.-Feb.) 1934. Kurzrok, Raphael, and O'Connell, C. P.: The Inhibition of Lactation During the Puerperium by Testosterone Propionate, Endocrinology **23**: 476 (Oct.) 1938.

37. Kurzrok, Raphael; Bates, R. W.; Riddle, Oscar, and Miller, E. G., Jr.: The Clinical Use of Prolactin, Endocrinology **18**: 18 (Jan.-Feb.) 1934.

38. Werner, A. A.: Experiment to Produce Lactation in Castrate Women, Endocrinology **19**: 144 (March-April) 1935.

breasts were variously prepared (or unprepared) by previous estrogenic treatment did not lactate after intramuscular injection of 200 units of prolactin daily for one to fourteen days; severe local and systemic reactions were produced in three of these women (two others reported on later). Evans [39] briefly reported seven successful tests with total doses of from 1,000 to 2,250 units. Hoffmann [40] noted that a total dose of 200 to 250 units given in two days may or may not increase the milk yield.

Two recent critical studies on the effects of twice-daily injections during two to four days, beginning on the sixth or seventh day post partum, led to somewhat different conclusions, though there were several obvious failures in both series of tests. Ross,[41] using intramuscular injections during two days, gave a total dose of 400 units to nine patients and 1,000 units to twelve others. Only the larger dose appeared to result in an appreciable increase in milk secretion, and only this dose produced any adverse local reactions; the latter involved redness and indurations varying in diameter from 2 to 15 cm., and a rise in temperature to 100 to 102 F. for two to six days. Considered of special significance was the observation that a higher proportion of the mothers given the larger dose were able to nurse their infants after discharge from the hospital. Stewart and Pratt,[42] giving a subcutaneous injection of 1,000 units daily from the sixth to the ninth postpartum day inclusive, got no significant increase in milk secretion from a group of fourteen patients whose secretion was less than 250 cc. on the fifth day. A similar group of ten mothers served as controls, and after leaving the hospital their nursing record was not significantly different from that of the group given prolactin. Stewart and Pratt conclude that the action of prolactin in animals is not analogous to that in women.

The most recent study is also the most complete and informative. Kenny and King [43] treated forty-three

39. Evans, E. I.: The Lactogenic Hormone of the Anterior Pituitary, Proc. Inst. Med. Chicago **11**: 282 (April) 1937.
40. Hoffmann, Friedrich: Ueber die Entstehung der Laktation, Zentralbl. f. Gynäk. **60**: 2882 (Dec. 5) 1936.
41. Ross, J. R.: Prolactin: Its Effect on the Secretion of Woman's Milk, Endocrinology **22**: 429 (April) 1938.
42. Stewart, H. L., Jr., and Pratt, J. P.: Effect of Prolactin on Mammary Gland Secretion, Endocrinology **25**: 347 (Sept.) 1939.
43. Kenny, Méave; King, Earl; Evers, Norman, and Hunan, W. J.: Effect of Prolactin in Nursing Women, Lancet **2**: 828 (Oct. 14) 1939.

women with prolactin for deficient lactation, beginning at different stages up to the third month post partum; forty-three other women, to whom other "galactogogues" were given or on whom routine methods of encouraging lactation were practiced, served as controls. In 74 per cent of the treated women and in only 21 per cent of the controls lactation became sufficient for the whole need of the baby until weaning at the sixth to the seventh month. The complete failures included 19 per cent of the treated women and 63 per cent of the controls. The total dose of prolactin was 900 units, which was given intramuscularly at the rate of two injections a day for five days, as follows: on the first and second days 300 units a day, on the third and fourth days 120 units a day and on the fifth day 60 units. No local or systemic ill effects were observed. The milk produced was of normal composition and quality. They recommended that treatment begin early in order that efficient nursing might be established before discharge from the hospital.

Other actions of prolactin have been reported from animal experimentation, though appropriate clinical applications have not yet been published. In adult birds prolactin has powerful antigonad action, made evident by extremely rapid and nearly complete atrophy of the testes [44] and by speedy inactivation and regression of the ovary.[45] In adult male rats 10 to 20 units of prolactin daily for eight to fifteen days does not decrease the weight of the testes.[46] In female mice [47] and rats [48] dosage with prolactin stops estrous cycles. Small amounts of luteinizing substance also stop the cycles of mice, and since luteinizing substance is a contaminant of many prolactin preparations it has been suggested [49] that this action on the cycles of rodents is

44. Riddle, Oscar, and Bates, R. W.: Concerning Anterior Pituitary Hormones, Endocrinology **17**: 689 (Nov.-Dec.) 1933.

45. Bates, R. W.; Lahr, E. L., and Riddle, Oscar: The Gross Action of Prolactin and Follicle-Stimulating Hormone on the Mature Ovary and Sex Accessories of Fowl, Am. J. Physiol. **111**: 361 (March) 1935. Bates, R. W.; Riddle, Oscar and Lahr, E. L.: The Mechanism of the Anti-Gonad Action of Prolactin, Am. J. Physiol. **119**: 610 (July) 1937.

46. Riddle, Oscar; Lahr, E. L.; Bates, R. W., and Moran, C. S.: Response of Adult Rat Testes, Sex Accessories and Adrenals to Injections of Prolactin, Proc. Soc. Exper. Biol. & Med. **32**: 509 (Dec.) 1934.

47. Dresel, I.: The Effect of Prolactin on the Estrous Cycle of Nonparous Mice, Science **82**: 173 (Aug. 23) 1935.

48. Lahr, E. L., and Riddle, Oscar: Temporary Suppression of Estrous Cycles with Prolactin, Proc. Soc. Exper. Biol. & Med. **34**: 880 (June) 1936.

49. Nathanson, I. T., and Fevold, H. L.: An Analysis of Amenorrhea by the Use of Commercial Prolactin and the Luteinizing Hormone, Endocrinology **22**: 86 (Jan.) 1938.

exercised by the luteinizing substance. Though it is clear that prolactin is, and luteinizing substance is not, effectively antigonad in birds, this action of prolactin is still uncertain in mammals.

Prolactin seems to be specifically involved in the onset or production of broodiness in the fowl,[50] the maternal or parental instinct in rats[51] and nesting behavior in fish.[52] Other anterior pituitary extracts, the urinary luteinizing substance, estrone (theelin), progesterone and extract of adrenal cortex all seem ineffective or inhibitory in the fowl, though phenol is active in fish, and pituitary luteinizing extract, intermedin, progesterone, desoxycorticosterone acetate and phenol are all fairly effective in rats.

Prolactin has marked calorigenic action in pigeons—normal, hypophysectomized or thyroidectomized—and its synergistic action with thyrotropin on heat production has been reported in normal doves.[53] The relation of prolactin to carbohydrate and fat metabolism is an important but still unsettled problem.[54] Splanchnomegaly exhibited in the liver, pancreas and intestine of the pigeon is associated with the action of prolactin.[55] Body weight and appetite are especially increased by prolactin in pigeons, and favorable effects of this substance on the growth of the dwarf mouse—with a

50. Riddle, Oscar; Bates, R. W., and Lahr, E. L.: Prolactin Induces Broodiness in Fowl, Am. J. Physiol. **111**: 352 (March) 1935.

51. Riddle, Oscar; Lahr, E. L., and Bates, R. W.: Maternal Behavior Induced in Virgin Rats by Prolactin, Proc. Soc. Exper. Biol. & Med. **32**: 730 (Feb.) 1935.

52. Noble, G. K.; Kumpf, K. F., and Billings, V. N.: The Induction of Brooding Behavior in the Jewel Fish, Anat. Rec. (supp. 1) **67**: 50 (Dec.) 1936.

53. Riddle, Oscar; Smith G. C.; Bates, R. W.; Moran, C. S., and Lahr, E. L.: Action of Anterior Pituitary Hormones on Basal Metabolism of Normal and Hypophysectomized Pigeons and on a Paradoxical Influence of Temperature, Endocrinology **20**: 1 (Jan.) 1936. Riddle, Oscar; Dotti, L. B., and Smith, G. C.: Blood Sugar and Basal Metabolism in Pigeons Following Administration of Prolactin and Cortin, Am. J. Physiol. **119**: 389 (June) 1937.

54. Young, F. G.: The Influence of Anterior Pituitary Extracts on the Glycemic Response to Insulin and Adrenalin in Rabbits, J. Physiol. **87**: 13P (June 10) 1936. Riddle, Oscar: Carbohydrate Metabolism in Pigeons, in Cold Spring Harbor Symposia on Quantitative Biology, Cold Spring Harbor, L. I., New York, The Biological Laboratory, 1937, vol. 5, p. 362. Long, C. N. H.: The Influence of the Pituitary and Adrenal Glands upon Pancreatic Diabetes, Harvey Lect. (1936-1937) **32**: 194, 1937. Bergman, A. J., and Turner, C. W.: Are the Lactogenic and Carbohydrate Metabolism Hormones Identical? Endocrinology **23**: 228 (Aug.) 1938. Young, F. G.: The Identity and Mechanism of Action of the Glycotropic (Anti-Insulin) Substance of the Anterior Pituitary Gland, Biochem. J. **32**: 1521 (Sept.) 1938.

55. Riddle, Oscar: Differentiating Some Functions of the Anterior Pituitary Hormones, Ann. Int. Med. **12**: 23 (July) 1933. Bates, R. W.; Riddle, Oscar; Lahr, E. L., and Schooley, J. P.: Aspects of Splanchnomegaly Associated with the Action of Prolactin, Am. J. Physiol. **119**: 603 (July) 1937.

synergistic effect on growth when prolactin is administered together with thyrotropin—have been reported.[56]

Large doses of ox pituitary prolactin (probably not entirely free from serum protein) injected daily into adult rabbits or young female monkeys for eighteen or more weeks resulted in the production of antiserums capable of inhibiting the growth response in the crop sac of the pigeon and probably of reducing milk secretion in lactating mice.[57] Preparations of prolactin from ox and sheep glands, apparently free from serum protein, were found to be antigenically indistinguishable, and the serum of rabbits given injections of prolactin from either source usually partly inhibited prolactin action on crop sacs.[58]

MAMMOGENIC HORMONES

The Mammogenic Response.—Numerous old and new studies clearly prove that hormones of the ovary play a part in the growth of the mammary gland—in most species an estrogen induces (directly or indirectly) duct growth, and an estrogen plus progesterone induces lobule-alveolar development. Androgens, too, were later proved capable of replacing estrogens in the induction of growth in the mammary parenchyma. Still more recently desoxycorticosterone acetate and some phenanthrene and stilbene compounds not now known to occur in the body are reported to cause duct development in normal and castrate males of the species studied.

In hypophysectomized animals, however, the use of estrogens or androgens to develop mammary tissue has usually, though apparently not always, resulted in failure. Such failures together with the recovery of potent lipoid extracts from the anterior lobes of pituitary glands which have been recently subjected to ovarian hormones—and other related facts—lead some investigators to believe that the pituitary gland secretes, in addition to its various hormones of protein nature, other (alcohol-ether soluble) hormone(s) with

56. Riddle, Oscar: Contemplating the Hormones, Endocrinology **19**: 1 (Jan.-Feb.) 1935. Bates, R. W.; Laanes, T., and Riddle, Oscar: Evidence from Dwarf Mice Against the Individuality of Growth Hormone, Proc. Soc. Exper. Biol. & Med. **33**: 446 (Dec.) 1935. Schooley, J. P.; Riddle, Oscar, and Bates, R. W.: Analysis of Pituitary Support of Growth of Body and Viscera in Pigeons, Anat. Rec. (supp.) **72**: 90 (Dec.) 1938. Bates, Riddle, Lahr and Schooley.[55]

57. Young, F. G.: The Production of Antisera to Preparations of Prolactin Containing the Glycotropic (Anti-Insulin) Factor of the Anterior Pituitary Gland, Biochem. J. **32**: 656 (April) 1938.

58. Bischoff, H. W., and Lyons, W. R.: Immunologic Investigation of Hypophysial Mammotropic Preparations, Endocrinology **25**: 17 (July) 1939.

specific ability to induce mammary growth. In this review one needs to consider only literature bearing on (1) the question of whether estrogens, androgens and available phenanthrenes act directly or indirectly on mammary tissue and (2) the intimately related question of the elaboration of specific mammogenic hormone(s) by the pituitary.

Direct or Indirect Action of Sterols.—Duct development was reported in 4 of 5 hypophysectomized male rats treated for fourteen days with 50 mouse units of estrone twice daily [59] and in mammary glands transplanted into similar rats.[60] Rapid mammary involution was observed in rats after hypophysectomy on the sixth day of lactation despite the injection of 100 micrograms of estrone daily; the conclusion was drawn that the pituitary is necessary for the action of estrone on the mammary gland.[61] Mammary involution would be expected to follow the withdrawal of prolactin and the generally adverse effects of hypophysectomy, and it was later reported [62] that the involution of the lobule-alveolar system of such rats is not rapid but requires at least as long as does its development. Houssay [63] observed teat and mammary enlargement in a male hypophysectomized dog given 10,000 international units of estrone daily for fifty-four days.

Estrone plus progestin was observed to cause further mammary development in hypophysectomized castrate rats than did estrone alone,[64] and the same was observed in guinea pigs similarly operated on.[65] In 4 hypophysectomized, ovariectomized rabbits given daily for fifteen days injections of 25 rat units of estradiol benzoate and 4 rabbit units of progestin, mammary development approximately equal to that in rabbits incompletely operated on or not operated on was

59. Ruinen, F. C.: Ueber den Angriffspunkt der Mamma-Wirkung von Menformon, Acta brev. Neerland. **2**: 161, 1932.

60. de Jongh, S. E.: Fortsetzung von Untersuchungen über den Angriffspunkt der Menformonwirkung auf den Mamma, Acta brev. Neerland. **3**: 99, 1933.

61. Selye, Hans; Collip, J. B., and Thomson, D. L.: Effects of Estrin on Ovaries and Adrenals, Proc. Soc. Exper. Biol. & Med. **32**: 1377 (May) 1935.

62. Turner, C. W.; in Allen, Edgar; Danforth, C. H., and Doisy, E. A.: Sex and Internal Secretions, ed. 2, Baltimore, Williams & Wilkins Company, 1939, chap. 11.

63. Houssay, B. A.: Secretion lactée provoquée par l'extrait antehypophysaire chez le chien, Compt. rend. Soc. de biol. **120**: 502, 1935.

64. Freud, John and de Jongh, S. E.: The Effect of Progestin upon the Mammary Gland of the Rat, Acta brev. Neerland. **5**: 47, 1935.

65. Nelson, W. O.: The Effect of Hypophysectomy upon Mammary Gland Development and Function in the Guinea Pig, Proc. Soc. Exper. Biol. & Med. **33**: 222 (Nov.) 1935.

reported.[66] Corner [67] had shown much earlier that in castrate rabbits with intact hypophyses progestin alone is without effect, and others [68] had observed that estrone plus progestin did cause development of the mammaries of such rabbits.

Later studies (since 1935) with estrogens on pituitaryless animals have more often given negative results on mammary growth, but the significance of these failures has been interpreted variously. Mammary growth in such rats was not stimulated by 25 to 500 international units of estradiol benzoate daily for fifteen to forty-five days,[69] by 20 to 100 units of estrone for twenty to twenty-five days [70] and only slightly after estrone,[71] even when the general condition of the rat was sustained with pituitary extract.[72] Selye and Collip [72] therefore reaffirmed the view that this action of estrone must be through the pituitary. Astwood and his associates [73] emphasized the great importance of reduced nutrition incident to hypophysectomy. They observed mammary regression in normal rats on restricted diet during fourteen days, although 5 micrograms of estrone was injected daily. Likewise, in hypophysectomized rats the duct system regressed under estrone dosage, and the authors concluded that such failures provide insufficient evidence that mammary growth is mediated by the pituitary. Other failures of estrogens to support or increase mammary growth in pituitaryless animals were observed in guinea pigs,

66. Asdell, S. A., and Seidenstein, H. R.: Theelin and Progestin Injection on Uterus and Mammary Glands of Ovariectomized and Hypophysectomized Rabbits, Proc. Soc. Exper. Biol. & Med. **32**: 931 (March) 1935.

67. Corner, G. W.: The Hormonal Control of Lactation: I. Non-Effect of Corpus Luteum; II. Positive Action of Extracts of the Hypophysis, Am. J. Physiol. **95**: 43 (Oct.) 1930.

68. Turner, C. W., and Frank, A. H.: The Effect of the Ovarian Hormones Theelin and Corporin upon the Mammary Growth of the Rabbit, Research Bulletin **174**; University of Missouri College of Agriculture, Agricultural Experiment Station, 1932, pp. 1-28.

69. Reece, R. P.; Turner, C. W., and Hill, R. T.: Mammary Gland Development in the Hypophysectomized Albino Rat, Proc. Soc. Exper. Biol. & Med. **34**: 204 (March) 1936.

70. Gomez, E. T., and Turner, C. W.: Hypophysectomy and Replacement Therapy in Relation to the Growth and Secretory Activity of the Mammary Gland, Research Bulletin 259, University of Missouri College of Agriculture, Agricultural Experiment Station, 1937, pp. 1-72.

71. Nelson, W. O., and Tobin, C. E.: The Effect of Estrone and of Pituitary Extracts on the Mammary Glands of the Rat, Anat. Rec. (supp. 1) **67**: 111 (Dec. 25) 1936.

72. Selye, Hans, and Collip, J. B.: Fundamental Factors in the Interpretation of Stimuli Influencing Endocrine Glands, Endocrinology **20**: 667 (Sept.) 1936.

73. Astwood, E. B.; Geschickter, C. F., and Rausch, E. O.: Development of the Mammary Gland of the Rat: A Study of Normal, Experimental and Pathologic Changes and Their Endocrine Relationships, Am. J. Anat. **61**: 373 (Sept.) 1937.

mice, rabbits, cats and ground squirrels.[74] Herold and Effkemann [75] reported no effect of estrogen in normal male and castrate female rats after severing the nerve connection between the pituitary and the midbrain and suggested that estrone acts by way of the diencephalon from which impulses reach the pituitary and cause it to secrete a mammary growth factor.

When the pituitary was removed during pregnancy, the effects on mammary growth were unexpected and important. In rats operated on during the second week mammary growth was unaffected, and the usual accumulation of metabolic products in the lobular cells occurred.[76] In guinea pigs operated on at the fortieth day, with mammaries examined nine to twelve days later, a condition resembling that of normal glands at parturition was found.[77] In mice it was observed that even after digital abortion of fetuses and hypophysectomy on the twelfth day normal mammary changes occurred if the placentas had been retained;[78] the presence of the ovaries had earlier been found unessential for mammary growth at this period.[79] These results recall earlier evidence for an influence of the placenta on both mammary growth and secretion in animals with intact pituitaries.[80] That this influence may result from the secretion of an estrogen by the placenta, or otherwise, is obvious.

Testosterone and other androgens have been observed to cause mammary development in normal or ovariectomized rats.[81] In hypophysectomized rats little or

74. Turner.[62] Lewis and Turner.[92]

75. Herold, L., and Effkemann, G.: Abhängigkeit der Follikelhormonwirkung auf die Brustdrüse von der nervösen Verbindung der Hypophyse zum Zwischenhirn, Klin. Wchnschr. **18**: 455 (April 1) 1939.

76. Jeffers, K. R.: Cytology of the Mammary Gland of the Albino Rat: II. Experimentally Induced Conditions, Am. J. Anat. **56**: 279 (March) 1935.

77. Desclin, L.: Influence de l'hypophyectomie sur la glande mammaire du cobaye gravide, Compt. rend. Soc. de biol. **131**: 837, 1939.

78. Newton, W. H., and Beck, Naomi: Placental Activity in the Mouse in the Absence of the Pituitary Gland, J. Endocrinol. **1**: 65 (June) 1939.

79. Newton, W. H., and Lits, F. J.: Criteria of Placental Endocrine Activity in the Mouse, Anat. Rec. **72**: 333 (Nov. 25) 1938.

80. Frankl, O.: Relation Between Placenta and the Secretion of Milk, Am. J. Obst. & Gynec. **6**: 399 (Oct.) 1923. Bradbury, J. T.: Study of Endocrine Factors Influencing Mammary Development and Secretion in the Mouse, Proc. Soc. Exper. Biol. & Med. **30**: 212 (Nov.) 1932.

81. Nelson, W. O., and Gallagher, T. F.: Some Effects of Androgenic Substances in the Rat, Science **84**: 230 (Sept. 4) 1936. Selye, Hans; McEuen, C. S., and Collip, J. B.: Effect of Testosterone on the Mammary Gland, Proc. Soc. Exper. Biol. & Med. **34**: 201 (March) 1936. Korenchevsky, Vladimir; Dennison, Marjorie, and Hall, Kathleen: The Action of Testosterone Propionate on Normal Adult Female Rats, Biochem. J. **31**: 780 (May) 1937. Nelson, W. O., and Merckel, C. G.: Effects of Androgenic Substances in the Female Rat, Proc. Soc. Exper. Biol. & Med. **36**: 823 (June) 1937.

nothing more than nipple growth is obtained.[82] In young male mice, not unoperated on, and in castrated male mice weighing from 15 to 25 Gm., the development of the mammary glands was accomplished with a wide variety of estrogens and androgens and with desoxycorticosterone acetate.[83] This result with the last named substance has special importance because (1) it adds the adrenal cortex to the few possible sources of mammogenic stimulation, (2) these cortical products are heat labile (in contrast with many estrogens) and more soluble in fat solvents, (3) such products are probably released from the cortex in increased amounts by estrone dosage in normal rats and pigeons but not or to a less extent after hypophysectomy,[84] (4) desoxycorticosterone (the only adrenal derivative hitherto tested) was observed in the study cited to have greater mammogenic potency per milligram than either of the five androgenic substances tested and (5) mice of just this type are used for assay of the mammogenic activity of the pituitary tissues and extracts to be described in the following section.

Mammogenic Potency of Pituitary Tissues and Extracts.—Implantation of pituitary tissue was followed by no appreciable mammary development in many tests made on rats.[85] In other tests, positive results in both ducts and lobules were secured on hypophysectomized male castrate and noncastrate guinea pigs following implantation of tissue from rats previously treated with

82. McEuen, C. S.; Selye, Hans, and Collip, J. B.: Role of Pituitary in Effect of Testosterone on the Mammary Gland, Proc. Soc. Exper. Biol. & Med. **36**: 213 (March) 1937. Noble, R. L.: Direct Gynecogenic and Indirect Estrogenic Action of Testosterone Propionate in Female Rats, J. Endocrinol. **1**: 184 (Sept.) 1939.

83. Van Heuverswyn, J.; Folley, S. J., and Gardner, W. U.: Mammary Growth in Male Mice Receiving Androgens, Estrogens and Desoxycorticosterone Acetate, Proc. Soc. Exper. Biol. & Med. **41**: 389 (June) 1939.

84. Miller, R. A., and Riddle, Oscar: Stimulation of Adrenal Cortex of Pigeons by Anterior Pituitary Hormones and by Their Secondary Products, Proc. Soc. Exper. Biol. & Med. **41**: 518 (June) 1939. Selye, Collip and Thomson.[81]

85. Schultze, A. B., and Turner, C. W.: Experimental Initiation of Milk Secretion in the Albino Rat, J. Diary Sc. **16**: 129, 1933. Weichert, C. K.; Boyd, R. W., and Cohen, R. S.: A Study of Certain Endocrine Effects on the Mammary Glands of Female Rats, Anat. Rec. **61**: 21 (Dec. 25) 1934. Nelson, W. O.: Endocrine Control of the Mammary Gland, Physiol. Rev. **16**: 488 (July) 1936. Loeb, Leo, and Kirtz, M. M.: The Effects of Transplants of Anterior Lobes of the Hypophysis on the Growth of the Mammary Gland and on the Development of Mammary Gland Carcinoma in Various Strains of Mice, Am. J. Cancer **36**: 56 (May) 1939.

estrogen.[86] In pituitaryless male and female guinea pigs a few tests with an extract of adrenal cortex (eschatin) alone or in combination with an estrogen (not named) indicated no mammary growth. Some further tests were reported, together with citations from the literature, indicating the ineffectiveness of thyroxin and of the recognized pituitary substances.[70] In a later study immature ovariectomized rats and spayed rabbits showed complete mammary development following implantation during twenty-five to thirty days of fresh (or acetone-dried) anterior pituitary tissue from pregnant cows.[87] In view of these results a new pituitary principle promoting growth in the mammary gland was postulated by Gomez and Turner and called "mammogenic hormone," or mammogen.

Nelson [88] reported that the mammary development of hypophysectomized immature female rats in which pituitaries from estrogen-treated rats of either sex had been implanted did not exceed or even equal that which followed implantation of normal rat pituitaries. He also announced his failure to confirm the claim of the existence of a specific "mammogenic hormone." Repeating this study, Reece and Leonard [89] also found no difference in the potency of pituitaries from untreated and estrogen-treated donors, but they noted that implanted glands of both types gave evidence of some growth when the test animals were compared with their untreated hypophysectomized control.

Albino mice were found to respond to pituitary implants and were considered most suitable for assay of mammogen.[90] The technic of this assay of the factor promoting duct growth involves the daily subcutaneous injection of macerated fresh anterior lobe tissue from pregnant cows or of mammogen-containing extracts for six days with autopsy on the seventh. The mouse unit is defined as the amount of tissue or extract

86. Gomez, E. T.; Turner, C. W., and Reece, R. P.: Growth of Mammary Gland of Hypophysectomized Guinea Pigs, Proc. Soc. Exper. Biol. & Med. **36**: 286 (April) 1937. Gomez and Turner.[70]

87. Gomez, E. T., and Turner, C. W.: Further Evidence for a Mammogenic Hormone in the Anterior Pituitary, Proc. Soc. Exper. Biol. & Med. **37**: 607 (Jan.) 1938.

88. Nelson, W. O.: Effect of Pituitary Implants on the Mammary Glands of Hypophysectomized Rats, Anat. Rec. (supp.) **72**: 117 (Dec. 25) 1938.

89. Reece, R. P., and Leonard, S. L.: Further Evidence for a Mammogenic Factor in the Rat Hypophysis, Proc. Soc. Exper. Biol. & Med. **42**:200 (Oct.) 1939.

90. Lewis, A. A.; Turner, C. W., and Gomez, E. T.: The Biological Assay of the Mammogenic Duct Growth Factor of the Anterior Pituitary, Endocrinol. **24**: 157 (Feb.) 1939.

required (per mouse) to produce definite signs of duct development in one or more glands of 50 ± 10 per cent of ten or more male albino mice weighing 15 to 25 Gm.[91] With this technic the mammogen content of the pituitaries from pregnant cows was found greatest at one hundred and fifty days; that of the dairy cow was greater than that of the pregnant beef cow; that of beef heifers with corpora lutea was 40 to 60 per cent greater than that of pregnant beef cows at the one hundred and fifty day peak; steer, bull and fetal pituitaries showed appreciable amounts of mammogen.[92] The authors suggested that estrogens lead to the production of a pituitary factor promoting duct growth, progesterone to a factor promoting lobule proliferation.

Lewis and Turner [93] observed that acetone and ether drying of prehypophysial tissue resulted in a loss of 60 per cent of its mammogen content. Extraction of the tissue with several volumes of hot ether–alcohol (1:3) resulted in a preparation (oily residue) containing 1 unit per 3 to 4 mg. and including practically 100 per cent of the potency of the fresh tissue. In their most recent publication [92] they have stated that their most potent preparation, tested in fourteen mice, gave positive results in 79 per cent at a dosage of 0.25 mg. per mouse. The estrogen content of some preparations was measured and considered far too low to have caused the mammary growth observed. The fact that fresh pituitary tissue containing mammogen caused both duct development and lobule hyperplasia, though the lipoid extracts cause only duct development, was regarded as evidence that the lipoid solvent separates a duct growth factor from a lobule growth factor.

91. Lewis, Turner and Gomez.[90] Lewis and Turner.[92]

92. Lewis, A. A., and Turner, C. W.: The Mammogenic Hormones of the Anterior Pituitary: I. The Duct Growth Factor, Research Bulletin 310, University of Missouri College of Agriculture, Agricultural Experiment Station, 1939, pp. 1-72.

93. Lewis, A. A., and Turner, C. W.: Chemical Concentration of Mammogen from Prehypophysial Tissue, Proc. Soc. Exper. Biol. & Med. **39**: 435 (Dec.) 1938.

CHAPTER VI

GONADOTROPINS OF THE ANTERIOR LOBE OF THE PITUITARY AND OF CHORIONIC TISSUE

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The concept of gonadotropic action of the anterior lobe of the pituitary gland arose from the experiments of Philip E. Smith in 1926, in which the treatment of immature rodents with implants of fresh anterior lobe produced follicular growth and the formation of corpora lutea. Similar effects were found at the same time in immature mice by Aschheim and Zondek, but Smith demonstrated that atrophy of the gonads follows hypophysectomy and that it may be prevented or the gonads repaired by treatment with fresh anterior pituitary implants.

Since this pioneer work, an amazing mass of literature concerning this subject has accumulated. It is beyond the scope of this chapter to consider all the work which has been reported. For such a comprehensive review the reader is referred to Van Dyke (1939).[1] In the present article attention will be directed to those observations which appear to be most firmly established and of greatest interest to one seeking to understand the action of the gonadotropic substances.

CLASSIFICATION

Two large groups of gonadotropins, differing in their qualitative physiologic action, are now known. The first consists of those obtained directly from the pituitary gland and, in addition, those probably of pituitary origin extracted from the blood and urine of normal men and women and of women who have undergone menopause (artificial or natural).

1. Van Dyke, H. B.: The Physiology and Pharmacology of the Pituitary Body, Chicago, University of Chicago Press, 1936, vol. 1; 1939, vol. 2.

The second major group of gonadotropins, the chorionic, derives its name from the fact that these substances are found in body fluids and tissues in the presence of living chorionic tissue, i. e., during normal pregnancy in women and in mares or when pathologic chorionic tissue is present. It is an accepted opinion that these gonadotropins originate from chorionic cells and are not anterior pituitary gonadotropins.

ANTERIOR PITUITARY GONADOTROPINS

Several gonadotropins from the anterior lobe of the pituitary have been postulated, but there is no general agreement as to their identity or their action. The greatest unanimity of opinion probably exists with respect to the so-called follicle-stimulating and luteinizing hormones, first postulated by Zondek, but the evidence as to the separate identity of these is not completely convincing to all investigators.

A great deal of the disagreement concerning the occurrence of these two gonadotropins as separate entities is due to the fact that at present proof of the identity of either substance depends on biologic differentiation. This differentiation is, in turn, complicated by lack of distinct end points. In many reports the interpretation of qualitative results is further complicated by the fact that intact animals were used, thus introducing as a factor the variable and unpredictable participation of the animal's own pituitary. Only the hypophysectomized rat or mouse affords standard test conditions.

The present status of the so-called follicle-stimulating and luteinizing hormones may be briefly summarized as follows:

A number of reactions of the ovaries and of the testes have been ascribed to each of these hormones. In the hypophysectomized female rat the purified follicle-stimulating extract causes growth and development of numerous follicles, associated with increase in ovarian weight. According to proponents of the dual hormone theory, it does not, if completely separated from the luteinizing factor, produce any lutein changes.

A secondary effect of the follicle-stimulating factor, presumably due to secretion of estrogen by the developing follicles, is the development of the secondary genitalia. However, Greep, Van Dyke and Chow

(1940) [2a] have recently described what is claimed to be a very pure preparation of the follicle-stimulating substance and have stated that although it stimulates follicular growth, it does not have any estrogenic effect, as indicated by lack of uterine and vaginal change. This has been confirmed by Fevold.[2b]

When administered to the hypophysectomized male rat, the follicle-stimulating extract produces a reaction analogous to that in the female. The epithelium of the seminiferous tubules is maintained or repaired, and sperm formation proceeds at least to the stage at which secondary spermatocytes appear. There is no effect on the testicular interstitial tissue, which remains atrophic, as indicated by its histologic appearance as well as by the atrophic state of the accessory sex glands.

The identity of the luteinizing hormone is particularly difficult to establish when its effects on the ovary are studied. According to proponents of the dual hormone theory, the luteinizing factor by itself has no positive effect on the ovary of the hypophysectomized rat. Follicles ripened to a certain degree are apparently necessary for the production of lutein changes by the luteinizing hormone. Such changes may be produced by administering an extract containing the latter factor to animals simultaneously with, or following, an extract containing the follicle-stimulating factor, in which case luteinization and sometimes ovulation result. The combination of the luteinizing and follicle-stimulating extracts also results in the "augmentation" phenomenon, the weight of the ovary increasing much beyond the sum of the increases produced by the two substances separately. It is stated [3] that the luteinizing hormone

2. (*a*) Greep, R. O.; Van Dyke, H. B., and Chow, B. F.: Separation in Nearly Pure Form of Luteinizing (Interstitial-Cell-Stimulating) and Follicle-Stimulating (Gametogenic) Hormones of the Pituitary Gland, J. Biol. Chem. **133**: 289 (March) 1940. Shedlovsky, T.; Rothen, A.; Greep, R. O.; Van Dyke, H. B., and Chow, B. F.: The Isolation in Pure Form of the Interstitial Cell-Stimulating (Luteinizing) Hormone of the Anterior Lobe of the Pituitary Gland, Science **92**: 178 (Aug.) 1940. (*b*) Fevold, H. L.: Synergism of the Follicle Stimulating and Luteinizing Hormones in Producing Estrogen Secretion, Endocrinology **28**: 33 (Jan.) 1941.

3. (*a*) Evans, H. M.; Simpson, M. E., and Pencharz, R. I.: An Anterior Pituitary Gonadotropic Fraction (Interstitial-Cell-Stimulating) Specifically Stimulating the Interstitial Tissue of Testis and Ovary, Cold Spring Harbor Symposia on Quantitative Biology, Cold Spring Harbor, L. I., New York, The Biological Laboratory, 1937, vol. 5. (*b*) Fevold, H. L.: Extraction and Standardization of Pituitary Follicle-Stimulating and Luteinizing Hormones, Endocrinology **24**: 435 (April) 1939.

stimulates the interstitial ovarian tissue.[4] Another action of this hormone is that of causing rapid involution of existing corpora lutea with concomitant decrease in ovarian weight.[5] The absence of this hormone may be the reason for the prolonged survival of preformed corpora in the hypophysectomized rat.

In the hypophysectomized male rat the luteinizing extract is reported to have a more distinct effect. Whereas in this form the follicle-stimulating substance stimulates only the tubular elements of the testis, the luteinizing factor produces growth and functional activity of the interstitial cells, as indicated by the histologic picture of the testis, as well as by the growth and development of the accessory sex glands, presumably as a result of secretion of androgen. If male rats are treated with a combination of follicle-stimulating and luteinizing substances, a synergism between the activities of these substances is noted, the responses being greater than with either fraction alone.[6]

Evidence has been offered enabling several authors to question the identity of two distinct pituitary gonadotropins or of a separate luteinizing factor. This evidence, beginning with the findings of Maxwell (1934) [7] is based on the fact that by manipulating the rate at which the administered gonadotropin reaches the ovary the reactions ascribed to the luteinizing factor by its proponents may be abolished or caused to appear. Thus Maxwell and others have demonstrated that by properly dividing the doses of unfractionated pituitary extract to be administered the incidence of lutein changes may be greatly diminished. Also, it has been adequately shown that addition of zinc sulfate ($ZnSO_4$) or copper sulfate ($CuSO_4$) or a variety of other nonspecific substances to the unfractionated extract causes at least

4. Evans, Simpson and Pencharz have claimed the separation of an additional gonadotropin which stimulates only the interstitial cells of the ovary and testis. This material, named interstitial cell-stimulating hormone, appears to be the same as the previously postulated luteinizing factor. Evans, H. M.; Simpson, M. E.; Tolksdorf, Sybylle, and Jensen, H.: Biological Studies of the Gonadotropic Principles in Sheep Pituitary Substance, Endocrinology **25**: 529 (Oct.) 1939. Fevold.[3b]

5. Bunde, C. A., and Greep, R. O.: Suppression of Persisting Corpora Lutea in Hypophysectomized Rats, Proc. Soc. Exper. Biol. & Med. **35**: 235 (Nov.) 1936.

6. Greep, R. O.: Pituitary Regulation of the Male Gonad, in Cold Spring Harbor Symposia on Quantitative Biology, Cold Spring Harbor, L. I., New York, The Biological Laboratory, 1937, vol. 5.

7. Maxwell, L. C.: The Quantitative and Qualitative Ovarian Response to Distributed Dosage with Gonadotropic Extracts, Am. J. Physiol. **110**: 458 (Dec.) 1934.

partial abolition of the luteinizing properties. All these treatments tending to decrease the rate of entrance of the injected gonadotropin into the blood stream also increase the weight response of the ovary. Furthermore, Saunders and Cole [8] have reported that zinc sulfate and egg albumin, if combined with the follicle-stimulating extract, are more effective in producing augmentation than is the luteinizing substance. However, it should be pointed out that the follicle-stimulating substance (and/or synergist [9]) which was used by these authors, of itself, produced corpora lutea in a great majority of the treated animals. It is not surprising, therefore, that addition of more luteinizing substance failed to produce augmentation.

The increased weight response and the decrease in the incidence of luteinization which result when the rate of entrance of the gonadotropin into the blood stream is decreased by the addition of nonspecific substances have been demonstrated only in the female rat. In these instances nonspecific substances have been substituted for luteinizing substance. However, the luteinizing fraction alone will cause interstitial activation in the male, and no one has yet demonstrated that nonspecific substances alone are able to simulate the effects of luteinizing substance on the interstitial cells and accessory sex glands of the hypophysectomized male rat.

Other evidence cited in support of the two hormone theory is that produced by Du Shane and his associates.[10] These investigators, using parabiotically united rats, castrated one of the parabionts (either male or female) and hypophysectomized the other (female). The ovaries of the hypophysectomized partner showed a high degree of follicular stimulation over a long period. They contained large follicles, many of them cystic, but no lutein changes were present. This correlates well with the concept that after castration the pituitary secretes large amounts of follicle-stimulating hormone. If there is

8. Saunders, F. J., and Cole, H. H.: On the Reliability of Present Methods for Characterizing Two Gonadotropic Hormones, Follicle-Stimulator and Luteinizer, Endocrinology **23**: 302 (Sept.) 1938.

9. The designation of the "synergist" as a separate and distinct gonadotropic fraction no longer seems justified since Evans has admitted that the pituitary fraction designated by this term is the same as the follicle-stimulating fraction.

10. Du Shane, G. P.; Levine, W. T.; Pfeiffer, C. A., and Witschi, E.: Experimental "Constant Oestrus" and the Notion of Antigonadotropic Hormones, Proc. Soc. Exper. Biol. & Med. **33**: 339 (Dec.) 1935.

only one pituitary gonadotropic hormone, it is not easily understandable why such ovaries, apparently exposed to effective gonadotropic stimulation over long periods, do not show lutein changes.

On the other hand, experiments have been performed that were identical except that hypophysectomized male instead of female rats were exposed to the gonadotropin from castrate parabiotic partners.[11] In these experiments the testes showed maintenance of interstitial cells and of accessory glands, a reaction presumably evoked by the luteinizing rather than by the follicle-stimulating hormone. Similarly Greep[12] simultaneously produced parabiosis of a hypophysectomized immature male rat, on one hand, and of a hypophysectomized female rat, on the other, with a third rat, a castrate. Thus a pair of testes and a pair of ovaries were simultaneously subjected to the same gonadotropin in identical dosage. Development of all the testicular elements was noted, while the ovaries showed only follicular stimulation.

The findings of Rowlands[13] are also interpreted to indicate two gonadotropic hormones. He treated rabbits chronically with preparations rich in luteinizing potency until antigonadotropic substances were demonstrable in the serums. These serums when injected together with unfractionated extracts containing both the follicle-stimulating and the luteinizing factor were able to neutralize selectively the luteinizing factor, leaving the follicle-stimulating one free to act. That this was not a matter of alteration of the rate of absorption of a gonadotropin by nonspecific substances was proved by the fact that serums from control animals did not possess this selective neutralizing ability. Furthermore, the selective inactivation was observed after injection of serum and gonadotropin at different sites, so that mixing, if any, presumably occurred after absorption.

11. Cutuly, Eugene; McCullagh, D. R., and Cutuly, Elizabeth: The Type and Degree of Gonadal Stimulation Induced in Hypophysectomized Male Rats Parabiotically Joined with Castrated, Cryptorchid, and Normal Partners, Endocrinology **21**: 241 (March) 1937. Cutuly, Eugene and Cutuly, Elizabeth C.: Inhibition of Gonadotropic Activity by Sex Hormones in Parabiotic Rats, ibid. **22**: 568 (May) 1938.

12. Greep, R. O.: Pituitary Function in Parabiotic Triplet Rats, Proc. Soc. Exper. Biol. & Med. **44**: 214 (May) 1940.

13. Rowlands, I. W.: Selective Neutralization of the Luteinizing Activity of Gonadotropic Extracts of Pituitary by Anti-Sera, Proc. Roy. Soc., London, s. B **126**: 76 (Sept. 23) 1938.

A great deal of chemical work has been done on the separation of the two hormones (Fevold;[14] Evans;[15] Wallen-Lawrence;[16] Van Dyke[17a]). Many of the preparations have been considerably purified, and data to indicate chemical homogeneity have been reported for at least one preparation.[2] Selective inactivation by enzymatic action has been employed to demonstrate the presence of two separate gonadotropic substances. However, in every case the demonstration of the distinctness of the hormone fractions has depended on one or more of the biologic tests described in foregoing paragraphs. Until the qualitative accuracy of the biologic end points have been more adequately demonstrated and until the luteinizing hormone-like effects of nonspecific substances are completely ruled out, it is not profitable, in relation to this question, to discuss the chemical differences which have been reported for the two fractions.

It has not been adequately shown that the best preparations of the follicle-stimulating and the luteinizing factor so far reported, if injected in high doses or over long periods, will not each produce the effects ascribed to the other. The difficulty of a distinct chemical separation of this type of substance makes understandable the contamination which occurs in even the best preparations made to date. It must be recognized, however, that the final solution of this problem will be achieved only when one or more active substances have

14. Fevold, H. L.: The Gonadotropic Hormones, in Cold Spring Harbor Symposia on Quantitative Biology, Cold Spring Harbor, L. I., New York, The Biological Laboratory, 1937, vol. 5. Fevold, H. L.; Lee, M.; Hisaw, F. L., and Cohn, E. J.: Studies in the Physical Chemistry of the Anterior Pituitary Hormones, Endocrinology **26**: 959 (June) 1940. Fevold[3b]

15. Jensen, H.; Simpson, Miriam E.; Tolksdorf, Sybylle, and Evans, H. M.: Chemical Fractionation of the Gonadotropic Factors Present in Sheep Pituitary, Endocrinology **25**: 57 (July) 1939. Evans, H. M.; Korpi, K.; Simpson, M. E.; Pencharz, R. I., and Wonder, D. H.: On the Separation of the Interstitial Cell-Stimulating, Luteinizing and Follicle-Stimulating Fractions of the Anterior Pituitary Gonadotropic Complex, Univ. California Publ. Anat. **1**: 255, 1936.

16. Wallen-Lawrence, Zonja: Proof of the Existence of a Follicle-Stimulating and a Luteinizing Hormone in the Anterior Lobe of the Pituitary Body, J. Pharmacol. & Exper. Therap. **51**: 263 (July) 1934.

17. (*a*) Chow, B. F.; Greep, R. O., and Van Dyke, H. B.: The Effects of Digestion by Proteolytic Enzymes on the Gonadotrophic and Thyrotrophic Potency of Anterior Pituitary Extract, J. Endocrinol. **1**: 440 (Dec.) 1939. Greep and others.[3] (*b*) Greep, R. O.; Van Dyke, H. B., and Chow, B. F.: Use of Anterior Lobe of Prostate Gland in the Assay of Metakentrin, Proc. Soc. Exper. Biol. & Med. **46**: 644 (April) 1941; Some Biological Properties of Metakentrin and Thylakentrin, Am. J. Physiol. **133**: P 303 (June) 1941. Chow, B. F.; Van Dyke, H. B.; Greep, R. O.; Rothen, A., and Shedlovsky, T.: Biochemical Aspects of Metakentrin (Interstitial Cell-Stimulating Hormone), J. Biol. Chem. **140**: xxvii (July) 1941.

been isolated in chemically pure form and when it is demonstrated whether one or more of the pure substances are necessary to account for all the physiologic responses. Progress in this direction is being made [17b] with substances claimed to be chemically pure,[2] and it may be hoped that an elucidation of this phase of the problem will be forthcoming in the near future.

In summary, the validity of the concept of two distinct pituitary gonadotropins depends at present chiefly on proof of the occurrence of the so-called luteinizing hormone. Proof based on the reactions of the rodent ovary is inadequate because similar reactions can be produced by manipulation of dosage and by use of nonspecific substances. The reaction of the testis affords better indications of the occurrence of a gonadotropic principle distinct from the follicle-stimulating or gametokinetic hormone, but even in this regard one encounters valid conflicting evidence. Therefore, a final answer to this important and intriguing question must await further clarifying work.

URINARY GONADOTROPINS

Considerable quantities of a gonadotropin are present in the blood and urine of ovariectomized (Fluhman) and postmenopausal (Zondek) women. The work of Hamburger [18] demonstrated this substance to be qualitatively different from the chorionic gonadotropin excreted by pregnant women. Experiments performed with extracts of relatively low potency led Smith and Engle, and Smith, Engle and Tyndale to consider this material as containing a follicle-stimulating or gametokinetic factor. More recently Tyndale, Levin and Smith [19] studied the effects of more potent and relatively nontoxic extracts of this gonadotropin. Normal and hypophysectomized immature rats were used. In the latter the extracts produced only follicular stimulation over a wide dose range. However, when larger doses, eight to ten times the minimal stimulating dose, were administered, luteinization was observed in all the animals. This material, like the pituitary follicle-

18. Hamburger, Christian: Studies on Gonadotropic Hormones from the Hypophysis and Chorionic Tissue, with Special Reference to Their Differences, Acta path. et microbiol. Scandinav., 1933, supp. 17.

19. Tyndale, H. H.; Levin, Louis, and Smith, P. E.: Responses of Normal and Hypophysectomized Immature Rats to Menopause Urine Injections, Am. J. Physiol. **124**: 174 (Oct.) 1938.

stimulating substance used by others, produces luteinization if enough of it is administered.

The results obtained when the same material was administered to intact immature rats afford an interesting comparison. Throughout the dose range corpora lutea were formed in the ovaries of some but not all of the test animals. The proportion of animals showing corpora increased with increased dose until, at precisely that dose which produced luteinization in the hypophysectomized animals, all the normal animals showed corpora in their ovaries. This indicates the role which the animal's own pituitary (even before sexual maturity) may play in the gonadotropic-gonadal relationship and the care which consequently must be exercised in the interpretation of qualitative results obtained by the use of intact animals.

That women with normal ovarian function excrete small amounts of a gonadotropin for a short time during the midinterval has been shown.[20] More recently Werner,[21] using the sensitive mouse uterus assay method,[22] has been able to show that this gonadotropin is excreted in a demonstrable amount throughout the menstrual cycle and that the amount is quite constant except for a sudden transient increase during the midinterval. This transient rise, probably related to the ovulatory process, is responsible for the occasional period of excretion noted by the earlier investigators, who used less sensitive assay methods and possibly less quantitative concentration procedures. Werner, in certain of his studies, also determined the excretion of pregnandiol and found that this substance, an indicator of progesterone secretion by the corpus luteum, frequently appears in the urine within two to five days after the "ovulatory" increase in gonadotropin excretion.

20. Kurzrok, Raphael; Kirkman, Irene J., and Creelman, Margaret: Studies Relating to the Time of Human Ovulation, Am. J. Obst. & Gynec. **28**: 319 (Sept.) 1934. Frank, R. T., and Salmon, U. J.: Gonadotropic Blood and Urine Cycles in Normal Menstruating Women, Proc. Soc. Exper. Biol. & Med. **32**: 1237 (May) 1935. Smith, G. V., and Smith, O. W.: The Urinary Excretion of Estrogenic and Gonadotropic Hormones During Menstrual Cycles, the Period of Conception and Early Pregnancy, New England J. Med. **215**: 908 (Nov. 12) 1936. D'Amour, F. E.; Funk, Dorothy, and Liverman, Helen: Daily Gonadotropic Hormone Tests During Fifty Complete Menstrual Cycles, Am. J. Obst. & Gynec. **37**: 940 (June) 1939.

21. Werner, S. C.: A Quantitative Study of the Urinary Excretion of Hypophysial Gonadotropin, Estrogen and Androgen of Normal Women, J. Clin. Investigation, to be published.

22. Levin, Louis, and Tyndale, H. H.: The Quantitative Assay of "Follicle-Stimulating" Substances, Endocrinology **21**: 619 (Sept.) 1937.

Although the exact nature of the gonadotropin excreted by normal women is not yet established, a limited study by D'Amour [23] indicates that this substance is very similar to that excreted following the menopause.

Normal men excrete a gonadotropin in somewhat larger amounts than do normal women. No careful day by day studies have been made, but the available data afford no indication of cyclic variation in this excretion.

The qualitative nature of the urinary gonadotropin of the normal male has been investigated recently in this laboratory.[24] Over a wide dose range this material produced only follicular stimulation in immature hypophysectomized female rats. Higher doses caused lutein changes. It therefore appears that the urinary gonadotropin of normal men is very similar to or identical with that excreted by ovariectomized or postmenopausal women.

It is of interest that although postclimacteric women excrete increased amounts of gonadotropin with fair regularity, several investigators have been unable to find increased excretion of gonadotropin in aged men with varying degrees of genital involution.

GONADOTROPIN OF HUMAN PREGNANCY

The gonadotropin of human pregnancy was discovered by Aschheim and Zondek. It appears in the blood and urine soon after the implantation of the egg,[25] and affords the basis for the Aschheim-Zondek and Friedman pregnancy tests. It has been reported to appear before the first missed menstrual period. The concentration of the gonadotropin in the blood and urine increases rapidly during the first part of pregnancy, reaching a high titer fifty to sixty days after the last menstrual period; it then declines rapidly to relatively low levels, which are maintained until a few days after parturition, when the substance disappears from the

23. D'Amour, F. E.: A Qualitative Study of Normal Gonadotropin, Am. J. Physiol. **127**: 649 (Nov.) 1939.

24. Leathem, J. H., and Levin, Louis: The Gonadotropic Action of Normal Male Urine Extract on the Ovaries of Normal and Hypophysectomized Immature Rats and of Immature Mice, Endocrinology **29**: 8 (July) 1941.

25. Engle, E. T.: Gonadotropic Substances of Blood, Urine and Other Body Fluids, in Allen, Edgar; Danforth, C. H., and Doisy, E. A.: Sex and Internal Secretions, Baltimore, Williams & Wilkins Company, 1939.

blood and urine. The same gonadotropin has been demonstrated in large amounts in the placenta, the concentration being roughly parallel to that in the blood and urine.

A gonadotropin has also been found during brief periods in the urine of pregnant rhesus monkeys and chimpanzees. A similar but not identical chorionic gonadotropin, to be discussed in a later section of this review, appears in the blood but not the urine of pregnant mares. Investigation of the tissues and body fluids of a rather extensive variety of other mammals has not as yet demonstrated gonadotropic activity.

The human chorionic gonadotropin differs qualitatively from other known gonadotropins. It is not anterior pituitary–like, and there is no longer any reason for the use of the designation "anterior pituitary–like," which was applied to this gonadotropin before its nature was well known. Neither is the designation "prolan A" as defined by Zondek any longer applicable to any fraction of this substance, since it is not under any condition a follicle-stimulating gonadotropin.

In the immature female rodent (rat or mouse) the growth of follicles (reaction 1), the appearance of hemorrhagic follicles (reaction 2, more constant in the mouse) and the formation of corpora lutea (reaction 3) follow exactly the description recorded by Aschheim and Zondek. Examination of the effects of this gonadotropin in the hypophysectomized rat prove that these reactions, though valid for the intact animal, are partially due to the participation of the animal's own pituitary. The age of the animal and the length of the period of regression following removal of the pituitary both affect the quality of the response.

In immature female rats treated immediately after hypophysectomy Leonard and Smith [26a] found that corpora lutea could be formed. This occurs only if maturing follicles are present in the ovary at the time

26. (a) Leonard, S. L., and Smith, P. E.: Responses of the Reproductive System of Hypophysectomized Rats to Injections of Pregnancy-Urine Extracts: II. The Female, Anat. Rec. **58**: 175 (Jan. 25) 1934. (b) Williams, P. C.: Effect of Stilbestrol on the Ovaries of Hypophysectomized Rats, Nature **145**: 388, 1940. Pencharz, R. I.: Effect of Estrogens and Androgens Alone and in Combination With Chorionic Gonadotropin on the Ovary of the Hypophysectomized Rat, Science **91**: 554 (June) 1940. Simpson, M. E.; Evans, H. M.; Fraenkel-Courat, H. L., and Li, C. H.: Synergism of Estrogens with Pituitary Gonadotropins in Hypophysectomized Rats, Endocrinology **28**: 37 (Jan.) 1941.

of hypophysectomy and if treatment is instituted immediately. In either case the interfollicular (interstitial) cells as well as the cells of the theca are caused to hypertrophy, and atretic follicles are converted into luteoid bodies. Estrogen is secreted (by the hypertrophied interstitial and thecal cells), as indicated by the vaginal cornification and estrus. In no instance has human chorionic gonadotropin been observed to cause follicular growth in hypophysectomized animals, whether adult or immature. Recent work [26b] has shown, however, that the synthetic estrogen stilbestrol synergizes with human chorionic gonadotropin in hypophysectomized immature female rats. The ovaries of such animals subjected to the combined treatment show development of fairly large follicles (sometimes hemorrhagic) and corpora lutea. The ovarian weight is markedly increased. The explanation for this synergism is not yet known, although the effect may be analogous to that of androgen on the testes of hypophysectomized rats.

In general, all other animals below primates which have been studied react as does the rat. Details of the species differences can be found elsewhere.[25] In the presence of a functional hypophysis, treatment with this chorionic gonadotropin results in stimulation of follicles and luteinization, frequently with ovulation. If the hypophysis is removed, estrus is induced by the secretion of the interfollicular, interstitial or thecal cells without follicular growth.

The Algerian baboon is reported to respond as do the lower animals (Courrier). The common laboratory rhesus monkey (Macacus mulatta), however, is quite different. Even in intact monkeys (rhesus) the human chorionic gonadotropin causes neither follicular growth, nor ovulation nor luteinization. If normal animals in the first part of the menstrual cycle are given adequate doses of an extract containing this substance, the estrogenically induced swelling and color of the sex skin disappears and the bleeding due to estrogen withdrawal follows. In immature females no follicular stimulation occurs, as judged by either the sex skin response or by ovarian structural changes. Small follicles are quickly forced into atresia with the formation of scar tissue, similar to corpora albicantia. Corpora

lutea may be formed in the intact animal if a follicle stimulator is added to the chorionic gonadotropin.

Geist reported on structural changes in human ovaries after treatment with human chorionic gonadotropin and stated that "apparently there is no stimulation of the follicle, rather an arrest of follicular development." Hamblen,[27] in several series, observed neither follicular growth, nor ovulation nor production of functional corpora lutea. That the structural changes of the human ovary after this treatment are mainly those of arrest or are degenerative in nature does not necessarily indicate that this substance is therapeutically without value in women.

The therapeutic value of this substance in gynecologic practice will be discussed in another chapter. It is pertinent to state here, however, that morphologic studies on human ovaries conform in general to the observations on the monkey.

As has been pointed out in the preceding paragraphs, human chorionic gonadotropin does not stimulate the gametogenic elements of the mammalian ovary but does produce some of the reactions ascribed to the luteinizing hormone of the pituitary gland. In the hypophysectomized male rat the analogy is not quite so clear. In such animals, as well as in intact immature male rats, the first and constant response to the gonadotropin of human pregnancy is a hypertrophy of the interstitial cell mass of the testis, as shown by an adequate series of hypophysectomized adult male rats studied by Smith and Leonard.[28] Results of other workers are in good general agreement, but recently Liu and Noble [35] published experiments which they claimed showed that spermatogenesis may be reinitiated in at least a few adult male rats even if the treatment with chorionic gonadotropin is begun as long as twenty-eight days after hypophysectomy.

If the treatment of mature animals is instituted immediately after hypophysectomy, spermatogenesis as well as the size of the accessory glands can be maintained. The characteristic maintenance of the interstitial cells and the production of androgen necessary

27. Hamblen, E. C.: Endocrine Gynecology, Springfield, Ill., Charles C. Thomas, Publisher, 1939.

28. Smith, P. E., and Leonard, S. L.: Responses of the Reproductive System of Hypophysectomized and Normal Rats to Injections of Pregnancy-Urine Extracts; I. The Male, Anat. Rec. 58: 145 (Jan. 25) 1934.

for the accessory glands are adequate. However, it has been demonstrated that androgen alone will maintain spermatogenesis in the hypophysectomized rat.[29] Nelson [30a] has stated that spermatogenesis in this animal can be maintained with androgen alone if treatment is begun within ten days after hypophysectomy. In view of this finding as well as of the fact that one pronounced effect of human chorionic gonadotropin is the stimulation of the testes to produce androgen, it is impossible to be certain at present whether the maintenance of spermatogenesis by means of this gonadotropin is due to a direct action on the tubules or to an effect secondary to the stimulation of androgen production. By analogy to the female, in which the human chorionic gonadotropin certainly is not a follicle stimulator and has no gametokinetic action, one might deduce that the gametogenic effect in the male is secondary to the stimulated production of androgen. Such a deduction is supported by the fact that if a considerable period is allowed to elapse between hypophysectomy and institution of treatment, neither androgen nor chorionic gonadotropin is effective in restoring spermatogenesis, even though the latter in such a case is able to stimulate the interstitial cells quite adequately and does to a certain extent cause the tubules to enlarge. In a recent preliminary report Nelson [30b] claimed that spermatogenesis may be reinitiated in the rat even if the androgen treatment is begun as long as twenty-eight days after hypophysectomy. However, it has not yet been proved whether the human chorionic gonadotropin does or does not have a direct effect on the spermatogenic elements.

It must be emphasized that the rat is a rather primitive, generalized type of animal and that the foregoing observations cannot be transferred directly to man. In the instance of androgenic maintenance of spermatogenesis it is evident that the rat is a favorable species, for the observation of this maintenance reaction does

29. Walsh, E. L.; Cuyler, W. K., and McCullagh, D. R.: The Physiologic Maintenance of the Male Sex Glands; The Effect of Androtin on Hypophysectomized Rats, Am. J. Physiol. **107**: 508 (Feb.) 1934. Nelson, W. O.: Some Factors Involved in the Control of the Gametogenic and Endocrine Functions of the Testis, in Cold Spring Harbor Symposia on Quantitative Biology, Cold Spring Harbor, L. I., New York, The Biological Laboratory, 1937, vol. 5.

30. Nelson, W. O.: (*a*) Re-Initiation of Spermatogenesis in Hypophysectomized Rats, Am. J. Physiol. **129**: P 430 (May) 1940; (*b*) Renewal of Sperm Formation in Hypophysectomized Rats, Anat. Rec. **79** (supp 2): 49 (March) 1941.

not hold for the monkey [31] or perhaps even for the guinea pig.[32]

The effect of human chorionic gonadotropin on the testes of the immature monkey is primarily one of stimulating the interstitial cell growth and the production of androgen. The tubules increase in size, but no spermatogenic effect in immature monkeys has been reported.[25]

Descent of the testes in monkeys accompanies the increase in androgen production following gonadotropin treatment. The therapeutic value of the gonadotropin of pregnancy urine in cryptorchidism is discussed in a subsequent chapter.

GONADOTROPIN OF PREGNANT MARE'S SERUM

Large quantities of a gonadotropin appear in the blood of the pregnant mare during the early stages of gestation, reaching a very high titer during the middle third of the pregnancy.[25] This gonadotropin, probably of chorionic origin, differs markedly from the other known gonadotropins in the respect that the physiologic responses it evokes are an interesting blend of those produced by the gonadotropic principles of human menopausal and human pregnancy urine. Very little if any of the gonadotropin is excreted in the urine of the mare. Similarly, if an extract of the gonadotropic substance is injected into laboratory animals, the gonadotropic factor is not excreted in the urine. After intravenous administration of high doses of such an extract to normal female monkeys, only traces of the gonadotropic factor appear in the urine. Its fate after injection into women is not known.

In intact immature female rats the gonadotropin of pregnant mare's serum causes follicular stimulation, followed by luteinization and marked increases in ovarian weight. It is also able to produce definite follicular stimulation in hypophysectomized rats, an action never evoked by human chorionic gonadotropin. In the hypophysectomized female rat the action of the equine gonadotropin is similar to that of the human

31. Smith, P. E.: Comparative Effects of Hypophysectomy and Therapy on the Testes of Monkeys and Rats, in Brouha, L.: Les hormones sexuelles, Paris, Hermann & Cie, 1938, pt. 3, L'hypophyse.

32. Scowen, E. F.: The Effects of Androsterone and Testosterone on the Testes of Hypophysectomized Guinea Pigs, Anat. Rec. **70** (supp. 3): 71 (March) 1938. Cutuly, E.: Androgen and Spermatogenesis in the Hypophysectomized Guinea Pig, Proc. Soc. Exper. Biol. & Med. **47**: 290 (June) 1941.

castrate or postmenopausal principle in ability to stimulate follicular growth and, with adequate doses, to produce granulosa luteinization. However, with the equine, though not with the human, gonadotropin thecal luteinization always accompanies even slight stimulation of follicles.[33]

When hypophysectomized male rats are given an extract of the equine gonadotropin, the responses, interstitial hypertrophy and tubular repair, are somewhat similar to those obtained with the gonadotropic material from the urine of pregnant women.[34] Liu and Noble [35] have recently administered human and equine chorionic material to hypophysectomized adult male rats. When treatment was instituted immediately after removal of the pituitary, the equine material was able to maintain spermatogenesis and accessory gland condition so that fertile matings were obtained.[34b] This is in agreement with the previously discussed observations of Smith and Leonard [28] with human chorionic gonadotropin. In the experiments of Liu and Noble the repair of the spermatogenic elements were not completely effective if treatment was delayed, even though larger doses of the mare material were employed. In connection with these observations it is instructive to recall again the fact that in rats androgen alone will maintain and even repair the spermatogenic elements.[30]

Studies on maintenance and repair of the testicular function of the hypophysectomized guinea pig by the gonadotropic extract from the serum of the pregnant mare have been made by Webster and Leathem.[36] The results were comparable to those of Liu and Noble with the rat. Maintenance of spermatogenesis as well as hypertrophy of the interstitial cells and accessory glands were obtained if treatment was started immediately after hypophysectomy. However, if treatment was delayed, only the interstitial cells and, consequently, the

33. Leathem, J. H.: Responses of Hypophysectomized Immature Female Rats to Mare Serum Hormone, Proc. Soc. Exper. Biol. & Med. **42**: 590 (Nov.) 1939.

34. (*a*) Smith, P. E.: Unpublished data. (*b*) Evans, H. M.; Pencharz, R. I.; Simpson, M. E., and Meyer, K.: Repair of the Reproductive Mechanism in Hypophysectomized Male Rats, in Lueschner, A. O., and Teggart, F. J.: Memoirs of the University of California, Berkeley, Calif., University of California Press, 1933, vol. 11, p. 301.

35. Liu, S. H., and Noble, R. L.: The Effects of Extracts of Pregnant Mare Serum and Human Pregnancy Urine on the Reproductive System of Hypophysectomized Male Rats, J. Endocrinol. **1**: 7 (June) 1939.

36. Webster, E. C., and Leathem, J. H.: Responses of Hypophysectomized and Normal Male Guinea Pigs to Mare Serum Hormone, to be published.

accessory glands were restored. The spermatogenic elements, though showing some improvement, were only incompletely restored.

In the female monkey the gonadotropic extract from pregnant mare's serum is a potent stimulator of follicles but produces no luteinization or fibrotic changes in the thecal wall.[25] In this respect the equine gonadotropin resembles the gonadotropic principles of the pituitary gland and human postmenopausal urine but differs markedly from the gonadotropin in the urine of pregnant women, which, if administered alone, does not cause follicular activation in the female monkey.

In the hypophysectomized adult male monkey the effects of the gonadotropic extract from the pregnant mare's serum are not similar to the effects in rodents. Smith [37] has shown that after postoperative involution of the testis the extract does not restore spermatogenic function even when the doses are high and the treatment prolonged. Smith has also pointed out that the longer treatment would probably give no better restorative effects because of the formation of antibodies against the gonadotropic substance.

The action of this substance on the gonads of men and women is discussed elsewhere.

Chemical experiments with the gonadotropin in pregnant mare's serum have yielded preparations of very high potency and considerable purity. However, even the purest of these preparations for which data are available produce physiologic effects identical with those of the crude, untreated material. This finding, if taken to indicate that the gonadotropin is a single entity, is difficult to reconcile with the claim [38] which has been made for the separation of the material into two fractions possessing qualitatively different physiologic activities. It may be that the results described in the latter report were due to a dosage phenomenon rather than to actual separation.

Any consideration of the effects of the gonadotropic extract from the serum of the pregnant mare must take into account the nonpermeability of the kidney to this material. As has been mentioned, the equine gonado-

37. Smith, P. E.: Presidental Address, read before the Twenty-Fourth Annual Scientific Session of the Association for the Study of Interna' Secretions, New York, 1940.

38. Evans, H. M.; Korpi, K.; Simpson, M. E., and Pencharz, R. I.: Fractionation of the Gonadotropic Hormones in Pregnant Mare Serum by Means of Ammonium Sulphate, Univ. California Publ. Anat. 1: 275, 1936.

tropin does not pass through the kidney into the urine as do other gonadotropins. It is not, therefore, surprising that the extract is as effective by intraperitoneal as by subcutaneous injection and by administration of a single dose as by administration of divided doses. In fact, there is evidence [39] that over short intervals a single intraperitoneal or intravenous injection is even more effective than if the same amount is divided among a number of injections. Because it is not excreted, one can presume that the gonadotropin remains in the blood stream in effective concentration, being relatively slowly inactivated or destroyed. This fact may account for some of the differences in physiologic action between this gonadotropin and its chorionic analog in the urine of pregnant women.

39. Pencharz, R. I.: Factors Influencing Ovarian Response of Normal and Hypophysectomized Rat to Pregnant Mare Serum, Proc. Soc. Exper. Biol. & Med. **42**: 525 (Nov.) 1939. Leathem, J. H.: The Mode of Administration as an Influence on the Effectiveness of Mare Serum in Hypophysectomized Immature Rats, Endocrinology **28**: 615 (April) 1941.

CHAPTER VII

THE ANTIHORMONES

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At the present time the term "antihormones" is most commonly applied to certain substances of unknown origin which appear in the serum of animals treated for long periods with certain anterior pituitary extracts, which apparently explain the observed gradual loss of responsiveness to such extracts and which are capable of making other animals, previously untreated, refractory to treatment with similar extracts. The demonstration of an antihormone of this type thus involves at least three groups of animals: first, those from which the original anterior pituitary extract is prepared; second, those to which this extract is administered until they become refractory to it and the antihormone is present in their serum; third, those to which the serum is administered and which then show partial or complete inhibition of their usual response to an anterior pituitary extract identical with or similar to that used in the first place. Extracts from endocrine organs other than the anterior lobe of the pituitary may also be found to decrease in efficacy when administered over long periods, and in one or two instances the serum of the treated animals may acquire inhibitory properties and be said to contain an antihormone. Various modifications of this term have been used in other senses as well. The subject has been reviewed several times within the last few years.[1]

1. Collip, J. B.: Recent Studies on Anti-Hormones, Ann. Int. Med. **9**: 150-161 (Aug.) 1935. Rambert, Paul: Le problème des antihormones, Paris méd. **1**: 345-351 (April 22) 1939. Collip, J. B.; Selye, Hans, and Thompson, D. L.: The Antihormones, Biol. Rev. **15**: 1-34 (Jan.) 1940. Thompson, K. W.: Antihormones, Physiol. Rev., to be published.

THE ANTITHYROTROPIC SUBSTANCE

When animals of various species are treated for long periods with anterior pituitary extracts containing the thyrotropic factor, the induced morphologic and physiologic signs of hyperthyroidism gradually wane and may indeed be replaced by signs of moderate hypothyroidism.[2] (In the English sparrow, however, no such refractory state has been observed.[3]) If the treatment is stopped, the animals may gradually regain their original sensitivity in from four to six months.[4]

Collip and Anderson[5] showed that the refractory state was not due to exhaustion of the thyroid under overstimulation, since thyroid tissue transplanted from a normal into a refractory animal was itself refractory; and they showed that injections of the serum of the resistant rats into untreated hypophysectomized rats prevented the normal response (increased metabolic rate) of the latter to treatment with the thyrotropic extract. They obtained similar antithyrotropic serums by treatment of guinea pigs, dogs, rabbits and horses. Long before this, Masay,[6] in a neglected paper, described cachexia in animals treated with the serum of rabbits or guinea pigs which had been pretreated with pituitary suspensions, comparing the condition with that following thyroidectomy and stating that it was accompanied by exophthalmos.

Collip and Anderson pointed out that their antithyrotropic substance had no power to inhibit the thyroid hormone, although it appeared to influence the metabolic rate rather than the structure of the thyroid

2. Aron, Max: Action combinée de la thyroxine et de l'extrait préhypophysaire sur la thyroïde chez le cobaye, Compt. rend. Soc. de biol. **104**: 96-98, 1930. Loeb, Leo, and Friedman, Hilda: Changes in Weight of Thyroid Gland of Young Guinea-Pigs Under the Influence of Acid Extract of Anterior Pituitary, Proc. Soc. Exper. Biol. & Med. **29**: 14-16 (Oct.) 1931; Long Continued Injections of Acid Extract of Anterior Pituitary on Thyroid Gland and Sex Organs, ibid. **29**: 172-174 (Nov.) 1931.

3. Miller, D. S.: Lack of Refractoriness to Prolonged Thyrotropin Administration in Birds, Proc. Soc. Exper. Biol. & Med. **38**: 453-455 (May) 1938.

4. Mahaux, Jacques: Action de l'hormone thyréotrope sur le métabolisme basal de lapins normaux et de lapins ayant présenté précédemment une période d'inversion d'action de l'hormone thyréotrope, Compt. rend. Soc. de biol. **132**: 97-99, 1939.

5. Collip, J. B., and Anderson, E. M.: Production of Serum Inhibitory to the Thyrotropic Hormone, Lancet **1**: 76-78 (Jan. 13) 1934. Anderson, E. M., and Collip, J. B.: Preparation and Properties of an Antithyrotropic Substance, ibid. **1**: 784-786 (April 14) 1934; Studies on the Physiology of the Thyreotropic Hormone of the Anterior Pituitary, J. Physiol. **82**: 11-25 (Aug. 24) 1935.

6. Masay, F.: L'acromégalie expérimentale, Bull. Soc. roy. d. sc. méd. et nat. de Bruxelles **64**: 338-346, 1906.

in their animals. But pretreatment with antithyrotropic serum was later found to prevent the histologic signs of thyroid activation [7] and the increase in thyroid weight [8] normally observed in young guinea pigs (or chicks [9]) treated with thyrotropic pituitary extract. Such tests are probably preferable to those based on metabolic rate, which may be influenced by the specific metabolic principle of the pituitary,[10] to which no resistance is developed. During continued treatment with thyrotropic pituitary extract the metabolic rate of the rat passes through a maximum about the end of the first week and by the fifth week has fallen to levels as low as those seen in hypophysectomized animals;[5] the antithyrotropic activity of serum has been found maximal at the fifth week in sheep [11] and at the tenth week in rabbits;[12] thereafter it declines. At the peak 2 cc. of the rabbit serum will inactivate a dose of thyrotropic pituitary extract otherwise sufficient to double the thyroid weight of immature guinea pigs.[12]

The antithyrotropic substance seems to show marked but not absolute species specificity; rabbits and guinea pigs which had become refractory to bovine thyrotropic extract still responded to similar extracts from anterior lobes of pig pituitaries, and vice versa;[13] and the anti-

7. (*a*) Eitel, Hermann, and Loeser, Arnold: Die Hemmung der Schilddrüsentätigkeit durch Tierblut, Klin. Wchnschr. **13**: 1742-1744 (Dec. 8) 1934; Die antithyreotrope Schutzkraft des Blutes, Arch. f. exper. Path. u. Pharmakol. **177**: 737-751, 1935. (*b*) Oudet, P.: Propriétés antithyréostimulantes du sérum d'animaux traités par la thyréostimuline préhypophysaire, Compt. rend. Soc. de biol. **123**: 1177-1179, 1936.

8. Harington, C. R., and Rowlands, I. W.: Fractionation of Antithyrotropic and Antigonadotropic Sera, Biochem. J. **31**: 2049-2054 (Nov.) 1937.

9. Cope, C. L.: The Young Chick as Test for the Thyrotropic Hormone, J. Physiol. **94**: 358-364 (Dec. 14) 1938.

10. Billingsley, L. W.; O'Donovan, D. K., and Collip, J. B.: The Specific Metabolic Principle of the Pituitary, Endocrinology **24**: 63-68 (Jan.) 1939.

11. Eitel, Hermann, and Loeser, Arnold: Die Bedeutung der Schilddrüse für die antithyreotrope Schutzkraft des Blutes, Arch. f. exper. Path. u. Pharmakol. **179**: 440-447, 1935.

12. Rowlands, I. W., and Parkes, A. S.: Study of Anti-Thyrotropic Activity, Proc. Roy. Soc., London, s. B **120**: 114-125 (May 1) 1936.

13. Eichbaum, Franz, and Kindermann, Viktor: Untersuchungen über die antigenen Funktionen von Hormonpräparaten; thyreotropes Hypophysenvorderlappenhormon, Ztschr. f. Immunitätsforsch. u. exper. Therap. **89**: 498-511 (Dec. 31) 1936. Eichbaum, Franz; Kindermann, E.; Oestreicher, F., and Reiss, M.: Zur Frage der Unwirksamkeit des thyreotropen Wirkstoffes bei andauernder Zufuhr, Endokrinologie **18**: 375-378, 1937. Oudet, P.: Recherches sur les propriétés anti-thyréostimulantes du sang d'animaux traités, durant une courte période, par un extrait purifié de préhypophyse, Compt. rend. Soc. de biol. **126**: 710-711, 1937.

serum obtained from rabbits treated with a bovine extract did not prevent the response of guinea pigs to a thyrotropic fraction from anterior lobes of human pituitaries.[14] It is also stated that purified thyrotropic fractions prepared by flavianic acid precipitation may be administered for long periods without development of a refractory state, and are effective in guinea pigs that have become refractory to cruder fractions prepared by salting out.[15] All these observations have naturally been regarded as evidence that the antithyrotropic substance is a true antibody, produced as a response to the foreign protein present in the thyrotropic extracts. Not easily reconciled with this view are the observations that an antithyrotropic serum, administered by itself, will depress the metabolic rate of the rat [5] or the rabbit [16] and flatten thyroid epithelium in the guinea pig (Eitel and Loeser [7a]). Still more serious is the objection that antithyrotropic substance has been detected in the serum of normal, untreated animals of various species, including the dog,[17] sheep (Eitel and Loeser [7a]), rabbit [18] and man,[19] which seems to prove it to be a normal constituent of the body, unless this is not the same substance as the one found in larger amounts in treated animals. Antithyrotropic activity has been recognized in the serum of animals treated with an anterior pituitary extract which had

14. Cope, C. L.: Effect of Antithyrotropic Serum on the Action of Human Thyrotropic Hormone, Lancet **1**: 888-890 (April 16) 1938.

15. Werner, S. C.: Prolonged Injection of a Thyrotropic Extract Without Development of Refractoriness, Proc. Soc. Exper. Biol. & Med. **34**: 390-392 (April) 1936; Antibody Nature of Refractoriness to Injections of Hypophyseal Extracts Containing Thyrotropic Hormone, ibid. **34**: 392-394 (April) 1936. Cutting, W. C.; Robson, G. B., and Emerson, Kendall, Jr.: Refractoriness from Pituitary Thyrotropic Extracts, Endocrinology **24**: 739-740 (May) 1939.

16. Mahaux, Jacques: Action du sérum de lapins à métabolisme abaissé par administration prolongée d'hormone thyréotrope sur le métabolisme basal de lapins normaux, Compt. rend. Soc. de biol. **132**: 99-102, 1939.

17. Loeser, Arnold: Die Bedeutung der Hypophyse für die antithyreotrope Schutzkraft des Blutes, Arch. f. exper. Path. u. Pharmakol. **180**: 458-465, 1936.

18. Scowen, E. F., and Spence, A. W.: Effect of Antithyrotropic Serum on the Thyroid Gland of Guinea-Pigs Treated with Thyrotropic Hormone, J. Physiol. **86**: 109-116 (Jan. 15) 1936.

19. (*a*) Collip, J. B., and Anderson, E. M.: Studies on the Thyrotropic Hormone of the Anterior Pituitary, J. A. M. A. **104**: 965-969 (March 23) 1935. (*b*) Herold, Ludolf: Nachweis und Auswertung von anti-thyreoiden Schutzstoffen im Blute von Basedowkranken und Schwangeren, Klin. Wchnschr. **13**: 1242-1244 (Sept. 1) 1934. (*c*) Eitel, Hermann: Die antithyreotrope Schutzkraft des Blutes gesunder und kranker Menschen, ibid. **17**: 1465-1467 (Oct. 15) 1938. Scowen and Spence.[18]

little or no thyrotropic potency.[20] The nature of the antithyrotropic substance is perhaps less important than the fact that this substance must be expected to appear in the serum of patients treated with thyrotropic anterior pituitary extracts for long periods.[21]

The antithyrotropic substance is destroyed by boiling [5] and is apparently associated with the pseudoglobulin of the serum; [8] it may be purified by adsorption.[22] Collip and Anderson,[19a] Loeser [17] and Gessler [23] all agree that it can be formed by hypophysectomized animals. It has been suggested that it cannot be formed by thyroidectomized animals (and even that it is identical with thyroxine [11]), but this has been denied.[24] Estrogens [25] may have some antithyrotropic activity, but this can hardly be relevant.

THE ANTIGONADOTROPIC SUBSTANCE

When chorionic gonadotropin (from human pregnancy urine or placenta) is administered for long periods to female rats, the weights of the ovaries increase at first but later decline to or below the normal level, as a refractory phase is entered; [25] a similar effect is observed when the ovaries are chronically stimulated by daily implantation of rat pituitary tissue.[26] In the refractory state the ovaries may be markedly atrophic and "wheel cells" may appear in the theca (a finding characteristic of complete absence of hypophysial stimuli), while typical "signet ring" castration cells

20. Young, F. G.: Preparation of Antisera to Preparations of Prolactin Containing the Glycotropic (Anti-Insulin) Factor of the Anterior Pituitary Gland, Biochem. J. **32**: 656-664 (April) 1938.

21. Spence, A. W., and Witts, L. J.: Substitution Therapy in Hypopituitarism, Quart. J. Med. **8**: 69-77 (Jan.) 1939.

22. Loeser, Arnold, and Trikojus, V. M.: Untersuchungen über die Gewinnung antithyreotroper Wirkstoffe aus Blut, Ber. d. naturforsch. Gesellsch. Freiburgi.-Br. **35**: 211, 1937.

23. Gessler, Carl: Activité antithyroïdienne de la folliculine; rôle de l'hypophyse, Arch. internat. de pharmacodyn. et de thérap. **55**: 267-281 (March 31) 1937.

24. Oudet, P.: Rôle éventuel de la sécrétion thyroïdienne dans la mise en jeu des propriétés antithyréostimulantes du sérum d'animaux traités par la thyréostimuline préhypophysaire, Compt. rend. Soc. de biol. **123**: 1180-1181, 1936. Chou, C. H.: Antithyrotropic Effect of Serum of Normal and Thyroidectomized Rabbits, Chinese J. Physiol. **12**: 155-162 (Sept. 15) 1937.

25. Zondek, Bernhard: Hormone des Ovariums und des Hypophysenvorderlappens, Berlin, Julius Springer, 1931. Collip, J. B.: Placental Hormones, Internat. Clin. **4**: 51-70 (Dec.) 1932. McPhail, M. K.: Effect on Reproductive Organs of the Rat of Prolonged Treatment with Ovary-Stimulating Substances, J. Physiol. **80**: 105-112 (Nov. 9) 1933.

26. Selye, Hans; Collip, J. B., and Thomson, D. L.: Loss of Sensitivity to the Gonadotropic Hormone of the Hypophysis, Proc. Soc. Exper. Biol. & Med. **31**: 566 (Feb.) 1934.

appear in the anterior lobe of the pituitary.[27] The refractory ovaries are not exhausted and incapable of responding to any stimulus, for those which have become refractory to the influence of chorionic preparations still respond to hypophysial preparations[28] and vice versa.[26] The serum of animals with such refractory ovaries will prevent the usual response of immature female rats to appropriate gonadotropic stimulation.[29] Analogous phenomena are observed in male rats and in other species.

The question whether the antigonadotropic substance is an immune body, produced in response to the injection of foreign protein, or is to be regarded in some other light—for example, as a normal constituent of the organism—has been eagerly debated, and much of the discussion has centered on the problem of specificity, which in this case is exceedingly complex. One has to consider (1) species specificity, the comparison of extracts from the hypophyses (or other organs) of different species; (2) extract specificity, the comparison of extracts made from the same material by different procedures; (3) hormone specificity, the comparison of extracts having different ratios of follicle-stimulating and luteinizing (interstitial cell-stimulating) activity, and (4) organ specificity—for example, the comparison of preparations from human pregnancy blood or serum with extracts from human hypophysis or menopausal urine (which has been little studied), or of preparations from the serum of pregnant mares with extracts from equine hypophysis.

Female rats which have become refractory to chorionic gonadotropin still respond to implants or

27. Collip, J. B.; Selye, Hans, and Thomson, D. L.: Histological Changes in the Hypophysis Produced by Chronic Administration of Hypophyseal Extracts, Proc. Soc. Exper. Biol. & Med. **31**: 682-683 (March) 1934. Collip, J. B.; Selye, Hans, and Williamson, J. E.: Changes in the Hypophysis and the Ovaries of Rats Chronically Treated with an Anterior Pituitary Extract, Endocrinology **23**: 279-284 (Sept.) 1938. Severinghaus, Aura E., and Thompson, K. W.: Cytological Changes Induced in Hypophysis by Prolonged Administration of Pituitary Extract, Am. J. Path. **15**: 391-412 (July) 1939.

28. Selye, Hans; Collip, J. B., and Thomson, D. L.: Loss of Sensitivity to Anterior-Pituitary-Like Hormone of Pregnancy Urine, Proc. Soc. Exper. Biol. & Med. **31**: 487-488 (Jan.) 1934.

29. Selye, Hans; Bachman, Carl; Thomson, D. L., and Collip, J. B.: Further Studies on Loss of Sensitivity to Anterior-Pituitary-Like Hormone of Pregnancy Urine, Proc. Soc. Exper. Biol. & Med. **31**: 1113-1115 (June) 1934. Twombly, G. H., and Ferguson, R. S.: Protective Substances in Sera of Animals Injected with Anterior-Pituitary-Like Hormone of Teratoma Testis Urine, ibid. **32**: 69-71 (Oct.) 1934. Bachman, Carl; Collip, J. B., and Selye, Hans: Anti-Gonadotropic Substances, ibid. **32**: 544-547 (Dec.) 1934.

extracts of animal hypophyses [28] or pregnant mare serum but not, it is claimed, to extracts from human hypophysis.[30] The serum of such rats is said to protect test animals against gonadotropic stimulation, not only that with chorionic gonadotropin but that with human hypophysial extracts [31] and even that with bovine hypophysial extracts; [32] it is more usually found, however, that preparations from animal pituitaries or pregnant mare serum are not inhibited.[33] Rowlands,[34] who carried out careful and extensive cross tests, concluded that human chorionic gonadotropin displayed species specificity but not organ specificity. It has been claimed that the serums of animals refractory to the gonadotropin of pregnant mare serum will confer resistance against gonadotropic preparations from the hypophyses of horses,[35] sheep or pigs [36] or from human pregnancy urine,[37] but this has been denied,[38] and Rowlands [34] concluded that both organ specificity and species specificity are displayed, only mare's serum being inhibited. Serums obtained by treatment with hypophysial extracts show little specificity; [34] they confer protection against extracts from other species [39] or pregnant mare serum,[40]

30. Kabak, J.: Species-Specificity of the So-Called Anti-Hormones, Bull. de biol. et de méd. expér. U. R. S. S. **1**: 342, 1936.

31. Brandt, Robert, and Goldhammer, Helene: Die Spezifizität der gonadotropen Hormone und ihrer Antiseren, Ztschr. f. Immunitätsforsch. u. exper. Therap. **88**: 79-90 (May 30) 1936. de Fremery, P., and Scheygrond, B.: Some Properties of the Gonadotropic Hormones of the Human Pituitary, Acta brev. Neerland. **7**: 133-135, 1937. Zondek, Bernhard, and Sulman, Felix: The Antigonadotropic Factor, Species Specificity and Organ Specificity, Proc. Soc. Exper. Biol. & Med. **36**: 712-717 (June) 1937.

32. Gegerson, H. J.; Clark, A. R., and Kurzrok, Raphael: Studies on Gonadotropic Antihormones, Proc. Soc. Exper. Biol. & Med. **35**: 193-195 (Oct.) 1936.

33. Fluhmann, C. F.: Species-Specificity in Production of Anti-Gonadotropic Substances, Proc. Soc. Exper. Biol. & Med. **32**: 1595-1596 (June) 1935.

34. Rowlands, I. W.: Specificity of Antigonadotropic Sera, Proc. Roy. Soc., London, s. B **124**: 503-521 (Jan. 14) 1938.

35. Simonnet, H., and Michel, E.: Recherches expérimentales sur la nature des antihormones, Compt. rend. Soc. de biol. **129**: 918-921, 1938.

36. Thompson, K. W., and Cushing, Harvey: Inhibition of Action of Pituitary Hormones by Animal Sera, Proc. Roy. Soc., London, s. B **121**: 501-517 (Jan. 1) 1937.

37. Guercio, Francesco, and Cazzola, Dino: Su alcune caratteristiche che permettono di distinguere gli antiormoni antigonadotropi dai comuni anticorpi, Atti Accad. med. lombarda **28**: 1, 1939.

38. Gustus, E. L.; Meyer, R. K., and Dingle, J. H.: Relationship of Precipitin Titers to Gonadotropic Inhibitory Action of Monkey Sera, Proc. Soc. Exper. Biol. & Med. **33**: 257-261 (Nov.) 1935.

39. Parkes, A. S., and Rowlands, I. W.: Inhibition of Ovulation in the Rabbit by Anti-Gonadotropic Serum, J. Physiol. **88**: 305-311 (Dec. 11) 1936. Thompson and Cushing.[36]

40. Collip, J. B.: Results of Further Experiments with the Anti-Maturity Hormone, Canad. M. A. J. **36**: 199-200 (Feb.) 1937.

though they may have little action against human chorionic gonadotropin.[41] Under some circumstances [42] it seems possible to obtain serums which inhibit the luteinizing rather than the follicle-stimulating hormone.[43]

Further evidence of a lack of specificity hard to reconcile with an immunologic interpretation may be found in observations that antigonadotropic serums not merely inhibit injected gonadotropic preparations but when administered alone prevent the action of the endogenous gonadotropic hormones and have effects comparable to those of hypophysectomy. Thus the antiserums may cause ovarian atrophy and suppress estrus in the female rat,[44] prevent ovulation following mating in the rabbit,[39] interrupt pregnancy in the rabbit [45] and dog [46] and produce gonadal atrophy in male rats.[45] It will be noted also that antihormones have been produced by injection of sheep pituitary extract into sheep [40] or implantation of rat pituitaries into rats [26] (though difficulty has been encountered in repeating the latter experiment [47]), in which there is no question of foreign protein. Moreover, although complement-fixing antibodies and precipitins may appear in the serum of animals treated with preparations from urine, nearly all of the many students of this subject agree that there is no parallelism between the immunologic titer and the quantity of antigonadotropic substance physiologically determined, and that the former depends on contam-

41. Collip.[40] Fluhmann.[38]

42. Guyénot, E.; Held, H., and Moszkowska, A.: Accoutumance aux hormones préhypophysaires et sérums protecteurs, Rev. suisse de zool. **44**: 153, 1937.

43. Rowlands, I. W.: Selective Neutralization of the Luteinizing Activity of Gonadotrophic Extracts of Pituitary by Antisera, Proc. Roy. Soc., London, s. B **126**: 76-87 (Sept. 23) 1938; Rate of Appearance of Anti-Luteinizing Activity in Serum of Rabbits Injected with Extract of Ox Pituitary Glands, J. Endocrinol. **1**: 172-176 (Sept.) 1939. Freud, J.: Notes on Refractoriness to the Action of Hormones, Acta brev. Neerland. **9**: 161-163, 1939.

44. Collip.[40] Thompson and Cushing.[36]

45. Rowlands, I. W.: Effect of Anti-Gonadotrophic Serum on Reproductive Organs of the Normal Animal, Proc. Roy. Soc., London, s. B **121**: 517-532 (Jan. 1) 1937.

46. Thompson, K. W.: Termination of Pregnancy of Dogs by Gonadotropic Antihormone, Endocrinology **24**: 613-616 (May) 1939.

47. Katzman, P. A.; Wade, N. J., and Doisy, E. A.: Effects of Chronic Implantation of Rats with Pituitaries of the Same Species, Endocrinology **21**: 1-7 (Jan.) 1937. Artemov, N. M.: Influence of Chronic Homoimplantations of the Pituitary Body upon the Genital System of Female Rats, Bull. de biol. et de méd. expér. U. R. S. S. **3**: 642-645, 1937.

inating proteins rather than on the gonadotropin itself.[48] If serums prepared by treatment with bovine pituitary extract are treated with bovine serum, the antibovine immune bodies are precipitated while the antigonadotropic activity remains.[32]

Other observations, however, favor an immunologic interpretation, or at least suggest that the antihormone is formed only under abnormal experimental conditions. Antigonadotropic activity has been ascribed to,[49] and denied[50] to, the serum of untreated human beings and animals, and was not found in human puerperal serum despite the high gonadotropic titer throughout pregnancy;[51] indeed, it is usually absent from the serum of patients long treated with preparations of chorionic gonadotropin,[52] though a positive result has recently been reported with pregnant mare serum.[53] Again when a castrate animal is linked with a normal one in parabiosis, the latter shows no sign of becoming refractory to the gonadotropic hormone produced in

48. Bachman, Carl: Immunological Studies of Anti-Gonadotropic Sera, Proc. Soc. Exper. Biol. & Med. **32**: 851-854 (March) 1935. Eichbaum, Franz, and Kindermann, Viktor: Untersuchungen über die antigenen Funktionen von Hormonpräparaten; gonadotropes Hypophysenvorderlappenhormon, Ztschr. f. Immunitätsforsch. u. exper. Therap. **86**: 284-299 (Nov. 13) 1935; **89**: 230-238 (Nov. 3) 1936. Demanche, R.; Laroche, Guy, and Simonnet, H.: Etude critique de la réaction de fixation du complément appliquée à la recherche des antihormones dans le sang, Compt. rend. Soc. de biol. **125**: 718-719, 1937. Guercio, Francesco, and Cazzola, Dino: Osservazioni sui rapporti tra siero antiormone e specificita zoologica, Arch. di fisiol. **39**: 409, 1939. Meyer, R. K., and Wolfe, H. R.: Gonadotropic Inhibitory Substances and Precipitins in the Blood of Monkeys Receiving Gonadotropic Hormone Preparations, J. Immunol. **37**: 91-102 (Aug.) 1939. van den Ende, M.: Precipitins in Antigonadotrophic Sera, J. Endocrinol. **1**: 156-171 (Sept.) 1939; Urinary Gonadotrophic Extracts and Anaphylaxis in Vitro, ibid. **1**: 356-365 (Nov.) 1939. Howell, K. M., and Soskin, Samuel: Antibody Response of Rabbits to Extracts of Human Pregnancy Urine and to Extracts of Normal Female Urine, Endocrinology **26**: 577-580 (April) 1940. Gustus, Meyer and Dingle.[38] Brandt and Goldhammer.[51] Sulman.[51]

49. Collip and Anderson.[19] Brandt and Goldhammer.[51] Sulman[51] Twombly.[56] Chen, Graham: Attempts to Produce Antigonadotropic Substance by the Use of Serum or Blood Extracts, Chinese J. Physiol. **11**: 329-333 (March 1) 1937. Guercio, F., and Cazzola, D.: Sulla Presenza di una Sostanza Antipreipofisaria (Antihormone Naturale) nel Sangue di Animali Non Trattati Preventivamente con Ormone, Arch. di fisiol. **40**: 473-491, 1940.

50. Fellows, M. D.: Antigonadotropic Hormone in Normal Human Blood Serum, Endocrinology **26**: 369-376 (March) 1940.

51. Sulman, Felix: Does the Gonadotropic Hormone Induce Antibodies or Antihormones? J. Exper. Med. **65**: 1-14 (Jan.) 1937.

52. Spence, A. W.; Scowen, E. F., and Rowlands, I. W.: Absence of Antigonadotropic Substances in the Blood Serum of Man Injected with Gonadotropic Extracts, Brit. M. J. **1**: 66-67 (Jan. 8) 1938. Saphir, William; Howell, Katharine M., and Kunstadter, R. H.: Human Serum Response to Gonadotropic Hormone, Endocrinology **24**: 182-186 (Feb.) 1939. Dorff, G. B.: Antihormone Studies in Boys Treated with Anterior Pituitary-Like Hormone, ibid. **22**: 669-673 (June) 1938. Sulman.[51] Brandt and Goldhammer.[51]

53. Rowlands, I. W., and Spence, A. W.: Production of Antigonadotrophic Activity in Man by Injection of Extract of Pregnant Mares' Serum, Brit. M. J. **2**: 947-950 (Nov. 11) 1939.

excess by the former,[54] though it may be passively protected by transfer of serum from an animal made refractory by injections.[55] It is claimed, too, that antigonadotropic substance is produced in response to injections of chorionic gonadotropin which has been inactivated by heat or aging or ultraviolet irradiation,[56] or of extracts from male urine practically devoid of gonadotropic activity;[57] Zondek and co-workers,[58] however, have maintained that in all such preparations there remains a residue of gonadotropic potency and also that the neutralization of the injected gonadotropin by the antihormone follows quantitative or stoichiometric laws quite unlike those governing antigen-antibody reactions, on the one hand, or enzyme reactions, on the other;[59] hence Zondek has abandoned his earlier view that the antihormone is a specific hormone-destroying enzyme belonging to the class of Abderhalden's abwehrfermente.

The antigonadotropic substance is divided between the euglobulin and pseudoglobulin fractions of the serum[8] and can be purified by iso-electric precipitation.[60] It does not arise from the gonads, as it can be formed by castrate animals; it has been urged that it is produced by the reticuloendothelial system, like true antibodies, since its formation is inhibited by "blockage" and splenectomy;[61] it has also been urged

54. Martins, T.: Sur la question des antihormones, Compt. rend. Soc. de biol. **119**: 753-755, 1935. Du Shane, G. P.; Levine, W. T.; Pfeiffer, C. A., and Witschi, E.: Experimental Constant Oestrus and the Notion of Anti-Gonadotropic Hormones, Proc. Soc. Exper. Biol. & Med. **33**: 339-345 (Dec.) 1935.

55. Kupperman, H. S.; Meyer, R. K., and Hertz, Roy: Effect of Antigonadotropic Sera upon Gonadotropic Secretion in Parabiotic Rats, Endocrinology **24**: 115-118 (Jan.) 1939.

56. Twombly, G. H.: Studies of the Nature of Antigonadotropic Substances, Endocrinology **20**: 311-317 (May) 1936.

57. de Fremery, P., and Scheygrond, B.: Inhibition of the Gonadotropic Activity of Pregnancy Urine Extract by the Serum of Rabbits Injected with an Extract of Male Urine, Nature, London **139**: 1015-1016 (June 12) 1937.

58. Zondek, Bernhard; Sulman, Felix, and Hochman, Abraham: Relationship between Inactivated Prolan and Antiprolan, Proc. Soc. Exper. Biol. & Med. **39**: 283-287 (Nov.) 1938.

59. Zondek, Bernhard; Sulman, Felix, and Hochman, Abraham: Quantitative Aspects of the Prolan-Antiprolan Reaction, Proc. Soc. Exper. Biol. & Med. **40**: 96-98 (Jan.) 1939.

60. Zondek, Bernhard; Sulman, Felix, and Hochman, Abraham: The Preparation of Concentrated Antigonadotropic Factor (Antiprolan), Biochem. J. **32**: 1891-1896 (Nov.) 1938. Thompson, K. W., and Melnick, J. L.: Electrophoresis of Antihormone Sera, Endocrinology **28**: 723-726 (May) 1941.

61. Gordon, A. S.; Kleinberg, William, and Charipper, H. A.: Relation of Reticulo-Endothelial System to Refractoriness Developed in Response to Gonadotropic Hormone, Proc. Soc. Exper. Biol. & Med. **36**: 484-486 (May) 1937. Gordon, A. S., and Charipper, H. A.: Effect of Splenectomy on Response to Pituitary Material and the Question of the Antihormone, ibid. **38**: 773-777 (June) 1938.

that the pineal gland contains an antigonadotropic substance.[62] Certain anterior pituitary extracts when injected intraperitoneally diminish the effect of simultaneous subcutaneous injections of extracts containing the follicle-stimulating substance, and are said to contain an "antagonist," which may be identical with the luteinizing (interstitial cell-stimulating) factor but is almost certainly distinct from the antigonadotropic substance found in serum

A curious and unexplained phenomenon which has been observed repeatedly is that, before antigonadotropic properties appear in the serum of a treated animal, there may be a phase during which the serum, far from inhibiting, augments the action of gonadotropic extract in a test animal.[63] It has been suggested that this "progonadotropic" effect represents the formation of an antihormone to the aforementioned "pituitary antagonist," but there is little direct evidence for this interpretation. The progonadotropic substance is found in the globulin fraction of serum and is active in hypophysectomized as well as in normal test animals and in males as well as in females.

ACQUIRED RESISTANCE TO OTHER PRINCIPLES

Animals become refractory to the action of prolactin, and an inhibitory substance may be demonstrated in their serum.[64] The diabetogenic effect of fresh anterior pituitary extracts in normal dogs does not persist in long treatment except with enormous doses; this might be due to increased production of insulin, but a similar phenomenon has been observed in depancreatized

62. Engel, P.: Zirbeldrüse und gonadotropes Hormon, Ztschr. f. d. ges. exper. Med. **94**: 333-345, 1934; Weitere Untersuchungen über die biologischen und chemischen Eigenschaften des antigonadotropen Hormons der Zirbeldrüse, ibid. **96**: 328-336, 1935.

63. Katzman, Wade and Doisy.[47] Collip.[40] Thompson, K. W.: Augmentary Factor in Animal Sera After Injections of Pituitary Extract, Proc. Soc. Exper. Biol. & Med. **35**: 640-644 (Jan.) 1937. Rowlands, I. W.: Pro-Gonadotropic Sera, Proc. Roy. Soc., London, s. B **124**: 492-503 (Jan. 14) 1938; Further Observations on Pro-Gonadotrophic and Antithyrotrophic Activity of Antisera to Extracts of the Anterior Pituitary Gland, J. Endocrinol. **1**: 177-183 (Sept.) 1939. Katzman, P. A.; Wade, N. J., and Doisy, E. A.: Progonadotropic Sera of Animals Treated with Hypophyseal Extracts, Endocrinology **25**: 554-567 (Oct.) 1939.

64. Young.[30] Strangeways, W. I.: Precipitin Tests and Anti-Prolactin Serum, J. Physiol. **93**: 47P-48P (July 2) 1938. Rowlands, I. W., and Young, F. G.: Capacity of Pituitary Preparations Containing the Thyrotrophic Hormone to Induce the Formation of Antisera, ibid. **95**: 410-419 (April 14) 1939. Bischoff, H. W., and Lyons, W. R.: Immunologic Investigation of Hypophyseal Mammotropic Preparations, Endocrinology **25**: 17-27 (July) 1939.

animals.[65] The glycostatic action on muscle glycogen cannot be maintained indefinitely, nor the growth-promoting action of purified extracts, nor the ketogenic effect; in the last case, there is some evidence that an antihormone is formed.[66] Glycotropic (anti-insulin) activity can, however, be maintained without refractoriness for long periods. The "tachyphylaxis" observed with pitressin need not be considered here.

According to Hartman[67] adrenal cortical extracts may be regarded as consisting of two fractions, one of which is life maintaining while the other ("Na factor") causes sodium retention in normal animals. Intravenous or intraperitoneal (but not subcutaneous) injections of the latter gradually lose in efficacy, but the refractory state produced yields to similar extracts from adrenals of another species. The serum of refractory animals, or its pseudoglobulin fraction, confers resistance against the adrenal extract originally used in fresh test animals (dogs). Such resistance can develop in adrenalectomized animals, and is not elicited by chemically pure preparations, such as corticosterone.

The practical importance of finding means to control metabolism in hyperthyroidism has led to many studies of "antithyroid" substances, not all of which can be discussed here. The "antithyroidin" of Möbius[68] was obtained from the blood of thyroidectomized sheep, and was followed by the "catechin" of Blum[69] from normal bloods, apparently identical with the preparation marketed as "tyronorman," which has been widely if not always critically used in the treatment of exophthalmic goiter[70] and which is said to possess anti-

65. Collip, J. B.: Some Recent Advances in the Physiology of the Anterior Pituitary, J. Mt. Sinai Hosp. **1**: 28-71 (May-June) 1934. Dohan, F. C., and Lukens, F. D. W.: Antihormone Effects in Pancreatic Diabetes, Proc. Soc. Exper. Biol. & Med. **42**: 167-171 (Oct.) 1939.

66. Black, P. T.; Collip, J. B., and Thomson, D. L.: Effect of Anterior Pituitary Extracts on Acetone Body Excretion in the Rat, J. Physiol. **82**: 385-391 (Oct. 17) 1934

67. Hartman, F. A.; Lewis, L. A., and Gabriel, J. E.: Further Studies on the Refractory State Developed Following Repeated Injections of Adrenal Extract, Endocrinology **26**: 879-885 (May) 1940.

68. Möbius, P. J.: Die Basedowsche Krankheit, Vienna, A. Hölder, 1906.

69. Blum, F.: Ueber die antithyreoidalen Eigenschaften des Blutes und das zugrundeliegende Katechin, Schweiz. med. Wchnschr. **63**: 777-781 (Aug. 12) 1933.

70. Herzfeld, Ernst, and Frieder, Alexander: Ueber das Katechin (Hemmungsstoff) der Schilddrüse und dessen therapeutische Verwendung bei Morbus Basedow, Deutsche med. Wchnschr. **59**: 84-86 (Jan. 20) 1933.

thyrotropic as well as antithyroid properties[71] but appears to be associated with the fat-soluble fractions of the blood rather than with the proteins. True antibodies appear in immunization with thyroglobulin but do not lead to refractoriness to the metabolic action of this substance.[72]

Parathyroid preparations soon lose their hypercalcemic activity when continuously administered, but there is no reason to believe that an antihormone is produced. Insulin may be given to diabetic patients for years without development of resistance, yet the phenomenon is sometimes encountered, and in one case the blood seemed to destroy or inhibit insulin;[73] the name "anti-insulin" has been used at various times, both for hyperglycemic factors in crude pancreatic extracts and for the glycotropic factor of the anterior lobe of the pituitary. With chemically pure estrogens, androgens, progesterone or epinephrine there is in general no sign of acquired resistance to the ordinary physiologic effects, though animals may display ability to adapt themselves to the damaging effects of excessive doses;[74] one is not justified in speaking of specific antihormones in these cases. Attempts to produce complement-fixing antibodies by using estrogens and androgens as haptens with pig serum as a carrier have succeeded, but the physiologic actions of these substances are not inhibited.[75]

Interesting preliminary results have been obtained by Wakerlin and Johnson[76] in studies of the renal

71. Schneider, E., and Widmann, E.: Die antithyreoidale Wirkung des Tyronormans, Deutsche Ztschr. f. Chir. **244**: 639-651, 1935. Glaubach, S., and Pick, E. P.: Ueber Prüfungsmethoden schilddrüsenwirksamer Stoffe und Gegenstoffe, Wien. klin. Wchnschr. **50**: 766-768 (May 22) 1937. Herold.[19]

72. Hektoen, Ludvig, and Schulhof, Kamil: Precipitin Reaction of Thyroglobulin, J. A. M. A. **80**: 386-387 (Feb. 10) 1923. Hektoen, Ludvig; Fox, H., and Schulhof, Kamil: Specificness in the Precipitin Reaction of Thyroglobin, J. Infect. Dis. **40**: 641-646 (June) 1927. Schulhof, Kamil: Effect of Antithyroglobulin Serum on the Physiological Action of Thyroglobulin, Am. J. Physiol. **93**: 175-177 (May) 1930. Rosen, S. H., and Marine, David: Immunity to Iodothyroglobulin Does Not Affect Its Physiological Action, ibid. **120**: 121-125 (Sept.) 1937.

73. Banting, F. G.; Franks, W. R., and Gairns, S.: Anti-Insulin Activity of Serum of Insulin-Treated Patient, Am. J. Psychiat. **95**: 562-564 (Nov.) 1938.

74. Selye, Hans: Studies on Adaptation, Endocrinology **21**: 169-188 (March) 1937.

75. Brandt, Robert, and Goldhammer, Helene: Antikörper gegen lipoide Hormone, Klin. Wchnschr. **17**: 1875-1877 (Dec. 19) 1936.

76. Johnson, C. A., and Wakerlin, G. E.: Antiserum for Renin, Proc. Soc. Exper. Biol. & Med. **44**: 277-281 (May) 1940. Wakerlin, G. E., and Johnson, C. A.: Reductions in Blood Pressures of Renal Hypertensive Dogs by Hog Renin, ibid. **46**: 104-112 (Jan.) 1941; Effect of Renin on Experimental Renal Hypertension in the Dog, J. A. M. A. **117**: 416-422 (Aug. 9) 1941.

pressor principle, renin. Renal extracts are administered intramuscularly for long periods to animals of *another* species; the serum of the treated animals acquires the power to inactivate renin inasmuch as fresh renal extracts give no acute pressor response in an etherized, nephrectomized dog when they have been incubated with such serum. When dogs made hypertensive by renal ischemia were treated with extracts of hog kidney, the appearance of "antirenin" in the serum was accompanied by a striking reduction in the elevated blood pressure; this suggested that, though the antigen must be heterologous, it is not specific and will inactivate the endogenous renin produced by the treated dog's own ischemic kidneys. This "antirenin" is presumably not identical with the antipressor substance demonstrable in extracts of normal kidney,[77] but neither is yet well enough known to be regarded as a probable addition to the armamentarium of therapeutics. Johnson and Wakerlin [78] have also recently reported on an antihormone to the posterior pituitary vasopressin.

77. Grollman, Arthur; Williams, J. R., Jr., and Harrison, T. R.: Reduction of Elevated Blood Pressure by Administration of Renal Extracts, J. A. M. A. **115**: 1169 (Oct. 5) 1940. Page, I. H.; Helmer, O. M.; Kohlstaedt, K. G.; Fouts, J. P., and Kempf, G. F.: Reduction of Arterial Blood Pressure of Hypertensive Patients and Animals with Extracts of Kidneys, J. Exper. Med. **73**: 7-41 (Jan.) 1941.

78. Johnson, C. A., and Wakerlin, G. E.: Antihormone for Vasopressin (Antivasopressin), Am. J. Physiol. **133**: P 341 (June 1) 1941.

CHAPTER VIII

DYSFUNCTIONS OF THE ANTERIOR LOBE OF THE PITUITARY AND THEIR TREATMENT

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Among the disturbances of the several functions of the anterior lobe of the pituitary, only two are susceptible to treatment with any assurance of success at present. These are dysfunctions in the supply of the growth-promoting and gonadotropic factors, which will be discussed in detail. Although the thyrotropic extracts of the anterior lobe of the pituitary have been shown to act in some persons, it has not yet been possible to secure sustained stimulation of the thyroid in hypothyroid patients.[1] Neither is there adequate evidence to warrant routine irradiation of the pituitary in an attempt to reduce production of the thyrotropic substance in patients with persistent thyrotoxicosis. The factors involved in pituitary stimulation of the adrenal glands are still further removed from immediate application in human therapy. The dysfunctions known under such terms as "basophilism," "Cushing's syndrome" or "the adrenogenital syndrome" will be discussed in another article of this series.[2] Therapy directed to the pituitary is to be considered in such cases only when a tumor is demonstrable, and then it should consist of hypophysectomy or destructive irradiation. The effects of anterior pituitary extracts on carbohydrate and fat metabolism in experimental animals probably have their counterparts in human clinical situations, but at present the most that can be done is to watch for evidences of disturbed sugar tolerance in patients with other anterior lobe dysfunctions and for stigmas of pituitary disorders in those who are known to have diabetes mellitus or recurrent severe hypoglycemia.

1. Thompson, W. O.; Thompson, P. K.; Taylor, S. G., III, and Dickie, L. F. N.: Influence of Pituitary in Thyroid Disease, West. J. Surg. 47: 4-9 (Jan.) 1939.

2. Section IV-a of outline for the book "Glandular Physiology and Therapy."

DYSFUNCTION IN SUPPLY OF GROWTH HORMONE

Gigantism.—Excessive activity of the anterior lobe of the pituitary in stimulating growth during childhood and early adolescence may so augment the rate of increase in stature that gigantism is produced before the closure of the epiphyses limits further elongation of the long bones. It seems unwise to use irradiation to limit this growth because of the danger of simultaneously interfering with other pituitary functions. Attempts to limit stature should not be undertaken until the child is over 5 feet (152.5 cm.) tall, for an alarmingly fast growth may subside spontaneously as stature approaches the usual adult range. Clinical experience in this field is still meager, but it is possible to observe the maturation of bones with films taken at intervals of six to twelve months and to stimulate maturation in the ossifying process when there is delayed epiphysial union. The physiologic factors which hasten this maturation are thyroid, androgen and estrogen. Occasionally these principles have been used in an attempt to expedite the union of epiphyses, because the effects of such substances on structures other than bone are temporary or are merely additive to the secretion of the glands in the child's body. Such a program is admittedly still experimental, and the dosage cannot be specified. This type of therapy should be undertaken only by a clinician with experience in judging the actions of the substances used.

Acromegaly.—When excessive pituitary secretion of the growth-promoting factor occurs after the union of the epiphyses, the bones and soft tissues undergo changes known as acromegaly. If this process is diagnosed while still active, it may be worth attempting to reduce pituitary activity. In cases of acromegaly associated with tumor of the pituitary the choice between surgical removal or irradiation needs to be made. When no neoplasm can be demonstrated, one may hesitate to employ destructive irradiation. Under such circumstances large doses of estrogen have been reported to give at least temporary benefit.[3] It has been supposed that this acts by inhibition of anterior lobe function. Use of testosterone for this purpose has

3. Kirklin, O. L., and Wilder, R. M.: Follicular Hormone Administered in Acromegaly, Proc. Staff Meet., Mayo Clin. **11**: 121-125 (Feb. 19) 1936.

not been reported. Since acromegaly without a tumor is most apt to occur at the climacteric and is frequently associated with increased growth of facial hair in women, testosterone is not recommended for female patients with acromegaly. The duration of active acromegaly is highly variable, and the need for therapy therefore questionable. The common clinical problem is merely the control or stopping of regional symptoms of tumor and headache, which does not involve endocrine therapy. The occurrence of goiter, myxedema, diabetes mellitus, diabetes insipidus or adrenal insufficiency as complications of acromegaly is of importance in understanding pituitary functions. These complications are treated as when they occur under any other circumstances.

Dwarfism.—Underactivity of the growth-promoting factor during childhood is the cause of certain types of dwarfism. If no other adequate cause for the retardment of growth can be found, the cause is assumed to be hypopituitarism. This conclusion is fortified whenever there are any other evidences of a reduction or an alteration of the functions of the same gland. Exact criteria for hypofunction of the anterior lobe in this responsibility are not available. It is therefore probable that application of potent extracts of the pituitary will not be uniformly successful in treating dwarfism. The materials which have been used are aqueous extracts, which contain inert proteins in addition to the desired active substance. The physiologic action is to increase anabolic processes in many tissues, thereby increasing the positive nitrogen balance and increasing the body weight by the laying down of protein-containing tissues, included in which is an increased amount of bone.[4] There is no uniform method for biologic standardization of these growth-promoting extracts, and it is therefore impossible to make any accurate comparison of the relative values of the several commercial brands in use. All are water soluble and act briefly; hence their efficiency in stimulating growth will be greater when they are given in divided doses at short intervals (daily) than when they are used in similar total amounts at longer intervals (alternate days to weekly). The manufacturers of

4. Lee, M. O., and Schaffer, N. K.: Anterior Pituitary Growth Hormone and the Composition of Growth, J. Nutrition 7: 337-363 (March) 1934.

some of these products suggest interrupted periods of injection, as if to avoid antihormone formation. This interruption is not necessary. It has been frequently suggested that growth will be more rapid if the injection of pituitary extract is augmented by feeding thyroid. This has been demonstrated in animals.[5] Caution is enjoined at this point, for promotion of growth is often undertaken as a late attempt to achieve normal stature before the epiphyses close, and thyroid therapy will certainly tend to expedite the maturing of the skeleton, which includes the union of epiphyses. It is therefore advised that except under most careful observation of the bones thyroid be avoided in treating dwarfism with pituitary extracts, unless there is evidence of hypothyroidism. Therapy for dwarfism should be continued for a minimum of three months to detect the beginning of results before it is abandoned as of no avail. When growth response is secured, the injections should be pursued until the stature is that desired for the age or until no gain is secured for at least three months and films of the femur show the epiphyses to be firmly united.

Pituitary Cachexia.—The failure of the anterior lobe of the pituitary to secrete the growth factor in adult life has not yet been definitely associated with any syndrome. It is usually assumed that pituitary cachexia (Simmonds' disease) is the consequence of a complete failure of the anterior lobe, with failure of all pituitary functions. The diagnostic confusion between anorexia nervosa and pituitary cachexia makes antemortem diagnosis uncertain. The reported results of the treatment of patients for these conditions are conflicting.[6] Benefits have been claimed for the use of various types of pituitary extracts and placental extracts, for other endocrine therapy, and for improvement of nutrition as well as for psychotherapy. Until the diagnostic riddle can be solved, therapy will remain uncertain. It is noteworthy that those who claim the best results for psychotherapy and improved nutrition admit that some patients may regain normal weight and be socially

5. Smith, P. E.: Increased Skeletal Effects in Anterior Pituitary Growth-Hormone Injection by Administration of Thyroid in Hypophysectomized Thyroparathyroidectomized Rats, Proc. Soc. Exper. Biol. & Med. **30**: 1252 (June) 1933.

6. Dick, G. F., and Dine, W. D.: Pituitary Extract in Simmonds' Disease, Endocrinology **22**: 703-707 (June) 1938. Richardson, H. B.: Simmonds' Disease and Anorexia Nervosa, Arch. Int. Med. **63**: 1-28 (Jan.) 1939.

rehabilitated but still show amenorrhea or other evidences of great reduction in gonadal function. This leaves presumptive evidence of pituitary hypofunction. Therefore the recent attempts to use endocrine therapy for pituitary cachexia have included administration of such diverse materials as growth-promoting pituitary extracts, gonadotropic substances, adrenal cortex extracts and desiccated thyroid. Obviously this, again, is an experimental field, in which dependable results cannot yet be expected.

DYSFUNCTION IN SUPPLY OF GONADOTROPIC HORMONES

Hypersecretion.—The only condition associated with excessive amounts of anterior pituitary gonadotropic hormones is the climacteric, whether occurring spontaneously or as the consequence of surgical ablation or irradiation of the gonads. There is no evidence that the occurrence of increased amounts of the gonadotropic hormones in the pituitary,[7] the blood or the urine[8] of such patients has any significance for the comfort or health of the patients. It was formerly thought that the relief from typical autonomic disturbances of the climacteric depended on the inhibition of this increased anterior lobe secretion.[9] It has been shown that such inhibition does not occur following the administration of doses of estrogen which provide relief[10] and that complete alleviation of the symptoms is totally unrelated to the presence of the typical climacteric level of gonadotropic substances in the urine. Therefore it is suggested that there is no clinical reason to attempt treatment of hypersecretion of the anterior pituitary gonadotropic hormones as such.

Hypogonadism in the Male.—The discussion of testicular disorders in another chapter[11] provides details which need not be repeated. There are three

7. Witschi, E., and Riley, G. M.: Quantitative Studies on the Hormones of Human Pituitaries, Endocrinology **26**: 565-576 (April) 1940.

8. Fluhmann, C. F.: Anterior Pituitary Hormone in Blood of Women: Preliminary Clinical Classification of Results in Non-Pregnant Individuals, Endocrinology **15**: 177-183 (May-June) 1931.

9. Jeffcoate, T. N. F.: Estrogenic Hormone Therapy, Brit. M. J. **2**: 671-676 (Sept. 30) 1939. Sevringhaus, E. L.: Endocrine Therapy in General Practice, Chicago, The Year Book Publishers, Inc., 1938, pp. 155-156.

10. Heller, C. G., and Heller, E. J.: Gonadotropic Hormone: Urine Assays of Normally Cycling, Menopausal, Castrated, and Estrin Treated Human Females, J. Clin. Investigation **18**: 171-178 (March) 1938.

11. Section II, B, 1 and 2 of outline for book "Glandular Physiology and Therapy."

fundamental types: infantilism, adiposogenital dystrophy and cryptorchism. Infantilism is the simple failure of testes to develop, and the therapy is commonly the use of gonadotropic substances. Adiposogenital dystrophy includes in addition to infantilism evident obesity, which is frequently but not always most marked about the lower part of the torso and the trochanteric region. The therapy required here is appropriate dietary reduction of weight plus a gonadotropic substance. In a few cases the reduction of weight has sufficed without endocrine therapy. This may mean that the adolescent development was delayed but eventually appeared, or it may indicate that the reduction in weight achieved some favorable alteration in the endocrine situation. The latter suggestion is entirely hypothetic. Obesity accentuated about the lower part of the abdomen and the trochanteric region but without hypogonadism is not justification for a diagnosis of adiposogenital dystrophy. Such obesity deserves treatment by dietary limitation and exercise but not by use of gonadotropic substances. Cryptorchism may be due to anatomic barriers to testicular descent, in which case the only endocrine problem is the reduced activity of a testicle which is not in the scrotum. That problem is dealt with elsewhere.[11] There are numerous cases in which delayed descent is associated with infantilism or with adiposogenital dystrophy. It is especially these patients who may benefit from endocrine therapy with the double purpose of accomplishing descent and stimulating development.

As explained by Engel,[12] there are three fundamentally different types of gonadotropic substances available for therapy: anterior pituitary extracts, concentrates from the serum of pregnant mares and concentrates from the urine of pregnant women. The last of these was the first to be used clinically, and it is still recognized as a potent stimulator of the interstitial tissue of the testicle, in response to which the testicle enlarges, the production of testosterone is increased, secondary sex characters are intensified, the genitalia are enlarged, and cryptorchid testes descend unless there is an anatomic barrier. Consequently there have been numerous reports of treatment of cryptorchism

12. Section I, 6 of outline for book "Glandular Physiology and Therapy."

with this chorionic gonadotropin. The limitations of this method are discussed by Thompson and Heckel.[18] The dosage employed by them and by Zelson [14] is from 100 to 1,000 units administered from three to seven times weekly. Positive results are to be expected in three months or less. The therapy should be watched carefully to avoid producing genitalia larger than normal for the age. The procedure is more frequently successful with bilateral cryptorchism, in the presence of other evidences of endocrine deficiency and before the age of 15. One great merit of such treatment is that it secures descent in that group of boys who do not require surgical treatment and, by failures, marks out for surgical intervention at an early age those who will require it.

Observations on boys treated with chorionic gonadotropin have led to the conviction that this substance does not stimulate all testicular functions. Spermatogenic tissue shows little response. Animal experiments show that anterior pituitary extracts or concentrates from the serum of pregnant mares will stimulate both interstitial and spermatogenic tissues. Therefore I consider it wiser to treat infantilism over long periods of time with one of these two gonadotropic materials, both of which are now available commercially. There is as yet no agreement about the methods of standardizing anterior pituitary gonadotropic extracts; hence the manufacturers' recommendations as to dosage will vary. The pregnant mare serum extracts will all appear in terms of an international unit during 1940, and the dosages will probably vary from 50 to 300 units given daily or on alternate days. Since these substances are water soluble, act quickly and for brief periods, they should be given at short intervals for maximum effect per unit. Since there is no recognized cycle of testicular growth or activity, these treatments may well be pursued without interruption until results are achieved. So little has been reported about the results of such treatment that prediction of the probable degree of success is uncertain.

Hypogonadism in the Female.—The discussion of ovarian dysfunctions and their treatment by Fluh-

13. Thompson, W. O., and Heckel, N. J.: Undescended Testes: Present Status of Glandular Treatment, J. A. M. A. **112**: 397-403 (Feb. 4) 1939.
14. Zelson, C.: Treatment of Cryptorchidism with Gonadotropic Substance, J. Pediat. **14**: 452-461 (April) 1939.

mann [15] includes the varieties of underactivity of the ovaries, their diagnosis and their therapy. When there is no evidence of primary disorder in the ovaries or of obvious disturbance from some other morbid process in the body, these ovarian hypofunctions are assumed to be a result of hypofunction of the anterior lobe of the pituitary in secreting gonadotropic hormones. Exact proof of this relation awaits far more study by hormone assays than is yet possible. The variety of clinical expressions of hypofunction is not surprising when one considers the three ovarian functions of ovulation, production of estrogen and production of progesterone, the last two of which may vary widely in intensity or duration. Further variables are the time and intensity of secretion of the two anterior pituitary gonadotropic hormones—the follicle-stimulating and the luteinizing hormone. That there is interaction of pituitary hormones on the ovaries and of ovarian hormones on the pituitary cannot be doubted. Knowledge of the quantitative relationships is essential to any completely rational therapy, as well as to diagnosis. At present endocrinologists have only the roughest ideas about these exact processes. Therefore therapy with gonadotropic materials is still crude.

The earliest therapy was with chorionic gonadotropin, but those who were previously enthusiastic have come to realize that the substance produced during pregnancy by chorionic tissue does not stimulate the ovaries of nonpregnant women to develop, to ovulate or to secrete more estrogen.[16] Stimulation of these functions has, however, followed the use of anterior pituitary extracts and concentrates from the serum of pregnant mares. Therefore these materials appear to be the only appropriate ones with which to stimulate hypofunctioning human ovaries. Chorionic gonadotropin is apparently a factor in sustaining the function of the corpus luteum during the first trimester of pregnancy, yet, curiously, there have been no systematic studies of the effect of this substance in the treatment of repeated abortion. This most obvious use of the chorionic gonadotropin might well be explored by gynecologists and obstetricians.

15. Fluhmann, C. F.: Ovarian Dysfunctions and Their Treatment, Section II, A, 6 of outline for book "Glandular Physiology and Therapy."

16. The Present Status of Therapy with Chorionic Gonadotropin, report of the Council on Pharmacy and Chemistry, J. A. M. A. 114: 487-489 (Feb. 10) 1940.

In recent years repeated abortion has been treated chiefly by the employment of progesterone. Although the gonadotropin in pregnant mare serum is probably chorionic in origin, it simulates anterior pituitary gonadotropin very closely. It is not yet justifiable to assume that mare serum concentrate provides a complete substitute in replacement therapy for human hypopituitarism.

In treating girls or women whose ovaries are to be stimulated, it is recommended [17] that small or moderate amounts be administered hypodermically at intervals of one or two days and that each course of injections be not longer than two weeks. The optimum time for such a course is the first two weeks of the menstrual cycle, when the development of a new follicle and growth of a new ovum normally occur. Interruption of the series of doses is advised to avoid continuous stimulation with small amounts of gonadotropin, which is believed to be the cause of polycystic ovaries. In those women who are amenorrheic or whose menses occur at longer intervals than two months without treatment, it may be wise to repeat such a course of pituitary injections, beginning every three weeks, until some semblance of cyclic function is established. The gage of success in such a program may be symptomatic improvement, restoration of more regular menstrual cycles, improved fertility or evidence of improvement in the endometrium or in the vaginal mucosa. Both diagnosis and therapy are rendered far more certain when treatment is preceded by biopsy of the endometrium [18] and study of stained smears of desquamated epithelium from the vagina.[19] A detailed study of each case is essential to early determination of the probable success of treatment.[20]

17. Campbell, R. E., and Sevringhaus, E. L.: Pituitary Gonadotropic Extracts for Treatment of Amenorrhea, Menorrhagia, and Sterility, Am. J. Obst. & Gynec. **37**: 913-928 (June) 1939.

18. Campbell, R. E.; Lendrum, F. C., and Sevringhaus, E. L.: Endometrial Histology and Pathology as Revealed by Biopsy Method, Surg., Gynec. & Obst. **63**: 724-731 (Dec.) 1936.

19. Papanicolaou, G. N., and Shorr, Ephraim: Action of Ovarian Follicular Hormone in the Menopause, as Indicated by Vaginal Smears, Am. J. Obst. & Gynec. **31**: 806-831 (May) 1936. Geist, S. H., and Salmon, U. J.: Evaluation of Human Vaginal Smear in Relationship to Histology of the Vaginal Mucosa, ibid. **38**: 392-399 (Sept.) 1939. Sevringhaus, E. L.: Treatment of Gonadal Hypofunction, Bull. New York Acad. Med. **16**: 53-82 (Feb.) 1940.

20. Sevringhaus, E. L., and Campbell, R. E.: Endocrinopathic Amenorrhea: Causes and Treatment, Am. J. Surg. **48**: 197-204 (April) 1940.

The frequency of anovulatory menstruation as a cause of irregularity of cycles, reduced fertility and glandular cystic hyperplasia of the endometrium has led to efforts to stimulate the ovaries to ovulation. The results of Hisaw and co-workers [21] suggested that this was to be accomplished by intravenous injection of a mixture of follicle-stimulating and luteinizing anterior pituitary extracts followed for several days by sustained hypodermic use of the same materials to stimulate secretion by the corpus luteum. This procedure has been tried by several investigators,[22] and a complete program of therapy has been reported by Siegler and Fein [23] with some promise of success. The most acceptable material is the concentrate of pregnant mare serum, which is given in doses of 100 to 300 international units hypodermically for several days to stimulate follicle development, followed by a single intravenous dose of 1,000 to 1,500 units to initiate ovulation, and then by four to six days more of hypodermic therapy to sustain function in the corpus luteum if ovulation occurs.

Since endocrinologists have no knowledge of the amount of gonadotropic hormone produced by the anterior lobe of the pituitary during health, they cannot tell how much is required for replacement therapy in deficiencies. Clinical results certainly indicate that even with the most potent commercial extracts 1.0 cc. daily cannot be regarded as a complete replacement for the secretion of the anterior lobe of the human pituitary. Owing to local reactions to the protein content of even the best preparations and to the cost of therapy, such treatment cannot be expected to be helpful save in the relief of partial deficiencies. If hypopituitarism resembles in chronicity hypothyroidism, diabetes mellitus or hypofunction of the adrenal cortex—a reasonable supposition in many ways—one must expect to use anterior lobe replacement therapy for months or years, not for just a few days. The paucity of cases in which gonadotropic therapy has been attended by striking success should not cause the clinician to doubt the ultimate importance of such treatment, although he

21. Hisaw, F. L.: Physiology of Menstruation in Macacus Rhesus Monkeys, Am. J. Obst. & Gynec. **29**: 638 (May) 1935.

22. Davis, M. E., and Koff, A. K.: Experimental Production of Ovulation in Human Subjects, Am. J. Obst. & Gynec. **36**: 183-189 (Aug.) 1939.

23. Siegler, S. L., and Fein, M. J.: Studies in Artificial Ovulation with the Hormone of Pregnant Mares' Serum, Am. J. Obst. & Gynec. **38**: 1021-1036 (Dec.) 1939.

may well decide to wait for better diagnostic methods, more potent and less expensive extracts and reports of longer periods of observation of patients now under treatment. Gonadotropic therapy is still in the experimental stage.

SUPPLY OF MAMMATROPIC HORMONE

The use of anterior pituitary extracts which are known to stimulate lactation by well developed mammary glands has not proved dependably successful in human subjects.[24] Details of experimental work on this problem are discussed in another chapter.[25] There is reason to hope for progress in this field of therapy. Better results have been achieved in the use of estrogens to prevent or interrupt lactation when clinical indications make this step advisable. For this purpose the patient is treated with high doses of estrogen, given intramuscularly, and the therapy should be successful within one to three days; if it is not, it should be stopped. Dosages have been reported as from 1 to 10 mg. of estrone (theelin) or estradiol benzoate per day. After the first few days there appears to be no need for further therapy. This suggests that the mammatropic hormone secretion which initiates lactation post partum is quickly reduced by therapy to a level which is ineffective in continuing secretion by the breasts. Success has been reported following oral use of 10,000 rat units of estradiol daily in divided doses for from one to four days.[26] The injection of testosterone propionate, 10 to 25 mg., twice daily for one or two days has also been found successful.[27] These methods do not require any additional application of local therapy to the breasts, nor do they cause complications or pain. It is believed that these varied types of endocrine therapy for suppression of lactation operate by inhibition of the secretion of the mammatropic hormone by the anterior lobe of the pituitary.

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24. Stewart, H. L., Jr., and Pratt, J. D.: Effect of Prolactin on Mammary Gland Secretion, Endocrinology **25**: 347-353 (Sept.) 1939.
25. Section I, 5 of outline for book "Glandular Physiology and Therapy."
26. Foss, G. L., and Phillips, P.: Suppression of Lactation by Oral Estrogen Therapy, Brit. M. J. **2**: 887-890 (Oct. 29) 1938.
27. Kurzrok, Raphael, and O'Connell, C. P.: Inhibition of Lactation During Puerperium by Testosterone Propionate, Endocrinology **23**: 476-478 (Oct.) 1938.

CHAPTER IX

THE NEUROHYPOPHYSIS

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The neurohypophysis consists of three poorly defined regions: (1) the neural lobe, (2) the infundibular stem and (3) the median eminence (or part thereof[1]). Because of the intimate contact of the neural and the intermediate lobe in man and most laboratory animals, these two parts of the pituitary gland are frequently classed together as the posterior lobe. However, with the clarification of the development and of the secretory capacity of the neural division of the hypophysis, it becomes expedient to abandon the use of a term based purely on gross anatomic relations.

The primary structural elements of the neurohypophysis are pituicytes and unmyelinated nerve fibers. The pituicytes are derived from the ependymal cells of the neural tube and are closely related to neuroglia. They have one or more cytoplasmic processes, and frequently these end in close relation to blood vessels or connective tissue.[2] The nerve fibers have their cells of origin in the supraoptic and tuberal nuclei. They sweep down the infundibular stem in dense bundles and spread out in the neural lobe, forming a rich network about the cells.[3]

ORIGIN AND PATH OF ESCAPE OF THE SECRETION

It was formerly believed that the so-called "posterior lobe hormones" (pressor, antidiuretic, oxytocic and

1. Weaver, T. A., Jr., and Bucy, P. C.: The Anatomical Relationships of the Hypophyseal Stem and the Median Eminence, Endocrinology **27**: 227 (Aug.) 1940.

2. Bucy, P. C.: The Pars Nervosa of the Bovine Hypophysis, J. Comp. Neurol. **50**: 505 (Aug.) 1930. Griffiths, Mervyn: The Relationship Between the Secretory Cells of the Pars Nervosa of the Hypophysis and Classical Neuroglia, Endocrinology **26**: 1032 (June) 1940.

3. Rasmussen, A. T.: Innervation of the Hypophysis, Endocrinology **23**: 263 (Sept.) 1938.

melanophore-dispersing principles) were elaborated by the pars intermedia. Support for this view came from the close association between the neural and the intermediate lobe in most species and from the reluctance of many investigators to ascribe a secretory role to cells of nervous origin.[4] However, during the past five years, convincing evidence has been adduced to prove that the pressor, antidiuretic and oxytocic hormones arise from the intrinsic elements of the neurohypophysis. In studies of species in which a pars intermedia is lacking (the cetaceans, the armadillo, the chicken and the South American manatee [5]) it has been shown that these three principles are present in extracts of the neural lobe, whereas the melanophore-dispersing factor is present only in extracts of the anterior lobe.[6] The colloid masses, or Hering bodies, frequently considered to be secretory antecedents migrating from the intermediate lobe into the neurohypophysis, are believed to be fixation artefacts.[7] Interruption of the nerve tracts to the neurohypophysis results in degenerative changes in the pituicytes and loss of pressor, antidiuretic and oxytocic activity.[8] Changes in the pituicytes have been correlated with secretory activity.[9] Tissue cultures of the neural lobe admixed with the intermediate lobe have pressor and melanophore-dispersing activity, while

4. Cushing, Harvey: Posterior Pituitary Activity from an Anatomical Standpoint, Am. J. Path. **9**: 539 (Sept.) 1933.

5. Valsõ, Jacob: Der Hormongehalt der Hypophyse des Blauwals (Balaenoptera sibbaldii), Klin. Wchnschr. **13**: 1819 (Dec. 22) 1934. Geiling, E. M. K.: The Hypophysis Cerebri of the Finback (Balaenoptera Physalis) and Sperm (Physeter Megalocephalus) Whale, Bull. Johns Hopkins Hosp. **57**: 123 (Sept.) 1935. Geiling, E. M. K., and Oldham, Frances K.: The Site of Formation of the Posterior Lobe Hormones, Tr. A. Am. Physicians **52**: 132, 1937. Oldham, Frances K.: McCleery, D. P., and Geiling, E. M. K.: A Note on the Histology and Pharmacology of the Hypophysis of the Manatee (Trichechus Inunguis), Anat. Rec. **71**: 27 (May 25) 1938. Oldham, Frances K.: The Pharmacology and Anatomy of the Hypophysis of the Armadillo, ibid. **72**: 265 (Nov. 25) 1938.

6. It is generally conceded that in those species possessing a discrete intermediate lobe, the melanophore-dispersing hormone is secreted by the cells thereof.

7. Gersh, Isidore, and Tarr, A. deL.: The So-Called Hyaline Bodies of Herring in the Posterior Lobe of the Hypophysis, Anat. Rec. **63**: 231 (Oct. 25) 1935.

8. Fisher, Charles, and Ingram, W. R.: The Effect of Interruption of the Supraoptico-Hypophyseal Tracts on the Antidiuretic, Pressor and Oxytocic Activity of the Posterior Lobe of the Hypophysis, Endocrinology **20**: 762 (Nov.) 1936.

9. Gersh, Isidore: The Structure and Function of the Aranchymatous Glandular Cells in the Neurohypophysis of the Rat, Am. J. Anat. **64**: 407 (May) 1939. Wang, Kun-jen: A Vagus Post-Pituitary Reflex: V. The Secretory Cells of the Pars Nervosa, Chinese J. Physiol. **13**: 405 (Dec.) 1938.

those of the intermediate lobe alone have only melanophore-dispersing activity.[10]

While the evidence points to the pituicytes as the secretory elements, the function of the nerve fibers is not as yet clear. There is evidence that a reflex connection exists between the vagus and the supraoptico-hypophysial tract.[11]

The neurohypophysis receives a fairly rich blood supply from the inferior hypophysial arteries. Its venous drainage is into the circular sinus.[12] Recent failures to detect the posterior lobe principles in cerebrospinal fluid do not support the view once advanced that these principles escape by way of the third ventricle.[13] Massive intravenous doses of pressor and oxytocic substances are excreted in part unchanged in the urine.[14] Efforts to demonstrate these factors in blood or urine under physiologic conditions have yielded contradictory results.[15]

CHEMISTRY OF EXTRACTS OF THE POSTERIOR LOBE

Although several commercial preparations of the posterior lobe of the hypophysis are available for clinical use, as yet none of the active principles have been isolated as chemically pure entities. Abel and his asso-

10. Geiling, E. M. K., and Lewis, M. R.: Further Information Concerning the Melanophore Hormone of the Hypophysis Cerebri, Am. J. Physiol. **113**: 534 (Nov.) 1935. Anderson, Evelyn, and Haymaker, Webb: Elaboration of Hormones by Pituitary Cells Growing in Vitro, Proc. Soc. Exper. Biol. & Med. **33**: 313 (Nov.) 1935.

11. Chang, H. C.: The Establishment of a Vagus-Post-Pituitary Reflex: a Review, Chinese M. J. **56**: 360 (Oct.) 1939. Sattler, D. G.: Vago-Neurohypophyseal Pressor Reflex, Proc. Soc. Exper. Biol. & Med. **44**: 82 (May) 1940.

12. Wislocki, G. B.: The Vascular Supply of the Hypophysis Cerebri of the Rhesus Monkey and Man, Proc. A. Research Nerv. & Ment. Dis., Proc. (1936) **17**: 48, 1938.

13. Van Dyke, H. B.; Bailey, Percival, and Bucy, P. C.: The Oxytocic Substance of the Cerebrospinal Fluid, J. Pharmacol. & Exper. Therap. **36**: 595 (Aug.) 1929. Friedman, Gertrude S., and Friedman, M. H.: An Examination of Cerebrospinal Fluid for Oxytocic Activity as Tested by the Rabbit Uterine Fistula Preparation, Am. J. Physiol. **103**: 244 (Jan.) 1933. Simon, Alexander: The Secretion of the Posterior Lobe of the Hypophysis After the Administration of Drugs, J. Pharmacol. & Exper. Therap. **49**: 375 (Nov.) 1933.

14. Larson, Edward: Tolerance and Fate of the Pressor Principle of Posterior Pituitary Extract in Anesthetized Animals, J. Pharmacol. & Exper. Therap. **62**: 346 (March) 1938; Fate of the Injected Oxytocic Principle of Posterior Pituitary in Anesthetized Dogs, ibid. **67**: 175 (Oct.) 1939.

15. Gilman, Alfred, and Goodman, Louis: The Secretory Response of the Posterior Pituitary to the Need for Water Conservation, J. Physiol. **90**: 113 (July 15) 1937. Walker, A. M.: Experiments upon the Relations Between the Pituitary Gland and Water Diuresis, Am. J. Physiol. **127**: 541 (Oct.) 1939.

ciates [16] have isolated a tartrate of high purity possessing pressor, oxytocic and antidiuretic properties. Kamm and co-workers [17] and more recently Stehle [18] and others have separated from pituitary extracts two fractions, pitressin and pitocin, which have been highly purified.

The treatment of posterior pituitary preparations with enzymes has given rise to results which are subject to different interpretations.[19] Gulland and his associates reported that an inactivating enzyme accompanies preparations of dipeptidase, aminopolypeptidase, trypsin and papain but that it is not identical with any of these. Larson confirmed the reports of earlier workers and expressed the opinion that the inactivation is due to the aminopeptidases of the tissue ereptases. He suggested that this enzymatic process may be one of the factors in the diminution of pressor response to successive doses of pituitary extract. Thus far, no enzyme acting in an acid range has been found that will destroy the active factors of the posterior lobe. Indubitably, other factors, besides enzymes, cause loss of activity, such as adsorption, hydrogen ion concentration, incubation time and temperature.[20]

Recent studies on electrophoresis indicate that both the pressor and the oxytocic principle are possibly

16. Abel, J. J.; Rouiller, C. A., and Geiling, E. M. K.: Further Investigations on the Oxytocic-Pressor-Diuretic Principle of the Infundibular Portion of the Pituitary Gland, J. Pharmacol. & Exper. Therap. **22**: 289 (Nov.) 1923.

17. Kamm, Oliver; Aldrich, T. B.; Grote, I. W.; Rowe, L. W., and Bugbee, E. P.: The Active Principles of the Posterior Lobe of the Pituitary Gland: I. The Demonstration of the Presence of Two Active Principles; II. The Separation of the Two Principles and Their Concentration in the Form of Potent Solid Preparations, J. Am. Chem. Soc. **50**: 573 (Feb.) 1928.

18. Stehle, R. L.: New Method for Separating Pressor and Oxytocic Substances from Posterior Lobe of Pituitary Gland, J. Biol. Chem. **102**: 573 (Oct.) 1933.

19. (*a*) Gulland, J. M., and MacRae, T. F.: The Oxytocic Hormone of the Posterior Pituitary: III. The Action of Plant Proteolytic Enzymes, Biochem. J. **27**: 1237, 1933; IV. The Action of Animal Proteolytic Enzymes, ibid. **27**: 1383, 1933. (*b*) Dale, H. H., and Dudley, H. W.: On the Pituitary Active Principles and Histamine, J. Pharmacol. & Exper. Therap. **18**: 27 (Aug.) 1921. (*c*) Thorpe, W. V.: Experiments on the Chemical Nature of the Oxytocic Principle in the Pituitary Gland, Biochem. J. **20**: 374, 1926. (*d*) Freudenberg, Karl; Weiss, Emil, and Biller, Hans: Notiz über Oxytocin, Ztschr. f. physiol. Chem. **233**: 172, 1935. (*e*) Heller, H., and Urban, F. F.: The Fate of the Antidiuretic Principle of Post Pituitary Extracts in Vivo and in Vitro, J. Physiol. **85**: 502, 1935. (*f*) Heller, H.: The State in the Blood and the Excretion in the Kidney of the Antidiuretic Principle of the Posterior Pituitary Extracts, ibid. **89**: 81 (Feb. 19) 1937.

20. Vaichulis, J. A.: The Effect on the Posterior Pituitary Principles of Incubating Beef Heads at Thirty-Seven Degrees Centigrade for Twenty-Four Hours, J. Pharmacol. & Exper. Therap. **63**: 37 (May) 1938, A Simple Method of Removing the Oxytocic Principle from the Posterior Lobe of the Beef Pituitary Glands, ibid. **66**: 37 (May) 1939.

amphoteric and are certainly basic in nature.[21] When such experiments are conducted in a medium below a certain p_H (the value of which differs in each report) the oxytocic substance migrates to the cathode. According to duVigneaud and his associates, the pressor substance migrates in the same direction at an even more rapid rate, since its concentration in the cathode vessel is greater than that of the oxytocic substance. Irving and duVigneaud repeated this experiment on the press juice from fresh glands with the same general result. This they advanced as evidence that the two principles exist as separate entities in the natural state.[22]

A preliminary study by Rosenfeld of sedimentation properties in the ultracentrifuge indicates that the pressor and oxytocic principles exist normally as relatively large molecules, which are broken down to smaller, physiologically active products by the usual methods of chemical extraction.[23]

Perhaps the most significant recent chemical finding has been the demonstration in three separate laboratories that the pressor and the oxytocic factor each possesses at least one reduction-oxidation system, presumably connected with the S-S group of cysteine.[24]

Analytic work by Stehle and others on highly purified preparations indicates a high amino acid content in both pressor and oxytocic principles. The amino acids found in largest amounts are cysteine, tyrosine and arginine.[25]

21. Das, N.; Ghosh, B. N., and Guha, B. C.: Bemerkung zur Elektrodialyse von Oxytocin, Ztschr. f. physiol. Chem. **238**: 131, 1936. Gulland, J. M.; Lucas, N. S.; Freeman, M., and Randall, S. S.: The Oxytocic Hormone—Ultra Violet Absorption Spectra, Adsorption, and Electrodialysis, Biochem. J. **29**: 2208, 1935. du Vigneaud, Vincent; Irving, G. W., Jr.; Dyer, H. M., and Sealock, R. R.: Electrophoresis of Posterior Pituitary Preparation, J. Biol. Chem. **123**: 45 (March) 1938.

22. Irving, G. W., Jr., and du Vigneaud, Vincent: The Differential Migration of the Pressor and Oxytocic Hormones in Electrophoretic Studies of the Untreated Press Juice of the Posterior Lobe of the Pituitary Gland, J. Biol. Chem. **123**: 485 (April) 1938.

23. Rosenfeld, Morris: The Native Hormones of the Posterior Pituitary Gland: The Pressor and Oxytocic Principles, Bull. Johns Hopkins Hosp. **66**: 398 (June) 1940.

24. Gulland, J. M., and Newton, S. S.: The Oxytocic Hormone of the Posterior Lobe of the Pituitary Gland: V. Recognition as an Oxidation-Reduction System, Biochem. J. **29**: 378, 1935. Sealock, R. R., and du Vigneaud, Vincent: Studies on the Reduction of Pitressin and Pitocin with Cysteine, J. Pharmacol. & Exper. Therap. **54**: 433 (Aug.) 1935. Freudenberg and others.[19d]

25. du Vigneaud, Vincent; Sealock, R. R.; Sifferd, R. H.; Kamm, Oliver, and Grote, I. W.: Some Chemical Properties of Highly Purified Preparations of Pitressin and Pitocin, J. Biol. Chem. **100**: xciv (May) 1933. Stehle, R. L., and Fraser, A. M.: The Purification of the Pressor and Oxytocic Hormones of the Pituitary Gland and Some Observations on the Chemistry of the Products, J. Pharmacol. & Exper. Therap. **55**: 136 (Oct.) 1935. Stehle, R. L., and Trister, S. M.: Additional Data Concerning the Chemistry of the Pressor and Oxytocic Hormones of the Pituitary Gland, ibid. **65**: 343 (April) 1939.

Many of the problems relating to the chemical and the physiologic actions of these substances must await final solution until the "mother substance" of Abel,[26] or the fractions derived therefrom or existing separately, are obtained as chemically pure compounds. These should be of astounding potency, judging from the high degree of physiologic activity exhibited by impure preparations.

At present there is available an international pituitary powder, against which all commercial preparations are standardized. The unit of pituitary potency is the activity contained in 0.5 mg. of the standard pituitary powder (U. S. P. XI).

PHYSIOLOGIC EFFECTS

Posterior pituitary extract (solution of posterior pituitary U. S. P. XI) exerts striking physiologic actions on the cardiovascular, respiratory and renal systems, on smooth muscle, on certain glandular structures and on the metabolism. The separation of pitressin and of pitocin from pituitary extracts necessitated apportioning these multiple pharmacodynamic actions. Pitressin elicits the cardiovascular, respiratory, renal, intestinal and certain metabolic effects, and pitocin the oxytocic action. Both substances cause hyperglycemia and act as antagonists to insulin. The mechanism of action of each in this respect is probably different, and the relative effectiveness depends on the species of animal used.[27]

CARDIOVASCULAR AND METABOLIC EFFECTS

In man, therapeutic doses of either solution of pituitary U. S. P. or of pitressin, given intramuscularly or subcutaneously, do not cause any significant rise of blood pressure, in spite of the marked pallor, which would lead one to infer that the arterial tension is elevated.[28] There is a decided but brief fall in the pulse rate, oxygen consumption and cardiac output,

26. Abel, J. J.: On the Unitary versus the Multiple Hormone Theory of Posterior Pituitary Principles, J. Pharmacol. & Exper. Therap. **40**: 139 (Oct.) 1930.

27. Ellsworth, H. C.: The Antagonism Between Posterior Lobe Pituitary Hormones and Insulin, J. Pharmacol. & Exper. Therap. **56**: 417 (April) 1936. Holman, D. V., and Ellsworth, H. C.: The Hyperglycemic Constituent of Posterior Pituitary Extract, ibid. **53**: 377 (March) 1935. Ellsworth, H. C.: The Action of Posterior Pituitary Hormone upon the Blood Sugar of the Rabbit, ibid. **55**: 435 (Dec.) 1935.

28. Moffat, W. M.: The Effect of Pituitrin Injections on Blood Pressure in Man, Am. J. M. Sc. **186**: 854 (Dec.) 1933.

which is followed by a more prolonged rise.[29] Pitocin causes only a slight increase in oxygen consumption and negligible changes in the circulation. The decreased cardiac output after the injection of solution of pituitary or of pitressin is due largely to coronary constriction, which may be obviated by the administration of epinephrine or ephedrine.[30] The subsequent elevation in cardiac output and pulse rate is due to the accumulation of catabolites during the period of decreased consumption of oxygen after the administration of these drugs. This accumulation of catabolites causes a condition of "oxygen debt," the liquidation of which is manifested in the later increase in oxygen consumption.

In trained unanesthetized dogs the effects of these drugs in larger doses per kilogram of body weight, given intravenously, are more intense but essentially similar to those found in man, except for the blood pressure.[31]

In experimental animals the blood pressure response to solution of pituitary and to pitressin is determined by several factors, such as dose, time between injections, type and depth of anesthesia and species of experimental subject. Small doses given to normal anesthetized dogs or cats cause peripheral vasoconstriction with a sharp rise of pressure. Repeated doses give a lessened response, and tolerance is easily acquired. Larger doses given to intact animals with or without anesthesia may cause a fall in pressure followed by a rise. The depressor effect is due to coronary constriction.[30] In experiments in which cardiac effects are eliminated by the use of the Gibbs artificial heart, large doses of solution of posterior pituitary or of pitressin invariably cause a sharp rise of pressure, and subsequent injections produce little or no effect. Such

29. Grollman, Arthur, and Geiling, E. M. K.: The Cardiovascular and Metabolic Reactions of Man to the Intramuscular Injection of Posterior Pituitary Liquid (Pituitrin), Pitressin, and Pitocin, J. Pharmacol. & Exper. Therap. **46**: 447 (Dec.) 1932.

30. Melville, K. I., and Stehle, R. L.: The Antagonistic Action of Ephedrine (or Adrenalin) upon the Coronary Constriction Produced by Pituitary Extract and Its Effect upon Blood Pressure, J. Pharmacol. & Exper. Therap. **42**: 455 (Aug.) 1931. Melville, K. I.: The Electrographic and Blood Pressure Changes Induced by Posterior Pituitary Extract (Postlobin-V) and the Influence of Ephedrine Thereon, ibid. **64**: 86 (Sept.) 1938; The Action of Pituitary Extract on the Blood Pressure of the Normal Unanesthetised Animal and the Effect of Ephedrine or Adrenaline Thereon, ibid. **47**: 355 (March) 1933. Ross, J. B.; Dreyer, N. B., and Stehle, R. L.: The Cardiac Action of Pituitary Extract (Posterior Lobe), ibid. **38**: 461 (April) 1930.

31. Geiling, E. M. K., and DeLawder, A. M.: Metabolic Changes Following the Intravenous Injection of Posterior Pituitary Extracts and Their Correlation with the Well Known Pharmacodynamic Action of the Drugs, Bull. John Hopkins Hosp. **51**: 1 (July) 1932.

experiments indicate that the depressor action is cardiac and that the tolerance factor is vested in the peripheral structures. In the fowl, pitocin gives a fall in pressure. This is a quantitative reaction and has been adapted as a method of assay for the oxytocic principle.[32]

RESPIRATORY EFFECTS

The respiratory changes are secondary to the circulatory effects. In unanesthetized animals there is a quickening of the respiratory rate interspersed with periods of cessation of breathing.[16]

RENAL EFFECTS: DIURETIC-ANTIDIURETIC ACTION

While the antidiuretic action of posterior pituitary is generally associated with the pressor fraction, recent studies by Heller suggest that it may be due to a separate entity, since heat inactivation of the pressor principle proceeds at a faster rate than that of the antidiuretic principle.[33] Therapeutic doses of either solution of pituitary or pitressin cause a marked antidiuretic effect lasting some hours in patients with diabetes insipidus or in normal subjects who have previously ingested water by mouth. This action is apparently a renal effect due to increased reabsorption of water by certain cells of the tubule.[34] Extrarenal effects, such as changes in the blood electrolytes [35] or inhibition of some hypothetic water center in the hypothalamus,[36a] are

32. Coon, J. M.: A New Method for the Assay of Posterior Pituitary Extracts, Arch. internat. de pharmacodyn. et de thérap. **62**: 79 (May) 31) 1939.

33. Heller, H.: The Effect of the Hydrogen Ion Concentration on the Stability of the Anti-Diuretic and Vasopressor Activities of Posterior Pituitary Extracts, J. Physiol. **96**: 337 (Aug. 14) 1939.

34. Burgess, W. W.; Harvey, A. M., and Marshall, E. K., Jr.: The Site of the Anti-Diuretic Action of Pituitary Extract, J. Pharmacol. & Exper. Therap. **49**: 237 (Oct.) 1933. Gersh, Isidore: Reabsorption of Water During Pituitary Anti-Diuresis, ibid. **52**: 231 (Oct.) 1934.

35. Stehle, R. L., and Bourne, W.: The Effect of Pituitary Extract on the Secretion and Composition of the Urine, J. Physiol. **60**: 229 (July) 1925.

36. (*a*) Molitor, H., and Pick, E. P.: Ueber die Bedeutung des Gewebswassers fur die Wirkung diuresebeeinflussender Arzneimittel: I. Der Einfluss von Flüssigkeitsanreicherung auf die Stärke der Pituitrinwirkung, Arch. internat. de pharmacodyn. et de thérap. **38**: 279, 1930. (*b*) Gilman, A., and Goodman, L.: The Secretory Response of the Posterior Pituitary to the Need for Water Conservation, J. Physiol. **90**: 113 (July 15) 1937. (*c*) Teel, H. M., and Reid, D. E.: Observations upon the Occurrence of an Antidiuretic Substance in the Urine of Patients with Pre-Eclampsia and Eclampsia, Endocrinology **24**: 297 (March) 1939. (*d*) Schaffer, N. K.; Cadden, J. F., and Stander, H. J.: Measurement of Antidiuretic Activity as Applied to Eclamptic Urine and Properties of Antidiuretic Substances in Rat Urine, Pituitary and Beef Liver, ibid. **28**: 701 (May) 1941. (*e*) Ham, G. C.: A Comparison of Pituitrin and the Antidiuretic Substance in Human Urine and Placenta, J. Clin. Investigation **20**: 439 (July) 1941. (*f*) Blazso, S., and Dubrausky, V.: Role of the Vasopressor and Antidiuretic Hormone of the Posterior Lobe of the Pituitary Gland in the Pathogenesis of Toxicoses of Late Pregnancy, Arch. f. Gynäk. **170**: 651 (Nov.) 1940.

probably of only secondary importance in the diuresis-inhibiting action of posterior pituitary.

A large number of controversial reports have dealt with the detection of posterior pituitary factors in the body fluids of normal animals and human beings under varying conditions. Recent work has indicated that such factors may be readily detected in the body fluids. Gilman and Goodman [36b] have shown that rats dehydrated through a lack of drinking water excrete an antidiuretic principle, apparently from the posterior lobe of the pituitary. Teel and Reid [36c] have demonstrated that concentrates of urine from pregnant women with toxemia have an antidiuretic effect. Antidiuretic substances have also been detected in the urine of rats and in beef liver by Schaffer, Cadden and Stander.[36d] These substances differ in chemical behavior from the antidiuretic factor in the urine of eclamptic women. The factor in human urine was apparently inactive, but its presence could be detected by the fact that the addition of the extracts containing it to pitressin augmented the antidiuretic activity of pitressin. Ham [36e] has likewise demonstrated that the antidiuretic factor in the urine of both rats and human beings is not identical with that of the posterior lobe of the pituitary but resembles the factor in placental extracts. Blazso and Dubrausky [36f] were unable to obtain any antidiuretic activity from the blood of patients with toxemia of pregnancy according to the method of Hoffmann and Anselmino, but they were able to detect such a factor in the urine according to the method of Gilman and Goodman.

A diuretic effect is best elicited with anesthetized rabbits rendered diuretic by feeding of greens, by rapid intravenous infusion of isotonic sucrose or by administration of phlorhizin. Unanesthetized animals with low urine flow may also respond in this way. This diuresis may be due to increased glomerular filtration or to decreased reabsorption of water by the tubules. According to Heller, the diuretic effect is due to the pressor and not to the antidiuretic principle.[37]

37. Heller, H.: The Action of the Anti-Diuretic Principle of Posterior Pituitary on the Urine Excretion of Anesthetized Animals, J. Physiol. 98: 405 (Aug.) 1940.

ACTION ON THE GASTROINTESTINAL TRACT

Variations in species of animal, in technic and in the portion of the gastrointestinal tract used account for the lack of concordance in the results obtained by different workers with preparations of the posterior pituitary principles. In the unanesthetized dog pitressin stimulates intestinal activity and causes defecation, while the oxytocic substance has an antagonistic influence in respect to these actions.[38]

Massive doses of posterior pituitary preparations give rise to gastric ulcers, due probably to local ischemic action by the pressor principle.[39]

OXYTOCIC EFFECTS

A number of workers[40] by in vitro and in vivo experiments on the uterus have made it clear that the nature and the degree of the reaction of the uterine musculature to posterior lobe preparations depend on (*a*) the species of the animal, (*b*) the phase of the menstrual or estrous cycle, (*c*) whether the uterus is gravid or nongravid and (*d*) the stage of pregnancy —early or late, in parturition or in the puerperium. Some of the variations in the uterine response become more intelligible when viewed in the light of the newer researches dealing with the effect on the uterus of the estrogenic and corpus luteum hormones and their interplay with the hormones from the anterior and posterior lobes of the pituitary. Briefly stated, the reaction of the uterine muscle to pituitary preparations is markedly affected by the nature of that ovarian, placental or anterior pituitary hormone whose influence is preponderant at the time of injection. During the early stages

38. Melville, K. I.: Pressor and Oxytocic Fractions of Posterior Pituitary Extract; Comparative Effects on Blood Pressure and Intestinal Activity, J. A. M. A. **106**: 102 (Jan. 11) 1936.

39. Dodds, E. C.; Noble, R. L., and Smith, E. R.: A Gastric Lesion Produced by an Extract of the Pituitary Gland, Lancet **2**: 918 (Oct. 27) 1934.

40. Knaus, Hermann: Die periodische Fruchtbarkeit und Unfruchtbarkeit des Weibes, Vienna, E. Maudrich, 1934. Robson, J. M.: Recent Advances in Sex and Reproductive Physiology, Philadelphia, P. Blakiston's Son & Co., 1934. Reynolds, S. R. M.: The Effect of Certain Calcium Salts on the Rhythmically Contracting and Quiescent Uterine Fistula, with Observations on the Action of Posterior Pituitary Extracts, Am. J. Physiol. **105**: 358 (Aug.) 1933. Moir, Chassar: Recording the Contraction of the Human Pregnant and Nonpregnant Uterus, Tr. Edinburgh Obst. Soc., 1933-1934, p. 93, in Edinburgh M. J., August 1934. Adair, F. L., and Haugen, J. A.: A Study of Suspended Uterine Muscle Strips in Vitro, Am. J. Obst. & Gynec. **37**: 753 (May) 1939. McLellan, Archibald: Response of Non-Gravid Human Uterus to Posterior Pituitary Extract, Lancet **1**: 919 (May 18) 1940.

of pregnancy the human uterus does not react to pitocin, probably because of the inhibitory effect of the luteal secretion. It does, however, respond to small doses of pitressin; whether the response is due to an effect of the drug per se or to mechanical factors remains a moot point. Later in the gestation period the reactivity to pitocin returns, and during parturition the uterus is very reactive to this substance and also to solution of pituitary. It is at this time that these drugs are mainly used by obstetricians. The influence of estrogenic substances in rendering the uterus more highly reactive comes into play here. In the puerperium, however, while involution is in progress, pitocin evokes little or no response.

In respect to the nongravid human uterus, both in vitro and in vivo studies indicate that pitressin is more effective than pitocin in eliciting contractions. According to Knaus, the uterus is highly reactive to posterior pituitary preparations during the first half of the menstrual cycle, but during the second half the reactivity is low. He attributed these changes to the liberation of progesterone following ovulation, and he presented these findings as evidence in support of the Ogino-Knaus theory of periodic fertility in women. Certain other investigators have been unable to confirm Knaus's findings.

For a more detailed discussion of the problem, the recent work of Reynolds should be consulted.[41]

The excised horns of the virgin guinea pig's uterus are very reactive to solution of pituitary and to pitocin; this action forms the basis of the official method of assay of these drugs.

CLINICAL USES

Solution of posterior pituitary was introduced into obstetrics by Blair Bell and Hofbauer.[42] Its use is now largely restricted to the control of postpartum bleeding in the third stage of labor and to the prevention or control of hemorrhage in therapeutic abortions. In some

41. Reynolds, S. R. M.: Physiology of the Uterus, New York, Paul B. Hoeber, Inc., 1939.

42. Bell, W. B.: The Pituitary Body and the Therapeutic Value of the Infundibular Extract in Shock, Uterine Atony, and Intestinal Paresis, Brit. M. J. 2: 1609, 1909. Hofbauer, J.: Hypophysenextrakt als Wehenmittel, Zentralbl. f. Gynäk. 35: 137, 1911.

clinics, both here and abroad, intranasal application or cautious use of small intramuscular doses following administration of castor oil and quinine is advocated for induction of labor. However, rupture of the uterus, fetal asphyxia or even death, laceration of the cervix with hemorrhage or infection, secondary atony of the uterus with thrombosis and embolism, and cardiac death from sudden overexertion have all occurred from the misuse of pituitary preparations during labor.[43] Its use during the second stage of labor, to hasten separation of the placenta and to decrease bleeding during the third stage, may lead to hourglass contraction of the uterus necessitating manual removal and hence increasing the dangers of puerperal fever. The use of posterior pituitary extract during the puerperium to hasten involution is the practice of some obstetricians in the belief that poor involution increases the chances of pelvic infection. Others believe that it is bad practice to aggravate a uterus that tends to wall off infection by reduced activity.

In the normal parturient patient, posterior pituitary in therapeutic doses usually produces no effect on the blood pressure. In the patient with eclampsia or preeclampsia, however, there are marked rise in blood pressure and decrease in urine volume.[44] Dieckmann and Michel have suggested "the pituitrin cold pressor test" (repeated cautious injections of pitressin) as an aid in detecting potential toxemia and in differentiating preeclampsia from the other forms of toxemia.

Posterior pituitary or its pressor fraction is of greatest value in the treatment of diabetes insipidus. This condition is characterized by persistent polyuria, the urine being of low specific gravity, and polydipsia, which is

43. Davis, M. E.: The Use and Abuse of Ergot and Pituitary, J. A. M. A. **109**: 1631 (Nov. 13) 1937. Sharkey, J. A.: Should Solution of Posterior Pituitary Be Used in the First and Second Stages of Labor? ibid. **115**: 1315 (Oct. 19) 1940. Pendleton, G. F.: Abuse of Solution of Posterior Pituitary During Early Labor, ibid. **115**: 1318 (Oct. 19) 1940. DeLee, J. B.: The Use of Solution of Posterior Pituitary in Modern Obstetrics, ibid. **115**: 1320 (Oct. 19) 1940.

44. Dieckmann, W. J., and Michel, H. L.: Vascular Renal Effects of Posterior Pituitary Extracts in Pregnant Women, Am. J. Obst. & Gynec. **33**: 131 (Jan.) 1937. Lambillon, Joseph: Contribution expérimentale et clinique à l'étude de la physiologie posthypophysaire de la grossesse et de ses rapports avec la gestose éclamptogène, Rev. belge sc. méd. **10**: 1 (Jan.) 1938. de Valera, E., and Kellar, R. J.: On the Effects of Intravenous Vasopressin on the Toxemias of Pregnancy, J. Obst. & Gynaec. Brit. Emp. **45**: 815 (Oct.) 1938.

secondary to the polyuria.[45] Both clinical and experimental studies support the view that diabetes insipidus is due to changes in the neurohypophysis resulting in loss of or diminution in the antidiuretic substance.[46] That the anterior lobe plays an important role in water metabolism is attested by the fact that complete hypophysectomy results in transient diabetes insipidus only. It has been suggested that the anterior lobe gives rise to a diuretic substance which normally antagonizes the antidiuretic action of the neurohypophysis. It is thought that this diuretic substance may act through the thyroid gland, probably through the intermediation of the thyrotropic principle of the anterior lobe.[47]

Other clinical uses for the pressor fraction are found in the treatment of paralytic ileus, in the allaying of postoperative distention[48] and in cholecystography, to reduce intestinal flatus.[49] The combination of pitressin with ephedrine has been advocated for the maintenance of blood pressure during spinal anesthesia.[50] The ephedrine, in addition to giving a transient rise in blood pressure, antagonizes the coronary constrictor action of the pitressin.[50] For the alleviation of the polyuria and polydipsia of diabetes insipidus, nasal insufflation of the dry powder has been used with success, while both clinical and experimental attempts have been made to prolong the action of subcutaneous or intramuscular

45. Richter, C. P.: The Primacy of Polyuria in Diabetes Insipidus, Am. J. Physiol. **112**: 481 (July) 1935.

46. (*a*) Fisher, Charles; Ingram, W. R., and Ranson, S. W.: Diabetes Inspidus and the Neurohumeral Control of Water Balance, Ann Arbor, Mich., Edward Brothers, Inc., 1938. (*b*) Berlinger, W.: Diabetes insipidus bei entzündlich-fibroser Atrophie der Neurohypophyse nach Unfall, Endokrinologie **20**: 305, 1938. (*c*) Baker, A. B., and Craft, C. B.: Bilateral Localized Lesions. The Hypothalamus with Complete Destruction of the Neurohypophysis in a Pituitary Dwarf with Severe Diabetes Insipidus, Endocrinology **26**: 801 (May) 1940. (*d*) Dandy, W. E.: Section of the Human Hypophyseal Stalk: Its Relation to Diabetes Insipidus and Hypophyseal Functions, J. A. M. A. **114**: 312 (Jan. 27) 1940.

47. Barnes, B. O.; Regan, J. F., and Bueno, J. G.: Is There a Specific Diuretic Hormone in the Anterior Pituitary? Am. J. Physiol. **105**: 559 (Sept.) 1933. Keller, A. D.: Hypophyseal Thyrotropic Mechanism Essential for Occurrence of Diabetes Insipidus in Its Maximal Form, Proc. Soc. Exper. Biol. & Med. **36**: 787 (June) 1937.

48. Potter, P. C., and Mueller, R. S.: Value of Pitressin in Abdominal Surgery with Special Reference to Dosage and Technique, Am. J. Surg. **43**: 710 (March) 1939. Frazier, W. D.: Use of Pitressin for Control and Relief of Distention, Am. J. Surg. **36**: 672 (June) 1937.

49. Kirklin, B. R., and Seedorf, C. E.: Use of Pitressin in Cholecystography, Proc. Staff Meet., Mayo Clin. **14**: 502 (Aug. 9) 1939.

50. Chaikoff, J. S.: Efficacy of the Combination of Ephedrine and Pitressin as Preanesthetic Medication in the Control of Blood Pressure During Spinal Anesthesia, Anesth. & Analg. **19**: 121 (May-June) 1940.

injections by administering the pituitary preparation with zinc salts or in oil.[51] Further work is required for a proper evaluation of these newer preparations.

DISEASES ASCRIBED TO HYPERSECRETION

In recent years, attempts have been made to associate preeclampsia and eclampsia with hypersecretion of the posterior lobe of the pituitary. However, attempts to demonstrate the presence of the active principles in the blood or urine of patients with these conditions have yielded variable results.[52] The possibility remains that the body becomes unusually sensitive to pituitary substances and support for this view comes from the work of Dieckmann and Michel, Lambillon and others.[44] Further study will be required to establish the role of the neurohypophysis in hypertension.[53]

HYPERSENSITIVITY TO POSTERIOR PITUITARY

Several cases of hypersensitivity to posterior pituitary have been reported.[54] The symptoms are those of shock: marked pallor, rapid pulse, fall in blood pressure, air hunger, sense of impending death and, in more severe cases, edema and semiconsciousness. These symptoms are alleviated by epinephrine.

51. Rutledge, D. I., and Rynearson, E. H.: Diabetes Insipidus: Treatment by Insufflation of Powdered Posterior Pituitary Substance, Proc. Staff Meet., Mayo Clin. **14**: 443 (July 12) 1939. Court, Donald, and Taylor, S. A.: Diabetes Inspidus Treated by Slowly Acting Pituitary Emulsion, Proc. Roy. Soc. Med. **32**: 1203 (Aug.) 1939. Greene, J. A., and January, L. E.: Diabetes Insipidus Treated by the Subcutaneous Administration of a Suspension of Pitressin Tannate in Oil, J. A. M. A. **115**: 1183 (Oct. 19) 1940. Stephens, D. J.: Zinc Salts and Oil in Prolongation of Therapeutic Effects of Pitressin in Experimental Diabetes Insipidus, Proc. Soc. Exper. Biol. & Med. **44**: 240 (May) 1940. Dodds, E. C.; Noble, R. L.; Rinderknecht, H., and Williams, P. C.: Prolongation of Action of the Pituitary Anti-Diuretic Substance and of Histamine, by Metallic Salts, Lancet **2**: 309 (Aug. 7) 1937.

52. Anselmino, K. J., and Hoffmann, F.: Ueber die pathologisch-anatomischen Grundlagen einer gesteigerten Hypophysenhinterlappenfunktion bei der Eklampsie und Nephropathie der Schwangeren, Zentralbl. f. Gynäk. **58**: 2363 (Oct. 6) 1934. Byrom, F. B., and Wilson, C.: The Alleged Pituitary Origin of the Eclamptic and Pre-Eclamptic "Toxemias" of Pregnancy, Quart. J. Med. **3**: 361 (July) 1934. Melville, K. I.: Anti-Diuretic Pituitary Substance in Blood, with Special Reference to the Toxemia of Pregnancy, J. Exper. Med. **65**: 415 (March) 1937. Teel, H. M., and Reid, D. E.: Occurrence of Anti-Diuretic Substance in Urine of Patients with Pre-Eclampsia and Eclampsia, Endocrinology **24**: 297 (March) 1939.

53. Liu, S. H.: Pathological States Produced by the Administration of Posterior Pituitary Pressor Principle, Chinese M. J. **55**: 448 (May) 1939.

54. McMann, Walter: Hypersensitivity to Solution of Posterior Pituitary, J. A. M. A. **113**: 1488 (Oct. 14) 1939. Pendleton, G. F.; Ball. W. G., and Rhode, William: Hypersensitivity to Pituitary Extract, J. Missouri M. A. **34**: 194 (June) 1937. Simon, F. A., and Ryder, C. F.: Hypersensitiveness to Pituitary Extracts, J. A. M. A. **106**: 512 (Feb. 15) 1936.

CONCLUSION

While, perhaps, one still cannot assign with certainty any specific roles to the powerful principles of the posterior lobe, nevertheless, in recent years much evidence, both clinical and experimental, has accumulated which indicates that the pressor substance plays an important role in water metabolism.[55] In regard to the oxytocic fraction, opinion is still divided as to whether or not it is a factor in the initiation of uterine contractions during labor. Of interest in this respect is the report by Fisher and co-workers that in cats suffering from experimental diabetes insipidus there developed striking disturbances in the mechanism of parturition.[56] The wide use of solution of posterior pituitary and of preparations of various fractions from the posterior lobe in both surgery and obstetrics justifies the furtherance of experimental studies to establish the chemical composition of the active principles, to determine their mechanism of action and to discover their role in the normal economy of the body.

55. Gersh, Isidore: Water Metabolism: Endocrine Factors, A. Research Nerv. & Ment. Dis., Proc. (1939) **20**: 436, 1940. Fisher, Ingram and Ranson.[46a]

56. Fisher, Charles; Ranson, S. W., and Magoun, H. W.: Dystocia in Diabetes Insipidus, Am. J. Obst. & Gynec. **36**: 1 (July) 1938.

CHAPTER X

PHYSIOLOGY OF THE OVARIES

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There are several important points about the ovaries which have emerged clearly under experimental attack. Once clearly defined, in many cases accentuated through experimental conditions, some of the phenomena stand out as normal in certain species but occur in others only occasionally. An instance is the formation of corpora lutea (accessory ones) in large follicles that have not ovulated. The ova are trapped inside, but the cells of the follicle take on luteal characteristics. Known for many years, this condition was first induced experimentally by unbalanced stimulation of the ovaries with anterior pituitary extracts.[1] It appears normally in the pregnant mare [2] and the pregnant porcupine [3] and occasionally in the nonpregnant

Since the outline for the second edition includes "The Estrogenic Substance" by Dr. Doisy, "Corpus Luteum Hormone" by Dr. Corner, and "Menstruation" by Dr. Bartelmez, this chapter on "The Physiology of the Ovaries" is designed as general in nature. An attempt is made to reunite considerations of the production of both eggs and hormones. W. U. Gardner and C. A. Pfeiffer have contributed criticisms and suggestions for improvement. Limitations as to length prevent inclusion of several important aspects of ovarian physiology. Many additional references will be found in: Allen, E.; Danforth, C. H., and Doisy, E. A.: Sex and Internal Secretions, edition 2, 1939.

1. Evans, H. M.; Meyer, Karl; Simpson, M. E.; Szarka, A. J.; Pencharz, R. I.; Cornish, R. E., and Reichert, F. L.: The Growth and Gonad-Stimulating Hormones of the Anterior Hypophysis, in Memoirs of the University of California, Berkeley, Calif., University of California Press, 1933, vol. 11, pp. 1-446. Evans, H. M., and Long, J. A.: Characteristic Effects upon Growth, Oestrus, and Ovulation Induced by the Intraperitoneal Administration of Fresh Anterior Hypophyseal Substance, Anat. Rec. **23**: 19 (Jan.) 1922. Engle, E. T., and Smith, P. E.: The Origin of the Corpus Luteum in the Rat as Indicated by Studies upon the Luteinization of the Cystic Follicle, ibid. **43**: 239 (Aug.) 1929. Fevold, H. L.; Hisaw, F. L., and Leonard, S. L.: The Gonad-Stimulating and the Luteinizing Hormones of the Anterior Lobe of the Hypophysis, Am. J. Physiol. **97**: 291 (May) 1931. Van Dyke, H. B., and Wallen-Lawrence, Zonja: Further Observations on the Gonad-Stimulating Principle of the Anterior Lobe of the Pituitary Body, J. Pharmacol. & Exper. Therap. **47**: 163 (Feb.) 1933.

2. Cole, H. H.; Howell, C. E., and Hart, G. H.: The Changes Occurring in the Ovary of the Mare During Pregnancy, Anat. Rec. **49**: 199 (May) 1931.

3. Mossman, H. W.: The Ovarian Cycle in the Porcupine, Erethizon Dorsatus, with Particular Regard to the Natural Occurrence of Unilateral Luteinization of Unruptured Follicles, Anat. Rec. **76** (supp. 2): 44 (Feb.) 1940.

monkey.[4] More careful observations may establish this as an occasional occurrence in women!

Another instance is the extreme development of the theca folliculi, the zone immediately surrounding the basement membrane about the outer layer of follicle cells, as described in the ovary of the pocket gopher by Mossman.[5] Although this tissue develops to a certain extent around growing follicles in the ovaries of most mammals, in the gopher it reaches extremes and apparently has an important secretory function accessory to that of the follicle, for it develops at puberty, when the symphysis pubis in this animal is resorbed to enlarge the birth canal. This resorption has been produced experimentally in males as well as in females, both gophers [6] and mice,[7] by injecting estrogen.

Another important point, which may now be mentioned as a fact rather than as an interesting possibility, is that a group of follicles, instead of just one, begins rapid growth which may result in ovulation of only one egg (or a few eggs in litter-bearing animals), the other follicles undergoing atresia and being resorbed. Loeb [8] emphasized this condition in his early studies of the guinea pig. It apparently needs the mass action of a group of follicles to assure ovulation of one (or a few), and this is apparently concerned with the secretory function of the follicles. The experiments of Hohlweg [9] and of Westman [10] are cited in this connection. Hohlweg reported precocious ovulation and the formation of corpora lutea following injections of estrogen in immature rats. Westman succeeded in destroying all of one ovary except one growing follicle. This was done in the

4. Corner, G. W.: Accessory Corpora Lutea in the Ovary of the Monkey, Macacus Rhesus, Anat. Rec. **76** (supp. 2): 16 (Feb.) 1940.

5. Mossman, H. W.: The Thecal Gland and Its Relation to the Reproductive Cycle: A Study of the Cyclic Changes in the Ovary of the Pocket Gopher, Geomys Bursarius (Shaw), Am. J. Anat. **61**: 289 (July) 1937.

6. Hisaw, F. L.: The Influence of the Ovary on the Resorption of the Pubic Bones of the Pocket Gopher, Geomys Bursarius, J. Exper. Zool. **42**: 411, 1925.

7. Gardner, W. U.: Pelvic Changes Occurring in Male Mice Receiving Large Amounts of Folliculin Benzoate, Proc. Soc. Exper. Biol. & Med. **33**: 104, 1935.

8. Loeb, L.: Ueber die Bedeutung des Corpus luteum für die Periodizität des sexuellen Zyklus beim weiblichen Säugetierorganismus, Deutsche med. Wchnschr. **37**: 17, 1911.

9. Hohlweg, W.: Veränderungen des Hypophysenvorderlappens und des Ovariums nach Behandlung mit grossen Dosen von Follikelhormon, Klin. Wchnschr. **13**: 92 (Jan. 20) 1934.

10. Westman, A.: Untersuchungen über die Abhängigkeit der Funktion des Corpus luteum von den Ovarialfollikeln und über die Bildungsstätte der Hormone im Ovarium, Arch. f. Gynäk. **158**: 476, 1934.

rabbit by means of a fine diathermy needle. The other ovary was then removed. The lone remaining follicle was unable to proceed through normal development. If, however, a single dose of estrogen was given at the time of the operation, this lone follicle did develop normally and ovulate. Later this experiment was repeated in hypophysectomized animals.[11] This investigation assigns a supportive secretory function (estrogenic) to other follicles than the favored ones, i. e., to the ones which develop only partially, and then die and are eliminated. The "favored follicle" truly "stands upon the shoulders of its contemporaries."

In a similar way it may be inferred that the partly developing follicles probably secrete estrogen, which provides a necessary supporting action for the formation and function of corpora lutea. This, too, has been tested by injecting estrogen and found to be true.[12] In fact, injections of estrogen prevent the rapid involution of the corpora lutea which follows removal of both (1) the anterior lobe of the pituitary[13] and (2) the pregnant uterus with its embryos and placenta.[14] The latter evidence may mean that pituitary support of luteal function is partly through a stimulus to follicular production of estrogen rather than entirely through a specific "luteinizer." The support of luteal function by estrogen is in line with Willard Allen's observation[15] that progesterone and estrogen together were more effective in producing progestational changes in the uterus than was progesterone alone. This synergism seems to occur in both ovarian and uterine functions.

That such an influence may be felt more strongly locally than systemically, i. e., in the same ovary more than in the other ovary (to which the stimulus must

11. Westman, A., and Jacobsohn, D.: Experimentelle Untersuchungen über die Bedeutung des Hypophysen-Zwischenhirnsystems für die Produktion gonadotroper Hormone des Hypophysenvorderlappens, Acta obst. et gynec. Scandinav. **17**: 235, 1937.

12. Westman, A., and Jacobsohn, D.: Ueber Oestrinwirkungen auf die Corpus luteum-Funktion, Acta obst. et gynec. Scandinav. **17**: 1 and 13, 1937. Heckel, G. P., and Allen, W. M.: Maintenance of the Corpus Luteum and Inhibition of Parturition in the Rabbit by Injection of Estrogenic Hormone, Endocrinology **24**: 137 (Feb.) 1939.

13. Robson, J. M.: Maintenance by Oestrin of the Luteal Function in Hypophysectomized Rabbits, J. Physiol. **90**: 435 (Sept.) 1937.

14. Greep, R. O.: Changes in the Corpora Lutea of Rabbits Following Hysterectomy During Pregnancy, Anat. Rec. **76** (supp. 2): 25 (Feb.) 1940.

15. Allen, W. M., and Heckel, G. P.: The Effect of Continued Injections of Progestin and Combinations of Oestrin and Progestin on the Endometrium of the Castrated Rabbit, Anat. Rec. **64** (supp. 3): 2 (March) 1936.

be carried by the blood stream), is shown by Mossman's [3] description of conditions during pregnancy in the porcupine. The porcupine bears only one young at a time and therefore usually ovulates only one ovum. However, several other follicles succeed in reaching large size. When the favored follicle ovulates, these others are transformed into accessory corpora lutea, similar in all respects to the true corpus except that they have the ova trapped inside. So far this is similar to conditions in the pregnant mare [2] and to Corner's [4] observations in the ovaries of 4 of 23 nonpregnant monkeys killed between ovulation and the end of menstruation. But the remarkable thing about the porcupine is that as pregnancy progresses the accessory corpora in the ovary which ovulated, and therefore the one which contains the true corpus, persist throughout pregnancy, while those in the other ovary disintegrate before midgestation.[16] This might be explained on the basis of a strong hormonal influence establishing the luteinization but diminishing as gestation progresses until some local influence from the true corpus sustains structures in that ovary but fails to reach systemically to sustain those in the other ovary.

During recent years while the endocrinology of reproduction has been making such rapid advances,[17] there has been a tendency to discuss ovarian hormones apart from other phases of ovarian physiology. However, the production of eggs is still the primary function of the ovaries. Phylogenetically, this was the first ovarian function, and it is probably still the only function of the ovaries in some of the lower forms. The production of eggs in the mammalian ovary must involve slow but continuous shifting of transient structures of changing size—many follicles forming, growing and dying as a few mature, ovulate and are transformed into corpora lutea. The corpora in turn function for brief periods, depending to some extent on the fate of the eggs previously ovulated from the follicles that nurture them, before they shrink and disintegrate. Therefore, as far as egg production is concerned, the ovary might be compared to "a slowly

16. In the pregnant mare the accessory corpora also do not persist throughout pregnancy.

17. Allen, Edgar; Danforth, C. H., and Doisy, E. A.: Sex and Internal Secretions, ed. 2, Baltimore, Williams & Wilkins Company, 1939.

boiling kettle" in which bubbles (follicles) are forming —from the surface, not from the bottom—sinking in as they grow to medium size, then reversing direction as they enlarge to rupture the surface at ovulation. But, as in any population, many follicles die before maturity; the mortality among ovarian eggs is very great.[18] In fact, this mortality is probably greater than formerly calculated, because new generations of eggs are differentiating from indifferent cells, during sexual maturity,[19] just as new sperm are continuously formed in the testes. This makes even more important the "selective elimination" to which growing eggs are subjected in the ovaries. These ova are eliminated not so much because they are defective as because there is only a limited supply of necessities, including anterior pituitary gonadotropic hormone, which is essential for their late growth. Many ova which would otherwise die can be brought to multiple ovulation by increased pituitary stimulation.[20] This is probably the key to the remarkable regenerative capacity of the ovary after removal of all but small parts[21] and to the compensatory hypertrophy of one ovary after removal of the other.

One important conclusion which follows is that the life span of ova in mammalian ovaries is usually a short one—in some of the small mammals it may be a matter of two or three weeks—almost as short as that of red blood corpuscles.[19b] This extensive development of transient structures involves constant rebuild-

18. Allen, Edgar; Kountz, W. B., and Francis, B. F.: Selective Elimination of Ova in the Adult Ovary, Am. J. Anat. **34**: 445 (Jan.) 1925.

19. (*a*) Allen, Edgar: Ovogenesis During Sexual Maturity, Am. J. Anat. **31**: 439 (May) 1923. (*b*) Evans, H. M., and Swezy, O.: Ovogenesis and the Normal Follicular Cycle in Adult Mammalia, in Memoirs of the University of California, Berkeley, Calif., University of California Press, 1931, vol. 9, p. 119. Evidence for ovogenesis is clearest in the adult mouse, in which, by use of the colchicine technic, hundreds of dividing cells, the first stage in ovogenesis, can be found at certain times in the estrous cycle (Allen, Edgar, and Creadick, R. N.: Ovogenesis During Sexual Maturity: The First Stage, Mitosis in the Germinal Epithelium, as shown by the Colchicine Technique, Anat. Rec. **69**: 191 [Sept.] 1937). Further search with improved technics will, I believe, furnish additional evidence in other mammals for this process, so fundamental in ovarian physiology.

20. Smith, P. E., and Engle, E. T.: Experimental Evidence Regarding the Rôle of the Anterior Pituitary in the Development and Regulation of the Genital System, Am. J. Anat. **40**: 159 (Nov.) 1927.

21. (*a*) Lipschütz, Alexander: On Some Fundamental Laws of Ovarian Dynamics, Biol. Rev. **2**: 263 (June) 1927. (*b*) Butcher, E. O.: Regeneration in Ligated Ovaries and Transplanted Ovarian Fragments of the White Rat (Mus Norvegicus Albinus), Anat. Rec. **54**: 87 (Sept.) 1932. (*c*) Van Wagenen, Gertrude, and Morse, A. H.: Personal communication to the author.

ing of the vascular nets of growing follicles and then further remodeling when these undergo atresia or ovulate to form corpora lutea.

To produce eggs in a majority of animals, especially birds, storage of nutriment seems necessary; therefore the ovaries must mobilize food for developing eggs. It seems probable that this must be done in competition with other bodily needs for nutriment in both growth and repair. In a general sense, this condition is reflected in the attainment of the major part of body growth before attainment of puberty presages mature ovarian function. Later during pregnancy in viviparous animals this competition for necessities between growing eggs in the ovary and bodily needs may be extended to include competition with the growing embryo and fetus, and during lactation, with function of the mammary glands. At first this competition is probably for things nutritional; later it may extend to include competition for extraovarian hormones, such as those of the anterior lobe of the pituitary, which are necessary for both body growth and reproductive function. This condition is accentuated by starvation, malnutrition or rapid growth of genital cancer. This sort of competition between organs or tissues must involve different rates of growth and metabolism, temporarily sustained.

The second function of the ovaries is the production of hormones. That this function is intimately tied up with growth processes is demonstrated by the striking growth of accessory genital organs of the female produced experimentally by injections of estrogen.[22] In fact, the sexual cycles, both estrous and menstrual, are fundamentally recurring waves of growth in the genital tract.[23] The ovarian estrogen is the essential hormone responsible for these cycles in nonpregnant females.[24] The luteal hormone is in a sense supplementary, acting apparently both as synergist and

22. Allen, Edgar, and Doisy, E. A.: An Ovarian Hormone: Preliminary Report on Its Localization, Extraction and Partial Purification, and Action in Test Animals, J. A. M. A. **81**: 819 (Sept. 8) 1923.

23. Allen, Edgar; Doisy, E. A.; Francis, B. F.; Gibson, H. V.; Robertson, L. L.; Colgate, C. E.; Kountz, W. B., and Johnston, C. G.: The Hormone of the Ovarian Follicle: Its Localization and Action in Test Animals, and Additional Points Bearing upon the Internal Secretion of the Ovary, Am. J. Anat. **34**: 133 (Sept.) 1924.

24. Allen, Edgar: The Menstrual Cycle of the Monkey, Macacus Rhesus: Observations on Normal Animals, the Effects of Removal of the Ovaries and the Effects of Injections of Ovarian and Placental Extracts into the Spayed Animals, Contrib. Embryol., (no. 98), **19**: 1, 1927.

antagonist, with and against the estrogenic hormone, in various reactions. It is now definitely established that the androgenic hormones may also play important supplementary roles in ovarian endocrine function.[25] In fact, the secretion of the ovaries is probably a mixture of estrogens, androgens and progesterone, functioning at minimal levels during relatively quiescent phases of the sexual cycle but with successive generations of follicles and corpora periodically intensifying their function and momentarily modifying and dominating the total hormone output. The final result must depend on the degree of dominance for a while before the balance swings back. This principle apparently involves not only ovarian tissues but also their interactions with other endocrine glands.

Since the blood stream is the common carrier for both nutritional and hormonal supplies, the matter of competition for limited quantities of these substances, amounting actually to a struggle for survival in some instances, seems most fundamental in any concept of ovarian physiology. The developing eggs in the mammalian ovary might truly be considered a crowded population in a life and death struggle for limited amounts of vital necessities, a struggle so severe that only 400 human eggs, of hundreds of thousands, may reach maturity and be ovulated during the reproductive life of the average woman.

Recent thorough studies of the ovaries in the bat by Guthrie and Jeffers [26] give a clear picture of this sort of competition between growing follicles. During the month of September, in this species, all but one of a group of follicles are elminated as that favored one, "the follicle of ovulation," grows to ovulation size. The inhibition of follicular growth during pregnancy and lactation may also be logically explained by competition for necessary pituitary secretions during this time by dominant uterine and mammary functions. Interrelations of endocrine glands seem best interpreted by inclusion of a concept of balance through competitive utilization of certain limited vital necessities by successively dominant tissues or organs. The rapid

25. Koch, F. C.: Biochemistry of Androgens, in Allen, Danforth and Doisy,[27] chap. 12.

26. Guthrie, M. J., and Jeffers, K. R.: Growth of Follicles in the Ovaries of the Bat, Myotis Lucifugus Lucifugus, Anat. Rec. 71: 477 (Aug.) 1938.

growth of mammary cancer may suppress the function of other genital tissues for the same reason.[26a]

The concept seems to take concrete form more readily when the ovary is considered an aggregate, or colony, of transient individuals. As an ontogenic beginning, the primordial germ cells migrate to the genital ridge from the "germ cell crescent" of the embryonic disk of the bird [27] or from the endoderm of the gut of the mammal.[28] If this germ cell migration is successful, the genital ridge differentiates into an ovary or a testis; if the "germ cells" are prevented from reaching the ridge, a "sterile ovary" without ova (or an atypical testis) results and the primordial germ cells fail in establishing themselves as such in other sites.[29] Therefore both the immigrant cells and the genital ridge environment are important in the differentiation of the ovary.[30]

Apparently these primordial immigrants are not the ancestors of the definitive ova, those which ovulate during adult life. However, because of their presence in the embryonic genital ridge, the overlying peritoneal cells divide, and daughter cells from these divisions differentiate into ova and migrate toward the massed primordial ova beneath. With them go sister cells, which become follicle cells—also from the germinal epithelium. The ova do not divide further; the follicle cells do, and as they proliferate they group around the eggs to form the small follicles. Many such groups are formed during embryonic life, but these follicles and their contained ova probably all die and are resorbed before sexual maturity. New generations of ova are added to replace them,[31] the new growth continuing into adult reproductive life.[19a]

Therefore the hereditary characters, the chromosomes, of the eggs which are ovulated are probably

26a. Allen, E., Diddle, A. W., Strong, L. C., Burford, T. H., and Gardner, W. U. The Estrous Cycles of Mice During Growth of Spontaneous Mammary Tumors and the Effects of Ovarian Follicular and Anterior Pituitary Hormones, Am. J. Cancer **25**: 291, 1935.

27. Swift, C. H.: Origin and Early History of the Primordial Germ-Cells in the Chick, Am. J. Anat. **15**: 483, 1914.

28. Allen, B. M.: The Embryonic Development of the Ovary and Testis of the Mammals, Am. J. Anat. **3**: 89 (June) 1904.

29. Willier, B. H.: Experimentally Produced Sterile Gonads and the Problem of the Origin of Germ Cells in the Chick Embryo, Anat. Rec. **70**: 89 (Dec.) 1937.

30. Willier, B. H.: Embryonic Development of Sex, in Allen, Danforth and Doisy,[17] chap. 13.

31. Arai, Hayato: On the Postnatal Development of the Ovary (Albino Rat), with Especial Reference to the Number of Ova, Am. J. Anat. **27**: 405 (Sept.) 1920.

not derived from the migrant primordial germ cells but are segregated in the peritoneum overlying the genital ridge. These cells have no chance to express their potentialities in the next generation unless the immigrant cells reach the site of the forming gonad and "beckon them in." This influence, this attraction, local in action, is probably in the nature of a secretion of the germ cells into the tissue fluids. It might be called a hormone if it were blood borne and its effects systemic. Since this influence probably moves by seepage through tissue fluids and is local, it is better classified as an "embryonic inductor" or "organizer." But hormones act similarly, as is shown in numerous endocrine experiments by more intense local than systemic actions.[32] This failure of the migrant primordial germ cells to become the immediate ancestors of the fertilized eggs that become our children does not mean a break in the chromosomal line of descent, for the undifferentiated cells of the germinal epithelium of the ovary are also descended from the same fertilized ovum. It is rather a temporary recognition of cousins while cells of the "true line" remain temporarily in undifferentiated obscurity.

Although the initial impulse of differentiation of sex in the embryo is undoubtedly genetic,[33] and the chromosomes of the primordial germ cells undoubtedly carry the determination of the genital ridge into the ovary or the testis, the further course of sexual differentiation, that of the accessory reproductive organs, seems to be taken over as an endocrine function of the developing gonad. There is evidence that in the female this embryonic ovarian hormone is estrogenic. At this time it is probably produced primarily by the partially growing follicles, for as yet interstitial and thecal tissues are scarcely developed at all, and corpus luteum participation is still far in the future.

Further development of the ovary consists of several successive "showers" or proliferations of cells, either in cords or in discrete masses, to form ova and follicle cells, and perhaps others which are not organized into follicles and which become interstitial cells.

32. Lyons, W. R., and Templeton, H. J.: Intravaginal Assay of Urinary Estrin, Proc. Soc. Exper. Biol. & Med. 33: 587 (Jan.) 1936.

33. Bridges, C. B.: Cytological and Genetic Basis of Sex, in Allen, Danforth and Doisy,[37] chap. 2.

One of the earlier "showers," which forms the medulla of the ovary, seems to carry potentialities for testis formation under certain conditions. Later "showers" give rise to the cells of the fetal ovarian cortex. Thus the ovary has potentialities for both sexes.[34] It is now definitely known that ovarian tissue after puberty can secrete androgenic substances under certain experimental conditions,[35] so that ovaries, as well as the adrenal cortex, may be an accessory source of masculinizing hormone in girls and women.

Apparently the genetic determination of sex involves a balance of genes in the chromosomes.[33] If the balance does not tip to a clear decision, sex intergrades may result. Apparently a similar balance exists between the androgenic and the estrogenic output of the gonads of both sexes. Sex intergrades have been produced by upsetting the usual endocrine balance.[36] Injections of androgens and estrogen early in embryonic life seem partly to reverse the sex as determined genetically or to block one potentiality and permit development in the direction of the opposite one. The transition of control by chromosomes to control by hormones needs much further study, but, since the convincing work of Stockard [37] on inheritance of endocrine imbalance in dogs and of Danforth [38] and Witschi [34] on factors determining type and color of plumage in birds, the significance of genic and endocrine dominance in influencing sex is clearly recognized.

When the consideration of embryonic life is extended to the mammal, ovarian hormones from the mother must be considered, for the placenta is by no means an absolute barrier against maternal hormones enter-

34. Witschi, Emil: Modification of Development of Sex in Lower Vertebrates and in Mammals, in Allen, Danforth and Doisy,[17] chap. 4.

35. Hill, R. T.: Ovaries Secrete Male Hormone: I. Restoration of the Castrate Type of Seminal Vesicle and Prostate Glands to Normal by Grafts of Ovaries in Mice, Endocrinology **21**: 495 (July) 1937.

36. Willier, B. H.; Gallagher, T. F., and Koch, F. C.: Sex-Modification in the Chick Embryo Resulting from Injections of Male and Female Hormones, Proc. Nat. Acad. Sc. **21**: 625, 1935. Burns, R. K.: The Effects of Crystalline Sex Hormones on Sex Differentiation in Amblystoma: I. Estrone, Anat. Rec. **71**: 447 (Aug.) 1938. Humphrey, R. R.: Studies on Sex Reversal in Amblystoma: III. Transformation of the Ovary of A. Tigrinum into a Functional Testis through the Influence of a Testis Resident in the Same Animal, J. Exper. Zool. **58**: 333, 1931.

37. Stockard, C. R.: The Physical Basis of Personality, New York, Norton & Company, 1931.

38. Danforth, C. H.: Relation of Genic and Endocrine Factors in Sex, in Allen, Danforth and Doisy,[17] chap. 6.

ing embryonic and fetal circulation.[39] Fortunately, sexual differentiation of the embryo one way or the other occurs fairly early in gestation. Although suppression of gonadal development by injecting hormones of the opposite sex has been accomplished in both directions, it usually must be done early, and higher levels of hormone must be attained than are found normally.[40]

There is now convincing evidence for the secretion of estrogen, progesterone and gonadotropins by placental tissues, and even by the chorion where no placenta is formed, as in the mare.[41] These products secreted during pregnancy are therefore predominantly fetal rather than maternal. In this connection the important point for ovarian physiology is that during pregnancy the endocrine function of the maternal ovaries is apparently "taken over" to a certain extent by the fetal membranes or the placenta. This is certainly true as far as the secretion of estrogen is concerned, for follicles seldom develop beyond the early antrum stage during pregnancy, yet large amounts of estrogens are excreted in pregnancy urine. In some animals, notably woman [42] and also the mare,[2] the true corpus luteum is no longer essential to the pregnancy after the first third of gestation.

A consideration of the physiology of the ovaries should include discussion of the growth of eggs, including storage of various kinds of yolk, the development and functions of egg membranes, both the fertilization membrane and the zona pellucida, the appearance of the corona radiata, the changes in the zona at the time of maturation and polar body formation, the withdrawal of processes of adjacent follicle cells from pores in the zona as the egg travels down the

39. Courrier, R.: Nouvelles recherches sur la folliculine; contribution à l'étude du passage des hormones au travers du placenta, Compt.rend. Acad. d. sc. **179**: 2192, 1924.

40. Greene, R. R.; Burrill, M. W., and Ivy, A. C.: Experimental Intersexuality: the Production of Feminized Male Rats by Antenatal Treatment with Estrogens, Science **88**: 130 (Aug.) 1938; Experimental Intersexuality: The Paradoxical Effects of Estrogens on the Sexual Development of the Female Rat, Anat. Rec. **74**: 429 (Aug.) 1939; Experimental Intersexuality: The Effect of Antenatal Androgens on Sexual Development of Female Rats, Am. J. Anat. **65**: 415 (Nov.) 1939. Hamilton, J. B., and Gardner, W. U.: Effects in Female Young Born of Pregnant Rats Injected with Androgens, Proc. Soc. Exper. Biol. & Med. **37**: 570 (Dec.) 1937.

41. Newton, W. H.: Some Problems of Endocrine Function in Pregnancy, in Allen, Danforth and Doisy,[17] chap. 10.

42. Asdell, S. A.: Growth and Function of Corpus Luteum, Physiol. Rev. **8**: 313 (July) 1928.

tube and the final dissolution of the zona in the uterus before implantation and increase in size of the segmenting ovum are possible—the latter being accomplished by shift of the hydrogen ion concentration of the uterine fluid to the acid side. Limitations of space exclude more than bare mention. Reference is made to the recent monograph of Pincus [43] for this phase of the subject.

One outstanding feature in the early growth of the ovum is its increase in size from deposition of yolk—the kind varying greatly with the species. In the pig there is much clear lipoid material; or there may be colorless yolk as in the rabbit, monkey or man, or pigmented yolk as in the carnivores. Yolk deposition in birds has been much studied. Precursors of definitive yolk can be stained cytologically by special dyes and their transport through (secretion by) the follicle cells demonstrated.[44]

As the ovum grows and cells multiply by mitotic division to form the follicle about it, all products of egg metabolism, both incoming and outgoing, must pass through these cells, because the blood supply lies beyond the follicular membrane (the basement membrane of the outer layer of follicle cells). At first, in the very small follicle, the limiting membranes between ovum and follicle cells and the basement membrane which separates follicular epithelium from stroma are hardly noticeable. As the follicle grows, they become much more prominent. The former becomes the tough, resistant capsule, the zona pellucida, through which processes from the inner layer of follicle cells extend to make contact with the surface of the ovum.

When the follicle of the mammalian ovary reaches a certain size, the liquor folliculi begins to form as an intercellular secretion. Cell-free liquor folliculi contains estrogen.[23] This is the best evidence that normally follicular cells secrete this hormone. The accumulation of follicular fluid is not necessary for secretion of estrogen, for solid granulosa cell tumors secrete this hormone.[45] The fact that other cells, such as theca,

43. Pincus, Gregory: The Eggs of Mammals, New York, Macmillan Company, 1936.

44. Guthrie, Mary J., and Jeffers, Katharine R.: A Cytological Study of the Ovaries of the Bats Myotis Lucifugus Lucifugus and Myotis Griscens, J. Morphol. **62**: 523, 1938.

45. Strong, L. C.; Gardner, W. U., and Hill, R. T.: Production of Estrogenic Hormone by a Transplantable Ovarian Carcinoma, Endocrinology **21**: 268, 1937.

"interstitial" or even luteal cells, may also secrete estrogen does not invalidate this evidence but merely indicates that they also share this secretion. When ova are destroyed by carefully graded, critical doses of roentgen rays,[46] and regenerating nonovular tissue secretes estrogen, these experiments do not disprove that follicles are normally the primary source of estrogen. They do prove that other tissues than follicles may undergo compensatory hypertrophy without follicular organization and retain this secretory function.

I think there is now evidence that all ovarian tissues may secrete estrogen—granulosa, theca, interstitial and luteal—but that follicular epithelium is probably normally the primary source. In the normal ovary the ova may be involved, for they are dynamic centers of the follicles. Primordial ova (without follicular appendages) may begin secretion of an "inductor." In birds the yolk contains estrogen.[47] The primary follicular fluid begins to form in partly developing follicles of mammalian ovaries, even after removal of the anterior lobe of the pituitary, as an intercellular secretion. The primary lakes of liquor folliculi first appear deep in the follicular epithelium. These follicles have very little theca, and the occurrence of interstitial cells is extremely variable in different species; therefore other possible sources do not exist at this time. Kingsbury[48] reported that interstitial cells are of variable and inconstant occurrence in ovaries of different species and are poorly developed before puberty even in the rabbit ovary, where later they are so conspicuous.

The changes in the follicle which precede ovulation, in fact, the changes which free the ovum from its attachment to the wall of the follicle and make its escape possible when the follicle ruptures, have been studied further in the rabbit by Pincus and Enzmann[49] and in

46. Parkes, A. S.: On the Occurrence of the Oestrous Cycle after X-Ray Sterilization: I. Irradiation of Mice at Three Weeks Old, Proc. Roy. Soc., London, s. B **100**: 172, 1926; IV. Irradiation of the Adult During Pregnancy and Lactation; and General Summary, ibid. **102**: 51, 1927.

47. Allen, Edgar; Whitsett, J. W.; Hardy, J. W., and Kneibert, F. L.: The Follicular Hormone of the Hen Ovary, Proc. Soc. Exper. Biol. & Med. **21**: 500 (May) 1924.

48. Kingsbury, B. F.: Atresia and Interstitial Cells of the Ovary, Am. J. Anat. **65**: 309 (Sept.) 1939.

49. Pincus, Gregory, and Enzmann, E. V.: The Growth, Maturation and Atresia of the Ovarian Eggs of the Rabbit, J. Morphol. **61**: 351, 1937.

the mouse by Snell and collaborators.[50] While the egg is still firmly attached by its cumulus to the follicle wall the nucleus is in resting condition.[51] When the ovum has become completely detached, the first maturation spindle has been completed and the first polar body formed. These phases of ovarian physiology would merit much more extensive discussion if limitations of space permitted.

The secretion of hormones by the ovaries is an ever expanding study. More than 40 estrogenic substances [52] have now been listed which act effectively in substituting for "the ovarian follicular hormone." Estradiol (dihydrotheelin) has been isolated from follicular contents and estrone (theelin) from sows' ovaries;[53] perhaps other estrogens are there. Freed and Soskin [54] postulated two estrogens with different properties from the ovaries of rats, one secreted by the granulosa and the other by the theca. Estrogens have been obtained from other than follicular sources: luteal tissue, ovaries without large follicles, and placental, chorionic and uterine tissues during pregnancy.[52]

Again there are several items of experimental evidence that show qualitative differences in the estrogenic content of tissues. For instance, luteal tissue from corpora of recent origin (corpora corresponding to eggs in the tubes) from human ovaries has a high estrogenic content.[55] In several other mammals estrogen is absent from luteal tissue or present only in traces. In working with fresh tissue from human ovaries (immediately after operative removal), demonstration of estrogenic response from liquor folliculi and from walls of follicles which contained normal ova is relatively

50. Snell, G. D.; Fekete, Elizabeth; Hummel, Katharine P., and Law, L. W.: The Relation of Mating, Ovulation and the Estrous Smear in the House Mouse to Time of Day, Anat. Rec. **76**: 39 (Jan.) 1940.

51. Robinson, A.: The Formation, Rupture, and Closure of Ovarian Follicles in Ferrets and Ferret-Polecat Hybrids, and Some Associated Phenomena, Tr. Roy. Soc. Edinburgh **52**: 302, 1918.

52. Doisy, E. A.: Biochemistry of Estrogenic Compounds, in Allen, Danforth and Doisy,[17] chap. 13.

53. Westerfeld, W. W.; Thayer, S. A.; MacCorquodale, D. W., and Doisy, E. A.: The Ketonic Estrogen of Sow Ovaries, J. Biol. Chem. **126**: 181 (Nov.) 1938.

54. Freed, S. C., and Soskin, Samuel: Complete and Incomplete Estrogenic Hormones Arising from Different Sites in the Rat's Ovary, Endocrinology **21**: 599 (Sept.) 1937.

55. Allen, Edgar; Pratt, J. P.; Newell, Q. U., and Bland, L. J.: Hormone Content of Human Ovarian Tissues, Am. J. Physiol. **92**: 127 (Feb.) 1930.

easy.[56] When degenerating eggs indicated that large follicles were atretic, negative results were frequent.

It is much more difficult to demonstrate progesterone than estrogen in human corpora lutea, as seen from the experiments of Pratt, Hamblen, Kamm and McGinty.[57] From excision of corpora at operation, however, there exists much evidence that is as good as actual recovery of this hormone.[58]

There is experimental evidence that in some mammals progesterone may be secreted by the follicle during the few hours before ovulation. Little is known about the modification in cell metabolism that must occur as the follicular cells, and perhaps to some extent those from the theca, transform to luteal cells.[59] There seems no obvious reason to object to a concept that postulates the secretion of follicular and later luteal hormones by the same cells at different phases of a life cycle of secretory activity.

The dividing line between follicle and corpus luteum was formerly placed at ovulation. This will not hold for accessory corpora as described earlier in this paper. The name "corpus luteum" is misleading, for only old corpora are truly "yellow bodies." Surely during the early stages of development from the large follicles a gradual transformation is indicated. Also, evidence is now accumulating to show that the large follicle may begin to change its secretion, perhaps toward the luteal type, before ovulation. The finding in mouse ovaries of a set of "intermediate follicular-luteal structures,"[60] in which part of the wall was luteal and part follicular (the part near the egg), has a bearing on this transformation. One line of evidence for this comes from the work of Young and collaborators.[61] They studied mating reactions (receptivity) of ovariectomized female guinea pigs treated by injection of estrogen and progesterone and showed that while a certain percentage

56. Allen, Edgar: Human Ova from Large Follicles: Including a Search for Maturation Divisions and Observations on Atresia, Am. J. Anat. **46**: 1 (July) 1930.

57. Pratt, J. P.; Hamblen, E. C.; Kamm, O., and McGinty, D. A.: The Human Corpus Luteum and Progestin, Endocrinology **20**: 741 (Nov.) 1936.

58. Allen, Edgar; Hisaw, F. L., and Gardner, W. U., in Allen, Danforth and Doisy,[17] chap. 8.

59. Hartman, C. G.: Studies on Reproduction in the Monkey and Their Bearing on Gynecology and Anthropology, Endocrinology **25**: 670 (Nov.) 1939.

60. Allen, Edgar; Smith, G. M., and Gardner, W. U.: Growth of Ovaries and Genital Tract in Response to Hormones as Studied by the Colchicine Technique, Anat. Rec. **67** (supp. 3): 3, (March) 1937.

would experience estrous behavior when given injections of estrogen alone, nearly all would react if first "primed" with estrogen and then given injections of progesterone. This secretion of progesterone by the preovulation follicle might be associated with the secretion of the watery secondary liquor folliculi. This secondary liquor is easily distinguishable by fixation and staining reactions from the primary liquor, which forms more gradually before late preovulation enlargement of the follicle begins.[51] But if the large follicle secretes progesterone, which had formerly been considered a specific secretion of the corpus luteum, one more of those artificial dividing lines between follicle and corpus luteum, used for the convenience of definition, becomes less distinct.

The question of secretion of specific hormones by certain recognized tissues needs further discussion. Before the study of the distribution of estrogen in ovarian tissues was completed, the substance was isolated from the placenta,[62] and ovariectomy during early pregnancy [63] showed that the placenta elaborated, rather than stored, estrogen. Further evidence for the secretion of estrogens by the placenta comes from the continuation of estrogenic effects of placental origin when the embryos are killed while the placentas are retained; i. e. (1) the continued growth of the mammary glands, (2) the continued support of luteal function and (3) the continued regulation of water balance (retention) typical of pregnancy.[41]

One of the most outstanding features in ovarian physiology continues to be the remarkable domination of late follicular development and ovulation by the anterior lobe of the pituitary.[64] Apparently follicles can develop to fairly large size—to almost the full size of the ovum with several layers of follicle cells and the formation of small amounts of follicular fluid—without need for anterior pituitary stimulation. This is true in the rat after complete removal of the anterior lobe of the pituitary. However, further follicular development

61. Young, W. C.; Dempsey, E. W.; Myers, H. I., and Hagquist, C. W.: The Ovarian Condition and Sexual Behavior in the Female Guinea Pig, Am. J. Anat. **63**: 457 (Nov.) 1938.

62. Allen, Edgar; Pratt, J. P., and Doisy, E. A.: The Ovarian Follicular Hormone, J. A. M. A. **85**: 399 (Aug. 8) 1925.

63. Waldstein, E.: Frühkastration in der Schwangerschaft, Zentralbl. f. Gynäk. **53**: 1305 (May 25) 1929.

64. Zondek, Bernhard, and Aschheim, S.: Hypophysenvorderlappen und Ovarium. Beziehungen der endokrinen Drüsen zur Ovarialfunktion, Arch. f. Gynäk. **130**: 1, 1927. Smith and Engle.[20]

and ovulation and also the formation of corpora lutea require anterior pituitary stimulation.[65] If additional follicle-stimulating hormone is present, many more follicles develop than normally. This shows that limitation of this particular hormone is a normal condition.

Many measurements of ovarian ova of mammals have shown that before much fluid is secreted and accumulated in the follicle as liquor folliculi, the egg has reached its full size. Since this stage of follicular development is reached in hypophysectomized animals, the growth of the egg and early growth of the follicle are independent of anterior pituitary hormones. Pituitary stimulation undoubtedly plays a primary part in stimulating later secretion of liquor folliculi. It also may be involved in the maturation of the ovum just before ovulation and in ovulation itself; Pincus thinks that this effect is on the follicle cells rather than on the ovum, because the initiation of the first maturation division occurs in vitro simply on explantation of ovarian eggs from the follicles.[43]

The conditions at puberty which initiate cyclic sexual activity are extremely important. This undoubtedly involves interaction between the ovaries and the pituitary. It is probable that at the approach of adolescence estrogen from partly developing follicles influences the anterior lobe of the pituitary, at first stimulating, then depressing pituitary secretion of the follicle-stimulating hormone. Clark[66] reported the content of this hormone to be higher in the female than in the male rat at puberty, although later the male pituitary is more potent than that of the female. Frank[67] reported that low doses of estrogen stimulate, while high doses depress, pituitary gonadotropic function. The atresia of the majority of the group of large follicles would permit the increased secretion of the pituitary which is so vital for the late development of large follicles of the next generation. A succession of several partial (anovulatory) estrous or menstrual cycles which result from these ovarian pituitary relation-

65. Smith, P. E., and White, W. E.: The Effect of Hypophysectomy on Ovulation and Corpus Luteum Formation in the Rabbit, J. A. M. A. **97**: 1861 (Dec. 19) 1931.

66. Clark, H. M.: A Prepubertal Reversal of the Sex Difference in the Gonadotropic Hormone Content of the Pituitary Gland of the Rat, Anat. Rec. **61**: 175, 1935; A Sex Difference in the Change in Potency of the Anterior Hypophysis Following Bilateral Castration in Newborn Rats, ibid. **61**: 193 (Jan.) 1935.

67. Frank, R. T.: The Sex Hormones; Their Physiologic Significance and Use in Practice, J. A. M. A. **114**: 1504 (April 20) 1940.

ships is the usual condition at adolescence of certain primates.[58] Puberty is usually not a sudden event but a gradual transition.[59] Hartman calls it a "staircase phenomenon." The conditions at puberty can be duplicated by injection experiments with estrogens. Several repetitions of a dose of estrogen subthreshold for an initial menstruation in the infantile monkey finally produce the first menstrual period.[68] Since effects of injected estrogens on the uterus itself appear to be transitory, the explanation probably must include interaction between ovarian estrogen and anterior pituitary gonadotropin.

Cystic follicles in the ovary have long worried the gynecologist. They are now easily produced experimentally by excessive or unbalanced stimulation with anterior pituitary gonadotropic extracts. In early stages of this condition a hyperestrogenic effect on the genital tract and mammary glands is usually present. Cystic follicles occur spontaneously in one genetic strain of rats,[69] which consequently remain for long periods in estrus (constant estrus rats), and Everett[70] has succeeded, by injections of progesterone, in inducing ovulation of these follicles.

Pfeiffer[71] has produced this condition of constant estrus by experimental modification of anterior pituitary gonadotropic function. One of the remarkable things about this experiment is that the effect is more than transitory; i. e., most endocrine effects disappear quickly after treatment with tropic substances is stopped, but in Pfeiffer's experiment altered anterior pituitary function continues after removal of the modifying factor. He transplanted testes from litter mates into 1 day old female mice. Since this was done in a closely inbred strain of mice, the grafted testes were vascularized and matured normally. The ovaries matured normally, and also the female accessory genital organs, but the animals were subject to "constant estrus," instead of "cyclic estrus," and did not recover from this condition even after the grafted testes were removed. Pfeiffer has

68. Allen, Edgar; Diddle, A. W.; Burford, T. H., and Gardner, W. U.: Ovarian Hormone Threshold for Experimental Menstruation in Monkeys, Am. J. Physiol. **117**: 381 (Nov.) 1936.

69. Everett, J. W.: Spontaneous Persistent Estrus in a Strain of Albino Rats, Endocrinology **25**: 123 (July) 1939.

70. Everett, J. W.: The Restoration of Cyclic Estrus and Ovulation in Persistent-Estrous Rats by Progesterone, Anat. Rec. **76** (supp. 2): 21 (Feb.) 1940.

71. Pfeiffer, C. A.: The Effects of an Experimentally Induced Endocrine Imbalance in Female Mice, Anat. Rec. **75**: 465, (Dec.) 1939.

explained these results as experimental modification by the testis grafts of the level or of the quality of anterior pituitary gonadotropic secretion. In rats similarly treated the injection of a pituitary extract containing the luteinizing principle induces ovulation; the animals mate and may become pregnant.[72] They often have difficulty in carrying pregnancy to term, but additional injections of the same extract make normal gestation and parturition possible.

The surgeon, confronted at laparotomy by cystic ovaries after symptoms of long duration, may think ovariectomy necessary, as it probably is in extreme cases. Experimental modification of anterior pituitary function, however, might remove the actual cause. Experiments of van Wagenen and Morse [73] point in this direction. By long-continued injections of an anterior pituitary extract containing the gonadotropic principles they succeeded in producing in monkeys extremely cystic ovaries, demonstrated by laparotomy. Treatment was then withdrawn. Recovery of normal ovarian function was shown when animals later mated, carried normal pregnancies to birth at term and nursed their offspring.

Another instance of the dependence of ovarian function on anterior pituitary hormone is the trigger mechanism of ovulation in the rabbit (also in the cat and ferret). Stimulation of the cervix uteri affects the pituitary, apparently through a nerve pathway; pituitary secretion within the next few hours induces ovulation about ten hours later. The time cited was proved by removal of the anterior lobe of the pituitary, which, if left intact for one hour after mating, functioned sufficiently to induce ovulation.[74] Then cytologic evidence for this transient pituitary function was discovered by Friedgood and Dawson [75] in the existence of cells which react to special stains only during the few hours when this transient function exists.

72. Pfeiffer, C. A.: Maintenance of Pregnancy in Constant Oestrous Female Rats, Anat. Rec. **76** (supp. 2): 45 (Feb.) 1940.

73. Van Wagenen, Gertrude, and Morse, A. H.: Pregnancy Following Induced Cystic Changes in the Ovaries, Lancet **1**: 1220 (May 28) 1938.

74. (*a*) Fee, A. R., and Parkes, A. S.: Studies on Ovulation: I. The Relation of the Anterior Pituitary to Ovulation in the Rabbit, J. Physiol. **67**: 383 (July) 1929. (*b*) Deanesly, R.; Fee, A. R., and Parkes, A. S.: Studies on Ovulation: II. Effect of Hypophysectomy on Formation of the Corpus Luteum, J. Physiol. **70**: 38 (Aug.) 1930.

75. Friedgood, H. B., and Dawson, A. B.: Cytologic Evidence of the Gonadotropic Activity of the Rabbit's Anterior Hypophysis, Endocrinology **22**: 674 (June) 1938.

Much work has been done in the last ten years on the physiology of ovulation itself. This began with the actual observation of ovulation by Walton and Hammond [76] in 1929. The process has since been photographed by moving pictures in several animals. While in the amphibia contraction of muscles in the wall of the follicle undoubtedly is of major force in ovulation,[77] in mammals increase in intrafollicular pressure is probably the dominant force. This is due to increase in secretion of the liquor folliculi, which forms rapidly in the preovulation follicle, and there is good evidence that this secretion is in response to pituitary stimulation. Events which directly precede ovulation include extreme stretching and "blowing out a pimple" in the weakest point of the follicle wall, a compression of the capillary net at this point, which leaves an avascular stigma, which finally breaks and through which follicular contents are extruded.[78] Before actual rupture of the follicle there may be a leakage of the more fluid follicular contents at this point, and then a rather sudden rupture and extrusion of the more viscous follicular contents, including the ovum with its cumulus of follicle cells still surrounding it.[78]

In some animals, notably the rabbit, the rupture of the follicle is accompanied by a remarkable change in electrical potential [79] as measured between the cervix and the symphysis pubis. In some other animals, which bear large litters, there may be a continued rise in electrical potential before ovulation with marked fluctuations in potential at the time the eggs are being ovulated,[80] but it may not be possible to associate a single rise in potential with the rupture of a specific follicle.[81a] Recent work by Boling and Burr [81b] indicates that contractions of the uterus and vagina, controlled

76. Walton, A., and Hammond, J.: Observations on Ovulation in the Rabbit, Brit. J. Exper. Biol. **6**: 190, 1928.

77. Rugh, R.: Ovulation in the Frog: II. Follicular Rupture to Fertilization, J. Exper. Zoöl. **71**: 163 (July) 1935.

78. Hill, R. T.; Allen, Edgar, and Kramer, T. C.: Cinemicrographic Studies of Rabbit Ovulation, Anat. Rec. **63**: 239 (Oct.) 1935.

79. Burr, H. S.; Hill, R. T., and Allen, Edgar.: Detection of Ovulation in the Intact Rabbit, Proc. Soc. Exper. Biol. & Med. **33**: 109 (Oct.) 1935.

80. Rogers, P. V.: Electric Potentials in Normal Castrate, and Theelin Treated Rats, Proc. Soc. Exper. Biol. & Med. **35**: 257 (June) 1936.

81. (*a*) Boling, J. L.; Barton, Dorothy S., and Burr, H. S.: Vaginal Electrical Correlates of the Estrous Cycle of the Rat, Anat. Rec. **76** (supp. 2): 8 (Feb.) 1940. (*b*) Boling, J. L., and Burr, H. S.: Factor Associated with Vaginal Electric Correlates of the Estrous Cycle of the Albino Rat, ibid. **79** (supp. 2): 9 (March 25) 1941.

by ovarian hormones, are involved in these changes in electrical potential in the rat.

Studies of changes in electrical potential have been made during the menstrual cycle in women.[82] In some instances the changes have been correlated with ovulation. At present it appears that alterations in potential, although occurring most frequently between successive periods, occur also at other times in the cycles in some women.[83] This indicates ovulation at other than the recognized usual time of ovulation in the cycle, or else the possibility that advanced development of follicles without ovulation may influence the rise in potentials.

Studies of the ovum and the follicles just preceding ovulation, especially those by Robinson and by Pincus, have greatly advanced the knowledge of the physiology of the ovary at this time. Reference is made to Pincus's book [43] for discussion of these stages.

During various phases of egg production, atresia, corpus luteum formation and retrogression, interstitial tissue appears. This is more abundant in some mammalian ovaries than in others; in some normal ovaries interstitial tissue may be very poorly developed or absent.[48] In broad definition interstitial tissue may include epithelioid cells between follicles or corpora—connective and vascular tissues being excluded. One source of formation of interstitial tissue is the proliferation of cells about a growing follicle to form the theca interna. This proliferation is readily gaged in normal ovaries by means of the drug colchicine, which shows proliferating follicles to have almost as many mitoses in the surrounding theca as among the granulosa cells of the follicles. Theca cells of growing follicles are really part of the follicle rather than "interstitial," but they become interstitial when atretic follicles undergo degeneration; in fact, Kingsbury stressed the principal derivation of interstitial cells in the ovary of the cat from thecal remains. As the follicle degenerates, the lipoid content of these cells increases. In this phase of its activity the theca of atretic follicles should not

82. Burr, H. S., and Musselman, L. K.: Bio-Electric Phenomena Associated with Menstruation, Yale J. Biol. & Med. **9**: 155 (Oct.) 1936. Rock, John; Reboul, Jean, and Wiggers, H. C.: The Detection and Measurement of the Electrical Concomitant of Human Ovulation by Use of the Vacuum-Tube Potentiometer, New England J. Med. **217**: 654 (Oct. 21) 1937.

83. Burr, H. S.; Musselman, L. K.; Barton, Dorothy S., and Kelly, Naomi B.: Bio-Electric Correlates of Human Ovulation, Yale J. Biol. & Med. **10**: 155 (Dec.) 1937.

be confused with the theca of rapidly growing follicles as described by Mossman in the pocket gopher. Other interstitial cells may be derived by invaginations from the germinal epithelium, certain nests of cells or "anovular follicles," or possibly by cell multiplication in situ. The term "interstitial cells" as proposed by Bouin carries the inference of secretion. Accumulation of lipoids in these cells has been interpreted by Kingsbury [48] as storage rather than secretion.

There is now a considerable accumulation of experimental evidence that pituitary secretion is necessary for the proper formation and function of corpora lutea. Although the pituitary seems in these ways to dominate the ovaries, there is adequate evidence to show that the secretions of the ovaries react forcibly on the pituitary. The effects of ovariectomy in inducing castration changes in the pituitary, the prevention of these changes by injected estrogens,[84] the tumors of the pituitary following excessive estrogenic stimulation,[85] all point to the importance of the influence of ovarian secretions on the pituitary.

Ovarian function during pregnancy was first studied because of the importance of the corpus luteum to continued gestation. It has been known for a long time that the growth of follicles is inhibited during pregnancy (and also during lactation). With the recognition of the placenta (and of the chorion in case no true placenta is formed) as a source of estrogen in the pregnant animal, its function compensatory to the ovaries was indicated. First it was shown that removal of the anterior lobe of the pituitary after mating prevented the formation and function of the corpora lutea.[74a] Then injections of estrogen in hypophysectomized animals were known to prevent involution of the corpora. Recently Greep [14] has shown that if the pregnant uterus is removed from the rabbit on the tenth or fifteenth day of gestation, the corpora involute, and new follicles begin growth. If estrogen is injected into such animals,

84. Fevold, H. L.: Follicle Stimulating and Luteinizing Hormones of Anterior Pituitary in Allen, Danforth and Doisy,[17] chap. 17.

85. Cramer, W., and Horning, E. S.: Experimental Production by Oestrin of Pituitary Tumors with Hypopituitarism and of Mammary Cancer, Lancet **1**: 247 (Feb. 1) 1936. McEuen, C. S.; Selye, Hans, and Collip, J. B.: Some Effects of Prolonged Administration of Oestrin in Rats, ibid. **1**: 775 (April 4) 1936. Gardner, W. U.: The Effect of Estrogen on the Incidence of Mammary and Pituitary Tumors in Hybrid Mice, Cancer Research **1**: 345 (May) 1941.

this involution of the corpora is prevented. However, Greep could not demonstrate estrogen in the rabbit placenta.

Snyder's [86] successful demonstration of stimulation of ovulation late in pregnancy, followed by superfetation, shows that follicular development is inhibited by deficiency of anterior pituitary hormone. He did this by injections of pregnancy urine in the rabbit late in gestation. This was one of the first experimental demonstrations of superfetation. This procedure usually prevented parturition.

A discussion of ovarian function would not be complete without citation of the evidence for secretion of androgen by the ovaries under certain conditions. It has been well established that androgens are excreted by females,[25] and at first the source was attributed to the adrenals, because of the masculinizing effects of tumors of the adrenal cortex. Hill [85] demonstrated clearly that ovarian tissue can, under certain conditions, secrete androgen. Using closely inbred strains of mice, he grafted ovaries in the ears of males. After the grafted ovaries had become vascularized, the testes were removed. In some cases, especially during a hot summer, the seminal vesicles and the prostate would undergo castrate atrophy; in others, especially during the winter, the accessory male organs were maintained in good functional condition. Hill [87] was then able to demonstrate an effect of temperature (the ovaries being implanted in the thin ears of the mouse were quickly subjected to environmental temperatures). When castrated males with ovaries grafted in their ears were living in warm temperatures, the seminal vesicles and prostates atrophied. When the same animals were removed to cool quarters, these accessory organs were maintained in functional condition, as shown by both organ weights and the appearance of intracellular secretory granules. After such proof one must consider the possibility of factors other than temperature modifying ovarian secretion from the estrogenic to the androgenic type—in fairly normal ovarian tissue as well as

86. Snyder, F. F.: The Prolongation of Pregnancy and Complications of Parturition in the Rabbit Following Induction of Ovulation near Term, Bull. Johns Hopkins Hosp. **54**: 1 (Jan.) 1934.

87. Hill, R. T.: Ovaries Secrete Male Hormone: III. Temperature Control of Male Hormone Output by Grafted Ovaries, Endocrinology **21**: 633 (Sept.) 1937.

in the clearly pathologic arrhenoblastoma. Mere hypertrophy in the absence of a clearly demonstrable tumor might well be sufficient to account for varying degrees of masculinization in young girls.

Studies of changes in bones of birds [88] show formation of new spicules of cancellous bone in the marrow cavities at the time when eggs are ripening in the ovaries—and extreme conditions of a similar nature induced experimentally in both birds [89] and mammals (mice)[90] by injections of estrogens emphasize the importance of estrogenic ovarian hormone on calcium metabolism and bone structure. That a similar mechanism may be involved in the normal pneumatization of bones in birds is indicated in recent studies of the chick by Bremer,[91] who emphasized the similarity of the osseous changes preceding this invasion of the bones by the air sacs to the pathologic condition osteitis fibrosa.

At the same time at which new bone is replacing marrow in the long bones and even in the axial skeleton of the mouse under estrogenic stimulation, bone is being resorbed from the symphysis pubis,[90] as occurs in normal mice toward the end of pregnancy.

The profound effect of the seasonal variation in the length of daylight on ovarian function of some birds and a few seasonally breeding mammals has been established.[92] Increased egg laying in fowls through the winter from increased lighting is a commonplace in modern farming. Benoit's [93] work in the duck, which conclusively demonstrated the influence of a light stimulus through the eyes to the anterior lobe of the pituitary and thence on the gonads, furnishes concrete experimental evidence. The work of Kirschbaum and

88. Kyes, P., and Potter, T. S.: Physiological Marrow Ossification in Female Pigeons, Anat. Rec. **60**: 377 (Nov.) 1934.

89. Landauer, W.; Pfeiffer, C. A.; Gardner, W. U., and Man, E. B.: Hypercalcification, -Calcemia and -Lipemia in Chickens Following Administration of Estrogens, Proc. Soc. Exper. Biol. & Med. **41**: 80 (May) 1939.

90. Gardner, W. U., and Pfeiffer, C. A.: Skeletal Changes in Mice Receiving Estrogens, Proc. Soc. Exper. Biol. & Med. **37**: 678 (Jan.) 1938.

91. Bremer, J. L.: The Pneumatization of the Bones of the Chick and the Association Activity of Theelin, Anat. Rec. **76** (supp. 2): 9 (Feb.) 1940.

92. Bissonnette, T. H.: The Influence of Light upon Pituitary Activity, Proc. A. Research in Nerv. & Ment. Dis., Proc. (1936) **17**: 361, 1938.

93. Benoit, Jacques: Stimulation of the Hypophysis and Genital Glands in the Duck by Electric Light: Effect of Thyroidectomy on the Testis and Liver, Anat. Rec. **67** (supp. 1): 81, 1936.

Pfeiffer [94] in sparrows, in which a differential response of ovaries and transplanted testes in the same animal to light was demonstrated, carries the solution further. Recently it has been shown that the timing of ovarian function—follicular maturation and ovulation—can be reversed in nocturnal animals, e. g., the rat [95] and the mouse,[50] by reversing day and night light conditions.

The physiology of the ovary at and after the time of the menopause is an extremely interesting subject which can only be touched on in the present chapter. One thing which is certain is that ovulation usually stops at the menopause. This eliminates consideration of the endocrine function of newly forming corpora lutea after this time. The secretion of old corpora might persist for several months in diminishing amounts. However, even after the menopause follicles develop partly for several years. Follicles may also become cystic at this time. But as a generalization it might be said that usually internal secretion of the postmenopausal ovary is definitely declining. Concurrently there is an increase in the amount of the gonadotropic substance which appears in the urine.[96] The same is true of the premature menopause following ovariectomy.[97]

One point of extreme importance in the endocrine function of the postmenopausal ovary is the possibility of a shift toward secretion of androgen at this time. Masculinization phenomena and hypertrichosis occurring in a few women after the menopause require the inclusion of consideration of this possibility.

Ten years after the menopause the ovary is represented by a mere fibrous fold of tissue, very small and obviously not very important from the point of view of production of either eggs or hormones. Apparently ovarian function declines while the gonadotropic function of the pituitary is still efficient.

94. Kirschbaum, A.; Pfeiffer, C. A; Van Heuverseyn, J. D., and Gardner, W. U.: Studies on Gonad-Hypophyseal Relationship and Cyclic Osseous Changes in the English Sparrow, Passer Domesticus L., Anat. Rec. **75**: 249 (Oct.) 1939.

95. Hemmingsen, A. M., and Krarup, N. B.: Rhythmic Diurnal Variations in the Oestrous Phenomena of the Rat and Their Susceptibility to Light and Dark, Copenhagen, Levin & Munksgaard.

96. Fluhmann, C. F.: The Endometrium in So-Called Idiopathic Uterine Hemorrhage, J. A. M. A. **93**: 1136 (Oct. 12) 1929.

97. Fluhmann, C. F.: The Significance of Anterior Pituitary Hormone in the Blood of Gynecologic Patients, Am. J. Obst. & Gynec. **20**: 1 (July) 1930; Anterior Pituitary Hormone in the Blood of Women: IV. A Preliminary Clinical Classification of Results in Non-Pregnant Individuals, Endocrinology **15**: 177, (May-June) 1931.

CHAPTER XI

THE ESTROGENIC SUBSTANCES

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ST. LOUIS

Although several investigators[1] contributed to the early development of research on estrogens, rapid progress did not begin until after the publication of the vaginal smear reaction[2] for the detection of these substances. The work was given additional impetus by the discovery of the high concentration of estrogens in the urine of pregnant women and in the urine of pregnant mares.[3] These discoveries, which provided excellent sources of the estrogens and the means of quantitative determination, were of paramount importance to the isolation of the estrogens and the determination of their structure, as well as to the introduction of an ample supply of estrogenic substances for therapeutic use. The subsequent availability of pure estrogens has permitted an investigation of their role in sex physiology, particularly with respect to the estrous and the menstrual cycle, and of their interrelationships with other internal secretions.

CHEMISTRY OF ESTROGENIC SUBSTANCES

At this point a brief review of the chemistry of both the natural and the synthetic estrogens may prove valuable to the reader. According to a system of nomenclature proposed by a group of British investigators,[4] the parent saturated hydro-

1. Adler, L.: Zur Physiologie und Pathologie der Ovarialfunktion, Arch. f. Gynäk. **95**: 349, 1912. Fellner, O. O.: Experimentell erzeugte Wachstumsveränderungen am weiblichen Genitale der Kaninchen, Zentralbl. f. allg. Path. u. path. Anat. **23**: 673, 1912. Herrmann, E.: Ueber eine wirksame Substanz im Eierstocke und in der Placenta, Monatschr. f. Geburtsh. u. Gynäk. **41**: 1, 1915. Frank, R. T., and Rosenbloom, J.: Physiologically Active Substances Contained in the Placenta and in the Corpus Luteum, Surg., Gynec. & Obst. **21**: 646, 1915.

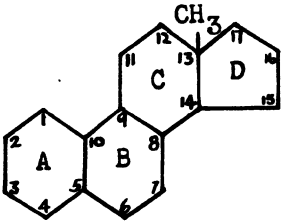
2. Allen, Edgar, and Doisy, E. A.: An Ovarian Hormone: Preliminary Report on Its Localization, Extraction and Partial Purification and Action in Test Animals, J. A. M. A. **81**: 819 (Sept. 8) 1923.

3. Aschheim, Selmar, and Zondek, Bernhard: Hypophysenvorderlappen Hormon und Ovarialhormon im Harm von Schwangeren, Klin. Wchnschr. **6**: 1322 (July 9) 1927. Zondek, Bernhard: Mass Excretion of Oestrogenic Hormone in the Urine of the Stallion, Nature, London **133**: 209 (Feb. 10) 1934.

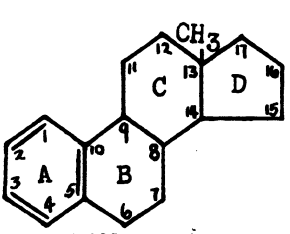
4. Adam, N. K.; Danielli, J. F.; Dodds, E. C.; King, H.; Marrian, G. F.; Parkes, A. S., and Rosenheim, O.: Nomenclature of the Oestrin Group, Nature, London **132**: 205 (Aug. 5) 1933.

carbon is named estrane, and the unsaturated hydrocarbon from which the natural estrogens are derived, $\Delta^{1,3,5}$-estratriene. The first natural estrogen isolated in a crystalline form was named theelin by Doisy;[5] according to its relationship to estrane, it is 3-hydroxy-17-keto-$\Delta^{1,3,5}$-estratriene. For convenience this name has been abbreviated to estrone. The Council on Pharmacy and Chemistry of the American Medical Association recognizes as nonproprietary names both estrone and theelin.[6]

Natural and Synthetic Estrogens.—Estrone, or theelin, $C_{18}H_{22}O_2$, the first pure estrogen to be isolated, was obtained in 1929 from human pregnancy urine.[7] Later it was obtained in crystalline condition from the urines of pregnant mares, stallions and men, from human placenta, from adrenal glands (probably beef) and from palm kernels. Its presence in sow ovaries was



Estrane



$\Delta^{1,3,5}$—estratriene

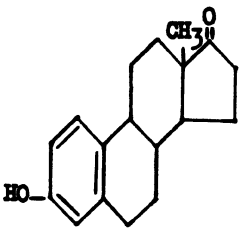
5. Veler, C. D.; Thayer, S. A., and Doisy, E. A.: The Preparation of the Crystalline Follicular Ovarian Hormone: Theelin, J. Biol. Chem. **87**: 357 (June) 1930.

6. The Nomenclature of Estrus-Producing Compounds, Report of the Council on Pharmacy and Chemistry, J. A. M. A. **107**: 1221 (Oct. 10) 1936.

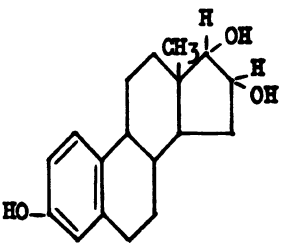
7. Doisy, E. A.; Veler, C. D., and Thayer, S. A.: Folliculin from the Urine of Pregnant Women, Am. J. Physiol. **90**: 329 (Oct.) 1929. Butenandt, Adolf: Ueber "Progynon" ein krystallisiertes weibliches Sexualhormon, Naturwissenschaften **17**: 878, 1929. de Jongh, S. E.; Kober, S., and Laqueur, Ernst: Ueber Identität des Brunsthormons (Menformon) aus Harn schwangerer Frauen und aus Harn trächtiger Pferde, Biochem. Ztschr. **240**: 247, 1931. Häussler, E. P.: Ueber das Vorkommen von α-Follikelhormon (3-oxy-17 keto-1,3,5-oestratriën) im Hengsturin, Helvet. chim. acta **17**: 531, 1934. Deulofeu, Venancio and Ferrari, J.: Krystallisiertes α-Follikelhormon aus Hengstharn, Ztschr. f. physiol. Chem. **226**: 192, 1934. Dingemanse, E.; Laqueur, Ernst, and Mühlbock, O.: Chemical Identification of Oestrone in Human Male Urine, Nature, London (supp.) **141**: 927 (May 21) 1938. Westerfeld, W. W.; MacCorquodale, D. W.; Thayer, S. A., and Doisy, E. A.: The Isolation of Theelin from Human Placenta, J. Biol. Chem. **126**: 195 (Nov.) 1938. Beall, D.: Isolation of Estrone from the Adrenal Gland, Nature, London **144**: 76 (July 8) 1939. Butenandt, Adolf, and Jacobi, H.: Ueber die Darstellung eines krystallisierten pflanzlichen Tokokinins (Thelykinins) und seine Identifizierung mit dem α-Follikelhormon, Ztschr. f. physiol. Chem. **218**: 104, 1933. Westerfeld, W. W.; Thayer, S. A.; MacCorquodale, D. W., and Doisy, E. A.: The Ketonic Estrogen of Sow Ovaries, J. Biol. Chem. **126**: 181 (Nov.) 1938. Schachter, Benjamin, and Marrian, G. F.: The Isolation of Estrone Sulfate from the Urine of Pregnant Mares, ibid. **126**: 663, 1938.

clearly indicated by physical, chemical and physiologic reactions. A conjugated form, estrone sulfate, has been isolated from mare's urine.

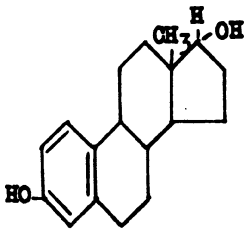
Estriol, or theelol, $C_{18}H_{24}O_3$, is obtained from human pregnancy urine,[8] human placenta and pussy willows (?). A conjugated form, estriol glycuronide, has been isolated from human pregnancy urine. The relationship of estriol to estrone was established on conversion of the former to the latter by dehydration.



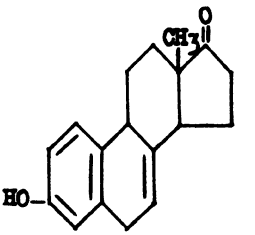
3-Hydroxy-17-keto-$\Delta^{1,3,5}$—estratriene
Estrone
Theelin



3,16,17-Trihydroxy-$\Delta^{1,3,5}$—estratriene
Estriol
Theelol



3,17-Dihydroxy-$\Delta^{1,3,5}$ estratriene
Estradiol
Dihydrotheelin



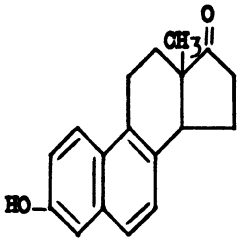
3-Hydroxy-17-keto-$\Delta^{1,3,5,7}$ — estratetraene
Equilin

Estradiol, or dihydrotheelin, $C_{18}H_{24}O_2$, can be obtained by reduction of estrone in two isomeric forms, due to the new asymmetric carbon atom 17. The alpha compound has been

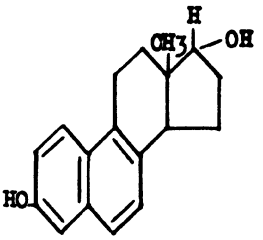
8. Marrian, G. F.: Observations on the Chemical Nature of Crystalline Oestrin, J. Soc. Chem. & Ind. **49**: 515, 1930. Doisy, E. A.; Thayer, S. A.; Levin, Louis, and Curtis, J. M.: A New Tri-Atomic Alcohol from the Urine of Pregnant Women, Proc. Soc. Exper. Biol. & Med. **28**: 88 (Oct.) 1930. Browne, J. S. L.: The Chemical and Physiological Properties of Crystalline Estrogenic Hormones, Canad. J. Research **8**: 180 (Feb.) 1933. Skarżyński, B.: An Oestrogenic Substance from Plant Material, Nature, London **131**: 766 (May 27) 1933. Cohen, S. L., and Marrian, G. F.: The Isolation and Identification of a Combined Form of Œstriol in Human Pregnancy Urine, Biochem. J. **30**: 57 (Jan.) 1936. Butenandt, Adolf, and Hildebrandt, F. M.: Ueber ein zweites Hormonkrystallisat aus Schwangerenharn und seine physiologischen und chemischen Beziehungen zum krystallisierten Follikelhormon, Ztschr. f. physiol. Chem. **199**: 243, 1931.

isolated from sow ovaries,[9] the urine of pregnant mares, human pregnancy urine and human placenta. The beta form has also been obtained from mare's urine.

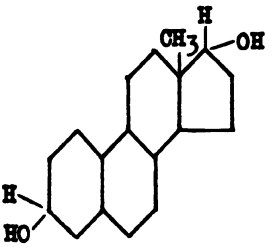
Several other natural estrogens have been isolated. In 1932 three additional compounds, equilin, hippulin and equilenin,[10] were obtained from the urine of pregnant mares. Subsequently, Wintersteiner and his collaborators isolated α-estradiol, β-estradiol and α-dihydroequilenin from the same source.



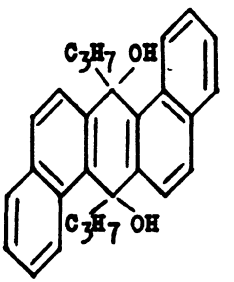
3-Hydroxy-17-keto-^{1,3,5,6,8}—estrapentaene
Equilenin



3,17-Dihydroxy-$\Delta^{1,3,5,6,8}$ — estrapentaene
Dihydroequilenin



Estranediol-3,17



9,10-Dihydroxy-
9,10-di-n-propyl-
9,10-dihydro-
1,2,5,6-dibenzanthracene

Owing to the minute concentration of estrogen and the large quantity of lipid contaminants, the isolation of estrogens from

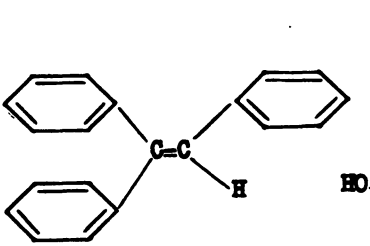
9. MacCorquodale, D. W.; Thayer, S. A., and Doisy, E. A.: The Isolation of the Principal Estrogenic Substance of Liquor Folliculi, J. Biol. Chem. **115**: 435 (Sept.) 1936. Wintersteiner, Oskar; Schwenk, Erwin, and Whitman, Bradley: Estrogenic Dihydroxy Compounds in Urine of Pregnant Mares, Proc. Soc. Exper. Biol. & Med. **32**: 1087 (April) 1935. Huffman, M. N.; MacCorquodale, D. W.; Thayer, S. A.; Doisy, E. A.; Smith, G. V., and Smith, O. W.: The Isolation of α-Dihydrotheelin from Human Pregnancy Urine, J. Biol. Chem. **134**: 591 (July) 1940. Huffman, M. N.; Thayer, S. A., and Doisy, E. A.: The Isolation of α-Dihydrotheelin from Human Placenta, ibid. **133**: 567, 1940.

10. Girard, André; Sandulesco, A.; Fridenson, A., and Rutgers, J. J.: Sur une nouvelle hormone sexuelle cristallisée retirée de l'urine des juments gravides, Compt. rend. Acad. d. sc. **194**: 909 (March 7) 1932; Sur une nouvelle hormone sexuelle cristallisée, ibid. **195**: 981 (Nov. 21) 1932. Hirschmann, H., and Wintersteiner, Oskar: The Isolation of Estrogenic Diols from the Urine of Pregnant Mares, J. Biol. Chem. **122**: 303 (Jan.) 1938.

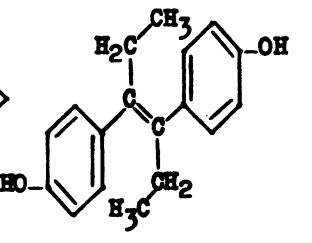
solid tissues did not proceed so rapidly. In 1930 Browne obtained estriol from human placenta, but it was not until 1938 that theelin and not until 1940 that α-dihydrotheelin were obtained from that tissue. α-Dihydrotheelin was isolated from sow ovaries in 1935, and the existence of approximately an equal concentration (about 6 mg. per ton) of theelin in sow ovaries was demonstrated in 1938.

Owing to the effect of certain types of adrenal tumors on sexual development, the isolation of estrone from adrenal extracts is interesting. Is estrone formed normally in the adrenal? If so, is this purposeful production, or is the substance merely a by-product of the steroid syntheses which seem to occur so profusely in the adrenal?

The synthesis of equilenin from simple starting materials by Bachmann [11] is one of the great accomplishments of the chemist



Triphenylethylene



4,4′-Dihydroxy-α, β-diethystilbene
Diethylstilbestrol

in the sex hormone field. Although partial syntheses have given progesterone and several androgens, the production of equilenin is the first total synthesis of a sex hormone.

In addition to the synthesis of the natural estrogen, equilenin, several compounds having estrogenic properties have been synthesized. The first compound which was prepared, 1-keto-1, 2, 3, 4-tetrahydrophenanthrene,[12] had relatively feeble activity,

11. (*a*) Bachmann, W. E.; Cole, Wayne, and Wilds, A. L.: The Total Synthesis of the Sex Hormone Equilenin, J. Am. Chem. Soc. **61**: 974 (April) 1939; **62**: 824 (April) 1940. (*b*) Marker, R. E.: Reduction of Naphtholic Steroids to Phenolic Steroids: Equilenin, ibid. **60**: 1897 (Aug.) 1938.

12. Cook, J. W.; Dodds, E. C., and Hewett, C. L.: A Synthetic Oestrus-Exciting Compound, Nature, London **131**: 56 (Jan. 14) 1933. Campbell, N. R.; Dodds, E. C.; Lawson, W., and Noble, R. L.: Biological Effects of the Synthetic Oestrogen Hexoestrol 4: 4′-Dihydroxy-γ: ξ-Diphenyl-n-Hexane, Lancet **2**: 312 (Aug. 5) 1939. Dodds, E. C.; Goldberg, L.; Lawson, W., and Robinson, R.: Synthetic Oestrogenic Compounds Related to Stilbene and Diphenylethane, Proc. Roy. Soc., London, s. B, **127**: 140 (May 18) 1939; Oestrogenic Activity of Certain Synthetic Compounds, Nature, London **141**: 247 (Feb. 5) 1938. Dodds, E. C.; Lawson, W., and Noble, R. L.: Biological Effects of the Synthetic Oestrogenic Substance 4: 4′-dihydroxy-α: β-Diethylstilbene, Lancet **1**: 1389 (June 18) 1938. Robson, J. M., and Schönberg, A.: Oestrous Reactions, Including Mating, Produced by Triphenyl Ethylene, Nature, London **140**: 196 (July 31) 1937.

but finally compounds of exceptional potency were obtained. 9, 10-Dihydroxy-9, 10-di-n-propyl-9, 10-dihydro-1, 2, 5, 6-dibenzanthracene and triphenylethylene have a fair degree of activity, but stilbestrol and hexestrol have potencies of the same magnitude as estrone. Similar to the natural estrogens, stilbestrol and hexestrol have phenolic properties.

Chemical and Physical Properties of the Natural Estrogens.— Since all of the natural estrogens are phenolic and all other known sex hormones are not phenolic, a separation can readily be effected through the solubility of the former in aqueous alkali. The relative insolubility of the estrogens in purified petroleum benzine U. S. P. (petroleum ether) has been important in their separation from other lipids.

In all of the estrogens ring A is aromatic and, consequently, the hydroxyl on carbon atom 3 is phenolic. The only other point of substitution in the hydrocarbon structure is at carbon atom 17, at which either a carbonyl or a secondary alcohol is present. If the latter is present, two stereoisomers are possible, depending on the relative position of the hydroxyl.

Two reagents have been used successfully to separate the ketonic from the nonketonic estrogens. Trimethylaminoacethydrazide chloride (Girard and Sandulesco [13a]) and carboxymethoxylamine (Wintersteiner [13b]) react with ketonic estrogens to form water-soluble derivatives. In addition to the reactions with these reagents, the ketonic compounds react with hydroxylamine, semicarbazide, acetylene and the Grignard reagents.

The phenolic hydroxyl reacts with a variety of compounds to produce esters; perhaps the acetate and benzoate are the best known. The 3, 17 dihydroxy estrogens can be converted into 3, 17 diesters, the 3-monoester or the 17-monoester. Esters of these types with several different acids have been synthesized by Miescher and Scholz.[14] The 3 benzoate and 3, 17 dipropionate of α-estradiol are used therapeutically.

Like simple phenols, the estrogens give color reactions with Millon's reagent, diazotized aromatic amines and Folin's phenol reagent. In addition, Kober [15a] has shown that under certain

13. (*a*) Girard, André, and Sandulesco, G.: Sur une nouvelle série de réactifs du groupe carbonyle, leur utilisation à l'extraction des substances cetoniques et la caracterisation microchimique des aldéhydes et cetones, Helvet. chim. acta **19**: 1095, 1936. (*b*) Wintersteiner, Oskar: Estrogenic Diols from the Urine of Pregnant Mares, in Cold Spring Harbor Symposia on Quantitative Biology, Cold Spring Harbor, L. I., New York, The Biological Laboratory, 1937, vol. 5, p. 25.

14. Miescher, K., and Scholz, C.: Ueber Ester der Follikelhormonreihe, Helvet. chim. acta **20**: 263, 1937; Ueber neue Verbindungen der Follikelhormonreihe, ibid. **20**: 1237, 1937.

15. (*a*) Kober, S.: Eine kolorimetrische Bestimmung des Brunsthormons (Menformon), Biochem. Ztschr. **239**: 209, 1931. (*b*) Cohen, S. L., and Marrian, G. F.: The Application of the Kober Test to the Quantitative Estimation of Oestrone and Oestriol in Human Pregnancy Urine, Biochem. J. **28**: 1603, 1934. (*c*) Bachman, Carl: Photometric Determination of Estrogens: A Modified Kober Reaction for Determining the Total Estrogens in a Mixture of Estrogenic Steroids, J. Biol. Chem. **131**: 455 (Dec.) 1939; A New Color Reaction for Estriol, ibid. **131**: 463 (Dec.) 1939.

conditions estrogens react with a mixture of phenol and sulfuric acid to give a red color, the intensity of which could be used for quantitative determinations. Cohen and Marrian[15b] proposed a method for the separation of estrone and estriol and determined the estrogen of each fraction by a modification of the Kober reaction. Additional modifications of the Kober reaction have been proposed by Bachman.[15c] Owing to the rapidity of execution and the accuracy of the results, it seems likely that in many instances these colorimetric analyses may displace determinations by bioassay.

The structure of the estrogens has been established through the contributions of several investigators. Butenandt, Weidlich and Thompson[16a] showed that rings A, B and C are fused together as in phenanthrene; Haworth and Sheldrick,[16b] that the hydroxyl in ring A is in the three position, and Cohen, Cook and Hewett,[16c] that the estrogens are perhydro derivatives of 1,2-cyclopentenophenanthrene having the angle methyl group on carbon atom 13 and the oxygen atom on carbon atom 17. Confirmation of the accepted views of structure has been effected by the splendid synthetic work of Bachmann and co-workers,[11a] in which a simple compound, 1-naphthylamine-6-sulfonic acid, has been converted by a series of reactions into equilenin. Since the conversion of equilenin into estrone had already been described by Marker, the structures of both estrone and equilenin are fully established.

BIOLOGIC REACTIONS OF THE ESTROGENS

As Edgar Allen[17] pointed out in the first edition of "Glandular Physiology and Therapy," the estrogens are primarily growth-promoting agents. Certain of the more important reactions will be considered in some detail. Similar to the natural estrogens, the synthetic estrogen, stilbestrol, produces almost all, if not all, of the effects of the natural estrogens.

The Vaginal Reaction.—Early work on the ovariectomized rat and mouse showed that injections of estrogen were followed by growth of the vaginal epithelium, the thickness increasing from two or three

16. (*a*) Butenandt, Adolf; Weidlich, H. A., and Thompson, H.: Neue Beiträge zur Konstitution des Follikel-hormons, Ber. d. deutsch. chem. Gesellsch. **66**: 601, 1933. (*b*) Haworth, R. D., and Sheldrick, George: Synthesis of Alkylphenanthrenes. 7-Hydroxyl: 2-Dimethylphenanthrene, J. Chem. Soc., London, 1934, pt. 1, p. 864. (*c*) Cohen, A.; Cook, J. W., and Hewett, C. L.: The Synthesis of Compounds Related to the Sterols, Bile Acids, and Oestrus-Producing Hormones. Experimental Evidence of the Complete Structure of Oestrin, Equilin and Equilenin, ibid. 1935, pt. 1, p. 445.

17. (*a*) Allen, Edgar: The Physiology of Estrogenic Principles, J. A. M. A. **104**: 1498 (April 27) 1935. (*b*) An excellent and extensive discussion of the physiology of the estrogens was given in Allen, Edgar; Danforth, C. H., and Doisy, E. A.: Sex and Internal Secretions, ed. 2, Baltimore, Williams & Wilkins Company, 1939.

to about fifteen cells.[18] The sloughing of squamous cells gave large cells with small nuclei or cornified cells typical of a response to estrogenic stimulation. This reaction has been used since 1923 in many assays of the estrogens. Without doubt the present advanced knowledge of the distribution and chemistry of the estrogens is due in large part to Edgar Allen's recognition of this reaction.

The work on vaginal smears has been extended to other species, but perhaps the most interesting development is the correlation by Papanicolaou [19a] of the change in the vaginal smear with other phenomena of the sex cycle of women. Subsequently, Papanicolaou and others [19b, c] have used the change in the smear as a guide to estrogenic therapy.

Estrogenic therapy for gonorrheal vaginitis [20] of prepuberal girls is based on the growth and thickening of the vaginal epithelium. Accompanying this growth, there is deposition of glycogen, with formation of a more acidic secretion. Biopsy specimens have shown the enormous growth that can be produced by proper medication with estrogens.

In the study of vaginal growth in animals the effects of colchicine [21] have yielded interesting results. Although this drug does not stimulate mitosis, it arrests dividing cells in the metaphase, thereby causing accumulation of many mitotic figures. This has permitted observation of the marked increase of mitosis due to the injection of estrogens.

18. Allen, Edgar; Francis, B. F.; Robertson, L. L.; Colgate, C. E.; Johnston, C. G.; Doisy, E. A.; Kountz, W. B., and Gibson, H. V.: The Hormone of the Ovarian Follicle; Its Localization and Action in Test Animals and Additional Points Bearing upon the Internal Secretions of the Ovary, Am. J. Anat. **34**: 133 (Sept.) 1924.

19. (*a*) Papanicolaou, G. N.: The Sexual Cycle in the Human Female as Revealed by Vaginal Smears, Am. J. Anat. (supp.) **52**: 519 (May) 1933. (*b*) Papanicolaou, G. N., and Shorr, Ephraim: The Action of Ovarian Follicular Hormone in the Menopause, as Indicated by Vaginal Smears, Am. J. Obst. & Gynec. **31**: 806 (May) 1936. (*c*) Werner, A. A.; Jones, Grey; Roberts, John; Broun, G. O.; Neilson, C. H., and Rothermich, N. O.: Effective Clinical Dosages of Theelin in Oil, Based on Study of Sixteen Castrate Women, J. A. M. A. **109**: 1027 (Sept. 25) 1937.

20. Lewis, R. M.: A Study of the Effects of Theelin on Gonorrheal Vaginitis in Children, Am. J. Obst. & Gynec. **26**: 593 (Oct.) 1933. Lewis, R. M., and Adler, E. L.: Gonorrheal Vaginitis: Results of Treatment with Different Preparations and Amounts of Estrogenic Substance, J. A. M. A. **106**: 2054 (June 13) 1936.

21. Allen, Edgar; Smith, G. M., and Gardner, W. U.: Accentuation of the Growth Effect of Theelin on Genital Tissues by Arrest of Mitosis with Colchicine, Anat. Rec. **67** (supp.): 49 (Dec.) 1936; Accentuation of the Growth Effect of Theelin on Genital Tissues of the Ovariectomized Mouse by Arrest of Mitosis with Colchicine, Am. J. Anat. **61**: 321 (July) 1937.

Uterine and Tubal Reactions.—The injection of estrogens produces cell division in both the epithelium and the muscular layers of the uterus. With the use of colchicine it has been possible to demonstrate clearly the increased cell division occurring in both the tubes and the uterus after injection of estrogens. In addition to the growth there is definite development of the uterine glands, with an increase in the amount of secretion in the lumens of the uterus and tubes. Markee's [22] experiments in which a piece of endometrium was transplanted to the anterior chamber of the eye furnished an opportunity to observe in the living animal the effects of injections of estrogens, ovariectomy and related procedures.

Early work indicated that the administration of liquor folliculi to ovariectomized rats produced changes in the uteri indistinguishable from those in the uteri of rats in normal estrus.[23] Since this has been confirmed, using pure crystalline estrogens, there can be no doubt that estrogens are responsible for the growth and hyperplasia observed during the estrous cycle of the rat and mouse. Moreover, Allen and others have shown that estrogens produce growth and hyperplasia in ovariectomized monkeys and that after cessation of injections bleeding occurs. This seems to be identical with the anovulatory menstruation of the monkey (Corner).

The increase in weight of the uterus following administration of estrogen was used for assay prior to the introduction of the vaginal smear. Recently, several investigators [24] have returned to the stimulation of uterine growth as a method of assay. However, it should be emphasized that with this procedure the animal can be used for only one assay.

22. Markee, J. E.: Menstruation in Intra-Ocular Endometrial Transplants in Macaca Mulatta, Anat. Rec. **64** (supp. 3): 32 (March) 1936.

23. Allen, Edgar; Francis, B. F.; Robertson, L. L.; Colgate, C. E.; Johnston, C. G.; Doisy, E. A.; Kountz, W. B., and Gibson, H. V.: The Hormone of the Ovarian Follicle; Its Localization and Action in Test Animals and Additional Points Bearing upon the Internal Secretions of the Ovary, Am. J. Anat. **34**: 133 (Sept.) 1924. Allen, Edgar: The Menstrual Cycle of the Monkey, Macacus Rhesus, Contrib. Embryol. **19**: 1, 1927.

24. Bülbring, Edith, and Burn, J. H.: The Estimation of Oestrin and of Male Hormone in Oily Solution, J. Physiol. **85**: 320 (Nov. 22) 1935. Dorfman, R. I.; Gallagher, T. F., and Koch, F. C.: Nature of Estrogenic Substance in Human Male Urine and Bull Testis, Endocrinology **19**: 33 (Jan.-Feb.) 1935.

Astwood and collaborators [25] have contributed an interesting analysis of the changes that occur in the uterus in response to the injection of estrogen. Six hours after the injection the uterine weight has increased appreciably, but the addition is due to water. Twenty-four hours later the composition of the uterus has been restored to normal, although the weight is greater than at the six hour interval.

In addition to the effects on the circulatory system and on uterine and tubal growth, estrogens have a pronounced effect in increasing uterine motility.[26]

The discrepancy between the observations of different investigators as to the effect of estrogens on pregnancy seems to have been satisfactorily explained by the experiments which showed that administration of estrogen shortly after coitus causes locking of the tubes, in rabbits and mice.[27] The fertilized ova remain in the tubes and do not continue their development.

Mammary Reaction.—Growth of both the nipple and the mammary gland is stimulated by estrogens.[28] Sand has used the development of the nipples of males as an indication of the survival of transplanted ovaries.

In several species the injection of estrogen stimulates growth of the ducts, but in the guinea pig both ducts and alveoli respond with increased development. In strains of female mice which usually acquire mammary cancer the malignant growth is prevented by early ovariectomy. Moreover, Lacassagne has shown that the males of strains in which the females are susceptible

25. Talbot, N. B.; Lowry, O. H., and Astwood, E. B.: Influence of Estrogen on the Electrolyte Pattern of the Immature Rat Uterus, J. Biol. Chem. **132**: 1 (Jan.) 1940.

26. Reynolds, S. R. M.: Action of Theelol (Tri-Hydroxy-Oestrin) on Uterine Fistulae in the Unanesthetized Rabbit, Proc. Soc. Exper. Biol. & Med. **30**: 1165 (May) 1933; Studies on the Uterus: VI. The Effect of Oestrin on the Uterine Fistula During Pseudo-Pregnancy, Am. J. Physiol. **98**: 230 (Sept.) 1931.

27. Smith, M. G.: Interruption of Pregnancy in the Rat by the Injection of Ovarian Follicular Extract, Bull. Johns Hopkins Hosp. **39**: 203 (Oct.) 1926. Levin, Louis; Katzman, P. A., and Doisy, E. A.: Effects of Estrogenic Substances and the Luteinizing Factor on Pregnancy in the Albino Rat, Endocrinology **15**: 207 (May-June) 1931. Burdick, H. O., and Pincus, Gregory: The Effect of Oestrin Injections upon the Developing Ova of Mice and Rabbits, Am. J. Physiol. **111**: 201 (Feb.) 1935. Whitney, Rae, and Burdick, H. O.: Tube-Locking of Ova by Oestrogenic Substances, Endocrinology **20**: 643 (Sept.) 1936.

28. Sand, K.: Transplantation der Keimdrüsen bei Wirbeltieren, in Bethe, Albrecht; von Bergmann, G.; Embden, Gustav, and Ellinger, Alexander: Handbuch der normalen und pathologischen Physiologie, Berlin, Julius Springer, 1926, vol. 74. Allen, Edgar, and Doisy, E. A.: The Induction of a Sexually Mature Condition in Immature Females by Injection of the Ovarian Follicular Hormone, Am. J. Physiol. **69**: 577, 1924, extensively reviewed by C. W. Turner in "Sex and Internal Secretions" (Allen, Danforth and Doisy [17b]).

to mammary cancer also show cancer in that gland if subjected to a course of estrogen injections.[29]

Reactions of the Anterior Lobe of the Pituitary.—Castration is followed by cytologic changes in the anterior lobe of the pituitary, and the cytologic changes can be reversed by administration of estrogens. In addition to the cytologic change following castration, the anterior lobe contains an increased quantity of gonadotropin.[30]

In young rats the administration of a large quantity of estrogen is followed by luteinization of the ovaries. According to one view, this is due to release of a luteinizing product of the anterior lobe of the pituitary. If the injection is continued over a longer period, the ovaries diminish in size, although the uterus and vagina show the effect of estrogenic stimulation. However, experiments have shown that the ovaries have suffered no permanent damage, since female rats which had been given daily injections for a month produced normal litters. Since this point has considerable importance in the treatment of gonorrheal vaginitis in girls, Allen and Diddle have studied the effect of prolonged treatment in monkeys. No permanent damage could be detected.[31]

29. Loeb, Leo: Internal Secretions as a Factor in the Origin of Tumors, J. M. Research **40**: 477 (Sept.) 1919. Cori, C. F.: The Influence of Ovariectomy on the Spontaneous Occurrence of Mammary Carcinomas in Mice, J. Exper. Med. **45**: 983 (June) 1927. Lacassagne, Antoine: Apparition de cancers de la mamelle chez la souris mâle, soumise à des injections de folliculine, Compt. rend. Acad. d. sc. **195**: 630 (Oct. 10) 1932. An excellent review of this topic by Leo Loeb appeared in the first edition of Glandular Physiology and Therapy: A Symposium, Chicago, American Medical Association, 1935.

30. Addison, W. H. F.: The Cell-Changes in the Hypophysis of the Albino Rat, After Castration, J. Comp. Neurol. **28**: 441, 1917. Hohlweg, Walter, and Dohrn, Max: Beziehung zwischen Hypophysenvorderlappen und Keimdrüsen, Wien. Arch. f. inn. Med. **21**: 337 (July 15) 1931. Nelson, W. O.: Effect of Oestrin and Gonadotropic Hormone Injections upon Hypophysis of the Adult Rat, Proc. Soc. Exper. Biol. & Med. **32**: 452 (Dec.) 1934. Halpern, S. R., and D'Armour, F. E.: Effects of Estrin upon Gonads, Mammary Glands and Hypophysis of the Rat, ibid. **32**: 108 (Oct.) 1934. Engle, E. T.: Effect of Daily Transplants of the Anterior Lobe from Gonadectomized Rats on Immatrue Test Animals, Am. J. Physiol. **88**: 101 (Feb.) 1929.

31. Hohlweg, W.: Veränderungen des Hypophysenvorderlappens und des Ovariums nach Behandlung mit grossen Dosen von Follikelhormon, Klin. Wchnschr. **13**: 92 (Jan. 20) 1934. Selye, Hans; Collip, J. B., and Thomson, D. L.: Effect of Oestrin on Ovaries and Adrenals, Proc. Soc. Exper. Biol. & Med. **32**: 1377 (May) 1935. Fevold, H. L.; Hisaw, F. L., and Greep, R.: Effect of Oestrin on the Activity of the Anterior Lobe of the Pituitary, Am. J. Physiol. **114**: 508 (Jan.) 1936. Hohlweg, Walter, and Chamorro, Antonio: Ueber die luteinisierende Wirkung des Follikelhormons durch Beeinflussung der luteogenen Hypophysenvorderlappensekretion, Klin. Wchschr. **16**: 196 (Feb. 6) 1937. Katzman, P. A.: A Note on the Effect of Theelin, Theelol and the Luteinizing Substance on Reproduction, Proc. Soc. Exper. Biol. & Med. **29**: 700 (March) 1932. Allen, Edgar, and Diddle, A. W.: Ovarian Follicular Hormone Effects on the Ovaries, Am. J. Obst. & Gynec. **29**: 83 (Jan.) 1935.

In addition to their effect in altering the gonadotropic output of the anterior lobe, injection of estrogen exerts a suppressing effect on the output of the lactogenic factor and indirectly on the secretion of milk.[32]

Effect on Calcium.—Although the effect on the serum calcium of birds has been a controversial point, it seems that massive quantities of estrogens do produce a striking increase. In the mouse, Gardner and Pfeiffer [33] have shown marked disturbances in calcium metabolism, in which the pelves show resorption and the long bones show an increase in density due to the replacement of marrow by bone spicules.

Effect on Fowls.—In some varieties of birds feathering is influenced by the sex hormones. In addition to the production of female feathering estrogens produce hypercalcification of bones and an increase in serum calcium and blood lipids. Although the increase in lipids due to the injection of estrogens is very marked, the values are not so high as those observed during the laying season.[34]

BIOASSAY OF ESTROGENS

Although an additional standard for the estrogen benzoates was provided by the Permanent Commission on Biological Standardization at the last meeting of the League of Nations (1935), it was the opinion of the participants that "as rapidly and as far as possible, the production of preparations of the oestrus-producing hormones should be limited to pure preparations of the different forms of the hormone and its chemically defined derivatives or of mixtures thereof, so that the activity may be indicated in exact weights, and indications in biological units may be abandoned." [35]

In my opinion it is not feasible to assay against a standard any estrogen other than the estrogen of which the standard is composed. Only two standards have been furnished: estrone

32. Nelson, W. O.: Endocrine Control of Mammary Gland, Physiol. Rev. **16**: 488 (July) 1936. Folley, S. J.: The Effect of Estrogenic Hormones on Lactation and on the Phosphatase of the Blood and Milk of the Lactating Cow, Biochem. J. **30**: 2262 (Dec.) 1936.

33. Gardner, W. U., and Pfeiffer, C. A.: Skeletal Changes in Mice Receiving Estrogens, Proc. Soc. Exper. Biol. & Med. **37**: 678 (Jan.) 1938.

34. Juhn, Mary, and Gustavson, R. G.: A Forty-Eight Hour Test for the Female Hormone with Capon Feathers as Indicator, Proc. Soc. Exper. Biol. & Med. **27**: 747 (May) 1930. Pfeiffer, C. A., and Gardner, W. U.: Skeletal Changes and Blood Serum Calcium Level in Pigeons Receiving Estrogens, Endocrinology **23**: 485 (Oct.) 1938. Lorenz, F. W.; Chaikoff, I. L., and Entenman, C.: Endocrine Control of Lipid Metabolism in Bird: The Effects of Estrin on the Blood Lipids of the Immature Domestic Fowl, J. Biol. Chem. **126**: 763 (Dec.) 1938.

35. Report of the Second Conference on the Standardization of Sex Hormones. League of Nations Quart. Bull. Health Organ. **4**: 618, 1935.

and α-estradiol monobenzoate. The members of the Commission realized that only preparations of estrone could be assayed satisfactorily against the estrone standard and only esters against the α-estradiol monobenzoate. To illustrate the discrepancies of assay when another estrogen is assayed against the estrone standard, estriol has been compared with estrone, mice being used for the assay. If each substance is given in a single injection of oil, the estrone is two hundred and sixty-six times as potent as estriol. If each is given in aqueous solution in four equal portions at intervals of twelve hours, the estrone is four times as potent as estriol. Depending on the solvent and on the division of the dose, estrone may be four or two hundred and sixty-six times as potent as estriol.

A similar situation exists with respect to the relation existing between estrone and α-estradiol. When each is given in aqueous solution in three portions during a nine hour period, the α-estradiol is ten times as active as estrone if rats are used for the assay but only twice as potent if mice are used.

In view of the past experience that each investigator has seemed unwilling to follow the assay procedure that a previous investigator has used, it appears that it would be wise to agree that since bioassay has served its purpose in guiding chemists to the preparation of pure estrogens, it should now be discarded, the products for therapeutic use labeled in metric units and their evaluation effected by the response of patients.

Metabolism of Estrogens.—A number of investigations have shown that the natural estrogens on administration to rats and mice are much less active when given orally than when given parenterally. It seems likely that the low potency following oral administration is not due to failure of absorption but rather to the fact that through the portal circulation the absorbed substance is offered first to the liver. Estrogens are rapidly destroyed by liver brei. Moreover, it has been shown that the heart-lung-liver preparation rapidly destroys estrogen, whereas the same system without the liver has little or no destructive action. A recent report gives interesting data on the effects of various tissue slices and of brei on estradiol, estrone and estriol.[36]

After parenteral administration, only a small proportion of estrogen can be recovered from tissues or excreta.[37] Even measures to hydrolyze possible conjugated and less active forms

36. (*a*) Zondek, Bernhard: Ueber das Schicksal des Follikelhormons (Follikulin) im Organismus, Skandinav. Arch. f. Physiol. **70**: 133, 1934. (*b*) Israel, S. L.; Meranze, D. R., and Johnston, C. G.: The Inactivation of Estrogen by the Liver. Observations on the Fate of Estrogen in Heart-Lung and Heart-Lung-Liver Perfusion Systems, Am. J. M. Sc. **194**: 835 (Dec.) 1937. (*c*) Heller, C. G.: Metabolism of the Estrogens, Endocrinology **26**: 619 (April) 1940.

37. Zondek[36a] Westerfeld, W. W.: The Inactivation of Oestrone, Biochem. J. **34**: 51 (Jan.) 1940. Marker, R. E.; Rohrmann, Ewald; Lawson, E. J., and Wittle, E. L.: The Isolation of Oestranediols from Human Non-Pregnancy Urine, J. Am. Chem. Soc. **60**: 1901 (Aug.) 1938.

did not appreciably increase the recovery. Two reactions which might explain the loss merit consideration: (1) oxidation and (2) reduction. The phenolic portion of the molecule (ring A) is a point of weakness at which oxidation can take place. In the urine of nonpregnant women Marker found two inactive reduced forms, estranediol A and estranediol B. Since these compounds have not been found in pregnancy urine, Marker believes that the metabolism of estrogens in the pregnant and the nonpregnant woman follows different pathways.

Several reports indicate that progesterone influences the metabolism of estrogen. A larger proportion of the injected estrogen is recovered when the organism has been treated with progesterone. Under these conditions a larger proportion of estrone or estradiol is converted to estriol. In the hysterectomized rabbit this change does not occur, thereby indicating that the uterus plays a part in the metabolism.[38]

ESTROGENIC THERAPY

The earlier attempts at estrogenic therapy with aqueous solutions did not yield results that were entirely satisfactory. This was due in part to the limitation of the solubility of estrogens in aqueous solution and to rapid absorption and excretion. This situation was met by using neutral triglycerides, in which the estrogens are much more soluble and from which absorption occurs more slowly. At the present time some physicians are experimenting with pellet therapy, in which a compressed tablet of estrogen is introduced directly under the skin. In spite of certain obvious objections, this form of administration does insure slow continuous absorption of the estrogen over a long period.[39]

From experiments on animals it seems that vaginal suppositories should be more effective than any other form of administration if it is desired merely to produce

38. Pincus, Gregory, and Zahl, P. A.: The Biogenesis of Primary Sex Hormones: The Fate of Estrins Injected into the Rabbit, J. Gen. Physiol. **20**: 879 (July) 1937. Smith, G. V., and Smith, O. W.: Observations Concerning the Metabolism of Estrogens in Women, Am. J. Obst. & Gynec. **36**: 769 (Nov.) 1938. Smith, G. V.; Smith, O. W., and Pincus, Gregory: Total Urinary Estrogen, Estrone and Estriol During a Menstrual Cycle and a Pregnancy, Am. J. Physiol. **121**: 98 (Jan.) 1938. Pincus, Gregory, and Graubard, Mark: Estrogen Metabolism in Cancerous and Non-Cancerous Women, Endocrinology **26**: 427 (March) 1940.

39. Deansley, R., and Parkes, A. S.: Factors Influencing the Effectiveness of Administered Hormones, Proc. Roy. Soc., London, s. B. **124**: 279 (Dec. 7) 1937. Salmon, U. J.; Geist, S. H., and Walter, R. I.: Inhibitory Effect of Implanted Estrogenic Hormone Crystals upon Post-Menopause and Castration Hypophysis of Women, Proc. Soc. Exper. Biol. & Med. **43**: 424 (Feb.) 1940. Bennett, H. G.; Biskind, Gerson, and Mark, Jerome: Subcutaneous Implantation of Compressed Crystalline Theelin Pellets in the Treatment of Menopausal Cases, Am. J. Obst. & Gynec. **39**: 504 (March) 1940.

growth of the vaginal epithelium. Possibly this form of therapy is ideal for gonorrheal vaginitis in that responses other than vaginal may be restricted.[40]

Although oral therapy may be more popular with patients, it seems that physicians have generally used preparations for parenteral use. No doubt, this was due in part to the prevailing evidence that in animals the natural estrogens were twenty or more times as active parenterally as enterally and in part to the desire on the part of the physician to secure more satisfactory control of therapy. Perhaps the recent experimentation with synthetic estrogens, ethinyl estradiol and stilbestrol,[41] which are approximately as active in ovariectomized animals when given by the oral route as the natural estrogens are on parenteral administration, will lead to an increased use of oral preparations. However, this will no doubt lead to an increase in self medication.

With every woman of 40 years a prospective patient and actually a high percentage sooner or later being treated with estrogens, an experiment of tremendous magnitude is in progress. The acute undesirable reactions from ethinyl estradiol and stilbestrol which have been reported by some physicians have served as a definite warning that caution should be exercised.[42] Furthermore, there seems during the last five years to be a definite trend toward massive dosage with both natural and synthetic estrogens. Perhaps this will eventually prove to be satisfactory, but in the meantime it does not seem wise to be too incautious. Within another five or ten years any subacute damage which may have occurred will begin to make its appearance.

The increased incidence of malignant change following administration of estrogens to cancer-susceptible strains of animals serves as a definite warning. While

40. Lyons, W. R., and Templeton, H. J.: Intravaginal Assay of Urinary Estrin, Proc. Soc. Exper. Biol. & Med. **33**: 587 (Jan.) 1936.

41. A report in which the results of stilbestrol therapy in 4,507 patients are summarized may be used by the reader to review the experiences recounted by the authors of sixty-four papers (Morrell, J. A.: Summary of Clinical Reports on Stilbestrol, J. Endocrinol. **1**: 419 (May) 1941). Additional information on the present status of stilbestrol is contained in the last chapter of this volume. (Present Status of Commercial Endocrine Preparation.)

42. Selye, Hans: On the Toxicity of Oestrogens with Special Reference to Stilbestrol, Canad. M. A. J. **41**: 48 (July) 1939. Shorr, Ephraim; Robinson, F. H., and Papanicolaou, G. N.: A Clinical Study of the Synthetic Estrogen Stilbestrol, J. A. M. A. **113**: 2312 (Dec. 23) 1939. Risks of Estrogens, editorial, Lancet **1**: 461 (March 9) 1940. Kurzrok, Raphael; Wilson, Leo, and Perloff, W. H.: The Action of Diethylstilbestrol in Gynecological Dysfunctions, Endocrinology **26**: 581 (April) 1940.

it is true that the dose per kilogram of body weight is much higher than the amounts commonly used therapeutically in the human patient and that the most pronounced carcinogenic reactions are obtained in susceptible animals, still it seems unwise to ignore the possibility of accelerating or starting the growth of cancer in susceptible persons. Certainly, the treatment of patients having cancer or of patients from families with a history of cancer should be undertaken with a full recognition of the possibility of untoward results.

CHAPTER XII

CORPUS LUTEUM HORMONE

GEORGE W. CORNER, M.D.
BALTIMORE

FUNCTION OF THE CORPUS LUTEUM

Speaking broadly, the endocrine function of the corpus luteum is now well understood. The gland is a part of the mechanism of pregnancy. When an ovum begins its journey through the fallopian tube, the follicle from which it took origin gives place to the corpus luteum, and this organ thereupon delivers into the blood stream a substance, progesterone, that has the property of causing extensive development of the endometrium, preparing the uterus for the reception and nutrition of the embryo.

Action on the Uterine Mucosa.—The action of the corpus luteum on the uterus is best known in the rabbit, because that species especially has been used for experiments with progesterone. About two days after the formation of the corpora lutea or after the beginning of a course of treatment with progesterone, rapid cell division begins in the epithelium, resulting in a downgrowth of the glands and ultimately in branching so profuse that the endometrium is crowded full of glands. The cells of the surface epithelium also increase in number by mitotic division so that the surface is folded. The epithelial cells, both on the surface and in the glands, become taller and acquire a more definitely columnar arrangement, presenting finally the appearance of secretory activity.

Accompanying this intense wave of epithelial proliferation there is marked engorgement of the endometrial blood vessels. By the fifth day the cells no longer divide, presumably because they have been converted to the secretory state, and the progravid condition is well established.

Because the implantation of the embryo (which these changes serve to promote) differs considerably in different animals, the progestational proliferation

differs also in detail. In the domestic pig, for example, with its superficial and noninvasive placentation, it is only the surface epithelium of the uterine lining which comes into contact with the embryonic tissues; therefore the effect of the corpus luteum is chiefly on the surface cells.

In the human and the other primate species, on the contrary, the implantation is invasive, and the progestational proliferation consequently affects chiefly the glands and the stroma, producing the well known "premenstrual" endometrium, in which the glandular epithelial cells become very high, with frayed surfaces, and the glands are dilated and acquire serrated contours. Glycogen is deposited in the epithelium, and the connective tissue cells of the stroma begin to enlarge in preparation for decidual change if pregnancy ensues.

By the time the fertilized ovum reaches the uterus, the endometrium is therefore already in a state of heightened secretory activity, by which (presumably) nutritive substances are provided for the embryo. The nature of this favorable chemical action is not yet clearly known; the experiments of Pincus[1] suggest that glutathione is secreted into the uterus and stimulates growth of the early embryo and of the endometrium itself.

When the embryo, thus nourished by the secretory activity of the endometrium, goes on to the stage of implantation, its requirements are met by another response of the uterus under the influence of the corpus luteum. The endometrium in some way is sensitized so that it responds to the presence of the embryo by forming the maternal part of the placenta (and the decidua, in those species in which the endometrial stroma becomes deciduous). This effect is well seen in the guinea pig, in which Loeb showed long ago that even in the nonpregnant animal the presence of corpora lutea in the ovaries is sufficient to induce in the uterus a special state during which any mechanical irritation (such as the presence of a small foreign body or a scratch) will produce a mass of decidual tissue exactly as if the embryos had been there to call it forth by the stimulus of their implantation.

1. Pincus, Gregory: The Metabolism of Ovarian Hormones, Especially in Relation to the Growth of the Fertilized Ovum, in Cold Spring Harbor Symposia on Quantitative Biology, Cold Spring Harbor, L. I., New York, The Biological Laboratory, 1937, vol. 5, p. 44.

Experiments have amply shown that removal of the corpus luteum during the early part of pregnancy causes failure of implantation of the embryo; if the embryo is already implanted, removal of the corpus causes destruction of the pregnancy.

Conversely, extracts of the corpus luteum that are administered to animals castrated during early pregnancy are able to replace the missing secretion and to maintain pregnancy.

Action on Uterine Muscle.—In addition to its progestational effect on the uterine mucosa, progesterone acts also on the uterine muscle, diminishing its spontaneous contractions and thus rendering the uterus quiescent. This probably also serves to facilitate attachment of the embryo, by inhibiting movements of the uterine wall which might hamper implantation at the critical time when the embryo is settling down at its site of attachment.

Although this action of progesterone varies considerably in different species, there is good evidence that it occurs in the human species.[2] This is important in connection with therapy, for it suggests the possibility that the substance may be useful not only for its specific effects on the endometrium but also for its sedative action on irritability of the myometrium.

So far as is known at present, the corpus luteum has no useful action except in pregnancy, but in most mammals (including the human species) the gland is formed in every cycle, in anticipation (so to speak) of pregnancy, and therefore in each human cycle the corpus luteum normally acts for about two weeks, bringing about the so-called premenstrual stages of the endometrium. When it retrogresses, menstruation ensues. Thus in the adult nonpregnant human female the corpus luteum is functioning about half the time, and takes part in the general physiology of the body to that extent. In animals with less frequent cycles the proportion of time during which the corpus luteum functions is much less; obviously, therefore, it is not necessary for general well-being.

PROGESTERONE

The substance elaborated by the corpus luteum which gives the effects described was first extracted in crude

2. Reynolds, S. R. M.: Physiology of the Uterus, with Clinical Correlations, New York, Paul B. Hoeber, Inc., 1939.

form from the ovaries of swine by Corner and Allen [3] and by Hisaw, Meyer, Weichert and Fevold [4] and was partially purified by Allen.[5] Investigations by Butenandt and Westphal,[6] by Slotta, Ruschig and Fels [7] and by Allen and Wintersteiner,[8] all of whom obtained the active factor in the pure state practically simultaneously, revealed that progesterone is a crystalline steroid having the formula $C_{21}H_{30}O_2$. It is closely related to the natural estrogens and androgens.

A number of other substances, closely related chemically to the ones just mentioned, have been found to have the same effect on the uterus as progesterone, but only in much larger doses. Pure progesterone exists in two different crystalline forms, one melting at 128 C. (α progesterone) and the other at 121 C. (β progesterone). It has been produced synthetically [9] from the vegetable sterol known as stigmasterol; the material now on the market is presumably made by similar methods from this or another sterol. By general agreement of the investigators concerned, the name "progesterone" is used for the pure principle; the term "progestin" is used to indicate the whole group of substances having similar action and to describe such a factor when present in a partially purified state or when not fully identified chemically.

Progesterone is administered by intramuscular injection, in solution in a vegetable oil; it cannot be given by mouth, because either it is not absorbed from the gastrointestinal tract or it is inactivated by the processes

3. Corner, G. W., and Allen, W. M.: Physiology of the Corpus Luteum: II. Production of a Special Uterine Reaction (Progestational Proliferation) by Extracts of the Corpus Luteum, Am. J. Physiol. **88**: 326 (March) 1929.

4. See Allen, W. M.: Biochemistry of the Corpus Luteum Hormone, Progesterone, in Allen, Edgar; Danforth, C. H., and Doisy, E. A.: Sex and Internal Secretions, ed. 2, Baltimore, Williams & Wilkins Company, 1939, chap. 15, p. 901-928.

5. Allen, W. M.: Physiology of the Corpus Luteum: V. The Preparation and Some Chemical Properties of Progestin, a Hormone of the Corpus Luteum Which Produces Progestational Proliferation, Am. J. Physiol. **92**: 174 (Feb.) 1930.

6. Butenandt, Adolf, and Westphal, U.: Zur Isolierung und Charakterisierung des Corpus-luteum-Hormons, Ber. d. deutsch. chem. Gesellsch. **67**: 1440, 1934.

7. Slotta, K. H.; Ruschig, H., and Fels, E.: Reindarstellung der Hormone aus dem Corpus-luteum, Ber. d. deutsch. chem. Gesellsch.. **67**: 1270, 1934.

8. Allen, W. M., and Wintersteiner, Oskar: Crystalline Progestin, Science **80**: 190 (Aug. 24) 1934.

9. Butenandt, Adolf; Westphal, U., and Cobler, H.: Ueber einen Abbau des Stigmasterins zu Corpus-luteum-wirksamen Stoffen; ein Beitrag zur Konstitution des Corpus-luteum-Hormons, Ber. d. deutsch. chem. Gesellsch. **67**: 1611, 1934. Fernholz, E.: Zur Synthese des Corpus luteum-Hormons, ibid. **67**: 1855, 1934.

of digestion. In 1939 pregneninolone, a substance closely related to progesterone, was found to be effective when given by mouth.[10a] This drug is now available commercially. It has been used to a limited extent in the therapy of various ovarian dysfunctions but has yet to be proved satisfactory.[10b] In some cases of dysmenorrhea it has been of some benefit.

In castrated rabbits progesterone readily produces progestational changes in the uterus; in rabbits castrated during early pregnancy it will protect the embryos by substituting for the product of their mother's corpora lutea and will maintain the pregnancy to full term. In guinea pigs it sensitizes the uterus to produce the maternal part of the placenta (Loeb's deciduoma).

In castrated women the premenstrual state of the endometrium can be produced by administering large doses of estrogen to bring the uterus back to its normal interval stage and then progesterone to effect the premenstrual changes. In monkeys progesterone has been shown to inhibit menstruation; but if a course of treatment with progesterone is given to the normal animal, or to a castrated animal suitably prepared with estrogen, menstruation inevitably follows discontinuance of the progesterone. Similar effects have been seen in human subjects. There is a considerable amount of evidence which indicates that progesterone is intimately involved in the metabolism of estrogens. Progesterone, according to Pincus and Zahl and the Smiths and their associates, prevents the excessive destruction of estrogens and facilitates a conversion of estrone to estriol in the rabbit and in the human being. This relationship is of special interest in studies of toxemias of pregnancy, in which the Smiths and their associates have demonstrated that the deficiency of progesterone is responsible for the imbalance of estrogens. On the basis of this work the administration of progesterone together with estrogens has been used in the experimental treatment

10. (*a*) Hohlweg, Walter, and Inhoffen, H. H.: Pregneninolon, ein neues per os wirksames Corpus luteum-Hormonpräparat, Klin. Wchnschr. **18**: 77 (Jan. 21) 1939. Clauberg, C., and Uestün, Ziya: Menstruation—per os erzeugt, Zentralbl. f. Gynäk. **62**: 1745 (Aug. 6) 1938. Emmens, C. W., and Parkes, A. S.: Some Biological Properties of Anhydro-Hydroxy-Progesterone, (Ethinyl Testosterone), J. Endocrinol. **1**: 332 (Nov.) 1939. (*b*) Soule, S. D.: Anhydro-Hydroxy-Progesterone in Dysmenorrhea, J. Clin. Endocrinol. **1**: 567 (July) 1941. Hamblen, E. C.; Cuyler, W. K.; Pattee, C. J., and Axelson, G. J.: Endocrine Therapy of Functional Meno-Metrorrhagia and Ovarian Sterility; Oral Use of Anhydro-Hydroxy-Progesterone and Estrogens, J. Endocrinol. **1**: 21 (March) 1941.

of toxemias of pregnancy and prophylactically in the treatment of pregnant diabetic women.

International Unit; Assay.—In 1935 a conference under the auspices of the League of Nations adopted an international standard of potency, which was defined as the amount of progestational activity present in 1 mg. of progesterone. This international unit has been generally adopted. The assay is usually performed by administering the preparation to be tested for five days to an adult female rabbit that has been mated and then castrated, or to an immature rabbit of about 600 Gm. weight which has been primed with five to ten daily doses of estrogen preceding the course of treatment with the test preparation. The progestational effect on the endometrium is estimated microscopically from sections of the uterus. Before the League of Nations standard was set up, various investigators were making use of other units. The Corner-Allen unit, based on use of the adult rabbit for assay and commonly cited in the American literature, is approximately equal to the international unit; the Clauberg unit, based on the immature rabbit, represents about 0.6 international unit.

Pregnandiol.—Endocrinologists owe to Venning and Browne [11] the demonstration that pregnandiol, a substance chemically related to progesterone and already known to exist in the urine of pregnant women, is actually derived from progesterone and represents the latter's excretion product. Pregnandiol as it appears in the urine is conjugated with glycuronic acid to form a water-soluble compound, sodium pregnandiol glycuronidate. Since each molecule of pregnandiol represents one molecule of progesterone, the amount of the former substance recovered from the urine in a given period gives at least a rough measure of the functional activity of the corpus luteum, although losses during excretion and during chemical manipulation, amounting perhaps to 50 per cent, prevent an exact measure of the progesterone output.

In the normal menstrual cycle pregnandiol appears in the urine about twelve days before the onset of menstruation (i. e., a day or two after ovulation). The excretion reaches a peak about one week before menstruation and usually ceases two or three days

11. Venning, E. H., and Browne, J. S. L.: Isolation of a Water-Soluble Pregnandiol Complex from Human Pregnancy Urine, Proc. Soc. Exper. Biol. & Med. 34: 792 (June) 1936.

before the onset of bleeding. The curve of pregnandiol excretion thus closely parallels the rise, activity and retrogression of the corpus luteum. From several investigations [12] one learns that the daily output at the peak, though variable, is of the order of 5 mg. of pregnandiol daily, and this may be conjectured to mean that the corpus luteum when most active produces something like 10 mg. of progesterone per day.

In pregnancy the output of pregnandiol begins to rise about the seventieth to the ninetieth day and in the ninth month may reach 60 to 100 mg. daily.[13] It is generally agreed that the large amount of progesterone indicated by these figures is not made by the corpus luteum but by the placenta.

CLINICAL POSSIBILITIES

Progesterone is now available in the drug trade, and the price, though still high, has been reduced sufficiently to make its use possible. Progesterone therapy is still, however, in the stage of experiment, and all the physician can do at present is to follow with caution the lead of the more critical clinical investigators as they progress. The clues given by its known effects on the endometrium and myometrium have naturally suggested its use in recurrent and threatened abortion, in dysmenorrhea, in after-pains of the early puerperium and in so-called functional uterine bleeding.

Recurrent Abortion.—Since the corpus luteum, as already shown, plays an essential part in promoting the growth and implantation of the early embryo and is necessary for maintenance of pregnancy during its earlier period, it is obvious that failure of the corpus luteum may be at times the cause of abortion. Such a result might be due to failure either of the progestational

12. (*a*) Venning, E. H., and Browne, J. S. L.: Studies on Corpus Luteum Function: II: Urinary Excretion of Sodium Pregnandiol Glucuronidate in the Human Menstrual Cycle, Endocrinology **21**: 711 (Nov.) 1937. (*b*) Hamblen. E. C.; Ashley, Catharine, and Baptist, Margaret: Sodium Pregnandiol Glucuronide: The Significance of Its Excretion in the Urine, ibid. **24**: 1 (Jan.) 1939. (*c*) Wilson, R. B.; Randall, L. M., and Osterberg, A. E.: Studies on Pregnandiol, Am. J. Obst. & Gynec. **37**: 59 (Jan.) 1939. (*d*) Stover, R. F., and Pratt, J. P.: Progestin Studies: Pregnandiol Excretion, Endocrinology **24**: 29 (Jan.) 1939. (*e*) Müller, H. A.: Die Pregnandiolausscheidung im Harn als Spiegelbild der Funktion des Corpus luteum, Klin. Wchnschr. **19**: 318 (April 6) 1940.

13. (*a*) Smith, G. V., and Smith, O. W.: Estrogen and Progestin Metabolism in Pregnant Women, Am. J. Obst. & Gynec. **39**: 405 (March) 1940. (*b*) Browne, J. S. L.; Henry, J. S., and Venning, E. H.: The Significance of Endocrine Assays in Threatened and Habitual Abortion, ibid. **38**: 927 (Dec.) 1939. Wilson and others,[12c]

("premenstrual") secretory reaction of the endometrium or to nonoccurrence of the quiescent state of the endometrium induced by progesterone or to both. Evidence has been obtained by Browne, Henry and Venning [13b] from estimations of pregnandiol excretion that in certain cases spontaneous abortion is indeed associated with diminished function of the corpus luteum. In such cases the condition should be relieved by treatment with progesterone. It is also possible that progesterone might be useful in preventing spontaneous abortion from other causes; by its property of inhibiting uterine motility it might act to reduce crampy uterine contractions no matter what their cause. There have been a number of favorable reports on progesterone therapy for recurrent abortion. Obviously, the results must be considered critically, for it is never possible to know in any individual case what would have happened without therapy. Statistical criteria for judging the results have recently been laid down by Malpas [14] and by MacGregor and Stewart.[15] Women who have aborted spontaneously three times are statistically almost certain to abort again if they become pregnant; such patients afford a real test of therapeutic effects, and if pregnancy is maintained, it is probable that the treatment was useful. The latest investigators rather cautiously agree that progesterone is a valuable agent in such cases.[16] Treatment should be begun as soon as pregnancy is suspected. Not less than 1 mg. daily is to be given, and preferably more, up to 5 or even 10 mg. If, as seems likely, the placenta supplies the hormone after about the third month, progesterone treatment is probably unnecessary thereafter.

Threatened Abortion.—It has been observed repeatedly that the cramps and bleeding which indicate a threatened abortion may subside after the injection of 1 mg. or more of progesterone, probably because this substance inhibits uterine motility and causes growth of the endometrial tissue. In such cases, if viability of the fetus is indicated by clinical signs and a urinary test for pregnancy (Aschheim-Zondek, Friedman) is positive, the patient should be put at rest and given progesterone

14. Malpas, Percy: A Study of Abortion Sequences, J. Obst. & Gynaec. Brit. Emp. 45: 932 (Dec.) 1938.

15. MacGregor, T. N., and Stewart, C. P.: Investigation of Cases of Recurrent Abortion and Their Treatment with Progesterone, J. Obst. & Gynaec. Brit. Emp. 46: 857 (Oct.) 1939.

16. Browne and others.[13b] MacGregor and Stewart,[15]

in ample dosage (not less than 1 international unit daily, and if possible, 5 or even 10) until the symptoms subside. After this, 1 unit daily may be given until it is thought safe to let the patient get up; thereafter less frequent doses are probably indicated until late in pregnancy. If symptoms recur, more intensive treatment may be resumed.

The advice to use relatively large doses, given in the foregoing paragraphs, follows the trend of sound clinical and laboratory experience and agrees, moreover, with the known rate of normal secretion by the human corpus luteum (see foregoing section on pregnandiol; during the third month, the most critical period for abortion, the normal patient is probably producing 15 to 40 mg. of progesterone.) Clinical results from very small doses, representing fractions of 1 unit per day, have been reported. It is perhaps possible that such small doses may at times serve by a trigger-like action or by redressing a delicate imbalance, but in the present state of knowledge 1 mg. per day is surely the least quantity that could conceivably affect the human body. There has never been any report of harmful effects from progesterone, in animals or women, even with large doses.

Dysmenorrhea.—Investigators have no satisfactory and comprehensive explanation of the cause of primary dysmenorrhea, and in all probability the condition is not a single clinical entity. The use of progesterone has sometimes been based on special hypotheses about endocrine factors in dysmenorrhea and sometimes on the hope that the substance may relieve pain by its property of reducing the irritability of the myometrium. Results must be judged critically, in view of the great importance of psychic and other general factors in such conditions. It is, however, reliably reported that a few sufferers from primary dysmenorrhea have received striking benefit and that about 25 to 50 per cent have been definitely helped by progesterone. Treatment should begin three or four days before the expected onset of menstrual pain. One or 2 international units per day may be given at first, but if necessary the dose should be pushed up to 5 units or more before abandoning the experiment.

A thorough, well controlled study of this question is greatly to be desired.

After-Pains.—As shown by Lubin, Clarke and Reynolds,[17] pain due to uterine contractions following parturition is promptly relieved by 1 international unit of progesterone. No harmful effects have been reported, although theoretically at least there is a possibility of harm from relaxation of the uterus.

Metrorrhagia and Menorrhagia; Amenorrhea.—In the normal menstrual cycle the corpus luteum is present during the second half of the interval; the menstrual flow begins when the corpus luteum degenerates. Administered progesterone in normal monkeys postpones natural menstruation, and in castrate monkeys suppresses the artificial menstruation produced by estrogen deprivation.[18] Progesterone has therefore been tried therapeutically in metrorrhagia and menorrhagia, and favorable results have been reported. In the special case of bleeding associated with glandular hyperplasia of the endometrium, another physiologic reaction may play a part. This disease is generally considered, on experimental grounds, to result from overaction of estrogenic hormones on the endometrium; progesterone may act beneficially either by cutting down the effectiveness of the estrogens, as it seems to do in the normal cycle,[18] or by producing changes of the premenstrual type and thus in some way counteracting the tendency to bleeding. Until one knows exactly why the uterus bleeds in normal menstruation and in functional uterine disease, one can hardly offer a more precise explanation of therapeutic hopes or results.

For the present the treatment of functional bleeding must be left to specialists in gynecologic endocrinology for cautious experimentation after skilled clinical analysis of each case. Because of the danger that abnormal bleeding may be due to a benign or a malignant tumor, the general physician should not administer progesterone for uterine hemorrhage.

As to amenorrhea, endocrinologists know as yet little about the hormonal relations of such disturbances of the cycle. Here again the use of ovarian products is still quite experimental and must be worked out by clinical specialists.

17. Lubin, Samuel; Clarke, F. J., and Reynolds, S. R. M.: The Relation of After-Pains to Uterine Contractions Following Administration of Progestin, Am. J. Obst. & Gynaec. **33**: 143 (Jan.) 1937.

18. Corner, G. W.: The Ovarian Hormones and Experimental Menstruation, Am. J. Obst. & Gynec. **38**: 862 (Nov.) 1939.

Other Conditions.—Now that progesterone is becoming generally available, it is inevitable that it will be tried, both rationally and irrationally, for all sorts of diseases; such efforts are to be received with caution. Already there are reports of its use in the treatment of certain types of rhinorrhea, arthritis and disturbances of the thyroid. "Corpus luteum therapy" for hyperemesis gravidarum, once attempted with orally administered glandular preparations but long abandoned, is being revived with progesterone. Serious attempts are being made to relieve toxemias of pregnancy, especially eclampsia, with this substance, though apparently thus far without statistically better results than can be achieved by conservative general treatment.

EMPIRIC PREPARATIONS

A number of corpus luteum preparations are still on the market in tablet form for oral administration or in aqueous solution, which are not assayed for progesterone and contain at most very small amounts of this factor. The methods of preparation or of administration are often such as to remove or to destroy the progesterone; if these preparations contain any other useful substance, it is something unknown to science. Their use is quite empiric, and they will no doubt become obsolete now that genuine assayed progesterone-containing products are available.

CHAPTER XIII

MENSTRUATION

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In the human species, menstruation is the only overt manifestation of rhythm in the female reproductive system. In other primates there may be additional and even more obvious indications of cyclic changes. Corner [1d] and Edgar Allen [1e] have recently summarized the evidence that the rhythm is maintained by a delicate balance of endocrines. We are not dealing with "an all or none" phenomenon but with a balanced system of forces which changes as a whole when any one factor changes. The oscillations of the system may have a high amplitude or they may become almost imperceptible. The system is accordingly variable in its manifestations. This is generally conceded; the difference of opinion is largely a matter of the range of variability that is to be regarded as normal. Many clinicians are unwilling to regard a cycle as normal unless its primary purpose is achieved; i. e., unless a fertilizable egg is produced. The fundamental controlling mechanism is nevertheless the same whether the optimal conditions which result in ovulation are achieved or not. In other words, there may be bleedings at regular intervals which are clinically indistinguishable from menstruation although the ovary has not responded to the extent of producing a ripe egg.

Physiologic extravasations of blood are a unique feature of the mammalian uterus. Before a scientific treatment of abnormal "functional" bleedings is possible it will be necesary to analyze the mechanisms which control normal bleedings. The analysis requires controls that can be applied only in experiments with animals. The problem seems at present to be centering

1. Markee, J. E.: Analysis of Rhythmic Vascular Changes in Uterus of Rabbit, (*a*) Am. J. Physiol. **100**: 374 (April) 1932; (*b*) Anat. Rec. **55** (supp.): 66 (March 25) 1933; (*c*) Contrib. Embryol. **27**: 223, 1940. (*d*) Corner, G. W.: The Ovarian Hormones and Experimental Menstruation, Am. J. Obst. & Gynec. **38**: 862 (Nov.) 1939. (*e*) Allen, Edgar: Physiology of the Ovaries, J. A. M. A. **116**: 405 (Feb. 1) 1941.

about the utero-ovarian vascular system, and some consideration of the vascular changes is definitely indicated in experiments with estrogen, progesterone and similar substances.[1c]

In the human species and in certain monkeys three normal types of endometrial bleedings may be seen:[2b]

1. Menstrual bleeding. This recurs at intervals of three to five weeks and is associated with rapid involution and some necrosis of the endometrium.

2. Implantation bleeding (placental sign of Long and Evans). This occurs soon after implantation of the ovum, in many mammals. In women and macaque monkeys it appears about four weeks after the last period,[2a,c] and when it involves an external show of blood it may be indistinguishable clinically from menstruation. In such cases the onset of labor is a month earlier than expected from the history.

3. Preovulatory bleeding associated with hyperemia. This is the familiar estrous bleeding of the dog and cow; in primates it is the rather uncommon "intermenstrual" bleeding (mittelschmerz). It has been seen in the macaque at about the time of ovulation and after large doses of estrogen.[1c]

The second and third types have received far less attention than they deserve.

The liberal definition of menstruation given here is justified by the finding that the ovaries and the uterus may present a very different appearance during successive periods in the same individual[1c] and at the same stage in different individuals. In the macaque there have now been observed a large series of transitions from the maxima of the breeding season to the minima of summer. There may be extreme hyperplasia and hypertrophy of the uterus associated with an active corpus luteum in one cycle. These processes may be much less obvious in the next cycle; occasionally it is impossible to tell from the endoemtrium whether or not ovulation has occurred. There are anovulatory cycles during which the endometrium grows more than in certain ovulatory cycles.[1c] In such cases it is necessary

2. Hartman, C. G.: (*a*) Uterine Bleeding as an Early Sign of Pregnancy in Monkey (Macacus Rhesus) Together with Observations on Fertile Period of Menstrual Cycle, Bull. Johns Hopkins Hosp. **44**: 155 (March) 1929; (*b*) Homology of Menstruation: New Observations of Intermenstrual Bleeding in Monkey, J. A. M. A. **92**: 1992 (June 15) 1929. (*c*) Contrib. Embryol. **23**: 1, 1932; (*d*) Endocrinology **25**: 670 (Nov.) 1939; (*e*) Endocrinology **26**: 449 (March) 1940.

to have serial sections of both ovaries to prove the absence of recent corpora lutea. In other words, ovulatory and anovulatory menstruation are not distinct types; cycles with much ovarian activity grade off imperceptibly into those with little. The "staircase phenomena" of Hartman [2c] gave us the first clear evidence of this.

At present, anovulatory cycles appear to be rare in women whose periods are "regular." [3] This may be due in part to the difficulty of demonstrating them. Usually it is necessary to have both ovaries removed early in a menstrual period which appeared as expected. Such cases are rare. Inspection of ovaries at operation and of fragments from endometrial biopsy specimens often lead to error, for the conditions in ovary and uterus may vary within wide limits, with transitions from ovulatory to anovulatory cycles, as the study of the macaque has abundantly shown.[2d] There are few who can diagnose an active corpus luteum histologically. The incidence of anovulatory cycles will not be known until physicians have a sure sign of ovulation which can be readily applied to large groups of women for twelve or more consecutive cycles.

Ovulation occurs a variable number of days after the onset of a menstrual period, and the duration of the cycle is likewise variable, both in different individuals and in the same individual at different times. Consequently, when one tries to puzzle out the succession of changes in a "typical" cycle, the menstrual history is of suggestive value only. Hitherto knowledge of the sequence of events has come from series of specimens arranged in an order that seemed logical to the investigator. Human ovaries have only occasionally been available, and it has been possible to exclude almost any given case as abnormal if the critic so desired. A large number of macaque uteri has now been studied, and one can say with assurance that the cyclic changes are fundamentally the same as in man.[4] One can therefore accept the recent observations of Markee[1c] as applicable to human conditions with but little modification. He has launched a new attack on the cycle by observing living endometrium transplanted to the anterior chamber of the eye in macaques. He could

3. Rock, J.; Bartlett, M. K., and Matson, D. D.: Am. J. Obst. & Gynec. 37: 1 (Jan.) 1939.
4. Corner, Hartman and Bartelmez: Unpublished data.

thus observe the same mucosa with a microscope day by day or minute by minute for many cycles in normal unanesthetized animals, and his experience has been extensive enough (432 menstrual cycles) so that he can speak with authority as to the essential features of the cycle. It is significant that the onset and duration of menstruation in these bits of endometrium coincide with the same events in the animal's uterus with its intact musculature. The observations agree with conditions observed in histologic sections, substantiating and extending them, but they do vastly more than this. They give one direct visual evidence of the actual sequence of events and the first satisfactory quantitative data on the same endometrium during a cycle and in different cycles. They exclude the nervous system as a significant factor in the control of the cycle. They furnish the basis for an analysis which includes all kinds of normal cycles. Markee calls it the "growth" rather than the "menstrual cycle." It may be subdivided as follows:

1. Postmenstrual phase of relative inactivity, which may vary in length from nothing to many days or even weeks.

2. Primary growth phase, which may or may not be associated with a growing follicle. The end of this phase is usually marked by a brief halt or even a regression in growth. The cause of this remains to be investigated.

3. Secondary growth phase, which reaches a maximum in the presence of an active corpus luteum. The endometrium may double in thickness, partly as a result of increasing edema.

4. Phase of involution. This is associated with a reduction in blood flow to the mucosa, and at the end there may be superficial congestion, leukocytosis and infiltration. The edema fluid is resorbed, and the involution may reach 60 per cent. Sooner or later there is persistent blanching of the surface due to constriction of the arteries which supply the surface.

5. Phase of bleeding. The constricted arteries open up, often one by one; almost at once there is extravasation, followed by bleeding from the surface and then by desquamation of the superficial zone.

6. Repair phase. This involves reestablishment of active circulation, rejuvenescence of the endometrium and restoration of the surface epithelium.

The vascular system of the endometrium plays a major role in this cycle. The peculiar coiled arteries are one of the most distinctive features of the primate endometrium. They pass with few or no branches in myometrium or basal mucosa to supply the superficial capillary bed [5] and are "end arteries." [6] At the beginning of the first growth period they are coiled in the mucosa basally. Capillaries pass out from them to the superficial reorganizing zone, which is rapidly increasing in thickness. The arterial channels gradually differentiate in this capillary bed and slowly approach the surface. Later the arteries grow more rapidly than the surrounding tissue, so that they become coiled throughout their length. Under the influence of the active corpus luteum they eventually differentiate as far as the surface epithelium.

The basal region of the endometrium has an independent supply of blood derived from small arteries and from anastomoses with the capillary bed of the myometrium. There is no sharp line of demarcation between this zone and that supplied by the coiled arteries. The drainage from the mucosa is by venules, which are predominantly radial, but with numerous anastomoses, which may enlarge into sinuses. Certain venules pass, without anastomoses, from the superficial to the deep veins of the endometrium. Lymphatics have been described, but their presence in the mucosa has not yet been proved.

Markee's [1b,c] observations on transplants show that there may be much variability in a given endometrium even in successive ovulatory cycles. His descriptions provide the basis for the following résumé of a typical cycle.

During the first three weeks the coiled arteries alternately dilate and constrict, so that as one looks at the surface it slowly "blushes" and then "blanches," a cycle occupying one to one minute and a half. After the second growth phase is well under way the vascular rhythm is occasionally disturbed, and in general the color of the transplant becomes paler. Presently the

5. Daron, G. H.: Am. J. Anat. **58**: 349 (March) 1936.
6. Jones, H. O., and Brewer, J. I.: Am. J. Obst. & Gynec. **38**: 839 (Nov.) 1939.

blood almost stops flowing through the dilated superficial vessels. Leukocytes begin to migrate into the stroma; the transplant is turning blue and manifestly shrinking. There is a loss of water.[7] With this involution, the tortuous arteries show buckling. These events herald the onset of menstruation. In the course of the next day one coiled artery after another clamps down, and for many seconds at a time not a single blood corpuscle moves in the superficial zone, although basally the blood is flowing freely. After several hours an artery here and there opens up, and almost at once blood pours out into the tissue from opened capillaries or arterioles. In the course of a few minutes a subepithelial hematoma develops, it soon ruptures, and dark blood slowly streams out over the surface. This blood does not clot. Necrosis sets in at the surface, and the ends of the coiled arteries are sealed by dead cells. Then fissures appear at the edges of blood-soaked areas; as they extend down into the tissue, small fragments are slowly loosened from the surface. An artery that has bled is not likely to bleed again during this flow, but if the animal is frightened or annoyed, the necrotic end may break and spout blood for minutes at a time. This blood promptly clots. Such conditions probably obtain in various metrorrhagias.[8]

At intervals during the next two days or so other arteries begin to bleed, and so the process is repeated, each time in a different region. By the third or fourth day the surface has become denuded and ragged; coiled arteries and glands project out from it, and veins are open. Slowly a little regurgitated blood oozes from an open vein for a period of many minutes. The wound surface looks clean, and most cells seem normal. Presently cells begin to migrate out in all directions from torn glands, and in two hours the surface epithelium is practically restored. During all the period of bleeding the circulation of blood has continued basally; now it is accelerated. Then the coiled arteries begin to develop capillary sprouts, which anastomose, and blood flows through them. The rejuvenation of the superficial zone is now under way, and a new growth cycle has begun.

7. Van Dyke, H. B., and Ch'en, G.: Am. J. Anat. 58: 473 (March) 1936.
8. von Mikulicz-Radecki, F.: Zentralbl. f. Gynäk. 53: 258 (Feb. 2) 1929.

THE MECHANISM OF MENSTRUATION [9]

It is not yet possible to enumerate all the hormonal factors concerned in the control of the menstrual cycle. The interactions of anterior hypophysis and ovary are essential [1d,e] but other endocrines may normally be involved. So far as the mechanism which brings about bleeding is concerned it would appear from what has been said that the question can be narrowed down to the factors which control the coiled arteries of the endometrium.

The hormone withdrawal hypotheses [9a] are fundamentally similar to the corpus luteum failure hypothesis of R. Meyer. That is, they attribute the menstrual changes to the elimination of a hormone or hormonal complex. It is now possible to say that ovarian hormone withdrawal initiates the characteristic premenstrual and menstrual behavior of the coiled arteries.[1c]

Various observations indicate that it is not the elimination of a specific hormone but an abrupt reduction in hormonal level which produces this type of bleeding. These data may be summarized as follows:

1. Hisaw [9f] found that a reduction of 50 per cent in the amount of estrogen administered to a spayed macaque results in menstrual bleeding. This has been abundantly confirmed and elaborated.[10] Markee [1c] has clearly demonstrated the importance of an abrupt change.

2. The thresholds in these reactions are vague and variable although it has been shown that in a spayed animal a definite minimal amount of estrogen is necessary to maintain the endometrium in a condition that will permit withdrawal bleeding.[11]

3. In keeping with this variability is the fact that various insults to the ovary such as unilateral ovariectomy or even resection may produce menstrual bleeding.

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9. The mechanism of menstruation is discussed at length by (*a*) Allen, Edgar; Hisaw, F. L., and Gardner, W. V., in Allen, Edgar; Danforth, C. H., and Doisy, E. A.: Sex and Internal Secretions, Baltimore, Williams & Wilkins Company, 1939. (*b*) Brewer, J. I.: Am. J. Anat. **61**: 429 (Sept.) 1937. (*c*) Corner.[1d] (*d*) Engle, E. T., and Smith, P. E.: Endocrinology **25**: 1 (July) 1939. (*e*) Hartman.[2b] (*f*) Hisaw, F. L.: Am. J. Obst. & Gynec. **29**: 638 (May) 1935. (*g*) Allen.[1e] (*h*) Hamblen, E. C.: Endocrine Gynecology, Springfield, Ill., Charles C. Thomas, Publisher, 1939.

10. (*a*) Corner, G. W.: Am. J. Physiol. **113**: 238 (Sept.) 1935. (*b*) Engle, E. T.: Am. J. Obst. & Gynec. **38**: 600 (Oct.) 1939. (*c*) Allen, Hisaw and Gardner.[9a] (*d*) Corner.[1d] (*e*) Engle and Smith.[9d] (*f*) Hisaw.[9f]

11. Engle, E. T.: Am. J. Obst. & Gynec. **38**: 600 (Oct.) 1939.

Similarly a profound systemic disturbance such as transection of the spinal cord initiates it.[12] As the level of the transection plays a part, it may be that the nerve supply of the ovary is involved in the reaction.

4. Bleeding during the course of treatment with estrogen may perhaps involve periodic hormone reduction through changes in renal thresholds.[13]

5. The reaction is not specific for any one hormone; the effects of estrogen, progesterone and androgen withdrawal are similar.[14]

The objection has been raised that the estrogen titer in the circulating blood may be high premenstrually and during menstruation [15] and the premenstrual fall in estrogen excretion does not necessarily militate against this. It is true that quantitative determinations in the blood hormones are fraught with many uncertainties, and some authors discard the findings on this basis; but it is always possible that in these cases there was a premenstrual fall from a still higher level.

Other factors besides ovarian hormones appear to be concerned in the production of menstrual bleeding. Markee [1c] showed that the effect of "estrogen deprivation" is essentially local. After spaying an animal which had transplants in both eyes, he administered estrogen, which brought about the typical vascular rhythm and endometrial growth. Then he inserted a crystal of estradiol into one eye and stopped the subcutaneous injections of estrogen. The transplants in the other eye began to shrink and in due time menstrual bleeding occurred there and in the uterus. But in the eye that had the crystal, growth continued, and no menstrual changes were to be seen. After the end of the experimental menstruation the injections of estrogen were resumed, and growth was seen in all transplants. When the crystal was removed, menstruation was induced only in the treated eye, while the estrogen level in the circulating blood was sufficient to maintain growth in the transplants of the other eye.

Markee [1c] has presented evidence that as soon as regression has set in, the menstrual process is con-

12. Van Wagenen, G.: Am. J. Physiol. **105**: 473 Aug.) 1933.
13. Zuckerman, S.: Proc. Zool. Soc., London. s. B **123**: 441, 1937.
14. Fluhmann, C. F.: Menstrual Disorders: Pathology, Diagnosis and Treatment, Philadelphia, W. B. Saunders Company, 1939. Corner.[2a] Engle.[10b] Allen.[9a]
15. Fluhmann, C. F.: Endocrinology **20**: 318 (May) 1936. Fluhmann.[14]

trolled within the endometrium itself. His hypothesis is that continued regression produces progressive buckling of the coiled arteries even in cycles with feeble growth. This retards the flow of blood superficially to such an extent that the mucosa is injured. As a result the tissue produces a vasoconstrictor to which the sphincters of the coiled arteries [5] are highly susceptible. They clamp down, and the resulting stasis brings on menstruation.

It may be that the reduction in hormonal levels activates a specific bleeding mechanism. The hypophysis as the source of such a factor seems to be ruled out.[11] Markee [1c] reported greater irregularity in the blush and blanch rhythm during the second growth period, and this supports the idea [16] that the same specific factor responsible for the rhythmic blanching also produces the protracted premenstrual blanching. In such a situation regression would be initiated when a definite threshold was reached. So far the hunt for a specific vasoconstrictor has failed. Brewer [17] presented evidence for a general vascular spasm premenstrually and suggested a substance resembling epinephrine as the agent. He attributed the premenstrual and menstrual changes to the gradual increase of such a vasoconstrictor in the blood.[18] Such a hypothesis assumes that in pregnancy the activated corpus luteum antagonizes the vasoconstrictor to such an extent that extravasation is confined to the region already damaged by the invading ovum.

Why should there be a periodic weakening of the superficial endometrium and an outpouring of blood? The most satisfactory explanation is that the process is an adaptation to provide nourishment and a foothold for the fertilized ovum during the period when the placenta is being established.[19]

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16. Bartelmez, G. W.: Contrib. Embryol. **24**: 141, 1933.
17. Brewer, J. I.: Am. J. Obst. & Gynec. **36**: 597 (Oct.) 1938.
18. Brewer, J. I.: The Menstrual Cycle, in Blumer, George: The Practitioner's Library of Medicine and Surgery, New York, D. Appleton-Century Company Inc., 1940.
19. Bartelmez, G. W.: Physiol. Rev. **17**: 28 (Jan.) 1937. Corner.[1d] Hartman.[2b] Brewer.[9b]

CHAPTER XIV

OVARIAN DYSFUNCTIONS AND THEIR TREATMENT

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The ovaries have three essential functions: First, they produce ova, the germ cells. Second, they elaborate or metabolize estrogenic substances, which play a primary role in the development of the female secondary sex characters and in the maintenance of the accessory genital organs. Third, in them is formed corpus luteum, an ovarian structure which appears at cyclic intervals and produces a hormone, progesterone, which is indispensable for reproduction.

Ovarian dysfunctions must be regarded as manifestations of deficiency or hyperactivity of one or more of these phenomena, but with the present knowledge a classification in terms of clinical syndromes is impossible. For this reason it is best to discuss the subject not on the basis of ovarian pathologic conditions but indirectly from evidences of gonadal disturbance obtained from the study of disorders of menstruation. It must be granted that there are disadvantages in doing this, since one must deal with symptoms rather than with disease entities, but it is the method of approach used by an overwhelming majority of clinicians. The only exceptions which may be made are in cases of a few fairly well defined clinical entities, such as precocious development, complete absence of ovarian function and metropathia haemorrhagica.

OVARIAN DYSFUNCTION DURING THE PREPUBERAL AND PUBERAL PERIODS

The first sign of active ovarian function is the development of the secondary sex characters. The earliest evidence appears at about the age of 8 years, when the breasts begin to develop and estrogenic substances become demonstrable in the blood and urine. The most striking manifestation, however, is the onset of the first menstrual period, the menarche. In this country, the average age for this event is between 13½ and 14½

years, and in the majority of instances it occurs between the ages of 10 and 16 years. The appearance of menstruation before the age of 9 is therefore regarded as precocious maturation and after the age of 16 as "delayed onset of the menses" or "primary amenorrhea."

1. *Pubertas Praecox.*—In true precocious menarche all the events of the normal physiologic process are reproduced. The secondary sex characters develop, there is an enhanced rate of body growth and the menses appear and recur at cyclic intervals. Of especial significance is the fact that the menstrual cycles are complete, with ovulation and corpus luteum formation, so that the subjects are able to become pregnant at an early age. In this category belong instances of gestation in girls from 6 to 12 years of age. The exact etiology of such a disturbance is not known. It must be of endocrine origin, but the only demonstrable abnormality is the appearance of normal physiologic phenomena at an earlier age. Obviously, no therapy is indicated, but a careful investigation of all such patients must be made to make sure that they do not belong to the second subgroup of this division.

2. *Pseudo Pubertas Praecox.*—The majority of instances of so-called precocious menarche in the literature are not true "pubertas praecox" but represent a definite pathologic entity. In such cases the secondary sex characters develop, the pelvic organs enlarge and uterine bleeding occurs. These changes, however, merely represent an estrogenic effect. Ovulation does not take place, there is no formation of corpus luteum and pregnancy is not possible. Following suitable treatment, there is a regression of these changes and uterine bleeding ceases, but if at least one ovary is present they recur later as part of the physiologic menarche.

The cause is found in tumors which produce excessive amounts of estrogen, and, although attention has been directed to lesions of the anterior lobe of the hypophysis, thymus and pineal gland, the only clear instances have been associated with tumors of the ovaries. The treatment consists of surgical removal of the new growths, which have been described as granulosa cell tumors, sarcomas, teratomas and carcinomas.

3. *Delayed Menarche and Primary Amenorrhea.*—These terms are employed synonymously, or an arbi-

trary distinction is made between them, according to the age of the patient. In the latter case, delayed menarche is applied to girls between the ages of 16 and 18 years and primary amenorrhea to those beyond the eighteenth year. Both terms refer to a delay in the appearance of the first menstruation, which often is accompanied by various disturbances in other changes of the adolescent period. The secondary sex characters are often imperfectly developed or absent. The breasts remain small, and there is no hair, or only a scant amount, over the pubic and axillary regions. There may be but little deposition of fat over the hips, shoulders and thorax. Of importance is the fact that the deficiency of ovarian function leads to an imperfect development of the genital organs, especially noteworthy in the case of the uterus, which persists in an "infantile type." At times alterations in body growth are striking, and the most characteristic finding is that the patients are tall and thin, with long lower extremities and span in proportion to the length of the trunk.

Many causes have been suggested for primary amenorrhea, and attention must be directed particularly to hereditary and constitutional factors, systemic disease, environment and malnutrition. It is likewise probable that primary deficiencies of the endocrine glands, notably the anterior lobe of the hypophysis, the thyroid and the gonads, are frequently responsible.

The treatment of primary amenorrhea has proved very disappointing. In recent years estrogenic and gonadotropic substances have been widely employed in the manner described in the section dealing with secondary amenorrhea. In spite of many favorable reports, the most successful approach probably lies in the employment of suitable dietary and hygienic measures, and, whenever clinical signs and a basal metabolic test so indicate, the use of thyroid substance.

SECONDARY AMENORRHEA

Under the term secondary amenorrhea are included instances of cessation of menstruation in women who possess one or both ovaries and an endometrium capable of normal function. The period of amenorrhea may vary from a few weeks to several years. When it becomes permanent in younger women it may be considered as a "premature menopause."

In many cases the exact mechanism concerned with amenorrhea is not clear, but the focal point of the disturbance is always the ovaries, which fail to undergo a normal cycle. This may be due to some influence exerted on the gonads directly or indirectly through interference with the gonadotropic function of the anterior lobe of the hypophysis.

There are many causes of functional amenorrhea. The occurrence of normal menstruation is dependent on general good health, and failure to menstruate may be the first indication of a systemic disease. It may occur in almost any form of ill health and is found in many instances of malnutrition, intoxications and wasting diseases such as tuberculosis and diabetes mellitus. Although the reproductive organs are primarily under the control of the endocrine glands, the glands themselves are subject to the autonomic nervous system, and various emotional disturbances may lead to amenorrhea. It is well known that shock, fright, fear, worry, sexual disharmony, changes of work, climate or environment, surgical operations and certain mental diseases frequently cause a cessation of menstruation. On the other hand, the majority of amenorrheic patients suffer from a primary endocrine disturbance. A small number of these present a definite condition, such as deficiency of the anterior pituitary lobe due to tumor growth or constitutional defect, hyperthyroidism, adrenal new growth or ovarian deficiency. In many cases the exact ductless gland at fault is not discernible with certainty, as the amenorrhea is not associated with clearcut clinical manifestations; these cases present a very difficult diagnostic problem.

The treatment of secondary amenorrhea primarily demands the employment of general measures and the eradication of any organic lesion which may be responsible for it. It is a symptom which calls for extensive investigation, and attention must be directed to the existence of systemic disease, endocrine disorder, psychic disturbance, malnutrition or an unfavorable living or occupational environment. If such a factor is discovered, it necessarily follows that the cure is altogether dependent on the rectification of the condition at fault.

Among general measures, special attention must be given to personal hygiene. Proper and sufficient food is necessary, and it may be advisable to supplement

the diet with vitamins and minerals. When amenorrhea exists with obesity an attempt must be made to reduce the patient's weight. A low basal metabolic rate requires the daily administration of suitable doses of thyroid substance. The patient also should take enough exercise in the open air, and at times an investigation of working hours and living conditions may give a clue to the correct therapeutic approach.

The specific treatment of amenorrhea must be directed toward a restoration of a complete ovario-uterine cyclic activity. Recent advances in knowledge of the mechanism of the menstrual cycle have shown conclusively the uselessness of emmenagogues and procedures which lead merely to an abnormal uterine hemorrhage.

The employment of low dosage irradiation of the ovaries has been championed by a number of authors both in Europe and in this country but has failed of wide clinical application owing to the individual variations in the narrow margin between safe and harmful dosages, and also because of the possibility of damage to the germ plasm which may manifest itself in later generations. Irradiation of the hypophysial area alone, in the hope of enhancing this gland's gonadotropic function, also has been advocated, but the results are not convincing.

The appearance of active biologic preparations of estrogenic substances was at once hailed as a possible cure for amenorrhea, and many favorable reports regarding their usage have been published. In some instances ridiculously small dosages have been employed, such as 100 to 500 international units taken by mouth. In others, a total of as much as 500,000 to 1,000,000 international units has been administered by intramuscular injections of from 10,000 to 50,000 international units two or three times a week. Many patients bleed cyclically during treatment or following each course of injections, but the infrequency of a permanent restoration of menstruation arouses a doubt as to the validity of many published reports. The employment of 5 mg. of diethylstilbestrol by mouth daily also may produce bleeding a few days after its discontinuance. Some authors justify this therapy on the ground that it may result in the growth of an atrophic uterus (temporary?). It is important to remember that the use of estrogenic substances is purely substitutive

therapy. Estrogens have no direct effect on the ovaries, and in the endometrium they merely stimulate growth and do not produce secretory changes, which is a function of the corpus luteum. Nevertheless, estrogens are widely used in the treatment of amenorrhea, and further judgment must remain *sub judice.*

The logical substance for the treatment of functional amenorrhea should be capable of stimulating the ovaries to normal function. For this reason attention is directed particularly to anterior pituitary or equine gonadotropins, but this method of approach is still in an experimental stage.

An international standard for equine gonadotropin has only recently been established, and there is none for hypophysial gonadotropin. Recent attempts with such substances have been inadequate, but attempts nevertheless are made by giving short courses of high doses of gonadotropin at intervals of a month. It is not possible to give any recommendations regarding dosage at the present time because of the lack of consistency in the available commercial preparations, but each manufacturer makes his suggestions for the use of his specific product.

Of importance is the fact that the employment of chorionic gonadotropin is not indicated. There is no evidence that this substance can lead to a normal development of graafian follicles in women. The only exception to this statement is that it may be used with the hypophysial follicle-stimulating principle with the object of enhancing the gonadotropic effect of the pituitary factor.

POLYMENORRHEA AND OLIGOMENORRHEA

The menstrual cycles of normal persons are subject to considerable variation in length, but at times there is such a marked shortening or lengthening of consecutive cycles that attention is directed to them as evidence of some disorder.

Polymenorrhea.—This condition is characterized by a shortening of the average length of the menstrual cycle. The usual course of events goes on both in ovaries and in endometrium, but the cycles last only from fourteen to twenty-five instead of the average thirty days. It is often stated that this abnormality is brought about by a hyperhormonal condition, that is by overactivity of the ovaries. This contention is open to

question, and on the basis of Schroeder's studies it is more likely that it results from an ovarian deficiency which manifests itself by a premature disintegration of the corpus luteum, thus leading to a shortening of the postovulatory phase of the cycle.

Although it is the least common type of menstrual irregularity and is found in women who are normal in all other ways, polymenorrhea is an important symptom pointing to a pathologic condition. It is found frequently in the preclimacterium or in ovarian deficiencies associated with hypothyroidism, and it may have a psychogenic origin and result from fear of pregnancy or infection or from one of various sex perversions. Most often it accompanies organic pelvic disease, especially pelvic inflammation, fibromyoma uteri or uterine retrodisplacement. In such cases the shortened cycles must be attributed to vascular disturbances which directly affect the ovaries.

In most instances polymenorrhea does not call for specific therapy, as it results from some systemic or pelvic disorder. Whenever there are no apparent local or general causes, treatment is very discouraging. The endocrine factors concerned are not clearly understood, and hormonal therapy has not proved effective. It is said that cycles may be prolonged by administering large doses of estrogens, such as from 50,000 to 100,000 international units, during the early part of the cycle, but this effect is not permanent.

Since menorrhagia frequently accompanies polymenorrhea, treatment should be directed primarily to this complication. Of importance also is the establishment of a correct diagnosis, since the occurrence of too frequent menses may be confused with the hemorrhage of metropathia haemorrhagica and midinterval (ovulation) bleeding.

Oligomenorrhea.—This condition is a prolongation of the menstrual cycle beyond average limits. It is closely related to amenorrhea, and it is difficult to differentiate between the two. A woman who menstruates at intervals of three or four months may be regarded as having either oligomenorrhea or recurring periods of amenorrhea.

Treatment of oligomenorrhea is not always justified, since this symptom may be compatible with normal good health. In certain instances, for example when

it is associated with sterility, it may be desirable to institute therapy, and in this case the problem should be approached exactly along the lines given for secondary amenorrhea.

HYPOMENORRHEA AND HYPERMENORRHEA

Although there is a marked variation in the length of successive menstrual cycles in women, there is a much greater constancy in the duration of the menses and the amount of loss of blood in each case. For this reason, any increase or decrease in the amount of blood lost during menstruation is of importance as indicative of a pathologic condition, but the exact role of the ovaries in such disturbances is not definitely understood.

Hypomenorrhea.—This term refers to a diminution of the amount of flow or a lessening of the duration of the menses. It is not a very frequent finding in cycles of average length. The cause, or causes, are difficult to determine, but generally it is attributed to endocrine disorders which lead to a functional ovarian deficiency. At times, also, it occurs from an endometrial deficiency, such as follows a subtotal hysterectomy with a resultant diminution of the total area of the uterine mucosa, or a partial destruction of the endometrium by injudicious curettage, chemical treatments or extensive infections.

This symptom often accompanies oligomenorrhea, and the most important indication for treatment is its occurrence in association with sterility. Endocrine therapy is directed, as in amenorrhea, toward either (1) stimulating the endometrium with estrogenic substances or (2) attempting to influence the ovaries with gonadotropic preparations. In either case the results have not been promising.

Hypermenorrhea, or Menorrhagia.—These terms are applied to the occurrence of a succession of unduly profuse or prolonged menstrual periods following complete ovulatory cycles. A flow of more than seven days' duration should be considered abnormal. Menorrhagia may be found with cycles of average length and is a frequent accompaniment of polymenorrhea. It is an important complication, as it may bring about serious consequences, even to the point of endangering the life of the patient.

It is probable that endocrine disorders may be etiologic factors, but it is difficult to determine the exact

mechanism by which profuse menses are produced. Theoretically, an excessive activity of normal ovarian function resulting in overgrowth of the endometrium prior to menstruation may result in excessive bleeding. This state of affairs is seen in some instances of hypothyroidism. As a matter of fact, menorrhagia in the vast majority of instances is observed in women with an organic pelvic disease, for example, chronic salpingitis, fibromyoma uteri, adenomyoma, subinvolution of the uterus, endometritis or a systemic condition, such as one of the various blood dyscrasias.

In all such cases, the treatment must depend on first combating the effect of the recurring hemorrhages by blood transfusions, the administration of liver extract and iron and a suitable dietary regimen. Whenever a systemic or local disturbance is noted it must be met with suitable therapeutic measures. In the total absence of apparent causative factors, local or general, various endocrine preparations may be employed, but the results are not as hopeful as they are in metropathia haemorrhagica. The use of the chorionic gonadotropin or of progesterone (as for metropathia haemorrhagica) has been widely advocated, but the results are favorable only in a limited number of instances. In many cases thyroid substance has proved of value, even in women with normal basal metabolic rates. Many other procedures have been advocated, with varying degrees of success, such as administration of solution of parathyroid, of moccasin snake venom or of insulin and irradiation of the spleen. Irradiation with roentgen rays or radium finds its greatest value in the treatment of women nearing the menopausal age, and for younger women the final resort is hysterectomy. In young or old, the possible existence of a carcinoma demands careful diagnostic precision, but a malignant condition of the uterus is not often found in instances of regular cyclic uterine hemorrhage.

ANOVULATORY MENSTRUATION

At certain times "menstruation" occurs without preceding ovulation, corpus luteum formation and secretory changes in the endometrium. It should be regarded as a type of ovarian deficiency, since it results from failure of ovulation to take place. Clinically, the patient has periods of bleeding indistinguishable from ovulatory menstruation, so that the diagnosis must be made from an examination of the endometrium.

The frequency and clinical significance of anovulatory menstruation in women are not determined, but they are possibly factors in some cases of sterility. If treatment seems desirable, the logical approach is to stimulate follicle growth with either anterior pituitary or equine gonadotropin. The gonadotropin should be given early in the cycle, a few days after the cessation of the menses.

METROPATHIA HAEMORRHAGICA

The term metropathia haemorrhagica is applied by Schroeder to a definite endocrine entity characterized by specific changes in the ovaries and endometrium and clinically of importance because it leads to profuse uterine bleeding.

The disease is found at all ages but is especially prone to occur shortly after the menarche and in the preclimacterium. The uterine hemorrhage may manifest itself in several ways. It may be periodic in occurrence and hence simulate menorrhagia. Frequently the bleeding occurs irregularly, or it may become continuous, appearing daily for weeks or months. In a considerable number of instances it sets in after a period of amenorrhea, in which case it may be confused with threatened or incomplete abortion.

In metropathia haemorrhagica the ovaries may be slightly enlarged and contain from a few to many cysts representing various stages of follicular atresia, the most characteristic feature being an absence of corpora lutea. There is thus a failure of ovulation in these patients, so that the endometrium is constantly stimulated by estrogens in the absence of the progesterone influence. The same result is obtained in the presence of granulosa cell tumor of the ovary.

The uterine mucosa reflects this abnormal stimulation from the ovary. It is usually thickened and edematous, and the surface has irregular prominences. Histologically, the normal cyclic changes are lacking, and it presents the abnormal development known as "hyperplasia of the endometrium."

The treatment of metropathia haemorrhagica consists in the first place of treating the resultant anemia with suitable means, such as the administration of iron and liver extract and the use of blood transfusions. Of the specific procedures to control the bleeding, five of them call for especial consideration: 1. Chorionic

gonadotropin may be given in large daily doses, such as from 500 to 1,000 international units, intramuscularly for from six to ten days, beginning with the onset of bleeding. The Council on Pharmacy and Chemistry of the American Medical Association recently held that the efficacy of this therapy is not clearly established, but many observers have reported favorable immediate results in as many as 75 per cent. of their cases. It is most effective in younger persons, but, although it may control the bleeding, recurrences are experienced at varying intervals. 2. Injections of progesterone also may be employed, from 1 to 3 international units being given daily until the bleeding is controlled. This substance often controls the bleeding in a dosage insufficient to stimulate definite secretory changes in the endometrium. Since this substance has no direct effect on the ovaries, it is not curative. 3. Although it must be regarded as an experimental procedure, the use of testosterone propionate is worthy of consideration and has given some encouraging results. It may be given by intramuscular injection in amounts of 25 mg. in oil twice weekly until a total of 250 to 400 mg. has been administered. There is some danger of inducing masculinizing effects when higher doses are employed. 4. Some authors claim that the administration of large doses of estrogen may suppress the bleeding temporarily, but such treatment is still on an experimental basis. 5. The operation of curettage is at times resorted to as an adjunct to endocrine therapy when there is a marked proliferation of the mucosa and polyp formation. It is also a valuable diagnostic procedure to eliminate the possibility of a coexistent carcinoma. 6. Roentgen and radium irradiation are indicated for patients in the climacteric because they do not respond readily to endocrine therapy. Radium is preferable, since a diagnostic curettage can be done at the time of its application. From 1,200 to 1,500 milligram hours in the uterine cavity has been found suitable. 7. Hysterectomy is the last resort in cases of uncontrollable metropathia haemorrhagica in young women, in which it is desired to avoid the castration produced by irradiation.

TOTAL ABSENCE OF OVARIAN FUNCTION

A complete loss of ovarian function follows surgical removal or destruction by irradiation of both gonads and is a sequence of the physiologic menopause. There

are many individual variations in the reaction to ovarian deprivation, and in some instances it produces numerous local and systemic disturbances.

The lack of ovarian stimulation immediately results in cessation of menstruation and in atrophy of the accessory genital organs. The breasts likewise undergo involution, and with the removal of the inhibitory influence of the ovaries there is an overproduction of the anterior pituitary gonadotropin, which appears in great amounts in the blood and urine. Estrogenic hormones are still demonstrable in the blood and urine after castration or the menopause, a fact which speaks for an extragonadal origin for these substances.

In most cases the loss of the ovaries produces few or no symptoms requiring therapy, but at times a long train of disturbing elements enter into the picture. The most frequent subjective symptom is "hot flushes," a result of vasomotor instability. Their incidence is usually placed at between 60 and 90 per cent of all cases. There also may be tachycardia, palpitation, dyspnea, choking sensations, insomnia and headaches. A certain number of women at the climacteric have an increase of blood pressure, but many authors attribute this condition to cardiovascular or renal disease, which is especially prevalent in this age group. At times, various psychoses may develop in unstable persons. The libido may undergo no change, although usually it is diminished. Hirsutism is occasionally found, and obesity frequently results.

The first step in the treatment of a patient with symptoms resulting from castration or the menopause is to direct attention to her general health. She should be encouraged to take plenty of exercise, preferably outdoors, and sufficient restful sleep. Hot baths or cold showers may be employed to advantage. A full life with vital interests, diversions and recreation should be encouraged. A suitable vacation or change of climate is often of great benefit. A correct diet is essential, and in the presence of obesity it should be controlled. Of importance is the patient's mental attitude, and the number and the intensity of the nervous and psychic manifestations of the climacteric may be greatly influenced by a helpful talk with a sympathetic physician. Women with intense psychic manifestations may require psychiatric supervision. In many cases mild sedatives, such

as bromides or phenobarbital, are of distinct value in controlling nervousness, insomnia and vasomotor symptoms.

The specific measures employed in the treatment of symptoms of the climacteric aim at a direct control of endocrine changes, and the most important is the administration of estrogenic substances. They have been instrumental in bringing relief to a high percentage of patients with vasomotor and nervous symptoms. Many authors advocate the continued employment of small doses, such as from 50 to 200 international units orally two or three times a day, increasing gradually until relief is obtained.[1] In cases of severe symptoms it is better to give short concentrated courses of estrogens by intramuscular injection. For instance, from 5,000 to 50,000 international units in oil may be given every three or four days for periods varying from two to five weeks. By this means relief is readily obtained, but it may be necessary to repeat such courses of treatment at intervals of a few months.

The usage of the synthetic hormone diethylstilbestrol has aroused considerable interest recently, and 0.5 mg. and 1.0 mg. given by mouth daily will relieve a large percentage of patients with climacteric symptoms. It is also effective by percutaneous administration or in the form of suppositories for local application. Physicians are warned that disagreeable reactions develop in 10 to 20 per cent of the patients after the administration of this substance, principally nausea, vomiting, dizziness and headaches.

Estrogenic substance performs another valuable service in the treatment of symptoms resulting from atrophy of the accessory genital organs, especially "senile vaginitis." In such cases, the substance is used in the form of vaginal suppositories containing 2,000 international units which can be inserted each evening by the patient herself.

Recently a number of authors have demonstrated that administration of the androgen testosterone is also effective in controlling postcastration and postmenopausal symptoms. This method has as yet been insufficiently studied, and two objections are apparent: First, the available preparations are very expensive and,

1. Some authorities favor giving a preliminary course of intramuscular injections. When relief is obtained, the estrogen is administered orally, and the daily dose is diminished until a "maintenance level" is determined.

second, overdosage may lead to undesirable masculinizing effects, such as facial hirsutism, enlargement of the clitoris and a change of the voice.

PREMENSTRUAL TENSION

During the premenstruum many women experience nervousness, irritability, tendency to cry, malaise, cold sensations, nausea or abdominal distention. In some instances these symptoms are markedly accentuated and cause patients to seek relief. Although psychic factors often are in the background, many authors believe that the symptoms result from an endocrine disturbance. Frank gave to this state the appropriate name of "premenstrual tension," and the term is now generally used in medical literature. Although no conclusive evidence in support of this concept had been advanced, he attributed the condition to an excess of estrogen in the blood and advocated the employment of magnesium sulfate in order to eliminate the hormone as speedily as possible.

The wide variety of therapeutic agents advocated for this distressing condition is a warning against premature explanation of its causation. If excess of estrogen is the cause, the administration of estrogenic substances should intensify the symptoms. This is often the case, but at times such a method of treatment brings relief. Some investigators administer progesterone, others testosterone, by intramuscular injection. Of especial interest is the suggestion made recently that premenstrual tension results from the generalized edema which is so often an accompaniment of this stage of the menstrual cycle. In order to offset this effect, it has been suggested that patients be placed on a salt-free diet and take 0.6 Gm. of ammonium chloride three times daily for two weeks before an expected period.

INTRINSIC DYSMENORRHEA

Pelvic pain occurring just before or at the time of menstruation in the absence of gross lesions has been termed intrinsic, primary, idiopathic, essential or functional dysmenorrhea. It is frequently complained of, but the exact incidence is not known, owing to the difficulty of interpreting the subjective symptom "pain" in different persons. There are many conflicting reports on the subject.

Intrinsic dysmenorrhea is essentially a disease of the third decade, but it may persist throughout menstrual life. It may have its inception at the menarche or after a few years of painless menstrual periods. The most common type of pain is sharp, cramping and intermittent, and is referred to the midline in the lower part of the abdomen, extending at times to the back or thighs. It is often accompanied by headache, backache, general pain, nervousness, irritability, nausea, vomiting, joint pains or gastrointestinal disorders. The time at which the pain begins varies considerably. It may start a few hours before the onset and continue during menstruation, or it may be present in the premenstruum and cease when the menses appear. In still other instances the patient suffers only during the flow. The duration is from a few hours to several days.

This subject is treated in this chapter in order to complete the general discussions included in the book "Glandular Physiology and Therapy," but this must not be interpreted as an admission that intrinsic dysmenorrhea has been proved to be ovarian dysfunction. The immediate factor producing dysmenorrhea is held to be a disordered or spasmodic contractility of the uterine musculature, but the basic cause is unknown, although many theories have been advanced. For instance, it has been suggested that intrinsic dysmenorrhea is the result of a congenital obstruction of the cervical canal, of purely psychogenic factors, of hypoplasia of the uterus, of overactivity of the abdominopelvic sympathetic nerve, of painful separation of a decidual cast or of constriction of the uterine arteries. There is likewise no lack of speculation regarding the role of the endocrine glands. It has been said that dysmenorrhea is caused by a deficiency of estrogens, an excess of estrogens, a lack of progesterone, an excess of progesterone, thyrotoxicosis, hypoglycemia and calcium deficiency.

In keeping with the vast array of theories advanced for the cause of dysmenorrhea are the countless therapeutic measures which have been recommended. It is impossible to record them all in this brief review, but mention may be made of some specific endocrine measures at present under investigation. In each instance favorable results have been reported, but the whole problem must be regarded as still in an experimental stage.

1. Estrogenic hormones are used in the belief that they may serve to overcome uterine hypoplasia and degenerative changes in the cervical ganglions and to produce dilatation of the endometrial blood vessels. Several methods of administration have been described. These substances have been given by mouth in daily doses of 100 to 500 units during the whole menstrual cycle. Some authors give intramuscular injections of 10,000 to 50,000 international units during the premenstruum. Still other investigators recommend the injection of 5,000 to 15,000 international units every three to four days over a period of three months, but such high dosages may disturb the cycle, lengthening it from one to three weeks.

2. The trial of progesterone is warranted because it has an inhibitory effect on the contractility of the uterine muscle. The minimal effective dose has not been ascertained, but ¼ to one or even more international units may be given intramuscularly two or three times a day, beginning a few days before an expected menstrual period. The advent of an orally effective progestin-like substance, pregneninolone, also presents possibilities, but no reports of well controlled studies are available.

3. Of especial interest have been the recent reports on the employment of testosterone propionate. This substance is given by intramuscular injection in doses of 10 or 25 mg. twice or three times a week over a period of one to two months. One wise recommendation limits the total dosage to 200 mg. given during one menstrual cycle and 150 mg. during the second.

4. The employment of chorionic gonadotropin was in vogue for a short time, but it is probably of no value beyond psychotherapeutic effects.

CHAPTER XV

OVARIAN TUMORS OF ENDOCRINE NATURE

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Not so many years ago the general concept of tumors was that they are collections of cells which have cut themselves off from all functional activity, living in a purely parasitic way. It is known now that this is not invariably true and that a considerable degree of functional activity may be retained by the cells of certain tumors, especially those of highly differentiated type. In the case of neoplasms of the endocrine glands such a persistence of function has long been recognized, probably because its manifestations are more striking, involving tissues and functions far removed from the tumor site itself. Many examples of functional endocrine tumors are now adducible as producing well established clinical syndromes. Among these may be mentioned adenoma of the eosinophilic, basophilic or chromophobic cells of the anterior lobe of the pituitary and certain tumors of the thyroid, parathyroid and adrenal (both cortex and medulla) glands and of the gonads, both male and female.

This article will concern itself only with certain functioning tumors of the ovary, long recognized as one of the endocrine glands of the body. The two ovarian hormones are estrogen and progesterone, produced by the follicle and the corpus luteum, respectively. Of these, estrogen may be considered the more fundamental, since it is responsible for the characteristic female secondary sex characters in addition to its role in menstruation and in pregnancy. Progesterone, on the other hand, plays an important role in the progestational portion of the cycle and in gestation itself. The fact, therefore, that certain ovarian tumors are characterized by estrogenic activity would at once suggest an origin from some phase of follicle development, as is indeed the case. The occasional cases in which progesterone effects are produced by ovarian tumors would in the

same way suggest the lutein cell as the source of the hormone, as it normally is, in the ovary at least. In addition to these, however, it will be seen that certain alien cells which may occur in the ovary may give rise to still other hormones, such as the androgenic principles and thyroxine.

The ovarian tumors which have been shown to possess endocrine activity are (1) granulosa cell carcinoma, (2) thecoma, (3) luteoma, (4) arrhenoblastoma, (5) adrenal ovarian tumors and (6) the so-called struma ovarii or thyroid tumor of the ovary. All of these, with the exception of the last two, are looked on as of dysontogenetic origin, arising from abnormalities in the early stages of gonadogenesis. For an appreciation of their endocrine effects, and even more for the interpretation of their varied histologic structure, some understanding of these early stages in the development of the ovary is absolutely essential. This subject can be set forth here in only the briefest fashion and with full appreciation of the fact that on certain points there is still considerable difference of opinion among embryologists.

At a very early embryonic phase there develops on the anterior or ventral surface of the wolffian body a grouping of cells which constitutes the anlage of the sex gland, testis or ovary, as the case may be. The first point to stress is that in this early, or undifferentiated, stage it is impossible to determine whether this nucleus of cells will differentiate as testis or as ovary. Incidentally, tumors may arise in later life from cells which in this undifferentiated phase are cut off from the germinal stream, as it were, while the surrounding cells undergo their later differentiation into ovarian or testicular structures. These tumors constitute the group designated as seminoma when in the testis and as dysgerminoma when in the ovary. The histologic structure of the male and female tumors is identical. As would be expected from their origin, they have no endocrine effect whatever.

At a later stage the cells in the gonadal area become differentiated and arranged in cords of zigzag appearance which converge toward the hilus, where in the male they link up with mesonephric structures to complete the testicular scaffolding. The cords become canalized to form the seminiferous tubules, and the wolffian duct itself becomes the vas deferens. From

the standpoint of the female, with which this article is more directly concerned, it is important to remember that the same cordlike differentiation occurs as in the male, though there is no link-up with mesonephric structures and the whole process is evanescent.

Over the fossil remains of this male type of differentiation, to put it figuratively, there then occurs a second differentiative process, the cells of the mesenchyme differentiating into cells of either granulosal or thecal type and arranging themselves in clusters about the germ cells to form the primitive follicles. In this typically female phase, however, cells may be left behind, especially in the region of the rete ovarii, which have persisted from the earlier male type of differentiation and which may therefore retain a potentiality to develop along masculine lines. It is from such male-directed cells in the ovarian medulla that, according to Meyer,[1] the masculinizing tumor, or arrhenoblastoma, has its origin. His explanation has been generally accepted, and it gives at least a satisfactory working theory of the histogenesis of these tumors. It is quite possible, however, that the real explanation goes deeper than this, for comparatively little is as yet known of the mechanism of sex differentiation. Especially suggestive is the close embryologic relationship of the ovarian medulla and the adrenal cortex, and the fact that the biologic effects of certain adrenal cortical lesions are so similar to those of arrhenoblastoma.

Robert Meyer,[1] to whom is due so much in the classification of these dysontogenetic tumors of the ovary, ascribes the origin of the second, or feminizing, group, the granulosa cell carcinoma, to masses of redundant granulosa cells or granulosa cell rests (granulosaballen) left over in the differentiative processes of early follicle formation. The evidence of recent years, however, indicates that this view must be modified. While it was formerly held that the follicular epithelium is derived from invagination of the germinal epithelium, the work of Fischel, Politzer and others has shown quite clearly that both granulosal and thecal elements are formed by differentiation of ovarian mesenchyme in situ, and these embryologic researches are borne out by studies on the experimental production of such tumors with roentgen

1. Meyer, Robert: Pathology of Some Special Ovarian Tumors and Their Relation to Sex Characters, Am. J. Obst. & Gynec. **22**: 697 (Nov.) 1931.

rays.[2] In other words, from the ovarian mesenchyme tumors may develop which morphologically are made up of either granulosal or thecal cells or of a mixture of the two. If a generic name were to be coined for this entire feminizing group, a good one would seem to be "feminizing ovarian mesenchymoma." Granulosa cell carcinoma and thecoma therefore have a common origin, and while they show certain structural differences, they produce similar feminizing effects and should therefore not be as sharply separated as they have been by some authors.

In certain granulosal or thecal tumors, either partial or complete luteinization has been noted. The folliculome lipidique described in 1910 by Lecène belongs to this group, and a considerable number of tumors of this group have been described. When the lutein transformation is complete, the tumor becomes a luteoma, and the majority of tumors coming under the latter designation are probably derived in this way. With lutein transformation of the granulosal or thecal cells, progesterone effects on the uterine mucosa may be noted. There are, however, many exceptions to this rule, and, although knowledge on this point is still very incomplete, it would seem that morphologic and functional lutein changes are not always parallel.

The subject of luteoma is further confused by the fact that masculinizing effects have been reported in association with certain tumors of this histologic group. The chief difficulty in the interpretation of such tumors has been to distinguish them from those of adrenal origin. Schiller [3] believes that, with the exception of a very few cases, the adrenal origin is the correct explanation for this masculinizing group, and with this view I agree.[4] Finally, in the group of ovarian tumors in which an origin from adrenal ovarian rests seems indubitable a masculinizing syndrome is produced which is practically identical with that brought about by arrhenoblastoma.[5]

2. Geist, S. H.; Gaines, J. A., and Pollack, A. D.: Experimental Biologically Active Ovarian Tumors in Mice, Am. J. Obst. & Gynec. **38**: 786 (Nov.) 1939. Traut, H. F.; Kuder, Alberta, and Cadden, J. F.: Study of Reticulum and of Luteinization in Granulosa and Theca Cell Tumors of the Ovary, ibid., p. 798.

3. Schiller, Walter: Zur Frage der Specificität vermannlichender Ovarialtumoren, Arch. f. Gynäk. **160**: 344 (Dec. 19) 1933.

4. Novak, Emil: Masculinizing Tumors of the Ovary (Arrhenoblastoma, Adrenal Ovarian Tumors), Am. J. Obst. & Gynec. **36**: 840 (Nov.) 1938.

5. Schiller.[3] Novak.[4]

It is obvious that knowledge of the histogenesis of the dysontogenetic functioning tumors of the ovary is still very incomplete, and hence it is not surprising that in certain cases there is difficulty, with difference of opinion as to the proper pathologic classification. A discussion of the gross and microscopic pathologic observations is beyond the scope of the present paper but may be found in many readily available papers, such as those of Schiller,[6] Novak and Brawner,[7] Novak,[4] Norris,[8] and Melnick and Kanter.[9]

CLINICAL CHARACTERISTICS OF GRANULOSA CELL CARCINOMA AND THECOMA

Granulosa cell carcinoma and thecoma may occur at any age, and the endocrine effects produced by them vary chiefly according to the age of the patient. They are not by any means uncommon, making up something like 10 per cent of all solid malignant tumors of the ovary. In the Laboratory of Gynecological Pathology of the Johns Hopkins Hospital we now have more than 80 specimens of this group. The endocrine effects are dependent on the production of estrogen by the tumor cells, as has been shown by hormone assays and by the demonstration of the estrous effects produced by injection or implantation of the tumor tissue in castrated animals.

Effects in Children.—A considerable group of granulosa cell tumors has been reported in children.[10] It is known that the development of secondary sex changes at puberty, as well as the initiation of the menstrual function, is due to the awakening of estrogenic function at that phase. The estrogenic function of a granulosa cell tumor which develops in infancy or childhood, therefore, brings about an abnormally early appearance of these puberal phenomena, constituting one group of cases, though numerically not the largest, of precocious puberty and precocious menstruation. The breasts become hypertrophic, more or less regular menstrua-

6. Schiller, Walter: Pathologie und Klinik der Granulosazelltumoren, Vienna, Maudrich, 1934.

7. Novak, Emil, and Brawner, J. N.: Granulosa Cell Tumors of the Ovary: Clinical and Pathological Study of Thirty-Six Cases, Am. J. Obst. & Gynec. **28**: 637 (Nov.) 1934.

8. Norris, E. H.: Arrhenoblastoma, Am. J. Cancer **32**: 1 (Jan.) 1938.

9. Melnick, P. J., and Kanter, A. E.: Theca Cell Tumors of the Ovary, Am. J. Obst. & Gynec. **27**: 41 (Jan.) 1934.

10. Novak, Emil: Granulosa Cell Ovarian Tumors as Cause of Precocious Puberty, Am. J. Obst. & Gynec. **26**: 505 (Oct.) 1933.

tion is initiated, genital and axillary hair growth appears, and the external genitalia assume the puberal type, with also hypertrophy of the uterus.

With removal of the tumor, these symptoms retrogress completely, a crucial demonstration of the causative role of the tumor hormones in their production. Indeed, in at least one recorded case in which such regression occurred a second reappearance of the precocious symptoms was found to be due to a recurrence of the tumor in the other ovary. Removal of the recurrent growth was followed by disappearance of the abnormal symptoms.

Effects During the Reproductive Epoch.—As might be expected, the effects of granulosa cell growths during reproductive life are far less striking than when they are projected against the bare estrogenic background of childhood. During menstrual life large amounts of estrogen are present in the circulation, and the secondary sex characters have long been present. The tumor, therefore, produces only a quantitative increase of estrogen, a "hyperestrogenism" like that found in many cases of functional bleeding and in at least some cases of amenorrhea. Menstruation may therefore be normal or excessive, or it may be absent for long periods.

Effects in Postmenopausal Life.—When such a tumor develops late in life, periodic uterine bleeding is usually observed and is interpreted by the patient as menstruation, though it is not associated with ovulation. The normal senile uterus is often hypertrophied to a size like that observed in reproductive life, and the endometrium often shows marked hyperplasia. Breast changes are not seen, presumably because of incapacity of the senile breast to respond to estrogenic stimulation as does the immature breast of the young child. Removal of the tumor not only brings about cessation of menstruation but may even be followed by vasomotor disturbances like those seen at the normal menopause (Novak; Dworzak; Schulze).

CLINICAL CHARACTERISTICS OF ARRHENOBLASTOMA

The masculinizing group of tumors is far less common than the feminizing, the total of reported cases being not over 60. As with other tumors of the dysontogenetic variety, there can be no question that the rarity

is not as great as published figures would indicate, for the reason that many pathologists and clinicians have not yet familiarized themselves with the characteristics of these neoplasms, so that the tumors often pass unrecognized.

Arrhenoblastoma occurs usually in young women and produces a train of symptoms characterized first by certain defeminization phenomena, followed by others which are definitely masculinizing. The history of a case of my own may be considered typical of the group. A woman of 35, previously of normal feminine type and with previously normal menstruation, had ceased menstruating eleven months before she was seen. There had been one full term pregnancy seven years previously. Shortly after the onset of her amenorrhea the breasts had begun to retrogress and at the time of examination were quite flat. She had lost slightly in weight, and her contour had lost the typical feminine curves. None of the thus far enumerated symptoms can be considered masculinizing. They are, however, to be interpreted as defeminizing, subtracting something from the characteristic feminine make-up.

Some months after the onset of amenorrhea, the patient noticed a rather heavy growth of downy hair over the face, extremities and abdomen, and this hypertrichosis had gradually increased. The previously high-pitched voice became deep and rough, and the patient had, in fact, been treated for many months for a supposed laryngitis. Still later the clitoris had become moderately enlarged. Pelvic examination revealed a rounded solid movable tumor about the size of a golf ball in the right ovary. Such a finding, plus the train of symptoms noted, left little doubt as to the diagnosis of arrhenoblastoma, which proved correct.

The removal of an arrhenoblastoma is followed by reappearance of menstruation, almost always about one month after operation. Other feminine characteristics, such as breast development and body contour, are soon restored. The positive masculinization phenomena are much slower to disappear, and regression is often incomplete. Hypertrichosis in some cases, as in the one described, may disappear within a few months, but the disappearance of the hair may be much slower and very incomplete. The same thing applies to the voice changes and the hypertrophy of the clitoris. If the latter is extreme, amputation may be necessary.

It should be remembered that mildly intersexual conditions of congenital origin are very common in women and that in such women simple ovarian tumors may develop in later life, possibly leading to an incorrect suspicion of arrhenoblastoma. With the latter, however, the abnormal sex phenomena occur characteristically in women who previously have been normal in this respect. The clinical test of the causal role of the tumor is the regression of the symptoms after removal of the growth. As with granulosa cell carcinoma, a double check has been possible in at least one case, in which such regression followed removal of an arrhenoblastoma, with reappearance of symptoms some years later because of a recurrence of tumor on the other side, and again disappearance of symptoms after removal of the second tumor.

ADRENAL TUMOR OF THE OVARY

The very rare adrenal tumor of the ovary is worth at least brief mention, if for no other reason than that it can produce a clinical syndrome identical with that of arrhenoblastoma. Only a small group of tumors of this type have been reported, apparently rising from adrenal tissue rests in ovaries.[11] Mention has already been made of the intimate embryologic relation between the anlagen of the adrenal cortex and the ovarian medulla, so that it is not surprising that certain adrenal tumors produce sex effects exactly similar to those of arrhenoblastoma. As already mentioned, it seems probable that most of the tumors reported as luteoma producing masculinization phenomena have probably been of adrenal origin.

THYROID TUMOR OF THE OVARY (STRUMA OVARII)

A rather rare type of ovarian tumor is the so-called struma ovarii, made up entirely or in part of thyroid tissue.[12] Such neoplasms are looked on as teratomas in which thyroid tissue has overridden and perhaps blotted out other teratomatous elements. The thyroid

11. Rottino, Antonio, and McGrath, J. F.: Masculinovoblastoma: Primary Masculinizing Tumor of the Ovary (So-Called Large Cell Variety—Hypernephroid—Luteoma), Arch. Int. Med. **63**: 686 (April) 1939. Saphir, William, and Parker, M. L.: Adrenal Virilism, J. A. M. A. **107**: 1286 (Oct. 17) 1936. Schiller.[8] Novak.[4]

12. Plaut, Alfred: Struma of Ovary, Arch. Path. **10**: 161 (July) 1930.

tissue in such tumors is quite typical histologically, and in some cases studies of the iodine content have added further confirmation on this point. Finally, in a few recorded cases the thyroid tissue of the tumor was apparently functionally active, producing clinical evidences of hyperthyroidism.[18]

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13. Moench, G. L.: Thyroid Tissue Tumors of Ovary, Surg., Gynec. & Obst. **49**: 150 (Aug.) 1929.

CHAPTER XVI

PHYSIOLOGY OF THE TESTIS

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The testicle performs two principal functions that make it the specific and primary organ of reproduction. The basic one is the formation of mature spermatozoa capable of fertilizing the egg: this is, of course, essential for the perpetuation of the race. The second function—the secretion of hormones—must be regarded as one supplementary to the formation of germ cells, since it contributes toward insuring delivery of mature spermatozoa in the proximity of mature eggs where fertilization can occur. Two concrete essentials are involved here: (1) the induction of behavioristic reactions, sometimes designated as sex drive or mating instincts, and (2) the provision of a vehicle of transportation for spermatozoa and of control of the ejaculate—either its discharge into the aquatic medium surrounding the egg in the case of lower vertebrates or its introduction into female passages by an organ of intromission. Just so far as either the formation of germ cells or the mating inclinations are defective the animal becomes deficient in its reproductive capacity; and whereas the two functions obviously cooperate and supplement each other toward the final goal, there appears to be an advantage in discussing them as though they were entirely separate.

I. SPERMATOGENIC ACTIVITY

Animals pass through variable periods in a juvenile state prior to attaining a stage of sexual maturity. Whereas at birth most systems assume their normal function, the production of germ cells is one of the last to be attained. Spermatozoa are produced during the second year of life in perhaps the majority of vertebrates, but in some they are produced both earlier and later. Thus, in the rat they make their appearance in the seminiferous tubules about thirty-five to forty days after birth; they are seen in the epididymis at approximately the fiftieth day, and the animal may successfully

inseminate females by about the seventieth to the eightieth day. In the guinea pig spermatozoa are found in the testis by about the fiftieth to the seventieth postnatal day and are first discharged in ejaculations, induced by electric stimulation on the head, from the fifty-fourth to the one hundred and sixteenth day.[1] In contrast to such early production of spermatozoa, in man mature germ cells do not occur until about the twelfth to the fifteenth year.

A great deal of variability occurs among vertebrates with regard to continuity in germ cell production. In probably the largest number of species spermatozoa are developed but once during the year and are present for relatively short periods; such animals are seasonal breeders. Others, including the rat, rabbit, guinea pig and man, which are continuous breeders, produce spermatozoa continuously.

Seasonal production of spermatozoa is quite regular throughout the classes of vertebrates; hence it must be considered the basic plan. A number of the lower vertebrates enter actively on spermatozoan differentiation in the late winter and early spring months in preparation for the usual spring breeding activity, but some produce spermatozoa in the fall months and carry them over the winter for use in the following spring. A notable exception to the ordinary course for birds is exhibited by the chicken which has been selectively cultivated for reproductive activity: Whereas in most birds the strictly seasonal activity of the testis rules, the cock, as well as the hen, manufactures mature gametes throughout the year.

Among mammals, the wild rodent of the Midwest (Citellus tridecemlineatus) has been rather carefully investigated by Wells.[2] This ground squirrel as observed in the Chicago area enters its subterranean burrow for a period of semihibernation usually in October and emerges above ground in April. Spermatogenic activity becomes evident during January, and on emergence most males have quantities of spermatozoa in the epididymis. Subsequent to the breeding season in April-May, testicular involution begins, and

1. Sayles, E. D.: Postnatal Development of Reproductive System in Male Guinea Pigs and Its Relation to Testis Hormone Secretion, Physiol. Zoöl. 12: 256 (July) 1939.

2. Wells, L. J.: Seasonal Sexual Rhythm and Its Experimental Modification in the Male of the Thirteen-Lined Ground Squirrel (Citellus Tridecemlineatus), Anat. Rec. 62: 409 (July 25) 1935.

frequently by June-July spermatozoa are absent and the testis is receding. Spermatozoa are, therefore, usually absent from about late June until March; during the low period the testicle may be one-twentieth the weight of the fully active organ.

The predominant characteristic among males of the vertebrate group as well as among the invertebrates is thus a periodic testicular activity, quite restricted to definite seasons and therefore controlled to some extent at least by environmental agencies. Definite cyclic periods, on the part of the females at least, still rule even in those forms that have become freed to a marked degree from the annual cycle; in such animals estrus cycles retain a controlled definiteness that speaks for adequate regulation but one more nearly restricted to internal control and less definitely influenced by environment. The males of these types usually show continuous testicular activity, and whether rhythms of sperm production occur in them is still to be shown. In the sparrow a definite diurnal rhythm is exhibited, in which spermatogenic divisions occur within a few hours after midnight, when the body temperature of the sleeping bird is low.[3]

The termination of germ cell production in a complete life history is an indefinite event, and it may be questioned whether in nature an animal lives to such an advanced age that spermatozoa are no longer produced. In man, on whom somewhat more exact information is available, it is well known that some males lack spermatozoa at the age of 50 years, whereas others may produce them abundantly up to the age of 90 years.[4] It is questioned whether the tendency to apply the term "senile" to men lacking spermatozoa either serves any useful purpose or represents the facts. Various organs may exhibit marked pathologic changes or atypical functions due to many different causes, and it is well known that testicular activity is in some respects a sensitive function influenced by many different bodily states.

Pituitary Factors Influencing Spermatogenesis.—The formation of spermatozoa is subject to many modifying controls. One important control, if not the most impor-

3. Riley, G. M.: Experimental Studies on Spermatogenesis in the House Sparrow, Passer Domesticus (Linnaeus), Anat. Rec. 67: 327 (Feb. 25) 1937.

4. Engle, E. T.: Male Reproductive System: Problems of Aging, Baltimore, Williams and Wilkins Company, 1939, chap. 15.

tant, is that exercised by the pituitary gland. As indicated somewhat earlier, the influence of the pituitary gland in control of the gonads was most convincingly demonstrated by Smith [5] and Smith and Engle [6] for the rat and mouse, and their observations have been abundantly confirmed on many species. Removal of the pituitary gland in the functional adult male leads immediately to involutionary changes in the testes and in the young male prevents the attainment of sperm formation. Implantations of fresh pituitary tissue or injections of a proper extract effect a satisfactory substitution and permit continuance of spermatogenic function. Prevention of the immediate spermatogenic involution following hypophysectomy has been accomplished by the administration of testis extracts, chemical androgens,[7] progesterone [8] and yeast extract,[9] but none of these substances afford reparative means if administration is delayed until the damage following hypophysectomy has occurred.

Stimulation of spermatogenesis, as evidenced by precocious formation of germ cells, has been attained by the administration of anterior pituitary materials or other gonadotropic agents in lower vertebrates,[10] in chicks [11]

5. Smith, P. E.: The Disabilities Caused by Hypophysectomy and Their Repair, J. A. M. A. **88**: 158 (Jan. 15) 1927; Hypophysectomy and a Replacement Therapy in the Rat, Am. J. Anat. **45**: 205 (March) 1930.

6. Smith, P. E., and Engle, E. T.: Experimental Evidence Regarding the Rôle of the Anterior Pituitary in the Development and Regulation of the Genital System, Am. J. Anat. **40**: 159 (Nov.) 1927.

7. Walsh, E. L.; Cuyler, W. K., and McCullagh, D. R.: The Physiologic Maintenance of the Male Sex Glands, Am. J. Physiol. **107**: 508 (Feb.) 1934. Nelson, W. O., and Gallagher, T. F.: Some Effects of Androgenic Substances in the Rat, Science **84**: 230 (Sept. 4) 1936. Nelson, W. O.: Some Factors Involved in the Control of the Gametogenic and Endocrine Functions of the Testis, in Cold Spring Harbor Symposia on Quantitative Biology, Cold Spring Harbor, L. I., New York, The Biological Laboratories, 1937, vol. 5, p. 123.

8. Nelson, W. O.: The Effect of Various Sex Hormones on the Testes of Hypophysectomized Rats, Anat. Rec. **67** (supp. 1): 110 (Dec. 25) 1936.

9. Hisaw, F. L.; Greep, R. O., and Fevold, H. L.: Pituitary-like Effects of Yeast Extracts, Anat. Rec. **67**: (supp. 1): 50 (Dec. 25) 1936.

10. Burns, R. K., and Buyse, Adrian: The Effect of an Extract of Mammalian Hypophysis upon the Reproductive System of Immature Salamanders After Metaphorphosis, J. Exper. Zoöl. **67**: 115 (Jan. 5) 1934. Forbes, T. R.: Effect of Injections of Pituitary Whole Gland Extract on Immature Alligator, Proc. Soc. Exper. Biol. & Med. **31**: 1129 (June) 1934. Turner, C. D.: The Effects of Antuitrin-S on the Male Genital Organs of the Lizard (Eumeces Laticeps) During Seasonal Atrophy, Biol. Bull. **69**: 143 (Aug.) 1935. Evans, L. T.: The Effect of Antuitrin-S on the Male Lizard, Anolis Carolinensis, Anat. Rec. **62**: 213 (June 25) 1935.

11. Schockaert, J. A.: Response of the Male Genital System of the Immature Domestic Duck to Injections of Anterior-Pituitary Substances, ibid. **50**: 381 (Oct. 25) 1931. Domm, L. V.: The Precocious Development of Sexual Characters in the Fowl by Homeoplastic Hypophyseal Implants: I. The Male, ibid. **51**: 20, 1931.

and in the seasonally active ground squirrel [12] but not in rats [13] or monkeys.[14]

The pituitary gland is thus one of the main factors in the control of spermatogenesis, and many other apparently separate phenomena in the modification of the testis appear to be secondary to the pituitary modification:

1. Seasonal testicular activity, in many respects at least, seems to be a problem of pituitary activity. In the ground squirrel the period of spermatogenic activity is a period of high gonadotropic activity of the pituitary, and the period of testicular inactivity one of low gonadotropic activity. Testes in such animals can be thrown into marked spermatogenic activity during the normal inactive period, provided pituitary materials are administered. Likewise, the marked stimulating power of light, especially on the bird, in which Rowan,[15] Bissonnette [16] and others have been able to cause production of spermatozoa in midwinter by gradually increasing the length of day by means of electric light, is principally through the eye. Benoit [17] demonstrated in the duck that light stimulates pituitary activity; hence the effect of light on the testes is only secondary, depending on pituitary activation.

2. Dietary phenomena, such as severe inanition or lack of vitamin B (the injury to the testes appears to be principally nutritional, rather than specifically related to the vitamin deficiency), induce spermatogenic involution.[18] That this is a secondary effect, due to a primary

12. Wells, L. J., and Moore, C. R.: Hormonal Stimulation of Spermatogenesis in the Testis of the Ground Squirrel, Anat. Rec. **66**: 181 (Sept. 25) 1936.

13. Moore, C. R.: Responses of Immature Rat Testes to Gonadotropic Agents, Am. J. Anat. **59**: 63 (May) 1936.

14. Engle, E. T.: Experimentally Induced Descent of the Testis in the Macacus Monkey by Hormones from the Anterior Pituitary and Pregnancy Urine, Endocrinology **16**: 513 (Sept.-Oct.) 1932.

15. Rowan, William: Experiments in Bird Migration; Manipulation of the Reproductive Cycle; Seasonal Histological Changes in the Gonads, Proc. Boston Soc. Nat. Hist. **39**: 151, 1929.

16. Bissonnette, T. H.: Experimental Modification of the Sexual Cycle in Males of the European Starling (Sturnus Vulgaris) by Changes in the Daily Period of Illumination and of Muscular Work, J. Exper. Zoöl. **58**: 281, 1931; Sexual Photoperiodicity, J. Heredity **27**: 170 (May) 1936.

17. Benoit, J.: Facteurs externes et internes de l'activité sexuelle; stimulation par la lumière de l'activité sexuelle chez le canard et la cane domestiques, Bull. biol. de la France et de la Belgique **70**: 487, 1936.

18. Moore, C. R., and Samuels, L. T.: The Action of Testis Hormone in Correcting Changes Induced in the Rat Prostate and Seminal Vesicles by Vitamine-B Deficiency or Partial Inanition, Am. J. Physiol. **96**: 278 (Feb.) 1931.

influence on the pituitary, is indicated by the low gonadotropic potency of pituitaries from such animals.[19]

3. Androgens as well as estrogens are injurious to testes, especially in young males, and the evidence is strong that the harmful influence is again secondary to inhibition of the pituitary gland.[20] Despite continued injections of harmful doses, no injury is apparent if at the same time the animal is provided with pituitary materials. Androgens administered to man induce severe reduction of sperm; recovery occurs on discontinuance of the treatment.[21]

Nonpituitary Influences.—Other agents harmful to spermatogenic activity do not clearly involve the pituitary gland:

1. Cryptorchism. Man, as well as other mammals, occasionally experiences retention of the testicle in the abdomen: Embryonic development has been imperfect to the extent that descent into the scrotum is not accomplished, but the organ shows as a defect only the lack of completed spermatogenesis.

It is now recognized that the failure in germ cell production in the undescended testis is merely the function of the higher temperature of the abdomen; the scrotum is a localized thermoregulator by virtue of its thin walls, modified skin, absence of subcutaneous fat, numerous sweat glands and ability to relax and separate the testicle from close contact with the body.[22] This conception is gained from a consideration of several different lines of evidence: (*a*) Simultaneous recording of temperatures in the abdomen and in the scrotum for rats, rabbits and guinea pigs reveals that scrotal tem-

19. Mason, K. E., and Wolfe, J. M.: The Physiological Activity of the Hypophysis of Rats Under Various Experimental Conditions, Anat. Rec. **45**: 232, 1930. Evans, H. M., and Simpson, M. E.: Subnormal Sex-Hormone Content of the Hypophysis of Animals with Inadequate Anti-neuritic Vitamine-B, ibid. **45**: 216, 1930.

20. Moore, C. R., and Price, Dorothy: Gonad Hormone Functions and the Reciprocal Influence Between Gonads and Hypophysis, with Its Bearing on the Problem of Sex Hormone Antagonism, Am. J. Anat. **50**: 13 (March) 1932.

21. Heckel, N. J.: The Influence of Testosterone Propionate upon Benign Prostatic Hypertrophy and Spermatogenesis: A Clinical and Pathological Study in the Human, J. Urol. **43**: 286 (Feb.) 1940. McCullagh, E. P., and McGurl, F. J.: The Clinical Use of Testosterone Propionate, ibid. **42**: 1265 (Dec.) 1939.

22. Moore, C. R.: Properties of Gonads as Controllers of Somatic and Psychical Characteristics: I. Testicular Reactions in Experimental Cryptorchidism, Am. J. Anat. **34**: 269 (Nov.) 1924; II. Heat Application and Testicular Degeneration: The Function of the Scrotum, ibid. **34**: 337 (Nov.) 1924. Moore, C. R., and Quick, W. J.: The Scrotum as a Temperature Regulator for the Testis, Am. J. Physiol. **68**: 70 (March) 1924.

peratures are from 1 to 8 degrees (C.) lower than abdominal temperatures. (*b*) In an adult, elevation and confinement of the active testes in the abdomen lead to complete disorganization of seminiferous tubules within five to seven days, with continual decline as long as the testes remain extrascrotal; return to the scrotum is followed by renewed formation of spermatozoa, provided the injury is not of too long standing. (*c*) A single exposure of the scrotum to a temperature 6 degrees (C.) above body temperature for a period of ten to fifteen minutes leads to degeneration of seminiferous tubules within a period of ten days; recovery of sperm formation follows if the injury has not been too severe.[23] (*d*) Insulation of the scrotum of a ram with woolen coverings, preventing the natural regulation of scrotal temperature, leads to degeneration of seminiferous tubules and loss of all sperm production. Such an animal, therefore, is sterilized by its own body heat through prevention of the function of the scrotal thermoregulatory capacity. (*e*) It has long been known that a testis graft located in any well vascularized portion of the body of any amphibian or bird will carry spermatogenesis to complete spermatozoon formation. In mammals this capacity is likewise present, subject to the limitations of adequate environmental temperature. A rat or a mouse testis graft will produce spermatozoa months after transplantation if the graft is scrotal in position or located in the anterior chamber of the eye;[24] each location provides a proper temperature.

In keeping with this principle, it is known that febrile states in man induce at least temporary loss of spermatozoa, and Mills[25] was able to correlate severity of testicular damage in soldiers dying in army camps with severity (high febrile states and duration) of disease—principally influenza and pneumonia; no specific toxicity was apparent.

2. Lack of vitamin E, a substance present in green vegetables, wheat germ and other products. This vitamin appears to be a specific requirement for sperm

23. Young, W. C.: The Influence of High Temperature on the Guinea Pig Testis: Histological Changes and Effects on Reproduction, J. Exper. Zoöl. **49**: 459 (Nov. 5) 1927.

24. Moore, C. R.: Testis Graft Reactions in Different Environments (Rat), Am. J. Anat. **37**: 351 (May) 1926. Browman, L. G.: Testicular Heterotransplantation in Rats and Mice, J. Exper. Zoöl. **75**: 283 (Feb. 5) 1937. Turner, C. D.: Intra-Ocular Homotransplantation of Prepuberal Testes in the Rat, Am. J. Anat. **63**: 101 (July) 1938.

25. Mills, R. G.: The Pathological Changes in the Testes in Epidemic Pneumonia, J. Exper. Med. **30**: 505 (Nov.) 1919.

production, since in its absence, though good nutritional states are maintained, sperm production fails.[26] The action of this dietary disorder is not so clearly indicated to involve pituitary dysfunction as that of a deficiency of vitamin B.

3. Irradiation. Roentgen rays and mesothorium are destructive to spermatogenesis if employed in large enough doses. Sterility is readily induced thereby and appears to be irreversible.

4. Alcoholism. Alcohol in excess has been shown to abolish spermatogenic activity in experimental animals and man.

5. Confinement. Especially in wild animals, this condition frequently induces sterility. It is generally known that cage confinement of dogs induces loss of sperm production. However, Huggins [27] and his associates recently noted ultimate adaptation and adjustment to such confinement; after a period of some four months their caged dogs regained the power of sperm production. This recovery is easily determined by subcutaneous injection of pilocarpine hydrochloride, which induces discharge of spermatozoa along with copious prostatic secretion. This phenomenon may later be proved to have pituitary involvements.

6. Vasoligation. This procedure, though held to produce spermatogenic atrophy by Bouin and Ancel [28] and especially by Steinach,[29] has been abundantly proved to be without such effect. Almost a century prior to the publication of this erroneous conception by Steinach, Sir Astley Cooper [30] proved on his own dog that ligation for a duration of several years did not interfere with spermatogenesis. A testis graft in which no outlet is present will produce spermatozoa in amphibia, birds and mammals. Occlusion of the vas deferens of months' and years' duration in the cock and in at least eight

26. Evans, H. M., and Bishop, K. S.: On the Existence of a Hitherto Unknown Dietary Factor Essential for Reproduction, J. A. M. A. **81**: 889 (Sept. 15) 1923. Evans, H. M.; Burr, G. O., and Althausen, T. L.: The Anti-Sterility Vitamine Fat Soluble E, in Memoirs of the University of California, Berkeley, University of California Press, 1927, vol. 8.

27. Huggins, Charles; Masina, M. H.; Eichelberger, Lillian, and Wharton, J. D.: Quantitative Studies of Prostatic Secretion, J. Exper. Med. **70**: 543 (Dec.) 1939.

28. Bouin, P., and Ancel, P.: Recherches sur les cellules interstitielles du testicule des mammifères, Arch. de Zool. Expér. **1**: 437, 1903.

29. Steinach, E.: Verjungung durch experimentelle Neubelebung der Alternden Pubertätsdruse, Arch. f. Entwchlngsmech. d. Organ. **46**: 557, 1920.

30. Cooper, A. P.: Observations on the Structure and Diseases of the Testis, London, Longman, 1830.

species of mammals, including man, fails to cause testis degenerations, and, indeed, congenital absence of an epididymis and vas deferens in an adult guinea pig did not prevent active sperm formation.[31] For further details of these phases of testicular function, see Moore.[32]

II. SECRETORY ACTIVITY

Although it has been known since biblical times that castration produces the eunuch—concrete evidence that the testes exert decided effects on the organism—it is less than a century since the clear demonstration of Berthold,[33] in 1849, that the effect is exerted through a humoral substance distributed by the circulation and not by prescribed nerve pathways. Berthold castrated the cock and noted that the bird became a capon. However, if after removal of the testis this organ was thrown back into the peritoneal cavity or was placed under the skin and persisted with good vascular connections, the bird remained a typical cock instead of becoming a capon. The site of the incorporated tissue was inconsequential, but vascular connections were all important. Incidentally, the transplanted testis continued to produce spermatozoa, and it remains essentially to the credit of Bouin and Ancel that, at about the beginning of the present century, they demonstrated that the masculinizing influence of the testis was independent of the active production of germ cells. Thus the testicle exerts its hormone-secreting capacity as a function secondary to germ cell formation, and although attempts have been made to relegate the entire secretory function to the interstitial cells of Leydig, there is much information to suggest that spermatogenic activity contributes to hormone secretion. Some writers have postulated that an entirely separate hormone is produced by the germinal epithelium, but this cannot be granted on the basis of the evidence presented.

Methods of Detection of Testicular Hormone.—The hormone-secreting capacity of the testes and the influence of this hormone in the organism have been studied rather intensively during the last two decades. Methods

31. Moore, C. R.: Supplementary Observations on Mammalian Testis Activity, Anat. Rec. **48**: 105 (Jan. 25) 1931.

32. Moore, C. R.: Biology of the Testes, in Allen, Edgar; Danforth, C. H., and Doisy, E. A.: Sex and Internal Secretions, Baltimore, Williams & Wilkins Company, 1939, chap. 7.

33. Berthold, A. A.: Transplantation der Hoden, Arch. f. Anat., Physiol. u. wissensch. Med. 1849, p. 42.

of detecting the active principles involved have been based largely on warding off or repairing changes occurring after castration, and as yet the only satisfactory indicator is the animal itself. Most of the classes of vertebrates have been employed in the study of testis hormone effects; invertebrates, in the main, do not show specific responses to castration. Certain species of fish exhibit characters representing responses to testis hormone, but these animals have not been used extensively as test objects for such substances. Amphibia, likewise, show certain characters of response to testis hormone—clasp reflexes, thumb or digital excrescences, dorsal fin fold growth and others—and some reactions have been proposed as means of detection of the hormone, without general adoption of the suggestions. Reptiles have been used but little in hormone studies, but birds have been used extensively.

The domestic cock when castrated shows immediate progressive shrinkage in comb and wattles, but hormone introduced by transplantation of testes or hypodermically injected restimulates, almost immediately, the growth of these head furnishings. Comb response in relation to testicular hormone subsequent to the early work of Berthold has been studied rather intensively by Pezard,[34] Benoit,[35] Caridroit,[36] Domm[37] and others. Gallagher and Koch[38] perfected the method of using such responses as quantitative indicators of the hormone activity in testicular tissue extracts, urine extracts and pure chemical androgens. Comb growth is perhaps the most frequently employed indicator for androgenic substances at the present time.

The use of mammals as indicators of testis hormone has depended in the main on changes occurring in the accessory reproductive organs after castration—these organs being the principal site in the organism of morphologic responses to the hormone.

34. Pezard, A.: Le conditionnement physiologique des caractères sexuels secondaires chez les oiseaux, Bull. biol. de la France et de la Belgique **52**: 1, 1918.

35. Benoit, J.: Le déterminisme des caractères sexuels secondaires du coq domestique. Étude physiologique et histo-physiologique, Arch. de zool. expér. et gén. **69**: 217, 1929.

36. Caridroit, F.: Étude histo-physiologique de la transplantation testiculaire et ovarienne chez les Gallinacés, Bull. biol. de la France et de la Belgique **60**: 135, 1926.

37. Domm, L. V.: New Experiments on Ovariotomy and the Problem of Sex Inversion in the Fowl, J. Exper. Zoöl. **48**: 31 (July 5) 1927.

38. Gallagher, T. F., and Koch, F. C.: The Quantitative Assay for the Testicular Hormone by the Comb Growth Reaction, J. Pharmacol. & Exper. Therap. **40**: 327 (Nov.) 1930.

1. Spermatozoa in the epididymis of the guinea pig are short lived in the absence of testis hormone, and this response has been employed to denote the presence of active substances in extracts of the testes.[39]

2. The guinea pig when stimulated by an electric current directed to the head ejaculates a coagulable substance and the reaction is a certain demonstration of function of the prostate gland and seminal vesicles. This function is dependent on the presence of testis hormone. The reaction and its modification by castration and by the injection of extracts containing testis hormone were described by Moore and Gallagher.[40]

3. The rat prostate gland responds to castration within four days by loss of certain definite morphologic features. The responses to extracts of testis hormone have been described by Moore, Price and Gallagher.[41]

4. Rat seminal vesicles demonstrate castration changes within forty-eight hours, and the changes can be clearly modified by administering testis hormone (Moore, Hughes and Gallagher).[42]

5. In a similar manner the vas deferens (Vatna) [43] and Cowper's gland (Heller [44]) have each proved sufficiently responsive to testis hormone to constitute within themselves responsible indicators of the presence of this substance.

Still other methods of detection of the testis hormone have been utilized. The seminal vesicles of the mouse have been used by Loewe and Voss,[45] and by Martins

39. Moore, C. R., and McGee, L. C.: On the Effects of Injecting Lipoid Extracts of Bull Testes into Castrated Guinea Pigs, Am. J. Physiol. **87**: 436, 1928.

40. Moore, C. R., and Gallagher, T. F.: Seminal Vesicle and Prostate Function as a Testis-Hormone Indicator; the Electric Ejaculation Test, Am. J. Anat. **45**: 39 (Jan.) 1930.

41. Moore, C. R.; Price, Dorothy, and Gallagher, T. F.: Rat-Prostate Cytology as a Testis-Hormone Indicator and the Prevention of Castration Changes by Testis-Extract Injection, Am. J. Anat. **45**: 71 (Jan.) 1930.

42. Moore, C. R.; Hughes, Winifred, and Gallagher, T. F.: Rat Seminal-Vesicle Cytology as a Testis-Hormone Indicator and the Prevention of Castration Changes by Testis Extract Injection, Am. J. Anat. **45**: 109 (Jan.) 1930.

43. Vatna, S.: Rat Vas Deferens Cytology as a Testis Hormone Indicator and the Prevention of Castration Changes by Testis Extract Injections, Biol. Bull. **58**: 322 (June) 1930.

44. Heller, R. E.: Cowper's Gland and Its Reaction to Castration and to Different Sex-Hormone Conditions, Am. J. Anat. **50**: 73 (March) 1932.

45. Loewe, S., and Voss, H. E.: Gewinnung, Eigenschaften und Testierung eines männliches Sexualhormons, Sitzungsber, d. Akad. d. Wissensch. Wien, Math.-Naturw. Kl., 1928, vol. 29, no. 20.

and Rocha e Silva.[46] The mouse adrenal exhibits rather definite changes in relation to testis hormone. Prostate secretion in the dog is readily stimulated by injection of pilocarpine hydrochloride, and Huggins and associates[27] have shown its loss through castration and reestablishment by injected androgens. In man, responses in eunuchs are now definitely known; many changes that involve penis growth, erections, ejaculations, voice changes, prostate growth and other related activities have been abundantly described within the last five years.[47]

Isolation of Testis Hormone.—Prior to 1927 the effects of testis hormone were studied almost entirely from changes caused by castration and from the effects of transplantation of testes, but in that year McGee and co-workers[48] succeeded in extracting from fresh testicles of the bull a substance that exerted comb growth–stimulating capacities in capons. As rapidly as short time indicators were developed in laboratory mammals, such extracts proved their capabilities of substituting for the mammalian testis secretions.[49] These active extracts, obtained in lipoid fractions after original extractions of the tissue in benzene, were further purified by Gallagher and Koch,[38] and their assay was established on a fairly satisfactory quantitative basis.

In 1929 Loewe and Voss[45] and Funk and Harrow[50] obtained comb growth–stimulating substances from human male urine. Through concentration of urinary extracts and purification, Butenandt obtained two pure crystalline substances, androsterone and dehydroandro-

46. Martins, T., and Rocha e Silva, A.: In The Seminal Vesicles of the Castrated Mouse; Test for the Testicular Hormones, Mem. do inst. Oswaldo Cruz, supp., p. 196.

47. McCullagh, E. P.; McCullagh, D. R., and Hicken, N. F.: Diagnosis and Treatment of Hypogonadism in the Male, Endocrinology **17**: 49 (Jan.-Feb.) 1933. Hamilton, J. B.: Treatment of Sexual Underdevelopment with Synthetic Male Hormone Substance, ibid. **21**: 649 (Sept.) 1937. Foss, G. L.: Effect of Testosterone Propionate on a Post-Puberal Eunuch, Lancet **2**: 1307 (Dec. 4) 1937. Vest, S. A., and Howard, J. E.: Clinical Experiments with the Use of Male Sex Hormones: Use of Testosterone Propionate in Hypogonadism, J. Urol. **40**: 154 (July) 1938. Kenyon, A. T.: The Effect of Testosterone Propionate on the Genitalia, Prostate, Secondary Sex Characters and Body Weight in Eunuchoidism, Endocrinology **23**: 121 (Aug.) 1938.

48. McGee, L. C.: The Effect of the Injection of a Lipoid Fraction of Bull Testicle in Capons, Proc. Inst. Med. Chicago **6**: 242, 1927. McGee, L. C.; Juhn, Mary, and Domm, L. C.: The Development of Secondary Sex Characters in Capons by Injections of Extracts of Bull Testes, Am. J. Physiol. **87**: 406 (Dec.) 1928.

49. Moore and McGee.[39] Moore and Gallagher.[40] Moore and others.[41] Moore and others.[42] Vatna.[43] Heller.[44]

50. Funk, C., and Harrow, B.: The Male Hormone, Proc. Soc. Exper. Biol. & Med. **26**: 325 (Jan.) 1929. Funk, C.; Harrow, B., and Lejwa, A., ibid. **26**: 569 (April) 1929.

sterone, which were definitely androgenic, and determined the structural formulas.[51] Starting from the suggested formula of androsterone, Ruzicka and co-workers[52] almost immediately produced this substance synthetically from cholesterol.

Comparative studies of the activity obtained with extracts from urine and from testes, carried on especially by the Laqueur laboratory in Amsterdam, Netherlands,[53] suggested that two different substances were involved, and Gallagher and Koch[54] demonstrated differential responses of the respective active principles to boiling alkali. In 1935 David and others[55] obtained from extracts of fresh testis a pure chemical substance, testosterone, that showed greater androgenic properties than androsterone. Testosterone likewise was produced synthetically at once by both the Butenandt[56] and the Ruzicka[57] group. Combinations of the parent substance, especially with certain fatty acids, greatly enhanced the androgenic effect in test organisms, and a large series of pure chemical compounds are now available that exert marked effects. One of the more active compounds is testosterone propionate, and this is the substance now most frequently used in clinical treatment. Its administration by injections is the more usual, but application of the substance as a cutaneous ointment reveals its ready penetration through the skin with the usual internal organ responses.[58] Whether the

51. Butenandt, Adolf: Ueber die chemische Untersuchung der Sexualhormone, Ztschr. f. angew. Chem. **44**: 905, 1931; Ueber die Chemie der Sexualhormone, ibid. **45**: 655, 1932. Butenandt, Adolf, and Dannenbaum, H.: Ueber Androsterone: III. Isolierung eines neuen Physiologisch unwirksamen Sterin-derivatives aus Mannerharn, seine Verknupfung mit Dehydroandrosterone und Androsterone, Ztschr. f. physiol. Chem. **229**: 192, 1934.

52. Ruzicka, L.; Goldberg, M. W.; Meyer, I.; Brungger, H., and Eichenberger, E.: Ueber die Synthese der Testikelhormons (Androsterone) und Stereoisomerer desselben durch Abbau hydrierter Sterine, Helvet. chim. acta **17**: 1395, 1934.

53. Laqueur, Ernst; David, K.; Dingemanse, E., and Freud, J.: Ueber männliches Hormon: Unterschied von Androsteron aus Harn und Testosteron aus Testis, Acta brev. Neerland. **5**: 84, 1935.

54. Gallagher, T. F., and Koch, F. C.: The Effect of Alkali on the Testicular Hormone, J. Biol. Chem. **104**: 611 (March) 1934.

55. David, K.; Dingemanse, E.; Freud, J., and Laqueur, Ernst: Ueber krystallinisches männliches Hormon aus Holden (Testosteron) wirksamer als aus Harn oder aus Cholesterin bereitetes Androsteron, Ztschr. f. physiol. Chem. **233**: 281, 1935.

56. Butenandt, Adolf, and Hanisch, Günter: Ueber Testosteron; Umwandlung des Dehydro-androsterons in Androstendiol and Testosteron, Ztschr. f. physiol. Chem. **237**: 89, 1935.

57. Ruzicka, L., and Wettstein, A.: Ueber die künstliche Herstellung des Testikelhormons Testosteron, Helvet. chim. acta **18**: 1264, 1935.

58. Moore, C. R.; Lamar, J. K., and Beck, Naomi: Cutaneous Absorption of Sex Hormones, J. A. M. A. **111**: 11 (July 2) 1938. Abarbanel, A. R.: Percutaneous Administration of Testosterone Propionate for Dysmenorrhea, Endocrinology **26**: 765 (May) 1940.

natural hormone secreted by the testis is represented by one of the forty or more active compounds now available is not certainly known, but it is believed that the naturally secreted hormone may be a substance at least closely allied to testosterone, perhaps in some especially effective chemical combination. It is not known whether the naturally secreted hormone is the same in different species of mammals or in the different classes of vertebrates, but several different pure chemical androgens are individually capable of effective substitution for the naturally secreted hormone in probably all vertebrates.

Periods of Hormone Secretion.—The time of onset of testis secretion of hormone cannot be stated more definitely than that the onset is largely responsible for the beginning of puberal development. Whether testes secrete active substances into the blood stream during embryonic life is yet to be demonstrated. In the rat secretion is evident by about the fortieth day after birth, from secretory development of the prostate gland and seminal vesicles,[59] and castration at birth induces delay in the development of these accessory reproductive structures.[60] In the guinea pig hormone secretion is evident by the thirtieth day, when ejaculation is induced by electrical stimulation.[1] In man, studies on the prostate by R. A. Moore [61] suggest a low grade secretion about the tenth to the thirteenth year. In animals whose breeding is constant, hormone secretion is continuous from its inception to its natural decline, but the latter event is less certain by date than the onset of secretion. Wiesner [62] failed to obtain a correlation between discontinuance of hormone secretion and old age in rats, and in man the evidence for termination of secretion suggests only a general decline at ages 60 to 70 years, with many evidences of good production in much later years.

The majority of vertebrates secrete testis hormone only at definite seasons, which usually coincide with their natural breeding periods. In a wild rodent secre-

59. Moore and others.[41] Moore and others.[43]

60. Price, Dorothy: Normal Development of the Prostate and Seminal Vesicles of the Rat with a Study of Experimental Post-Natal Modifications, Am. J. Anat. **60**: 79 (Nov.) 1936.

61. Moore, R. A.: The Evolution and Involution of the Prostate Gland, Am. J. Path. **12**: 599 (Sept.) 1936; The Histology of the Newborn and Prepuberal Prostate Gland, Anat. Rec. **66**: 1 (Aug. 25) 1936.

62. Wiesner, B. P.: The Experimental Study of Senescence, Brit. M. J. **2**: 585 (Sept. 24) 1932.

tion of testis hormone is limited to a period of about three months, and at other times of the year such accessory reproductive organs as the seminal vesicles, prostate and Cowper's gland remain essentially in the condition observed in the castrate, but capable of responding at any time to introduced hormone.[2] The natural period of rut, as well as of less definitely seasonal forms of the mating instinct, is conditioned by the hormone.

Modification of the Secretion of Testis Hormone.— In many respects the secretion of hormone and the formation of germ cells run parallel, and conditions which modify one function act likewise on the other; this, however, is not universally true, as will be mentioned later.

Removal of the pituitary gland or loss or diminution in its function precludes or diminishes secretion of hormone by the testis. Involutionary changes in the accessory reproductive organs are quite similar after ablation of the pituitary gland and after castration. In the opposite direction, the administration of fresh pituitary substance or of the gonadotropic principle from other sources results in a marked precocious stimulation of the production of testis hormone or in an intensification of secretion already under way. Some of the indications for precocious hormone secretion are (*a*) the production of secretion granules in the epithelium of the rat seminal vesicle appreciably earlier than such granules appear in normal animals,[60] (*b*) increases in the gross fresh weight of the seminal vesicles of treated rats by 3,000 to 5,000 per cent,[18] (*c*) marked stimulation of the growth of comb and wattles in young chicks, and attempts at crowing and treading pen mates two weeks after hatching [63] and (*d*) tremendous increases in the size of all accessory organs of reproduction in ground squirrels artificially stimulated during the period of low sexual activity.[2]

A recognition of the fundamental influence of the pituitary gland on the secretion of hormone in the testis as well as on spermatogenesis suggests that hypogonadal states may represent fundamentally hypopituitary activity. Furthermore, since it has been estab-

63. Domm, L. V., and Van Dyke, H. B.: Precocious Development of Sexual Characters in the Fowl by Daily Injections of Hebin, Proc. Soc. Exper. Biol. & Med. **30**: 349 and 351 (Dec.) 1932.

lished that there is a definite correlation between pituitary activity and testis activity in wild rodents whose breeding is seasonal, one is justified in assuming that the problem of seasonal reproductive activity is essentially a problem of seasonal control of the pituitary function. It would appear that an outstanding difference between mammals in which reproductive activity is continuous and those in which it is seasonal is that one group has a continuously active pituitary and the other a gland the activity of which is influenced by the season.

In view of the conditions portrayed, it is natural that attention should be directed to the problem of pituitary control. Present information is here very limited, but some suggestions may be mentioned. Environmental influences appear to play a decisive role in the seasonal control of the pituitary, but different elements of this complex may be active in different species. Light has been demonstrated to be an effective agent, particularly in the bird, but also in some mammals. Rowan's original demonstration [15] that the progressive daily addition of a few minutes extra light caused testicular activity to be stimulated in midwinter has been extended to show not only that the effective site of this influence is the eye but also that direct illumination of the pituitary gland through the orbit is effective in stimulating testicular activity; the illuminated pituitary possesses much higher gonadotropic activity than the nonilluminated ones.[7] Reproductive stimulation has likewise followed treatments of fish with light,[64] also treatments with temperature changes.[65] Failure of hormone secretion during straight inanition and during deficiency in vitamin B requirements is believed to be a secondary effect, due to primary inactivity of the pituitary.

The hormone-secreting capacities of the testicle in the total absence of spermatogenic activity was first emphasized by Bouin and Ancel.[28] Undescended testes, which are devoid of germ cell activity, exert nevertheless the typical masculinizing tendencies of normal organs; but, contrary to earlier ideas, such germ cell–free testes do not secrete more hormone than normal

64. Hoover, E. E.: Experimental Modification of the Sexual Cycle in Trout by Control of Light, Science **86**: 425 (Nov. 5) 1937.

65. Craig-Bennett, A.: The Reproductive Cycle of the Three-Spined Stickle-Back, Gasterosteus Aculeatus, Linn., Phil., Tr. Roy. Soc., London, s. B. **219**: 197, 1930.

organs, but considerably less. In like manner, testis grafts devoid of spermatogenic activity may exert masculinizing influence, as do also testes injured through irradiations. Vasectomy does not modify the hormone-secreting capacities,[66] nor does it cause degeneration of the germ cell–producing capacities; the operation is, however, an effective measure for insuring sterility by virtue of preventing egress of spermatozoa.

Thus it becomes evident that the two testicular functions are closely parallel and that practically all conditions that stimulate or depress germ cell formation likewise modify hormone secretion. It is true, however, that sperm formation may precede evidence of hormone secretion in approaching puberty or during the annual period of development. It is also true that hormone can be produced in the absence of germ cell activity, as in cryptorchism, in transplantation of testes, after irradiation of testes and particularly in pathologic adrenal involvement.[67]

From the foregoing discussion it will be appreciated in general (1) that testis hormone is not stored in the body but is rather quickly utilized, broken down or excreted and that (2) if it is to be completely effective it must be maintained in the body in concentrations that will affect the organs with the highest thresholds of response. Since different organs exhibit different thresholds, it is possible to maintain one organ in a functional state and not another.

Effects of Testis Hormone.—The effects of testis hormone in the organism are represented by the differences between castrated and normal males, and since investigators do not yet know all the effects of castration, to that extent they fail to understand the effects of the hormone. In general, the two large categories of effects may be stated as (1) the conditioning of the male organism to show sexual responses to the female and (2) the development and maintenance of the accessory organs of reproduction in a functional state, making possible effective insemination. Brief mention of some particular effects may be in order.

66. Poynter, H.: Testis Hormone Secretion in the Rat Under Conditions of Vasectomy or Isolation, Anat. Rec. **74**: 355 (July 25) 1939.

67. Wilkins, L.; Fleischmann, W., and Howard, J. E.: Macrogenitosomia Praecox Associated with Hyperplasia of the Androgenic Tissue of the Adrenal and Death from Cortico-Adrenal Insufficiency, Endocrinology **26**: 385 (March) 1940.

Treatment of embryos in the process of sexual differentiation with pure chemical androgens induces modification in the reproductive system, especially in females. Such modification in mammals has been described for guinea pigs,[68] rats,[69] mice[70] and opossums.[71]

Treatment of prepuberal young males induces precocious development of accessory reproductive organs. This follows treatment in amphibia, birds, mammals and even man.

Gonadal hormones from either sex react on the pituitary gland and effectively reduce the amount of gonad-stimulating hormone available to the organism. Castration enhances, and the administration of androgen or of estrogen lowers, the gonad-stimulating effects of the pituitary. Thus testes are probably never active to the full extent of their powers, and this reciprocal interaction between gonad and pituitary secretions appears to be a fundamental element in the regulation of the two glands, hence of the reproductive cycles.

Androgens introduced into castrated males rebuild the accessory organs into a functional state or, if introduced at the time of castration, prevent castration changes from appearing.

The effects of androgens on the normal testicle to a large extent have been found to be harmful, rather than stimulating as was once anticipated. The evidence points to the mechanism of action as an indirect effect on the testes by virtue of a suppressive action on the pituitary, rather than a direct action on the sex glands.[20] It has been found that the harmful influence is absent if either fresh pituitary tissue or gonadotropic agents are

68. Dantchakoff, V.: L'hormone mâle adulte dans l'histogénèse sexuelle du mammifère, Compt. rend. Soc. de biol. **123**: 873, 1936.

69. Greene, R. R.; Burrill, M. W., and Ivy, A. C.: The Experimental Production of Intersexuality in the Female Rat, Am. J. Obst. & Gynec. **36**: 1038 (Dec.) 1938.

70. Raynaud, A.: Intersexualité obtenue expérimentalement chez la souris femelle par action hormonale, Bull. biol. de la France et de la Belgique **72**: 297, 1938. Turner, C. D.; Haffen, Rita, and Struett, Helen: Some Effects of Testosterone on Sexual Differentiation of Female Albino Mice, Proc. Soc. Exper. Biol. & Med. **42**: 107 (Oct.) 1939.

71. Moore, C. R.: Modification of Sexual Development in the Opossum by Sex Hormones, Proc. Soc. Exper. Biol. & Med. **40**: 544 (April) 1939; On the Role of Sex Hormones in Sex Differentiation in the Opossum (Didelphys Virginiana), Physiol. Zool. **14**: 1, 1941. Burns, R. K.: The Differentiation of Sex in the Opossum (Didelphys Virginiana) and Its Modification by the Male Hormone Testosterone Propionate, J. Morphol. **65**: 79 (July 1) 1939; Sex Differentiation During the Early Pouch Stages of the Opossum (Didelphys Virginiana) and a Comparison of the Anatomical Changes Induced by Male and Female Sex Hormones, ibid. **65**: 497 (Nov. 1) 1939.

supplied. Injurious effects on the testes from the administration of androgens have been reported in rats, dogs, ducks, cocks, guinea pigs and man. On the other hand, it has been pointed out earlier in this paper that androgens, as well as progesterone and yeast extract, exerted a protective action on spermatogenesis immediately following hypophysectomy though none of these substances had a reparative action if administered following damage to the testicles from hypophysectomy.

SUMMARY

The testes function in producing mature spermatozoa and in secreting a hormone that induces mating desire and stimulates the function of accessory reproductive organs; function of the latter insures transfer of spermatozoa to localities in which mature eggs are to be found.

Sperm production in the majority of vertebrates is seasonal, but in many others it is continuous. It appears that pituitary gland function exercises the basic control; hence seasonal influences appear to operate largely through this gland.

The testes are labile organs and easily influenced by a number of different conditions. Whereas many agents or conditions affect both spermatogenesis and the secretion of hormone, the former may be in abeyance while the latter continues.

Naturally secreted testis hormone is believed to be closely similar, if not identical, to testosterone or some compound of it. It is unknown whether testis secretion is the same in different species of vertebrates, but testosterone compounds are androgenic in practically all vertebrate males.

Precocious secretion of testis hormone can be stimulated by the administration of pituitary materials or other gonadotropic substances; hence testes probably never secrete to their full capacity.

Testis hormone is not stored in the body. Its effect is to condition the mating drive and the function of the proper accessory reproductive glands. The rate of its secretion is believed to be controlled by a reciprocal action between it and pituitary secretions.

In the majority of cases studied the injection of androgens into intact males is harmful rather than beneficial to the testes.

CHAPTER XVII

THERAPEUTICS OF TESTICULAR DYSFUNCTION

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The first major advancements in endocrinologic knowledge came from studies pertaining to the testis. Aristotle[1] recognized that the absence of the testis is responsible for the changes observed in castrate animals and man. In 1849 Berthold[2] concluded that the testis produces an internal secretion, since capons with an implanted testis have comb growth and other characteristics of the cock. Thereafter, the study of testicular function lagged behind that of other ductless glands until, as a result of intensive study[3] during the last decade, the status of androgenic substances shifted abruptly from that of relatively unknown materials to that of highly active crystalline compounds that can be prepared synthetically and are available for therapeutic use.

The androgens of chief interest in therapeusis are testosterone, a substance isolated from testis tissue (of the bull[4]), and androsterone and dehydroisoandrosterone, which are excreted in human urine.[5] Clinically,

The following members of the group engaged in study of problems pertaining to androgenic substances gave aid and opinions which were responsible for some of the data presented: Drs. Gilbert Hubert, Judson Gilbert, Edgar Allen, Edward Edwards, Ralph Dorfman and Hubert Catchpole.

1. Aristotle: Historia Animalium, translated by D'Arcy Thompson in Works of Aristotle, translated into English under the editorship of J. A. Smith and W. D. Ross, London, Oxford University Press, 1910, vol. 4.

2. Berthold, A. A.: Transplantation der Hoden, Arch. f. Anat., Physiol. u. wissensch. Med., 1849, pp. 43-46.

3. (*a*) McGee, L. C.; Juhn, M., and Domm, L. V.: The Development of Secondary Sex Characters in Capons by Injections of Extracts of Bull Testes, Am. J. Physiol. **87**: 406-435 (Dec.) 1928. (*b*) Butenandt, A., and Hanisch, G.: Ueber Testosteron: Umwandlung des Dehydroandrosterons in Androstendiol und Testosteron; ein Weg zur Darstellung des Testosterons aus Cholesterin, Ztschr. f. physiol. Chem. **237**: 89-97, 1935. Ruzicka, L., and Wettstein, A.: Ueber die künstliche Herstellung des Testikelhormons, Testosteron (Androsten-3-on-17-ol), Helvet. chim. acta **18**: 1264-1275, 1935.

4. David, K.; Dingemanse, E.; Freud, J., and Laquer, Ernst: Ueber krystallinisches männliches Hormon aus Hoden (Testosteron) wirksamer als aus Harn oder aus Cholesterin bereitetes Androsteron, Ztschr. f. physiol. Chem. **233**: 281-282, 1935.

5. Butenandt, A., and Tscherning, K.: Ueber Androsteron, ein krystallisiertes männliches Sexualhormon, Ztschr. f. physiol. Chem. **229**: 167-184, 1934.

testosterone is the compound used at present almost exclusively. For intramuscular injections, the propionic ester of testosterone is utilized, as it provides prolonged action. The nonesterified form may be found more effective percutaneously and in implanted pellets, but these modes of administration are still largely in the experimental stage.

The rationale in therapy with androgens is either that of substitution in conditions characterized by deficiency of testicular secretions, or that of utilization of pharmacologic actions in states in which actual deficiency of testicular secretions is not present or is in doubt. For a proper understanding of the uses, limitations and dangers of androgens, it is essential to appreciate (1) the relationship between the testis and the pituitary, (2) the sequelae of castration and of eunuchoidism (underdevelopment of the testis) and, conversely, (3) the bodily functions and morphologic changes promoted by androgens.

RELATIONSHIP BETWEEN THE TESTIS AND THE ANTERIOR LOBE OF THE PITUITARY GLAND

The testis has two rather distinct functions, spermatogenic and endocrinous. Both functions are induced by, and dependent for maintenance on, gonadotropins from the anterior lobe of the pituitary. In turn, testicular secretions limit the production by the pituitary of gonad-stimulating material.[6] Thus, a loss of testicular function leads to increased secretion of gonadotropins, whereas the administration of exogenous androgens suppresses production of the gonadotropic substances. Therefore, titers of urinary gonadotropins may serve to indicate whether the atrophy or underdevelopment of the testis is primarily referable to testicular hyposecretion or is secondary to lesions of the pituitary or of the brain stem. From the standpoint of replacement therapy, however, such methods of distinguishing between primary and secondary hypogonadism in the male are of limited value. Primary hypogonadism is usually obvious, as for example after bilateral orchiectomy or overt damage of the testes, such as an interruption of the blood supply during repair of hernia. Even if the eunuchoidism is secondary, no means of initiating spermatogenesis is known at present.

6. Moore, C. R.: Biology of the Testes, in Allen, E.; Danforth, C. H., and Doisy, E. A.: Sex and Internal Secretions, Baltimore, Williams and Wilkins Company, 1939, chap. 1.

CASTRATION AND EUNUCHOIDISM [7]

The extent of the abnormality resulting from insufficiency of testicular secretion depends on the degree of secretory deficiency and on the age of the patient at the onset of the condition. There are two distinct types, that in which the onset of deficiency occurred before sexual maturity and that in which it was delayed until after sexual maturity.

Prepuberal Onset of Testicular Insufficiency.—Prepuberal deficiency of testicular secretion gives rise to the eunuchoid state, in which the genitalia remain small and the skeleton is characterized by extraordinary length of the long limb bones, associated with delay of epiphysial union. The voice retains a high pitch, and the larynx, although larger than that of a child, does not have the prominence of the thyroid cartilage or the size of that of the adult male. The beard is usually composed of only fine hair, shaving being more a matter of desire than of necessity. The bodily proportions, a lack of muscular development and in some cases a characteristic distribution of adipose tissue—along with the lack of beard and manly voice—have given rise to incorrect allusions to femininity.

Postpuberal Onset of Testicular Insufficiency.—Postpuberal interruption of testicular function does not produce all the sequelae observed following the prepuberal onset of testicular insufficiency. Eunuchoid proportions of the long bones are not observed if the epiphyses are already closed. The secondary sexual characters show regression, but such organs as the larynx and genitalia do not return to an immature state. An adult character of voice is retained [8] and, with variation from subject to subject, a limited degree of sexual ability and desire.

Phenomena Characteristic of Castration and Eunuchoidism.—Castrate and eunuchoid persons are excellent subjects for study of both the phenomena of testicular insufficiency and the effects produced by androgens. The widespread physiologic effects of "sex hormones" described in the following paragraphs serve

7. In eunuchoidism, a eunuch-like state, the testes are present but do not secrete properly; the range of patients is from those like castrates to those more like normal men.

8. Hamilton, J. B., and Hubert, G.: Vocal Changes in Eunuchoidal and Castrated Men upon Administration of Male Hormone Substance, Am. J. Physiol. **128**: 372-373 (May) 1940.

to dispel the prevalent erroneous notion that the influence of testicular secretions extends only in the sphere of reproductive purposes:

1. Absence of spermatozoa and testicular secretions: The most obvious derangements in the castrate man are the lack of spermatozoa and the low levels of androgens in the bodily fluids. According to Gallagher and co-workers,[9] normal men excrete in the urine an average of about 66 international units of androgen per twenty-four hours, whereas the amount of androgenic material excreted by castrate and eunuchoid men is much less.[10]

2. Integument: The skin is soft, and the face viewed from a distance appears to be that of a young person. Closer inspection reveals the absence of deep furrows and in the older men the presence of numerous fine wrinkles. The skin is characteristically of a pasty, sallow color, due to lack of cutaneous pigments[11] rather than to anemia. Spectrophotometric analyses[12] indicate that the volume of blood in the skin and the percentage of oxygenated hemoglobin are less than in normal men. In certain areas like the buttocks, however, which contain a goodly amount of "venous" blood (a high proportion of reduced hemoglobin), there is more blood than in normal men. Melanin is present in less than average amounts.

The skin and hair are dry and the sebaceous secretions apparently diminished. Acne does not occur in the person who does not mature sexually.[13]

Dermal appendages are affected, especially the hair. The beard of the eunuchoid is soft and not entirely unlike that of an adolescent, whereas the postpuberal castrate retains many hairs of large diameter and may require shaving once or more each week. The axillary and pubic hair is of fine texture and of limited amount. Other hair on the trunk and that of the limbs is not of the coarse, thick type typical of secondary sexual hair. Eyebrows are present but are less bushy, and there is lacking the hair between the eyebrows that was present before postpuberal castration and that returns on androgenic therapy. None of the eunuchoids in our series were bald,

9. Gallagher, T. F.; Peterson, D. H.; Dorfman, R. I.; Kenyon, A. T., and Koch, F. C.: The Daily Urinary Excretion of Estrogenic and Androgenic Substances by Normal Men and Women, J. Clin. Investigation **16**: 695-703 (Sept.) 1937.

10. Kenyon, A. T.; Gallagher, T. F.; Peterson, D. H.; Dorfman, R. I., and Koch, F. C.: The Urinary Excretion of Androgenic and Estrogenic Substances in Certain Endocrine States: Studies in Hypogonadism, Gynecomastia and Virilism, J. Clin. Investigation **16**: 705-717 (Sept.) 1937.

11. Hamilton, J. B., and Hubert G.: Photographic Nature of Tanning of the Human Skin as Shown by Studies of Male Hormone Therapy, Science **88**: 481 (Nov. 18) 1938.

12. Edwards, E.; Hamilton, J. B.; Duntley, S. Q., and Hubert, G.: Cutaneous Vascular and Pigmentary Changes in Castrate and Eunuchoid Men, Endocrinology **26**: 119-128 (Jan.) 1941.

13. Hamilton, J. B.: Male Hormone Substance: A Prime Factor in Acne, J. Clin. Endocrinol. **1**: 570-592 (July) 1941.

although in some of the families other male members were bald; the hair over each temple extended well toward the lateral edge of the eyebrow.

3. Adiposity: In both eunuchoid and castrate men adipose tissue may be deposited subcutaneously, being particularly prominent about the mammae and over the trochanter and mons pubis. Such adiposity is absent, however, in many castrate or eunuchoid persons, some of whom have had marked testicular insufficiency for as long as forty years.

4. Voice: In the eunuchoid the vocal pitch and range are high, for example, a range from D above middle C to the third E above middle C, with the pitch in conversation F above middle C (frequency per second: range, 294 to 1,319; pitch, 349). The mature voice of the postpuberal castrate is largely maintained, as shown by the range of one patient from the second G below middle C to E above middle C, with a pitch during low conversation of B below middle C (frequency per second: range, 98 to 330; pitch, 247).[8]

5. Circulation: A characteristic derangement of the blood content of the skin of the castrated man was described under item 2 in this list. Hot flushes, similar to those in some women at the menopause or after bilateral oophorectomy, occur frequently and if severe are followed by sweating. With four exceptions, the systolic blood pressure in 14 of the patients in our series has been only slightly more than 100 mm. of mercury. The chief complaint of many patients is fatigue and inability to carry on work. The data pertaining to circulatory fitness in such patients are scanty as yet, and there is difficulty in distinguishing which disturbances are primarily circulatory and what roles metabolic and other factors play. It is unwise to do more than indicate that derangements of the circulatory system occur and that administration of testosterone results, possibly indirectly, in circulatory changes in castrate men.

6. Genitourinary system: In the eunuchoid man the external genitalia are indicative of the underdeveloped state of the internal genitalia. The penis is not unlike that of a newborn child, the scrotum a flat band, without pendulousness or saclike form, and the epididymis so tiny as to defy attempts by palpation to establish its contour. The prostate and seminal vesicles may be so small as to be recognized on rectal palpation only with difficulty and uncertainty. In eunuchoidism of this severity the urinary stream is of small diameter and may not be of great force. In the man castrated after sexual maturation the genitalia remain large save for the scrotum, the size of which is apparently dependent in part on the enclosed organs.[14] In white men the skin of the scrotum and penis loses much of the dark brown color, and the raphe is not prominent.

14. Hamilton, J. B., and Hubert, G.: Differential Diagnosis of Pseudocryptorchidism and True Cryptorchidism, Endocrinology 21: 644-648 (Sept.) 1937.

In general, penile erections are of limited number and completeness. Notable exceptions occur. Two men surgically castrate for twenty-two and thirteen years, respectively, had erections that would permit satisfactory intercourse despite obvious organic and functional evidence of castration, including extremely low levels of urinary androgenic activity (8 and 5 international units, respectively, per twenty-four hours). Such facts serve as an argument against any theory that erectile capacity is due to androgens of extragonadal source. The ejaculate in 4 instances amounted to only a minim.

7. Behavior: Judgment as to irregularities of behavior in these patients is fraught with error, for the mere appreciation that sexual defects are present is in itself psychologic trauma of a high degree. Many authors classify the eunuch or eunuchoid man as sullen or untrustworthy, but detailed examination reveals different individual traits and not necessarily a particular personality pattern.[15]

Caution must be exercised in distinguishing the effects of senility from those that can be properly accredited to testicular insufficiency. In advanced age any decrease in gonadal function is blended closely in ordinary experience with matters in the domain of geriatrics. This has led to the deplorable, and perhaps wishful, thought that restoration of testis function produces rejuvenation.

Therapeutic Indications.—The use of androgens is clearly indicated after bilateral orchiectomy or in severe eunuchoidism. A few patients are able to compensate for their defects and make fairly adequate adjustments, but the majority complain of fatigability, sexual incapacity and other sequelae of testicular insufficiency.

Widely divergent opinions are held, however, regarding the management of the boy in his early teens who is not exhibiting the usual signs of sexual maturation. There is no uniform age for the onset of puberty, and unfortunately the armamentarium does not include as yet a technic for differentiation with certainty between boys with delayed maturation and those who will never mature properly.

The benefits from early treatment with endocrine preparations must be questioned and compared with the limitations and dangers imposed. Many phenomena characteristic of testicular insufficiency disappear while the patient receives treatment, but the only permanent changes thought to be avoided by the early adoption

15. Carmichael, H. T., and Kenyon, A. T.: Eunuchoidism: A Psychiatric and Endocrine Study of Six Cases, Arch. Neurol. & Psychiat. 40: 717-742 (Oct.) 1938.

of endocrine measures are the skeletal proportions of eunuchoidism. Possibly escape might be had from some of the psychologic trauma experienced by the sexually immature, but it is debatable whether early acceptance of a need for continued replacement therapy is more comforting than the hope of eventual establishment of normal physiologic functions.

In opposition to hastily undertaken procedures is the fact that a large percentage of the boys with less evidence of sexual maturation than their associates do eventually mature. The misconception has arisen that endocrine treatment will encourage the body to assume normal reproductive functions. Suffice it to say, there exists no basis for the belief that temporary use of gonadotropins or androgens will result in the initiation and continuance of normal testicular activities. Instead, possible dangers have been indicated by the report of precocious closure [16] of the epiphyses following administration of large amounts of androgens.

Moreover, it must be remembered that in some instances sexual immaturity is complicated by hypothyroidism or other conditions not amenable to treatment with androgens or gonadotropins.

In my opinion the rationale for the early use of gonadotropic or androgenic therapy in males with less than average sexual maturity at the age of adolescence is the prevention of eunuchoid changes that would otherwise be permanent. The known preventable defects are chiefly skeletal and are believed to be due to prolongation of the period of growth beyond the ordinary chronologic time of epiphysial union, not to any augmentation of growth at an early time. Thus it seems possible to control skeletal growth even if endocrine treatment is withheld until the probability of normal body function has been excluded. Extended delay until the patient is stigmatized by a markedly eunuchoidal stature is not countenanced. In brief, the facts now available suggest that there is little gain and that there may be harm in the early administration of gonadotropic or androgenic preparations to boys with

16. Howard, J. E., and Vest, S. A.: II. Further Observations on Testosterone Propionate in Adult Hypogonadism, and Preliminary Report on the Implantation of Testosterone, Am. J. M. Sc. **198**: 823-837 (Dec.) 1939. McCullagh, E. P., and McGurl, F. J.: The Effects of Testosterone Propionate on Epiphyseal Closure, Sodium and Chloride Balance and on Sperm Counts, Endocrinology **26**: 377-384 (March) 1940.

delayed sexual maturation save when dwarfism or some other complication requires attention.

Drug, Dose and Route of Administration.—Both gonadotropins and androgens have been used in the therapy of eunuchoidism. Obviously gonadotropins depend, for their effects, on the capacity of the testis to secrete androgens and hence are of no avail in the case of the castrate or of the person whose testes are not responsive.[17] Moreover, since gonadotropins do not initiate spermatogenesis, direct therapy with a stable crystalline androgenic drug is preferable to stimulating testicular secretion to a variable degree with an extract containing proteins. If in the future more satisfactory preparations of gonadotropic substances are developed and proved capable of initiating spermatogenesis, this choice of management is subject to modification.

Pharmaceutic houses prepare ampules with 25 mg. of testosterone propionate per cubic centimeter, an amount which has been stated to be a satisfactory daily dose.[18] In our experience 20 mg. of testosterone propionate in 1 cc. of oil injected intramuscularly six or seven times a week has been satisfactory in all but a single instance. The injection of 20 mg. three times a week has not been adequate for good clinical effect and has not been sufficient to maintain urinary androgens at the average levels found in normal men.

Deanesly and Parkes[19] demonstrated that subcutaneous implantation of testosterone in the form of compressed pellets is economical and requires replacement only at long intervals. A more prolonged and even course of stimulation is obtained than with other methods.[20] A trocar has been described[21] for use with the human subject, but no discussion has been

17. (*a*) Hamilton, J. B.: Treatment of Sexual Underdevelopment with Synthetic Male Hormone Substance, Endocrinology **21**: 649-654 (Sept.) 1937. (*b*) Eidelsberg, J., and Ornstein, E. A.: Observations on the Continued Use of Male Sex Hormone Over Long Periods of Time, ibid. **26**: 46-53 (Jan.) 1940.

18. Kenyon, A. T.: The Effect of Testosterone Propionate on the Genitalia, Prostate, Secondary Sex Characters, and Body Weight in Eunuchoidism, Endocrinology **23**: 121-134 (Aug.) 1938.

19. Deanesly, R., and Parkes, A. S.: Factors Influencing the Effectiveness of Administered Hormone, Proc. Roy Soc., London, s. B **124**: 279-298 (Dec. 7) 1937.

20. Hamilton, J. B., and Dorfman, R. I.: Influence of the Vehicle upon the Length and Strength of the Action of Male Hormone Substance, Testosterone Propionate, Endocrinology **24**: 711-719 (May) 1939.

21. Vest, S. A., and Howard, J. E.: Clinical Experiments with Androgens: IV. A Method of Implantation of Crystalline Testosterone, J. A. M. A. **113**: 1869-1872 (Nov. 18) 1939.

given of the important matter of the surface area of the pellets. We have observed satisfactory stimulation in the eunuch following implantation of 4 pellets of testosterone each of which is 5 mm. in diameter and 7 mm. in length, with a total surface area of about 750 sq. mm. and a total weight of 960 mg. The dangers in treatment with even small pellets are apparent, and it is regrettable if women or other persons who do not require intensive treatment are subjected to long-continued influence of androgenic substances.

On oral administration, testosterone propionate is absorbed from the gastrointestinal tract [22] and a degradation product, androsterone, is excreted by the kidney,[23] but the clinical benefit is slight.[22] Apparently such substances are inactivated by the liver.[24] Methyltestosterone by mouth exerts an androgenic influence but curiously produces only minor elevation of the titer of urinary androgenic activity.[25] Quantities about three to four times greater in weight than those of testosterone propionate given intramuscularly are necessary to induce somewhat equivalent androgenic effects. Per unit of weight methyl testosterone for oral use is not as expensive as androgen prepared in sterile oil vehicles and can be taken without inconvenience by the patient; these features serve to counterbalance the expense of the larger amounts of androgens required in oral than in intramuscular administration.

Androgens secreted by the testes and those absorbed from intramuscular or subcutaneous routes of administration are favored by pathways of blood flow to escape for some time the influence of the liver. The use of rectal suppositories emphasizes the expected difficulties intrinsic in this mode of administration.

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22. Dorfman, R. I., and Hamilton, J. B.: Urinary Excretion of Androgenic Substances After Intramuscular and Oral Administration of Testosterone Propionate to Humans, J. Clin. Investigation **18**: 67-71 (Jan.) 1939.

23. (*a*) Callow, N. H.: The Isolation of Two Transformation Products of Testosterone from Urine, Biochem. J. **33**: 559-564 (April) 1939. (*b*) Dorfman, R. I.; Cook, J. W., and Hamilton, J. B.: Conversion by the Human of the Testis Hormone, Testosterone, into the Urinary Androgen, Androsterone, J. Biol. Chem. **130**: 285-295 (Sept.) 1939.

24. Biskind, G. R., and Mark, J.: The Inactivation of Testosterone Propionate and Estrone in Rats, Bull. Johns Hopkins Hosp. **65**: 212-217 (Aug.) 1939. Pfeiffer, C. A.: Sexual Differences of the Hypophyses and Their Determination by the Gonads, Am. J. Anat. **58**: 195-225 (Jan.) 1936.

25. Dorfman, R. I., and Hamilton, J. B.: Concerning the Metabolism of Testosterone to Androsterone, J. Biol. Chem. **133**: 753-760 (May) 1940.

Percutaneous administration has been publicized, but information is lacking concerning proper doses and intervals between applications that would maintain fairly constant levels of the drug in body tissues and fluids. The simplicity of this method of application, which permits self treatment by the patient, admits by the same expedient that control of the medication is subject to the whims of the patient.

Results from the Administration of Testosterone Propionate.—Pronounced masculinization and disappearance of many of the phenomena characteristic of testicular insufficiency are obtained [26] even in persons castrate for more than two decades or in those whose eunuchoid state has persisted until the middle years of life. Within an hour there are changes in the blood volume and pigments of the skin.[12] Erectile ability may be enhanced, often within a matter of several hours, and spontaneous erections are particularly frequent during the first days after the beginning of treatment.

The genitalia, with the exception of the testes, are stimulated to considerable development. In the eunuchoid man the amount of growth is major, but with severe eunuchoidism, especially in older persons, long-continued treatment is required before the genitalia attain a size approaching that of normal men.

The levels of androgenic substances in the urine are elevated and may be within the range found in normal men.[22] This is largely due to the presence of androsterone.[27] Body weight may be increased by several pounds. In 4 eunuchoids [28] 1 to 4.5 Gm. of nitrogen were retained per day, the decreased level of urinary nitrogen being reflected in the urea fraction, without evidence of change in the nitrogenous components of the blood; there was also retention of urinary sodium, amounting to from 0.33 to 0.55 Gm. daily, and usually of chloride. Muscular development and in some men strength are markedly increased.

The skin becomes flushed and of a darker color. Pigmentary abnormalities give way to a trend in the

26. Foss, G. L.: Effect of Testosterone Propionate on a Post-Puberal Eunuch, Lancet **2**: 1307-1309 (Dec. 4) 1937. Kenyon and co-workers.[28] Hamilton.[27a]

27. Callow.[22a] Dorfman and others.[22b] Dorfman and Hamilton.[22]

28. Kenyon, A. T.; Sandiford, I.; Bryan, A. H.; Knowlton, K., and Koch, F. C.: The Effect of Testosterone Propionate on Nitrogen, Electrolyte, Water and Energy Metabolism in Eunuchoidism, Endocrinology **23**: 135-153 (Aug.) 1938.

direction of the type and amount found in normal men. Thus there is increase in the volume of blood (save in areas like the buttocks), in the percentage of oxyhemoglobin and to a lesser extent in the amount of melanin and related substances.[12]

Increased oiliness of skin and hair becomes noticeable. In many but not all patients an acneform response appears after a latent period of some weeks.[13]

Growth of secondary sexual hair is rapid, the beard becoming stiffer within a few weeks, the axillary and pubic hair more coarse. Later the trunk and limbs acquire long thick hairs.

The vocal range and pitch of the eunuchoid approach those of the mature man. In the postpuberal castrate the pitch used in speaking may be lowered a tone or so and the voice sound hoarse. The mucous membrane of the larynx appears congested and rough, but the change in vocal pitch and range in the eunuchoid can ensue without marked prominence of the tracheal cartilage or great enlargement of the laryngeal cartilages.

The testes do not assume normal function. Testosterone affords essentially substitution therapy so that administration of the substance must be continued if the results are to be maintained. Maturation of voice and to some extent of the accessory reproductive organs is retained, but otherwise regressive changes ensue on cessation of the medication. Obviously, the cost of continued replacement therapy is a matter of deep concern.

OTHER STATES OF TESTICULAR DYSFUNCTION

Cryptorchism.—Despite evidence to the contrary from short time experiments,[29] it appears that in long-continued cryptorchism less than normal amounts of androgens are produced,[30] but this is scarcely the main issue. More pertinent are the facts that the later stages of spermatogenesis do not ensue properly in testes not

29. Moore, C. R., and Gallagher, T. F.: Seminal-Vesicle and Prostate Function as a Testis-Hormone Indicator: The Electric Ejaculation Test, Am. J. Anat. 45: 39-69 (Jan.) 1930. Jeffries, M. E.: Hormone Production by Experimental Cryptorchid Rat Testes as Indicated by the Seminal-Vesicle and Prostate-Cytology Tests, Anat. Rec. 48: 131-139 (Jan. 25) 1931.

30. Nelson, W. O.: Some Factors Involved in the Control of the Gametogenic and Endocrine Functions of the Testis, Cold Spring Harbor Symposia on Quantitative Biology, Cold Spring Harbor, L. I., New York, The Biological Laboratory 1937, vol. 5, pp. 123-135.

in the scrotum [31] and that approximately 11 per cent of all testicular tumors are found in retained testes.[32] Since the incidence of undescended testes in the adult male population is about 0.23 per cent, the correlation of tumor and imperfect descent is about forty-eight times greater than expected from chance association. There is no proof that the ectopic position is responsible for the high incidence of tumors in ectopic testes, but because of improper function, an untoward environment and the possibility of unobserved development of tumor, it is desirable that ectopic testes be transferred to the scrotum.

False versus True Retention: Intermittent retraction of the testicle continues to be commonly confused with true retention. This wrongly diagnosed condition would respond to any form of treatment, even to injection of saline solution or to mere choice of a more propitious moment for the examination. One cannot but be skeptical of optimistic reports of cases in which the testes descended in from three hours to three days, an alacrity that would put to shame a fast-growing tumor. Adoption of the following technic, full details of which are given elsewhere,[14] or of some similar method would allow differentiation between false and true retention and would provide a basis for comparison of the results in cases reported by different investigators:

The subject is placed as much at ease as possible while casual observation and brief palpation for the testes are done. A hot water bag wrapped in a single layer of flannel and containing water at about 115 F. is then put on the scrotum, groin and perineum. The patient is covered with blankets sufficient to insure warmth. Children in particular are reassured by the painlessness of the mock examination, and they can be examined thoroughly after the heat has been applied for thirty minutes to induce relaxation of those muscles in the scrotum and groin which cause retraction of the testis.

A carefully taken history and the status of scrotal development provide valuable confirmatory evidence.

31. Fukui, N.: On the Action of Heat Rays upon the Testicle: An Historical, Hygienic and Endocrinological Study, Acta scholae med. univ. imp. in Kioto **6**: 225-258, 1923. Moore, C. R.: Properties of the Gonads as Controllers of Somatic and Psychical Characteristics: VIII. Heat Application and Testicular Degeneration; the Function of the Scrotum, Am. J. Anat. **34**: 337-357 (Nov.) 1924.

32. Gilbert, J. B., and Hamilton, J. B.: Incidence and Nature of Tumors in Ectopic Testes, Surg., Gynec. & Obst. **71**: 731-743 (Dec.) 1940.

Correction of True Retention: Recognized procedures include the time-honored reliance on nature, the administration of endocrine products and orchiopexy. Reliance on nature to effect descent of the testes has in its favor tradition and the avoidance of rash procedures but has been criticized on the ground that, if descent is not spontaneous, irremediable changes may occur before corrective measures are applied. A final decision cannot be made in view of the fact that there are at present no thoroughly reliable data regarding the incidence of descent at puberty in cases of true cryptorchism. The available records are open to criticism on the ground that they include intermittently retracted testes. A well considered and substantiated body of opinion among surgeons, endocrinologists and pediatricians is necessary before the final choice is made between waiting a few years for the effect of natural puberty and alternative use at an early date of aggressive measures. It is urged that in the preparation of data a distinction be made between unilateral and bilateral cryptorchism, since in cases in which one testis has descended satisfactorily a state of endocrine deficiency, such as hypothyroidism, has been in my experience less frequent than in bilateral retention.

The commonly used endocrine preparations are chorionic gonadotropins, urinary extracts possessing luteinizing activity in the female. Enthusiastic reports have been made since these substances were first employed by Shapiro [33] and the method demonstrated experimentally in monkeys by Engle.[34] Critical study has shown that about one of every five retained testes responds to this treatment.[35] Androgens have been tested as an alternate choice, since presumably a gonadotropin exerts some of its actions by stimulating testicular secretion and since certain of the effects produced with luteinizing preparations can be duplicated with androgens. The results are discouraging,[36] how-

33. Shapiro, B.: Klinische Studien über die Wirkung des Hypophysenvorderlappens auf den männlichen Genitalapparat, Ztschr. f. klin. Med. **114**: 610-622, 1930.

34. Engle, E. T.: Experimentally Induced Descent of the Testis in the Macacus Rhesus Monkey by Hormones from the Anterior Pituitary and Pregnancy Urine, Endocrinology **16**: 513-520 (Sept.-Oct.) 1932.

35. Thompson, W. O., and Heckel, N. J.: Undescended Testes: Present Status of Glandular Treatment, J. A. M. A. **112**: 397-403 (Feb. 4) 1939.

36. Hamilton, J. B., and Hubert, G.: Effect of Synthetic Male Hormone Substance on Descent of Testicles in Human Cryptorchidism, Proc. Soc. Exper. Biol. & Med. **39**: 4-5 (Oct.) 1938.

ever, indicating definitely that the administration of androgens is of little value in producing descent of testes.

In my opinion, endocrine substances should be expected, on rational grounds, to effect descent only in instances in which the body levels of these substances are low and there is no mechanical adhesion or other permanent barrier to progression of the testicle. The advisability of their use before the age of puberty must be evaluated with regard to the function and fate of the ectopic testis (with the realization, however, that the high incidence of testicular tumors is not found until some time after puberty), the dangers of excessive and long-continued administration of powerful endocrine compounds and the comparative effectiveness of exogenous and endogenous gonadotropins. Some surgeons are of the opinion that under endocrine stimulation the testes descend or, failing that, the scrotum and cord structures are stimulated to such growth that orchiopexy is more easily accomplished. In any selection of methods of choice in the management of testicular retention, data furnished by the surgeons must weigh heavily, since cryptorchism remains, despite some enthusiasm for treatment with endocrine products, primarily and in most cases a surgical problem.

Sterility.—For purposes of brief discussion, sterility may be subdivided into factors related to spermatogenesis and factors related to transportation and deposition of sperm in the female reproductive tract.

Spermatogenesis is dependent on gonadotropic stimulation by secretions of the anterior lobe of the pituitary, residence of the testes in a site like the scrotum and satisfactory vascularization. It is now believed that spermatozoa capable of fertilizing human ova under normal conditions generally have at least a minimal count [37] per unit volume and only a certain proportion of abnormal forms.[38]

In the rat, spermatogenesis can be maintained by androgens and stimulated or maintained by gonado-

37. Hotchkiss, R. S.; Brunner, E. K., and Grenley, P.: Semen Analyses of Two Hundred Fertile Men, Am. J. M. Sc. **196**: 362-384 (Sept.) 1938.

38. Moench, G. L.: The Relation of Certain Seminal Findings to Fertility, with Special Reference to Sperm Concentration and the Significance of Testicular Epithelial Cells in Semen, Am. J. Surg. **47**: 586-596 (March) 1940.

tropins. In the primate, however, spermatogenesis is more difficult to control.[39] Claims have been advanced that under certain optimum conditions gonadotropins from urine [40] and androgens [41] can increase the number of spermatozoa in man. It would appear, however, that with androgens the number of spermatozoa is decreased rather than increased [42] and that no endocrine substance now available has been proved adequate for the stimulation of spermatogenesis in the eunuchoid man. The somewhat negative observations with present technics argue only for conservative methods of treatment and do not preclude the possibility that in the future the use of endocrine substances will be applicable to the problem.

Difficulty of intromission is common, although in many of the purported cases it is questionable that the interest is solely that of fertility. Testosterone propionate increases the capacity for erections in men with deficient testicular secretions, but the ability to obtain erections depends on more than the presence in the body of androgens. Vigorous erections have been observed repeatedly in persons with pronounced organic and functional signs of testicular insufficiency and with undisputedly low titers of urinary androgenic activity. Moreover, even though the elicitation of erections in the young and the middle-aged castrate or eunuchoid man is abetted by androgens, it is not to be assumed that, because of the inexorabilities of age, old men present a comparable situation of testicular insufficiency. Of old men with the symptom complex of benign hypertrophy of the prostate who were given testosterone propionate in trial therapy, the capacity for erection was not augmented in all, and when present it proved to be distracting and ill adapted to the needs of both husband and wife.

39. Smith, P. E.: Comparative Effects of Hypophysectomy and Therapy on the Testes of Monkeys and Rats, in Brouha, L.: Les hormones sexuelles, Paris, J. Hermann, 1938, pt. 3, pp. 201-208.

40. Huberman, J.; Israeloff, H. H., and Hymowitz, B.: Effects on Spermatogenesis of a Follicle-Stimulating Extract Obtained from Menopausal or Castrate Urines, Endocrinology **21**: 67-71 (Jan.) 1937. Heckel, N. J.: The Gonadotropic and the Gonadotropic-like Factor in the Treatment of Male Sterility, ibid. **22**: 111-114 (Jan.) 1938.

41. Rubinstein, H. S.: The Induction of Sexual Maturity in the Genitally Hypoplastic Adult Through the Use of Testosterone Propionate, J. A. M. A. **111**: 1818-1821 (Nov. 12) 1938. Rubinstein, H. S., and Kurland, A. A.: Effect of Testosterone Propionate on Spermatogenesis in Human, South. M. J. **32**: 499-503 (May) 1939.

42. Heckel, N. J.: The Influence of Testosterone-Propionate upon Benign Hypertrophy and Spermatogenesis: A Clinical and Pathological Study in the Human, J. Urol. **43**: 268-308 (Feb.) 1940.

. *Precocious or Abnormal Function of the Testis.*—Hypersecretory states of many endocrine glands have been recognized, but thus far not of the testis—even though in the past few years such states might have been simulated as a result of the administration of large amounts of crystalline androgens to patients. Abnormal and precocious function of the testis is recognized. Certain testicular tumors produce enormous amounts of gonadotropic substances,[48] which serve for diagnosis and for ready identification of the presence and functional state of metastases. With more complete study of cases of abnormal testicular function, it may be that, in addition to precocious function, in which the effects seem to be largely due to stimulation by androgens at an early age, hypersecretory states will be distinguished. It must be recalled, however, that the human body possesses a great capacity for rapid inactivation of large amounts of androgenic substance.[22]

States in Which Dysfunction of the Testis Is Dubious or Unproved and Conditions in Which Androgens Have Been Tried Because of Pharmacologic Actions.—Perhaps the most widely known trial of testosterone for its pharmacologic actions unassociated with testicular functions is that in certain gynecologic disorders, but testosterone has also been employed in men in whom testicular insufficiency was questionable. The relation of the prostate to testicular secretions and the reactions of this gland to both estrogens and androgens led inevitably to tests of the usefulness of testosterone therapy in men with benign hypertrophy of the prostate. In evaluating claims of beneficial results it must be remembered that spontaneous improvement may occur and that androgens induce in some persons a sense of well-being entirely apart from the capacity to pass a stream of urine. Improvement in control of the urinary stream during administration of testosterone may not be due necessarily to any decrease in the size of the prostate and may, in a few instances, be accompanied by an apparent increase in the size of the prostate.[44] Further study would be more enlightening if in

43. Ferguson, R. S.: Quantitative Behavior of Prolan A in Teratoma Testis, Am. J. Cancer **18**: 269-295 (June) 1933.

44. Hamilton, J. B., and Gilbert, J.: New Conception of Etiological Factors in the Production of Symptoms Observed in Benign Prostatic Hypertrophy: Mechanism of Control by Male Hormone Substance, Tr. West. Branch Soc., Am. Urol. A. **7**: 144-145, 1938.

reports on the treatment of patients with the symptoms of benign hypertrophy of the prostate investigators would state not just the percentage of patients benefited but also the extent to which the claimed improvement correlates with the urologic condition and its changes, if any, in each patient.

The development of muscle and greater dynometric strength when the castrate is given androgen and, indeed, the difference in this regard between the average man and woman have suggested the trial use of androgens in certain types of muscular weakness. In 2 men who had myotonia atrophica and genital atrophy a limited amount of improvement was obtained on administration of testosterone,[45a] viz., a status approximately 25 per cent of that of the normal male. Regression followed discontinuance of treatment. No report has appeared in which androgens have been shown to be of value in the correction of muscular conditions not accompanied by diminished testicular secretion.

Material acceleration of body growth has been obtained by the use of androgens in persons whose epiphyses are not closed.[45b,c] Observations in this regard are too scanty as yet to allow definition of the most advantageous procedures with regard to dosage, duration of treatment and suitable types of cases and the possibility that overdosage may produce inhibition of growth.

The interrelations of sexual and behavioral spheres have already induced studies of the use of testosterone in various psychiatric conditions. In this regard it is unfortunate that the concept of rejuvenation has appealed to some investigators. Careful examination is necessary to distinguish any direct influence on behavior from effects due to recognition of the organic and functional changes produced by the androgen. One cannot but urge that controlled experiments be made, including the injection of plain oil and the withholding from the patient of information that the drug is a "sex hormone."

45. (*a*) Hesser, F. H.; Langworthy, O. R., and Vest, S. A.: Muscle Strength in Myotonia Atrophica (Dystrophia Myotonica) Improved by Testosterone Propionate, Endocrinology **26**: 241-243 (Feb.) 1940. (*b*) Rapfogel, I.: The Effect of Testosterone Propionate upon Skeletal Development of a Eunuch, ibid. **27**: 179 (Aug.) 1940. (*c*) Shay, H.; Gershon-Cohen, J.; Paschkis, K., and Fels, S. S.: Influence of Testosterone Propionate on Somatic Growths in the White Rat, ibid. **28**: 877 (June) 1941.

The effects of testosterone on vascular dynamics will undoubtedly lead to trials of this substance in certain of the peripheral vascular diseases.

With respect to the states in which testicular insufficiency is dubious and androgens are utilized for their pharmacologic actions, recommendation must be withheld. These matters require further and critical study. It is probable that in conditions in which the use of testosterone may, perhaps, eventually be acceptable, no sweeping utility is to be expected; for example, only a selected type of muscular weakness would be benefited.

LIMITATIONS, CONTRAINDICATIONS AND DANGERS

The foregoing list of phenomena influenced in sundry manners by androgens is limited by space and by the boundaries of present knowledge, but it serves to indicate the widely ramifying influence of these substances on bodily economy and to give sober reflection to the physician who might feel constrained to use carelessly the active and valuable androgens now available.

The use of large doses in a child can result in closure of the epiphyses and in development of male secondary sexual characters. In the sexually mature person exogenous androgen depresses reproductive functions, such as spermatogenesis [42] and gonadotropic secretion in the pituitary.[46]

If large doses are employed in women, masculinization results. This includes hirsutism, hoarseness and deepening of the voice, interruption of reproductive function and growth of the clitoris and of the body musculature. These changes seem to be largely temporary, but permanent modification is obtained experimentally if the female is subjected to the influence of much androgen at an early age, especially prenatally.[47]

The temporary use of endocrine preparations does not stimulate an endocrine gland to function thereafter of its own accord. Testosterone is used in replacement therapy or for its pharmacologic actions. Treatment is often long continued, and the cost of the therapy is a major consideration.

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46. Hamilton, J. B., and Wolfe, J. M.: The Effect of Synthetic Androgen upon the Gonadotropic Potency of the Anterior Pituitary, Endocrinology **22**: 360-365 (March) 1938.

47. Greene, R. R., and Ivy, A. C.: The Experimental Production of Intersexuality in the Female Rat with Testosterone, Science **86**: 200-201 (Aug. 27) 1937. Dantchakoff, V.: Sur la faculté des tissus induits par l'hormone mâle, d'édifier de nouvelles structures chez l'embryon de cobaye femelle, Compt. rend. Soc. de biol. **124**: 516-518, 1937.

There is latent danger in that conditions which need immediate attention of a specialized nature may be allowed to continue untreated because the patient experiences a sense of well-being on receiving testosterone. Moreover, euphoria is not uncommon and should be guarded against by strict insurance that the patient gets rest and does not overexert. Stimulation of an older man with androgens may cause him to feel younger and to attempt to lead the life of a younger man. The situation is to some extent like that of pouring new wine into old bottles.

CHAPTER XVIII

THE FUNCTION OF THE ADRENAL CORTEX

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When the adrenal glands are removed from laboratory animals, symptoms of deficiency develop, which may be summarized as follows: The first notable change is usually a loss of appetite, which is soon followed by nausea, vomiting, increased peristalsis and eventually bloody diarrhea. Associated with these changes are profound weakness of the muscles and a listless stupor, or, in some animals, restlessness and marked salivation, with clonic movements and general convulsions similar to those observed in hypoglycemia induced by insulin. There is a gradual decrease in body temperature and a decrease in the basal metabolic rate.

Continuance of the state of adrenal deficiency is invariably associated with a decrease in blood pressure to the death level, an increase in the hematocrit reading and a progressive decrease in the volume of the circulating blood. Soon after removal of the adrenal glands there is a marked and continuous increase in the concentrations of nonprotein nitrogen and potassium and a decrease in the concentrations of sodium and chloride in the blood serum.

The concentration of dextrose in the blood and of glycogen in the liver depends on whether the animal ingests food. With a daily intake of food the blood sugar may be within normal limits, but if an adrenalectomized animal does not eat, the blood sugar level drops rapidly and glycogen disappears from the liver.

The administration of an extract from the adrenal cortex promptly reverses the trend of the changes outlined. After a short interval the profound weakness and prostration are relieved, and within a few hours the concentrations of sodium and chloride increase. The potassium and nonprotein nitrogen in the serum decrease more slowly to normal. If the deficiency has

been allowed to progress nearly to the point of death, treatment with an extract from the adrenal cortex for several days may be required before the appetite returns to normal and the animal can be considered fully restored.

One of the most interesting and important observations is that of the influence of sodium and potassium salts. The administration of potassium salts rapidly aggravates the condition and may cause the death of the adrenalectomized animal. The administration of a diet high in sodium chloride and sodium citrate or sodium bicarbonate with a low content of potassium has such a beneficial influence that the amount of extract of adrenal cortex required may be greatly reduced, and eventually the animal may be maintained in a normal condition without the administration of any cortical substance.

Although the concentrations of the constituents of the blood in such an animal may be within normal limits, and although the appetite, weight and body temperature and the general condition may be normal, the animal cannot withstand stress and may rapidly succumb to a high intake of potassium salts, a low environmental temperature, violent exercise or, in particular, a sudden withdrawal of sodium salts from the diet.

After the successful preparation of an extract of adrenal cortex which would maintain the life of adrenalectomized animals, investigators immediately became interested in a study of its mode of action. A wide divergence of opinion soon became evident. The regulation of carbohydrate metabolism as the prepotent action of the adrenal was suggested and vigorously upheld. Changes in blood pressure and in the volume of the blood and the distribution of electrolytes and water seemed of primary importance to another group. A possible relation between the adrenal cortex and mineral metabolism, including renal function and the excretion of sodium and potassium, was investigated by still others.

Early in the investigation Hartman and his associates[1] suggested the name "cortin" for the hormone of the adrenal cortex, and until quite recently all

1. Hartman, F. A.; Brownell, K. A.; Hartman, W. E.; Dean, G. A., and MacArthur, C. G.: The Hormone of the Adrenal Cortex, Am. J. Physiol. 86: 353-359 (Sept.) 1928.

physiologic effects produced by an extract of the adrenal cortex were ascribed to the vital principle of the gland. In 1934 crystalline compounds were separated, and by 1938 several crystalline compounds, shown in the structural formulas, had been isolated, some of which showed marked physiologic activity. These compounds were designated as cortin-like, which indicated physiologic activity of the same quality but quantitatively inferior. The chemical investigation of the gland has now been carried to the point where the active extract can be separated into fractions either as crystalline compounds or as purified amorphous material, and the physiologic investigation of this series of individual compounds has raised an important question. Does the adrenal cortex elaborate one compound which can be regarded as the vital hormone of the gland, or does it prepare a number of hormones, each with a special and specific function? The results presented in this paper answer this question and indicate that no one compound can be regarded as the vital or essential hormone which can produce all the physiologic effects of the gland. Some of the physiologic activities which are influenced by the adrenal cortex are indicated in the following outline:

PHYSIOLOGIC ACTIVITIES AFFECTED BY THE ADRENAL CORTEX

The physiologic activities affected by the adrenal cortex are carbohydrate metabolism, capacity of muscle to respond to stimulation of (1) long duration and (2) short duration, distribution of electrolytes, renal function, growth of young animals (rats), atrophy of adrenal and thymus glands of normal rats, and resistance to stress.

The physiologic processes associated with the first four items can be considered in relation to the site of action and the compounds or fraction from the adrenal cortex as shown in the accompanying table. Britton and Silvette[2] have made an extensive study of the influence of the adrenal cortex on carbohydrate metabolism and have shown that glycogen in the liver and dextrose in the blood serum may be reduced to low levels after removal of the adrenal

2. Britton, S. W., and Silvette, H.: The Adrenal Cortex and Carbohydrate Metabolism, in Cold Spring Harbor Symposia on Quantitative Biology, Cold Spring Harbor, L. I., New York, The Biological Laboratory, 1937, vol. 5, pp. 357-359.

cortex. Long and Lukens,[3] Evans[4] and Grollman[5] and others tried to demonstrate an effect on carbohydrate metabolism by extracts of the adrenal cortex through the use of phlorhizin, hypophysectomized-depancreatized animals and partially depancreatized rats. Rats which were partially depancreatized when young might have glycosuria after they had increased in weight, since the small amount of pancreas remaining could not furnish sufficient insulin for the adult animal. If these rats were adrenalectomized the glycosuria disappeared, and if they were given sodium chloride or

Physiologic Processes Associated with Glyconeogenesis, Muscle Activity, Distribution of Electrolytes and Renal Function in Relation to the Site of Action and the Compound or Fraction from the Adrenal Cortex

Primary Physiologic Activity	Primary Site of Action	Compounds or Fractions from Adrenal Cortex
Glyconeogenesis	Liver	Corticosterone and its derivatives with an atom of oxygen on C_{11}
Muscle efficiency	Muscles	
Distribution of electrolytes	Extracellular fluid Intracellular fluid	Desoxycorticosterone
Renal function	Kidney	Amorphous fraction

sufficient cortical substance to maintain life, glycosuria did not reappear. At the time these experiments were carried out by Long and others, excessive amounts of cortical extract were not administered, since it did not appear necessary to give more than was required to maintain life. Finally Long, Fry and Thompson[6] showed that pure crystalline compound B, or corticosterone, produced glycosuria in partially depancreatized rats, and that the same results could be produced with

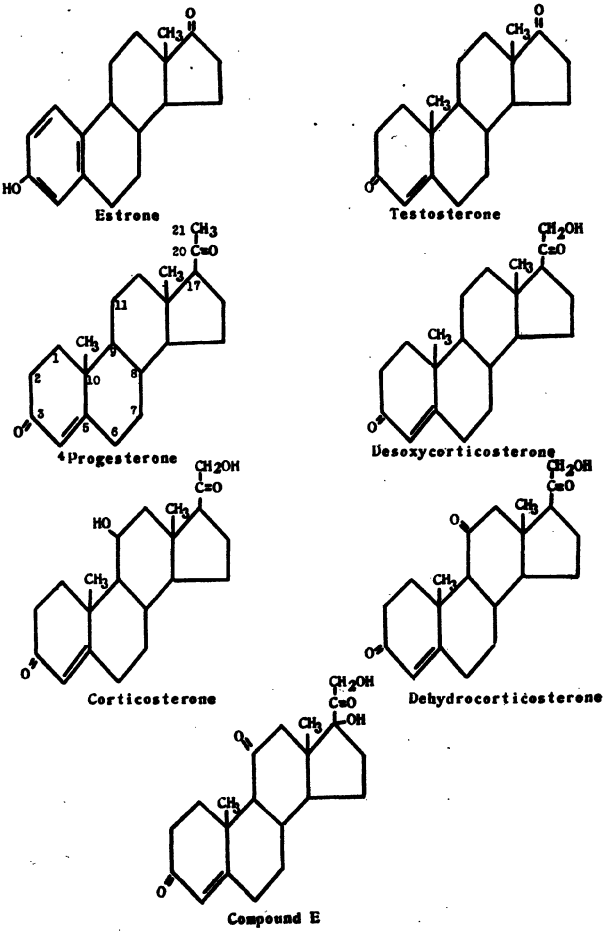
3. Long, C. N. H., and Lukens, F. D. W.: The Effects of Adrenalectomy and Hypophysectomy upon Experimental Diabetes in the Cat, J. Exper. Med. **63**: 465-490 (April) 1936. Long, C. N. H.: Studies on the "Diabetogenic" Action of the Anterior Pituitary, in Cold Spring Harbor Symposia on Quantitative Biology, Cold Spring Harbor, L. I., New York, The Biological Laboratory, 1937, vol. 5, pp. 344-353.

4. Evans, Gerald: The Adrenal Cortex and Endogenous Carbohydrate Formation, Am. J. Physiol. **114**: 297-308 (Jan.) 1936.

5. Grollman, Arthur: The Relation of the Adrenal Cortex to Carbohydrate Metabolism, Am. J. Physiol. **122**: 460-471 (May) 1938.

6. Long, C. N. H.; Fry, E. G., and Thompson, K. W.: The Effect of Adrenalectomy and Adrenal Cortical Hormones upon Pancreatic Diabetes in the Rat, Am. J. Physiol. **123**: 130 (July) 1938.

large amounts of an extract from the adrenal cortex. No distinction was made by Long and his associates between the effect of the crystalline compound B and that of the whole extract. The evidence indicated that



Structural formulas of adrenal compounds and of chemically related steroids.

larger amounts of the whole extract were required to produce glycosuria than to maintain life, but this conclusion suggests the question whether the maintenance of life and the effect on carbohydrate metabolism are

both due to the action of one vital hormone. This is one interpretation.

Another interpretation is that the adrenal cortex produces a series of compounds, some of which maintain life but have little effect on carbohydrate metabolism; other compounds have a marked effect on carbohydrate metabolism. In the extracts which were first used those compounds essential for maintenance of life were present in adequate amount, but there was a smaller quantity of those which affect carbohydrate metabolism. By the use of large amounts of extract a sufficient amount of the compounds that affect glyconeogenesis was given to produce glycosuria.

Long, Katzin and Fry [7] clearly demonstrated that the adrenal cortex increases the rate of glyconeogenesis and that the administration of pure crystalline compounds A, B and E resulted in the conversion of protein to carbohydrate. Recently Wells [8] has shown wide divergence in the response of the various fractions of the adrenal cortex in respect to glyconeogenesis. Marked activity is possessed only by those compounds in which an atom of oxygen is attached at C_{11}. Desoxycorticosterone is much less efficient, and Wells has now shown that the amorphous fraction has little activity in respect to the conversion of protein to carbohydrate.

In 1936 Ingle [9] published a method for the assay of cortical extracts based on the efficiency of muscles of adrenalectomized rats maintained with the extracts. The method has been used extensively during the past few years, and several compounds have been tested in regard to their effect on the efficiency of muscles stimulated over long periods. The work of Ingle [10] shows that only compounds which possess an oxygen atom at C_{11} have a favorable influence and that desoxycorticosterone and the amorphous fraction, although highly active in other respects, have little effect on the efficiency of muscle.

In the summer of 1938 desoxycorticosterone, furnished by Professor T. Reichstein, was standardized in

7. Long, C. N. H.; Katzin, B., and Fry, Edith G.: The Adrenal Cortex and Carbohydrate Metabolism, Endocrinology **26**: 309-344 (Feb.) 1940.

8. Wells, B. B.: The Influence of Crystalline Compounds Separated from the Adrenal Cortex on Gluconeogenesis, Proc. Staff Meet., Mayo Clin. **15**: 294-297 (May 8) 1940.

9. Ingle, D. J.: Work Capacity of the Adrenalectomized Rat Treated with Cortin, Am. J. Physiol. **116**: 622-625 (Aug.) 1936.

10. Ingle, D. J.: Personal communication to the author.

my laboratory and found to be at least six times more active than corticosterone in the maintenance of a normal level of blood urea and a normal condition in the adrenalectomized dog.[11] The observations of the early experiments have been confirmed and extended:[12] Desoxycorticosterone and its acetate will increase the concentration of sodium and decrease the concentration of potassium in the serum of normal rats more than any other product from the adrenal cortex.

At the same time Loeb and his associates[13] found that the concentration of potassium in the dog can be depressed so low that paralysis can be produced. These striking effects are qualitatively different from those of the amorphous fraction, which is also concerned with the distribution of inorganic ions and water but which does not modify the normal distribution of electrolytes in the blood serum.[12]

During the past several years the compound most active in the maintenance of life after adrenalectomy has been designated as the vital hormone, as the salt and water hormone, as the essential hormone and as cortin. Investigation of the amorphous fraction which is left after removal of the crystalline compounds has indicated that, although on the basis of weight it is many times more efficient than any other product from the adrenal cortex, it nevertheless has little effect on glyconeogenesis and on efficiency of muscle. To designate this fraction as the essential hormone, therefore, is misleading, but for maintenance of renal function, which appears to be its primary effect, it shows an extraordinary degree of activity. Between 1 and 2 micrograms per kilogram of body weight is sufficient for the daily dose of an adrenalectomized dog.

In 1930 Hartman and Thorn[14] showed that the growth of young rats was retarded by adrenalectomy, and they proposed a method for the standardization of extracts from the adrenal cortex on this basis. The

11. Reichstein, T., and von Euw, J.: Ueber Bestandteil der Nebennierenrinde. Isolierung der Substanzen Q (Desoxy-Corticosteron) und R sowie weiterer Stoffe, Helvet. chim. acta **21**: 1197-1210, 1938.

12. Wells, B. B., and Kendall, E. C.: A Qualitative Difference in the Effect of Compounds Separated from the Adrenal Cortex on Distribution of Electrolytes and on Atrophy of the Adrenal and Thymus Glands of Rats, Proc. Staff Meet., Mayo Clin. **15**: 133-139 (Feb. 28) 1940.

13. Kuhlman, Daniel; Ragan, Charles; Ferrebee, J. W.; Atchley, D. W., and Loeb, R. F.: Toxic Effects of Desoxycorticosterone Esters in Dogs, Science **90**: 496-497 (Nov. 24) 1939.

14. Hartman, F. A., and Thorn, G. W.: A Biological Method for the Assay of Cortin, Proc. Soc. Exper. Biol. & Med. **28**: 94-95 (Nov.) 1930.

method has since been used extensively to measure the activity of adrenal products, particularly by Grollman.[15] It will be considered in more detail later, but here one may state that various fractions separated from the adrenal cortex have been shown to exert widely varying effects on the rate of growth.[12]

When an extract of the adrenal cortex is given to normal rats, the adrenal and thymus glands become atrophied within a week or ten days.[16] Investigations of this response by administration of the various fractions from the adrenal have shown that the effect is highly specific.

Atrophy of the adrenal is produced by corticosterone and those compounds with an oxygen atom at C_{11}, but the same amount of desoxycorticosterone given over the same interval does not produce atrophy of this gland; the thymus may be slightly hypertrophied.[12] Little if any effect is produced by the administration of enormous amounts of the amorphous fraction free from corticosterone and related compounds. The differences in the physiologic response to the various fractions from the adrenal cortex are perhaps most marked in the effects on growth and on atrophy of the adrenal and thymus glands. It is obvious that these results could be obtained only after separation of the extract into the various constituents, since each fraction produces such highly specific qualitative effects.

RESISTANCE TO STRESS

The influence of the various compounds of the adrenal cortex on resistance to stress as given in the preceding outline has been investigated only in part. Little is known in regard to the specific action of the various compounds on bacterial toxins or on histamine. A recent article by Perla and his associates [17a] indicates the

15. Grollman, Arthur: The Comparative Activity of Desoxycorticosterone and Other Crystalline Derivatives and of Purified Extracts of the Adrenal Cortex, J. Pharmacol. & Exper. Therap. **67**: 257-264 (Nov.) 1939.

16. Ingle, D. J.: Atrophy of the Thymus in Normal and Hypophysectomized Rats Following Administration of Cortin, Proc. Soc. Exper. Biol. & Med. **38**: 443-444 (May) 1938. Ingle, D. J.; Higgins, G. M., and Kendall, E. C.: Atrophy of the Adrenal Cortex in the Rat Produced by the Administration of Large Amounts of Cortin, Anat. Rec. **71**: 363-372 (July 25) 1938.

17. (*a*) Perla, David; Friedman, D. G.; Sandberg, Marta, and Greenberg, S. C.: Prevention of Histamine and Surgical Shock by Cortical Hormone (Desoxycorticosterone Acetate and Cortin) and Saline, Proc. Soc. Exper. Biol. & Med. **43**: 397-404 (Feb.) 1940.

favorable influence of a combination of sodium chloride and desoxycorticosterone in protection against histamine. Sodium chloride alone was more effective than desoxycorticosterone alone, but the best result was produced by the simultaneous administration of sodium chloride and desoxycorticosterone.

An exudate produced by inflammatory tissue has been shown by Menkin [17b] to increase the permeability of capillaries. This investigator has recently found that an extract of the adrenal cortex will wholly prevent or in part inhibit this effect. The injections of the exudate and the extract in the skin can be at the same time as a mixture or the hormones of the adrenal cortex will protect the injected area if the extract is given several minutes or hours before the exudate.

Some preliminary experiments indicate that the compounds with an oxygen atom attached at C_{11} have a favorable action against the toxic effects of thyroxine in adrenalectomized rats. This work will be continued in order to demonstrate the effect of other fractions in regard to resistance to thyroxine.

Jensen and Grattan [18] have recently shown that corticosterone acetate will prevent convulsions after the injection of insulin. They also tried the whole extract, but since corticosterone acetate produced a favorable effect, it seems probable that corticosterone and related compounds in the whole extract are responsible for the increased resistance to insulin.

The striking response of adrenalectomized rats to phlorhizin when treated with various compounds and fractions of the adrenal cortex has been presented by Wells.[8] It is highly significant that in this experimental condition, which is a sensitive index for glyconeogenesis, those compounds with an oxygen atom on C_{11} will permit the animal to live with a markedly increased rate of glyconeogenesis. Desoxycorticosterone and the amorphous fraction are not sufficient for maintenance of life under these conditions. A high percentage of adrenalectomized rats given desoxycorticosterone and phlorhizin died in convulsions.

17. (*b*) Menkin, Valy: Effect of Adrenal Cortex Extract on Capillary Permeability, Am. J. Physiol. **129**: 691 (June) 1940.

18. Jensen, H., and Grattan, J. F.: The Identity of the Glycotropic (Anti-Insulin) Substance of the Anterior Pituitary Gland, Am. J. Physiol. **128**: 270-276 (Jan.) 1940.

Evans [19] has shown that when the percentage of oxygen in the inspired air was reduced glycogen was deposited in the livers of normal rats but not in the livers of adrenalectomized rats. Since compounds with an oxygen atom attached to C_{11} are involved in glyconeogenesis, it seems probable that these are the compounds responsible for the effects noted by Evans.

Selye and Schenker [20] have recently standardized the conditions first suggested by Hartman and his associates [21] with regard to the influence of low temperature on adrenalectomized rats. Since it seemed of interest to investigate this test by the use of purified crystalline compounds and the amorphous fraction, adrenalectomized rats were subjected to low temperature, and it was found that as little as 16 micrograms of corticosterone, about 20 micrograms of compound E and about 13 micrograms of the amorphous fraction were sufficient to maintain life.[22] This indicates that compounds with an oxygen atom on C_{11} and those found in the amorphous fraction were about equally active.

No experimental work has been carried out to determine the specificity of the various fractions with regard to the resistance of adrenalectomized rats to the injection of water.

STANDARDIZATION OF EXTRACTS OF THE ADRENAL CORTEX

The separation of the extract from the adrenal cortex into crystalline compounds and the amorphous fraction permits the identification of the compound responsible for the physiologic activity which has been the basis of each of the several methods that have been used for standardization. Among the many methods which have been proposed, six will be considered.

1. *Survival of Adrenalectomized Rats.*—This criterion is not specific for any compound or group of compounds. Even progesterone has been shown to

19. Evans, Gerald: The Effect of Low Atmospheric Pressure on the Glycogen Content of the Rat, Am. J. Physiol. **110**: 273-277 (Dec.) 1934.

20. Selye, Hans, and Schenker, Victor: A Rapid and Sensitive Method for Bioassay of the Adrenal Cortical Hormone, Proc. Soc. Exper. Biol. & Med. **39**: 518-522 (Dec.) 1938.

21. Hartman, F. A.; Brownell, Katherine A., and Crosby, A. A.: The Relation of Cortin to the Maintenance of Body Temperature, Am. J. Physiol. **98**: 674-686 (Nov.) 1931.

22. Kendall, E. C.: The Function of the Adrenal Cortex, Proc. Staff Meet., Mayo Clin. **15**: 297-304 (May 8) 1940.

increase the survival time of adrenalectomized ferrets,[23] and all of the compounds which possess any physiologic activity will permit the survival of adrenalectomized rats. The test does show the presence of some compound which can replace, at least in part, the secretion of the gland, but it is quite nonspecific for the identification of any particular compound.

2. *Growth of Young Rats.*—When the various fractions are administered to adrenalectomized young rats, those compounds with an oxygen atom on C_{11} retard or actually suppress the growth of the animals whereas the amorphous fraction will bring about an almost normal rate of growth, and desoxycorticosterone acetate may cause a gain in weight greater than that in a normal control.[24] Such a response is not a satisfactory criterion for the identification of any compound or group of compounds. With each solution tested, the rate of growth would be determined by the algebraic sum of the retarding influence of some of the compounds and the beneficial effects of others.

3. *Stimulation of Muscle.*—The Everse and de Fremery test [25] is based on the response of muscle to a short stimulation. The normal animal will react characteristically both as to the duration of time and as to the vigor of the response to the stimulation. Adrenalectomized rats either will not respond at all or will respond in a much more limited way before the muscle is exhausted. It has been found that desoxycorticosterone acetate produces an effect about ten times greater than that of corticosterone.[11] Compound E has been shown to have little activity.[26] This criterion, therefore, has but little application, and a negative result does not mean lack of cortical activity, for activity may be indicated by other criteria.

4. *Prolonged Stimulation of Muscle.*—This test, which has been developed by Ingle [27] and is not related

23. Gaunt, Robert, and Hays, H. W.: The Life-Maintaining Effect of Crystalline Progesterone in Adrenalectomized Ferrets, Science **88**: 576-577 (Dec. 16) 1938.

24. Wells, B. B., and Kendall, E. C.: The Influence of Corticosterone and C_{17}Hydroxydehydrocorticosterone (Compound E) on Somatic Growth, Proc. Staff Meet., Mayo Clin. **15**: 324-328 (May 22) 1940. Wells and Kendall.[12]

25. Everse, J. W. R., and de Fremery, P.: On a Method of Measuring Fatigue in Rats and Its Application for Testing the Suprarenal Cortical Hormone, Acta brev. Neerland. **11**: 152-153, 1932.

26. Reichstein, T.: Ueber Bestandteil der Nebennierenrinde: VI. Trennungsmethoden, sowie Isolierung der Substanzen F. a. H und J, Helvet. chim. acta **19**: 1107-1126, 1936.

27. Ingle, D. J.: Work Performance of Adrenalectomized Rats Treated with Corticosterone and Chemically Related Compounds, Endocrinology **26**: 472-477 (March) 1940. Ingle.[9]

to the short stimulation of muscle, is highly specific for those compounds which have an oxygen atom attached to C_{11}. Compound E apparently is the most active; desoxycorticosterone manifests little activity, and the amorphous fraction in amounts many times that required for maintenance of a normal condition in an adrenalectomized dog is without effect. The high specificity of the compounds which have an oxygen atom attached to C_{11} suggests that this test may be used for the quantitative determination of this group of compounds.

5. *Resistance to Low Temperature.*—This criterion is nonspecific, since corticosterone, compound E and the amorphous fraction have all been shown to be about equally active.

6. *Maintenance of Adrenalectomized Animals.*—This method of standardization [28] is not the same as number 1, since survival is not the basis of the test. That amount of material is determined which is required to hold the constituents of the blood within a normal range and to maintain the appetite, weight and normal appearance of the animal. The test becomes highly specific for the amorphous fraction, since this substance will maintain an adrenalectomized dog in excellent condition when as little as 1 to 2 microns per kilogram of body weight is used. Desoxycorticosterone is very active when tested with this method; about 15 micrograms per kilogram of body weight is required to maintain the adrenalectomized dog in normal condition.

FUNCTION OF THE ADRENAL CORTEX

The quantitative investigation of the physiologic response to several compounds separated from the adrenal cortex clearly shows that no one compound can produce all of the known effects of the extract. For glyconeogenesis and maintenance of the efficiency of muscle, corticosterone, compound E and their derivatives with an oxygen atom on C_{11} are necessary. For the most marked effect on the distribution of electrolytes, desoxycorticosterone is required and for maintenance of normal renal function the amorphous fraction is the most efficient.

28. Pfiffner, J. J.: Swingle, W. W., and Vars, H. M.: The Cortical Hormone Requirement of the Adrenalectomized Dog, with Special Reference to a Method of Assay, J. Biol. Chem. **104**: 701-716 (March) 1934.

These three typical physiologic responses can all be produced, at least in part, by the single substance compound E. For this, however, 10 mg. is required in order to maintain normal renal function in the adrenalectomized dog.

If the structure of compound E is altered by the substitution of an atom of hydrogen instead of a hydroxyl group on C_{17}, the compound is dehydrocorticosterone. Only 2.5 mg. of this is required to maintain normal renal function, and it is about as active as compound E in its effect on glyconeogenesis and on the efficiency of muscle. It does not modify the normal concentration of electrolytes in the serum.

If the structure of dehydrocorticosterone is modified by substitution of two atoms of hydrogen instead of an atom of oxygen at C_{11}, the compound is desoxycorticosterone. Three-tenths mg. of this compound will maintain normal function of the kidney, but the effect on glyconeogenesis is very small, and the compound has little effect on the efficiency of muscle. Comparison of these three compounds shows how intimately the physiologic effects are related to the chemical structure.

The close connection between the many physiologic effects as well as the interrelation between carbohydrate metabolism and the distribution of water and inorganic ions is well shown by nonspecific therapy, such as that with dextrose and a diet which contains a low concentration of potassium and a high concentration of sodium chloride and of sodium bicarbonate or sodium citrate. With this nonspecific therapy and without any product of the adrenal cortex it is possible to maintain adrenalectomized dogs in normal condition so far as the concentration of electrolytes in the serum is concerned.[29] Glycogen in the liver and dextrose in the blood are held within normal limits by the carbohydrate in the food. Such animals are to all appearances normal, but they do not possess ability to withstand stress. If the administration of sodium chloride is stopped even for a short time, they rapidly pass into a condition of crisis from adrenal deficiency.

29. Kendall, E. C.: A Chemical and Physiological Investigation of the Suprarenal Cortex, in Cold Spring Harbor Symposia on Quantitative Biology, Cold Spring Harbor, L. I., New York, The Biological Laboratory, 1937, vol. 5, pp. 299-310.

Finally, it is possible to maintain dogs for at least seven days without food, provided sodium chloride is given in the drinking water and potassium salts are withheld. This result can be interpreted only by the primary importance of the regulation of the distribution and excretion of inorganic ions. If the changes in mineral metabolism, which have been well established, were secondary to disturbances in carbohydrate metabolism, it would be possible to maintain a normal condition by the administration of large amounts of dextrose, whether or not sodium chloride was supplied. It has been shown repeatedly by many investigators that this is not possible. On the other hand, when the loss of sodium and the retention of potassium in the adrenalectomized animal are corrected by the administration of large amounts of sodium chloride, glyconeogenesis can proceed at a rate sufficient to maintain the metabolism of carbohydrate within normal limits.

CONCLUSION

It has been shown that the adrenal cortex does not elaborate any single substance which can be described as the vital hormone of this gland. An extract of the adrenal cortex contains a surprisingly large number of closely related steroid derivatives which have specific effects, qualitatively different one from the other. Substitution therapy in adrenalectomized animals is inadequate unless the compounds which influence glyconeogenesis and the efficiency of muscles are given together with the compounds that influence renal function and the distribution of water and electrolytes.

CHAPTER XIX

ADRENAL CORTEX INSUFFICIENCY

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CLINICAL PICTURE OF ADRENAL CORTEX INSUFFICIENCY

The syndrome of adrenal cortex insufficiency was first described by Thomas Addison, in his classic treatise, published in 1855. This symptom complex as observed in Addison's disease is characterized by an insidious onset and progressive development of fatigability and asthenia to the point of utter exhaustion, associated with varying degrees of anorexia, nausea and vomiting. Very occasionally diarrhea and abdominal pain are present. The symptoms may appear in the course of weeks, months or even years. At times, however, the tempo of the disease may be greatly accelerated. This may occur either spontaneously or in response to a withdrawal of sodium salts from the diet, or in association with an acute infection, and may, in the course of a few days, terminate fatally. These abrupt episodes of adrenal insufficiency are termed "crises." In a few instances the symptoms and signs of hypoglycemia may dominate the clinical picture, particularly among patients receiving salt or desoxycorticosterone acetate therapy.

Pigmentation and arterial hypotension are the physical signs usually exhibited by patients with adrenal cortex insufficiency. The pigmentation is brownish and may be either diffuse or blotchy in distribution. It is most typically observed in the mucous membranes of the buccal cavity. However, there are patients in whom no pigmentation develops. Hypotension is almost invariably present among patients seeking medical aid, but under emotional stress the blood pressure may momentarily rise to normal or even to abnormal levels. During crises it falls to strikingly low levels, and the patient presents a picture of dehydration and shock.

PATHOLOGIC PHYSIOLOGY

In the past decade great advances have been made in the clarification of certain mechanisms involved in the development of adrenal cortex insufficiency. These have been discussed extensively elsewhere in this series, but, since they constitute the basis of present therapy, it is essential that those with clinical significance be considered briefly at this point.

Salt and Water Metabolism.—In 1927 Baumann and Kurland [1] and Marine and Baumann [2] showed that the sodium concentration in the blood of adrenalectomized cats was decreased and that a solution of sodium chloride prolonged life more effectively than did solutions of other electrolytes or of dextrose. In 1932 and 1933 it was shown by the writer [3] that the concentration of sodium in the blood of patients suffering from uncompensated adrenal cortex insufficiency was decreased and that replacement of the sodium ion relieved this condition. It was also shown that withdrawal of the sodium ion from the diet of patients with Addison's disease precipitated acute adrenal cortex insufficiency.[4] It was then observed in dogs that the loss of sodium following adrenalectomy results from increased excretion of sodium ions by the kidney.[5] These observations have been amply confirmed,[6] and Harrop and associates [7] and Allers [8] independently pointed out that

1. Baumann, E. J., and Kurland, S.: Changes in the Inorganic Constituents of Blood in Suprarenalectomized Cats and Rabbits, J. Biol. Chem. **71**: 281 (Jan.) 1927.

2. Marine, David, and Baumann, E. J.: Duration of Life After Suprarenalectomy in Cats and Attempts to Prolong It by Injections of Solutions Containing Sodium Salts, Glucose and Glycerol, Am. J. Physiol. **81**: 86 (June) 1927.

3. (*a*) Loeb, R. F.: Chemical Changes in the Blood in Addison's Disease, Science **76**: 420 (Nov. 4) 1932; (*b*) Effect of Sodium Chloride in Treatment of a Patient with Addison's Disease, Proc. Soc. Exper. Biol. & Med. **30**: 808 (March) 1933.

4. (*a*) Harrop, G. A.; Weinstein, Albert; Soffer, L. J., and Trescher, J. H.: The Diagnosis and Treatment of Addison's Disease, J. A. M. A. **100**: 1850 (June 10) 1933. (*b*) Harrop, G. A.: Diagnosis and Treatment of Addison's Disease, ibid. **101**: 388 (July 29) 1933. Loeb.[3b]

5. Loeb, R. F.; Atchley, D. W.; Benedict, Ethel M., and Leland, Jessica: Electrolyte Balance Studies in Adrenalectomized Dogs with Particular Reference to the Excretion of Sodium, J. Exper. Med. **57**: 775 (May) 1933.

6. Harrop, G. A.; Soffer, L. J.; Ellsworth, Read, and Trescher, J. H.: Studies on Suprarenal Cortex: III. Plasma Electrolytes and Electrolyte Excretion During Suprarenal Insufficiency in the Dog, J. Exper. Med. **58**: 17 (July) 1933. Harrop and others.[4]

7. Harrop, G. A.; Soffer, L. J.; Richardson, W. N., and Strauss, Margaret: Studies on the Suprarenal Cortex: IV. The Effect of Sodium Salts in Sustaining the Suprarenalectomized Dog, J. Exper. Med. **61**: 839 (June) 1935.

8. Allers, W. D.: Influence of Diet and Mineral Metabolism on Dogs After Suprarenalectomy, Proc. Staff Meet., Mayo Clin. **10**: 406 (June 26) 1935.

adrenalectomized dogs could be maintained in good health indefinitely if sodium salts were administered in sufficient amounts, particularly if the potassium content of the diet was reduced. A disturbance in water metabolism is intimately related to that of the concentration of sodium ions in adrenal cortex insufficiency. Thus, there is a tendency for water to be lost as the insufficiency progresses up to the point where a fall in blood pressure causes oliguria.[5] This is associated with a consistent decrease in plasma volume. However, water is not, as a rule, lost from the body as rapidly as is sodium. This is shown by the fact that the sodium concentration of the blood decreases. It has been shown that acute adrenal cortex insufficiency may be induced in a patient with Addison's disease by restricting the intake of fluid, despite the administration of salt. Under these conditions a crisis associated with a fall in blood pressure, collapse and a decrease in plasma volume without a decrease in blood sodium appeared in a patient studied by Willson and Sunderman.[9] A loss of sodium without a decrease in plasma volume, i. e., without a simultaneous loss of water, does not regularly give rise to the picture of adrenal cortex insufficiency.

Potassium Metabolism.—An increase in the concentration of the potassium ion was first observed in animals by Baumann and Kurland,[1] in 1927, and in human subjects with adrenal insufficiency by the writer in 1932.[3] This disturbance is closely related to disturbances in sodium and water metabolism. In general, as sodium is lost from the body, potassium in the blood serum tends to increase and the ability of the body to excrete potassium ions is impaired. Kendall and associates[10] and Zwemer and Truszkowski[11] emphasized the susceptibility of adrenalectomized animals to the injection or ingestion of potassium salts. Wilder and his collaborators[12] emphasized the danger attendant

9. Willson, D. M., and Sunderman, F. W.: Studies in Serum Electrolytes: The Effect of Water Restriction in a Patient with Addison's Disease Receiving Sodium Chloride, J. Clin. Investigation **18**: 35 (Jan.) 1939.

10. Allers, W. D.; Nilson, H. W., and Kendall, E. C.: Studies on Adrenalectomized Dogs: The Toxic Action of Potassium, Proc. Staff Meet., Mayo Clin. **11**: 283 (April 29) 1936.

11. Zwemer, R. L., and Truszkowski, R.: Potassium: A Basal Factor in the Syndrome of Cortico-Adrenal Insufficiency, Science **83**: 558 (June 5) 1936.

12. Wilder, R. M.; Kendall, E. C.; Snell, A. M.; Kepler, E. J.; Rynearson, E. H., and Adams, Mildred: Intake of Potassium: An Important Consideration in Addison's Disease: A Metabolic Study, Arch. Int. Med. **59**: 367 (March) 1937.

on the ingestion of large amounts of potassium salts by patients with Addison's disease. Despite the deleterious effects of the potassium ion in adrenal cortex insufficiency, the concept that death from this insufficiency results from potassium poisoning is untenable.

Renal Function.—Marshall and Davis [18] were the first to point out, in 1916, an increase in nonprotein nitrogen in the blood of adrenalectomized animals. In patients with adrenal cortex insufficiency an increase in nonprotein nitrogen, inorganic phosphorus and sulfate may occur. Furthermore, decreases in urea clearance may be present even in the absence of nitrogen retention. These disturbances have been shown by Stahl, Kuhlmann and Urban [14] to be associated with hemoconcentration and presumably with a decrease in renal blood flow. Observations on creatinine clearance in adrenal cortex insufficiency by Margitay-Becht and Gömöri [15] led to the same inference. The disturbances in electrolyte and water metabolism already discussed are to a large extent referable to a derangement of normal renal activity [16] other than that resulting in decreased renal blood flow. It seems probable that this derangement is related to tubular reabsorption.

Carbohydrate Metabolism.—Porges,[17] in 1909, was the first to report the presence of hypoglycemia in patients suffering from Addison's disease and in adrenalectomized animals. In recent years the studies of Britton and Silvette,[18] Long and his colleagues [19] and Kendall and his associates [20] have shown that a decrease

13. Marshall, E. K., Jr., and Davis, D. M.: The Influence of the Adrenals on the Kidneys, J. Pharmacol. & Exper. Therap. **8**: 525 (Sept.) 1916.

14. Stahl, Jules; Kuhlmann, D., and Urban, M.: A propos du mécanisme de l'insuffisance rénale au cours de l'insuffisance surrénalienne expérimentale, Compt. rend. Soc. de biol. **127**: 1286, 1938.

15. Margitay-Becht, A., and Gömöri, P.: Die Nierenfunktion bei der Addisonschen Krankheit, Ztschr. f. d. ges. exper. Med. **104**: 22, 1938.

16. Harrison, H. E., and Darrow, D. C.: Renal Function in Experimental Adrenal Insufficiency, J. Clin. Investigation **17**: 505 (July) 1938.

17. Porges, O.: Ueber Hypoglykämie bei Morbus Addison sowie bei nebennierenlosen Hunden, Ztschr. f. klin. Med. **69**: 341, 1909-1910.

18. Britton, S. W., and Silvette, Herbert: The Apparent Prepotent Function of the Adrenal Glands, Am. J. Physiol. **100**: 701 (May) 1932.

19. Long, C. N. H.; Katzin, B., and Fry, E. G.: The Adrenal Cortex and Carbohydrate Metabolism, Endocrinology **26**: 309 (Feb.) 1940.

20. (*a*) Kendall, E. C.: The Function of the Adrenal Cortex, Proc. Staff Meet., Mayo Clin. **15**: 297 (May 8) 1940. (*b*) Sprague, R. G.: The Influence of Extract of the Adrenal Cortex on Glycogenesis in Fasting Rats, Proc. Staff Meet., Mayo Clin. **15**: 291 (May 8) 1940. (*c*) Wells, B. B.: The Influence of Crystalline Compounds Separated from the Adrenal Cortex on Gluconeogenesis, Proc. Staff Meet., Mayo Clin. **15**: 294 (May 8) 1940.

in glycogen storage in the liver and in sensitivity to insulin are also characteristic of adrenal cortex insufficiency.[21] The disturbances in carbohydrate metabolism vary considerably in different species, and they vary greatly in intensity in persons with Addison's disease, but they may be of sufficient gravity to prove fatal. It is generally believed that the abnormalities of carbohydrate metabolism result from an increase in the utilization of carbohydrate and a decrease in gluconeogenesis.[22] The disturbances in carbohydrate metabolism as seen in Addison's disease vary independently of the disturbances in salt and water metabolism.[23] It has been amply established by the work of Long and associates,[19] Thorn,[24] Kendall [20a] and our own laboratory [25] that various steroids isolated from the adrenal cortex exhibit striking differences in their effect on carbohydrate and electrolyte metabolism.

Nitrogen Metabolism.—Nitrogen retention and a decrease in the excretion of ammonia appear in the development of severe adrenal cortex insufficiency and are probably in part due to decrease in renal blood flow. Following the administration of various extracts of adrenal cortex there is often a temporary increase in the urinary excretion of nitrogen perhaps due to improvement in renal function. On the other hand, it has been shown by Long and co-workers [19] and Kendall and his associates [20a, b] that the nitrogen excretion of diabetic rats or of fasting normal rats may be increased by the administration of certain adrenal steroids. This increase has been shown to be due to gluconeogenesis from body protein, which may be the primary effect, but which possibly arises in response to a decrease in the utilization of carbohydrate.

Adynamia.—This manifestation of adrenal cortex insufficiency, which plays a major part in the subjective manifestations of Addison's disease, has been exten-

21. This view of an intimate relation between carbohydrate metabolism and the function of the adrenal cortex has been amply confirmed and is contrary to that expressed by the writer in the first edition of this book.

22. Long and others.[19] Kendall and others.[20]

23. Loeb, R. F.; Atchley, D. W., and Parson, William: The Significance of Certain Chemical Abnormalities Found in the Blood in Addison's Disease, Tr. A. Am. Physicians **52**: 228, 1937.

24. Thorn, G. W.: Personal communication to the author.

25. Ferrebee, J. W.; Ragan, Charles; Atchley, D. W., and Loeb, R. F.: A Comparison of Certain Effects of Desoxycorticosterone Acetate, Corticosterone and Cortical Extract in a Patient with Addison's Disease, Endocrinology **27**: 360 (Sept.) 1940.

sively studied by Ingle [26] in rats. From the results of his work it is certain that adynamia results chiefly from the disturbances in carbohydrate metabolism and to a lesser extent from the loss of salt and water and decrease in blood flow. Whether or not other factors play a part is not known.

Arterial Hypotension.—The fall in blood pressure to shock levels during crises of adrenal cortex insufficiency is dependent to a large extent on the disturbances in salt and water metabolism. The chronic but milder degrees of hypotension may also be dependent on the same abnormality, since the blood pressure may be raised by the administration of sodium salts as well as by the administration of desoxycorticosterone acetate, which primarily affects the metabolism of salt and water. Whether other factors contribute to hypotension is not known.

Miscellaneous Disturbances.—Among these, pigmentation occupies a prominent position. This abnormality, although not universally present in Addison's disease, is extremely common, yet its mechanism remains totally obscure. Sensitivity to change in temperature, particularly to cold, and sensitivity to pain stimuli, which are characteristics of patients and animals in adrenal cortex insufficiency, may possibly be correlated with the disturbances in carbohydrate and in electrolyte metabolism, as indicated by the studies of Kendall.[20a] The moderate lowering of the basal metabolic rate occasionally observed is probably on a similar complex basis. The mechanisms acting in the production of hypercalcemia, elevation of serum phosphatase, retention of bromsulphalein and gynecomastia, all of which are sometimes present in addisonian patients, are not known. Focal and diffuse neurologic disorders in adrenal cortex insufficiency result probably from hypoglycemia or from decreased cerebral blood flow or from both.

In 1933 Swingle and collaborators [27] suggested that a hormone of the adrenal cortex had an effect on the peripheral vascular bed. The evidence provided was not convincing. However, the fact that an extract of the adrenal cortex may effect prompt relief in dogs suffer-

26. Ingle, D. J.: The Work Performance of Adrenalectomized Rats Treated with Corticosterone and Chemically Related Compounds, Endocrinology **26**: 472 (March) 1940.

27. Swingle, W. W.; Pfiffner, J. J.; Vars, H. M.; Bott, P. A., and Perkins, W. M.: The Function of the Adrenal Cortical Hormone and the Cause of Death from Adrenal Insufficiency, Science **77**: 58 (Jan. 13) 1933.

ing from severe adrenal cortex insufficiency without causing demonstrable changes in the blood volume or the electrolyte pattern [28] suggests the possibility of action either on tissue of the nervous system or perhaps on the capillaries. Furthermore, Menkin [29] has recently shown that extract of adrenal cortex inhibits the dilatation of capillaries which usually results from the cutaneous injection of leukotaxine or exudates. The significance of these observations in relation to adrenal cortex insufficiency is not yet apparent.

TREATMENT OF ADRENAL CORTEX INSUFFICIENCY

The treatment of adrenal cortex insufficiency and that of diabetes mellitus have much in common. In both diseases therapy should be directed toward (1) the specific replacement of the hormone or hormones lacking, (2) the correction of the physiologic disturbances resulting from hormonal deficiencies and (3) the avoidance of those factors known to intensify the abnormalities present. The treatment of adrenal cortex insufficiency as exemplified by Addison's disease is also analogous to that of diabetes in that often a few simple measures suffice to control the disease, whereas at times the failure to institute vigorous therapeutic measures promptly may result in a fatal outcome which might have been avoided. Thus, in a patient whose symptoms and signs of adrenal cortex insufficiency are mild, ingestion of a moderate amount of salt in addition to that present in the diet, moderate limitation of the potassium content of the diet, avoidance of extremes of heat and cold, as well as avoidance of mental and physical strain, may be adequate to maintain moderately good health for months or years. On the other hand, when a crisis develops either in the natural course of the disease or because of salt withdrawal, diarrhea, acute infection, surgical intervention or other factors, an immediate intensive attempt to restore base and water by all possible means becomes imperative to avoid fatal peripheral circulatory collapse.

In recent years the treatment of Addison's disease has been advanced materially through (1) the introduction of salt therapy, (2) the restriction of potassium

28. Stahl, Jules; Atchley, D. W., and Loeb, R. F.: Observations on Adrenal Insufficiency, J. Clin. Investigation **15**: 41 (Jan.) 1936.
29. Menkin, Valy: Effect of Adrenal Cortex Extract on Capillary Permeability, Am. J. Physiol. **129**: 691 (June) 1940.

salts, (3) the elaboration of extracts of the adrenal cortex and (4) the synthesis and availability of desoxycorticosterone esters. The role of each of these must be considered in detail.

Role of Salt and Diet.—With the ingestion of 7 to 20 Gm. of salt in addition to that of the diet, many patients with Addison's disease can be restored to moderately good health for months or years, as stated. Most of these patients take their salt in enteric-coated 1.0 Gm. tablets distributed at frequent intervals during the course of the day. Others prefer to take their salt in the form of an approximately 1 per cent solution. Only a few are able to tolerate capsules. Wilder and associates have suggested that part of the sodium be taken in the form of citrate or bicarbonate to avoid chloride retention. In the writer's experience this has rarely proved necessary.

It should be pointed out that before the advent of desoxycorticosterone many patients in severe addisonian crises recovered as a result of the parenteral administration of liberal amouts of physiologic solution of sodium chloride. This treatment not only restored base but also lowered the blood potassium and nonprotein nitrogen to normal, improved renal function, raised the blood pressure and relieved peripheral circulatory collapse.

Wilder and his associates [12] have properly advocated the restriction of potassium in the diet of the salt-treated patients with adrenal cortex insufficiency, and Sister Mary Victor [30] has carefully outlined the technic of the preparation of this type of diet.[31] In view of the capricious appetites of most patients with Addison's disease, the rigid restriction of the intake of potassium becomes more a theoretical than a practical consideration. In patients who cannot be maintained in good health through the ingestion of large amounts of salt and moderate restriction of the intake of potassium, treatment with desoxycorticosterone esters is indicated. The treatment of hypoglycemic episodes occurring in Addi-

30. Mary Victor, Sister: Directions for the Planning of Diets Low in Content of Potassium, Proc. Staff Meet., Mayo Clin. 12: 424 (July 7) 1937.

31. The following foods should be rigidly avoided: soups, broths, gravies, catsup, dried fruits and vegetables, bran and molasses. The consumption of milk, meat and leguminous vegetables should be moderately restricted. Meats and vegetables should be cut into small pieces and cooked in 6 to 8 volumes of water.

son's disease is best accomplished by the ingestion of a glass of orange juice or two lumps of sugar. If the patient is unable to cooperate, 50 cc. of a 25 per cent solution of dextrose may be given intravenously. Infusions of large amounts of dextrose are apt to give rise to hyperglycemia, followed in the course of two to four hours by a recurrence of hypoglycemia.

Desoxycorticosterone Acetate.—Since Steiger and Reichstein [32] synthesized desoxycorticosterone, this natural steroid of the adrenal cortex has become available for the treatment of patients and has initiated a new era in the therapy of adrenal cortex insufficiency. This particular substance, as has been suggested in another chapter, affects almost exclusively electrolyte and water metabolism, as has been demonstrated by the studies of Thorn and his associates,[33] and Cleghorn and his co-workers,[34] Levy Simpson [35] and Loeb and his collaborators [36] (shown in the accompanying chart). It causes striking retention of sodium salts with restoration of the blood sodium level to normal. It also causes retention of water with increase in plasma and interstitial fluid volume and consequent gain in weight. It lowers the serum potassium, at times to abnormally low levels, and temporarily increases the excretion of this ion. It restores renal function and increases the excretion of nitrogen when nonprotein nitrogen has been retained. Desoxycorticosterone esters also cause a decrease in the concentration of total protein, calcium

32. Steiger, Marguerite, and Reichstein, T.: Partial Synthesis of a Crystallized Compound with the Biological Activity of the Adrenal Cortical Hormone, Nature **139**: 925 (May 29) 1937.

33. (*a*) Thorn, G. W.; Howard, R. P.; Emerson, Kendall, Jr., and Firor, W. M.: Treatment of Addison's Disease with Pellets of Crystalline Adrenal Cortical Hormone (Synthetic Desoxy-corticosterone Acetate) Implanted Subcutaneously, Bull. Johns Hopkins Hosp. **64**: 339 (May) 1939. (*b*) Thorn, G. W.; Howard, R. P., and Emerson, Kendall, Jr.: Treatment of Addison's Disease with Desoxy-Corticosterone Acetate, a Synthetic Adrenal Cortical Hormone (Preliminary Report), J. Clin. Investigation **18**: 449 (July) 1939. (*c*) Levy Simpson, S.: Discussion on Recent Developments in the Treatment of Addison's Disease, Proc. Roy. Soc. Med. **32**: 685 (Dec. 13) 1938.

34. Cleghorn, R. A.; Fowler, J. L. A., and Wenzel, J. S.: The Assay of Desoxycorticosterone Acetate and Its Use in the Treatment of Addison's Disease, J. Clin. Investigation **18**: 475 (July) 1939.

35. Levy Simpson, S.: The Use of Synthetic Desoxycorticosterone Acetate in Addison's Disease, Lancet **2**: 557 (Sept. 3) 1938.

36. (*a*) Loeb, R. F.; Atchley, D. W.; Ferrebee, J. W., and Ragan, Charles: Observations on the Effect of Desoxycorticosterone Esters and Progesterone in Patients with Addison's Disease, Tr. A. Am. Physicians **54**: 285, 1939. (*b*) Ferrebee, J. W.; Ragan, Charles; Atchley, D. W., and Loeb, R. F.: Desoxycorticosterone Esters: Certain Effects in the Treatment of Addison's Disease, J. A. M. A. **113**: 1725 (Nov. 4) 1939. (*c*) Anderson, E.; Haymaker, W., and Henderson, E.: Successful Sublingual Therapy in Addison's Disease, J. A. M. A. **115**: 2167 (Dec.) 1940.

and cholesterol in the serum, probably because of the hemodilution associated with an increase in plasma volume.

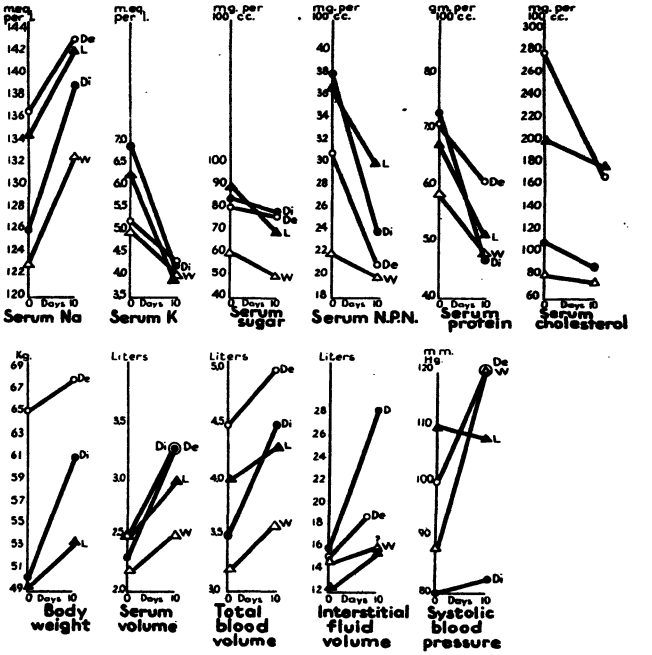
There are great variations in the responses to desoxycorticosterone esters in patients suffering from adrenal cortex insufficiency. These are important to recognize and as yet impossible to anticipate. For example, in studies at the Presbyterian Hospital,[36b] in New York, one patient became markedly edematous and gained 11 Kg. in ten days, during which time he received 10 Gm. of salt a day and 19 mg. of desoxycorticosterone propionate daily. On the other hand, another patient receiving the same diet and the same amount of salt gained but 2 Kg. in thirty days, during which time he received 25 mg. of the same synthetic product each day. Normal persons on this regimen gain but minimal amounts of weight.

The arterial blood pressure of patients with Addison's disease who are treated with desoxycorticosterone esters rises. In conjunction with salt solution administered during crises, the drug may cause a rise in pressure from shock levels in the course of a few hours. In other patients the rise in blood pressure may be gradual over a period of two to four weeks. At times hypertension may appear. Thus, in four of the patients studied by us there has been a rise in blood pressure to 175/100, 160/92, 160/110 and 146/108 respectively. Whether or not these patients had underlying hypertensive disease, masked by Addison's disease, is not known. It is also not known whether the rise in blood pressure is dependent on factors other than the correction of the disturbances of electrolyte and water metabolism.

An important fact to be borne in mind is that desoxycorticosterone esters have no demonstrable effect on the carbohydrate metabolism of patients with Addison's disease. No definite effect on the fasting blood sugar, dextrose tolerance curves or respiratory quotients has been observed. Furthermore, spontaneous and severe hypoglycemia may appear in patients receiving enough synthetic desoxycorticosterone to correct disturbances of electrolyte and water metabolism, just as it may appear in patients receiving adequate amounts of salt alone. Recent studies [37] of patients receiving other steroids of the adrenal cortex, e. g., corticosterone

37. Thorn.[24] Ferrebee and others.[25]

and compound E of Kendall, as well as cortical extract, indicate that massive doses of these substances have some effect on carbohydrate metabolism. Unfortunately, the amounts of these steroids necessary to produce a significant effect are not available for clinical use, and the amount of cortical extract required to elevate the blood sugar and prevent hypoglycemia is in most cases almost prohibitive.



Effects produced in patients with Addison's disease in ten days by a desoxycorticosterone derivative. These patients received large doses, i. e., about 190 mg., during the initial ten days of treatment.

There is no definite evidence that the pigmentation of the patient with Addison's disease is influenced by desoxycorticosterone esters. It is true that patients under treatment with the synthetic compound and salt appear lighter in color, but it seems likely that this change should be ascribed to rehydration rather than to depigmentation. I have not seen pigment spots in the oral mucous membranes disappear under any form of treatment. Possibly more definite knowledge concern-

ing pigment metabolism will be forthcoming when patients have been under treatment for longer periods.

Subjectively, desoxycorticosterone esters tend to increase the sense of well-being, the strength, the appetite and the outlook on life of patients with Addison's disease. A number of patients resume work, and many others are able, from the standpoint of strength, to take up normal activities. It must be remembered, however, that the majority of patients with Addison's disease suffer from tuberculous infection, and this constitutes a therapeutic problem in itself.

Administration of Desoxycorticosterone Esters.—Two general technics for the treatment of adrenal cortex insufficiency with desoxycorticosterone acetate or propionate have been developed. Thorn and his co-workers [33a] and Levy Simpson [33c] have prepared and implanted subcutaneously, with great success, pellets of a crystalline preparation (synthetic desoxycorticosterone acetate). Others [38] have administered desoxycorticosterone esters subcutaneously or intramuscularly in solutions of peanut or sesame oil. Desoxycorticosterone is only sparingly soluble in water and is essentially without effect when patients with Addison's disease take it by mouth.

The preparation of pellets for implantation presents two problems. The first is that of sterility, which is difficult to obtain, because high temperatures destroy the compound. The second problem is concerned with the pressure under which pellets are prepared. If they are too solidly packed, their solubility is slight. If they are too loosely packed, absorption is apt to be irregular and, indeed, if they crumble, absorption may be very rapid, and serious overdosage may ensue. Thorn [33a] has prepared and standardized his pellets so that from each is dissolved 0.3 to 0.4 mg. of desoxycorticosterone acetate daily. After the daily requirement of the substance is determined in the patient with Addison's disease who takes about 4 Gm. of salt a day in addition to that of a normal diet, by means of daily subcutaneous injections of desoxycorticosterone acetate, the pellets are implanted subcutaneously below the scapula. Enough pellets are used so that the number implanted will yield about two thirds of the subcutaneous requirement. This procedure allows a margin of

38. Wilder, R. M.: Progress in Treatment of Addison's Disease, Proc. Staff Meet., Mayo Clin. 15: 273 (May 1) 1940. Levy Simpson.[33c] Ferrebee and others.[33b]

safety in that if the pellets produce symptoms or signs of overdosage, the salt intake may be reduced. If the pellets are not quite enough to maintain the sodium concentration of the blood at a normal level, the salt intake may be increased, or supplementary doses of desoxycorticosterone acetate may be injected, or more pellets may be implanted.

When patients are treated with daily subcutaneous injections instead of pellets, they are trained in the technic of injection as are diabetic patients who take insulin. The patients in our experience [86b] do best on a normal diet of average salt content, and the dose of desoxycorticosterone ester is adjusted under these conditions.

Patients should be under close observation during the regulation of their dosage. They should be weighed daily and their blood sodium, serum protein, potassium, nonprotein nitrogen and sugar should be determined initially if possible. The vital capacity, the venous pressure and the size of the heart as determined by roentgen examination should be observed from time to time, as these examinations and auscultation of the chest afford the best evidence of impending cardiac insufficiency, a complication occasionally occurring in the course of treatment.

The maintenance requirements of desoxycorticosterone acetate or propionate vary greatly in different patients. In our series the dose necessary to maintain the normal blood electrolyte pattern has varied from 1 mg. to 7 mg. daily. In recent months Anderson et al.[86c] have reported favorable results in the treatment of Addison's disease by means of sublingual instillation of a 10 per cent solution of desoxycorticosterone acetate in propylene glycol. The reports of this form of treatment merit its continued clinical trial.

In the presence of a crisis or at the beginning of treatment in the presence of moderately severe insufficiency, larger doses may be required for two or three days. A dose of 15 mg. may perhaps be given safely twice a day, provided the patient is carefully watched for evidence of overdosage. In the presence of a severe crisis the administration of desoxycorticosterone acetate should be supplemented by one or two daily infusions of 1,500 cc. of physiologic solution of sodium chloride until the patient is able to take food and fluid by mouth. No further definite rules can be advanced for the treat-

ment of the patient in a crisis, as the desoxycorticosterone and salt requirements differ widely not only in different patients but also in the same individual at different times.

Surgical Procedures.—Before the advent of desoxycorticosterone acetate, major surgical operations in patients with Addison's disease almost invariably proved fatal. At the present time an operation may be undertaken with comparative safety if the disturbances of electrolyte and water metabolism are first corrected with synthetic desoxycorticosterone. No specific regimen for anteoperative therapy can be outlined, but in some instances the injection of 25 mg. of desoxycorticosterone acetate four hours before operation and perhaps 1 liter of saline solution serves to prevent serious depletion and consequent shock. In the postoperative period the patient must be watched for evidence of adrenal cortex insufficiency, including hypoglycemia, as well as for manifestations of overdosage. The amounts of desoxycorticosterone and salt must be adjusted to these indications.

Complications.—Desoxycorticosterone esters which produce the striking salutary effects described in patients with adrenal cortex insufficiency may at times give rise to dangerous complications. Among the first 18 patients whom we [36] treated with the synthetic substance at the Presbyterian Hospital there were 15 in whom edema developed, varying from transient puffiness of the face or ankles to massive anasarca. In 5 of these patients there developed also varying degrees of respiratory distress and a sense of tightness of the chest associated with roentgen evidence of dilatation of the heart, predominantly on the right side. In some of these patients there occurred an elevation of venous pressure, roentgen and clinical evidence of pulmonary congestion and a decrease in vital capacity. Cardiac insufficiency developing as a consequence of desoxycorticosterone therapy has been observed also by others. This complication of therapy is fortunately reversible in most instances but may prove fatal. The mechanisms responsible for the development of cardiac insufficiency have not been definitely established. Arterial hypertension cannot be of great importance, as congestive failure appeared in 3 patients in whom the systolic pressure was below 110 mm. of mercury. An increase in plasma volume and a decrease in the serum

potassium concentration may be of importance. It is of interest that Ragan,[39] at the Presbyterian Hospital, has been able to produce cardiac dilatation in adrenalectomized dogs by massive doses of desoxycorticosterone acetate and sodium chloride, but he has observed no effect in normal animals.

Not only have patients under treatment with desoxycorticosterone esters suffered from varying degrees of cardiac insufficiency, but in our series 6 of 20 patients given this therapy in the last twelve months have died. Of these, 1 died of cardiac insufficiency, 1 died of disseminated tuberculosis and 1 died at home presumably of adrenal cortex insufficiency when the dose of the synthetic compound was reduced excessively for the relief of mild symptoms of cardiac insufficiency. Two other patients died suddenly at home without the cause of death being determined. Finally, a patient died suddenly while under observation in the hospital. She had mild cardiac insufficiency and mild hypoglycemia (blood sugar 62 mg. per hundred cubic centimeters). Her blood sodium and potassium had been normal three days before death, and at autopsy no adequate cause for her sudden demise was determined. Our experiences are entirely similar to those recently reported by Wilder [38] and by Thompson and co-workers.[40]

Thus, it is apparent that while desoxycorticosterone therapy has proved exceedingly useful, it is not devoid of danger. Furthermore, it should not be considered a panacea in the treatment of the numerous disturbances present in adrenal cortex insufficiency. It has been shown to have no demonstrable effect on carbohydrate metabolism in patients with adrenal cortex insufficiency and no definite effect on pigmentation. It does not completely relieve the symptom of adynamia, as might be anticipated from Ingle's study.[26] Finally, a number of patients, apparently adequately controlled from the standpoint of electrolyte and water metabolism, died rather suddenly and for no obvious cause. Excessive doses of desoxycorticosterone cause a periodic paralysis in dogs associated with a sharp decrease in blood potassium and partial replacement of intramuscular potassium by sodium. This paralysis is relieved by the administration of potassium chloride. It

39. Ragan, Charles: Unpublished data.
40. Thompson, W. O.; Thompson, Phebe K.; Taylor, S. G., and Hoffman, W. S.: Treatment of Addison's Disease with Desoxycorticosterone Acetate, abstr. J. A. M. A. 114: 688 (Feb. 24) 1940.

is possible that sudden weakness appearing in patients treated with synthetic hormone may, at times, have a similar basis.

Adrenal Cortex Extract.—Commercial preparations of adrenal cortex have, in large amounts, been shown to have definite effects on water and electrolyte [41] as well as carbohydrate metabolism. The effects are, however, small, and it seems unlikely, as stated before, that these extracts in the doses usually administered have significant clinical value. On theoretical grounds, extract of adrenal cortex should prove of greater value than desoxycorticosterone, as it contains other steroids which are known to have greater and more diversified action, particularly in relation to carbohydrate metabolism. Unfortunately, the concentrations of these steroids are not sufficiently high to prove of real value in small doses. On the basis of our studies [25] it does not seem probable that doses of less than 5 cc. of cortical extract contain clinically significant quantities of steroids that would affect the carbohydrate metabolism. Consequently, the injection of 5 cc. amounts of extract, even in conjunction with the administration of desoxycorticosterone acetate, does not seem indicated in the maintenance of the patient.

Despite these criticisms, it seems probable that 50 to 75 cc. at least of commercial extract given in divided doses in the course of twenty-four hours in addition to saline infusions and with repetition of the dosage the next day may prove more satisfactory in treating serious crises than will desoxycorticosterone acetate alone.

Although cortical extracts have been shown to be active when administered by mouth, the amounts necessary are probably at least 3 to 4 times that of the parenteral dose. Consequently oral administration is of doubtful clinical value.

Studies on adrenalectomized animals have shown that a number of preparations of adrenal cortex extract have demonstrable potency when administered orally. Pfiffner and his co-workers [41a] estimated that 10 cc. of their preparation given by mouth was equivalent to

41. Thorn, G. W.; Garbutt, H. R.; Hitchcock, F. A., and Hartman, F. A.: The Effect of Cortin on the Sodium, Potassium, Chloride, Inorganic Phosphorus and Total Nitrogen Balance in Normal Subjects and in Patients with Addison's Disease, Endocrinology **21**: 202 (March) 1937.

41a. Pfiffner, J. J.; Swingle, W. W., and Vars, H. M.: The Cortical Hormone Requirements of the Adrenalectomized Dog, with Special Reference to a Method of Assay, J. Biol. Chem. **104**: 701 (March) 1934.

about 1 cc. given parenterally. Thorn [41b] demonstrated that the glycerin extract of 1,000 Gm. of adrenal glands administered by mouth daily to a 10 Kg. adrenalectomized dog maintained the animal in good health. Grollman[41c] has also reported activity of a charcoal adsorbate of adrenal cortex extract. On the basis of these and similar findings the oral administration of extracts has been recommended in the treatment of patients. In view of the relative inactivity of the usual cortical extracts administered parenterally in doses of 5 cc. or less, it is unlikely that considerably larger amounts given by mouth have any significant therapeutic effect. The observations of the writer support this view. Thus one patient was given oral doses of a commercial glycerin extract costing from $5 to $15 a day over a period of some months. The extract was given in addition to salt and had little if any clinical effect. This patient's disease is now easily controlled by small doses of desoxycorticosterone acetate. Another patient whose disease was controlled both by desoxycorticosterone acetate in doses of 1 to 2 mg. daily and by about 15 Gm. of salt was given 15 tablets of an oral preparation in conjunction with vitamin C daily. When the salt intake was reduced to 5 Gm. a day, symptoms of acute insufficiency developed in three days with a fall in sodium from 136 to 129 m. eq. per l. despite the large dose of oral extract. A third patient taking 6 tablets of the same preparation and 3 Gm. of salt a day in addition to her diet was admitted to the hospital because of progressive weakness, anorexia and nausea. Her blood sodium on admission was 124.8 m. eq. per liter. A single infusion of salt solution, the discontinuation of the oral cortical extract preparation and an increase in the daily salt intake to 12 Gm. a day for four days resulted in clinical improvement associated with a rise in blood sodium to 135 m. eq. per liter. It is the writer's opinion that, in the presence of established adrenal insufficiency, cortical extract given by mouth has no place in treatment, particularly now that desoxycorticosterone acetate is available.

Numerous attempts have been made to transplant an adrenal gland or the cortical cells of a gland from one

41b. Thorn, G. W.; Emerson, K., Jr., and Eisenberg, H.: Oral Therapy in Adrenal Insufficiency, Endocrinology **23**: 403 (Oct.) 1938.
41c. Grollman, A.: The Adrenals, Baltimore, Williams & Wilkins Company, 1936.

person to another in the treatment of Addison's disease. These efforts have not met with success up to the present time, and the procedure is not to be recommended. Ingle has devised an ingenious method for the successful transplantation of the adrenal glands of a newborn rat into another young rat, but this procedure is so far only of academic interest. Auslender [42] has recently reported successful heterotransplants of adrenal cortex free from medullary cells. These observations have not been confirmed.

TREATMENT OF MISCELLANEOUS CONDITIONS

Preparations of the adrenal cortex have been employed in recent years in the treatment of various conditions ranging from nonspecific fatigue to surgical shock. At the present time it is difficult to appraise the value or wisdom of administering these preparations in the absence of demonstrable adrenal cortex insufficiency, since their continued administration is known to cause atrophy of the adrenal glands of normal animals.[43] However, Perla and his co-workers [44] have offered evidence suggesting that anteoperative treatment with saline solution and desoxycorticosterone acetate prevents surgical shock, and Scudder [45] has advocated the use of large quantities of extract of adrenal cortex in the treatment of that condition. These studies receive theoretical support from the observations of McAllister [46] and of Ragan and his associates.[47] These workers have shown that desoxycorticosterone esters administered three to six hours before operation prevent

42. Auslender, E. M.: Immediate and Late Results of Transplantation of the Suprarenal Cortex in Fourteen Cases in Addison's Disease, Novy khir. arkhiv. **42**: 375, 1938.

43. Ingle, D. J.; Higgins, G. M., and Kendall, E. C.: Atrophy of the Adrenal Cortex in the Rat Produced by Administration of Large Amounts of Cortin, Anat. Rec. **71**: 363 (July 25) 1938. Wells, B. B., and Kendall, E. C.: A Qualitative Difference in the Effect of Compounds Separated from the Adrenal Cortex on Distribution of Electrolytes and on Atrophy of the Adrenal and Thymus Glands of Rats, Proc. Staff Meet., Mayo Clin. **15**: 133 (Feb. 28) 1940. Unpublished data from the Presbyterian Hospital.

44. Perla, David; Freiman, D. G.; Sandberg, Marta, and Greenberg, S. S.: Prevention of Histamine and Surgical Shock by Cortical Hormone (Desoxycorticosterone Acetate and Cortin) and Saline, Proc. Soc. Exper. Biol. & Med. **43**: 397 (Feb.) 1940.

45. Scudder, John: Shock: Blood Studies as a Guide to Therapy, Philadelphia, J. B. Lippincott Company, 1940.

46. McAllister, F. F.: The Effect of Ether Anesthesia on the Volume of Plasma and Extracellular Fluid, Am. J. Physiol. **124**: 391 (Nov.) 1938.

47. Ragan, Charles; Ferrebee, J. W., and Fish, G. W.: Effect of Desoxycorticosterone Acetate upon Plasma Volume in Patients During Ether Anesthesia and Surgical Operation, Proc. Soc. Exper. Biol. & Med. **42**: 712 (Dec.) 1939.

the usual decrease in plasma volume accompanying ether anesthesia and surgical operation. On the basis of the extensive and conflicting reports which have been published, it may be stated that the clinical value of adrenal cortical preparations in the treatment of surgical shock is certainly equivocal.

Weil and Browne [48] reported that the urinary substances protecting young adrenalectomized rats from the fatal effects of exposure to cold are not excreted by normal human beings but appear in the urine following febrile illnesses and other forms of stress. Although the significance of this report is not yet apparent, it does not justify indiscriminate administration of preparations of the adrenal cortex, particularly as it has not been demonstrated that these protective substances are of adrenal origin.

48. Weil, Paul, and Browne, J. S. L.: A Cortin-like Action of Extracts of Human Urine, Am. J. Physiol. **126**: P652 (July) 1939.

CHAPTER XX

THE ADRENAL MEDULLA

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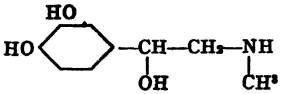
Epinephrine has been studied extensively as a physiologic and as a pharmacologic agent. The subject has wide ramifications, and only what appear to be the most significant findings can be discussed in the space available.

ISOLATION OF EPINEPHRINE

The staining reactions described by Vulpian (green color with ferric chloride) and by Henle (brownish yellow color with dichromates) offered the first evidence for the presence in the medulla of the adrenal gland of a substance absent from most other tissues. Following the observation by Oliver and Schäfer [1] of the remarkable pressor effect of extracts of the adrenal medulla, intensive efforts were made to isolate the blood pressure-raising principle. Takamine [2] and Aldrich [3] isolated the free base, while Abel [4] reported the isolation of a crystalline substance, which was later shown to be a benzoyl derivative formed during isolation. The name "epinephrine," proposed by Abel for this derivative, was subsequently adopted as "official" for the natural substance. The term "adrenalin" is still trademarked in this country.

The correct empiric formula was determined by Aldrich [5] and eventually shown, through the efforts of many workers and synthesis by Stolz [6] and by Dakin,[7a] to represent the following structure.



Present address Medical Research Division, Sharp & Dohme, Glenolden, Pa.

1. Oliver, G., and Schäfer, E. A.: J. Physiol. **18**: 230, 1895.
2. Takamine, J.: J. Physiol. **27**: xxix, 1901.
3. Aldrich, T. B.: Am. J. Physiol. **5**: 457, 1901.
4. Abel, J.: Bull. Johns Hopkins Hosp. **13**: 29, 1902.
5. Aldrich, T. B.: J. Am. Chem. Soc. **27**: 1074, 1905.
6. Stolz, F.: Ber. d. deutsch. chem. Gesellsch. **37**: 4149, 1904.
7. (*a*) Dakin, H. D.: Proc. Roy. Soc., London, s. B **76**: 491, 498, 1905. (*b*) Bloor, W. R., and Bullen, S. S., J. Biol. Chem. **138**: 727, 1941.

Many methods have been devised for the determination of epinephrine, but the accuracy of most of these is seriously interfered with by the presence of various tissue constituents. The most sensitive technic for the determination of epinephrine in blood appears to be that of Bloor.[7b]

INNERVATION OF MEDULLARY CELLS

It has been known since the work of Elliott,[8] and his work has since been repeatedly confirmed, that the innervation of the adrenal medulla is of the preganglionic type, a finding consistent with the view that the medullary cells are modified ganglion cells. Recent work by Young [9] confirms the essential absence of postganglionic fibers in the adrenal medulla (cat) and emphasizes the large number of roots which send fibers to the medulla; these fibers arise from at least the sixth to the sixteenth segment of the spinal cord and pass without synaptic interruption to the cells they innervate. The greater splanchnic nerve conducts the majority of these fibers to the adrenal gland, where a network is formed in the connective tissue; after penetrating the cortex, without innervating it, the fibers end in intimate relationship with the medullary cells. Young found that each nerve fiber innervates a definite number of cells; in this manner units are formed which may conceivably be recruited much like the units of voluntary muscle.

Nerve Control of the Secretion of Epinephrine.—The preganglionic fibers supplying the adrenal medulla, like those to the sympathetic ganglions, are of the cholinergic type; i. e., on stimulation they produce a substance pharmacologically indistinguishable from acetylcholine (Feldberg and co-workers [10]). A liberation of epinephrine from the adrenal medulla can be shown to follow a suitable injection of acetylcholine. The effects of splanchnic stimulation and of the injection of acetylcholine are greatly increased by a previous injection of physostigmine, a drug known to inhibit the enzyme (esterase) responsible for the inactivation of acetylcholine. After the injection of physostigmine, acetylcholine when perfused through the isolated adrenal gland increases considerably the output of epinephrine (Heard and Welch [11]).

The adrenal medulla, like the sympathetic ganglions, is not paralyzed by atropine in small doses. Nicotine, however, the typical effect of which is to paralyze all autonomic ganglions after transient stimulation of them, causes the adrenal medulla to fail to respond to splanchnic stimulation, after a short period of augmented secretion of epinephrine.

8. Elliott, T. R.: J. Physiol. **46**: 285, 1913.
9. Young, J. Z.: J. Anat. **73**: 540, 1939.
10. Feldberg, W.; Minz, B., and Tsudzimura, H.: J. Physiol. **81**: 286, 1934.
11. Heard, R. D. H., and Welch, A. D.: Biochem. J. **29**: 998, 1935.

An increased discharge of epinephrine from the adrenal medulla has been demonstrated under a variety of conditions, among which may be mentioned stimulation of sensory nerves, states of fear and rage, muscular activity, asphyxiation, hemorrhage, hypoglycemia, application of excessive heat or cold and administration of convulsant drugs (strychnine, picrotoxine), inhalation anesthetics (chloroform and ether), morphine (in cats and dogs) and histamine (for literature, see Trendelenburg[12]). In many of these cases the epinephrine content of the adrenal medulla (which represents a balance between the rate of new formation and the rate of discharge) is found to be diminished.

Sectioning of the splanchnic nerves, the principal efferent arm of the reflex arc, abolishes or greatly diminishes the discharge of epinephrine. The central connections, however, are not definitely known. Stimulation of certain hypothalamic areas is followed by an increase in secretion of the hormone,[13] as well as by other manifestations of sympathetic hyperactivity. The prolonged hyperglycemia and hyperlactacidemia which follow the piqûre of Claude Bernard are due in large part to liberation of epinephrine from the adrenal medulla.

Whether there is a basal rate of secretion of epinephrine has perhaps not been unequivocally established, but it is probably safe to conclude that so long as nerve impulses reach the medullary cells epinephrine is liberated. The physiologic signficance of such secretion of epinephrine appears to be small, since denervation of the adrenals or even complete sympathectomy does not lead to notable functional disturbances under normal environmental conditions. The increased discharge of epinephrine under conditions of stress is regarded by Cannon and his school[14] as an emergency function; it serves to reinforce the activity of the sympathetic nervous system and causes a variety of circulatory and metabolic adjustments which (by a priori reasoning) are assumed to be useful to the organism.

The evidence presented may be summarized by saying that the adrenal medulla consists of modified sympathetic ganglion cells under the control of cholinergic preganglionic fibers arising in the thoracicolumbar portion of the spinal cord, and that secretion of epinephrine is normally dependent on the degree of activity of these fibers.

SYMPATHOMIMETIC ACTION OF EPINEPHRINE

The action of epinephrine on a given tissue may be inhibitory or excitatory; it is identical, however, with the effect produced by stimulation of the sympathetic fibers supplying the tissue, provided the fibers are of the adrenergic type, i. e., on stimu-

12. Trendelenburg, P.: Die Hormone, Berlin, Julius Springer, 1929, vol. 1, p. 313.
13. Houssay, B. A., and Molinelli, E. A.: Compt. rend. Soc. de Biol. **93**: 1454, 1925.
14. Cannon, W. B.: Ergebn. d. Physiol. **27**: 380, 1928.

lation, produce an epinephrine-like substance.[15] A change in the effect of sympathetic stimulation—for example, from an inhibitory effect on the uterus of the nonpregnant cat to an excitatory effect in the pregnant animal—is paralleled by a similar change in the response to epinephrine. Some of the more important "sympathomimetic" actions of epinephrine are represented in constriction of arterioles, acceleration of the heart rate, contraction of the radial muscle of the iris and of the nictitating membrane, salivary secretion, relaxation of bronchial musculature, inhibition of the small intestine and inhibition of the uterus and bladder (in certain species).

When the sympathetic fibers supplying smooth muscle or other tissues are cut and sufficient time is allowed for degeneration of the peripheral segments, the action of epinephrine is not abolished;[16] this indicates that epinephrine does not act on the nerve endings of these fibers. It is incorrect to conclude, however, that epinephrine acts directly on smooth muscle fibers; a number of drugs act in this manner (for instance, barium ions), but their effect does not parallel that of sympathetic stimulation. Elliott[17] suggested that the union of the sympathetic nerve fiber with the muscle fiber causes the development of a special structure, the "myoneural junction," which is neither nervous nor contractile but which renders the associated cell peculiarly sensitive to epinephrine. When this structure has once developed as the result of innervation,[18] it remains intact despite denervation and the cell retains its sensitiveness to epinephrine. Clark[19] has shown that the amount of epinephrine taken up by isolated tissues when a measurable effect is produced is so small that only a small fraction (less than 1 per cent) of the cell surface could be occupied. This leads to the concept that epinephrine acts on certain specific receptors in the cell, which (in the case of smooth muscle) are in intimate relation with the contractile mechanism.

Transmission of Nerve Impulses.—It is generally assumed that there exists discontinuity between the termination of the nerve fibers and the muscle cell, and the question arises how stimulation of sympathetic nerve fibers can produce the same effect as epinephrine. Two theories have been proposed for

15. Some sympathetic fibers, e. g., those innervating the sweat glands of some species, are cholinergic, and the effect of their stimulation is therefore "mimicked" by the injection of acetylcholine chloride and not by that of epinephrine hydrochloride.

16. As a matter of fact, it is greatly increased. When one eye is sympathetically denervated and the other kept as a control, doses of intravenously injected epinephrine hydrochloride which dilate the pupil of the denervated eye have no effect on the normal eye. Denervated structures (eye, heart, blood vessels) have been used as sensitive indicators of an increased discharge of epinephrine from the adrenals.

17. Elliott, T. R.: J. Physiol. **32**: 401, 1905.

18. It is of interest that placental vessels, which have no nerve supply, respond to epinephrine and acetylcholine very weakly. This supports Elliott's idea of the development of a special receptor substance under the influence of innervation.

19. Clark, A. J.: The Mode of Action of Drugs on Cells, Baltimore, William Wood & Company, 1933.

the transmission of nerve impulses, one electrical and the other chemical; only the latter, which had its inception in the work of Loewi,[20] is within the scope of this review.[21]

According to the humoral transmission theory, an epinephrine-like substance is liberated at the termination of the sympathetic "adrenergic" fibers. This chemical mediator (which seems to be identical with epinephrine) reaches the receptor mechanism of the cell by diffusion; there it combines with a specific substance E if the effect is excitatory and with a specific substance I if the effect is inhibitory. Cannon and his school [22] have shown that the products of these combinations, "sympathin E" and "sympathin I," may escape from the site of their formation and be carried by the blood stream to distant tissues, where they produce effects which differ from each other and from effect of epinephrine. For example, sympathin E released by stimulation of the hepatic nerve fibers has a pressor action and causes contraction of the nictitating membrane but, unlike epinephrine, produces no significant pupillary dilatation or relaxation of the uterus of the nonpregnant cat. Certain drugs (ergot alkaloids, yohimbine and synthetic dioxane derivatives), which abolish the pressor effect of epinephrine have little effect on the rise in blood pressure produced by sympathin E (released as the result of stimulation of hepatic or lumbar nerve fibers). It has been shown recently that stimulation of sympathetic nerve fibers supplying the rabbit ear results in liberation not only of an epinephrine-like substance but also of a compound closely resembling histamine,[23] both detectable in the venous blood of the ear. This suggests that the "sympathin E" and "sympathin I" effects may conceivably be resultants of the release of more than one pharmacologically active substance.

When adrenergic nerve trunks are stimulated in vitro, an epinephrine-like substance diffuses into the surrounding medium (Lissák [24]). Several authors who found an epinephrine-like substance in extracts of various tissues probably obtained this material from the adrenergic fibers contained in the tissue, since, as Cannon and Lissák have shown, degeneration of the sympathetic fibers supplying a tissue (heart or liver) results in the disappearance of the sympathomimetic substance. Lissák [26] reported that when the preganglionic (cholinergic) fibers of the superior cervical ganglions (cat)

20. Loewi, O.: The Humoral Transmission of Nervous Impulse, in Harvey Lectures, 1932-1933, Baltimore, Williams & Wilkins Company, 1934.

21. It may be pointed out that these two theories are not mutually exclusive if it is conceded that the receptor mechanism of the smooth muscle cell may be stimulated (or inhibited) electrically as well as by a specific chemical substance.

22. Cannon, W. B., cited by Rosenblueth, A.: Physiol. Rev. **17**: 514, 1937.

23. Lambert, E. H., and Rosenthal, S. R.: Proc. Soc. Exper. Biol. & Med. **44**: 235, 1940.

24. Lissák, K.: Am. J. Physiol. **126**: 564, 1939; **127**: 263, 1939.

25. Footnote deleted.

26. Lissák, K.: Am. J. Physiol. **125**: 778, 1939.

are cut, there occurs, after a week or two, a disappearance of the acetylcholine which is normally present, while a substance with the properties of epinephrine remains in the ganglion. These observations show that adrenergic neurons are characterized by the presence of epinephrine (or a substance closely resembling this principle in its pharmacologic and chemical properties) in cell bodies, axons and their terminations and by the liberation of epinephrine during nerve activity. The situation is similar to that obtaining in cholinergic neurons, in which acetylcholine is the characteristic substance that acts on the receptor mechanism of the (muscle or gland) cell. However, a collection of specially differentiated cells containing acetylcholine and displaying an auxiliary function comparable to that which the adrenal medulla bears to the adrenergic system has not been demonstrated.

An integral part of the humoral transmission theory (if it is to account for the observed bioelectric phenomena) is not only the formation of the chemical mediator but also the inactivation of this principle at the site of action. Observations concerning the formation, stabilization and destruction of epinephrine will be reviewed briefly.

FORMATION OF EPINEPHRINE

The most likely precursor of epinephrine (see table, no. 1) is phenylalanine (table, no. 2), one of the amino acids known to be indispensable at least for growth (rats). The introduction of two hydroxyls (in positions 3 and 4) into the benzene ring is an essential step in the synthesis of epinephrine from phenylalanine. The body readily introduces the first hydroxyl, converting phenylalanine to tyrosine (table, no. 3), but the mechanism of insertion of the second hydroxyl has remained obscure. An enzyme, tyrosinase, which has the specific property of introducing this second hydroxyl into typrosine, converting it to dihydroxyphenylalanine (dopa) (table, no. 4), occurs in plants but has not been found in mammalian tissues. Recently, however, Arnow [27] has shown that ultraviolet radiation in vitro converts tyrosine into dihydroxyphenylalanine, a reaction which is catalyzed by Fe^{++} ions and by ascorbic acid (Rothman [28]). Ascorbic acid, in the absence of ultraviolet radiation, exerts a similar effect on synephrine (epinephrine minus the meta-hydroxy group), converting it to epinephrine. These reactions offer possible non-enzymatic mechanisms for the introduction of the meta-hydroxy group into epinephrine precursors. The mechanism of formation of melanin, the pigment of human skin [29] is a related problem. Heard and Raper,[30] in a study of

27. Arnow, L. E.: J. Biol. Chem. **120**: 151, 1937; Science **87**: 308, 1938.

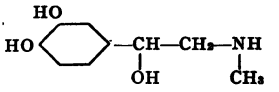
28. Rothman, S.: Proc. Soc. Exper. Biol. & Med. **44**: 485; **45**: 52, 1940.

29. The possible relation between the abnormal pigmentation and the destruction of the adrenal medulla in Addison's disease has often been pointed out.

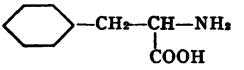
30. Heard, R. D. H., and Raper, H. S.: Biochem. J. **27**: 36, 1933.

Epinephrine and Related Compounds

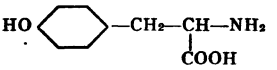
1. Epinephrine (Adrenalin)



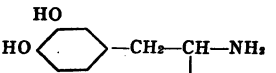
2. Phenylalanine



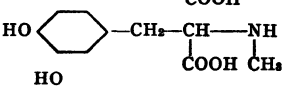
3. Tyrosine



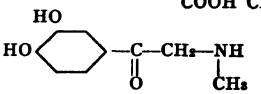
4. Dihydroxyphenylalanine (Dopa)



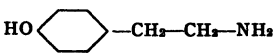
5. N-methyl tyrosine



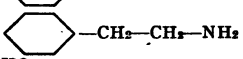
6. Adrenalone



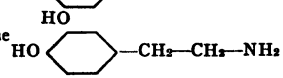
7. Tyramine



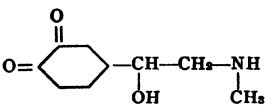
8. Phenylethylamine



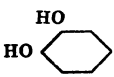
9. Dihydroxyphenyl-ethylamine (Dopamine)



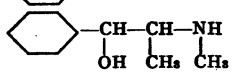
10. Quinone of epinephrine



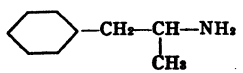
11. Catechol



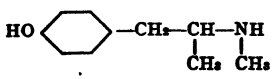
12. Ephedrine



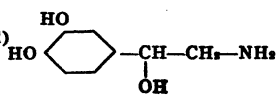
13. Benzedrine (Amphetamine)



14. Veritol



15. Norepinephrine (Arterenol)



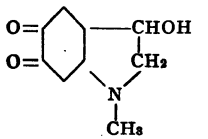
the oxidation of N-methyl tyrosine (table, no. 5) by tyrosinase, found, in addition to melanin, a small amount of pressor substance, the activity of which was increased by hydrogenation, suggesting that adrenalone (table, no. 6) had been formed. One possible pathway of the synthesis of epinephrine might therefore be the formation of dihydroxyphenylalanine, the oxidation of the β-carbon of the side chain with formation of a hydroxyl group, methylation of the amino group and finally decarboxylation. Enzymes responsible for these reactions have not been isolated from mammalian tissues. Incubation of adrenal medullary tissue with a number of possible precursors of epinephrine (tyramine [table, no. 7],[31] phenylethylamine [table, no. 8],[32] dihydroxyphenylethylamine (dopamine) [table, no. 9] [33]) has not, so far, yielded results of great value in the elucidation of the synthesis of epinephrine.

STABILIZATION AND DESTRUCTION OF EPINEPHRINE

It has long been known that epinephrine is much more stable in blood than in Ringer's solution and that many other tissues contain substances which exert a protective action on the hormone. Several authors have studied the stabilizing effect of amino acids on epinephrine. This is not accomplished through the formation of complexes, as was suggested by Wiltshire,[34] but through the amino acids serving as hydrogen donors to the quinone of epinephrine (table, no. 10), a portion of which is thereby reduced to epinephrine (Welch [35]). In this manner the rate of destruction of epinephrine is decreased. Much more effective than amino acids are glutathione and ascorbic acid, two reducing substances which are present in most tissues; they prevent the irreversible oxidation of epinephrine by molecular oxygen [35] and by the widely distributed cytochrome—cytochrome oxidase system.[36] The high concentration of ascorbic acid and of glutathione in the adrenal gland may be related to the stabilization of the hormone either in the gland or in the blood leaving it. Heard and Welch [11] showed that perfusates of the adrenal gland always contain ascorbic acid; in such perfusates the pressor activity remains unchanged as long as reduced ascorbic acid is present. With completion of the oxidation of ascorbic acid, the red color of oxidized epinephrine appears, followed by a progressive decrease in pressor activity.[37]

31. Schuler, W., and Wiedemann, A.: Ztschr. f. physiol. Chem. **233**: 235, 1935.
32. Devine, J.: Biochem. J. **34**: 21, 1940.
33. Vinet, A.: Compt. rend. Acad. d. Sc. **210**: 552, 1940.
34. Wiltshire, M. O. P.: J. Physiol. **72**: 88, 1931.
35. Welch, A. D.: Am. J. Physiol. **108**: 360, 1934.
36. Green, D. E., and Richter, D.: Biochem. J. **31**: 596, 1937.
37. Addition of ascorbic acid to isolated tissues does not intensify or prolong the action of epinephrine, presumably because considerable amounts of reducing substances are present in the tissues to begin with. It seems necessary to assume that there exists in the tissues a mechanism for the destruction of epinephrine which is not inhibited by reducing substances.

It is very unlikely that nonenzymatic auto-oxidation of epinephrine represents a mechanism by which epinephrine is inactivated in the tissues. In vitro it has been shown that heavy metal ions and an alkaline reaction favor this auto-oxidation. Ball and Chen [38] showed that there is first formed an extremely unstable quinone (table, no. 10); apparently this undergoes internal oxidation-reduction and rearrangement to form an unstable indole derivative, called adrenochrome: [36]



Various oxidizing agents form adrenochrome or derivatives of it (e. g., iodoadrenochrome) from epinephrine. The red color which develops in solutions of epinephrine is probably due, at least in part, to the formation of adrenochrome, a substance which is pharmacologically inactive. Green and Richter [36] have demonstrated the oxidative formation of adrenochrome from epinephrine through catalysis by hematin derivatives and by the cytochrome—cytochrome oxidase system. Of particular significance is the observation that the sympathomimetic 3, 4 dihydroxybenzene derivatives most rapidly destroyed in vivo are also those most rapidly oxidized by this system, which may well be concerned with the physiologic inactivation of epinephrine.[39]

In addition to this enzyme system which attacks the catechol (table, no. 11) portion of the molecule, there is present in mammalian tissues another enzyme, amine oxidase,[40] which attacks a multitude of amines, converting them to aldehydes and ammonia (in the case of epinephrine, methylamine is formed instead of ammonia). The oxidation of epinephrine by this enzyme is apparently not inhibited by reducing substances.

Ephedrine (table, no. 12), benzedrine (table, no. 13), veritol (table, no. 14) (and other substances related to epinephrine, with a methyl group attached to the carbon adjacent to the nitrogen) are not attacked by this enzyme but are able to compete with epinephrine and the sympathetic mediator for amine oxidase, thus protecting them from destruction. Gaddum and Kwiatkowski [41] demonstrated that epinephrine and the sympathetic mediator are strikingly potentiated by ephedrine in their action on the blood vessels of the rabbit ear and explain this effect as due to the inhibition of amine oxidase. In

38. Ball, E. G., and Chen, T.: J. Biol. Chem. **102**: 691, 1933.

39. It is of interest that epinephrine can replace flavoprotein as a carrier in certain enzyme systems which reduce diphosphopyridine nucleotide (e. g., lactic dehydrogenase); this property is dependent on the oxidation of epinephrine to adrenochrome.

40. Blaschko, H., Richter, D., and Schlossmann, H.: Biochem. J. **31**: 2187, 1937.

41. Gaddum, J. H., and Kwiatkowski, H.: J. Physiol. **94**: 87, 1938.

agreement with this interpretation are the observations that denervation of blood vessels and of the iris, procedures which abolish the formation of the sympathetic mediator, greatly diminish the action of ephedrine and that the action of ephedrine is restored when epinephrine is present in the circulation.[42] Morton and Tainter,[43] in a detailed study of the action of ephedrine on blood vessels, found most of the results compatible with the view that this substance inhibits amine oxidase. Recent experiments by Chang-Shaw Jang [43b] throw some doubt on the validity of the general conclusion that ephedrine and related phenylisopropylamine derivatives owe their activity exclusively to the inhibition of amine oxidase. Beyer [43c] has shown that amphetamine (table, no. 13), which cannot be attacked by the cytochrome-cytochrome oxidase system or by amine oxidase, nevertheless undergoes some destruction in the body.

Richter [44] reported recently that orally administered d-epinephrine and l-epinephrine are mainly eliminated as conjugated derivatives in the urine, from which he concludes that conjugation with sulfate is the main physiologic process by which epinephrine is inactivated in the body.

Several other enzymes are capable of oxidizing epinephrine (e. g., catechol oxidase, peroxidase, tyrosinase), but these have rarely if ever been found in mammalian tissues. Bacq [45] claimed that a catechol oxidase is present in the smooth muscle of the uterus, but this has not been confirmed by Lissák [46] and others. The evidence available at present points to the cytochrome—cytochrome oxidase system—and to amine oxidase as the enzymes principally concerned with the physiologic inactivation of epinephrine.

RELATION BETWEEN CHEMICAL STRUCTURE AND ACTION

In a large number of investigations, among which those of Barger and Dale [47] may be mentioned particularly, it has been made clear that nearly all compounds with relatively simple substitution of the phenylethylamine skeleton (table, no. 8) possess some sympathomimetic action. The role of the various groupings may be summarized as follows: The catechol portion (table, no. 11) of the molecule enables it to undergo oxidation to a quinone, with indole rearrangement, adrenochrome formation and loss of physiologic activity. The greater

42. The nictitating membrane seems to be an exception, since the action of ephedrine is not abolished by denervation and since ephedrine has only a minor effect in prolonging or enhancing the action of epinephrine on this structure.

43. (*a*) Morton, M. C., and Tainter, M. L.: J. Physiol. **98**: 263, 1940. (*b*) Chang-Shaw Jang: J. Pharmacol. & Exper. Therap. **70**: 347, 1940. (*c*) Beyer, K. H.: J. Pharmacol. & Exper. Therap. **71**: 304, 1940.

44. Richter, D.: J. Physiol. **98**: 361, 1940.

45. Bacq, Z. M.: J. Physiol. **92**: 28P, 1938.

46. Lissák, K.: Science **87**: 371, 1938.

47. Barger, G., and Dale, H. H.: J. Physiol. **41**: 19, 1910.

stability of many of the synthetic sympathomimetic compounds in clinical use may be attributed to the absence of one or both of the hydroxyls found on the benzene ring of epinephrine.

The hydroxyl group on the carbon atom adjacent to the benzene ring confers optical activity on the molecule; this is of significance since the naturally occurring or l-epinephrine is approximately fifteen times as active as the d-isomer. It may be pointed out here that none of the numerous synthetic compounds related to epinephrine has a pressor activity greater than that of the naturally occurring principle, although some possess definite advantages for one or another therapeutic use. Another possible role for the hydroxyl group in the side chain might be related to ester formation. There is some evidence for the presence of a precursor of epinephrine in the adrenal medulla, and an ester involving this group might be expected to yield epinephrine readily.

The significance of the methyl group in epinephrine is obscure; the pressor activity of the demethylated compound (norepinephrine or arterenol [table, no. 15]) differs but little from that of epinephrine. Barger and Dale[47] pointed out that N-methyl epinephrine-like compounds excel as "inhibitors," while the corresponding unmethylated amines are often superior in producing "augmentation." This has led to the suggestion that sympathin E might be norepinephrine, but the results obtained by Gaddum and Kwiatkowski[41] do not support this view.

HEMODYNAMIC EFFECTS OF EPINEPHRINE

The blood pressure response to intravenously injected epinephrine hydrochloride is characterized by a very short latent period, a dependence of the magnitude of the response on the dose (within certain limits) and a rapid return to normal. When epinephrine is liberated from the adrenals or when epinephrine hydrochloride is injected intravenously, it must pass through the right chambers of the heart, the pulmonary circulation and the left chambers of the heart before reaching the systemic vascular bed. It is of importance, therefore, that the blood vessels of the lung are relatively insensitive to the constrictor action of epinephrine and that the coronary vessels of the heart are actually dilated by the drug. The additional work of the heart during a rise in blood pressure could not be sustained without the increased oxygen supply that the dilated coronaries afford. Similarly, the greatly increased flow of blood through a working skeletal muscle is not interfered with by pressor doses of epinephrine hydrochloride and the flow through resting muscle may actually be increased by small doses.

The most intensive effect of epinephrine is on the arterioles, which are richly endowed with smooth muscle supplied with sympathetic (adrenergic) nerves. The constriction of these vessels, particularly in the splanchnic area, causes increased resistance to the circulation of the blood, as a result of which the blood pressure begins to rise. In the excised heart epinephrine causes an increase in rate and contractility; in the intact animal this effect of epinephrine is less marked, because the rising blood pressure, through stimulation of the pressor receptors of the carotid sinus and the aorta and the resultant vagal reflex, depresses the cardiac rate. When the pressor reflex mechanism is abolished by section of the vagi or by atropinization, a given dose of epinephrine hydrochloride causes a much greater rise in blood pressure and acceleration of the heart rate than when the reflex mechanism is intact.

During the abrupt initial rise in blood pressure the output of the heart probably decreases; this is followed, however, by an increase in cardiac input and output when the blood expressed from the contracted splanchnic area and the spleen has reached the left cavities of the heart, and by a marked increase in the rate of blood flow. As a result of the changes outlined, the circulatory system may be said to operate at a "higher level" with increases in blood flow, circulating blood volume and blood pressure.

The effect of a single intravenous injection of epinephrine hydrochloride is of short duration. Continuous injection of the drug may be used to maintain blood pressure at an elevated level for long periods, the level depending on the amount injected per unit of time. The minimal rate of constant intravenous injection (in milligrams per kilogram per minute) which causes a rise in blood pressure is 0.00005 in man, 0.0005 in dogs, cats and rabbits and 0.001 in rats (Cori [48]).[49] These figures show that the vascular system of man is more sensitive to the action of epinephrine than that of other species. In the laboratory animals mentioned, subcutaneously injected epinephrine hydrochloride does not cause a rise in blood pressure; in man, however, such injection of the drug (0.5 to 1 mg.) is generally followed

48. Cori, C. F.: Physiol. Rev. 11: 143, 1931.

49. It should be emphasized that epinephrine hydrochloride when injected in doses too small to raise blood pressure may nevertheless cause well defined vascular changes; i. e., the vessels of the skin may contract while those of muscles are dilated.

by a rise in systolic blood pressure [50] (10 to 30 mm. of mercury), in pulse rate (10 to 20 beats per minute), in minute volume of the heart (20 to 70 per cent) and in the volume of respiration (50 to 100 per cent), effects which reach their maximum after one-half hour and disappear after one to two hours; extrasystoles are often noted.

METABOLIC EFFECTS OF EPINEPHRINE

The chief metabolic changes which result from the administration of epinephrine are increases in blood sugar, blood lactic acid and basal metabolic rate. Procedures which cause an increased discharge of epinephrine from the adrenals (see a preceding section) lead to the same metabolic changes. The maximal rate at which epinephrine is discharged from the adrenals during splanchnic stimulation in cats has been shown to be 0.003 mg. per kilogram per minute; [51] this procedure causes a decided rise in blood pressure. It should be emphasized that metabolic changes may be produced in unanesthetized dogs, rabbits and cats by intravenous injection at the rate of 0.0002 mg. per kilogram per minute, i. e., at a rate which does not cause a rise in blood pressure. Furthermore, subcutaneous injection of epinephrine hydrochloride in these species causes hyperglycemia and glycosuria without changes in blood pressure. In man, epinephrine hydrochloride injected intravenously at the rate of 0.00005 mg. per kilogram per minute causes a rise in blood sugar as well as in lactic acid and in basal metabolic rate.[52] In all species this rise is detectable a few minutes after the start of the injection, continues while the injection is being made and slowly subsides following the cessation of the injection. The lowest rate of intravenous injection which causes a rise in blood sugar also causes a rise in blood lactic acid. Within a certain range there is a parallelism between rate of injection and magnitude of response.

A single intravenous injection, even of a large dose, produces metabolic changes of only moderate intensity, while the same dose when injected slowly during two hours causes marked hyperglycemia and hyperlactacidemia. For example, a rabbit responded to a

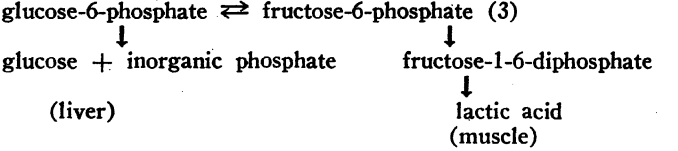
50. The diastolic blood pressure often decreases in man following the subcutaneous injection of epinephrine hydrochloride, because of vasodilatation in certain vascular areas, particularly in muscle.

51. Cannon, W. B., and Rapport, D.: Am. J. Physiol. **58**: 308, 1922.

52. Cori, C. F., and Buchwald, K. W.: Am. J. Physiol. **95**: 71, 1930.

single intravenous injection of 0.03 mg. per kilogram with a maximal rise in blood sugar of 60 mg. and in blood lactic acid of 10 mg. per hundred cubic centimeters; the same dose when injected at a rate of 0.00025 mg. per kilogram per minute for one hundred and twenty minutes (0.03 mg. per kilogram) caused a rise in blood sugar of 180 mg. and in lactic acid of 32 mg. per hundred cubic centimeters. In the latter case the conditions are comparable to subcutaneous injection, a procedure which, owing to continuous absorption of the drug from the subcutaneous depot, causes intense and prolonged hyperglycemia in rabbits.[53] These observations emphasize the fact that epinephrine is rapidly inactivated in the body and that a prolonged effect is dependent on a continuous supply of the drug. Since epinephrine is quite stable in the blood itself, this inactivation must occur in other tissues, presumably at the site of action of the drug. The liver has been shown to be an important organ for the inactivation of epinephrine, but rapid inactivation occurs even in hepatectomized animals.

Epinephrine accelerates the rate of enzymatic breakdown of glycogen in liver and muscle, the end products of this breakdown being dextrose in the former and lactic acid in the latter case.[54] The formation of blood sugar and lactic acid from glycogen follows a common initial pathway, namely:



The first of the aforementioned reactions represents a reversible enzymatic equilibrium; it has been shown that the direction in which the reaction proceeds (i. e., whether it goes to the right or to the left) is determined by the relative concentrations of inorganic phosphate and glucose-1-phosphate. The second reaction, the

53. In hypophysectomized animals the absorption of subcutaneously injected epinephrine hydrochloride is so slow that hardly any hyperglycemia occurs; intravenous injection of the drug has the same hyperglycemic action in these animals as in normal animals (Russell, J. A., and Cori, G. T.: Am. J. Physiol. 119: 167, 1937).

54. Ergot alkaloids inhibit glycogenolytic action in the liver but have no effect on lactic acid formation in muscle.

enzymatic conversion of glucose-1-phosphate to glucose-6-phosphate, accelerates the breakdown of glycogen by disturbing the equilibrium of the first reaction. In liver glucose-6-phosphate is acted on by a phosphatase which splits the ester to glucose and inorganic phosphate; in muscle, which does not contain an active phosphatase, the ester is converted to lactic acid.[55]

The formation of lactic acid is secondary to the accumulation of hexosemonophosphate. When isolated frog muscles are immersed in Ringer's solution containing epinephrine hydrochloride in a concentration of 1 : 10,000,000, there occur a decrease in glycogen, an increase in hexosemonophosphate and a corresponding decrease in inorganic phosphate; only a small amount of lactic acid is formed.[56] The same changes are observed in mammals when epinephrine is injected subcutaneously, except that a greater part of the muscle glycogen which is broken down is converted to lactic acid. The decrease in the inorganic phosphate of the blood which is observed in mammals following the injection of epinephrine hydrochloride is due to the accumulation of hexosemonophosphate in muscle. The drop in serum potassium which takes place, after a short initial rise, may also be connected with the accumulation of hexosemonophosphate.

In postabsorptive rats subcutaneously injected epinephrine hydrochloride caused a decrease in muscle glycogen and an increase in hepatic glycogen three hours after injection. When the course of this reaction was studied, it was found that fifteen minutes after the injection of epinephrine hydrochloride the hepatic glycogen had decreased, that it reached the original level after one hour and that it exceeded the original level three hours after the injection; muscle glycogen, which decreased initially, remained low throughout the period of observation. The explanation of these findings is that the lactic acid which is formed from glycogen in muscle escapes into the blood stream and is carried to the liver, where it is converted back to glycogen. Initially the rate of glycogen breakdown in the liver exceeds that of glycogen formation, but later this is reversed, so that the end effect of an injection of epinephrine hydrochloride is a redistri-

55. Cori, C. F.: Endocrinology **26**: 285, 1940.
56. Hegnauer, A. H., and Cori, G. T.: J. Biol. Chem. **105**: 691, 1934.

bution of glycogen, an increase in hepatic glycogen at the expense of muscle glycogen.[48] The same changes in the distribution of glycogen, with somewhat different time relations, have been observed in rabbits.

The immediate effect of epinephrine on the blood sugar level depends on the content of glycogen in the liver. The hyperglycemic response is much smaller in animals with low hepatic glycogen than in those with large glycogen stores. The maintenance of a high blood sugar level for longer periods depends on the supply to the liver of material from which new carbohydrate (liver glycogen and blood sugar) can be formed. Injections of epinephrine do not cause increased excretion of nitrogen in the urine, which indicates that protein is not the source of the newly formed carbohydrate. Balance experiments in normal animals have shown that the decrease in muscle glycogen is of sufficient magnitude to account for the newly formed carbohydrate in the liver. In depancreatized dogs injections of epinephrine hydrochloride cause excretion of a large amount of extra sugar in the urine, which is largely derived from muscle glycogen. One may conclude that epinephrine is able to maintain an elevation of the blood sugar level partly by accelerating the breakdown of liver glycogen and partly by accelerating the transformation of muscle glycogen to lactic acid, which serves as a source of new carbohydrate.

The mobilization of glycogen by epinephrine is of importance during hypoglycemia. It has been shown that hypoglycemia evokes an increased discharge of epinephrine from the adrenals.[57] Animals with denervated adrenals are hypersensitive to the hypoglycemic action of insulin; convulsions are produced with smaller doses and the original blood sugar level is regained more slowly than in animals in which the mechanism for the discharge of epinephrine is intact. These experiments demonstrate the participation of epinephrine in the regulation of the blood sugar level under conditions of stress. Under more normal conditions medullisadrenalectomized and sympathectomized dogs are able to regulate their blood sugar as efficiently as animals that have not been operated on.[58]

57. Cannon, W. B.; McIver, M. A., and Bliss, S. W.: Am. J. Physiol. **60**: 46, 1924.

58. Brouha, L.; Cannon, W. B., and Dill, D. B.: J. Physiol. **95**: 431, 1939.

Epinephrine increases the oxygen consumption from 15 to 30 per cent in the fasting, in the postabsorptive and in the dextrose-fed animal. In the first the extra calories are derived exclusively from oxidation of fat; in the postabsorptive animal, in which a mixture of carbohydrate and fat is being oxidized, epinephrine also increases the metabolism without much change in the proportion of the foodstuffs burned. During absorption of dextrose in previously fasting rats epinephrine actually decreases oxidation of carbohydrate and the extra calories are furnished by oxidation of fat. The calorigenic action of epinephrine may represent, in part at least, the cost of reconversion of lactic acid to carbohydrate, a process which is known to require energy.[48]

The elevation of the blood sugar level during epinephrine action does not signify that oxidation of carbohydrate is correspondingly increased.[59] In man, Conn and associates [60] found that at a blood sugar level of 200 mg. per hundred cubic centimeters produced by injection of epinephrine hydrochloride, less dextrose was oxidized than when no epinephrine was given and the blood sugar was 80 mg. per hundred cubic centimeters. When the blood sugar was raised to the same level in one instance by injecting epinephrine hydrochloride and in another by feeding dextrose, twice as much sugar was oxidized in the latter as in the former case. Courtice and co-workers [61] found that in man under postabsorptive conditions epinephrine hyperglycemia is not associated with an increase in oxidation of sugar, and the same result was obtained by Dill and collaborators.[62] Lundsgaard and associates [63] reported that the increase in utilization of dextrose which occurs in a perfused hind limb preparation of a dog at a high blood sugar level is prevented by epinephrine.

The difference in the action between epinephrine and insulin is illustrated in the following experiments, in

59. The lactic acid formation results in a lowering of the carbon dioxide–combining power of the blood. Experiments in which respiratory metabolism is measured have to be extended to a time when the acid-base equilibrium has returned to the original level; or the proper corrections for the extra carbon dioxide given off have to be made.

60. Conn, J. W.; Conn, E. S., and Johnston, M. W.: J. Nutrition **19**: (supp.) 16, 1940.

61. Courtice, F. C.; Douglas, C. G., and Priestley, J. G.: Proc. Roy. Soc: London, s. B **127**: 41, 1939.

62. Dill, D. B.; Johnson, R. E., and Daly, C.: Am. J. M. Sc. **198**: 702, 1939.

63. Lundsgaard, E.; Nielson, N. A., and Øskov, S. L.: Skandinav. Arch. f. Physiol. **81**: 11, 1939.

which these substances were injected into rats immediately after the completion of absorption of a sugar meal.[64] The changes which occurred during the first three hours after the injection are expressed in milligrams per hundred grams of rat.

Epinephrine, in spite of a raised level of blood sugar, has little effect on oxidation of carbohydrate and increases mainly the disappearance of muscle glycogen, which is partly oxidized and partly converted to lactic acid and hence to liver glycogen and blood sugar. Insulin doubles oxidation of carbohydrate, and the material drawn on is mainly blood sugar derived from liver glycogen. The opposite effects of epinephrine and insulin on the blood sugar level are clearly reflected in the rate at which blood sugar is oxidized in the tissues. Insulin enables the tissues to oxidize blood sugar at an increased rate, even when the concentration

	Liver Glycogen	Glycogen in Rest of Body	Tissue Sugar	Carbohydrate Oxidized	Blood Sugar per 100 Cc.
Controls	— 49	—167	—22	220	—45
Epinephrine	+ 26	—298	+ 8	263	+16
Insulin	—141	—188	—47	434	—89

is below normal; epinephrine prevents the increase in oxidation of blood sugar which is normally associated with a rise in the concentration of blood sugar.

Anesthetics, particularly barbiturates, greatly enhance the effect of epinephrine on the utilization of blood sugar, as shown in the following experiments. Unanesthetized rats metabolized 98 per cent, and when treated with epinephrine, 88 per cent, of the dextrose supplied them in four hours. During amytal anesthesia 91 per cent was utilized, but when epinephrine was injected during amytal anesthesia, only 33 per cent was utilized, and the rest of the sugar was excreted in the urine.[65] The mechanism by which barbiturates enhance the inhibitory effect of epinephrine on the utilization of dextrose is not known.

THERAPEUTIC USES OF EPINEPHRINE

Extensive use is made of the vasoconstrictor action of epinephrine hydrochloride; the drug is applied locally to congested mucous membranes and to bleeding sur-

64. Cori, C. F., and Cori, G. T.: J. Biol. Chem. **79**: 321, 1928.
65. Cori, G. T.: Am. J. Physiol. **95**: 285, 1930.

faces; it is injected in combination with various local anesthetic agents to reduce blood flow through the tissues receiving the injection and thus to delay absorption. This use permits a minimal concentration of the anesthetic compound to be employed, reducing the danger of systemic effects; it also prolongs the duration of local anesthesia and reduces bleeding following incision.

Epinephrine usually gives relief in a variety of allergic disorders which are thought to be due to the liberation of a histamine-like substance; it counteracts the urticaria and other manifestations of serum sickness; it counteracts the acute circulatory disturbance (nitritoid crisis) which is sometimes seen following an injection of any of the arsphenamines. In cases of angioneurotic glottis edema the drug may be life-saving. In asthmatic attacks epinephrine usually gives prompt relief, due to its dilator action on the bronchial musculature. In shock the drug is of little value, and may actually be harmful; the arterioles, on which epinephrine principally acts, are believed to be maximally constricted as a result of the state of shock, while the constrictor action on the dilated capillaries is too feeble to displace the blood which stagnates in the splanchnic area. However, Kabat and Freedman [66] present evidence for a definitely favorable effect of "slow" epinephrine (epinephrine in peanut oil or with gelatin, given intramuscularly) in experimental shock in cats.

Spectacular is its occasional successful use in resuscitating persons in whom the heart has apparently ceased to beat as a result of drowning, carbon monoxide poisoning or accidents during anesthesia. In such cases, justifying heroic measures, intracardiac injections of epinephrine hydrochloride have sometimes resulted in the initiation of cardiac contractions. Epinephrine is used in Stokes-Adams disease to increase the idioventricular rate.

Intravenous injections of epinephrine hydrochloride are potentially dangerous, particularly in patients with hypertension. The sudden rise in blood pressure may cause acute cardiac dilatation in a weakened heart; it may lead to rupture of atheromatous blood vessels and intensify the severity of internal hemorrhages; it is often followed by a prolonged period of low blood pressure. In animals, paralysis of the central nervous system

66. Kabat, H., and Freedman, A. M.: Proc. Soc. Exper. Biol. & Med. 46: 385, 1941.

and edema of the lung are terminal symptoms of an overdose of epinephrine, and similar symptoms have been observed in man. Under certain conditions injections of epinephrine hydrochloride have been shown to cause fatal ventricular fibrillation; best known of conditions predisposing to such an occurrence is light chloroform anesthesia.

Most of the desirable systemic actions of the drug can be secured by subcutaneous administration; this is safer than intravenous injection, and the action is of longer duration. It should be mentioned, however, that absorption of epinephrine hydrochloride from subcutaneous tissues may be deficient in persons with failing circulation. Massage of the injected area has a marked effect on the rate of absorption, as shown by the immediate blood pressure response. If a prolonged systemic action is desired, epinephrine suspended in oil may be injected subcutaneously or intramuscularly.[67] For certain therapeutic purposes, intravenous injection at a constant rate (in contrast to single intravenous injections) has been used but it requires a special apparatus, the addition of some stabilizer to prevent in vitro oxidation of the drug, and repeated control of blood pressure.

67. Keeney, E. L.: Bull. Johns Hopkins Hosp. **62**: 227, 1938; Am. J. M. Sc. **198**: 815, 1939.

CHAPTER XXI

THE ADRENOGENITAL SYNDROME

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The advances made in adrenal and sex endocrinology during the past decade have materially broadened and to some extent rationalized our conceptions about the essential nature of the adrenogenital syndrome. In keeping with the general intent of this series, the endocrinologic and biochemical aspects have been preferentially treated in this article, at the expense of the clinical and pathologic features. A brief description of the latter aspects will suffice as a background for the topics more specifically discussed here.[1]

The adrenogenital syndrome in the broadest sense of the term comprises all conditions in which the abnormal changes in the sexual sphere are referable to organic or functional disturbances in the adrenal cortex. It is far more frequently seen in females, in whom the changes consist in the appearance of the male secondary sex characteristics and the repression of female characters and function: adrenal virilism. In general, the earlier in life the lesion develops, the more pronounced is the masculinization; hence in very young children, in whom the damage is probably prenatal, the picture is that of pseudohermaphroditism of various degrees, so that there is often some doubt about the true sex. If the onset occurs later in prepubertal life, the masculinization is usually less complete, and may overlap with signs of isosexual precocious maturity (enlargement of clitoris and labia majora, appearance of pubic hair and hirsutism, and sometimes also growth of breasts and appearance of menstruation). It is only natural that in many

1. Recent books and reviews that may be consulted in this connection are: (*a*) Grollman, Arthur: The Adrenals, Baltimore, Williams & Wilkins Company, 1936. (*b*) Young, H. H.: Genital Abnormalities, Hermaphroditism and Related Adrenal Diseases, Baltimore, Williams and Wilkins Company, 1937. (*c*) Broster, L. R.; Allen, C.; Vines, H. W. C.; Patterson, J.; Greenwood, A. W.; Marrian, G. F., and Butler, G. C.: The Adrenal Cortex and Intersexuality, London, Chapman and Hall, 1938. (*d*) Haymaker, Webb, and Anderson, Evelyn: The Syndromes Arising from Hyperfunction of the Adrenal Cortex: The Adrenogenital and Cushing's Syndromes—A Review, Internat. Clin. **4**: 245 (Dec.) 1938. (*e*) Cahill, G. F.: The Adrenogenital Syndrome and Adrenocortical Tumors, New England J. Med. **218**: 803 (May 12) 1938.

cases in which the imbalance is of a more latent character the onset of puberty is required to bring the maleness in appearance (contour of body, distribution of hair, failure of breasts to develop) into full prominence. Menstruation remains in abeyance. If the syndrome becomes established after normal puberty, or later in life, hirsutism is usually the first change noted, followed by irregularity or cessation of the menstrual cycle, changes in the body contour and usually mammary atrophy and enlargement of the clitoris.

In males the juvenile form of the syndrome gives rise to precocious sexual maturity with genital enlargement and hirsutism, often associated with rapid increase in stature and muscular development, or with obesity. In adult men the disease is rare, and then the tendency is more frequently toward feminization, with enlargement of the breasts and genital atrophy, than toward increased virility.

Besides the changes in the sexual sphere and the acne of the face, back and chest, which is almost invariably present, other less constant features occur with various degrees of frequency. Such are obesity or abnormal distribution of fat, purple striae, hypertension, osteoporosis and frank or latent diabetes. All the latter conditions are also encountered in Cushing's syndrome (basophil adenoma of the pituitary). The original definition of this syndrome lists among its symptoms genital dystrophy and depression of sexual functions but not signs of sex reversal, such as virilism; later, however, it became increasingly clear that no sharp line of demarcation could be drawn between this condition and the adrenogenital syndrome proper. The frequent findings of malignant adrenocortical tumors in cases of either condition add to the difficulty of classification. There has been an increasing tendency in recent years to ascribe the symptoms of Cushing's disease to a primary hyperfunction of the adrenal cortex, while others adhere to the original view, which holds that the fundamental endocrine disturbance is in the pituitary. Whatever interpretation may be correct, there is hardly any doubt that the adrenal cortex is somehow involved in most, if not all, cases of this syndrome. This is probably true also of the "diabetes of bearded women," first described by Achard and Thiers, in which the chief symptoms are hirsutism of the face, irregularity or absence of menstruation, obesity and glycosuria. Since

other features of Cushing's disease are frequently present, the condition is now usually regarded as a variation of the latter syndrome.

The condition of the adrenal cortex in the adrenogenital syndrome bears no definite relation to the type and severity of the symptoms. The gland may be grossly normal, slightly or considerably hyperplastic or, more rarely, may be adenomatous or carcinomatous. The reported absence of structural abnormalities, as well as the fact that adrenal hyperplasia is a relatively common finding entirely apart from the syndrome, makes it necessary to assume that the disturbance in these cases arises on a functional rather than on a structural basis. The often confirmed remission of the symptoms after unilateral adrenalectomy when the removed gland was normal in size or only slightly enlarged seems to justify this view. Neither do all neoplasms of the cortex give rise to sexual changes. Among the symptom-producing tumors, malignant carcinoma predominates. This type of tumor is found in cases of juvenile and adult virilism, of isosexual precocity in boys and girls, of feminization in adult males and of Cushing's syndrome. It is clear from this that cortical neoplasms may cause changes in the direction of masculinity as well as of femininity, which has led to the distinction, on purely clinical grounds, between androgenic and estrogenic tumors.

Grollman,[1a] who has denied the existence of estrogenic tumors, postulated that all masculinizing tumors arise from a special "androgenic" tissue, which is functionally distinct from the rest of the cortex. In fetal and early postnatal life this tissue comprises a part of the reticular zone; later it undergoes involution and then is confined to a thin juxtamedullary layer or merely a few scattered cells in this location. More recently, however, the author seems to have discarded this hypothesis (Gersh and Grollman[2]) and now attributes virilism either to the inclusion on the cortex of testicular rests which become functional or to a derangement in steroid metabolism resulting in the formation of androgens. Vines and co-workers [1c] have attempted to correlate virilism with the presence of histologic elements showing an abnormal (red) staining

2. Gersh, I., and Grollman, Arthur: The Relation of the Adrenal Cortex to the Male Reproductive System, Am. J. Physiol. **126**: 368 (June) 1939.

reaction with the ponceau fuchsin stain. The fuchsinophil material is not confined to any particular cell type, nor to the juxtamedullary zone, and therefore does not seem to bear any relationship to Grollman's androgenic tissue. In the series of Broster and Vines the reaction was positive in normal-sized and hyperplastic adrenals in 34 of 36 cases of virilism. Among the tumors, only those causing masculinizing symptoms gave the reaction. These observations have been confirmed by others, though Cahill [3] could find an excess of fuchsinophil material only in carcinoma, and not in adenomatous or non-neoplastic glands.

ENDOCRINE BASIS OF THE ADRENOGENITAL SYNDROME

An interesting theory concerning the influence of the adrenal cortex on sexuality has been developed by Vines and co-workers,[1c] based chiefly on a study of the fuchsinophil reaction in human fetal adrenals, which cannot be rendered here in detail. In brief, the cortex is conceived as "a potentially bisexual accessory sex gland, largely controlled by the pituitary and capable of secreting simultaneously androgens or estrogens, the one or the other being in excess." In the fetal adrenals of both sexes there occurs an androgenic phase shortly after the differentiation of the gonads and accessory sex organs has been completed. In the female this phase is of much shorter duration than in the male, but it nevertheless "interrupts the continuity of development along female lines and thereby introduces an element of instability," while in the male it serves to reenforce and stabilize the genetic maleness. In the female an overproduction of adrenal androgen during the androgenic phase or a failure of the latter to be terminated by inhibitory influences normally present (pituitary?) may then lead to various degrees of fetal masculinization. If the androgenic factor becomes dominant early in fetal life (before the twentieth week), the female gonad itself, which is then still in a plastic state, may become inverted (pseudohermaphroditism). Adolescent virilism is conceived as resulting from later fetal masculinization, which does not affect the gonad, and remains latent till puberty. Other forms of the syndrome are simply referable to an overproduction of adrenal androgenic or estrogenic factors, with the primary stimulus either

3. Cahill, G. F.: Adrenal Cortical Syndromes and Adrenal Tumors, Tr. Am. A. Genito-Urin. Surgeons **31**: 111, 1938.

residing in the gland itself (tumors) or in the pituitary (Cushing's syndrome).

The fundamental premise of this hypothesis, namely, that the adrenal can elaborate androgens and estrogens, is now well established by chemical research. There is also some reason to believe that this secretion is subject to, if not necessarily regulated by, stimuli from the pituitary. The exact mechanism involved in the pathologic overproduction of these hormones can only be guessed. The existence in the cortex of a special tissue secreting sex hormones which is stimulated to overfunction in disease must be regarded as highly hypothetic. The substances responsible for the symptoms may be formed by some aberration of cortical steroid metabolism; or, since there is evidence that the gland normally gives rise to androgens and perhaps estrogens, the pathologic change may consist merely in an increased rate of production. There are indications that the metabolism of cortical steroids, other than androgens, is affected in virilism. Indeed, Haymaker and Anderson [1d] expressed the opinion that Cushing's syndrome differs from the adrenogenital syndrome proper mainly in that overproduction of the cortical hormones acting on electrolyte and carbohydrate metabolism supervenes in the former and that of sex hormones in the latter. In support of this view they quoted their own findings on the presence of a life-maintaining factor in the blood and urine of patients with Cushing's disease,[4] on serum electrolyte changes in this disease which are opposite to those observed in adrenal cortex insufficiency,[5] and on the diabetes often associated with the syndrome.

ANDROGENS IN THE ADRENAL CORTEX

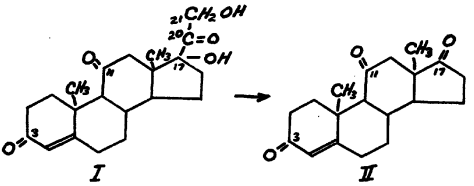
In 1936 Reichstein [6] isolated from beef adrenals an unsaturated triketone, adrenosterone (II), which exhibited androgenic potency in the capon comb test equivalent to one fifth of that of androsterone. More recently a saturated compound, structurally related to adrenosterone, 11-hydroxyisoandrosterone (formula II minus double bond, hydroxyl groups instead of keto

4. Anderson, Evelyn, and Haymaker, Webb: Adrenal Cortical Hormone in Blood and Urine of Patients with Cushing's Disease, Proc. Soc. Exper. Biol. & Med. **38**:610 (June) 1938.

5. Anderson, Evelyn; Haymaker, Webb, and Joseph, Michael: Hormone and Electrolyte Studies of Patients with Hyperadreno-Cortical Syndrome (Cushing's Syndrome), Endocrinology **23**: 398 (Oct.) 1938.

6. Reichstein, T.: "Adrenosteron." Ueber die Bestandteile der Nebennierenrinde II, Helvet. chim. acta **19**: 223, 1936.

groups at C_3 and C_{11}), was also found in the gland.[7] Its androgenic potency is only one thirtieth of that of androsterone.[8] Both these compounds have also been prepared in the laboratory by oxidative degradation of certain pregnane derivatives occurring in the adrenal cortex. The formation of adrenosterone from 11-dehydro-17-hydroxycorticosterone (I), a compound possessing weak life maintenance activity, is shown in the formulas below.



The cleavage of the side chain can also be effected, as Mason[9] has shown, by weak alkali. It is conceivable that similar reactions resulting in the production of androgenic ketones may also occur biologically and that this represents the pathway by which the cortical steroids possessing the requisite groups at C_{17} and C_{20} are degraded in the organism. Pregnane compounds of this type, which thus, from the chemical point of view, may be considered as potential precursors of androgens, are relatively abundant among the steroids of the adrenal cortex. It is interesting in this connection that one of these compounds, the 17-hydroxyprogesterone recently isolated by Pfiffner and North,[10] is by itself androgenic, its potency in the rat test equaling that of androsterone. On oxidation it yields Δ_4-androstenedione, which, a close relative of testosterone, is still more effective in the castrated rat. This suggests that 17-hydroxyprogesterone may owe its androgenic properties to degradation in the treated animal to androstenedione, although it is of course equally possible that the original molecule may be androgenic as such.

7. Reichstein, T., and von Euw, J.: Ueber Bestandteile der Nebennierenrinde: XX. Isolierung der Substanzen Q (Desoxycorticosteron) und R sowie weiterer Stoffe, Helvet. chim. acta **21**: 1197, 1938.

8. Reichstein, T.: Ueber Bestandteile der Nebennierenrinde: IV. Helvet. chim. acta **19**: 402, 1936.

9. Mason, H. L.: Chemical Studies of the Suprarenal Cortex: V. Conversion of Compound E to the Series Which Contains Four Atoms of Oxygen and to Adrenosterone by the Action of Calcium Hydroxide, J. Biol. Chem. **124**: 475 (July) 1938.

10. Pfiffner, J. J., and North, H. B.: 17-β-Hydroxyprogesterone, J. Biol. Chem. **132**: 459 (Feb.) 1940; **139**: 855 (June) 1941.

EXCRETION OF SEX HORMONES AND RELATED STEROIDS IN THE ADRENOGENITAL SYNDROME

Androgens.—Owing to the expense and difficulty of the capon comb growth method of assaying urine for androgens, quantitative data on the excretion of these substances are yet too scant to permit satisfactory correlation with the clinical and pathologic observations. The colorimetric method when used, as by Callow,[11] with proper caution regarding procedure and interpretation may prove of great value in this respect. The colorimetric method has now been adopted in various modifications by a number of authors for clinical use. There is, however, some agreement on the point that malignant cortical tumors are often associated with inordinately high levels of androgen excretion regardless of the sex of the patient and the type of the symptoms. Of particular interest in this connection is a report by Crooke and Callow[12] on 2 cases of adrenal carcinoma classed as pituitary basophilism (the patients were a 25 year old man and a 6 year old girl); the authors contrasted the relatively enormous amounts of androgen excreted in these cases with the essentially normal amounts in 2 other cases of the syndrome not associated with adrenal tumor. But exceptions undoubtedly occur, as in a case of carcinoma listed by Kenyon and co-workers[13] in which the figure was not far above the normal range. Moderately elevated or essentially normal values of urinary androgen seem to prevail in

11. (*a*) Callow, N. H.; Callow, R. K., and Emmens, C. W.: Colorimetric Determination of Substances Containing the Grouping -CH_2.CO- in Urine Extracts as an Indication of Androgen Content, Biochem. J. **32**: 1312 (Aug.) 1938.

11. (*b*) Talbot, N. B.; Butler, A. M., and MacLachlan, E. A.: Alpha and Beta Neutral Ketosteroids (Androgens); Preliminary Observations on Their Normal Urinary Excretion and Clinical Usefulness of Their Assay in Differential Diagnosis, New. Eng. J. Med. **223**:369 (Sept.) 1940. Talbot, N. B.; Wolfe, J. K.; MacLachlan, E. A., and Berman, R. A.: Chromatographic Separation and Colorimetric Determination of Alcoholic and Non-alcoholic 17-Ketosteroids in Extracts of Human Urine, J. Biol. Chem. **139**: 521 (June) 1941. Fraser, R. W.; Forbes, A. P.; Albright, F.; Sulkowitch, H., and Reifenstein, E. C., Jr.: Colorimetric Assay of 17-Ketosteroids in Urine, J. Clin. Endocrinology **1**: 234 (March) 1941. Friedgood, H. B., and Whidden, H. L.: Colorimetric Determination of Crystalline and Urinary Ketosteroids: Clinical Usefulness of This Method, Endocrinology **27**: 258 (Aug.) 1940.

12. Crooke, A. C., and Callow, R. K.: The Differential Diagnosis of Forms of Basophilism (Cushing's Syndrome) Particularly by the Estimation of Urinary Androgen, Quart. J. Med. **8**: 233 (July) 1939.

13. Kenyon, A. T.; Gallagher, T. F.; Peterson, D. H.; Dorfman, R. I., and Koch, F. C.: The Urinary Excretion of Androgenic and Estrogenic Substances in Certain Endocrine States: Studies in Hypogonadism, Gynecomastia and Virilism, J. Clin. Investigation **16**: 705 (Sept.) 1937.

cases of adolescent or adult virilism in which there is no evidence of a malignant tumor.[14] In the series of Broster, in which information about the condition of the gland was available through unilateral adrenalectomy, Patterson and Greenwood [1c] determined the so-called "free male hormone" (the androgenic substance extractable without previous acid hydrolysis of the urine), because this entity was found to be entirely absent from normal female urine. There was no particular correlation between the amounts of free androgen excreted and the size of the gland or the degree of virilism; however, in almost all cases in which free androgen was present, the gland showed a strong fuchsinophil reaction. The operation effected an immediate reduction of the free androgen in the urine and the virtual disappearance of this substance in later postoperative stages. Similarly, the excessive (total) androgen observed in cases of tumor of the adrenal was seen to decline to lower values after operation.[12]

The androgen content of hyperplastic glands seems to be so low that in most cases it is not demonstrable at all (Patterson and Greenwood [1c]). Slot [15] found none, and Crooke and Callow [12] only comparatively small quantities of androgen (0.2 to 0.35 international unit per gram of non-necrotic tissue) in tumors which had given rise to excessive amounts in the urine. Obviously, only an insignificant portion of the androgen produced is stored at the site of origin. This is reminiscent of the situation in normally functioning endocrine glands which produce steroid hormones (testis, ovarian follicle, corpus luteum).

Estrogens.—Abnormally high amounts of estrogen seem to be excreted sometimes in virilism with or without adrenal neoplasm [16] but more often in those cases of adrenal tumor classed as instances of Cushing's

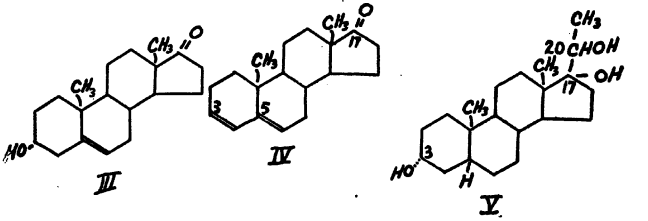
14. (*a*) Levy Simpson, S.; de Fremery, P., and Macbeth, Alison: The Presence of an Excess of Male (Comb Growth and Prostate Stimulating) Hormone in Virilism and Pseudohermaphroditism, Endocrinology **20**: 363 (May) 1936. (*b*) Glass, S. J., and Bergman, H. C.: Subclinical Adrenogenital Syndrome, ibid. **23**: 625 (Nov.) 1938. (*c*) Callow, R. K.: The Significance of the Excretion of Sex Hormones in the Urine, Proc. Roy. Soc. Med. **31**: 841 (May) 1938. (*d*) Kenyon and others.[13]

15. Slot, W. J. B.: The Relation of Sex Hormones in a Case of Virilism by Hypernephroma, Acta med. Scandinav. **89**: 371, 1936.

16. (*a*) Saphir, William, and Parker, M. L.: Adrenal Virilism, J. A. M. A. **107**: 1286 (Oct. 17) 1936. (*b*) McGavack, T. H.: Masculinizing and Non-Masculinizing Carcinomata of Cortex of Adrenal, Endocrinology **26**: 396 (March) 1940. (*c*) Levy Simpson and others.[14a]

syndrome.[17] Frank [17a,b] considered inordinately high amounts (1,000 to 10,000 mouse units per day) as characteristic for cortical carcinoma, since he did not observe them in cases in which the gland was merely adenomatous or hyperplastic (Frank test). It has to be pointed out, however, that by no means all cases of carcinoma exhibited this feature,[18] so that only a strongly positive test may be regarded as diagnostically significant.

Excretion Products.—The excessive androgen titer of the urine examined chemically by Callow [19] in 2 cases of tumor was shown to be chiefly due to its content of dehydroisoandrosterone (III), which was isolated in extraordinarily high yields (110 and 60 mg. per liter).



III IV V

This author suggested that the small amounts of this substance excreted by normal men and women have their source likewise in the adrenal. A related compound, $\Delta_{3,5}$-androstadiene-17-one (IV), accounted roughly for the moderately increased urinary androgen in a case reported by Burrows and co-workers.[20] The patient was a man exhibiting symptoms of feminization, caused by a metastasized adrenal tumor. Since the compound was not found in the urine of patients with cancer in other locations, its derivation from the adrenal and not from the testes, which were atrophied, is fairly certain.

17. Frank, R. T.: (*a*) A Suggested Test for Functional Cortical Adrenal Tumor, Proc. Soc. Exper. Biol. & Med. **31**: 1204 (June) 1934; (*b*) A Suggested Test for Cortical Adrenal Carcinoma, J. A. M. A. **109**: 1121 (Oct. 2) 1937. (*c*) Graef, Irving; Bunim, J. J., and Rottino, Antonio: Hirsutism, Hypertension and Obesity Associated with Carcinoma of the Adrenal Cortex, Arch. Int. Med. **57**: 1085 (June) 1936. (*d*) Hare, D. C.; Ross, J. M., and Crooke, A. C.: Cortical Carcinoma of the Suprarenal, Lancet **2**: 118 (July 20) 1935. McGavack.[16b]

18. Cahill, G. F.; Loeb, R. F.; Kurzrok, Raphael; Stout, A. P., and Smith, F. M.: Adrenal Cortical Tumors, Surg., Gynec. & Obst. **62**: 287 (Feb., no. 2A) 1936. Walters, Waltman, and Kepler, E. J.: Adrenal Cortical Tumors and Their Treatment, Ann. Surg. **107**: 881 (June) 1938. Crooke and Callow.[12] Kenyon and others.[13] Slot.[15] Hare and others.[17d]

19. Crooke and Callow.[13] Callow.[14c]

20. Burrows, H.; Cook, J. W.; Roe, E. M. F., and Warren, F. L.: Isolation of $\Delta_{8,9}$-Androstadiene-17-one from the Urine of a Man with a Malignant Tumor of the Adrenal Cortex, Biochem. J. **31**: 950 (June) 1937.

The estrogen excretion in this case was very high (3,000 international units per liter). The compound concerned was in all probability estrone (theelin). The relative preponderance of the heterosexual hormone in this rare type of sex inversion is of interest.

From the studies of Butler and Marrian [21] it appears that the excretion of steroids in the syndrome is qualitatively as well as quantitatively altered from the normal. From the urine of patients with symptoms of virilism, caused by cortical hyperplasia, four steroids were isolated. One of these is a pregnane-3,17,20-triol (V) stereoisomeric with similar triols which occur in the gland itself (Reichstein). The authors definitely established the absence of this triol from the urine of normal men, and of normal and pregnant women; they also showed that it disappeared from the urine of the patients after removal of the hyperplastic gland. It is therefore an abnormal excretion product specifically associated with the syndrome and probably derived from the diseased gland. Later another triol of this type, stereoisomeric with the first, was isolated from the pathologic urines; furthermore, two stereoisomers of androsterone, isoandrosterone and 3α-hydroxyetiocholan-17-one, were present. It should be mentioned that among the four isolated compounds only isoandrosterone is known to be (weakly) androgenic; 3α-hydroxyetiocholanone, which was later shown to occur also in normal urine,[22] is physiologically inactive; and the two pregnanetriols should lack androgenic action for structural and stereochemical reasons.[23]

21. Butler, G. C., and Marrian, G. F.: The Isolation of Pregnane-3,17,20-triol from the Urine of Women Showing the Adrenogenital Syndrome, J. Biol. Chem. **119**: 565 (July) 1937; The Isolation of 3(α)-Hydroxyetiocholane-17-one, 3(β)-Hydroxyetioallocholane-17-one (Isoandrosterone), and a New Triol from the Urine of a Woman with an Adrenal Tumor, ibid. **124**: 237 (June) 1938. "Hyperplasia" should be substituted for "tumor" in foregoing title; see correction in Nature, London, **142**: 400 (Aug. 27) 1938.

22. (*a*) Callow, Nancy H., and Callow, R. K.: The Isolation of 17-Ketosteroids from the Urine of Normal Women, Biochem. J. **33**: 931 (June) 1939. (*b*) Callow, Nancy H.: The Isolation of Two Transformation Products of Testosterone from Urine, ibid. **33**: 559 (April) 1939.

23. Recently Wolfe, Fieser and Friedgood published a careful quantitative study on the composition of the 17-keto steroid fraction from the urine of a girl with a corticoadrenal tumor. Androsterone was present in about the same amounts as in normal female urine (0.3 mg. per liter), while the levels of 3-α-hydroxyetiocholan-17-one (13 mg. per liter) and of dehydroisoandrosterone (88 mg. per liter) represented tenfold and hundredfold increases, respectively, above normal. Furthermore, Δ3,5-androstadien-17-one (25 mg. per liter) and 3-α-hydroxyandrosten-17-one, a new isomer of dehydroisoandrosterone in which the position of the double bond is still undetermined, were isolated. (Wolfe, J. K.; Fieser, L. F., and Friedgood, H. B.: Nature of the Androgens in Female Adrenal Tumor Urine, J. Am. Chem. Soc. **63**: 582 [Feb.] 1941.)

It was also noted by Butler and Marrian [23a] and by Venning, Weil and Browne [24] that their patients with virilism excreted pregnanediol. These patients had primary or secondary amenorrhea; the diol could therefore not have been derived, as in normal cyclic women, from the progesterone secreted by a functional corpus luteum. Since the adrenal cortex is known to elaborate progesterone,[25] and since the excretion of pregnanediol ceased after operation,[24] there can be little doubt as to the adrenal origin of the compound in these cases.

ADRENAL CORTEX AS A NORMAL SOURCE OF SEX HORMONES

It can now be considered as certain that at least a part of the androgens excreted by normal subjects is derived from an extragonadal source, which as far as present knowledge goes can be only the adrenal cortex. The urine of eunuchs and of ovariectomized women shows small but definite amounts of androgenic activity.[26] According to Callow,[14c] the figures may even overlap the normal range. Hirschmann [27] succeeded in identifying, by actual isolation, the androgens excreted by ovariectomized women as androsterone and dehydroisoandrosterone. The stereoisomer of the former, 3α-hydroxyetiocholan-17-one, which is physiologically inactive, was likewise present. The yields were only little lower than those reported by the Callows when they isolated the same three compounds from the urine of normal women [22a] and men.[22b] It would seem from these results that the contribution of the adrenal to the normal excretion of androgen is much greater than was hitherto suspected.[27a]

The presence of residual estrogenic activity in the urine of male and female castrates [26] invites an analo-

23a. Butler and Marrian.[21] Broster and others.[16]

24. Venning, Eleanor H.; Weil, P. G., and Browne, J. S. L.: Excretion of Sodium Pregnanediol Glucuronidate in the Adrenogenital Syndrome, J. Biol. Chem. **128**: cvii (June) 1939.

25. Beall, D.: The Isolation of Progesterone and 3,20-Allopregnanolone from Ox Adrenals, Biochem. J. **32**:1957 (Nov.) 1938.

26. Literature in Parkes, A. S.: Source of Androgenic and Oestrogenic Substances in the Urine, Lancet **2**: 902 (Oct. 16) 1937.

27. Hirschmann, H.: Androgens from the Urine of Ovariectomized Women, J. Biol. Chem. **130**: 421 (Sept.) 1939; **136**: 483 (Nov.) 1940.

27a. This is true also of the androgen excretion in males, since the Callows later demonstrated the presence of the aforementioned compounds in the urine of eunuchs. The level of dehydroisoandrosterone equaled that of normal male urine, while the excretion of the other two steroids was lower than in normal males. The data gives support to Callow's earlier suggestion that dehydroisoandrosterone, at any rate, may be wholly derived from the adrenals. (Callow, H. N., and Callow, R. K.: Biochem. J. **34**: 276 [March] 1940.)

gous interpretation. The possibility that these small quantities may be derived from foodstuffs appears to have been ruled out by Eng.[28] The ability of the adrenal cortex to elaborate, if not to secrete, estrogens cannot be doubted, since crystalline estrone (theelin) has been isolated from beef glands.[29]

The still controversial question whether the adrenal cortex, with its potential faculty to secrete androgens and estrogens, actually plays a part, beside the gonads, in the regulation of normal sex functions must be treated here summarily. Gersh and Grollman [2] denied any normal "androgenic function" on the ground that the effect of castration on the accessory sex organs of male rats and mice is not modified by the absence of the adrenal, but this has been disputed by Burrill and Greene,[30] who contend that the partial maintenance, in early prepubertal life, of the ventral prostate of the young castrated rat is due to an androgenic secretion from the adrenals. A positive response of the accessory organs was also observed by Hodler [31] in castrated male guinea pigs receiving beef adrenal implants or extracts. Furthermore, Davidson and Moon [32] were able to produce significant enlargement of the seminal vesicles and prostates of castrated rats by means of adrenotropic anterior pituitary preparation, which also caused hypertrophy of the adrenal cortices. Since the effect could not be elicited in the absence of the adrenals, it is clear that this gland had been stimulated to secrete an androgenic principle. Similar results have been obtained by Hodler [31] in male guinea pigs, while in normal and ovariectomized females a masculinizing effect (hypertrophy of the clitoris) was observed. That a secretion of estrogens may be evoked by the same stimulus would appear from the experiments of Moon,[33] who induced

28. Eng, H.: Follikulin im kastrierten Organismus, Klin. Wchnschr. **15**: 349 (March 7) 1936.

29. Beall, D.: Isolation of Oestrone from the Adrenal Glands, Nature, London **144**: 76 (July 8) 1939; J. Endocrinology **2**: 81 1940.

30. Burril, M. W., and Greene, R. R.: Further Studies on the Andromimetic Function of the Immature Rat Adrenal, Endocrinology **26**: 645 (April) 1940.

31. Hodler, D.: Surrénales et masculinisation, Arch. d'anat., d'histol. et d'embryol. **24**: 1, 1937.

32. Davidson, C. S., and Moon, H. D.: Effect of Adrenocorticotropic Extract on Accessory Reproductive Organs of Castrated Rats, Proc. Soc. Exper. Biol. & Med. **35**: 281 (Nov.) 1936; Davidson, C. S.: ibid. **36**: 703 (June) 1937.

33. Moon, H. D.: Effect of Adrenocorticotropic Hormone on Sexual Development of Spayed Rats, Proc. Soc. Exper. Biol. & Med. **37**: 36 (Oct.) 1937.

vaginal opening and signs of estrus in immature spayed rats by administration of an adrenotropic extract.

It must be conceded that all these results, except perhaps those of Burrill and Greene, reveal a potential availability rather than an active, functional secretion of androgens and estrogens from the adrenal cortex. Further work is needed to clarify this issue. At any rate it would not seem, from the present evidence, that these secretions play an important part functionally in the normal adult organism. The biochemist is perhaps tempted to take the more limited view that they are incidental by-products of cortical steroid metabolism, which, to judge from the number of compounds isolated from the gland (most of which are physiologically inactive), seems to be highly complex and ramified.

CHAPTER XXII

CLINICAL SIGNIFICANCE OF HORMONE ASSAYS

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Studies of the hormonal content of urine, blood and tissues are of significant value only when considered in relation to the clinical history, physical findings and other laboratory data of the patient. There are few tests which when considered apart from other evidence can be used safely for diagnostic purposes. In the past several years intensive investigations have pointed to the variability and complexity of tests performed for the purpose of diagnosing endocrine conditions. The results are complicated by the fact that the different laboratories vary in their technics and that there are factors of many sorts over which little control can be exercised and which interfere with the accumulation of uniform data. Definite progress has been made, however, by the use of biochemical procedures, in determining the quantities of certain hormonal substances. This advancement has been especially marked in assaying the numerous steroids of the ovary, testis and adrenal cortex.

In recent years, biochemical and physiologic studies have indicated hitherto undreamed of relationships of such steroids. At the present time, while much of this investigation is of little practical value, it points to the possibility that the future will reveal an understanding of certain dysfunctions which up to the present time have remained obscure. While most of such investigations require extensive laboratory facilities, it is definitely worth while that the physician follow the reports of investigators who have such facilities in order to appreciate more fully not only the diagnostic value of the results but the possibility of understanding the etiology of the disturbances and the bases for various therapeutic procedures.

ANTERIOR LOBE OF THE PITUITARY

The pituitary is a remarkably complex organ with numerous hormonal secretions. Tests for the function of the anterior lobe of this organ should therefore be

aimed at a specific function, avoiding conclusions concerning the general activity of the gland on the basis of results obtained on the secretion of one component.

The gonadotropic factors of the anterior lobe of the pituitary have been given most attention by experimenters, probably because dysfunctions of the reproductive organs are the most common endocrine disturbances encountered and because bioassays for these factors are relatively simple. Numerous reports on analyses of urine and blood for gonadotropic substances have appeared in the past few years. The results of these investigations have not been of great practical importance as yet, since efforts have been directed mainly at obtaining normal values, and it is only lately that fairly accurate data on normal individuals have been obtained. The recently devised mouse or rat uterus test for gonadotropin may result in more satisfactory studies of the excretion of gonadotropic factors.[1]

It is necessary, of course, to determine the values of the gonadotropic substances excreted in normal individuals before conclusions may be drawn as to any possible dysfunctions. It seems fairly certain that in children little or no gonadotropic substance is excreted except before the onset of puberty, when appreciable amounts may be found, appearing somewhat later in boys than in girls.[1b] Methods in which urine is concentrated a hundredfold are necessary to detect the gonadotropic activity.[2] In the normally menstruating woman, according to the evidence presented in the earlier literature, the excretion of gonadotropin is insignificant except at the midinterval stage, on the average between the twelfth and sixteenth days.[3] It was assumed by several investigators that the peak in excretion at the middle of the menstrual cycle indicated ovulation. Others were

1. (a) Levin, L., and Tyndale, H. H.: The Quantitative Assay of "Follicle Stimulating" Substances, Endocrinology **21**: 619 (Sept.) 1937. (*b*) Nathanson, I. T.; Towne, L. E., and Aub, J. C.: Normal Excretion of Sex Hormones in Childhood, Endocrinology **28**: 851 (June) 1941.

2. Katzman, P. A., and Doisy, E. A.: A Quantitative Procedure for Determining Normal Excretion of Prolan, Proc. Soc. Exper. Biol. & Med. **30**: 1188 (June) 1933.

3. Frank, R. T.: Sex-Endocrine Factors in Blood and Urine in Health and Disease, in Glandular Physiology and Therapy, Chicago, American Medical Association, 1935, chap. 16, p. 219. Frank, R. T., and Salmon, U. J.: Gonadotropic Blood and Urine Cycles in Normal Menstruating Woman, Proc. Soc. Exper. Biol. & Med. **32**: 1237 (May) 1935. Kurzrok, R.; Kirkman, I. J., and Creelman, M.: Studies Relating to the Time of Human Ovulation, Am. J. Obst. & Gynec. **28**: 319 (Sept.) 1934. Freed, S. C.: Gonadotropic Substance in Urine of Normal Children, Proc. Soc. Exper. Biol. & Med. **33**: 35 (Oct.) 1935.

unable to confirm these results.[4] From the data of the latter observers it was concluded that an increased excretion of gonadotropin could be obtained at any point during the month, but that a consistent rise in output preceded the menstrual flow. There is additional evidence that an increased amount of gonadotropin is not always found at the midinterval. For instance, D'Amour and co-workers [5] reported on daily tests for urinary gonadotropin in 50 complete menstrual cycles; their results were as follows: during (*a*) 3 cycles there was no response, (*b*) 21 cycles there was one response between the thirteenth and sixteenth days, (*c*) 2 cycles there was one positive response on the nineteenth day, (*d*) 13 cycles there were two responses with a six to twelve day interval, (*e*) 2 cycles there were two responses very close together and (*f*) 9 cycles there were three responses at fairly regular intervals. These workers concluded that either ovulation may occur more than once during the menstrual cycle or ovulation is not directly dependent on the amount of gonadotropin in circulation. Von Haam [6] investigated the daily gonadotropin excretion of 3 women and his conclusions are quite similar. It is therefore apparent that the interpretation of assays for gonadotropin in menstruating women is not, as yet, clear of confusing elements.

Such determinations are of some value in regard to nonpregnant women with amenorrhea. It is well established that in the complete absence of ovarian function in adult women there is an increased excretion of gonadotropin.[7] Thus, in amenorrhea due to ovarian failure, spontaneous menopause or surgical castration, increased amounts of gonadotropin are present in blood

4. Albright, F.; Halsted, J. A., and Cloney, E.: Studies on Ovarian Dysfunction: I. The Hormonal "Measuring Sticks" Available for Clinical Use and Values Obtained on Normal Individuals, New England J. Med. **212**: 192 (Jan. 31) 1935. Smith, G. V., and Smith, O. W.: The Urinary Excretion of Estrogenic and Gonadotropic Hormones During Menstrual Cycles, the Period of Conception and Early Pregnancy, ibid. **215**: 908 (Nov. 12) 1936.

5. D'Amour, F. E.; Funk, D., and Liverman, H.: Daily Gonadotropic Hormone Tests During Fifty Complete Menstrual Cycles, Am. J. Obst. & Gynec. **37**: 940 (June) 1939.

6. Von Haam, E.: The Direct and Indirect Determinations of Estrogenic and Gonadotropic Hormones, Am. J. Clin. Path. **10**: 205 (March) 1940.

7. Zondek, B.: Ueber die Hormone des Hypophysenvorderlappens; Follikelreifungshormon (Prolan A)—Klimakterium—Kastration, Klin. Wchnschr. **9**: 393 (March 1) 1930. Fluhmann, C. F.: Significance of Anterior Pituitary Hormone in Blood of Gynecologic Patients, Am. J. Obst. & Gynec. **20**: 1 (July) 1930.

and urine. The increase in urinary gonadotropin may be detected within as little as three days following ovariectomy.[8] Even in patients without ovaries, however, the excretion fluctuates significantly from day to day, necessitating repeated tests at times, before conclusions may be drawn as to whether or not any particular patient has ovarian failure. Such tests are of some value, therefore, in determining whether the pituitary or the ovary is at fault in cases of amenorrhea. If the absence of menstruation is due to failure of the pituitary, little or no gonadotropin will be excreted; if the ovaries are at fault, this substance will be found in most cases on thorough investigation. Similarly, there may be some increase in the amounts of gonadotropin excreted in males in whom testicular function is low or absent if the pituitary is normal, but these amounts are not easily measured. Possibly the more sensitive tests in which rat or mouse uteri are used will yield more information in questionable states of testicular activity or borderline hypogonadism.

It has been reported that the thyrotropic principle of the anterior lobe may be detected in the urine and blood of animals. The amount is apparently increased following thyroidectomy, analogous to the increase in gonadotropin excreted after castration. The use of thyrotropic hormone assays in the clinic has been somewhat limited owing to the variability of the results of different investigators. In myxedema an increased elimination of thyrotropic substance has been observed by several investigators. The blood of such patients is also claimed to be rich in this principle while little, if any, is found in the opposite state of hyperthyroidism. With regard to acromegaly, there is a difference of opinion as to whether there is increased excretion, while in Simmonds's disease the excretion is claimed to be below normal. Practical use of this test is not very great inasmuch as considerable technical facilities are required. A further complication has arisen, since recent work indicates that the thyrotropic principle may be separated into two factors, one which stimulates the growth of the thyroid gland and one which stimulates the secre-

8. Salmon, U. J.; Geist, S. H., and Walter, R. I.: Inhibitory Effect of Implanted Estrogenic Hormone Crystals upon Post-Menopause and Castration Hypophysis of Women, Proc. Soc. Exper. Biol. & Med. 43: 424 (Feb.) 1940.

tion of the thyroid hormone. Further work will be necessary in order to correlate such findings with clinical disturbances.[9]

The detection of other principles of the pituitary in the tissues of the body is still highly experimental. The status of the parathyrotropic hormone has not progressed beyond that of several years ago, at which time a few reports indicated the presence of such a principle in the urine of women in pregnancy and in that of patients with hyperplasia of the parathyroid glands. Several investigators have been able to detect a contrainsular hormone, a principle of the anterior lobe which antagonizes the action of insulin. Serum or urine extracts have been administered to animals with the induction of resistance to insulin. Speculation on this subject indicates that a substance like the diabetogenic substance or contrainsular factor may be responsible for the insulin resistance of some patients with diabetes.[10] The secretion of this substance is stimulated by the ingestion of fat and reduced by the ingestion of carbohydrates. The lactogenic hormone is found increased in women post partum.[11] Lyons [12] has indicated that the lactogenic substance can be demonstrated in the urine of normally menstruating women. These findings have not been utilized as yet for clinical studies.

POSTERIOR LOBE OF THE PITUITARY

Recent work has renewed interest in studies of posterior lobe hormones in the body fluids of animals. Gilman and Goodman [13] have shown that rats deprived of water will excrete the antidiuretic principle of the posterior lobe. There has been a considerable amount of work on the subject of antidiuretic and pressor principles in the body fluids and their detection in eclampsia, hypertension and other states. Much of the earlier work has never been satisfactorily confirmed and, according to a number of investigators, has been rather adequately disproved. More recent investigations

9. A discussion of this phase will be found in the section on adrenotropic, thyrotropic and parathyrotropic factors, chapter 3.

10. Himsworth, H. P.: The Mechanism of Diabetes Mellitus (Goulstonian Lecture), Lancet **2**: 65 (July 8) 1939.

11. Lyons, W. R., and Page, E.: Detection of Mammotropin in the Urine of Lactating Women, Proc. Soc. Exper. Biol. & Med. **32**: 1049 (April) 1935.

12. Lyons, W. R.: Personal communication to the author.

13. Gilman, A., and Goodman, L.: The Secretory Response of the Posterior Pituitary to the Need for Water Conservation, J. Physiol. **90**: 113 (July 15) 1937.

indicate the possibility for study of the activity of the posterior lobe in clinical states. Thus, Teel and Reid [14] have shown that concentrates of urine from women with eclampsia or preeclampsia had powerful antidiuretic effects when injected into rats. Normal pregnancy urine extracts had no such effect, although when pregnant women were deprived of water they excreted an antidiuretic substance.

GONADS

The last few years have seen considerable progress in the investigations on the excretion of sex hormones. Refinements of method have made assays more dependable, but by far the greatest progress has been due to the biochemical studies in this field. Especially significant has been the work on the relationship of the various steroids of the gonads and adrenal cortex. At the present time much of the data is complicated and confusing. Many factors influence the excretion of these principles. It is acknowledged that the quantitative excretion of these substances does not necessarily indicate the activity of the various glands, since several of these steroids, for instance, are elaborated by more than one tissue. Consideration must be made of the rate of the utilization of these substances, of their conjugation and of their destruction in the liver and metabolic fate. It is, furthermore, no longer accurate to refer to "male" or "female" sex hormone excretions. Androgens are excreted by both women and men; estrogens are similarly excreted by both sexes. In addition, both androgens and estrogens have been isolated from the adrenal cortex, as has been progesterone. These compounds have a basic skeletal structure and many have common physiologic properties to a varying degree. It is obvious, therefore, that simple biologic tests have only a limited value. There is also evidence that certain androgens arise chiefly from the gonads while others arise from the adrenal cortex. These can be detected by biochemical procedures only. The possibility of differentiating between tumors of the ovaries, pituitary and adrenals may, therefore, be likely and, in spite of the complexities of the present studies, it is most encouraging that from the accumulation of data may

14. Teel, H. M., and Reid, D. E.: Observations upon the Occurrence of an Antidiuretic Substance in the Urine of Patients with Pre-Eclampsia and Eclampsia, Endocrinology **24**: 297 (March) 1939.

come definite practical help to the clinician in the diagnosis and treatment of certain endocrine diseases.

Ovaries.—In order to appreciate data on estrogen assays, it is essential that the factors determining the excretion of these substances be understood. The greater portion of endogenous or exogenous estrogen is destroyed by the liver and cannot be recovered. The metabolism of estrogens in some animals may be influenced by progesterone. According to Pincus and Zahl,[15a] and Smith and Smith,[15b] the corpus luteum or exogenous progesterone prevents excessive destruction of estrogens. In addition, the liver conjugates much of the estrogens to glycuronides and possibly other compounds, the so-called combined estrogens. Hydrolysis of the urine with hydrochloric acid is necessary to free the estrogens from such a combination. The intrinsic metabolism of estrogens is quite complicated. According to Pincus and Zahl, estradiol (dihydrotheelin) in the rabbit is converted to estrone (theelin), a reversible reaction, while estrone is converted to estriol (theelol) in the presence of the uterus, an irreversible reaction, which is facilitated by progesterone. These relationships may therefore account for the finding in human urine of estrone, estriol and estradiol. It should be understood that other steroids may be converted in part to estrogens. The estrogen potency of hydrolized human urine may be further increased by the use of zinc-hydrochloric acid-hydrolysis according to Smith and Smith.[15c] These workers indicate that this process converts metabolic derivatives of estrogens to their original active form. The significance of this for clinical purposes is as yet undetermined.

Despite the numerous factors influencing the excretion of estrogens, the activity of the ovaries may be measured to some extent by examining the estrogenic substances of the urine. Results with blood assays for estrogens are most unsatisfactory, owing to the small amounts available in circulating blood. Pioneer work in the field of urinary estrogen assays was done by Robert Frank and co-workers. This group described

15. (*a*) Pincus, G., and Zahl, P. A.: The Biogenesis of Primary Sex Hormones: I. The Fate of Estrins Injected into the Rabbit, J. Gen. Physiol. **20**: 879 (July) 1937. (*b*) Smith, G. V., and Smith, O. W.: Observations Concerning the Metabolism of Estrogens in Women, Am. J. Obst. & Gynec. **36**: 769 (Nov.) 1938. (*c*) Smith, O. W., and Smith, G. V.: The Increased Estrogenic Potency of Human Urine After Zinc-Hydrochloric Acid Hydrolysis, Endocrinology **28**: 740 (May) 1941.

a biphasic curve of estrogen excretion in the normal woman. One peak appeared at the midperiod and the other before the menses, with a rapid drop in estrogen excretion preceding the flow. A number of competent investigators have been able to confirm this work.[16] Despite the fact that the assay method used by Frank and co-workers did not at first utilize the hydrolysis of urine for liberating bound estrogens, recent elaborate studies based on daily urine specimens have indicated that most normally menstruating women have a more or less sharply defined biphasic curve for estrogen excretion.[17] The curves resemble only approximately those which Frank described. There is considerable variation in the shapes of the consecutive monthly graphs obtained from a woman. The peaks vary in their height, and the distance between the two peaks may show a considerable variation. It is significant that the day to day excretion of estrogens may fluctuate tremendously, and pooling urine for two or three days levels off a peak or obscures the shape of a curve. Gustavson and associates[17] considered that the first peak might indicate ovulation and the second peak activity of the corpus luteum. With this conception, these investigators stated that since there is no constant relationship between the two peaks and the next menstruation, the time of ovulation may vary considerably in the same subject and the corpus luteum may require varying lengths of time to reach full development. Otherwise they must conclude that the peaks of estrogen excretion have no particular relationship to ovulation or corpus luteum activity. Furuhjelm[18a] reached the same conclusion on the basis of urinary assays of estrogens in normal women. Nevertheless, Werner[18b] offers a somewhat different interpretation

16. (*a*) Gallagher, T. F.; Peterson, D. H.; Dorfman, R. I.; Kenyon, A. T., and Koch, F. C.: Daily Urinary Excretion of Estrogenic and Androgenic Substances by Normal Men and Women, J. Clin. Investigation **16**: 695 (Sept.) 1937. (*b*) Smith, G. V.; Smith, O. W., and Pincus, G.: Total Urinary Estrogen, Estrone and Estriol During a Menstrual Cycle and a Pregnancy, Am. J. Physiol. **121**: 98 (Jan.) 1938. (*c*) Von Haam, E., and Rothermich, N. O.: Excretion of Gonadotropic and Estrogenic Hormones in Urine During Normal Menstrual Cycle, Proc. Soc. Exper. Biol. & Med. **44**: 369 (June) 1940.

17. Gustavson, R. G.; Mason, L. W.; Hays, E. E.; Wood, T. R., and D'Amour, F. E.: The Quantitative Determination of Estrogenic Substances in Normal Female Urine During the Menstrual Cycle, Am. J. Obst. & Gynec. **35**: 115 (Jan.) 1938.

18. (*a*) Furuhjelm, M.: Excretion of Oestrogenic and Androgenic Substances in the Urine of Women, Acta obst. et gynec. Scandinav. (supp. 1) **20**: 1, 1940. (*b*) Werner, S. C.: Quantitative Study of Urinary Excretion of Hypophyseal Gonadotropin, Estrogen and Androgen of Normal Women, J. Clin. Investigation **20**: 21 (Jan.) 1941.

on the basis of his results in measuring simultaneously the estrogen and gonadotropin excretions in normal women. He confirmed much of the previous work in finding that there is a sudden increase in the amount of gonadotropin excreted at the midinterval and frequently at other times during the cycle, and that the estrogen excretion curve has peaks at midinterval and during the premenstrual period. He observed, however, that in almost every curve at the midinterval a peak of gonadotropin excretion coincided with a peak of estrogen excretion. The uniformity of the results and the finding of pregnandiol shortly thereafter suggested to him that ovulation occurred at these times.

A recent study by Darby and Childs [19] revealed the interesting fact that there are seasonal fluctuations in the estrogen excretion of normal menstruating women, the greatest excretion usually occurring in the spring.

With the development of biochemical procedures for the assaying of estrogens, studies in this field have revealed interesting data. The investigations of the Smiths and their associates [16b] have been most extensive in this regard. According to this group, estriol is excreted by normal women in addition to those who are pregnant. Estriol was found in the urine in greatest quantities during the luteal phase, which probably accounts for the second peak of estrogen excretion, while estrone was most abundant during the follicular phase. The total estrogenic activity of urinary extracts could not be accounted for by adding the estrogenicity of these two substances. It was concluded that the remaining activity was due to an "x" estrogen, and it was suspected that this "x" estrogen was estradiol. This estrogen was detected in greatest quantities in the follicular phase of the menstrual cycle. It has been recently demonstrated conclusively that the "x" estrogen in pregnancy urine is estradiol,[20] and there remains little doubt that estradiol is present in the nonpregnant woman's urine. David [21] has confirmed this work by also identifying estradiol in pregnancy urine. This brilliant series of investigations on fundamental ovarian

19. Darby, H. H., and Childs, D.: Seasonal Fluctuation in Estrogen Excretion, Science **93**: 115 (Jan. 31) 1941.

20. Huffman, M. N.; MacCorquodale, D. W.; Thayer, S. A.; Doisy, E. A.; Smith, G. V., and Smith, O. W.: The Isolation of α-Dihydrotheelin from Human Pregnancy Urine, J. Biol. Chem. **134**: 591 (July) 1940.

21. David, K. G.: Die Identifizierung von Oestradiol im Schwangerenharn, Acta brev. Neerland. **10**: 30, 1940.

physiology was anticipated in animal work to some extent by the demonstration of MacCorquodale and associates that both estrone and estradiol are present in sows' ovaries, and by the work of Freed and associates, who postulated on the basis of histologic changes that rat ovaries secrete two or more estrogens.

In young girls the estrogen excretion is insignificant until the time of puberty.[22] About one and one-half years before puberty, this estrogen excretion becomes cylic in girls and the intensity of these cycles increases gradually until complete sexual development.[1b] In menopausal women, likewise, the estrogen excretion is small, but approximately 5 rat units of estrogen are consistently found in the urine of menopausal and castrate women. It has been suggested that this estrogen is derived from the adrenal cortex. In cases of the common ovarian dysfunctions estrogen determinations have not been satsfactory for clinical use. In such conditions as dysmenorrhea or metrorrhagia there is no typical pattern in the curve of estrogen excretion, although early reports indicated that there might be an increased yield in the urine of women with functional bleeding. Frank [23a] and associates demonstrated that in women who complain of premenstrual distress, nervousness, psychic disturbances, headaches, gastrointestinal upsets and other symptoms there is a rise of the serum level of estrogens preceding menstruation. A study of the estrogen excretion in women with amenorrhea has some significance. Frank has obtained in such cases three types of curves, representing (1) absence of excretion, (2) diminished or almost normal excretion and (3) increased excretion of estrogenic substances. He has suggested that other factors besides an abnormal elaboration of estrogen by the ovaries may be responsible for functional amenorrhea and has hinted that a disturbance of the ratio between estrogens and androgens might be a factor. Albright and Halsted [23b] have described similar curves of estrogen excretion in amenorrhea.

22. Dorfman, R. I.; Greulich, W. W., and Solomon, C. I.: The Excretion of Androgenic and Estrogenic Substances in Urine of Children, Endocrinology **21**: 741 (Nov.) 1937.

23. (*a*) Frank, R. T.: The Sex Hormones: Their Physiologic Significance and Use in Practice, J. A. M. A. **114**: 1504 (April 20) 1940. (*b*) Albright, F., and Halsted, J. A.: Studies of Ovarian Dysfunction: II. The Application of the "Hormonal-Measuring Sticks" to the Sorting Out and to the Treatment of the Various Types of Amenorrhoea, New England J. Med. **212**: 250 (Feb. 7) 1935.

A high estrogen excretion has been suspected and found in cases of certain ovarian tumors, principally those of the granulosa cell type. Actually a number of assays of granulosa cell tumors themselves revealed surprisingly low yields of estrogen to account for the extreme degree of endometrial hyperplasia usually found. Palmer [24] reported the greatest amount of estrogen in a granulosa cell tumor: an equivalent of 2,000 international units of estrone per kilogram of fresh tissue. The urine contained correspondingly large amounts of estrogen. Other ovarian tumors, including different types of cysts, have been analyzed for estrogens, but no consistent results have been obtained.

Assays of androgen in the urine of women have been made as a method of measuring ovarian function. Theoretically, it is possible for the ovaries to secrete androgen since this action has been demonstrated in the mouse under certain experimental conditions. Furthermore, an ovarian tumor (arrhenoblastoma) in woman secretes androgens, as evidenced by its masculinizing effect, and in addition the hilus of the normal ovary contains cells with potentially androgenic properties. Androgen assays indicate that essentially only small amounts, if any, are secreted by the ovary, since women who have had their ovaries removed or who have spontaneous menopause excrete considerable amounts of androgens. The androgens are probably derived from the adrenal cortex. Nevertheless, there is some evidence that women with ovarian dysfunctions excrete abnormal amounts of androgen. Women excrete varying quantities of androgens which may approximate the amounts found in the urine of normal males [25] but which are on the average somewhat less. The excretion of androsterone and other 17-ketosteroids is rather uniform throughout the menstrual cycle. Callow and Callow [26]

24. Palmer, A.: Estrogenic Hormone in the Urine and Tumor of a Patient with a Granulosa Cell Tumor of the Ovary, Am. J. Obst. & Gynec. **37**: 492 (March) 1939.

25. (*a*) Kenyon, A. T.; Gallagher, T. F.; Peterson, D. H.; Dorfman, R. I., and Koch, F. C.: Urinary Excretion of Androgenic and Estrogenic Substances in Certain Endocrine States: Studies in Hypogonadism, Gynecomastia and Virilism, J. Clin. Investigation **16**: 705 (Sept.) 1937. (*b*) Dingemanse, E.; Borchardt, H., and Laqueur, E.: Capon Comb Growth-Promoting Substances ("Male Hormones") in Human Urine of Males and Females of Varying Ages, Biochem. J. **31**: 500 (April) 1937. (*c*) Baumann, E. J., and Metzger, N.: Colorimetric Estimation and Fractionation of Urinary Androgens, Endocrinology **27**: 664 (Oct.) 1940. (*d*) Gallagher and others.[25a]

26. Callow, N. H., and Callow, R. K.: The Isolation of 17-Ketosteroids from the Urine of Normal Women, Biochem. J. **33**: 931 (June) 1939.

claimed the excretion was somewhat higher at the beginning of the menstrual cycle. Gallagher and associates [16a] could detect no pattern in the monthly excretion of androgens, the daily yield fluctuating from 1.3 to 4.6 milliequivalents of androsterone according to the capon method. Furuhjelm [18a] reported that the androgen excretion curve in normal women paralleled roughly the peaked estrogen excretion except that the androgen values were high during menstruation, at which time the estrogen excretion was very low. Hamblen, Cuyler and Baptist [27] reported a lowering of androgen excretion during menstrual bleeding. Werner,[18b] however, found a consistent output of androgens throughout the menstrual cycle, there being only a minor variation from the mean even over several menstrual cycles. According to Hamblen and associates,[28] studies on androgen excretion in patients with ovarian dysfunctions have revealed significant findings. Hamblen, Pattee and Cuyler [28a] found that during the menopause the average daily amount of androgen excreted was elevated from the normal of 3.4 to 8.4 mg. In hypo-ovarianism the values were ·7.0 mg. daily and in menorrhagia about 6.0 mg. In women whose breasts were recurrently painful with the menstrual cycle the daily androgen excretion averaged 5.1 mg., in women with functional dysmenorrhea 6.6 mg. and in women with "menstrual headaches" 5.8 mg. It is interesting that estrogen administration lowered these values somewhat.

In cases of virilism without gross pathologic changes in the adrenals, there is little increase in the androgen excretion.[29] In such cases the androgen output may occasionally be greater than normal but is considerably less than the yields observed in cases in which the adrenal cortex shows lesions.

Assays for progesterone in the blood or urine of man and other animals have until recently never been practical, owing to the fact that these fluids contain insufficient amounts of this substance for testing by the usual

27. Hamblen, E. C.; Cuyler, W. K., and Baptist, M.: Urinary Androgens and Uterine Bleeding, Endocrinology **27**: 16 (July) 1940.

28. (*a*) Hamblen, E. C.; Pattee, C. J., and Cuyler, W. K.: Alteration of Urinary Excretion of Androgens by Estrogenic Therapy, Endocrinology **27**: 734 (Nov.) 1940. (*b*) Hamblen, E. C.: Rationale of Androgen Therapy in Gynecology, J. Clin. Endocrinol. **1**: 180 (Feb.) 1941.

29. (*a*) Talbot, N. B.; Butler, A. M., and Maclachlan, E. A.: Alpha and Beta Neutral Ketosteroids (Androgens): Preliminary Observations on Their Normal Urinary Excretion and the Clinical Usefulness of Their Assay in Differential Diagnosis, New England J. Med. **223**: 369 (Sept. 5) 1940. (*b*) Kenyon and co-workers.[29a] (*c*) Baumann and Metzger.[29c]

biologic methods. Several liters of blood or urine would be required to furnish sufficient progesterone to induce an endometrial response in a single rabbit by the Corner-Allen method. Recently a more sensitive method has been used to detect progesterone in a few cubic centimeters of blood.[30] The material to be assayed is introduced directly into the rabbit uterus, and the endometrial response is obtained with relatively minute amounts of progesterone. Haskins [31] has reported finding progesterone in the serum of pregnant women. No practical applications have been as yet reported.

Within the past few years progesterone secretion has been measured by an indirect method. It has been conclusively shown that pregnandiol is a metabolic derivative of progesterone.[32] This substance occurs in the urine of normal women and in increased amounts during pregnancy. In the urine it occurs as sodium pregnandiol glycuronide, although there are small amounts of free pregnandiol, perhaps because of spontaneous hydrolysis of the urine specimen. In the normal cycle, sodium pregnandiol glucuronide is present in the urine during the corpus luteum phase. The daily excretion of this substance varies considerably, but the average is about 3 to 4 mg. daily. The total amount of this substance in the urine during a luteal phase may be from 3 to 60 mg. On the occurrence of menstruation, pregnandiol disappears from the urine. Pregnandiol assays are of value at the present time in investigations of the causes of ovarian dysfunctions. The wide fluctuations in the excretion of this substance prevent the quantitative application of such assays for practical purposes.[33]

30. McGinty, D. A.; Anderson, L. P., and McCullough, N. B.: Effect of Local Application of Progesterone on Rabbit Uterus, Endocrinology **24**: 829 (June) 1939. de Allende, I. L. C.: Blood Progesterone During Sexual Cycle of Macaca Rhesus: Quantitative Assay, Proc. Soc. Exper. Biol. & Med. **44**: 534 (June) 1940. Haskins, A. L., Jr.: Modification of the Intrauterine Assay Method for Progesterone, Endocrinology **27**: 983 (Dec.) 1940.

31. Haskins, A. L.: Assay of Blood of Pregnant Women for Progestin, J. Clin. Endocrinol. **1**: 65 (Jan.) 1941.

32. Venning, E. H., and Browne, J. S. L.: Studies on Corpus Luteum Function: I. The Urinary Excretion of Sodium Pregnanediol Glucuronidate in the Human Menstrual Cycle, Endocrinology **21**: 711 (Nov.) 1937; Study of Metabolism of Crystalline Progesterone, ibid. **27**: 707 (Nov.) 1940.

33. Hamblen, E. C.; Ashley, C., and Baptist, M.: Sodium Pregnanediol Glucuronide: The Significance of Its Excretion in the Urine, Endocrinology **24**: 1 (Jan.) 1939. Buxton, C. L.: Pregnanediol Determination as an Aid in Clinical Diagnosis, Am. J. Obst. & Gynec. **40**: 202 (Aug.) 1940. Bachman, C.; Leekley, D., and Hirschmann, H.: Excretion of Sodium Pregnanediol Glucuronidate in Urine of Normal Human Pregnancy, J. Clin. Investigation **19**: 801 (Nov.) 1940.

There is a definite relationship between the metabolism of progesterone and the state of the endometrium. At one time it was claimed that the endometrium was necessary for the conversion of progesterone to pregnandiol; later it was shown that the endometrium is not essential to this reaction [34] but aids materially in the conversion of progesterone to pregnandiol. The role of the endometrium in the metabolism of the ovarian steroids may prove to be of considerable significance in those common ovarian disorders which still remain unexplained. In addition to the state of the endometrium, other factors influencing the conversion of progesterone to sodium pregnandiol glycuronide and the excretion of the substance are the liver, kidney and its metabolic relationships with other steroidal hormones. Since progesterone is also elaborated by the adrenal cortex, pregnandiol may be found in minute quantities in the urine of castrate women and in that of men.[35]

Testes.—The activity of the testes may be measured within certain limits by the excretion of androgens in the urine. At the present time the values for the androgens excreted are in somewhat of a confused state inasmuch as the recent data have been obtained with biochemical assay methods rather than the capon method. The colorimetric methods of assay cannot distinguish between the ketosteroids which do not have androgenic activity and those which do. The values obtained with colorimetric procedures are usually two to three times as great as those with the use of the capon assay method. The presence of interfering substances and other chromogenic materials has resulted in variations in results as obtained by different investigators. Nevertheless, for practical purposes there appears to be satisfactory correlation between the capon and the colorimetric assays. The androgens found in normal male urine are principally androsterone and dehydroandrosterone. The latter has about one-fourth the androgenic activity of androsterone, but both give

34. Buxton, C. L., and Westphal, U.: Recovery of Pregnandiol in Urine of Men Treated with Progesterone, Proc. Soc. Exper. Biol. & Med. **41**: 284 (May) 1939. Hamblen, E. C.; Cuyler, W. K., and Hirst, D. V.: Urinary Excretion of Pregnandiol Complex by Males: II. Following Intramuscular Administration of Progesterone, Endocrinology **27**: 172 (Aug.) 1940.

35. Engel, L. L.; Thorn, G. W., and Lewis, R. H.: Urinary Excretion of Steroid Compounds: Normal Male Subjects, J. Biol. Chem. **137**: 205 (Jan.) 1941. Hirschmann, H.: Steroids of Urine of Ovariectomized Women, ibid. **136**: 483 (Nov.) 1940.

an equal intensity of color when tested by the Zimmerman colorimetric reaction. The ketosteroid etioallocholanolone is found in quantities approximately equal to those of androsterone. It gives a color reaction but has little androgenic activity. In terms of milligrams of androsterone, the normal male excretes about 7 mg. daily when tested by the capon method.[16a] Androsterone is apparently a metabolic derivative of the true testicular hormone, testosterone,[36] but about one third of the excreted androsterone is probably derived from the adrenal cortex.[36a,b,c] Dehydroandrosterone amounts to about 5 per cent of the ketosteroids, but this is increased to as much as 15 per cent of the total in normal males between the ages of 20 and 27.[36d]

The excretion of androgens in children is from 0.3 to 2 milliequivalents of androsterone. Likewise, the androgen excretion in males past the age of 40 is diminished.[36b] In castrates and eunuchs there is a distinct decrease in androgen excretion to about one third of normal.[37] Studies of borderline hypogonadism have not been intensive as regards androgen excretion. The differential diagnosis of psychic and organic impotence by means of androgen assays would be valuable to clinicians. Disorders of sperm formation are not likely to result in significant changes in androgen excretion inasmuch as androgenic substances are more concerned with the function of the accessory sex organs.

Males also excrete estrogenic substances in small amounts equivalent to about 10 micrograms of estrone daily. This amount is decreased considerably in eunuchs and castrates, indicating that a good part of the estrogen is a metabolic derivative of testosterone. This contention is supported by the demonstration that injections of testosterone raise the yield of urinary estrogens in both castrate and normal men.[36a,c]

A theory has been evolved that a relative excess of estrogens is responsible for the production of prostatic

36. (*a*) Dorfman, R. I.: Fate of Testosterone in the Human, Proc. Soc. Exper. Biol. & Med. **45**: 739 (Nov.) 1940. (*b*) Kochakian, C. D.: Excretion of Male Hormones, Endocrinology **21**: 60 (Jan.) 1937. (*c*) Callow, N. H.; Callow, R. K., and Emmens, C. W.: The Effect of the Administration of Testosterone Propionate on the Urinary Excretion of Compounds Allied to the Steroid Hormones, J. Endocrinol. **1**: 99 (June) 1939. (*d*) Baumann, E. J., and Metzger, N.: Colorimetric Estimation and Fractionation of Urinary Androgens, Endocrinology **27**: 664 (Oct.) 1940.

37. Callow, N. H., and Callow, R. K.: Excretion of Androgens by Eunuchs: The Isolation of 17-Ketosteroids from the Urine, Biochem. J. **34**: 276 (March) 1940. Kenyon and others.[36a]

adenoma. Dingemanse and Laqueur [38] were unable to confirm this theory, although they found significant variations from the normal steroid excretion in males with prostatic adenoma. The estrogen-androgen ratios of such patients average 1 : 3, compared with an average ratio of 2 : 1 in normal males of the same age.

In understanding testicular disorders gonadotropin assays are often helpful. Certain neoplasms of the testes elaborate gonadotropic substance. Seminoma, a tumor of the germinative epithelium, secretes little or no gonadotropin. Teratoma, with or without chorionic tissue, elaborates varying amounts, from several hundred rat units daily to as much as a million rat units. This gonadotropin is similar, if not identical, with the chorionic gonadotropin of pregnancy. The course of a patient who has had such a tumor removed may be followed by examinations of the urine for gonadotropin at intervals following the operation.

ADRENAL CORTEX

The adrenal cortex elaborates numbers of steroids. These have widely varying properties, although many of them are similar in their behavior. For instance, some of the steroids may have androgenic, estrogenic or even progestational activity. Others have marked effects on salt and water metabolism, capillary permeability, and carbohydrate, fat and protein metabolism. The maintenance of life and health in adrenalectomized animals is probably due to a combination of the various activities of the adrenal steroids, although there are a number of these compounds which can alone maintain the life of adrenalectomized animals for considerable time. There are, however, certain metabolic weaknesses in these surviving animals. For instance, desoxycorticosterone may maintain the life of adrenalectomized animals or patients with Addison's disease, but it cannot adequately alleviate hypoglycemic reactions which may occur, since it has little or no role in regulating carbohydrate metabolism.

At the present time, assays for the urinary 17-ketosteroids are of some value in detecting types of adrenal

38. Dingemanse, E., and Laqueur, E.: The Content of Male and Female Hormone in the Urine of Patients with Prostatic Hypertrophy, J. Urol. 44: 530 (Oct.) 1940; abstracted, J. Clin. Endocrinol. 1: 89 (Jan.) 1941.

dysfunction.[39] Thus, while the alpha ketosteroid, androsterone, is chiefly a product of the testes, the beta ketosteroids, such as dehydroisoandrosterone, are exclusively products of the adrenal cortex. Under certain conditions beta ketosteroids are greatly increased in quantity—for instance, in patients with hyperplasia, adenoma or carcinoma of the adrenal cortex. Cushing's syndrome due to pituitary basophilism may be clinically quite similar to the adrenogenital syndrome resulting from neoplasm or hyperplasia of the adrenal cortex. An analysis of the androgen excretion is of distinct value in differentiating between the pituitary and the adrenal type of Cushing's syndrome. Talbot and associates have concluded:

> The beta neutral ketosteroids arise solely from substances produced by the adrenal cortex. It follows as a corollary that the rate of excretion of beta ketosteroids may be an index of at least one aspect of adrenocortical activity. The assay of the alpha, beta and total neutral ketosteroids gives specific information which may be useful in differentiating adrenocortical hyperplasia, Cushing's syndrome without adrenal tumor, and adrenocortical carcinoma.

Other ketosteroids have been found to be excreted in large amounts in adrenal cortex hyperactivity.[40] An increase in steroids which maintain life was demonstrated in the blood stream of a patient with hyperfunction of the adrenal cortex (Cushing's syndrome).[41] In some cases of adrenal carcinoma large amounts of estrogens may also be found excreted in the urine.[42] Venning and associates have detected large amounts of pregnandiol in two cases of adreno-genital syndrome.[42b]

39. Crooke, A. C., and Callow, R. K.: The Differential Diagnosis of Forms of Basophilism (Cushing's Syndrome), Particularly by Estimation of Urinary Androgens, Quart. J. Med. **8**: 233 (July) 1939. Levy-Simpson, S.; de Fremery, P., and Macbeth, A.: The Presence of an Excess of "Male" (Comb-Growth and Prostate-Stimulating) Hormone in Virilism and Pseudo-Hermaphroditism, Endocrinology **20**: 363 (May) 1936. Talbot and others.[29a]

40. Butler, G. C., and Marrian, G. F.: Chemical Studies on the Adreno-Genital Syndrome: I. The Isolation of 3 (a)-Hydroxyetiocholane-17-one, 3 (B)-Hydroxyetioallocholane-17-one (Isoandrosterone), and a New Triol from the Urine of a Woman with an Adrenal Tumor, J. Biol. Chem. **124**: 237 (June) 1938; correction, Nature, London **142**: 400 (Aug. 27) 1938.

41. Anderson, E.; Haymaker, W., and Joseph, M.: Hormone and Electrolyte Studies of Patients with Hyperadrenocortical Syndrome (Cushing's Syndrome), Endocrinology **23**: 398 (Oct.) 1938.

42. (*a*) Frank, R. T.: A Suggested Test for Functional Cortical Adrenal Tumor, Proc. Soc. Exper. Biol. & Med. **31**: 1204 (June) 1934. (*b*) Venning, E. H.; Weil, P. G., and Browne, J. S. L.: Excretion of Sodium Pregnandiol Glucuronidate in the Adreno-Genital Syndrome, J. Biol. Chem. **128**: cvii (June) 1939.

In precocious puberty unrelated to the adrenal cortex there is little change in the excretion of beta ketosteroids.

Weil and Browne [43] have been able to detect significant amounts of life-maintaining steroids in the urine of normal persons. These substances were found in increased amounts under certain conditions, chiefly when the subjects had been placed under stress, as in infections, operations and exposure to cold. Seventeen-ketosteroid excretion is low or absent in Addison's disease as well as in cases of anterior lobe insufficiency.[43b] The significance of these findings is complicated by the fact that Fraser and associates have shown that malnutrition or debility of a non-specific nature frequently results in subnormal amounts of 17-kerosteroid excretion. In addition to these findings, these workers have detected changes of 17-kerosteroid excretion in the following conditions: liver disease, low; Addison's disease, absent in females and low in males; adrenal cortex tumor, high; Cushing's disease (pituitary basophilism), high and normal; precocious puberty due to a lesion of the hypothalamus, normal; pituitary insufficiency, very low; acromegaly, normal or low; arrhenoblastoma of the ovary, normal; hypothyroidism, low or absent; hyperthyroidism, slightly subnormal, and psychic impotence, normal.[43c]

PREGNANCY

The excretion of the various sex hormones in pregnant women differs considerably from that in normal women. It is acknowledged that the placenta has a prominent role in the elaboration of many of these hormones. In addition to increases in amounts of substances which are present in normal persons, an entirely different one appears: chorionic gonadotropin or the anterior pituitary–like hormone. This is the substance which is responsible for the widely used pregnancy tests involving gonadal stimulation; it is not found in the absence of chorionic tissue. Within a few days after

43. (*a*) Weil, P., and Browne, J. S. L.: The Excretion of Cortin After Surgical Operation, Science **90**: 445 (Nov. 10) 1939. (*b*) Callow, N. H.; Callow, R. K., and Emmens, C. W.: 17-Ketosteroid, Androgen and Oestrogen Excretion in the Urine of Cases of Gonadal or Adrenal Cortical Deficiency, J. Endocrinol. **2**: 88 (May) 1940. (*c*) Fraser, R. W.; Forbes, A. P.; Albright, F.; Sulkowitch, H., and Reifenstein, E. C., Jr.: Colorimetric Assay of 17-Ketosteroids in Urine, J. Clin. Endocrinol. **1**: 234 (March) 1941.

the first missed period, chorionic gonadotropin appears in the body fluids and may be detected by injecting the urine into rabbits or rats. The concentration of this substance increases rapidly, and from twenty to fifty days after the last missed period tremendous quantities may be found; as much as several hundred thousand rat units have been demonstrated in a liter of pregnancy urine for a short time around this period.[44] This occurrence has been described as an explosion, since it appears and disappears rapidly. After this peak, the daily gonadotropin excreted remains at a fairly constant level, from 3,000 to 10,000 rat units, until the termination of pregnancy, after which it disappears from the body within four or five days. The most reliable and practical of pregnancy tests is the well known Friedman test, which has withstood the test of time and is considered one of the most useful biologic methods available to the clinician. Chorionic gonadotropin is elaborated, in addition, by the chorionic tissue of the hydatid mole and by chorioepithelioma. Quantitative assays may be useful in the detection of such tissue, the quantitative Aschheim-Zondek method being used. Several years ago it was considered that high values for chorionic gonadotropin were diagnostic of these growths. It has been conclusively demonstrated, however, that normal or even low values may occasionally be found when the uterus contains either of these growths. Although high titers of chorionic gonadotropin after the third month of pregnancy are excellent evidence for the diagnosis of these conditions, nevertheless, the finding of normal values or even low ones does not rule out the occurrence of a hydatid mole or a chorionic tumor. The clinical history and physical examination should be carefully considered under these circumstances. Tests for chorionic gonadotropin are also of value in cases in which such tumors have been removed, since metastases are capable of elaborating the gonadotropin, and they may be traced by testing the urine at intervals.

The excretion of estrogens in pregnancy is somewhat complicated, since there are at least three estrogenic substances found in the urine, each of which occurs both free and combined with glycuronic acid. The placenta is responsible for the elaboration of most of the estro-

44. Evans, H. M.; Kohls, C. L., and Wonder, D. H.: The Gonadotropic Hormone in the Blood and Urine of Early Pregnancy: Normal Occurrence of Transient Extremely High Levels, J. A. M. A. **108**: 287 (Jan. 23) 1937.

gens of pregnancy. The total estrogen content of pregnancy urine rises gradually from the first missed menses to term, at which time the excretion amounts to as high as 100,000 rat units daily. Patients differ greatly, and the daily excretion of any given one fluctuates considerably also. The Smiths [45] and their associates have studied, both biologically and gravimetrically, the excretion of estrogens through partitioning the total estrogen into its three components, estrone, estriol and estradiol. Their work is in agreement with that of Cohen, Marrian and Watson [46] that about 90 per cent of the estrogenic activity in the late months is due to estriol. The Smiths and their associates found that at about two months of pregnancy the ratio between estrone and estriol was 1 : 2 but that at nine months it was about 1 : 15. Estradiol is excreted at a fairly uniform rate throughout pregnancy, averaging about 0.13 mg. daily. The amounts of estrone and estriol constantly increase until term. There is, however, a sudden rise of estradiol in the urine at term, with a disappearance of estrone from the urine. At this time estriol is also present in lesser amounts. According to these investigators, the changes in estrogen pattern result from a reduction in the progesterone output at term. The lack of this substance is claimed to be responsible for the depressed conversion of estrone to estriol and the increased destruction of estrogens. A theory of parturition has been advanced by Cohen, Marrian and Watson, who demonstrated that large amounts of combined estrogens are spontaneously altered to the free or active state immediately preceding parturition, thus accounting for the sudden contractility of the uterus.

Progesterone is elaborated during the first three months by the corpus luteum of the ovary. After the third month the placenta takes over the function of secreting progesterone. Assays for pregnandiol indi-

45. Smith, G. V., and Smith, O. W.: Estrogen and Progestin Metabolism in Pregnant Women, with Especial Reference to Pre-Eclamptic Toxemia and the Effect of Hormone Administration, Am. J. Obst. & Gynec. **39**: 405 (March) 1940. Smith, O. W.; Smith, G. V., and Schiller, S.: Estrogen and Progestin Metabolism in Pregnancy; Spontaneous and Induced Labor, J. Clin. Endocrinol. **1**: 461 (June) 1941. Smith, G. V., and Smith, O. W.: Estrogen and Progestin Metabolism in Pregnancy; The Endocrine Imbalance of Preeclampsia and Eclampsia, J. Clin. Endocrinol. **1**: 470 (June) 1941. Smith, G. V., and Smith, O. W.: Estrogen and Progestin Metabolism in Pregnancy; Effect of Hormone Administration in Preeclampsia, J. Clin. Endocrinol. **1**: 477 (June) 1941.

46. Cohen, S. L.; Marrian, G. F., and Watson, M.: Excretion of Oestrin During Pregnancy, Lancet **1**: 674 (March 23) 1935.

cate that at about the hundredth day there may be a drop in excretion due to a lag in elaboration of progesterone by the placenta.[47] It is significant that habitual abortion is most common at this time, and the theory has been advanced that the decrease in progesterone content of the blood renders the uterus sensitive and contractile. Pregnandiol excretion in normal pregnancy is not uniform but varies from day to day and among individuals. At the fifth week of pregnancy about 8 mg. (as sodium pregnandiol glycuronide) is excreted daily and at term this rises to an average of about 80 mg. Removal of the corpus luteum in pregnancy in 2 cases did not result in a decrease in the pregnandiol excreted.[48a,b] Ovariectomy in one pregnant patient resulted in a significant drop in pregnandiol excretion although gestation remained undisturbed.[48c]

The toxemias of pregnancy have been investigated through hormone excretion chiefly by the Smiths and their associates. This group has demonstrated that during a toxemia or even preceding the development of such a state there is a definite disturbance of the ratio between estrogens and gonadotropin.[45] Their evidence indicates that the content of chorionic gonadotropin in the blood serum and the urine is increased, while the blood and urine concentration of estrogens is significantly lowered. They have maintained also that this imbalance may be counteracted by the administration of large doses of estrogens and progesterone. More recently they have claimed that the lowered pregnandiol excretion during toxemias is an important factor in the altered estrogen metabolism in these states. Partitioning of the estrogens during toxemias revealed that the estrone fraction dropped to very low values or disappeared entirely, the estriol fraction decreased considerably, but the estradiol fraction increased. Such changes were explained by the failure of estrone conversion to estriol caused by lack of progesterone, which also allowed an increase in destruction of the estrogens. It has already been pointed out that these investigators

47. Browne, J. S. L.; Henry, J. S., and Venning, E. H.: The Significance of Endocrine Assays in Threatened and Habitual Abortion, Am. J. Obst. & Gynec. **38**: 927 (Dec.) 1939.

48. (*a*) Browne, J. S. L.; Henry, J. S., and Venning, E. H.: The Corpus Luteum Hormone in Pregnancy, J. Clin. Investigation **16**: 678 (July) 1937. (*b*) Jones, H. W., and Weil, P. G.: The Corpus Luteum Hormone in Early Pregnancy, J. A. M. A. **111**: 519 (Aug. 6) 1938. (*c*) Seegar, G. E., and Delfs, E.: Pregnandiol Excretion Following Bilateral Oophorectomy in Early Pregnancy, ibid. **115**: 1267 (Oct. 12) 1940.

demonstrated similar changes in estrogen concentrations at parturition. Taylor and Scadron [49] obtained lowering of estrogen and elevation of gonadotropin levels in only a few of their patients with toxemias. They believed that these occasional changes may have been due to hepatic or renal disturbances rather than to any defect in hormone metabolism. Other evidence, which does not support the theories of the Smiths and their associates on estrogen metabolism of normal and abnormal pregnancy, was contributed by Hain.[50] This worker recovered large amounts of combined estrogen before or even during parturition in normal women. Furthermore, large amounts of pregnandiol were also found preceding or during normal labor in some women as well as in an eclamptic woman. Hain was unable to subscribe to the theory of deconjugation of estrogens as the cause of labor. Nevertheless, Weil [51] has observed lowered pregnandiol excretion in toxemia and Taylor and Scadron [49] have reported a similar observation in a small series of cases. Bachman and associates have also obtained low yields of pregnandiol in preeclampsia and hypertensive disorders of pregnancy where there was proteinuria, but not in uncomplicated chronic hypertension damage.[51b] The excretion of androgens in pregnancy was found to be normal by several workers.[28b]

MISCELLANEOUS OBSERVATIONS

There are numerous reports on hormone excretion in a variety of conditions. Most of these show little uniformity in the results obtained. For example, Geschickter and associates [52] have claimed that in chronic cystic mastitis there is an increased estrogen content of the mammary tissue. They have also indicated recently that pregnandiol determinations

49. Taylor, H. C., Jr., and Scadron, E. N.: Hormone Factors in the Toxemias of Pregnancy, with Special Reference to Quantitative Abnormalities of Prolan and Estrogens in the Blood and Urine, Am. J. Obst. & Gynec. **37**: 963 (June) 1939.

50. Hain, A. M.: The Excretion of Oestrogen and Pregnanediol by Pregnant and Parturient Women: Normal and Toxaemic Cases, J. Endocrinol. **2**: 104 (May) 1940.

51. (*a*) Weil, P. G.: The Excretion of Pregnandiol in the Toxemias of Pregnancy, Science **87**: 72 (Jan. 21) 1938. (*b*) Bachman, C.; Leekley, D., and Hirschmann, H.: The Urinary Excretion of Pregnandiol Glucuronidate in the Hypertensive Disorders of Pregnancy, J. Clin. Endocrinol. **1**: 206 (March) 1941.

52. Geschickter, C. F.; Lewis, D., and Hartman, C. G.: Tumors of the Breast Related to the Oestrin Hormone, Am. J. Cancer **21**: 828 (Aug.) 1934.

revealed a deficiency of progesterone in this condition.[53] Taylor [54] was unable to find any increase in urinary estrogens in women with this disturbance. Yolton and Rea were unable to detect any change in the androgen or estrogen excretion in men with cancer of the mammary gland.[54b] In regard to acne vulgaris, which has long been suspected to be the result of some endocrine disturbance, numerous assays have been reported. It has recently been demonstrated that in patients with this disorder there is decreased excretion of estrogens and normal excretion of androgens.[55] This relative predominance of androgens has been considered the etiologic factor for the changes in the skin. An excellent review on the relationship of androgens to acne has recently appeared.[55b] There have been several reports of an altered estrogen-androgen ratio in homosexuality. None of this evidence has been adequately confirmed. It is of considerable interest that in males with cirrhosis of the liver there is increased excretion of free estrogens due to failure of the liver to conjugate them with glycuronic acid. In those patients with cirrhosis who have testicular atrophy there is little androgen excretion.[56] This decrease in the androgen-estrogen ratio is believed to be responsible for the hyperplasia of the breast tissue in such patients. Assays in cases of hemophilia revealed no abnormality of estrogen excretion, apropos the theory advanced several years ago that a deficiency of estrogen accounted for this disturbance in males. Pincus and Pearlman have indicated that in cancerous men and women the 17-ketosteroid excretion is decreased and does not have the sex difference which occurs in normal men and women. The possibility exists that this may be of a nonspecific nature associated with the general debility of the patients.[56b]

53. Bucher, N. L. R., and Geschickter, C. F.: Corpus Luteum Studies: Pregnandiol and Estrogen Output in Urine of Patients with Chronic Cystic Mastitis, J. Clin. Endocrinol. **1**: 58 (Jan.) 1941.

54. (*a*) Taylor, H. C., Jr.: The Relation of Chronic Mastitis to Certain Hormones of the Ovary and Pituitary and to Coincident Gynecological Lesions: I. Theoretical Considerations and Histological Studies, Surg., Gynec. & Obst. **62**: 129 (Feb.) 1936. (*b*) Yolton, N., and Rea, C.: Excretion of Androgens and Estrogens in Males with Mammary Carcinoma, Proc. Soc. Exper. Biol. & Med. **45**: 54 (Oct.) 1940.

55. (*a*) Lawrence, C. H., and Werthessen, N. T.: Endocrine Dyscrasia of Acne Vulgaris in Women, Endocrinology **27**: 755 (Nov.) 1940. (*b*) Hamilton, J. B.: Male Hormone Substance: A Prime Factor in Acne, J. Clin. Endocrinol. **1**: 570 (July) 1941.

56. (*a*) Glass, S. J.; Edmondson, H. A., and Soll, S. N.: Sex Hormone Changes Associated with Liver Disease, Endocrinology **27**: 749 (Nov.) 1940. (*b*) Pincus, G., and Pearlman, W. H.: Alcoholic and Non-Alcoholic Ketosteroids and the Zimmerman Color Reaction, Science **93**: 163 (Feb. 14) 1941.

Physicians will be disappointed if they expect simple or casual hormone assays to furnish them with significant information except in a few isolated cases. Intensive and carefully controlled investigations are essential in contributing to knowledge of normal or abnormal endocrine physiology. The rapid strides made in the past few years are encouraging, and it is anticipated that definite practical aid will be available to the clinician with the accumulation of reliable data on the assays of hormones and related compounds.

CHAPTER XXIII

THE ASSAY OF GONADOTROPINS AND OF GONADAL HORMONES

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As in other physical states, the goal of therapy in gonadal dysfunction is to reproduce a normal condition in an abnormal body. This requires quantitative knowledge of the normal concentration of the hormones concerned in the body tissues and fluids and quantitative knowledge of their concentration in the agents used for replacement. In both cases the development of suitable assay procedures is essential.

The problem is not simple. Biologic methods, with all their attendant difficulties, must still be used in assaying many of these principles. Metabolic processes may alter certain of the substances both chemically and biologically. Because the actions of the substances are multiple, a confusingly large number of methods, based on different responses, with correspondingly many units, have been proposed. The space available permits discussion of only a few of the problems, and the same limitation prevents reference to much of the enormous amount of work done.

GENERAL PROBLEMS INVOLVED IN BIOASSAYS

Criteria which determine the suitability of a biologic method of assay are:

1. Objectivity. The weight of an object is the same in one laboratory as in another, and therefore a good balance is more reliable than the most conscientious investigator's judgment.
2. Sensitivity. There should be a large increase in response following a small increase in dose; this obviously makes for greater accuracy.
3. Simplicity. When assays must be done frequently and expense is a consideration, a complicated or expensive procedure, no matter how accurate, will usually fail in practice.

4. Individual variation. The unfortunate property of test animals to differ greatly in their individual responses to drug or glandular treatment has been emphasized by every one engaged in assay work. The greater this variation, the larger the number of animals which must be employed.

These are the most fundamental factors. However, it is apparent, no matter which method is chosen, that all details of technic must be duplicated each time the assay is performed, in order that results may be reproducible. Such details are: type and age of animals, duration of injection period, route of administration, single or divided dose, menstruum, and purity of substance being assayed.

THE ASSAY OF GONADOTROPINS

Gonadotropins Concerned.—The gonadotropic preparations used clinically originate chiefly from three sources: the urine of pregnant women, the serum of pregnant mares and the anterior lobe of the pituitary. Large amounts of a gonadotropic substance are excreted by castrate and menopausal women, but this source has not been exploited commercially. Normal women throughout the midinterval of the menstrual cycle also excrete a gonadotropic substance similar to that found in castrate women, but the small amount renders this source unimportant. There is considerable agreement that the gonadotropin in the urine of pregnant women is entirely luteinizing, while that in the serum of pregnant mares is largely, if not entirely, follicle stimulating. Separation of anterior pituitary extracts into pure or nearly pure follicle-stimulating and luteinizing fractions has been accomplished, and some of the commercial preparations are nearly entirely follicle stimulating in effect. Hypophysectomized animals are essential in determining that the separation has been made.

Assay Methods.—Since no chemical methods are available, these are based on biologic responses. Suitable reactions are discussed in reference to the established criteria in the following paragraphs:

1. Increase in weight of seminal vesicles. This method is applicable to preparations of the gonadotropin in the urine of pregnant women and of that in pregnant mare's serum. It is completely objective; the sensitivity is moderate; with practice the process is simple, requiring not more than thirty seconds for dissection of the

vesicles and a little more for weighing. The structures can be cleanly and completely removed. The individual variation, i. e., difference between the maximum and the minimum response on a given dose, is about 100 per cent.

2. Increase in weight of ovaries. This method is applicable to preparations of gonadotropins from all three sources and is completely objective. The sensitivity varies with the gonadotropin concerned, being low for that of pregnancy urine, low at low doses for that of pregnant mare's serum but higher with larger doses, and moderate for anterior pituitary gonadotropin. It is simple, with practice the ovaries can be cleanly and completely dissected, and the time required is little longer than for seminal vesicles. The individual variation is somewhat greater than with the seminal vesicle method.

3. Increase in weight of uteri. This method is applicable to all three types of gonadotropins; it is completely objective and about as easy as the previous ones. The great absolute weight of the uteri is an advantage, making exact weighing less important. The sensitivity is great, a six-fold increase in weight being obtained on either pregnancy urine or pregnant mare's serum preparations, with doses which will only double the weight of the seminal vesicles and hardly affect the ovarian weight at all. The individual variation is great, being in some studies 300 to 400 per cent, which makes the use of large numbers of animals necessary. One disadvantage is the fact that the weight curve reaches a maximum and then declines with larger doses.

4. Vaginal cornification. This method is the simplest and is applicable to preparations of gonadotropins from all three. It is reasonably objective, provided one sets as his standard a full estrus smear, i. e., the complete disappearance of leukocytes and their replacement by epithelial cells. The sensitivity is great and appears to parallel rather closely the uterine weight method.

5. Luteinization. This method is unsatisfactory in practically every respect. Unless microscopic sections are prepared, which takes time and labor, the degree of luteinization is difficult to determine; macroscopic examination fails on the score of objectivity. The method is insensitive and individual variation still rather great.

From the foregoing discussion the following conclusions may be drawn: Organ weight methods have the advantage of objectivity; however, the ovarian response is rather insensitive and the seminal vesicle response decidedly insensitive to anterior pituitary preparations. This leaves the uterine weight method as applicable to preparations of gonadotropins from all three sources and as being both objective and sensitive. Individual variation is great, but that is characteristic to some degree of all biologic assays. Vaginal cornification is simplest and reasonably objective with practice. Since both uterine weight and vaginal estrus can be determined simultaneously, i. e., on the same animals, a combination of the two might be employed. However, it must be remembered that both responses are also produced by estrogens, and the absence of the latter from the material being assayed must be assured.

Assay of Commercial Gonadotropins and Establishment of International Standards.—Several studies of commercial preparations have been reported.[1] Usually (less rarely with anterior pituitary preparations) it is found that these materials contain the biologic activity stated on the label to be present, according to the method of assay employed. However, all such studies emphasize the fact that no comparison of activity is possible because of the lack of uniformity of the assay methods employed. This situation has been measurably relieved by the establishment of international standards and the definition of international units for the gonadotropin in the urine of pregnant women[2] and that in pregnant mare's serum.[3] This improvement has resulted from the work of the Commission of Biological Standardization of the Health Organization of the League of Nations and is to be highly commended. For both substances, contributions from a number of laboratories

1. D'Amour, F. E., and D'Amour, Marie C.: The Potency of Certain Commercial Hormone Preparations, Endocrinology **22**: 583 (May) 1938. Gaensbauer, Ferdinand, and Bradbury, J. T.: The Comparative Potency of Commercial A. P. L. Preparations, ibid. **24**: 867 (June) 1939. D'Amour, F. E.: The Potency of Certain Commercial Hormone Preparations: Second Study, ibid. **26**: 88 (Jan.) 1940. Goldman, S. F., and Cinberg, B. L.: The Potency of Pituitary Gonadotropins, ibid. **27**: 524 (Sept.) 1940.

2. Memorandum on the International Standard for the Gonadotrophic Substance of Human Urine of Pregnancy (Chorionic Gonadotrophin) Established by Department of Biological Standards, National Institute of Medical Research, Bull. Health Organ., League of Nations **8**: 884, 1939.

3. Memorandum on the International Standard for the Gonadotrophic Substance of Pregnant Mares' Serum Established by Department of Biological Standards, National Institute for Medical Research, Bull. Health Organ., League of Nations **8**: 898, 1939.

were pooled and diluted to a proper concentration, the final product representing the International Standard Preparation. In the case of preparations of pregnancy urine gonadotropin, the international unit (I. U.) is defined as "the specific gonadotrophic activity of 0.1 mg. (100 gamma) of the standard preparation"; in the case of preparations of pregnant mare serum gonadotropin the international unit is 0.25 mg. of the standard preparation. In both cases the following recommendation for use of the standard preparation in biologic assays is made:

> The only tests for the comparative determination of gonadotrophic activity, in units as above defined, on which the Conference has evidence to justify recommendation, depend on:
>
> (*a*) The observation of a direct or indirect gonadotrophic effect, shown by morphological changes in the gonads;
>
> (*b*) The observation of secondary changes in the accessory reproductive organs, in animals not deprived of their gonads. When this type of test is used, the absence of substances directly causing such changes in the accessory reproductive organs should be assured by control tests on animals deprived of their gonads.

In more recent publications [4] the data obtained from the assay of materials making up the pools are submitted to statistical analysis. It is recommended that for preparations of pregnancy urine gonadotropin the vaginal cornification test be employed. No specific recommendation is made for preparations of pregnant mare serum gonadotropin.

The Council on Pharmacy and Chemistry of the American Medical Association has recommended the adoption of international units in expressing the potency of Council-approved preparations. It is desirable to know just how much biologic activity is represented by 1 international unit of either material and also how the activities of the two compare over a range of doses. In one such study [5] it was found that the biologic activity of 1 international unit of each was approximately the same, but at higher levels of dose the ovarian response was much greater with the gonadotropin of pregnant mare serum than with that of pregnancy urine.

4. Emmens, C. W.: Analysis of Assays Carried Out in Various Laboratories on Contributions Offered Towards International Standard Preparation of Gonadotrophic Substance of Urine of Pregnancy, Bull. Health Organ., League of Nations **8**: 862, 1939. Emmens, C. W.: Analysis of Assays Carried Out in Various Laboratories on Separate Contributions Offered Towards International Standard Preparation for Gonadotrophic Substance of Pregnant Mare's Serum, ibid. **8**: 887, 1939.

5. D'Amour, F. E., and D'Amour, Marie C.: A Comparison of the International Gonadotrophin Standards, Endocrinology **27**: 68 (Jan.) 1940.

As regards the anterior pituitary gonadotropin, the Commission of Biological Standardization has not felt that complete separation into follicle-stimulating and luteinizing fractions was sufficiently assured to warrant establishing international standards for materials from this source. Confusion will therefore continue to exist in respect to the activity and unitage of anterior pituitary preparations.

While the establishment of international units for preparations of gonadotropins from two of the three sources is no doubt a long step toward uniformity, it must not be supposed that this will solve the problem completely. Such standards have been used in estrogen standardization for several years. However, one still finds in the labels on well known commercial estrogenic preparations mention of "rat units," "active biological units" and "vaginal canalization units." Oral administration has also introduced a confusing factor in that a preparation containing the stated number of international units when assayed subcutaneously produces a much smaller biologic response when administered orally. Percutaneous administration presents a similar difficulty. Consideration must also be given to the possibility of deterioration on standing, as well as to variability of response with the use of different vehicles, i. e., aqueous vs. oil suspensions. Some of these, and no doubt other problems, may be anticipated in the gonadotropin sphere. Some clinicians do not yet recognize the fact that preparations of the gonadotropins come from three different sources and possess different properties, and the failure to establish international units for the anterior pituitary gonadotropins may, if the gonadotropins from the three sources are confused, reduce the value of the establishment of such units for the other two.

Summary.—Gonadotropins can be assayed only by biologic methods whose accuracy is dependent on careful control of many factors. Of the methods available, that of vaginal cornification appears to be the most highly recommended. International units have been established for the gonadotropin prepared from the urine of pregnant women and for that from pregnant mare's serum but not for the anterior pituitary gonadotropins. Although the establishment of international units has eliminated much confusion, the use of these units is no guaranty of uniformity of results in practice.

THE ASSAY OF GONADAL HORMONES

The development of reliable methods of assay has led to the isolation of estradiol (dihydrotheelin) from the follicle fluid of the sow,[6] progesterone from the corpus luteum of the sow[7] and testosterone from the testicle of the bull.[8] As a result of metabolic changes in the body, these primary principles are altered chemically and eventually are found in the urine as degradation products which in some instances are conjugated with glycuronic acid. These conjugated compounds, which are soluble in water and insoluble in most of the immiscible solvents, have lost much of their biologic activity. Androgenic and estrogenic conjugates occur in the urine of both sexes. The adrenal cortex may compensate for regression of gonadal activity by secreting androgens and estrogens.[9] These findings have complicated the problem of analysis and the task of interpretation.

Since crystalline estrogens, androgens and progesterone are available, biologic assays are necessary only to confirm the dosages stated on the labels of commercial preparations. So far as we know, clearcut distinctions have not been made in the clinical use of various estrogens and androgens. It is of course recognized that the greater solubility of estriol (theelol) in water affects the rate of absorption. The occurrence of these substances in the blood and urine has led to the hope that a quantitative determination of these products would be useful in furnishing more complete information about normal physiologic mechanism, provide a basis for the diagnosis of pathologic conditions and possibly offer a guide to therapy. It must be admitted that the task is difficult because of its complicated nature. Two general sorts of methods are used for the quantitative assay of these substances in blood and urine—biologic methods and chemical methods. Space does not permit a complete discussion of the details of these methods.

6. MacCorquodale, D. W.; Thayer, S. A., and Doisy, E. A.: The Isolation of the Principal Estrogenic Substance of Liquor Folliculi, J. Biol. Chem. **115**: 435 (Sept.) 1936.

7. Allen, W. M., and Wintersteiner, O.: Crystalline Progestin, Science **80**: 190 (Aug. 24) 1934.

8. David, K.; Dingemanse, E.; Freud, J., and Laqueur, E.: Ueber krystallinisches männliches Hormon aus Hoden (Testosteron), wirksamer als aus Harn oder aus Cholesterin bereitetes Androsteron, Ztschr. f. physiol. Chem. **233**: 281, 1935.

9. Callow, Nancy H.; Callow, R. K., and Emmens, C. W.: 17-Ketosteroid, Androgen and Oestrogen Excretion in the Urine of Cases of Gonadal or Adrenal Cortical Deficiency, J. Endocrinol. **2**: 88 (May) 1940.

Urinary Estrogens.—Estrone (theelin) and estriol (theelol) are found in human urine almost entirely conjugated with glycuronic acid. The glycuronides must be hydrolyzed by acidifying the urine and heating before the estrogens can be extracted with immiscible solvents. Long boiling results in destruction of the estrogens. A compromise therefore has to be established between complete hydrolysis and minimum destruction.[10] Marrian[11] adjusted the p_H to about 1 and then heated the material in an autoclave at 120 C. for two hours.

Two biologic methods are used to assay these extracts for their total estrogenic content: the growth of the uterus of the immature rat or mouse; the growth of the uterus of the castrate rat or mouse. Bülbring and Burn[12] found that the increase in the weight of the uterus varies as the logarithm of the dose. Astwood[13] has proposed a six hour assay, which depends on the rapid imbibition of water by the uterus of the immature rat during the first six hours the animal is under the influence of an estrogen. The vaginal smear method of Allen and Doisy is more generally used. The accuracy of this method of assay has been studied carefully.[14] By direct application of the preparation to

10. Gallagher, T. F.; Peterson, D. H.; Dorfman, R. I.; Kenyon, A. T., and Koch, F. C.: The Daily Urinary Excretion of Estrogenic and Androgenic Substances by Normal Men and Women, J. Clin. Investigation **16**: 695 (Sept.) 1937. Gustavson, R. G.; Mason, L. W.; Hays, E. E.; Wood, Thomas, and D'Amour, F. E.: The Quantitative Determination of Estrogenic Substances in Normal Female Urine During the Menstrual Cycle, Am. J. Obst. & Gynec. **35**: 115 (Jan.) 1938.

11. Marrian, G. F.: The Conjugated Estrogens, in Cold Spring Harbor Symposia on Quantitative Biology, Cold Spring Harbor, L. I., New York, The Biological Laboratory, 1937, vol. 5, p. 16.

12. Bülbring, Edith, and Burn, J. A.: The Estimation of Oestrin and of Male Hormone in Oily Solution, J. Physiol. **85**: 320 (Nov. 22) 1935.

13. Astwood, E. B.: A Six-Hour Assay for the Quantitative Determination of Estrogen, Endocrinology **23**: 25 (July) 1938.

14. (*a*) D'Amour, F. E., and Gustavson, R. G.: A Critical Study of the Assay of Female Sex Hormone Preparations, J. Pharmacol. & Exper. Therap. **57**: 473 (Dec.) 1930. (*b*) Kahnt, L. C., and Doisy, E. A.: Vaginal Smear Method of Assay of Ovarian Hormone, Endocrinology **12**: 760 (Nov.-Dec.) 1928. (*c*) Coward, K. H., and Burn, J. H.: The Variation in the Unit of Oestrus-Producing Hormone, J. Physiol. **63**: 270 (Aug.) 1927. (*d*) Marrian, G. F., and Parkes, A. S.: The Assay of Estrin, ibid. **67**: 389 (July) 1929. (*e*) Allan, H.; Dickens, F., and Dodds, E. D.: The Standardization of the Water Soluble Estrus Producing Hormone, ibid. **68**: 348 (Jan.) 1930. (*f*) de Jongh, S. E.; Laqueur, E., and de Fremery, P.: Die Konzentrationswirkungskurve des Follikelhormons (Menformon), Biochem. Ztschr. **250**: 448, 1932. (*g*) Hain, A. M., and Robson, J. M.: Comparative Assay of Estrone in the Rat and the Mouse, J. Pharmacol. & Exper. Therap. **57**: 337 (Aug.) 1936. (*h*) Emmens, C. W.: Reports on Biological Standards: V. Variables Affecting the Estimation of Androgenic and Oestrogenic Activity, Medical Research Council Special Report Series, no. 234, London, His Majesty's Stationery Office, 1939.

the vagina, Lyons[15] was able to detect 1/200 of a rat unit of estrone. Subcutaneous injections of divided doses of an aqueous solution or suspension have been used by most workers, although maximum absorption appears to be obtained by the intraperitoneal route. Unfortunately, the increase in sensitivity toward estrone and estriol with increase in the number of injections is not the same in the mouse. Emmens[14h] found that 7 micrograms of estriol gave a 50 per cent response when the substance was given in two injections, but that only 0.16 microgram was required when four injections were given during the same period. With estrone, 0.1 microgram gave a 50 per cent response with two injections, and 0.067 microgram gave the same response with four injections. It is therefore of the utmost importance to repeat various methods of assay for total estrogenic content in exactly the manner reported by the original author if the results are to be comparable. The observations also show that the separation of the different estrogens before assaying the extract would give much more fundamental data.

Substances which do not possess estrogenic activity will sometimes accentuate the activity of the estrogens, and therefore a crude preparation may exhibit greater activity than the pure estrogen.

Attempts to follow the day by day total excretion of estrogens have been made by a number of workers.[16] Two peaks of excretion occur, one at the midinterval, presumably related to ovulation, and the second following the activity of the corpus luteum.

The separation and purification of the various estrogens depend on dividing the extract into (*a*) acidic substances, which are extracted from an ether solution with sodium carbonate, (*b*) stronger phenolic sub-

15. Lyons, W. R., and Templeton, H. J.: Intravaginal Assay of Urinary Estrogen, Proc. Soc. Exper. Biol. & Med. **33**: 587 (Jan.) 1936.

16. Gustavson, R. G., and Green, D. F.: The Quantitative Determination of the Amount of Estrogenic Substances Excreted Daily in the Urine of the Normal Human Female, J. Biol. Chem. **105**: xxxiv (May) 1934. Gustavson, R. G.; Hays, E. E., and Wood, T. R.: The Quantitative Determination of Estrogenic Substances in Normal Female Urine During the Menstrual Cycle, ibid. **119**: xlii (June) 1937. Yerby, L. D.: Relation of Urinary Excretion of Estrone to Menstrual Cycle of Normal Woman, Proc. Soc. Exper. Biol. & Med. **36**: 496 (May) 1937. Palmer, A.: Hormones in Urine of a Normal Non-Pregnant Woman, ibid. **37**: 273 (Oct.) 1937. Smith, G. V., and Smith, O. W.: Urinary Excretion of Estrogenic and Gonadotropic Hormones During Menstrual Cycles, Period of Conception and Early Pregnancy, New England J. Med. **215**: 908 (Nov. 12) 1936. Gallagher, T. F.; Peterson, D. H.; Dorfman, R. I.; Kenyon, A. T., and Koch, F. C.: The Daily Urinary Excretion of Estrogenic and Androgenic Substances by Normal Men and Women, J. Clin. Investigation **16**: 695 (Sept.) 1937.

stances, which are extracted from the ether solution with tenth-normal sodium hydroxide, and (*c*) weaker phenolic substances, which are extracted from the ether solution with normal sodium hydroxide.

Colorimetric Assays.—In view of the time, expense and inherent difficulties in biologic assays, colorimetric reactions are being studied. Absorption spectra may be used for the qualitative detection of the estrogenic and androgenic substances and are useful as a supplementary aid in the quantitative estimation of these substances. Callow and co-workers [17] indicated the value of spectrum analysis in pointing out that colorimetric estimation can replace the biologic assay provided due regard is given to the occasional presence of interfering compounds, which may be revealed when the absorption spectrum is examined. These workers studied and determined the absorption spectrums of seventeen androgenic and estrogenic substances or immediately related compounds.

A second method is the development of color through specific reactions and the comparison of this color with a standard, using the photoelectric colorimeter with carefully chosen filters.

Voss [18] studied the color reactions of estrogens with 1-nitroso-2-naphthol. The color obtained depends on the estrogen used. Schmulovitz and Wylie [19] used the orange color developed when the estrogens are coupled with diazotized p-nitroaniline. David [20] noted a typical blue color when estriol crystals were treated with sulfuric acid followed by arsenic acid. The reaction lacks sensitivity and cannot be used with impure extracts, since, according to Pincus and collaborators,[21] a bluish cloudy suspension always occurs.

Perhaps the earliest work on colorimetric determination of the sex hormones was that by Kober,[22] in which

17. Callow, Nancy H.; Callow, R. K., and Emmens, C. W.: Colorimetric Determination of Substances Containing the Grouping —CH_2CO— in Urine Extracts as an Indication of Androgen Content, Biochem. J. **32**: 1312 (Aug.) 1938.

18. Voss, Karl: Farbreaktionen der Sexualhormonen, Ztschr. f. physiol. Chem. **250**: 218, 1937.

19. Schmulovitz, M. J., and Wylie, J. B.: The Chemical Diagnosis of Pregnancy by Detection of Estrin in Urine, J. Lab. & Clin. Med. **21**: 210 (Nov.) 1935.

20. David, K.: Characteristic Color Reactions of Marrian Crystals (Trihydroxy Estrin), Acta brev. Neerland. **4**: 64, 1934.

21. Pincus, Gregory; Wheeler, Grace; Young, Genevieve, and Zahl, P. A.: The Colorimetric Determination of Urinary Estrin, J. Biol. Chem. **116**: 253 (Nov.) 1936.

22. Kober, S.: Eine kolorimetrische Bestimmung des Brusthormons (Menformon), Biochem. Ztschr. **230**: 209, 1931.

he used equal parts of concentrated sulfuric acid and phenolsulfonic acid in reaction with estrone. Cohen and Marrian [23] found it necessary to modify this method. The variation in tint of the final color makes it necessary to use a Lovibond tintometer to analyze and determine separately the intensities of the components. The authors expressed the belief that the estrone and estriol present in human pregnancy urine may be separated and estimated with a reasonable degree of accuracy by their modification of the Kober reaction. The separation of the estrone and estriol is not quantitative but is sufficiently complete to permit detection of any abnormal amounts of either compound in pregnancy urine. With "synthetic urines" of known estrone and estriol content, they observed a 13 per cent loss of estriol but a nearly quantitative recovery of estrone. Their work showed the necessity of removing pregnandiol and cholesterol.

Pincus and co-workers [21] used the phenosulfonic acid colorimetric method and obtained reliable results with the estrone and estriol fractions of human pregnancy urine. Their results check particularly well with those of biologic assays on urines from women in the sixth to ninth months of pregnancy, though the estrone fraction even in late pregnancy may give "over estimates."

Venning and co-workers [24] modified the method of Cohen and Marrian and attained an accuracy of ± 5 per cent when 5,000 micrograms of estrogens was present per liter of urine. They obtained poor results from early pregnancy and nonpregnancy urines, in which the estrogens are present in relatively small amounts. They used a photoelectric colorimeter in making their measurements.

Bachman [25a] used a diluted sulfonic acid reagent and carried out the reaction at 150 C. He found that the use of the modified reaction made feasible an examination of the chromogenic properties of estrogen, α-estradiol, and estriol and established conditions under which the total content of a mixture of these three may be determined.

23. Cohen, S. L., and Marrian, G. F.: A Critical Study of the Application of the Kober Colorimetric Reaction, Biochem. J. **28**: 1603, 1934.

24. Venning, Eleanor H.; Evelyn, K. A.; Harkness, E. V., and Browne, J. S. L.: The Determination of Estrin in Urine with the Photoelectric Colorimeter, J. Biol. Chem. **120**: 225 (Aug.) 1937.

25. Bachman, Carl: Photometric Determination of Estrogens: (*a*) I. Modified Kober Reaction for Determining Total Estrogens in Mixture of Estrogenic Steroids, J. Biol. Chem. **131**: 455 (Dec.) 1939; (*b*) II. New Color Reaction for Estriol, ibid. **131**: 463 (Dec.) 1939.

Bachman [25b] developed a new reaction for estriol. This estrogen is heated at 150 C. with sodium paraphenolsulfonate in phosphoric acid. A stable violet pink color is obtained.

Hormones from the Corpus Luteum.—Progesterone, the secretion of the corpus luteum, is reduced from a diketone to a dihydroxy compound called pregnandiol, which occurs in normal and pregnancy urine conjugated with glycuronic acid. It is difficult to prove the presence of progesterone in blood or urine. Venning has shown that it is possible to determine the quantity of pregnandiol with a fair degree of accuracy.[26] Pregnandiol occurs in increasing quantities in the urine following ovulation and disappears just previous to menstruation.[27] During the first two months of pregnancy the excretion remains on the level of the luteal phase of the cycle, then rises until a maximum is attained, in the eighth month, and falls abruptly before parturition.[28]

Urinary Androgens.—The practical biologic methods of assaying androgens are limited to the growth of the comb in the capon and the increase in the weight of the seminal vesicle in the rat or the mouse. Since the androgens occur in conjugated forms, it is necessary to hydrolyze the urine before extracting with immiscible solvents. Here the period of boiling must be limited to fifteen minutes if losses by destruction are to be avoided.[29] The problem of assaying the crude extract from urine is fraught with many problems.[30]

The growth of the comb of the capon has been used for assaying androgens. The test may be made more sensitive if the material is applied directly to the comb, provided the estrogens are first removed, since they have an inhibiting effect on comb growth. Frank and Klempner,[31] Voss [32] and others have used the increase

26. Venning, Eleanor H.: Gravimetric Method for the Determination of Sodium Pregnandiol Glucuronidate (an Excretion Product of Progesterone), J. Biol. Chem. **119**: 473 (July) 1937.

27. Venning, Eleanor H., and Browne, J. S. L.: Studies on Corpus Luteum Function: I. Endocrinology **21**: 711 (Nov.) 1937.

28. Browne, J. S. L.; Kenny, J. S., and Venning, Eleanor H.: The Corpus Luteum Hormone in Pregnancy, J. Clin. Investigation **16**: 678 (July) 1937.

29. Peterson, D. H.; Gallagher, T. F., and Koch, F. C.: The Effect of Acid Hydrolysis on the Yield of Androgenic and Estrogenic Activities from Human Urine, J. Biol. Chem. **119**: 185 (June) 1937.

30. (*a*) Allen, Edgar; Danforth, C. H., and Doisy, E. A.: Sex and Internal Secretions, ed. 2, Baltimore, Williams & Wilkins Company, 1939, p. 877. (*b*) Emmens.[14b]

31. Frank, R. T., and Klempner, E.: The Comb of the Baby Chick as a Test for the Male Sex Hormones, Proc. Soc. Exper. Biol. & Med. **36**: 763 (June) 1937.

32. Voss, H. E.: Die örtliche Wirkung von Sexualhormonen, Klin. Wchnschr. **16**: 769 (May 29) 1937.

in weight of combs of baby chicks. The lack of a base line from which to measure is an objection to these methods.

The response of the seminal vesicle is so complicated that it is practically valueless as the basis of an assay method. Foreign substances, not in themselves androgenic, augment the effect of androgens present, as shown by many workers.[30a] These so-called x substances do not affect comb growth.

In the early work on the testicular hormone it was assumed that the androgenic material which could be extracted from normal urine was "the male hormone" and that the biologic assay of urinary extracts would give an index to the level of testicular secretion. However, androgenic activity is not specific to one substance, and at least two related compounds with androgenic activity, androsterone and transdehydroandrosterone, are present in the mixture of neutral compounds which can be prepared from urine. Further, these two compounds occur in the urine of both sexes. This distinction between hormones and excretory transformation products is confirmed by the discovery that testosterone is degraded in the human body to give androsterone and the stereoisomeric, inactive compound aetiocholan-3(α)-ol-17-one.[33] Androgenic substances in the urine may be derived from the adrenal cortex, since quantities of androgens and related steroids have been isolated from the urine in cases of adrenal disease.[34] For these reasons, the comb growth–promoting activity of urinary extracts, which consist of a complex mixture of degradation products, may not be an index of the production of androgens. Zimmermann [35] investigated the colors produced when urinary extracts are treated with m-dinitrobenzene. This method is based on the nonspecific reaction between the $CH_2C=O$ group and m-dinitrobenzene in alkaline solution. The temperature, duration of reaction, alkalinity of the solution, relative amount of reactants and intensity of light to which the solution

33. Callow, Nancy H.: The Isolation of Two Transformation Products of Testosterone from Urine, Biochem. J. **33**: 559, 1939.

34. Burrows, H.; Cook, J. W.; Roe, E. M. F., and Warren, F. L.: Isolation of $\Delta 3{:}5$ Androstadiene-17-One from the Urine of a Man with a Malignant Tumour of the Adrenal Cortex, Biochem. J. **31**: 950, 1937. Callow, R. K.: Isolation of the Male Hormone Present in the Urine of a Patient with an Adrenal Tumor, Chemistry & Industry **55**: 1030, 1936.

35. Zimmermann, W.: Colorimetrische Bestimmung der Keindrüsenhormone, Ztschr. f. Physiol. Chem. **245**: 47, 1936.

is exposed must be carefully controlled.[36] This reaction is given by a number of compounds of the 17-ketosteroid type. The color given in this reaction bears no relation to the biologic activity of the compound. It may be possible that the measure of these ketosteroids, the biologically active and the inactive, may be of more significance than an assay of biologically active urinary androgens. Wu and Chou [37] applied the reaction to a large series of urines. Callow and co-workers [17] showed close spectrographic similarity of the colors given by neutral fractions of urinary extracts and by pure compounds. Comb growth-promoting activity of urinary extracts, expressed in terms of the international standard, is only roughly proportional to the content of 17-ketosteroid compounds.

Androgens and Estrogens in Blood.—Androgens occur in the blood in such minute quantities that quantitative assays at the moment seem impossible. Attempts to assay the estrogen content of blood have been the subject of a number of papers. While some progress has been made, the limited quantity of blood which may be drawn for analysis and the small quantity of estrogen to be assayed make this problem even more difficult than the analysis of urine.

Summary.—It is obvious from the foregoing considerations that a great deal of research must be carried on before a satisfactory solution will be obtained to the problem of the analysis of urine and blood for sex hormones.

36. Baumann, E. J., and Metzger, N.: Colorimetric Estimation and Fractionation of Urinary Androgens, Endocrinology **27**: 664 (Oct.) 1940.

37. Wu, H., and Chou, C. Y.: Colorimetric Methods for Determination of Sex Hormones in Human Urine, Chinese J. Physiol. **11**: 413 (May 15) 1937.

CHAPTER XXIV

THE PHYSIOLOGY OF THE THYROID GLAND

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The thyroid is a gland of internal secretion whose chief function, so far as is known, is the elaboration and storage of its own peculiar hormone, namely, thyroglobulin, or the amino acid thyroxin contained therein. Originally it was a gland taking part in digestion. In the course of evolutionary changes, it has lost its connection with the alimentary tract. As Means [1] aptly said, "For a rôle in digestion it has substituted a rôle in growth and metabolism." To fulfil these functions, it is endowed with tremendous capacities for increasing or decreasing its activities, manifested by changes in size, blood supply, microscopic appearance and hormone content. In man the thyroid attains its relative maximum size just prior to puberty, corresponding to the time of maximum load on the organ from factors of growth and development. Simple colloid goiter, so common at this age, is the clinical manifestation of this physiologic fact.

HISTOPHYSIOLOGY OF THE THYROID

The gland is composed of a number of so-called follicles or acini, each one of which is a secretory unit. The average diameter, according to Wilson,[2] is 300 microns, but according to Jackson [3] it is somewhat smaller. The wall of the follicle consists of epithelium one cell deep. In normal glands the cell is about as high as it is wide (about 15 microns). The height of the epithelium has become a useful index of the functional activity of the thyroid.[4] The epithelium becomes flat when thyroid function is diminished and columnar when thyroid function is increased.

1. Means, J. H.: The Thyroid and Its Diseases, Philadelphia, J. B. Lippincott Company, 1937.
2. Wilson, G. E.: The Thyroid Follicle in Man: Its Normal and Pathological Configuration, Anat. Rec. **37**: 31 (Nov.) 1927.
3. Jackson, J. L.: The Shape and Size of the Human Thyroid Follicle in Health and Disease, Anat. Rec. **48**: 219 (Feb.) 1931.
4. Rawson, R. W., and Starr, Paul: Direct Measurement of Height of Thyroid Epithelium; Method of Assay of Thyrotropic Substance; Clinical Application, Arch. Int. Med. **61**: 726 (May) 1938.

The cells of the follicle make the thyroid colloid; the lumen is the storehouse. The body taps this stored secretion as needed. Such a mechanism is obviously adapted to cope with great functional variations. The essential component of the follicle is the thyroid cell, which is fundamentally a secretory cell. Close study of this cell reveals certain characteristics, in addition to height, which betray its state of activity.

The importance of the mitochondria in relation to the functional activity of the cell was first noted by Goetsch.[5] Numerous experimenters have confirmed this. Cramer and Ludford[6] showed that as the cell passes from an inactive to a very active phase there occur heightening of the cell, diminution of colloid in the follicle and increase in the number and size of the mitochondria.

The Golgi apparatus is another cytoplasmic structure of physiologic significance. In the salamander Uhlenhuth[7] correlated the state of the Golgi apparatus to the secretory phases of the cell. In the guinea pig Krogh and Okkels[8] observed the changes after an injection of a thyrotropic extract of the anterior lobe of the pituitary. There occur, after two hours, hyperemia of the whole gland, diminution in the size of the follicles, disappearance of colloid and great hypertrophy of the Golgi apparatus. This is maintained for about seventeen hours. These histologic changes are paralleled by an increase in metabolic rate. Similar cytologic changes were observed by Severinghaus[9] in the thyroids of other species. The latter also noted that colloid droplets first appear in close proximity to the enlarged Golgi apparatus.

Oxidase granules were described by Okkels,[10] who related their distribution to thyroid function. In normal

5. Goetsch, E.: Functional Significance of Mitochondria in Toxic Thyroid Adenomata, Bull. Johns Hopkins Hosp. **27**: 129 (May) 1916.

6. Cramer, W., and Ludford, R. J.: Cellular Activity and Cellular Structure as Studied in the Thyroid Gland, J. Physiol. **61**: 398 (June) 1926.

7. Uhlenhuth, Eduard: The Golgi Apparatus in the Thyroid Gland of Amphibians, in Its Relation to Excretion Polarity, Quart. J. Micro. Sc. **76**: 615, 1934.

8. Krogh, Marie, and Okkels, Harald: Sur l'histophysiologie du corps thyroïde. Stades initiaux de la sécrétion thyroïdienne, Compt. rend. Soc. de biol. **112**: 1694 (May) 1933. Okkels, Harald: Studies on the Thyroid Gland: I. On the Histology and Cytology of Normal and Abnormal Thyroids in Man, Acta path. et microbiol. Scandinav. **9**: 1, 1932.

9. Severinghaus, Aura E.: Cytological Observations on Secretion in Normal and Activated Thyroids, Ztschr. f. Zellforsch. u. mikr. Anat. **19**: 653, 1933.

10. Okkels, Harald: Stades initiaux de la sécrétion thyroïdienne. Les granulations oxydasiques, Compt. rend. Soc. de biol. **116**: 251 (May) 1934.

animals they are chiefly in the base of the cells. After injection of a thyrotropic extract they increase in numbers in the cells, invade the colloid and later become numerous in the interfollicular spaces as the colloid becomes vacuolated.

There is only one kind of cell in the follicle. It presents different appearances because it is observed in different stages of secretory activity. Recently Nonidez [11] described "parafollicular" cells, derived from parenchymal cells but occupying a position independent of the follicles. He suggested two possible functions for these cells: Either they represent a second type of secretory cell or they absorb something from the intrafollicular colloid and deliver it into the perifollicular blood vessels.

A good deal of controversy exists as to the direction in which the thyroid cells secrete—into the follicle or into the circulation. Means,[1] after going over the evidence thoroughly, favored the view that secretion can be in either direction. "Very likely, direct secretion into vessels occurs only when, in the face of great demand, the follicular storehouse has been emptied." Recently Algire,[12] employing a special method of transilluminating the thyroid gland of the living salamander, observed that, following injections of a thyrotropic anterior pituitary substance, there occur, in sequence, the gradual enlargement of a granule into a colloid droplet, the formation of new colloid droplets in proximity to the apical end of the nucleus, and the movement of colloid droplets from the apical end of the cell to the basal position, presumably for direct secretion into the vessels.

How the stored colloid gets into the circulation is also a moot question. It has been suggested [13] that it

11. Nonidez, J. F.: Further Observations on Parafollicular Cells of Mammalian Thyroid, Anat. Rec. **53**: 339 (Aug.) 1932.

12. Algire, G. H.: The Study of the Living Thyroid Gland, Bull. School Med., Univ. Maryland **23**: 211 (April) 1939.

13. Hirschlerowa, Z.: Mikroskopisch-anatomische Untersuchungen an der Amphibienschilddrüse mit besonderer Berücksichtigung ihres Golgi-Apparates, Ztschr. f. Zellforsch. u. mikr. Anat. **6**: 234 (Aug.) 1927. Uhlenhuth, Eduard; Schwartzbach, S. S., and Thompson, G. P.: Die Physiologie der Thyreoaktivator bei Amphibien: II. Die Strukturveränderungen der Schilddrüse der mit Vorderlappen eingespritzten Salamander, Endokrinologie **16**: 9, 1935. McLendon, J. F.: The Release of Colloid from the Thyroid Gland by Centrifugal Force, Endocrinology **24**: 82 (Jan.) 1939.

passes through intercellular channels. This view was opposed by Severinghaus,[9] who expressed the belief that the apexes of the cells push into the colloid in a pseudopodial-like manner, for the purpose of absorbing it. The colloid is then actively passed through the cells to the perifollicular vessels. The suggestion by Hertz (cited by Means [1]) that endothelial leukocytes transport colloid from the follicle into the interfollicular spaces affords another explanation. The parafollicular cells of Nonidez, as indicated in a foregoing paragraph, may be engaged in a similar function. Recently Williams,[14] observing living thyroid follicles implanted in rabbits' ears, found that all follicles go through repeated cycles of activity. These cycles are divided into four stages—secretion, secretion and colloid release, partial collapse and recuperation. Secretion is toward the lumen. He found no evidence of active secretion across the base of the cell. In the second stage, when the follicle is filled with colloid, a droplet is pinched off and comes to lie within the follicular wall. The droplet slowly disappears, presumably by diffusion across the thin wall, but is never extruded into the interfollicular spaces. In other follicles a whole section of wall may become thin and colloid diffuse across it. No vacuoles are seen at the periphery of the colloid. The finding of proteolytic enzyme activity in thyroid colloid by De Robertis [15] confirms Williams' observation.

Since diffusion of a large molecule like thyroglobulin across a living semipermeable membrane is not likely, the presumption is that thyroglobulin is probably first digested (enzymatically) and that the resultant small fragments then diffuse through the follicle wall. This mechanism suggested by Williams, in contrast to the others, is consistent with my own observation [16] that thyroglobulin is not detectable in the peripheral blood or the blood of the thyroid vein of the normal person or of the patient with thyroid disease.

14. Williams, R. G.: Microscopic Studies of Living Thyroid Follicles Implanted in Transparent Chambers Installed in the Rabbit's Ear, Am. J. Anat. **62**: 1 (Nov.) 1937.

15. De Robertis, E.: Proteolytic Enzyme Activity of Colloid Extracted from Single Follicles of the Rat Thyroid, Anat. Rec. **80**: 219 (June) 1941.

16. Lerman, Jacob: Iodine Components of the Blood: Circulating Thyroglobulin in Normal Persons and in Persons with Thyroid Disease, J. Clin. Investigation **19**: 555 (July) 1940.

BLOOD SUPPLY AND INNERVATION OF THE THYROID

The blood supply of the thyroid is abundant. The blood volume of normal man (about 5 liters) moves through his thyroid about once an hour. There is a rich capillary network intimately connected with the parenchymal cells. Modell [17] recently described arteriovenous anastomoses and so-called "muscle cushions" in the thyroids of dogs. Williams,[15] on the other hand, was unable to see such structures in the living tissue. Their function is undoubtedly to regulate the flow of blood to the follicles and thus control the delivery of secretion to the body.

There is also a lymph plexus in close proximity to the follicles. A small portion of the thyroid's secretion leaves by this channel.

The thyroid is supplied by a rich network of autonomic nerve fibers, sympathetic from the cervical ganglions and parasympathetic from the vagus. It is still debated whether these fibers are secretory or vasomotor. Although bilateral cervical sympathectomy results in a decrease in metabolic rate, the weight of evidence favors the view that the autonomic nerve fibers are not secretory and that secretory activity of the thyroid is governed indirectly by vasomotor control (Nonidez;[18] Cahane and Cahane;[19] Uotila[20]). On the other hand, there is good evidence that secretory activity of the pituitary can be influenced directly through nerve channels (Thomson and Collip;[21] Friedgood and Pincus[22]). This has been confused with direct nervous control of thyroid secretion. There is also evidence that the hypothalamohypophysial pathways

17. Modell, Walter: Observations on the Structure of the Blood Vessels Within the Thyroid Gland of the Dog, Anat. Rec. **55**: 251 (Feb. 25) 1933.

18. Nonidez, J. F.: Innervation of the Thyroid Gland: III. Distribution and Termination of the Nerve Fibers in the Dog, Am. J. Anat. **57**: 135 (July) 1935; Nervous "Terminal Reticulum": Critique; Observations on Thyroid and Liver, Anat. Anz. **84**: 1 (March) 1937.

19. Cahane, Mares, and Cahane, Tatiana: Sur l'existence des centres nerveux infundibulaires réglant la fonction du corps thyroïde, Acta med. Scandinav. **94**: 320, 1938.

20. Uotila, U. U.: Rôle of Cervical Sympathetics in the Regulation of Thyroid and Thyrotropic Function, Endocrinology **25**: 63 (July) 1939.

21. Thomson, D. L., and Collip, J. B.: The Hormones, in Luck, J. M.: Annual Review of Biochemistry, Stanford University, Calif., Stanford University Press, 1933, vol. 2, p. 231.

22. Friedgood, H. B., and Pincus, Gregory: Studies on Conditions of Activity in Endocrine Organs: XXX. The Nervous Control of the Anterior Hypophysis as Indicated by Maturation of Ova and Ovulation After Stimulation of Cervical Sympathetics, Endocrinology **19**: 710 (Nov.-Dec.) 1935.

may control thyroid function by their influence over the pituitary. Uotila [23] recently demonstrated that under normal circumstances section of the pituitary stalk does not affect the thyrotropic function; the thyroid consequently remains normal. However, under certain stresses, such as exposure to cold, animals in which the pituitary stalk has been sectioned fail to display the expected hypertrophic changes in the thyroid.

THYROID HORMONE

One of the outstanding peculiarities of the thyroid is that it contains iodine in organic combination, a fact discovered by Baumann in 1896. The normal human thyroid contains about 0.186 per cent of iodine in terms of dried gland, the variation being 0.05 to 0.45 per cent (Gutman, Benedict, Baxter and Palmer [24]). Of the total iodine store in the body, the thyroid possesses about one third to one fourth. Seidell and Fenger [25] demonstrated a seasonal variation in the storage of iodine—lower in the early spring and higher in the late summer. Locality and food habits also play a role in determining the iodine content of the thyroid. Fenger [26] found iodine in fetal thyroids as early as the third month. It was demonstrated by Marine and Lenhart [27] that the iodine store varies inversely with the degree of hyperplasia.

Other structures of the body contain iodine, but the thyroid differs from them in its affinity for this element. It rapidly traps any extra iodine entering the circulation up to its maximum capacity, the amount trapped depending on the degree of thyroid activity (hormone depletion). This fact is the basis of the so-called iodine tolerance test in hyperthyroidism, introduced by Elmer.[28] Thyroid has the same affinity for iodine in

23. Uotila, U. U.: On the Rôle of the Pituitary Stalk in the Regulation of the Anterior Pituitary, with Special Reference to the Thyrotropic Hormone, Endocrinology **25**: 605 (Oct.) 1939.

24. Gutman, A. B.; Benedict, Ethel M.; Baxter, Blanch, and Palmer, W. W.: The Effect of Administration of Iodine on the Total Iodine, Inorganic Iodine, and Thyroxine Content of the Pathological Thyroid Gland, J. Biol. Chem. **97**: 303 (July) 1932.

25. Seidell, A., and Fenger, F.: Seasonal Variation in the Iodine Content of the Thyroid Gland, J. Biol. Chem. **13**: 517, 1913.

26. Fenger, F.: On the Iodine and Phosphorus Contents, Size and Physiological Activity of the Fetal Thyroid Gland, J. Biol. Chem. **14**: 397, 1913.

27. Marine, David, and Lenhart, C. H.: Further Observations on the Relation of Iodine to the Structure of the Thyroid Gland in the Sheep, Dog, Hog and Ox, Arch. Int. Med. **3**: 66 (Feb.) 1909.

28. Elmer, A. W.: Iodine Tolerance Test for Thyroid Insufficiency, Endocrinology **18**: 487 (July-Aug.) 1934.

vitro as in vivo (Marine and Feiss;[29] Rabinowitch and Frith[30]). Using radioactive iodine, Hertz, Roberts, Means and Evans[31] found that the normal rabbit's thyroid takes up iodine rapidly and becomes saturated within ten to fifteen minutes after an intravenous injection of iodine. Furthermore, certain types of hyperplastic glands are less able to utilize small amounts of iodine than are normal glands, although their capacity for taking up iodine from large doses is several times that of the normal gland. This may explain the need for relatively large amounts of iodine to induce a remission in the syndrome of exophthalmic goiter.

Preceding Baumann's discovery, it became apparent that the thyroid made a hormone. The syndrome associated with spontaneous atrophy of the thyroid, described by Gull[32] in 1874, was soon recognized as identical with the one due to total removal of the thyroid as noted by the Reverdins[33] in 1882 and by Kocher[34] in 1883. Knowledge of the curative value of thyroid substitution soon followed. In 1891 Murray[35] cured patients with hypothyroidism by subcutaneous injection of sheep's thyroid, and in 1892 Mackenzie[36] and also Fox[37] found that oral administration was equally effective.

Attempts at chemical isolation of the thyroid principle began with the discovery by Oswald[38] and by Hutchison[39] that the iodine of the thyroid is bound to a globulin (iodothyroglobulin) which is physiologi-

29. Marine, David, and Feiss, H. O.: The Absorption of Potassium Iodide by Perfused Thyroid Glands and Some of the Factors Modifying It, J. Pharmacol. & Exper. Therap. **7**: 557, 1915.

30. Rabinowitch, I. M., and Frith, A. B.: Iodine Studies: The Avidity of the Thyroid Gland for Various Iodine Compounds in Vitro, J. Clin. Investigation **1**: 473 (June) 1925.

31. Hertz, Saul; Roberts, A.; Means, J. H., and Evans, R. D.: Radioactive Iodine as an Indicator in Thyroid Physiology: Iodine Collection by Normal and Hyperplastic Thyroids in Rabbits, Am. J. Physiol. **128**: 565 (Feb.) 1940.

32. Gull, W. W.: On a Cretinoid State Supervening in Adult Life in Women, Tr. Clin. Soc. Lond. **7**: 180, 1874.

33. Reverdin, J.-L.: Accidents consécutifs à l'ablation totale du goitre, Rev. med. de la Suisse Romande **2**: 539 (Sept.) 1882.

34. Kocher, Theodore: Ueber Kropfextirpation und ihre Folgen, Arch. f. klin. Chir. **29**: 254, 1883.

35. Murray, G. R.: Note on the Treatment of Myxoedema by Hypodermic Injections of an Extract of the Thyroid Gland of a Sheep, Brit. M. J. **2**: 796 (Oct.) 1891.

36. Mackenzie, H. W. G.: A Case of Myxoedema Treated with Great Benefit by Feeding with Fresh Thyroid Glands, Brit. M. J. **2**: 940 (Oct.) 1892.

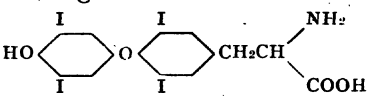
37. Fox, E. L.: A Case of Myxoedema Treated by Taking Extract of Thyroid by the Mouth, Brit. M. J. **2**: 941 (Oct.) 1892.

38. Oswald, A.: Die Eiweisskörper der Schilddrüse, Ztschr. f. physiol. Chem. **27**: 14, 1899.

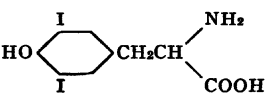
39. Hutchison, R.: The Chemistry of the Thyroid Gland and the Nature of Its Active Constituent, J. Physiol. **20**: 474, 1896.

cally active and equal to whole thyroid. Other proteins of the thyroid do not contain iodine and are physiologically inert. It is the only protein present in colloid; it is also present in the follicle cells. The molecular weight of thyroglobulin was determined by Heidelberger and Svedberg [40] to be in the neighborhood of 700,000.

The early attempts at chemical purification of the colloid brought only partial success. Among others, Baumann, Müller and also Oswald obtained fractions of high iodine content and high physiologic potency by acid hydrolysis. In 1915 Kendall [41] isolated a pure crystalline substance containing 65 per cent iodine and possessing all the pharmacologic characteristics of whole thyroid. He named it "thyroxin." Harington [42] in 1926 demonstrated that Kendall's substance is an amino acid containing four iodine atoms—a hydroxyphenyl ether of tyrosine having the structural formula:



Later Harington and Barger [43a] suggested that thyroxin is formed in the organism by the oxidative coupling of diiodotyrosine. It was not until 1939 that von Mutzenbecher [43b] succeeded in obtaining thyroxin from diiodotyrosine in vitro. The latter compound was isolated in pure chemical form from thyroid colloid by Harington and Randall.[44] It has the structural formula:



These two iodine-containing amino acids are the only ones, so far as present knowledge goes, to be found in the thyroid. Of the total iodine present in normal thyroid, about 30 per cent is in the form of thyroxin and about 70 per cent in the form of diiodotyrosine.

40. Heidelberger, Michael, and Svedberg, The: The Molecular Weight of Thyroglobulin, Science **80**: 414 (Nov. 2) 1934.

41. Kendall, E. C.: The Isolation in Crystalline Form of the Compound Containing Iodin Which Occurs in the Thyroid: Its Chemical Nature and Physiological Activity, Tr. A. Am. Physicians **30**: 420, 1915.

42. Harington, C. R.: Chemistry of Thyroxine: I. Isolation of Thyroxine from the Thyroid Gland, Biochem. J. **20**: 293, 1926; II. Constitution and Synthesis of Desiodo-Thyroxine, ibid. **20**: 300, 1926.

43. (*a*) Harington, C. R., and Barger, G.: Chemistry of Thyroxine: Constitution and Synthesis of Thyroxine, Biochem. J. **21**: 169, 1927. (*b*) Von Mutzenbecher, P.: Ueber die Bildung von Thyroxin aus Dijodtyrosin, Ztschr. f. Physiol. Chem. **261**: 253, 1939.

44. Harington, C. R., and Randall, S. S.: Observations on the Iodine-Containing Compounds of the Thyroid Gland: Isolation of dl-3: 5-di-Iodotyrosine, Biochem. J. **23**: 373, 1929.

PHYSIOLOGIC POTENCY

What determines the physiologic potency of whole thyroid gland and how it is related to that of pure thyroxin are matters of physiologic interest. They are also of practical importance from the point of view of standardization of thyroid preparations. Several methods for measuring physiologic potency are available, for example, the Gudernatsch tadpole test and the Hunt acetonitrile test. However, the most convenient and most precise method of assaying thyroid for physiologic potency is by observing the effect on the rate of gaseous metabolism. In the past ten years my co-workers and I have assayed many and a variety of preparations, using as the index of activity the rate of increase of the basal metabolism of patients with full blown myxedema, from their low level, when the material is administered in daily doses for approximately two weeks. The basis for this technic has been discussed extensively by Means.[1]

Following the work of Kendall and of Harington on the isolation and nature of thyroxin, it was believed that thyroxin was the only active substance contained in thyroid and that measurement of the activity of a thyroid preparation could be obtained by measuring its thyroxin content. In other words, the calorigenic potency of a thyroid preparation could be better ascertained from its thyroxin content than from its total iodine content. Direct comparison between thyroxin and whole thyroid is impossible, because the former is absorbed irregularly from the gastrointestinal tract and the latter cannot be given parenterally. This difficulty was solved when Harington and Salter [45] isolated a polypeptide of thyroxin containing 50 per cent iodine, which Salter, Lerman and Means [46] found to be equally active whether given by mouth or intravenously. These investigators then found that thyroxin and the polypeptide of thyroxin are physiologically equal on the basis of their iodine content. On the other hand, the activity of whole thyroid containing a standard amount of thyroxin iodine is much greater than that of thyroxin polypeptide containing the same standard amount of

45. Harington, C. R., and Salter, W. T.: The Isolation of l-Thyroxine from the Thyroid Gland by the Action of Proteolytic Enzymes, Biochem. J. **24**: 456, 1930.

46. Salter, W. T.; Lerman, Jacob, and Means, J. H.: The Calorigenic Action of Thyroxin Polypeptide, J. Clin. Investigation **12**: 327 (March) 1933.

thyroxin iodine (Means, Lerman and Salter[47]). Whole thyroid containing total organic iodine equal to standard thyroxin iodine gives a standard response. Moreover, Lerman and Salter,[48] observing the maintenance requirements of myxedematous patients on several brands of thyroid which contained different amounts of total iodine and of thyroxin iodine, concluded that total organic iodine, rather than thyroxin iodine, determines the calorigenic activity of whole thyroid.

Palmer and Leland,[49] however, using intact guinea pigs as test objects, came to the conclusion that thyroxin iodine, rather than total organic iodine, determines physiologic potency. Krogh and Lindberg,[50] using the same kind of test animal, came to similar conclusions. On the other hand, Meyer and Wertz,[51] using thyroidectomized rats, and White and collaborators,[52] using intact guinea pigs, have supported the contentions of Means, Lerman and Salter. In the past few years I have been comparing the effects of whole thyroid and thyroxin in normal and in thyroidectomized rabbits.[53] The results thus far emphasize the difficulty of using herbivorous animals in testing the effect of thyroid by mouth. In some instances the calorigenic response to thyroid by mouth equals that to thyroxin given intravenously in equi-iodine dosage; in others the calorigenic response to thyroid by mouth is much lower. These discrepancies are understandable when one realizes that stasis of food in the rabbit's stomach, frequently lasting eighteen to thirty-six hours, may result in destruction of ingested thyroglobulin.

The results obtained by Means and co-workers led to a puzzling paradox. The organic iodine of the

47. Means, J. H.; Lerman, Jacob, and Salter, W. T.: The Rôle of Thyroxin Iodine and Total Organic Iodine in the Calorigenic Action of Whole Thyroid Gland, J. Clin. Investigation **12**: 683 (July) 1933.

48. Lerman, Jacob, and Salter, W. T.: Maintenance Requirements of Myxedema Patients: Clinical and Chemical Assay of Commercial Thyroid Preparations, J. Pharmacol. & Exper. Therap. **50**: 298 (March) 1934.

49. Palmer, W. W., and Leland, J. P.: Comparative Calorigenic Action of Normal and Pathological Thyroid Glands Administered in Equi-Thyroxine Doses, J. Clin. Investigation **14**: 619 (Sept.) 1935.

50. Krogh, Marie, and Lindberg, Anna-Louise: Studies on Thyroid Gland; Physiological Activity of Iodine in Thyroxin and in Normal and Pathological Thyroid Glands, Acta path. et microbiol. Scandinav. **9**: 21, 1932.

51. Meyer, A. E., and Wertz, Anne: The Calorigenic Efficiency of Thyroid Material in Relation to Thyroxine and to Iodine Content, Endocrinology **24**: 683 (May) 1939.

52. White, Julius; McGinty, D. A.; Anderson, L. P., and White, Florence R.: The Influence of Desiccated Thyroid, Thyroid Concentrate and Thyroxine on the Oxygen Consumption of the Guinea Pig, Endocrinology **24**: 693 (May) 1939.

53. Lerman, Jacob: Unpublished data.

thyroid is divided between thyroxin and diiodotyrosine. When separated, the latter is inert. Yet the activity of the whole thyroglobulin molecule is apparently due to all its iodine. It was suggested by Harington and Salter [45] that the high activity of whole thyroid iodine as compared with the thyroxin derived from it is due to such factors as chemical combination (peptide linkage) or optical activity of thyroxin. However, the polypeptide of thyroxin is not more potent than crystalline thyroxin in terms of iodine (Salter, Lerman and Means [46]), and l-thyroxin, which occurs naturally in thyroglobulin, is no more active than d-thyroxin (Salter, Lerman and Means [54]).

The synthesis of an active protein from the inert diiodotyrosine peptone by Salter and Lerman [55] may explain this paradox on the basis of Harington's theoretic concepts. According to Harington,[56] there is a special linkage in the thyroglobulin molecule between thyroxin and diiodotyrosine which is ruptured in the initial stage of isolation of thyroxin. He has visualized this linkage as follows: "It may be supposed that the iodine which reaches the thyroid is first introduced into the molecule of tyrosine to form 3,5-diiodotyrosine. The latter will then fulfil a dual role; part of it will be converted into thyroxine . . . and another part will be linked with the thyroxine so formed, together with other anino-acids, to form the true active principle of the gland."

PHYSIOLOGY OF THYROID HORMONE

Knowledge of the physiologic action of thyroid has been obtained by four different methods. The first method consists in observing the effects produced by destruction of the gland—by disease or by surgical ablation—in the experimental animal and in man. The second method consists in observing the effect of feeding the gland to subjects deprived of it. The third method consists in observing the effects of an excess of active substance, spontaneous or induced. The final method, and the most recent, consists in observing the effect on the thyroid of removal of other glands or of

54. Salter, W. T.; Lerman, Jacob, and Means, J. H.: The Calorigenic Action of D- and L-Thyroxin, J. Clin. Investigation **14**: 37 (Jan.) 1935.

55. Salter, W. T., and Lerman, Jacob: The Genesis of Thyroid Protein: Clinical Assays of Artificial Thyroid Protein in Human Myxedema, Endocrinology **20**: 801 (Nov.) 1936.

56. Harington, C. R.: Biochemical Basis of Thyroid Function, Lancet **1**: 1199 (May 25); 1261 (June 1) 1935.

administration of other glands. Without a doubt, the thyroid hormone plays a vital role in the economy of the body. Its various functions are to be considered under several headings, but it must be emphasized that they are undoubtedly all interrelated.

Calorigenic Action.—Magnus-Levy first recognized that the thyroid plays a role in the regulation of heat production, or, its equivalent, oxygen consumption and carbon dioxide production. In other words, the rate of energy exchange, or metabolism, is regulated by the thyroid. This calorigenic action is most conveniently measured by determining the consumption of oxygen over a period of time. When the organism is deprived of all thyroid, the metabolism drops to about 40 to 45 per cent below normal in about 60 to 80 days. The rate of decline is predictable. It may be represented by the exponential equation $y = C + Ae^{-kt}$ which describes a decay curve.[57] When thyroid or thyroxin is administered, the metabolism rises, the rate of increase and level attained depending on the dosage used. In general the response to thyroid is inversely proportional to the initial level of metabolism—it is greater at low levels than at high levels for any given dose. At high environmental temperatures and humidity the effect of thyroxin is exaggerated (Schmidt and Schmidt[58]).

The means by which thyroid accelerates oxidative processes is not absolutely certain. It is not to be explained by increased muscular activity and is independent of adrenal activity (Aub, Bright and Uridil[59]). It involves every tissue of the body. Ample proof is available that the metabolism of tissue excised from thyroidectomized animals is low, and that of tissue excised from hyperthyroid animals, high. The experiments of Meyer, McTiernan and Aub[60] indicate that the calorigenic action is independent of the nervous

57. Means, J. H., and Lerman, Jacob: The Curves of Thyroxine Decay in Myxedema and of Iodine Response in Thyrotoxicosis: Their Similarity and Its Possible Significance, Ann. Int. Med. **12**: 811 (Dec.) 1938. Boothby, W. M., and Baldes, E. J.: Activation and Decay Curves of Thyroxin, Proc. Staff Meet. Mayo Clin. **1**: 166, 1926.

58. Schmidt, L. H., and Schmidt, I. G.: The Relation of Environmental Temperature to the Action of Thyroxine, Endocrinology **23**: 553 (Nov.) 1938.

59. Aub, J. C.; Bright, E. M., and Uridil, J.: Studies upon the Mechanism of the Increased Metabolism in Hyperthyroidism, Am. J. Physiol. **61**: 300 (July) 1922.

60. Meyer, O. O.; McTiernan, C., and Aub, J. C.: The Effect of Thyroxin upon the Metabolism of Isolated Normal and Malignant Tissue, J. Clin. Investigation **12**: 723 (July) 1933.

system, since tissues denervated prior to administration of thyroid show elevated metabolism just as do tissues left intact. Moreover, it is not necessary that the animal be intact for the hormone to exert its characteristic effect. Canzanelli and Rapport [61] reviewed the results published on the effect of thyroid in vitro and reported that although thyroxin does not alter the oxygen consumption of tissues in vitro, thyroglobulin increases their metabolism considerably. The effect of diiodothyronine is inconstant and that of thyronine and that of diiodotyrosine negligible.

In its action the hormone, according to Zondek,[62] is in the nature of a catalyst, exerting its effect directly on the cell. Its action differs from that of an enzyme, which can accelerate reactions in an unorganized system. The hormone, to exert its effect, requires a living intact cell. On the other hand, Dye [63] concluded that an excess or a scarcity of thyroxin leads to an increase or a decrease in the amount, potency and effectiveness of the respiratory catalysts of cells, rather than that the thyroid hormone acts as an independent catalyst of itself. Such a role is also suggested by the observation of Scharles, Robb and Salter [64] that thyroxin produces a marked increase of liver amylase in the fasting animal.

Not only do thyroglobulin and thyroxin possess this calorigenic effect, but other substances, closely related to thyroxin, also have this property, but to a less extent. According to Harington,[56] any change in the molecule of thyroxin diminishes its physiologic activity. Thus Canzanelli and Rapport [65] found that in the dog tyrosine in large doses has a calorigenic effect, but this is only one two-thousandth of that of thyroxin. I have given large doses of tyrosine (1.5 Gm. daily, equivalent to the tyrosine content in 40 Gm. of thyroid protein) to 2 patients with myxedema without observing any meta-

61. Canzanelli, Attilio, and Rapport, David: The Effect of Thyroglobulin and Related Substances on the O_2 Consumption of Liver in Vitro, Endocrinology **21**: 779 (Nov.) 1937. Canzanelli, Attilio; Guild, Ruth, and Rapport, David: Further Observations on the Effect of Thyroglobulin and Thyroxin on the O_2 Consumption of Tissues in Vitro, ibid. **25**: 707 (Nov.) 1939.

62. Zondek, Hermann: The Diseases of the Endocrine Glands, translated by C. Prausnitz, ed. 3, Baltimore, William Wood & Company, 1935.

63. Dye, J. A.: The Action of Thyroxin on Tissue Respiration, Am. J. Physiol. **105**: 518 (Sept.) 1933.

64. Scharles, F. H.; Robb, P. D., and Salter, W. T.: Liver Amylase: The Effect of Nutrition and of Hormones, Am. J. Physiol. **111**: 130 (Feb.) 1935.

65. Canzanelli, Attilio, and Rapport, David: The Comparative Effects upon Metabolism of Intravenously Injected Tyrosine, Diiodotyrosine, Diiodothyronine and Thyroxine, Am. J. Physiol. **103**: 279 (Feb.) 1933.

bolic change.[66] The former investigators also reported that diiodotyrosine is seven and a half times as active as tyrosine, diiodothyronine fifteen times as active as diiodotyrosine and thyroxin seventeen times as active as diiodothyronine. Thompson and co-workers [67] found some calorigenic action in diiodotyrosine when it was given to patients with myxedema, but only one ten-thousandth of that of thyroxin. Lerman and Salter [68] found that crystalline 3,5-diidothyronine and an iodothyronine-like substance derived from artificial iodoprotein each has an activity about one thirtieth to one fortieth of that of whole thyroid in terms of iodine.

Thus one must consider a molecule containing two atoms of iodine attached to a tyrosine nucleus as the minimum requirement for a substance having thyroxin-like physiologic properties. As indicated before, Harington [56] has claimed that in the body tyrosine is converted into diiodotyrosine, which is then elaborated into diiodothyronine and then into thyroxin. That such changes take place is suggested by the work of Salter and Lerman,[55] already referred to, on the formation of active protein from the inert diiodotyrosine peptone, which on rediegestion yields "thyroxin-like" and "diiodotyrosine-like" peptones. The production of thyroid from diiodotyrosin by von Mutzenbecher [43b] also confirms Harington's viewpoint. The genesis of thyroid hormone may then be viewed as consisting of two processes: one, the building up of colloidal molecules from simpler polypeptide chains; the other, the combination of iodinated tyrosine residues to form iodinated thyronine residues. The mechanism of the second process is suggested by the work of Cohn, Salter and Ferry,[69] who showed that under certain conditions the phenolic groups of diiodotyrosine may undergo conjugation to form an iodized thyronine nucleus.

An important finding in this connection is that of Lerman and Salter,[68] Abelin [70] and Ludwig and von

66. Lerman, Jacob: Unpublished data.

67. Thompson, W. O.; Alper, J. M.; Thompson, Phebe K., and Dickie, Lois F. N.: The Effect of Diiodotyrosine on the Basal Metabolism in Myxedema, J. Clin. Investigation **13**: 29 (Jan.) 1934.

68. Lerman, Jacob, and Salter, W. T.: The Relief of Myxedema with Proteins of Extrathyroidal Origin, Endocrinology **25**: 712 (Nov.) 1939.

69. Cohn, E. J.; Salter, W. T., and Ferry, R. M.: The Amphoteric Properties of Globin and Iodized Globin, J. Biol. Chem. **123**: xxiv (May) 1938.

70. Abelin, I.: Nichtschilddrüsenstoffe mit Schilddrüsenwirkung: Weitere Erfahrungen über die Gewinnung schilddrüsenähnlich wirkender Substanzen aus künstlich jodiertem Eiweiss, Arch. f. exper. Path. u. Pharmakol. **181**: 250, 1936.

Mutzenbecher [71] that the simple process of iodinating protein by means of a gentle chemical reaction generates thyroid-like activity. It may be that simply the introduction of iodine into the tyrosine nucleus in the protein chain results in thyroid hormone. The significance of this is that the thyroid may not be the factory for the hormone but merely the storehouse for its distribution.

Action on Growth, Maturation and Differentiation of Tissue.—Congenital athyreosis in the human subject results in the dwarfism and juvenile habitus of the cretin. When thyroid is administered, growth is resumed. Conversely, a child suffering from thyrotoxicosis tends to be definitely taller than the average for the age. The failure of the cretin to attain the adult habitus is evidence that the thyroid plays a role in the maturation of the organism. In adult animals, according to Marine,[72] thyroidectomy produces little objective change, but in young ones thyroidectomy leads to stunted physical, mental and sexual development. This effect is more strikingly seen in the metamorphosing amphibia. Gudernatsch [73] showed that tadpoles metamorphose at an accelerated rate when thyroid is administered. Similar results have been obtained by Uhlenhuth [74] in the salamander. In both instances the animals mature to adulthood but do not change much in size.

The effect on the growth of individual tissues is seen in the retarded skin, hair and nail change in myxedema and in the delayed bony development in childhood athyreosis, i. e., delay in the appearance of ossification centers as well as in epiphysial union. In contrast, thyroxin stimulates growth of epiphysial cartilage in rabbits (Coryn [75]) and markedly accelerates the rate of eruption of incisor teeth in rats (Karnofsky and Cronkite [76]).

71. Ludwig, W., and von Mutzenbecher, P.: Ueber die Entstehung von Thyroxin durch Jodierung von Eiweiss, Ztschr. f. physiol. Chem. **244**: iv, 1936.

72. Marine, David: Physiology and Principal Interrelations of the Thyroid, J. A. M. A. **104**: 2250 (June 22) 1935.

73. Gudernatsch, J. F.: Fütterungsversuche an Amphibienlarven, Zentralbl. f. Physiol. **26**: 323 (June) 1912.

74. Uhlenhuth, Eduard: Relation Between Thyroid Gland, Metamorphosis, and Growth, J. Gen. Physiol. **1**: 473 (March) 1919.

75. Coryn, G.: Recherche expérimentale sur l'influence des glandes endocrines sur l'histologie du cartilage de conjugaison, Ann. d'anat. path. **16**: 27 (Jan.) 1939.

76. Karnofsky, D., and Cronkite, E. P.: Effects of Thyroxine on Eruption of Teeth in Newborn Rats, Proc. Soc. Exper. Biol. & Med. **40**: 568 (April) 1939.

The growth-promoting action of the thyroid is, in all probability, a phase of its action on metabolic processes in general. According to Hammett,[77] the thyroid hormone is concerned more with growth by increase in cell size than with growth by increase in cell number. It is essentially different from the growth hormone of the anterior lobe of the pituitary. However, there appears to be a synergism between the thyroid and the growth hormone of the anterior lobe of the pituitary. According to Evans, Simpson and Pencharz,[78] thyroid, which promotes normal growth of thyroidectomized animals, does not have this effect on hypophysectomized animals. Conversely, the growth produced by the growth hormone of the anterior lobe of the pituitary, although not dependent on the presence of the thyroid, is greater when the thyroid is intact. It is of interest that Sternheimer [79] obtained changes in liver glycogen, sugar and protein by the single injection of thyroxin which are consistent with those seen in growing tissues. These changes take place before the rise in oxygen consumption.

Action on the Distribution of the Water, Salts and Colloids of the Body.—When the organism is deprived of the thyroid, there takes place storage of water, salts and protein. In 1925 Boothby and associates [80] showed that in human myxedema a large amount of extra protein—so-called "deposit protein"—is stored in the body. It is contained in the body fluids and not in the cell protoplasm. When thyroid is administered, this extra deposit protein is quickly oxidized and eliminated in the urine along with the extra salts and water held in combination with the protein. Consequently, an appreciable diuresis is a characteristic finding when active hormone is administered to myxedematous patients for the first time. In fact, the diuretic action of thyroid may also be striking in nor-

77. Hammett, F. S.: Studies of the Thyroid Apparatus: XXIX, The Rôle of the Thyroid Apparatus in Growth, Am. J. Physiol. **76**: 69 (March) 1926.

78. Evans, H. M.; Simpson, M. E., and Pencharz, R. I.: Relation Between the Growth Promoting Effects of the Pituitary and the Thyroid Hormone, Endocrinology **25**: 175 (Aug.) 1939.

79. Sternheimer, Richard: The Effect of a Single Injection of Thyroxin on Carbohydrates, Protein and Growth in the Rat Liver, Endocrinology **25**: 899 (Dec.) 1939.

80. Boothby, W. M.; Sandiford, I.; Sandiford, K., and Slosse, J.: The Effect of Thyroxin on the Respiratory and Nitrogenous Metabolism of Normal and Myxedematous Subjects: I. A Method of Studying the Reserve or Deposit Protein with a Preliminary Report of the Results Obtained, Tr. A. Am. Physicians **40**: 195, 1925.

mal persons, and is made use of in the treatment of nephrosis. The work of Byrom [81] indicates that the diuresis produced in patients with myxedema is accompanied by a loss chiefly of sodium salts, whereas in normal persons the loss is chiefly of potassium salts. Consequently, in the former the fluids are derived largely from extracellular sources whereas in the latter the fluids are derived largely from intracellular sources. This confirms Boothby's findings, mentioned earlier in this paragraph. Byrom has suggested that the abnormally collected protein in myxedema is in the nature of a mucoprotein derived from the ground substance of the cell. Since fetal tissue, like myxedematous tissue, contains an excess of mucin, it seems that one function of the thyroid is to provide the cells with a "mature" type of environment.

Soon after Boothby's work became known, Thompson [82] described a significant reduction in plasma volume in myxedema and a return to normal on treatment with thyroid. These results have been confirmed by Gibson and Harris,[83] who made the additional finding that the blood volume in thyrotoxicosis tends to be above normal. Along with the reduced plasma volume there is an increased concentration of plasma protein, with a corresponding increase in spinal fluid protein (Thompson and co-workers [84]). On administration of thyroid both the plasma and the spinal fluid revert to normal. In thyroidectomized animals the changes in the blood are similar to those in myxedema.

The metabolism of various inorganic salts is bound up with the state of thyroid activity. Aub and collaborators [85] showed that in hypothyroidism there is a diminished rate of exchange of calcium and phosphorus, the amounts eliminated in the urine and stool being less than in the normal person. The actual concentrations in the blood are not greatly altered. The reverse holds

81. Byrom, F. B.: The Nature of Myxedema, Clin. Sc. **1**: 273 (Nov.) 1934.

82. Thompson, W. O.: Studies in Blood Volume: The Blood Volume in Myxedema, with a Comparison of Plasma Volume Changes in Myxedema and Cardiac Edema, J. Clin. Investigation **2**: 477 (Aug.) 1926.

83. Gibson, J. G., Jr., and Harris, A. W.: Clinical Studies of the Blood Volume: V. Hyperthyroidism and Myxedema, J. Clin. Investigation **18**: 59 (Jan.) 1939.

84. Thompson, W. O.; Thompson, Phebe K.; Silveus, Esther, and Dailey, Mary E.: The Cerebrospinal Fluid in Myxedema, Arch. Int. Med. **44**: 368 (Sept.) 1929.

85. Aub, J. C.; Bauer, Walter; Ropes, M., and Heath, C.: The Relation of the Thyroid Gland to Calcium Metabolism, Tr. A. Am. Physicians **42**: 344, 1927.

when thyroid is administered and in spontaneous hyperthyroidism. These effects, according to Low, Wilson and Aub,[86] are not brought about by changes in phosphatase activity in the bones. Talbot,[87] however, described low phosphatase in children with untreated hypothyroidism, associated with delayed osseous development. Thyroid treatment repairs these abnormalities. In the growth period the effect of thyroid on calcium metabolism may be different from that in later life. Thus Maroney and Johnston[88] found that retention of calcium and nitrogen was increased by the administration of thyroid to a cretin and to an adolescent after thyroidectomy. When administered in large doses thyroid may actually lead to premature cessation or retardation of growth (Smith and McLean[89]).

The metabolism of other inorganic salts is affected in the same manner as that of calcium and phosphorus. The blood levels of sodium and chloride in hypothyroidism are within normal limits,[90] but the urinary excretion of chlorides, according to Stephens[91] and Dr. Patricia Smith (personal communication), is low. These findings help differentiate myxedema due primarily to dysfunction of the thyroid from that due primarily to dysfunction of the pituitary.[92] In the latter, blood sodium and chloride are low and their excretion in the urine high (adrenal cortex insufficiency). The administration of thyroid restores the excretion of salt in primary hypothyroidism to normal, but in hypothyroidism secondary to pituitary dysfunction, by increasing the already excessive loss of salt, it may throw the patient into an addisonian crisis.

It should be indicated at this point that thyroid hormone also has an important control over blood lipoids. The blood cholesterol is elevated in hypothyroidism and

86. Low, M. B.; Wilson, R. O., and Aub, J. C.: Phosphatase Activity of the Bones and Kidneys in Thyrotoxicosis, Proc. Soc. Exper. Biol. & Med. **31**: 447 (Jan.) 1934.

87. Talbot, N. B.: Influence of Thyroid Hormone on Serum Phosphatase, Endocrinology **24**: 872 (June) 1939.

88. Maroney, J. W., and Johnston, J. A.: Effect of Thyroid on Nitrogen and Calcium in the Growth Period, J. Pediat. **13**: 937 (Oct.) 1938.

89. Smith, E. E., and McLean, F. C.: Effect of Hyperthyroidism upon Growth and Chemical Composition of Bone, Endocrinology **23**: 546 (Nov.) 1938.

90. Lerman, Jacob: Unpublished data.

91. Stephens, D. J.: Chloride Excretion in Hypothyroidism, Proc. Soc. Exper. Biol. & Med. **43**: 742 (April) 1940.

92. Means, J. H.; Hertz, Saul, and Lerman, Jacob: The Pituitary Type of Myxedema: or Simmonds' Disease Masquerading as Myxedema, Tr. A. Am. Physicians **55**: 32, 1940.

decreased in hyperthyroidism. In fact, there is a close reciprocal relationship between basal metabolic rate and blood cholesterol.[93] On the experimental side Schmidt and Hughes [94] found that hyperthyroidism produces no change in cholesterol. Thyroidectomy, however, produces a rise in which the normal ratio of free to esterified cholesterol is preserved. Thyroxin merely restores the normal level. In the rabbit I have been unable to find any change in blood cholesterol after complete thyroidectomy.[95] This finding may be due to the fact that the fat content of the rabbit's diet is low.

Action on Carbohydrate Metabolism.—Coggeshall and Greene [96] showed that thyroid feeding depletes hepatic glycogen even though the animals are on a diet high in carbohydrate. The conclusion was drawn that thyroid hormone not only depletes hepatic glycogen but also injures the liver so that it is unable to store glycogen. Conversely, thyroidectomy leads to excessive storage of glycogen in the liver. One frequently observes aggravation of latent or mild diabetes mellitus with the onset of hyperthyroidism. If myxedema and diabetes coexist, the relief of the myxedema intensifies the diabetes; on omission of thyroid the diabetes becomes milder. In fact, total thyroidectomy has been used in the treatment of diabetes mellitus.

Barnes [97] reported that feeding thyroid to the "Houssay dog" does not increase the mild glycosuria. He suggested that thyroid may exert its influence on carbohydrate metabolism through the pituitary which, in turn, may act on the adrenals. Similarly, thyroidectomy in pancreatectomized animals does not affect the diabetes in the way pituitarectomy (Houssay) or adrenalectomy (Long) does (Long;[98] Dohan and Lukens [99]). On the

93. Hurxthal, L. M., and Hunt, H. M.: Clinical Relationships of Blood Cholesterol, with a Summary of Our Present Knowledge of Cholesterol Metabolism, Ann. Int. Med. **9**: 717 (Dec.) 1935.

94. Schmidt, L. H., and Hughes, H. B.: The Free and Total Cholesterol Content of Whole Blood and Plasma as Related to Experimental Variations in Thyroid Activity, Endocrinology **22**: 474 (April) 1938.

95. Lerman, Jacob: Unpublished data.

96. Coggeshall, H. C., and Greene, J. A.: The Influence of Desiccated Thyroid Gland, Thyroxin, and Inorganic Iodine, upon the Storage of Glycogen in the Liver of the Albino Rat Under Controlled Conditions, Am. J. Physiol. **105**: 103 (July) 1933.

97. Barnes, B. O.: The Effects of the Endocrine Glands on the Carbohydrate Metabolism. A Working Hypothesis, Am. J. Physiol. **109**: 5 (July) 1934.

98. Long, C. N. H.: Recent Advances in Carbohydrate Metabolism with Particular Reference to Diabetes Mellitus, Ann. Int. Med. **9**: 166 (Aug.) 1935.

99. Dohan, F. C., and Lukens, F. D. W.: The Effect of Thyroidectomy upon Pancreatic Diabetes in the Cat, Am. J. Physiol. **122**: 367 (May) 1938.

other hand, Soskin and co-workers [100] reported that the administration of thyroxin to hypophysectomized dogs helps maintain their normal blood sugar during periods of fasting, probably by increasing protein catabolism.

Action on the Nervous System.—The action of the thyroid on the nervous system is best observed in states of excess or of scarcity of the hormone. In the former case the person has emotional instability, increased irritability and sometimes gross disturbance in cerebration. In the latter case the person lives at a low emotional level, reacts sluggishly and cerebrates slowly; the memory is poor, and there is diminished sensory acuity. The effect of the thyroid on the vegetative nervous system is manifested by increase in vasomotor activity, peristaltic activity and activity of the sweat glands in thyroid intoxication and by the reverse of these phenomena in thyroid deprivation. An objective approach to evaluate this function of the thyroid was made by Ross and Schwab.[101] They found that the cerebral cortical alpha rate, as obtained by the electroencephalogram, is low in myxedema and returns to normal with the administration of thyroid. There is a good correlation between the alpha rate and the state of metabolism.

Action on the Muscular System.—In thyrotoxicosis there are changes in skeletal muscle which vary from mild myasthenia to advanced muscular atrophy, with corresponding degenerative changes in the muscle fibers. In hypothyroidism there is hypotonicity. Histologically, there is interstitial edema of muscle fibers. Spontaneous creatinuria is a characteristic finding in hyperthyroidism; thyroid administration in myxedematous and normal people initiates creatinuria. According to Richardson and Shorr,[102] creatine tolerance is decreased in thyrotoxicosis, and as thyrotoxicosis declines creatine tolerance rises. Thorn [103] also found decreased creatine tolerance in hyperthyroidism and increased tolerance in myxedema. A useful correlation between total basal

100. Soskin, Samuel; Levine, R., and Heller, R. E.: Rôle of the Thyroid in the Carbohydrate Disturbance Which Follows Hypophysectomy, Am. J. Physiol. **125**: 220 (Feb.) 1939.

101. Ross, D. A., and Schwab, R. S.: The Cortical Alpha Rhythm in Thyroid Disorders, Endocrinology **25**: 75 (July) 1939.

102. Richardson, H. B., and Shorr, Ephraim: The Creatin Metabolism in Atypical Graves' Disease, Tr. A. Am. Physicians **50**: 156, 1935.

103. Thorn, G. W.: Creatine Studies in Thyroid Disorders, Endocrinology **20**: 628 (Sept.) 1936.

caloric output (a gage of the level of thyroid activity) and creatinine excretion (a gage of active muscle mass) has been obtained by Talbot, Worcester and Stewart,[104] confirming the earlier results of Palmer, Means and Gamble.[105]

Action on the Circulatory System.—The action of the thyroid on the circulation may be accounted for chiefly by its other actions, already discussed. A heightening in metabolism produced by thyroid calls forth an increased mass movement of blood. This is forthcoming from the more rapid heart action, the peripheral dilatation and the increased stroke volume of the heart. In hypothyroidism the reverse of these activities is observed. According to Zondek,[62] there is also a shift in the oxygen dissociation curve of the blood which facilitates delivery of oxygen to the tissues as metabolism rises. Increased cardiac irritability and tone in thyrotoxicosis and lowered tone in myxedema may be due to direct action of the thyroid hormone on the cardiac musculature. The cardiac enlargement so characteristic of myxedema is due to a combination of diminished cardiac tonus and myxedematous infiltration of the cardiac musculature. When thyroid is administered, edema disappears, tonus increases and heart size shrinks (Lerman, Clark and Means[106]).

Miscellaneous Actions of Thyroid Hormone.—Several actions of the thyroid are difficult of classification, but as knowledge improves their true significance should become clear.

Tolerance to some types of drugs is in some manner related to thyroid function. According to Benedict[107] (and my co-workers and I confirm it), patients with myxedema tolerate morphine poorly. It is our impression that such patients also tolerate digitalis poorly. On the other hand, patients with hyperthyroidism tolerate morphine and other sedative drugs extremely well. These findings may be attributed to the metabolic action of thyroid. In the same category is the acetonitrile

104. Talbot, N. B.; Worcester, Jane, and Stewart, Ann: New Creatinine Standard for Basal Metabolism and Its Clinical Application, Am. J. Dis. Child. **58**: 506 (Sept.) 1939.

105. Palmer, W. W.; Means, J. H., and Gamble, J. L.: Basal Metabolism and Creatinine Elimination, J. Biol. Chem. **19**: 239 (Oct.) 1914.

106. Lerman, Jacob; Clark, R. J., and Means, J. H.: The Heart in Myxedema. Electrocardiograms and Roentgen-Ray Measurements Before and After Therapy, Ann. Int. Med. **6**: 1251 (April) 1933; Further Observations on the Heart in Myxedema, ibid. **8**: 82 (July) 1934.

107. Benedict, Edward B.: Morphine in Myxedema, J. A. M. A. **94**: 1916 (June 14) 1930.

reaction of Hunt [108]—the increased resistance of mice to acetonitrile, produced by the feeding of thyroid—and the increase in fluorine toxicity in animals receiving thyroid (Phillips, English and Hart; [109] Wilson and De Eds [110]).

In the field of animal husbandry, two types of speed-ups attributable to thyroid have been recently reported. The rate of egg laying by hens receiving thyroid is increased above normal (Winchester [111]); also the production of milk and milk fat by cows is increased by thyroid (Graham [112]) and reduced by thyroidectomy.

Althausen and Stockholm [113] have shown that absorption from the gastrointestinal tract is regulated by the level of thyroid activity. Thus the absorption of various sugars is slow in thyroidectomized animals and rapid in hyperthyroid ones. In the light of these results dextrose tolerance tests in patients with myxedema and in patients with exophthalmic goiter acquire new significance.

The thyroid controls the blood-forming organs in a manner that is not clearly understood. In hyperthyroidism an excess of mononuclear cells in the blood stream is a frequent finding (Hertz and Lerman [114]); in myxedema moderate anemia is common (Lerman and Means [115]).

BLOOD IODINE

Of the normal blood iodine of about 8 to 10 micrograms per hundred cubic centimeters of blood, a small portion is due to circulating inorganic iodine, representing exogenous material; the remainder is due to organic iodine and may represent the amount of circulating

108. Hunt, R.: The Influence of Thyroid Feeding upon Poisoning by Acetonitrile, J. Biol. Chem. **1**: 33, 1905.

109. Phillips, P. H.; English, Honora, and Hart, E. B.: The Augmentation of the Toxicity of Fluorosis in the Chick by Feeding Desiccated Thyroid, J. Nutrition **10**: 399 (Oct.) 1935.

110. Wilson, R. H., and De Eds, F.: The Synergistic Action of Thyroid on Fluorine Toxicity, Endocrinology **26**: 851 (May) 1940.

111. Winchester, C. F.: Influence of Thyroid on Egg Production, Endocrinology **24**: 697 (May) 1939.

112. Graham, W. R., Jr.: The Effect of Thyroidectomy and Thyroid Feeding on the Milk Secretion and Milk Fat Production of Cows, J. Nutrition **7**: 407 (April) 1934.

113. Althausen, T. L., and Stockholm, M.: Influence of the Thyroid Gland on Absorption in the Digestive Tract, Am. J. Physiol. **123**: 577 (Sept.) 1938.

114. Hertz, Saul, and Lerman, Jacob: The Blood Picture in Exophthalmic Goitre and Its Changes Resulting from Iodine and Operation: A Study by Means of Supravital Technique, J. Clin. Investigation **11**: 1179 (Nov.) 1932.

115. Lerman, Jacob, and Means, J. H.: Treatment of the Anemia of Myxedema, Endocrinology **16**: 533 (Sept.-Oct.) 1932.

hormone. The concentration of iodine in the blood fluctuates. Diet, exercise, medication and season are among the factors influencing the level of blood iodine (Salter [116]). The level fluctuates with thyroid function—it is lowered in myxedema and raised in thyrotoxicosis.

The nature of the organic iodine in the blood has been a puzzle. Lunde, Closs and Pedersen [117] separated total iodine into alcohol-soluble and alcohol-insoluble fractions. According to Salter,[116] such separations are artificial. Recent experiments indicate that most of the iodine is bound to protein but can be extracted from the protein by butyl alcohol (Trevorrow [118]). This extract, in turn, can be separated into "thyroxin-like" and "diiodotyrosine-like" fractions. Salter has suggested the following classification of iodine fractions in the plasma: (1) "I" iodine, nonprecipitable and presumably inorganic, and (2) "P" iodine, the maximum amount precipitated with the protein. The "P" fraction may then be separated into a "T" (thyroxin-like) fraction and a "D" (diiodotyrosine-like) fraction. Indeed, Trevorrow [118] found the ratio of "T" to "D" blood iodine the same as the ratio of these fractions in human thyroids.

That the blood contains circulating hormone is indicated by the fact that the oxygen consumption of mouse liver is increased by the addition of blood from patients with exophthalmic goiter and decreased by blood from myxedematous patients as compared with normal blood (Salter and Craig [119]). The failure of crystalline thyroxin to increase the consumption of oxygen is presumptive evidence that the circulating hormone is not the same as thyroxin. Canzanelli and Rapport [61] also found that thyroxin does not increase the metabolism of tissues in vitro, whereas thyroglobulin does. The phenomena observed by Salter and Craig are "thyroglobulin-like" rather than "thyroxin-like." In addition Perkin has been unable to recover thyroxin

116. Salter, W. T.: Iodine Metabolism or Fluctuations in Body Iodine, Physiol. Rev. **20**: 345 (July) 1940.

117. Lunde, G.; Closs, K., and Pedersen, O. C.: Untersuchungen über den Jodstoffwechsel: III. Untersuchungen über den Blutjodspiegel bei der primären Thyreotoxikose, Biochem. Ztschr. **206**: 261, 1929.

118. Trevorrow, V.: Studies on the Nature of the Iodine in Blood, J. Biol. Chem. **127**: 737 (March) 1939.

119. Salter, W. T., and Craig, F. N.: Vicarious Metabolic Response: The Oxygen Consumption of Surviving Tissues in Plasma from Hyperthyroid Organisms, J. Clin. Investigation **17**: 502 (July) 1938.

from the organic iodine fraction in his method of separating organic and inorganic iodine in the blood.[120] He is able to recover injected crystalline thyroxin from the organic fraction by the same technic after a lapse of three days. He concludes that the "thyroxin-like" fraction in the blood is not the same as crystalline thyroxin.[121]

As indicated, I^{16} have been unable to detect any thyroglobulin in the serum of normal persons or of patients with myxedema or hyperthyroidism. Consequently, it seems unlikely that intact thyroglobulin is represented in the organic iodine fraction.

RELATION OF THE THYROID TO OTHER ENDOCRINE ORGANS

It is obvious that in a complex mechanism like the body the thyroid is only one link in the elaborate coordinating system of glands. It acts on its endocrine partners and is in turn acted on by them, particularly by the central gland—the pituitary. The thyroid may influence other endocrine glands in several ways: (1) by a specific influence on all endocrine glands, (2) by a specific influence on the pituitary, (3) by a calorigenic effect on all endocrine glands and (4) by a calorigenic effect on the pituitary.

The effect of the thyroid on the pituitary is especially significant. Through this mechanism the thyroid indirectly controls the function of all glands and organs regulated by the pituitary. The thyroid, in turn, is controlled by the secretions of other glands or organ metabolites by way of the blood stream or by way of the nervous system. It is of interest that Foot, Baker and Carrel,[122] cultivating human thyroids in toto in the Lindbergh apparatus, showed that the histologic picture reached by the gland seems determined not by the phase of activity which existed while it resided in the body but by the nature of the perfusate used during its existence in vitro. In other words, it seems that humoral factors reaching the gland are more important than the condition inherent in the gland.

120. Perkin, H. J., and Hurxthal, L. M.: The Fractionation of the Iodine of the Blood in Thyroid Disease, J. Clin. Investigation **18**: 733 (Nov.) 1939.

121. Perkin, H. J.: Personal communication to the author.

122. Foot, N. C.; Baker, L. E., and Carrel, Alexis: The Behavior of Abnormal Human Thyroid Tissue Cultivated in the Lindbergh Apparatus, J. Exper. Med. **70**: 39 (July) 1939.

Pituitary.—Inasmuch as the pituitary-thyroid relationship is discussed fully in another chapter, I shall merely touch on the high lights. Knowledge of this relationship probably began with the observation of Rogowitsch [123] that in rabbits total thyroidectomy causes pituitary hypertrophy. It was shown by Houssay and associates [124] that the compensatory hyperplasia following subtotal thyroidectomy does not take place in the absence of the hypophysis. Moreover, according to the recent review by Means,[125] there is more thyrotropic activity in the blood and urine of myxedematous patients and patients who have undergone thyroidectomy, and less in the blood and urine of thyrotoxic patients before operation, than in the blood and urine of normal persons.

There is considerable experimental evidence that the thyroid hormone exercises a depressing effect on the thyrotropic function of the anterior lobe of the pituitary (Aron and co-workers;[126] Kuschinsky;[127] Marine;[72] Uotila[128]). Uotila found that thyroxin produces atrophy of the thyroid to the same degree that hypophysectomy does and attributed the atrophy to the depressing effect of thyroxin on the anterior lobe of the pituitary. It is of interest in this respect that the oxygen consumption of thyroid tissue excised from thyroxin-treated animals (Belasco and Murlin[129]) and of thyroid tissue bathed in thyroglobulin solution (Galli-Manini[129]) is diminished.

Conversely, numerous investigators have shown that pituitarectomy results in involution of the thyroid. The

123. Rogowitsch, N.: Die Veränderungen der Hypophyse nach Entfernung der Schilddrüse, Beitr. z. path. Anat. u. z. allg. Path. **4**: 453, 1888.

124. Houssay, B. A.; Biasotti, A., and Magdalena, A.: Hypophyse et thyroïde. Hypophyse et hypertrophie compensatrice de la thyroïde, Compt. rend. Soc. de biol. **110**: 142 (May) 1932.

125. Means, J. H.: Diseases of the Thyroid Gland, New England J. Med. **221**: 820 (Nov. 23) 1939.

126. Aron, Max; Van Caulaert, C., and Stahl, J.: L'équilibre entre l'hormone préhypophysaire et l'hormone thyroïdienne dans le milieu intérieur à l'état normal et à l'état pathologique, Compt. rend. Soc. de biol. **107**: 64 (May) 1931.

127. Kuschinsky, G.: Ueber die Bedingungen der Sekretion des thyreotropen Hormons der Hypophyse, Arch. f. exper. Path. u. Pharmakol. **170**: 510, 1933.

128. Uotila, U. U.: The Regulation of Thyrotropic Function by Thyroxin After Pituitary Stalk Section, Endocrinology **26**: 129 (Jan.) 1940.

129. Belasco, I. J., and Murlin, J. R.: The Effect of Thyroxin and Thyrotropic Hormone on the Basal Metabolism and Thyroid Tissue Respiration of Rats at Various Ages, Endocrinology **28**: 145 (Feb.) 1941. Galli-Manini, C.: Effect of Thyrotropic and Thyroid Hormone upon the Oxygen Consumption (Q_{O_2}) of Guinea Pig Thyroid, Endocrinology (in press).

syndrome described by Simmonds in 1914 represents the equivalent in man of total hypophysectomy. The essential symptoms are progressive wasting, weakness, subnormal metabolism, hypotension, hypoglycemia and cessation of sexual activity. Pathologically, there is hypoplasia of all the endocrine glands. In most instances the picture of full blown athyreosis is not present. In a few cases, however, as recently described by Means, Hertz and Lerman,[92] the dominant feature is myxedema, i. e., pituitary myxedema. These patients require treatment by replacement with preparations of the various tropic substances in proper combination. Administration of thyrotropic extract alone (Bulger and Barr [130]) or of thyroid alone (Means, Hertz and Lerman [92]) will aggravate the condition due to adrenal cortex insufficiency and result in death. Thyroid treatment should be accompanied by therapy aimed to protect the adrenals, namely, the administration of large amounts of salts, anterior pituitary adrenotropic extract or extract of adrenal cortex, and gonadotropic extracts or androgen or estrogen.

The experiments of P. E. Smith, B. M. Allen and others have emphasized the role of the anterior lobe of the pituitary as the regulator of the thyroid. That the thyrotropic principle of the anterior lobe of the pituitary regularly causes thyroid hyperplasia, increased metabolism and other evidences of increased thyroid function is now well established. This action is probably directly on the thyroid cells, because isolated thyroid tissue may be stimulated to increase metabolism by an extract of anterior lobe containing the thyrotropic factor. Williams [14] observed that the secretory cycle of thyroid follicles implanted in the rabbit's ear is accelerated by giving the animal thyrotropic material.

The relation of the posterior lobe to the thyroid is less clear than that of the anterior lobe. Mahoney and Sheehan [131] produced experimental diabetes insipidus in dogs by placing a clip on the pituitary stalk, and were then able to abolish the diabetes by total thyroidectomy. Feeding thyroid caused a return of the polyuria, which could then be abolished by injecting a

130. Bulger, J. A., and Barr, D. P.: Metabolic Studies of Pituitary Insufficiency, Endocrinology **20**: 137 (March) 1936.

131. Mahoney, William, and Sheehan, Donald: The Effect of Total Thyroidectomy upon Experimental Diabetes Insipidus in Dogs, Am. J. Physiol. **112**: 250 (June) 1935.

solution of posterior pituitary. Apparently the diuretic action of thyroid may be antagonized by the antidiuretic action of the posterior lobe of the pituitary. In the rat, however, Swann and Johnson [132] found little influence by the thyroid on the diabetes insipidus following removal of the posterior lobe of the pituitary. Thyroid administration does not affect the rat's fluid exchange.

Adrenal.—In Addison's disease there is often a moderate drop in basal metabolism. This is not due to any gross disturbance in the function of the thyroid, nor is there any histologic disturbance found in the thyroid at autopsy. In the experimental animal, on the other hand, Marine and Baumann [133] and Davis and Hastings [134] found that removal of the adrenals causes a rise in metabolism. Again, as Means, Hertz and Lerman [92] emphasized, patients with hypofunction of the adrenal cortex, be it primary or secondary to pituitary disease, may be thrown into a crisis of adrenal failure by administration of thyroid. Zondek [62] stated that thyroidectomized animals survive total adrenalectomy longer than those possessing thyroids. Thyroid administration produces enlargement of the adrenal cortex (Uotila; [128] Ingle and Higgins [135]). Similarly Hoen, Langefeld and Oehme [136] showed that a large dose of desoxycorticosterone administered to guinea pigs antagonizes the rise in metabolism caused by thyroxin and also hinders the hypertrophy of the adrenal cortex seen in induced hyperthyroidism. On the other hand, Bock [137] showed that an extract of adrenal cortex plus thyroxin accelerates metamorphosis of tadpoles and axolotls at a greater rate than thyroxin alone. The administration of salt to hypophysectomized rats, according to Evans and co-workers,[138] produces

132. Swann, H. G., and Johnson, P. E.: Thyroid Function in Diabetes Insipidus in the Rat, Endocrinology **24**: 397 (March) 1939.

133. Marine, David, and Baumann, E. J.: Influence of Glands with Internal Secretions on Respiratory Exchange: Further Data on Effect of Suprarenal Insufficiency (by Removal) in Rabbits, J. Metabolic Research **2**: 1 (July) 1922.

134. Davis, J. E., and Hastings, A. B.: Relationship of Adrenal and Thyroid Glands to Excised Muscle Metabolism, Am. J. Physiol. **105**: 110 (July) 1933.

135. Ingle, D. J., and Higgins, G. M.: The Effect of Thyroxine on the Extent of Regeneration in the Enucleated Adrenal Gland of the Rat, Endocrinology **23**: 419 (Oct.) 1938.

136. Hoen, A.; Langefeld, H., and Oehme, C.: Über die Beziehungen zwischen Schilddrüse und Nebennieren, Endokrinologie **21**: 305, 1939.

137. Bock, K. A.: Die Einwirkung von Nebennierenrindenextrakt auf den Ablauf der Thyroxinmetamorphose bei Froschlarven und beim Axolotl, Klin. Wchnschr. **17**: 1311 (Sept.) 1938.

138. Evans, H. M.; Luck, J. M.; Pencharz, R. I., and Stoner, H. C.: The Calorigenic Action of Amino Acids in the Hypophysectomized Animal, Am. J. Physiol. **122**: 533 (May) 1938.

a rise in their reduced metabolism, whereas the injection of salt into normal animals reduces their metabolism. Interestingly enough, the metabolism of one of our patients with pituitary myxedema was elevated fifteen to twenty points by therapeutic procedures aimed only at raising the levels of the blood sodium and chloride.

In myxedema the excretion of 17-ketosteroids in the urine—important evidence of adrenocortical function—is low (1 to 2 mg. in twenty-four hours). In pituitary myxedema the urine is practically free of ketosteroids. In the one the low excretion is probably due to the nonspecific effect of retarded metabolism of adrenal cells; in the other the absence of excretion is due to specific depression of adrenocortical function.

Experimental results from different sources all agree that administration of thyroid causes increased sensitivity of the organism to epinephrine. In the human subject this fact is the basis of the Goetsch test for hyperthyroidism. At one time the author measured the response of the metabolism, pulse rate and blood pressure of patients with hyperthyroidism and with myxedema to a standard amount of epinephrine given intravenously. The changes were much greater in the group with hyperthyroidism than in the myxedematous group.[139] This exaggerated sensitivity of patients with hyperthyroidism to epinephrine, so suggestive of underfunction of the adrenal medulla, is at variance with the statement of Crile [140] that overfunction of the adrenal medulla is important in the genesis of exophthalmic goiter.

Gonads.—The evidence of a truly specific interrelationship between the thyroid and the gonads is conflicting. In hyperthyroidism the libido may be increased; in myxedema, diminished. This is not necessarily an expression of endocrine imbalance, but rather of the level of nervous irritability. In myxedema the gonadal function is present but low. This is indicated by the low urinary excretion of 17-ketosteroids (partly of gonadal origin in the male) and of estrogen. Administration of thyroid increases the output of these substances. In cretinism retardation of genital development

139. Lerman, Jacob: Unpublished data.

140. Crile, George: The Comparative Anatomy of the Thyroid-Adrenal-Sympathetic System, with Discussion of Unique Function of Adrenal-Sympathetic System, Tr. Am. A. Study Goiter, 1934, p. 23.

is the rule. Similarly, thyroidectomy in young rabbits leads to sexual retardation.

Either hyperthyroidism or hypothyroidism may decrease fertility and produce abortion. The striking benefit obtained by us in the treatment of habitual abortion with thyroid is discussed by Means.[1] Similarly King and Herring [141] reported benefit from the treatment of habitual abortion with thyroid.

The characteristic menstrual pattern in thyrotoxicosis is oligomenorrhea or amenorrhea, and in myxedema before the menopause, menorrhagia. These symptoms may be explained on the basis of the nonspecific action of thyroid hormone on gonadal cells, or, in the light of recent experiments, on the basis of a specific effect on the gonads. Several investigators have shown that thyroxin causes degeneration of the ovaries. Uotila [128] found that thyroxin produces atrophy of the seminal vesicles of the rat, probably by depressing the output of gonadotropins by the anterior lobe of the pituitary. Smelser [142] explained such findings by an increase in the threshold of response to gonadotropic and androgenic hormones in animals with hyperthyroidism. Tyndale and Levin [143] concluded that thyroid hormone exerts an inhibiting action on the gonads directly. In any case the action of thyroxin may be such as to produce a state of low estrogenic function. In myxedema the reverse of this situation will be true—a state of relative "hyperestrinism" (metropathia hemorrhagica) is produced.

Recently we have observed several patients with myxedema due to primary dysfunction of the thyroid who had amenorrhea not attributable to the menopause. The hormonic balance, as far as the usual tests show, was not unusual. On administration of thyroid the ovarian cycles of these patients became normal. The most likely explanation is that the ovarian cycle in such cases is normal but the flux of hormones is at a low level (as a result of the low metabolism of the cells).

141. King, E. L., and Herring, J. S.: Hypothyroidism in the Causation of Abortion, Especially of the "Missed" Variety, J. A. M. A. **113**: 1300 (Sept. 30) 1939.

142. Smelser, G. K.: Testicular Function and the Action of Gonadotropic and Male Hormones in Hyperthyroid Male Rats, Anat. Rec. **73**: 273 (March) 1939.

143. Tyndale, H. H., and Levin, Louis: Ovarian Weight Responses to Menopause Urine Injections in Normal, Hypophysectomized and Hypophysectomized Thyroxin-Treated Immature Rats, Am. J. Physiol. **120**: 486 (Nov.) 1937.

Consequently the phases of endometrial congestion and proliferation are insufficient to produce clinical menstrual bleeding. Thyroid, by stimulating the ovary, increases the production of estrogen and progestin, and the normal cycle returns. This conception fits with the observations of Grumbrecht [144] that thyroid increases the weight of ovaries of infantile rats receiving a constant dose of gonadotropic substance, the increase in weight being proportional to the dose of thyroid.

The evidence of gonadal influence on the thyroid is less conclusive. Gessler [145] showed that estrogen lowers the basal metabolic rate of normal guinea pigs and hypophysectomized rats, and Sherwood [146] found that estrogen reduces the duration of thyroid intoxication of rats. The creatinuria induced by thyroxin administration is reduced or completely abolished by testosterone propionate (Jailer [147]). According to Smelser,[142] the amount of androgen necessary to stimulate accessory sex organs of castrates is increased fivefold in thyroxin-treated animals. In fact, Starr and Patton [148] induced remissions in exophthalmic goiter by injecting an extract of pregnancy urine. They suggested that the excess of estrogen produced by the gonadotropic material from pregnancy urine depressed the thyroid directly. On the other hand, Marine [72] stated that "total removal of the gonads in the dog, rabbit and rat usually leads to a slow involution of the thyroid in about one month and to slight reduction of total metabolism." Moreover, it has been noted by several workers that estrogen causes a rise in metabolism in normal and in ovariectomized animals; androgen also is said to stimulate the thyroid (Nathanson and collaborators [149]). These descrepancies may be explained by the observation of Pincus and

144. Grumbrecht, P.: Die Anwendung von Schilddrüsenpräparaten bei Periodenstörungen, Zentralbl. f. Gynäk. **63**: 1942 (Sept. 2) 1939.

145. Gessler, C.: Influence of Folliculin on the Basal Metabolic Rate, Arch. internat. de pharmacodyn. et de thérap. **54**: 263 (Oct. 31) 1936.

146. Sherwood, T. C.: The Relation of Estrogenic Substance to Thyroid Function and Respiratory Metabolism, Am. J. Physiol. **124**: 114 (Oct.) 1938; Effect of Estrogenic Substance on Experimentally Hyperthyroid Male Rats, Endocrinology **29**: 215 (Aug.) 1941.

147. Jailer, J. W.: Effect of Testosterone Propionate on Creatinuria of Experimental Hyperthyroidism in Male and Female Monkeys, Endocrinology **29**: 89 (July) 1941.

148. Starr, Paul, and Patton, Helen: The Effect of Pregnancy Urine Extract and Ovarian Follicular Hormone on Hyperthyroidism, Endocrinology **18**: 113 (Jan.-Feb.) 1934.

149. Nathanson, I. T.; Brues, A. M., and Rawson, R. W.: Effect of Testosterone Propionate upon Thyroid and Parathyroid Glands of Intact Immature Female Rats, Proc. Soc. Exper. Biol. & Med. **43**: 737 (April) 1940.

Werthessen [150] that injections of estrogen into animals over a short time (five to ten days) lead to thyroid enlargement whereas injections over a longer time (twenty days or more) lead to thyroid involution. Such observations plus the finding of atrophy of the interstitial cells in a patient with myxedema [151] led Marine to suggest that gonadal atrophy may play a role in the etiology of myxedema. In our own experience castration does not lead to the development of thyroid disease.

Pancreas.—The interrelationship of the pancreas and the thyroid has already been touched on. The well known deleterious effect of thyroid on the severity of diabetes mellitus may not be due to a specific antagonism between thyroid and insulin but may be the result of the characteristic action of thyroid in increasing metabolic rate. Some observers believe that the thyroid and pancreas are antagonistic. Bodansky [152] reported that thyroidectomized sheep are more sensitive to insulin than normal animals. Conversely, thyroid administered to thyroidectomized rabbits decreases the hypoglycemic action of insulin. He attributed these results to the action of thyroid in promoting glycogenolysis. In our own experience the action of insulin on the blood sugar in myxedema is as follows: The drop in blood sugar from the fasting level requires forty-five to sixty minutes, against a normal of twenty to thirty minutes; the level of depression may be as low as in the normal or lower; the return of the blood sugar to fasting levels requires two hours or more as against a normal of ninety minutes. These results may easily be explained by the fact that all metabolic processes (including the burning of sugar, storage of glycogen and mobilization of epinephrine) are slow in hypothyroidism.

Parathyroid.—There is little evidence of interrelationship between the thyroid and the parathyroid glands. The effect of thyroid on the calcium and phosphorus metabolism is undoubtedly due to the rate of turnover of these elements between bones, blood and kidneys. Consequently, thyroid administration (Aub and asso-

150. Pincus, Gregory, and Werthessen, N.: Continued Injection of Oestrin into Young Rats, Am. J.. Physiol. **103**: 631 (March) 1933.

151. Marine, David: Changes in the Interstitial Cells of the Testes in Gull's Disease, Arch. Path. **28**: 65 (July) 1939.

152. Bodansky, A.: Effect of Thyroidectomy upon the Reaction of Sheep to Insulin, Proc. Soc. Exper. Biol. & Med. **21**: 46 (Oct.) 1923.

ciates [153]) or spontaneous thyrotoxicosis (Cope and Donaldson [154]) may raise the level of blood calcium in hypoparathyroidism.

Thymus.—It is well known that the endocrinology of the thymus is yet to be discovered. A relationship between the thyroid and the thymus is suggested by the work of Gudernatsch,[73] who found that tadpoles fed thymus show acceleration of growth and failure of metamorphosis whereas those fed thyroid show the reverse. Speidel [155] noted that feeding thyroid to tadpoles causes proliferation of the thymus. The enlarged thymus in the patient with exophthalmic goiter is a well known finding. Similarly thyroidectomy hastens involution of the thymus (Marine and co-workers; [156] Chiodi [157]). On the other hand, Richter and Wislocki [158] reported evidence contradicting the aforementioned observations. In hypophysectomized rats, whereas the thyroid and adrenals are hypoplastic, the thymus and lymph nodes are hyperplastic.

THYROID AND NUTRITION

It has long been known that thyroid hypertrophy and hyperplasia develop in animals on a high fat or a high protein diet. Rabbits kept on a cabbage diet (Chesney, Clawson and Webster [159]) or a diet of alfalfa hay and oats (Marine and Baumann [160]) and rats kept on a

153. Aub, J. C.; Albright, Fuller; Bauer, Walter, and Rossmeisl, Elsie: Studies of Calcium and Phosphorus Metabolism: VI. In Hypoparathyroidism and Chronic Steatorrhea with Tetany with Special Consideration of the Therapeutic Effect of Thyroid, J. Clin. Investigation **11**: 211 (Jan.) 1932.

154. Cope, Oliver, and Donaldson, G. A.: Relation of Thyroid and Parathyroid Glands to Calcium and Phosphorus Metabolism: Study of a Case with Coexistent Hypoparathyroidism and Hyperthyroidism, J. Clin. Investigation **16**: 329 (May) 1937.

155. Speidel, C. C.: Studies of Hyperthyroidism: II. The Significance of Changes in the Thymus Glands of Thyroid-Treated Frog Tadpoles, Am. J. Anat. **37**: 141 (March) 1926.

156. Marine, David; Manley, O. T., and Baumann, E. J.: Influence of Thyroidectomy, Gonadectomy, Suprarenalectomy, and Splenectomy on Thymus Gland of Rabbits, J. Exper. Med. **40**: 429 (Oct.) 1924.

157. Chiodi, H.: Acción de la tiroidectomía y suprarrenalectomía sobre el timo de ratas albinas castradas, Rev. Soc. argent. de biol. (no. 4) **14**: 322 (July) 1938.

158. Richter, C. P., and Wislocki, G. B.: Anatomical and Behavior Changes Produced in the Rat by Complete and Partial Extirpation of the Pituitary Gland, Am. J. Physiol. **95**: 481 (Nov.) 1930.

159. Chesney, A. M.; Clawson, T. A., and Webster, B.: Endemic Goitre in Rabbits; Incidence and Characteristics, Bull. Johns Hopkins Hosp. **43**: 261 (Nov.) 1928.

160. Marine, David, and Baumann, E. J.: Further Studies on the Etiology of Goiter: The Effect of Cyanides, Tr. A. Am. Physicians **47**: 261, 1932.

soybean diet (Sharpless, Pearsons and Prato[161]) develop thyroid enlargement. Similarly, animals deficient in iodine or in vitamin D have hyperplastic glands. Conversely, the thyroids of undernourished animals undergo involution—the cells become flat and the follicles distended with colloid (Rabinovitch;[162] Stephens[163]). Stephens offered the interesting suggestion that the changes in inanition are due to suppression of thyrotropic hormone.

In general, the metabolism of vitamins, as well as the metabolism of ordinary food substances, is regulated by the thyroid. Consequently, the need for vitamins parallels the rate of metabolism. Himwich and co-workers[164] showed that vitamin B spares the weight loss induced by thyroid. In patients with hyperthyroidism vitamin B in the form of yeast does not cause reduction in metabolism but improves the appetite and causes weight gain.[165] The author recently observed in a patient, a lifelong "imbiber," that acute peripheral neuritis developed as soon as hyperthyroidism developed. According to Drill[166] the liver and kidneys of hyperthyroid animals are depleted of their vitamin B_1 content and abnormal liver function is produced by thyroid feeding when the yeast content of the diet is inadequate.

There is a marked reduction in the vitamin C content of tissues of rats made toxic with thyroid (Sure and Theis[167]), and excretion of vitamin C is diminished in patients with hyperthyroidism (Lewis[168]).

Several investigators have reported an antagonism between vitamin A and thyroxin. The literature on

161. Sharpless, G. R.; Pearsons, Janice, and Prato, Geneva S.: Production of Goiter in Rats with Raw and with Treated Soy Bean Flour, J. Nutrition **17**: 545 (June) 1939.

162. Rabinovitch, J.: Effect of Underfeeding on Proliferative Activity of Thyroid Gland in Guinea Pig, Am. J. Path. **5**: 87 (Jan.) 1929.

163. Stephens, D. J.: The Effect of the Thyrotropic Principle of the Anterior Pituitary on the Thyroid of the Undernourished Guinea Pig, Endocrinology **26**: 485 (March) 1940.

164. Himwich, H. E.; Goldfarb, Walter, and Cowgill, G. R.: Studies in the Physiology of Vitamins: XVII. The Effect of Thyroid Administration upon the Anorexia Characteristic of Lack of Undifferentiated Vitamin B, Am. J. Physiol. **99**: 689 (Feb.) 1932.

165. Lerman, Jacob, and Hertz, Saul, cited by Means.[1]

166. Drill, V. A.: The Effect of Experimental Hyperthyroidism on the Vitamin B_1 Content of Some Tissues, Am. J. Physiol. **122**: 486 (May) 1938. Drill, A. V., and Hays, H. W.: Hyperthyroidism and Liver Function in Relation to B Vitamins, Proc. Soc. Exper. Biol. & Med. **43**: 450, 1940.

167. Sure, Barnett, and Theis, R. M.: Influence of Hyperthyroidism on Vitamin C Content of Various Endocrines and Tissues, Endocrinology **24**: 672 (May) 1939.

168. Lewis, R. A.: The Effect of Hyperthyroidism upon the Metabolism of Vitamin C, Bull. Johns Hopkins Hosp. **63**: 31 (July) 1938.

this subject was reviewed by Wohl and Feldman,[169] who reported pathologic dark adaptation in hyperthyroidism; they attributed this to rapid destruction of vitamin A. They also reported pathologic dark adaptation in myxedema, which they attributed to failure of conversion of carotene to vitamin A in the absence of thyroid hormone. Similarly, carotene diminishes the metabolic effect of thyroid (Smith and Perman[170]). On the other hand, Sure and associates[171] reported marked loss of weight of the thyroid and of other endocrine glands in vitamin A deficiency and in riboflavin deficiency, and Cutting and Robson[172] found that none of the vitamins (A, B_1, B_2, C and D) have any effect on the metabolic rate of guinea pigs with experimental hyperthyroidism induced by a thyrotropic substance.

169. Wohl, M. G., and Feldman, J. B.: Vitamin A Deficiency in Disease of the Thyroid Gland: Its Detection by Dark Adaptation, Endocrinology **24**: 389 (March) 1939.

170. Smith, D. C., and Perman, J. M.: Effect of Thyroxin Combined with Carotene upon the O_2 Consumption of Cats, Endocrinology **27**: 110 (July) 1940.

171. Sure, Barnett; Theis, R. M., and Harrelson, R. T.: Vitamin Interrelationships: I. Influence of Avitaminosis on Ascorbic Acid Content of Various Tissues and Endocrines, J. Biol. Chem. **129**: 245 (July) 1939.

172. Cutting, W. C., and Robson, G. B.: Effects of Various Agents on the Metabolic Rate in Experimental Hyperthyroidism, J. Pharmacol. & Exper. Therap. **66**: 389 (Aug.) 1939.

CHAPTER XXV

THYROID DYSFUNCTIONS AND THEIR TREATMENT

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CHICAGO

CLASSIFICATION OF DISEASES [1]

A. HYPOFUNCTION

1. Primary
   - (*a*) Cretinism
     - (1) Sporadic
     - (2) Endemic
   - (*b*) Myxedema
     - (1) Spontaneous
     - (2) Postoperative
     - (3) Postinfectious
     - (4) During administration of iodine
2. Secondary
   - (*a*) Hypopituitarism
   - (b) Addison's disease

B. HYPERFUNCTION

1. Exophthalmic goiter (Symmetric toxic goiter)
2. Toxic adenoma (Nodular toxic goiter)
3. Mixed type

C. USUALLY NO DISORDER OF FUNCTION

1. Simple goiter (Symmetric nontoxic goiter, colloid goiter)
2. Nontoxic adenoma (Nodular nontoxic goiter)
3. Anomalies of development
   - (*a*) Substernal goiter
   - (*b*) Pyramidal lobe
   - (*c*) Lingual goiter
   - (*d*) Thyroglossal cyst
   - (*e*) Lateral aberrant thyroid tissue
   - (*f*) Thyroid tissue in teratoma

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1. Thompson, W. O.: Endocrinology, in Allen, E. van N.: Specialties in Medical Practice, New York, Thomas Nelson & Sons, 1940, vol. 2. chap. 9.

4. Thyroiditis
   (*a*) Suppurative: acute and chronic
   (*b*) Nonsuppurative: acute and chronic
   (*c*) Struma lymphomatosa
   (*d*) Riedel's struma
   (*e*) Chagas' disease (Trypanosoma cruzi)
5. New growths
   (*a*) Primary
      (1) Papillary adenocarcinoma—30 per cent
      (2) Carcinoma in adenoma (malignant adenoma)—38 per cent
      (3) Diffuse adenocarcinoma—30.4 per cent
      (4) Squamous epithelioma—0.8 per cent
      (5) Sarcoma—0.8 per cent
   (*b*) Secondary
      Metastases from other organs (rare)

ANATOMY AND PHYSIOLOGY

The chief object of this chapter is to discuss disorders of thyroid function. In order to do this intelligently, it is necessary to bear in mind a few pertinent facts about the anatomy and physiology of the gland. The thyroid normally lies in the front of the neck just above the manubrium. In the normal adult it weighs about 25 to 40 Gm. According to the needs of the body, it supplies an internal secretion of which thyroxin is an integral amino acid. It appears to be capable of producing its hormone at the time of birth, and under normal circumstances its maximum weight is reached about the time of puberty. The gland contains numerous follicles, lined by epithelium and surrounded by a rich network of capillaries and lymphatics. The active secretion is stored in the colloid, to be supplied to the body according to demand. The height of the cells surrounding the acini is related to the amount of colloid which the latter contain, the cells being cuboid when the acini are distended with colloid and columnar when the acini contain little or no colloid. The maximum storage capacity of the gland for iodine is about 5 mg. per gram of dried gland, or a total of about 25 to 40 mg. for the normal gland.[2] Whenever the iodine content drops below 1 mg. per gram of dried gland hyperplasia appears.

2. Marine, David, and Lenhart, C. H.: The Pathological Anatomy of the Human Thyroid Gland, Arch. Int. Med. 7: 506 (April) 1911.

The thyroid elaborates thyroxin, or its equivalent,[3] at the rate of about 0.3 mg. (0.2 mg. of iodine) per day,[4] which means that in the allotted three score years and ten the normal gland elaborates about 8 Gm. In the tissues outside the thyroid in a normal man there is the equivalent of about 10 to 14 mg. of racemic thyroxin.[5] These figures suggest that the iodine requirement of the normal thyroid is probably not more than 0.2 mg. per day and explain why so little iodine is required to prevent simple goiter and to produce a maximum reduction in basal metabolism in most patients with exophthalmic goiter (from 6 to 24 mg. daily). The maximum effect of thyroxin is dependent on the integrity of the molecule, and slight alterations greatly reduce, modify or abolish it.[6] In the interpretation of the presence or absence of hypothyroidism it is important to bear in mind that it takes about seventy to ninety days for the basal metabolism to drop from normal to its lowest level (— 40 to — 50 per cent) after omission of thyroid, and a still longer period for the full blown picture of myxedema to develop.

HYPOFUNCTION

Hypothyroidism may be primary or secondary. The primary type is caused by loss of functioning thyroid tissue, produced by operative removal, infection or primary atrophy of unknown cause. The gland is usually largely replaced by scar tissue. Raising the basal metabolism to normal results in a complete cure. The secondary type is caused by lack of adequate stimulation of the thyroid. The gland is commonly smaller than normal but may be normal in size or even greatly enlarged. The gland tends to be in a resting state and to show flat or low cuboidal epithelium with storage of colloid. Raising the basal metabolism to normal with desiccated thyroid may produce some improvement, but there is left a residue of symptoms, e. g., amenorrhea, which is not corrected by correcting

3. Salter, W. T.: The Endocrine Function of Iodine, Cambridge, Mass., Harvard University Press, 1940. Gutman, A. B.; Benedict, Ethel M.; Baxter, Blanch, and Palmer, W. W.: The Effect of Administration of Iodine on the Total Iodine, Inorganic Iodine, and Thyroxine Content of the Pathological Thyroid Gland, J. Biol. Chem. **97**: 303 (July) 1932.

4. Thompson, W. O.; Thompson, P. K.; Taylor, S. G., III; Nadler, S. B., and Dickie, L. F. N.: The Pharmacology of the Thyroid in Man, J. A. M. A. **104**: 972 (March 23) 1935.

5. Kendall, E. C.: Thyroxine, New York, Chemical Catalog Company, Inc., 1929. Thompson and others.[4]

6. Harington, C. R.: The Thyroid Gland: Its Chemistry and Physiology, New York, Oxford University Press, 1933. Thompson and others.[4]

the hypothyroidism. The primary type is seen in patients with cretinism or spontaneous myxedema and occasionally following subtotal thyroidectomy. The secondary type may occur in patients with hypofunction of the anterior lobe of the pituitary and in some patients with Addison's disease.

CRETINISM

Cretinism may be sporadic or endemic. Endemic cretinism is an end result of untreated endemic goiter lasting over a period of several generations. "Sporadic cretinism" is the term used to denote the cretinoid state that occasionally appears in the offspring of apparently healthy parents. In this country cretinism has been sporadic, but in Switzerland and some other mountainous areas it has been endemic. In this country endemic goiter has not lasted long enough in the goitrous areas to result in endemic cretinism. However, cretinism was endemic in many domestic animals in these areas before the diets of such animals were supplemented with iodine. Cretinism is simply myxedema of infancy. Added to the characteristic signs and symptoms of the disease are defects in the development of the skeleton and brain. There may be great disproportion between the two. In an older cretin the cerebration may be good and the development of the skeleton poor, or the reverse may be true. The skeleton can recover from a longer absence of thyroid secretion than the brain. The brain doubles in size during the first year of life by virtue of an increase in white matter, and an absence of thyroid function for only a few months results in permanent damage, the extent varying with the duration of the deficiency. On the other hand, a cretin may remain untreated until the age of 4 years and yet show growth of the skeleton to almost normal proportions. Differences in the susceptibility of the brain and skeleton to absence of thyroid function in the early years of life account for the marked discrepancies between mental and skeletal development observed in cretins.

It is obvious that early diagnosis is important, and yet it is difficult. At the time of birth the concentration of thyroxin in the child is normal if the concentration in the mother is normal. As previously shown, it then takes three months for the effects of absence

of thyroid function to become evident. Determinations of metabolism in infants and young children are not satisfactory and are carried out in only a few clinics in this country. In most instances, before the diagnosis is made, irreparable damage has been done to the brain. Cretinism should be suspected at the first appearance of lethargy, constipation and failure to grow in length or to nurse properly. If treatment is instituted as soon as the signs and symptoms appear, almost completely normal development results. After the disease is well advanced, with appearance of the typical cretinoid facies, dry scaly skin and pot belly, the diagnosis is easy, but it is usually too late to make the child normal. Even then the diagnosis is often missed.

There is a great tendency to feed too large doses of thyroid, which produce nervousness, excessive perspiration and tachycardia and often lead to discontinuance of treatment for long periods. As a result, it is rare to see a cretin who has been treated continuously from the time the diagnosis was made. The dose of thyroid usually required varies from $\frac{1}{10}$ grain (0.006 Gm.) of the U. S. P. material at 6 months to about 1 grain (0.064 Gm.) daily at the time of puberty. If the condition is not recognized for several years, the development of the brain may be so primitive that cerebral inhibition is lacking, and treatment may stimulate the patients so much that they may become very unruly. For this reason some cretins in institutions for the insane are untreated.

Prevention.—There is some evidence that animals with hypothyroidism are more liable to give birth to cretinous offspring than animals with normal thyroid function. It would therefore seem desirable to prevent conception in myxedematous women for several months after their thyroid function has been restored to normal and to watch their offspring carefully for the development of hypothyroidism. All women in goitrous areas should receive iodine throughout pregnancy.

MYXEDEMA

Myxedema denotes marked hypothyroidism in childhood and in adult life. It is more common in women than in men (4:1) and occurs most frequently during the fourth, fifth and sixth decades of life. The characteristic myxedematous appearance usually does not

manifest itself until the metabolism drops to between —20 and —25 per cent. There are, however, a few striking exceptions to this rule. The cause is unknown in most instances, and the myxedema is spoken of as spontaneous. It may follow thyroidectomy, the spontaneous disappearance of exophthalmic goiter or an infection of the thyroid; in rare instances it may be produced by the preoperative or postoperative administration of iodine in patients with exophthalmic goiter. It is characterized by a slowing up of all reactions in the body, so that patients who have it become virtually hibernating. Outstanding among the manifestations are:

1. Puffiness of the face and eyelids, producing the typical myxedematous facies.
2. Swelling of the tongue and larynx, producing the typical hoarse, slow, slurred speech.
3. Dryness and roughness of the skin.
4. Falling out of hair all over the body, producing areas of alopecia (sometimes complete baldness), commonly almost complete absence of axillary and pubic hair, and sparseness and moth-eaten appearance of the eyebrows.
5. Poor memory, slowing of all mental reactions and dulness of the sensorium.
6. Constipation.
7. Reduction in basal metabolism.

In complete absence of thyroid function the basal metabolism drops to between —40 and —50 per cent, although all degrees of hypothyroidism are seen. All reactions that go on with a normal thyroid function also appear to go on in its absence, but at a slower rate. It takes between two and three months after the omission of treatment for the basal metabolism to drop to its lowest point, several more weeks or months for the characteristic myxedematous appearance to develop, and much longer for the changes in the skin and hair to reach their maximum. In some instances careful histories suggest that the disease may be present in early life in a form so mild it is not recognized and that under the stress and strain of living it gradually becomes more marked.

Too much emphasis has been placed on the supraclavicular pads of fat in the past. They are of no value in diagnosis. The thyroid hormone does not exercise

a specific influence on fat metabolism, and hypothyroidism is not to be regarded as a cause of obesity. The increased weight is caused largely by deposition of water, and patients rarely lose more than 20 pounds (9 Kg.) during treatment. The weight lost consists mostly of water containing nitrogen and electrolytes. Boothby and co-workers [7] showed that the edema of myxedema is an albuminous deposit with about the composition of egg white.

Changes in the Blood.—In patients with myxedema any or all of the following changes may be present in the blood:

1. Secondary anemia, which is sometimes accompanied by eosinophilia and basophilia.
2. A decrease in the total quantity of circulating plasma.
3. An increase in the concentration of protein in the plasma.
4. A corresponding decrease in the concentration of water in the plasma.
5. A decrease in the concentration of chloride in the plasma and a corresponding increase in the concentration of bicarbonate, the total base usually remaining constant.
6. An increase in the concentration of cholesterol in the plasma.[8]

Changes in the Circulation.—The following changes are noted in the circulation:

1. A marked decrease in the minute volume of the heart and a prolongation of the circulation time. The opposite changes are noted in hyperthyroidism. These changes are associated with alterations in the mechanical work required of the heart and may represent adaptations of the circulation to variations in the demand for oxygen.

2. Myxedematous changes in the heart muscle, interfering with its efficiency. The transverse diameter of the heart is often increased, the sounds become weak, the pulse pressure drops, all of the complexes in the

7. Boothby, W. M.; Sandiford, I.; Sandiford, K., and Slosse, J.: The Effect of Thyroxin on the Respiratory and Nitrogenous Metabolism of Normal and Myxedematous Subjects: I. A Method of Studying the Reserve or Deposit Protein with a Preliminary Report of the Results Obtained. Tr. A. Am. Physicians **40**: 195, 1925.

8. Hurxthal, L. M.: Blood Cholesterol in Thyroid Disease: II. Effect of Treatment, Arch. Int. Med. **52**: 86 (July) 1933.

electrocardiogram are reduced in amplitude, and the T waves in leads 1, 2 or 3 may be inverted. The change in the heart muscle may so interfere with cardiac efficiency that decompensation develops, with characteristic pitting edema of dependent portions of the body (so-called myxedema heart). The decompensation and edema in this condition do not clear up with digitalis but promptly disappear with thyroid.

Difference Between Myxedema and Cardiac Edema.—In myxedema there is general increase in intracellular water. When pitting develops it usually is the result of superimposed cardiac edema. In cardiac edema there is marked increase in intercellular water, chiefly in dependent portions of the body. It is not associated with the increased deposition of protein seen in patients with myxedema.

Changes in the Cerebrospinal Fluid.—In myxedema there is usually a well marked increase in the concentration of protein in the cerebrospinal fluid, while the opposite change is observed with toxic goiter.[9] Values as high as 300 mg. per hundred cubic centimeters have been observed in cases of myxedema, compared with the normal of 25 to 40 mg. per hundred cubic centimeters. No other significant change has been noted in the fluid. The high concentration of protein in the cerebrospinal fluid, like that of the cholesterol in the blood, is sometimes of diagnostic value, especially in regard to cretins, although the values for both vary too much for purposes of routine diagnosis. The cerebrospinal fluid findings in myxedema resemble those in some cases of brain tumor and are similar to those in diabetes mellitus.

Changes in Calcium Metabolism.—In hypothyroidism the urinary excretion of calcium is diminished, and the bones sometimes show an increase in density, whereas in hyperthyroidism the opposite changes are noted.[10] In rare instances the decalcification of the skeleton in toxic goiter is so marked that spontaneous fractures occur. These alterations in the metabolism of calcium in thyroid disease occur without corresponding changes

9. Thompson, W. O.; Thompson, P. K.; Silveus, E., and Dailey, M. E.: The Protein Content of the Cerebrospinal Fluid in Myxedema, J. Clin. Investigation **6**: 251 (Oct.) 1928.

10. Aub, J. C.; Bauer, Walter; Heath, Clark, and Ropes, Marion: Studies of Calcium and Phosphorus Metabolism: III. The Effects of the Thyroid Hormone and Thyroid Disease, J. Clin. Investigation **7**: 97 (April) 1929.

in the concentration of calcium in the serum, in contrast to the changes in hypoparathyroidism and hyperparathyroidism.

Sexual Changes.—There is commonly decrease in libido, decrease in axillary and pubic hair and often sterility. The menstrual history is variable. The flow may be excessive, simulating incomplete abortion; there may be scantiness of menstruation with prolongation of the intermenstrual period, or there may be complete absence of menstruation. In hyperthyroidism there is often scantiness of menstruation with or without prolongation of the intermenstrual periods.

Pathologic Anatomy.—The gland is usually largely replaced by scar tissue. In some instances, however, a goiter is actually present, suggesting either inadequate stimulation of the thyroid or enlargement of a diseased gland in an attempt to supply an adequate amount of secretion. The gland in such a patient may enlarge during the period of myxedema and decrease in size when thyroid is administered. There is some separation of the fibers of skeletal and cardiac muscles and of the cells of the skin by the myxedematous condition.

Differential Diagnosis.—Myxedema may be confused with chronic nephritis, Simmonds's disease and pernicious anemia. In both myxedema and chronic nephritis there may be secondary anemia, hypertension and some albuminuria. However, the typical myxedematous appearance is absent in patients with chronic nephritis. In nephrosis the basal metabolism may be as low as in myxedema, but the marked general pitting edema, the reduction in the concentration of protein in the serum, the reversal of the albumin-globulin ratio, the marked albuminuria and the absence of the myxedematous facies make it comparatively easy to distinguish the two diseases. In Simmonds's disease there may be marked lowering of the basal metabolism and amenorrhea, but the cachexia and the absence of the myxedematous appearance and of roughness and dryness of the skin usually make it possible to exclude myxedema. In pernicious anemia the lemon yellow tint of the skin, the characteristic blood findings and the absence of the myxedematous facies readily make it possible to exclude myxedema. In rare instances the two diseases coexist, and then appropriate treatment must be given for each.

As the use of basal metabolism apparatus becomes more widespread, there is a tendency for hypothyroidism to be detected in its early stages more commonly than before. However, the diagnosis of myxedema is still often not made until the disease is well advanced and then only when the patient visits a special clinic. Alterations in the basal metabolism must be correlated with the clinical picture. There are patients with basal metabolism of — 20 to — 25 per cent who do not appear myxedematous and who are not improved by the administration of thyroid. The cause of the low basal metabolism in them is obscure.

In Addison's disease there is often moderate depression of the basal metabolism, and in rare instances myxedema is present. Patients are often improved by the administration of thyroid, but it is not known at present whether the adrenal cortex affects the thyroid directly or indirectly.

TREATMENT OF PRIMARY HYPOTHYROIDISM

The following directions and considerations are important in the management of the primary type of hypothyroidism:

1. Administer the minimum amount of desiccated thyroid necessary to maintain a normal level of metabolism. The average dose required for maintenance in thyroidless adults is from 1½ to 2 grains (0.096 to 0.128 Gm.) of thyroid U. S. P. (0.18 to 0.23 per cent iodine) daily. In cretins, as previously stated, the dose varies from 1/10 grain at the age of 6 months to 1 grain daily at the age of puberty.

2. Begin with an inadequate dose (1 grain daily in most adults under 40 years of age) and increase the amount gradually after several weeks until the correct amount is being administered.

3. Avoid a large initial dose in all cases, particularly if the patient shows arteriosclerosis and coronary disease, because of the danger of precipitating coronary thrombosis. For such a patient the initial dose should not exceed ½ grain (0.032 Gm.) daily. If angina develops during treatment, the metabolism should be adjusted at a somewhat subnormal level. Besides coronary accidents, the administration of large initial doses of desiccated thyroid or of thyroxin, as advocated in some clinics, may produce symptoms of thyroid intoxi-

cation, including aching and marked tenderness of muscles, sometimes a fever (as high as 104 F.), occasionally nausea and rarely vomiting. By raising the metabolism slowly, these unpleasant symptoms are avoided and the danger of accidents is greatly minimized.

4. Make changes in the dose slowly, because of the slow adjustment. At least two months is required for complete adjustment to any dose.

5. In recording the dose of thyroid always state the iodine content.

6. Do not expect thyroxin to revive untreated patients after they have become almost moribund; the action of the drug is too slow. Such an emergency should be prevented by early treatment.

7. Do not administer thyroxin intravenously except in the rare instances in which there is a question whether or not the patient is taking the dose of thyroid prescribed. Thyroxin works well when given in alkaline solution by mouth but possesses no advantage over desiccated thyroid.

When secondary anemia is present, it is usually not corrected by the administration of thyroid alone but requires in addition the administration of large doses of iron.

Extravagant claims are made for various preparations of thyroid from which the toxic material is supposed to have been removed. In my experience these claims have appeared to be without foundation: such preparations have possessed no advantage over desiccated thyroid. As a matter of fact, all of the symptoms of intoxication associated with the administration of large doses of desiccated thyroid have been associated also with the administration of the pure substance, thyroxin.

TREATMENT OF SECONDARY HYPOTHYROIDISM

The treatment of secondary hypothyroidism is also best carried out at present by the administration of thyroid, although this represents only part of the treatment. In the patient with hypopituitarism the thyroid may be stimulated by administering the thyrotropic factor of the pituitary, but, apparently because of development of immunity, the effect lasts only a few weeks even though the treatment is continued. Some improvement in the stimulation type of treatment may be expected with improvement in anterior pituitary extracts.

LOW BASAL METABOLISM WITHOUT MYXEDEMA

Reference has already been made to a group of patients with low basal metabolism in whom the administration of thyroid does not produce improvement. In the interpretation of the low rates in such patients it may be pointed out that the total metabolism may be altered in a variety of ways which do not involve any change in thyroid function. The increases effected by epinephrine, muscular exercise, dinitrophenol, dinitroorthocresol and diiodothyronine occur too quickly to be the result of any change in thyroid function. In a patient with myxedema the metabolism may be raised to normal with dinitrophenol or dinitroorthocresol without any change in the myxedematous condition.[4] The possibility must therefore be entertained that some unknown factor or factors outside the thyroid may directly affect oxidation. In some persons normal thyroid function may be present in spite of basal metabolism 20 to 25 per cent below normal. In such persons a normal level of metabolism would represent a thyrotoxic one. These considerations may explain occasional instances in which clinical relief from thyrotoxicosis appears to have been associated with a reduction of the basal metabolism from a standard normal to a low level. However, the greatest caution must be exercised in the interpretation of all such apparent instances of improvement if needless thyroidectomy is to be avoided.

Unless the basal metabolism is depressed more than 25 per cent below normal, myxedema is usually not present and the symptoms are indefinite, making it difficult to tell by the clinical picture alone whether or not hypothyroidism is present. A therapeutic test with thyroid must therefore be made. Hypothyroidism of either the primary or the secondary type may be considered to exist in a patient with low basal metabolism when clinical improvement is produced by raising the basal metabolism to normal. When no improvement occurs, it may be concluded that hypothyroidism is not present and that the administration of thyroid is futile.

TOXIC GOITER

There is some question as to whether or not there are two types of toxic goiter. Plummer[11] attempted

11. Plummer, H. S.: The Clinical and Pathologic Relationships of Hyperplastic and Non-Hyperplastic Goiter, J. A. M. A. **61**: 650 (Aug. 30) 1913.

to differentiate two types (exophthalmic goiter and toxic adenoma) on the basis of function. According to his hypothesis, there is with toxic adenoma simple hyperthyroidism, whereas with exophthalmic goiter there is simple hyperthyroidism plus production of an abnormal product. This abnormal product, which he thought might be a deficiently iodinized thyroxin, accounted for the peculiar emotional instability with exophthalmic goiter, which distinguished it from toxic adenoma. Up to the present time, observations on the calorigenic and clinical effects of compounds closely related to thyroxin (notably thyronine and diiodothyronine) have not supported Plummer's hypothesis. There do, however, appear to be certain differences between the two diseases. In the patient with exophthalmic goiter the goiter and the thyrotoxic symptoms usually appear abruptly at about the same time, whereas in the patient with toxic adenoma the thyrotoxic symptoms commonly appear insidiously after goiter has been present for several years (fourteen and a half years on the average).[12] In general, emotional instability is more marked in patients with symmetrically enlarged goiters than in patients with nodular goiters.

The terms used to denote the types of toxic goiter are misnomers. This is particularly true of the syndrome described as exophthalmic goiter, which may be present in a patient without a goiter and without exophthalmos. However, even though the thyroid is not enlarged, it is usually firmer than normal. To produce descriptive terms that are more accurate, the American Association for the Study of Goiter has proposed that "symmetric toxic goiter" and "nodular toxic goiter" be substituted for "exophthalmic goiter" and "toxic adenoma," respectively. Similarly, they have suggested that simple goiter and nontoxic adenoma be called "symmetric nontoxic goiter" and "nodular nontoxic goiter," respectively.

Cause.—The cause of toxic goiter is not known. Among the most plausible possibilities are overproduction of the thyrotropic factor by the anterior lobe of the pituitary, some disorder of the sympathetic nervous system, a disturbance of some center controlling thyroid

12. Haines, S. F.: Adenomatous Goiter with Hyperthyroidism, Tr. Third Internat. Goiter Conf. & Am. A. Study Goiter, 1938, p. 198.

function in the base of the brain and some abnormality inherent in the thyroid itself. Great attention has been focused in recent years on the interrelations of the thyroid and the pituitary.[13] It is definitely established that integrity of thyroid function is dependent on integrity of pituitary function and that hypothyroidism and hyperthyroidism may be secondary to corresponding states in the anterior lobe of the pituitary, e. g., chromophobe adenoma and acromegaly, respectively. In acromegaly the basal metabolism may show a reduction during the administration of iodine just as in exophthalmic goiter. In patients with normal or slightly depressed basal metabolism all of the symptoms of toxic goiter except exophthalmos may be produced by administration of an anterior pituitary extract containing the thyrotropic factor.[14] The severity of toxic goiter may be increased in the same manner. The thyrotropic factor is inert in the absence of thyroid tissue capable of function. However, in spite of all these convincing observations, there is so far no positive proof that the pituitary is concerned in the clinical disorder of toxic goiter.

Incidence with Regard to Age and Sex.—Exophthalmic goiter occurs most frequently in persons between the ages of 15 and 50.[15] Both types of toxic goiter are more common in women than in men, in the proportion of 4 or 5 to 1. The sex ratio varies from place to place and from time to time and to some extent with age, the difference in incidence in the two sexes being less marked in the very young and the very old. The cause for the greater frequency of the disease in women is unknown.

Pathologic Anatomy.—In exophthalmic goiter the gland is characterized by papillary projections into the acini, decrease in the amount of colloid and in the iodine content and often by increase in the lymphoid tissue between the follicles. These changes are associated with

13. Collip, J. B.: Inhibitory Hormones and Principle of Inverse Response, Ann. Int. Med. 8: 10 (July) 1934. Uhlenhuth, E.: Thyreoactivator Hormone: Its Isolation from Anterior Lobe of Bovine Pituitary Gland and Its Effects on Thyroid Gland, Tr. Am. A. Study Goiter, 1936, p. 25.

14. Thompson, W. O.; Thompson, P. K.; Taylor, S. G., III, and Dickie, L. F. N.: The Influence of the Pituitary in Thyroid Disease, West. J. Surg. 47: 4 (Jan.) 1939.

15. Means, J. H.: The Thyroid and Its Diseases, Philadelphia, J. B. Lippincott Company, 1937.

increase in the concentration of iodine in the blood and increase in the excretion of iodine in the urine.[16] In some instances thick bands of fibrous tissue are observed throughout the gland, and the capsule may be adherent to surrounding tissues. Often there is proliferation of lymphoid tissue throughout the body, together with persistence of the thymus. According to Warthin, certain persons are born with this so-called thymicolymphatic, or Graves' constitution and are predisposed to the development of toxic goiter. Relative lymphocytosis in the blood has been described and much was made of it by Kocher. When iodine is administered, the papillary projections decrease or disappear, the colloid and iodine content of the gland increase, and the organic iodine content of the blood decreases. These changes are usually associated with decrease in the basal metabolism. The mechanism of the action of iodine is unknown. In a few instances the response to iodine may be reversible, the administration of inadequate doses being associated with increase in basal metabolism and the administration of adequate doses immediately afterward with decrease.[17] The minimum dose of iodine that would produce any effect in most patients in Boston was about 0.75 mg. per day, and the minimum dose that would produce a maximum effect was about 6.0 mg. per day. For some patients a larger dose is required to produce a maximum effect. In Chicago as much as 24.0 mg. per day has been necessary in some patients.

It has been pointed out by Marine [2] that the histologic structure of the thyroid and its response to iodine are the same in simple goiter and in exophthalmic goiter. In the individual nodules of glands with toxic adenoma the same changes may be observed as are seen in glands with exophthalmic goiter, but they are not so characteristic. In toxic adenoma the goiter is commonly of long standing, and various degenerative changes are seen, including thick bands of scar tissue, colloid degeneration, hemorrhage and formation of cartilage and bone.

16. Curtis, G. M., and Puppel, I. D.: The Iodine Metabolism in Thyroid Disease, Tr. Third Internat. Goiter Conf. & Am. A. Study Goiter, 1938, p. 367.

17. Thompson, W. O.; Thompson, P. K., and Cohen, A. C.: The Range of Effective Iodine Dosage in Exophthalmic Goiter: IV. The Effect on Basal Metabolism of the Daily Administration of About 0.75 Mg. of Iodine, Arch. Int. Med. **49**: 199 (Feb.) 1932.

Signs and Symptoms.—The more common signs and symptoms of toxic goiter are:

1. Goiter (absent in rare instances, although the gland is always firmer than the normal)
2. Nervousness
3. Tremor
4. Tachycardia
5. Palpitation
6. Dyspnea
7. Loss of weight
8. Increase in basal metabolism
9. Exophthalmos (in about from 60 to 70 per cent of patients with exophthalmic goiter)
10. Various other ocular signs, the most important being puffiness of the eyelids, lid lag (von Gräfe) and poor convergence (Möbius)
11. Systolic thrill and bruit, usually most marked over the superior poles (not present in adenomatous goiter)
12. Emotional instability
13. Muscle weakness
14. Early fatigue
15. Increased perspiration
16. Diarrhea (in about one third of the cases, usually the more severe ones)
17. Pressure symptoms (one quarter of the cases)
18. Nausea and vomiting (rare and unfavorable signs)

Crisis.—Sometimes in either the treated or the untreated disease a condition known as a crisis develops. A crisis is characterized by loss of appetite, nausea, vomiting, gradually increasing heart rate, marked weakness, loss of weight and sometimes by diarrhea and fever. If no treatment has previously been given, a crisis can often be controlled by administration of iodine, but this condition may appear during treatment or fail to respond to all known therapeutic measures and result in the death of the patient before operative procedures can be carried out. A crisis may develop during the postoperative period and under these circumstances usually indicates inadequate preoperative preparation. In a crisis the cause of the disease is presumably acting with great intensity.

Differential Diagnosis.—Toxic goiter may have to be distinguished from nontoxic goiter, neurocirculatory asthenia, emotional disturbances from other causes, acromegaly and pulmonary tuberculosis. In nontoxic goiter, neurocirculatory asthenia and emotional disturbances from causes other than thyrotoxicosis, the basal metabolism is normal. In the patient with simple goiter the thyroid gland is softer than that of the patient with toxic goiter, and a bruit is usually not heard. In acromegaly there may be a goiter, high basal metabolism and exophthalmos, but the characteristic skeletal changes readily make it possible to distinguish the two diseases. The roentgenologic findings, fever and poor appetite readily distinguish pulmonary tuberculosis, which does not very often present a problem in diagnosis. In the leukemias and polycythemia vera the metabolism may be elevated, but these diseases rarely present problems in diagnosis.

Whenever there is any doubt about the diagnosis, it is important to determine (1) the level of the basal metabolism and (2) the effect of administration of iodine on this level.

Treatment.—There are three ways of treating toxic goiter: (1) by subtotal resection of the gland after suitable preparation of the patient, (2) by roentgen irradiation of the gland and (3) medically (chiefly by iodine and rest).

It is generally agreed that subtotal resection of the gland after suitable preparation of the patient represents the best method of treatment.[18] Treatment, therefore, involves an intelligent combination of surgical and medical measures. Roentgen ray therapy is valuable in a few selected cases in which operation is contraindicated, but its results are too delayed and too uncertain to make it practical in most instances. In a few cases in which the symptoms are mild the disease may be held in check by iodine alone until it disappears, but this form of treatment must be carried out with the greatest care because the disease is characterized by remissions and relapses and a mild form of the disease may at any time become a severe one.

The outcome of operation is determined by the condition of the patient at the time of operation and by the skill of the surgeon. Both factors are important,

18. Lahey, F. H.: Surgery in Hyperthyroidism, Tr. Third Internat. Goiter Conf. & Am. A. Study Goiter, 1938, p. 297.

but our data indicate that the condition of the patient is the more important.[19] The operation is an elective one and should be carried out only when the condition of the patient justifies this. As already pointed out, postoperative crises usually mean inadequate preoperative preparation. Thyroidectomy is never an emergency operation and when done as such commonly results in the death of the patient. With some patients it may be necessary to take several months for adequate preparation. The preoperative management should be carried out in the medical service of a hospital by a specially trained internist. The internist should continue his observation of the patient in the immediate postoperative period as well as later, because many of the problems that arise are medical in nature.

The following points are important in preparing patients for operation:

1. The diet administered should be sufficiently high in calories to produce a gain in weight (usually 4,000 to 5,000 calories daily).

2. The condition of the patient must be improved by the administration of iodine. Within wide limits the size of the dose and the form in which it is administered are not important. For routine purposes it is desirable to administer 5 minims (0.3 cc.) of compound solution of iodine or 1 grain (0.06 Gm.) of sodium or potassium iodide three times a day. It used to be thought that operative procedures should be carried out as soon as the basal metabolism showed its maximum reduction during the administration of iodine, because of the danger of a serious exacerbation of the disease with continued administration. The danger of this occurrence has been greatly exaggerated, and it is well established that maximum clinical improvement from iodine does not necessarily coincide with maximum reduction in basal metabolism. My associates and I rarely recommend operative procedures until about one week has elapsed after the time of maximum reduction in basal metabolism and do not hesitate to delay operation as long as is necessary to improve the condition of the patient.

3. Rest is very important, but patients must not be confined to bed. Some activity should be allowed and

19. Thompson, W. O.; Taylor S. G., III; Meyer, K. A., and McNealy, R. W.: Experiences in Treating Toxic Goiter in a Large Public Hospital, Ann. Int. Med. 12: 217 (Aug.) 1938.

encouraged in order to preserve muscle tone, except in the presence of a crisis or of cardiac decompensation. Patients should be prepared in the hospital, away from the stress and strain of the home environment.

4. Roentgen ray treatment should be considered for patients with high metabolic rates refractory to iodine.

5. Patients with cardiac decompensation should be conditioned for operation by the administration of digitalis.

It is usually unwise to operate when:

1. The patient fails to gain or is losing weight.

2. Emotional instability and muscle weakness are pronounced.

3. The basal metabolism is plus 60 per cent or higher in spite of the administration of iodine.

4. The disease is increasing rapidly in severity.

5. Less than two weeks has elapsed since an infection of the upper respiratory tract cleared up.

6. Cardiac decompensation is present.

Each patient presents an individual problem, and there are, of course, exceptions to these rules. For example, often, if a patient is gaining weight steadily, we do not hesitate to operate even though the basal metabolism remains 60 or 70 per cent above normal in spite of the administration of iodine. Gain in weight is the single most important sign in gaging the risk of operation. With it there are usually a reduction in emotional instability and an increase in strength. Whenever there is any doubt, it is best to err on the side of conservatism. Operation can always be delayed, but after a postoperative crisis has set in, often nothing will stop it. Unless cardiac decompensation is present, the criteria of operability are the same in the presence of organic heart disease as in its absence.

When the patient cannot be prepared properly by the procedures outlined, sufficient improvement to justify the carrying out of operative procedures may be produced by roentgen ray treatment of the thyroid (eight to twelve treatments given at weekly intervals).

Immediate Preoperative Preparation.—It is important:

1. To make a careful search for an infection of the upper respiratory tract just before the patient goes to the operating room.

2. To administer a carbohydrate meal from six to eight hours before operation, to prevent the development of acidosis in the postoperative period.

3. To administer the regular dose of iodine with this meal.

4. To institute, twenty-four hours before the scheduled time of operation, some program suitable for the control of emergencies when the disease is complicated by diabetes.

Immediate Postoperative Treatment.—It is important:

1. To observe the wound carefully for early detection of excessive bleeding.

2. To observe the patient carefully to detect respiratory difficulty as soon as it arises, either from laryngeal or from tracheal obstruction. When only one vocal cord is paralyzed, serious respiratory difficulty usually does not develop.

3. To secure the services, for the first forty-eight hours, of a specially trained nurse who will report trouble as soon as it arises.

4. To have available facilities for emergency passage of a life-saving tube and performance of a tracheotomy. A tracheotomy is rarely necessary, but when it is necessary it usually has to be done in a hurry.

5. To administer intravenously a suitable combination of salt and dextrose for prolonged or excessive vomiting, a thyroid crisis or a circulatory collapse (1 liter of 5 per cent dextrose in physiologic solution of sodium chloride every six hours).

6. To administer iodine until the patient's discharge from the hospital, to control any residual thyrotoxicosis.

7. To search for parathyroid tetany on the second to the fourth postoperative day and, if observed, to control it with suitable measures.

In most instances it is desirable to allow patients out of bed within twenty-four to forty-eight hours after thyroidectomy, to prevent their becoming bedridden and to shorten their postoperative course.

Deaths.—By the application of the principles outlined, my associates and I [19] were able to reduce the operative mortality at the Cook County Hospital during the period from 1932 to 1937 from 10.8 to 1.6 per cent. About

40 per cent of the deaths in our series were caused by sudden respiratory difficulty, about 30 per cent by pneumonia and about 20 per cent by crises. However, after 1934 there was not a single death from a crisis, a fact which we attribute to more adequate preoperative preparation of the patients. The deaths from sudden respiratory difficulty were usually associated with bilateral paralysis of the vocal cords with or without pressure on the recurrent laryngeal nerves from hard blood clots under the strap muscles, and in one instance death was caused by pressure from a hematoma on a collapsible trachea. Preoperative infections of the upper respiratory tract appear to play an important role in the development of postoperative pneumonia. The mortality from postoperative pneumonia can now be greatly reduced by chemotherapy.

Results of Treatment.—Following subtotal thyroidectomy the basal metabolism in about 70 per cent of cases drops to normal in from ten to fourteen days and remains normal for the rest of the patient's life. In about 20 to 25 per cent of cases it finally drops to a subnormal level, although the development of complete myxedema is rare. In about 5 to 10 per cent of cases the disease persists or recurs. A true recurrence is rare; in most cases postoperative thyrotoxicosis represents persistence of the disease.

Most patients showing low basal metabolism are improved by the administration of thyroid. The treatment of the persistent or recurrent disease is the same as that of the untreated disease, unless some complication, such as paralysis of a vocal cord, makes it undesirable to carry out further operative procedures. Two points are important in determining whether or not the disease has been eliminated by operation:

1. A basal metabolic rate of plus 15 per cent or higher during the administration of iodine from ten to fourteen days after operation usually means that the disease has not been abolished. On the other hand, if the basal metabolism is normal from ten to fourteen days after operation and iodine is being administered, it is uncertain whether the disease is still present or not.

2. If the basal metabolism remains within normal limits for as long as two months without the administration of iodine, a cure can almost be assured.

SIMPLE GOITER

In simple goiter there is symmetric enlargement of the thyroid, usually associated with no disturbance of function, although in some instances there is mild hypothyroidism. Like most thyroid diseases, it is more common in women than in men and is more common during periods of increased stress on the thyroid, namely, at puberty and during pregnancy. As the incidence in the population increases, the ratio of male to female patients increases. The response of simple goiter and exophthalmic goiter is the same histologically to administration and withdrawal of iodine, but the causes of the two diseases are entirely different. Simple goiter is not commonly a precursor of exophthalmic goiter. The cause of exophthalmic goiter is unknown. The most important cause of simple goiter is a deficiency of iodine. Other factors are involved in this type of goiter. Its production in a variety of ways and the greater susceptibility of some persons as demonstrated by its tendency to occur in families are consistent with this point of view. However, most of the causes appear to act by rendering iodine unavailable to the thyroid. Iodine is very effective in the prevention of simple goiter, and there is a close parallelism between the iodine content of the soil and the incidence of simple goiter in various parts of the world.[20] The incidence has been high in the Himalayan Mountain regions of South Central Asia; the Alpine, Pyrenean and Carpathian Mountain regions of Europe; the Andean Plateau and Southeastern Brazil in South America; while in North America its incidence has been high in the St. Lawrence River Basin and Great Lakes district, the Dakotas and adjacent Canadian provinces, and the Pacific Northwest including Oregon, Washington and British Columbia.

Pathologic Anatomy.—The histologic picture of the gland depends largely on the duration of the iodine deficiency. When the gland is subjected to an inadequate supply of iodine, it first uses up the iodine it has stored in its own colloid. Marine and Lenhart [2] have shown that when the iodine content of the gland drops below 1 mg. per gram of dried gland, in simple goiter papillary projections into the acini appear, and the epithelium begins to change from

20. McClendon, J. F.: Iodine and the Incidence of Goiter, New York, Oxford University Press, 1939.

columnar to cuboidal. The extent of these changes depends on the degree of depletion of colloid. A similar quantitative relationship is observed in patients with exophthalmic goiter. According to Marine and Lenhart,[2] the gland increases in size in a steplike fashion. After a period of hyperplasia, the subject may receive a supply of iodine, whereupon the gland stores colloid, the papillary projections disappear, the epithelium reverts to cuboidal form and the gland is in a resting or involutional stage. Then again come a deficiency and an abundance of iodine, and the cycle is repeated. These pathologic considerations readily explain why in the treatment of simple goiter the gland usually does not decrease in size. In fact, at first it may increase in size and then remain stationary. Prevention of further increase in size is all that can be promised from treatment. However, in rare instances a marked regression in size does occur under the influence of iodine. Another type of change may occur. Groups of acini may become much more distended with colloid than surrounding acini, which become compressed and disintegrate and are replaced by scar tissue forming a nodule surrounded by a capsule. Wegelin and Rienhoff have suggested that many nodules appear in this way, and it would seem that the development of adenomatous goiter is commonly an end result of simple goiter.

Treatment.—An effort should be made to cause a reduction in size by first giving a large excess of iodine (5 minims of compound solution of iodine or 1 grain of potassium iodide or sodium iodide daily) for two or three months. If no reduction in size occurs, a much smaller dose should be administered, such as the amount in iodized salt or 1 minim (0.06 cc.) of compound solution of iodine once a week. If there is associated hypothyroidism, a marked decrease in the size of the goiter may occur during the administration of desiccated thyroid.

Instead of giving iodine in small daily doses, Marine and Kimball [21] have shown that it is possible to prevent simple goiter by giving an excess of iodine for two or three weeks twice a year. The explanation for this is simple. The storage capacity of the thyroid for iodine is about 5 mg. per gram of dried gland, or a

21. Marine, David, and Kimball, O. P.: The Prevention of Simple Goiter in Man, J. Lab. & Clin. Med. 3: 40 (Oct.) 1917.

total of about 25 mg. for the average normal thyroid. If all of this iodine were available for the production of thyroxin, it would yield 37.5 mg. of thyroxin, which is enough to maintain normal thyroid function for one hundred and fourteen days. On the same basis, a second period of excess of iodine would result in the formation of a normal supply of the thyroid hormone for a total of two hundred and twenty-eight days. The additional iodine in the food and water would in most instances be adequate for maintaining normal thyroid function throughout the year.

Prophylaxis.—It is more important to prevent simple goiter than to treat it after it has developed. This is best done by the universal use of iodine in goitrous areas, preferably in the form of iodized salt. It has been previously pointed out that the iodine requirement of the normal thyroid probably does not exceed 0.2 mg. daily. The iodized salt used in this country contains about 200 mg. of iodine per kilogram (0.02 per cent), which would mean that a person who takes 5 Gm. of salt daily would get 1 mg. of iodine. This amount may be greater than is necessary. The iodized salt used in Switzerland contains only one fortieth of this amount and yet has proved effective except at puberty and during pregnancy. Marine has advocated the use of salt containing 10 mg. of iodine per kilogram, one twentieth of the amount now being used. More work needs to be done in this country on the prophylactic value of doses of iodine smaller than are now being used.

Importance of Prophylaxis.—Goiter presents a problem on every continent and affects not only man but also domestic animals. The prevention of simple goiter means the elimination of endemic cretinism, which in the past has caused great economic loss in both man and domestic animals. Its prevention also means a great reduction in the incidence of nontoxic adenoma and therefore presumably of toxic adenoma and of carcinoma. It therefore represents a public health problem of the first magnitude in many parts of the world.

NONTOXIC ADENOMA (NODULAR NONTOXIC GOITER)

Adenoma of the thyroid may develop at any point from the base of the tongue to the diaphragm. The growths vary in size from microscopic nodules to

nodules many centimeters in diameter, are commonly multiple and, as a rule, lie in the usual location of the thyroid. Some substernal extension is common. Occasionally the whole adenomatous goiter lies in the mediastinum, and rarely a substernal nodule is encountered with the remainder of the thyroid in the usual location.

The diagnosis rarely presents any problem except when the goiter is confined to the substernal space or when thyroid nodules are confused with parathyroid nodules.

Pathologic Anatomy.—The most common type of adenoma is that arising in simple goiter. In this type of goiter the hyperplastic and colloid phases (colloid adenoma) of simple goiter are encountered. Various types of degenerative changes may be observed in goiters of long standing. Several acini may become greatly distended with colloid; their walls may rupture and coalesce, giving rise to large gelatinous masses. Thick bands of scar tissue, hemorrhage and formation of cartilage and bone are also noted.

Nodules may arise from fetal rests. These are of two types. One contains columns of cells (embryonic adenoma) and the other small, closely packed acini (fetal adenoma). It has been suggested that the more undifferentiated the type of cell in an adenoma the more malignant the carcinoma arising from it.

Treatment.—Routine removal of all nodules of the thyroid has been suggested in some quarters in order to prevent development of carcinoma and prevent toxic adenoma. Among persons past the age of 45, thyrotoxicosis develops in about 1 in 4 and carcinoma in about 1 in 200. If all nodules were routinely removed, the mortality, except in leading centers, would probably be higher than the mortality from carcinoma. The incidence of tetany (less than 1 per cent) and of paralysis of the vocal cords (perhaps 5 per cent) must also be taken into consideration. In the best hands, it is probably justifiable to remove all nodules routinely. In any event, it is desirable to remove all nodular goiters with any substernal extension, all those which have recently increased in size, all those that are very large, those which produce pressure symptoms and those which are accompanied by enlargement of the lymph glands in the neck. Subtotal thyroidectomy should be done even

though there appears to be only a single nodule, because there are commonly smaller nodules (often microscopic) throughout the gland. When the enlargement is slight, it is perhaps best to let the patient himself make the decision, after all the facts have been placed before him. If he is carefully followed, thyrotoxicosis can be detected as soon as it appears, further enlargement can usually be prevented by the administration of iodine, and he may wish to run the risk of carcinoma developing.

ANOMALIES OF DEVELOPMENT

Anomalies of development can readily be understood if the embryologic origin of the thyroid as an evagination of the primitive pharynx is borne in mind.[22] The thyroid tissue may fail to descend in whole or in part or may migrate to too low a level. Thus, it may be found in the nasopharyngeal, lingual, intralingual, sublingual, prelaryngeal, intratracheal, intraesophageal and mediastinal areas, and in very rare instances, as low as the diaphragm (goiter plongeant). The origins of lingual goiters, pyramidal lobes and substernal goiters are thus readily accounted for. Thyroglossal cyst is readily accounted for by the thyroid cells, in their descent, carrying down some pharyngeal cells, which give rise to a cyst. Such a cyst may communicate with the pharynx by way of the thyroglossal duct, which has failed to obliterate as it does during normal development, and with the skin through a permanent fistulous opening produced by spontaneous rupture or surgical incision. Such an opening is in the midline between the isthmus of the thyroid and the hyoid bone. There is some difference of opinion about the origin of lateral aberrant thyroid tissue. According to some observers, it arises from thyroid cells which come from the so-called fifth branchial pouch (ultimobranchial body).[23] According to hypothesis, these cells normally descend and fuse with the anlage from the pharynx but take no part in the formation of the parenchyma of the thyroid. Failing to descend they may give rise to embryonic rests, which later develop into lateral aberrant thyroid tissue. This gives rise to tissue of a low grade of malignancy, which is usually of the

22. Norris, E. H.: The Early Morphogenesis of the Human Thyroid Gland, Am. J. Anat. **24**: 443 (Nov.) 1918.

23. Leech, J. V.; Smith, L. W., and Clute, H. M.: Aberrant Thyroid Glands, Am. J. Path. **4**: 481 (Sept.) 1928.

nature of papillary cystadenoma. According to other observations, lateral aberrant thyroid tissue arises by separation from the main body of the thyroid. In rare instances thyroid tissue is found in teratoma, especially in the ovary. The thyroid tissue in teratoma may function. There is an instance on record in which it was responsible for the symptoms of exophthalmic goiter.

THYROIDITIS

Thyroiditis is a rare disease and may be acute, subacute or chronic and may be suppurative or nonsuppurative.[24] It may arise from a distant focus of infection or from a focus in neighboring structures of the neck. Infection may be introduced at the time of operation and give rise to postoperative thyroiditis. Any organism may infect the gland. The milder forms of infection may go unrecognized. Redness, swelling and tenderness of the skin over the thyroid, together with fever, suggest the presence of thyroiditis. Recurrent attacks of the noninflammatory variety may be noted, although one attack may completely destroy the gland and result in the development of myxedema. Tuberculosis of the gland is very rare and always secondary to tuberculosis elsewhere. Infection with pyogenic organisms may result in single or multiple abscesses or suppuration of the whole gland, usually associated with fever and often with a sudden onset. Abscesses should be drained because of the danger of spread of an infection into the mediastinum and along the fascial layers of the neck. Abscesses of the thyroid may compress the trachea, and relief may follow tracheotomy. The redness, swelling, tenderness and fever that sometimes accompany necrosis of thyroid tissue in patients with carcinoma may lead to a mistaken diagnosis of thyroiditis. The end result of thyroiditis is usually hypothyroidism, the extent of which is related to the amount of gland destroyed.

Struma lymphomatosa[25] and Riedel's struma are classified under the heading of chronic thyroiditis. Struma lymphomatosa occurs in older people and almost entirely in women. It is characterized by a rapid, firm swelling of the gland. It is usually bilateral,

24. Clute, H. M.: Thyroiditis—Simple, Suppurative and Chronic, Tr. Am. A. Study Goiter, 1931, p. 136.

25. Joll, C. A.: The Pathology, Diagnosis and Treatment of Hashimoto's Disease (Struma Lymphomatosa), Brit. J. Surg. 27: 351 (Oct.) 1939.

and the shape of the gland conforms more or less to the normal contour. The gland is hard and divided into lobules by dense bands of fibrous tissue. It shows numerous areas of lymphoid tissue with germinal centers and a few multinuclear giant cells. The gland may compress the trachea, although tracheotomy is rarely necessary. Subtotal thyroidectomy is indicated.

In Riedel's struma there is rapid, firm swelling of the gland, producing hard, woody thyroiditis. About half of the patients are men. In about half of the patients the process is unilateral. The gland is irregular and bound down to surrounding structures, giving rise, as a rule, to a preoperative diagnosis of carcinoma. However, there is no involvement of the skin or of the regional lymph nodes. As the disease progresses, there may develop compression of the trachea with asphyxia, hoarseness, dysphagia and involvement of the great vessels. Tracheotomy is necessary in about one fifth of the cases. Microscopically, the gland is largely replaced with dense fibrous tissue containing an occasional lymphoid follicle and some foreign body giant cells. As much of the mass should be removed surgically as possible.

Various types of involvement of the thyroid by Trypanosoma cruzi have been described in South America by Chagas.

NEW GROWTHS

The best review of the subject is given by Pemberton.[26] The ratio of malignant to benign tumors of the thyroid at the Mayo Clinic is about 4.9 per cent. Carcinoma of the thyroid may occur at any age but is most common between the ages of 40 and 70 years. The average age for men is about 53 years and for women about 48 years. The ratio of males to females in the experience of the Mayo Clinic is about 1 to 1.74.

Carcinoma of the thyroid almost always arises from a preexisting adenoma, although in rare instances it may develop in a gland involved in exophthalmic goiter. The diagnosis is not suspected before operation in about 60 per cent of the cases.

Papillary adenocarcinoma is of a low grade of malignancy and carcinoma in adenoma is of a slightly higher

26. Pemberton, J. deJ.: Malignant Lesions of the Thyroid Gland: A Review of Seven Hundred and Seventy-Four Cases, Tr. Third Internat. Goiter Conf. & Am. A. Study Goiter, 1938, p. 154.

grade, while diffuse adenocarcinoma represents "the acute fulminating malignant growths of the thyroid gland."

Squamous epithelioma and sarcoma are very rare and very highly malignant. It is uncertain whether they arise from extensions from the esophagus, trachea or thyroglossal duct or directly from the thyroid by metaplasia of the epithelium.

Malignant change of the thyroid is most commonly confused with Riedel's struma, struma lymphomatosa and hemorrhagic adenoma. In the early stages it can be diagnosed only on histologic examination.[27] As the disease progresses, "the history of recent increase in the size of a pre-existing adenoma, the recent development of a tumor of the thyroid gland, a complaint of a sense of pressure in the neck, often out of proportion to the size of the tumor and the finding on palpation of a thyroid tumor that is firmer, more nodular and relatively more firmly fixed than that usually encountered in benign goiters, are all suggestive evidence. . . . Hoarseness and a fixed vocal cord in the absence of syphilis, aortic aneurysm or mitral stenosis are almost pathognomonic of malignancy." There is uncertainty about what constitutes microscopic evidence of malignancy. Invasion of blood vessels appears to be an absolute criterion but, according to Pemberton,[26] is not necessary for diagnosis, anaplasia and dedifferentiation being adequate evidence, as they are for carcinoma elsewhere in the body.

Whenever feasible, the tumor should be removed, and if complete removal is impossible, the operation should be followed by roentgen ray or radium treatment. If the growth is inoperable, radiation therapy is used alone. Complete fixation to surrounding structures of the neck and the presence of distant metastases are contraindications to operation. Enlargement of cervical lymph nodes also constitutes a contraindication except in the case of the slowly growing papillary adenocarcinoma.

27. Graham, Allen: Nodular Goiters: Their Relation to Neoplasia, Am. J. Surg. 7: 163 (Aug.) 1929.

CHAPTER XXVI

THE PARATHYROIDS—PHYSIOLOGY AND THERAPEUTICS

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NORMAL AND PATHOLOGIC PHYSIOLOGY OF PARATHYROID GLANDS

The parathyroid hormone has a marked influence on the metabolism of calcium and phosphorus. Its primary action, in my opinion, for reasons which cannot be gone into, is on phosphorus metabolism, and the changes in the metabolism of calcium are dependent on the preceding changes in the metabolism of phosphorus.[1]

Calcium Metabolism.—The metabolism of calcium is considerably simpler than that of phosphorus. Over 99 per cent of the body's calcium is in the form of a calcium phosphate-calcium carbonate compound which is deposited in the organic matrix of bone and teeth. In addition, there are small amounts of calcium in body fluids. The calcium in the blood except for a negligible part is in two forms, calcium ions and calcium proteinate.[2] The parathyroid hormone apparently regulates the level of calcium ions. There is considerable circumstantial evidence that the stimulus for the parathyroid glands to produce more hormone is a serum calcium ion level below normal. Even when calcium is absent from the diet, a considerable amount is excreted in the urine, since the normal serum calcium value (10.5 mg. $\pm$ 1 mg. per hundred cubic centimeters) is above the threshold (circa 7 mg.) for calcium excretion. Calcium is also excreted in the feces. There is evidence that fecal calcium represents not only calcium which has not been absorbed by the gastrointestinal tract but calcium which has been excreted into the gastrointestinal tract.[3] The latter portion, however, is of small quantitative significance except in hyperthyroidism.[3] Two other routes of calcium loss from the body are the lactating breast and the placenta. Since the amount of calcium which can be held in the body fluids

1. Albright, Fuller, and Ellsworth, R.: Studies on the Physiology of the Parathyroid Glands: I. Calcium and Phosphorus Studies on a Case of Idiopathic Hypoparathyroidism, J. Clin. Investigation **7**: 183 (June) 1929.

2. McLean, F. C., and Hastings, A. B.: Clinical Estimation and Significance of Calcium-Ion Concentration in the Blood, Am. J. M. Sc. **189**: 601 (May) 1935.

3. Aub, J. C.; Bauer, Walter, C., and Ropes, M.: Studies of Calcium and Phosphorus Metabolism: III. The Effects of the Thyroid Hormone and Thyroid Disease, J. Clin. Investigation **7**: 97 (April) 1929.

is small and quite constant, it follows that if the calcium intake is greater than the calcium output the balance must for the most part be represented by the calcium deposited in bones or possibly in teeth. The converse must be true if the patient is in negative calcium balance.

Under normal conditions both calcium deposition and calcium absorption from bone are going on at all times. One process, to be sure, may be greater than the other, depending on the calcium balance as a whole. There is no such metaplasia of tissue in the teeth. It may be that there is a very slight interchange of calcium in the adult tooth, but, in my opinion, for all practical purposes this may be disregarded. When the tooth is being laid down there can be acalcification (i. e., absence of calcification) if the calcium metabolism in the body is faulty; once the tooth is formed, however, there is no decalcification. If tooth decay occurs during pregnancy, and it apparently does, the mechanism is not mobilization of calcium from the tooth to the blood stream, to the placenta, to the child.

Phosphorus Metabolism.—Phosphorus, likewise, is found in large amounts in bones and teeth (calcium to phosphorus ratio, approximately 2 to 1), and in smaller amounts in body fluids. In the serum one is here concerned chiefly with phosphorus in the form of inorganic phosphate. It is customary to speak of this as "serum phosphorus" rather than to use the more clumsy expression "serum phosphorus as phosphate." The normal level for adults is 3.5 mg. plus or minus 0.5 mg. It is higher in children. There are, in addition, in the body many organic phosphate compounds, such as phosphoprotein, phospholipids and various phosphate esters, which liberate phosphate ions on hydrolysis. Thus, a positive phosphorus balance does not necessarily mean that phosphates are entering the bones.

Relation of Calcium Metabolism to Phosphorus Metabolism in Disease of the Parathyroids.—If one stops substitution therapy with parathyroid extract in a parathyroidectomized patient, four cardinal metabolic changes occur. There is first an immediate decrease in the phosphorus excreted in the urine; second, the serum phosphorus level rises; almost simultaneously the serum calcium level falls; finally, with the fall in serum calcium there is diminished excretion of calcium in the urine.[1] If one administers parathyroid extract to a normal person these same four metabolic functions are altered in the opposite directions; i. e., one obtains hyperphosphaturia, hypophosphatemia, hypercalcemia and hypercalciuria. There are two schools of thought as to the mechanism by which these four cardinal metabolic effects are mediated. One school holds that the hormone acts on bone tissue (notably osteoclasts) and tends to dissolve or otherwise remove calcium phosphate deposits from the bone.[4] Such a theory might explain the hypercalcemia,

4. Thomson, D. L., and Collip, J. B.: The Parathyroid Glands. Physiol. Rev. **12**: 309 (July) 1932. Jaffe, H. L.: Hyperparathyroidism (Recklinghausen's Disease of Bone), Arch. Path. **16**: 63 (July) 1933.

hypercalciuria, and hyperphosphaturia; it does not explain the hypophosphatemia unless one hypothesizes some secondary adjustment. The theory to which I adhere is based on the hypothesis that the hormone affects phosphates in the circulating body fluids in such a way that their excretion in the urine is increased. This would explain the immediate hyperphosphaturia on administration of parathyroid extract and the resulting hypophosphatemia. Furthermore, because of the lowered level of serum phosphorus, it is contended that the serum would be less saturated with respect to calcium phosphate and there would be an increased tendency for calcium phosphate to enter the serum from the gastrointestinal tract or from the bone. This would lead to hypercalcemia, and finally the hypercalcemia would lead to hypercalciuria. It will be impossible to discuss the pros and cons of these two opposite and still unproved theories in the present article.

HYPOPARATHYROIDISM

Causes.—A decrease in the number of, or absence of, the parathyroid glands is most commonly due to accidental removal of some or all of these organs during thyroidectomy. Very rarely, the condition occurs idiopathically. Just why all four glands should disappear idiopathically is of considerable academic interest. In an autopsy in such a case all four glands were found to be present and grossly to have a normal appearance. However, histologic sections showed that the epithelial cells had been entirely replaced by fat cells.[5] An intermediate stage in which the epithelial elements were much diminished and replaced by fat tissue has been described in a patient suffering from anterior pituitary deficiency.[6] In hypopituitarism, however, one does not find clinical evidence of hypoparathyroidism, and I do not believe that idiopathic hypoparathyroidism is due to lack of some hormone from the anterior lobe of the pituitary. There is considerable evidence that the parathyroid may be functionally deficient shortly after birth and that convulsions in an infant may be a manifestation of hypoparathyroidism.[7] Of academic interest is a case recently reported of hypoparathyroidism in a child born of a mother with

5. Drake, T. G.; Albright, Fuller; Bauer, Walter, and Castleman, Benjamin: Chronic Idiopathic Hypoparathyroidism: Report of Six Cases with Autopsy Findings in One, Ann. Int. Med. **12**: 1751 (May) 1939.

6. Castleman, Benjamin, and Hertz, Saul: Pituitary Fibrosis with Myxedema, Arch. Path. **27**: 69 (Jan.) 1939.

7. Bakwin, Harry: Tetany in Newborn Infants: Relation to Physiologic Hypoparathyroidism, J. Pediat. **14**: 1 (Jan.) 1939.

hyperparathyroidism.[8] This suggests that the infant's parathyroids became compensatorily hypoplastic in intrauterine life.

Pathologic Physiology.—The most important chemical findings are the low level of serum calcium and the high level of serum phosphorus. In aparathyroidism the serum calcium may be as low as 5.0 mg. and the serum phosphorus as high as 12.0 mg. per hundred cubic centimeters. There should be no calcium excreted in the urine except in the very mild forms, as the serum calcium should be below the threshold for excretion of calcium (see earlier comment on this). The phosphorus excreted in the urine is often within normal limits. One expects hypophosphaturia only when the patient is adjusting from one degree to a lesser degree of parathyroid function.

Symptoms.—The most striking clinical feature of hypoparathyroidism is the increase in neuromuscular excitability dependent on the hypocalcemia, producing the symptom complex known as tetany. This phase of the subject is so well covered in all the textbooks that it will be mentioned only in passing here. The features of tetany are: carpopedal spasm, positive Chvostek, Trousseau and Erb's signs, laryngeal spasm and epileptic seizures, especially if the condition has been present a long time or if it occurs in very young children. Cataracts are present in almost all cases of long-standing duration, although it may take a slit lamp examination to find them. In many cases, however, they are sufficiently severe that their removal is required. Calcification of brain tissue is not an infrequent finding.[9] I have seen this in a case in which no symptoms referable to the central nervous system had been observed and in a case in which there had been epileptic seizures. When hypoparathyroidism develops before the teeth have entirely formed, one finds aplasia or hypoplasia of the teeth from that point in their development at which the hypoparathyroidism came in.[10] Thus, if the disease develops in a child at about the twelfth

8. Friderischsen, C.: Therapy in a Suckling with Latent Osteitis Fibrosa in the Mother, Lancet **1**: 85 (Jan. 14) 1939.

9. Eaton, L. M., and Haines, S. F.: Symmetrical Cerebral Calcification Associated with Parathyroid Insufficiency, Proc. Staff Meet., Mayo Clin. **14**: 48 (Jan. 18) 1939.

10. Albright, Fuller, and Strock, M. S.: Association of Acalcification of Dentine with Hypoparathyroidism in Rats and the Cure of Same with Parathormone, with Some Correlated Observations in Man, J. Clin. Investigation **12**: 974 (Sept.) 1933.

year of age, the teeth will be entirely normal except for blunting of the root ends of the cuspids, premolars and second molars and hypoplasia of the crowns of the third molars. Those are the last parts of the teeth to form. Changes of the nails and hair have been described,[11] but they are not present in all cases and their significance remains obscure. The bones in hypoparathyroidism are definitely more dense than normal, which is of considerable academic but of no clinical importance.[11]

Differential Diagnosis.—Other conditions which cause tetany may be confused with hypoparathyroidism. For all practical purposes one may consider that there are two causes of tetany: hypocalcemia and alkalosis. As far as I am aware, there is no evidence that the tetany caused by alkalosis is due to some secondary change in the availability of calcium ions.

The nonparathyroid causes for hypocalcemia are rickets or its adult form osteomalacia, steatorrhea, and renal insufficiency with phosphate retention. In rickets or osteomalacia the low value for serum calcium is characteristically coupled with a low, or in some instances normal, value for serum phosphorus. High values for serum phosphorus are most unusual. The serum phosphatase level, which is always normal or even low in hypoparathyroidism, is high in both rickets and osteomalacia. Steatorrhea, of which sprue is an example, probably leads to hypocalcemia, because vitamin D, being fat soluble, is dissolved in the unabsorbed fat; hence the condition results in hypovitaminosis D; the chemical findings in the blood are therefore those of osteomalacia. In addition, one may find evidence of a lack of other fat-soluble vitamins, notably vitamin K (hemorrhagic diathesis) and vitamin A (night blindness, keratosis pilaris).[12]

In renal insufficiency one finds phosphorus retention with a compensatory lowering of the serum calcium level. There is, however, in renal insufficiency as compared with hypoparathyroidism a lesser degree of hypocalcemia for the same degree of hyperphosphatemia. The probable reason why the serum can hold more calcium in proportion to the phosphorus in renal insufficiency is that there is almost invariably an associated

11. Howard, J. E.: Idiopathic Hypoparathyroidism, Ann. Int. Med., to be published.

12. Albright, Fuller, and Stewart, J. D.: Hypovitaminosis of All Fat-Soluble Vitamins Due to Steatorrhea: Report of a Case, New England J. Med. **223**: 239 (Aug. 15) 1940.

acidosis. Furthermore, one seldom meets tetany in renal insufficiency with hypocalcemia because of the associated acidosis, which inhibits tetany. If one controls the acidosis, tetany may develop.

The commonest cause of tetany due to alkalosis is hyperventilation, usually due to some emotional disturbance. The diagnosis, though often missed, is very simple. The respirations are often quite deep but not so strikingly rapid; there may be a past history of similar attacks under emotional stress; the condition responds quickly to holding the breath or rebreathing in a paper bag; the urine, characteristically, is alkaline and contains normal amounts of calcium. The presence of calcium in the urine can be quickly verified by the aid of the Sulkowitch solution, which will be discussed under "Treatment." In hyperventilation the carbon dioxide–combining power of the serum may be only slightly reduced. The most significant change in the blood is a lowering of the carbon dioxide content of the arterial blood.[13] A less common form of the tetany due to alkalosis is that following ingestion of large amounts of an alkali, in which one encounters an alkaline urine, calcium in the urine, high carbon dioxide–combining power of the serum and possibly a high value for serum total base. In alkalosis due to excessive loss of gastric contents, one again encounters an alkaline urine, calcium in the urine and an increased carbon dioxide–combining power of the serum, also a marked lowering of the serum chloride value.

Treatment.[14]—The tetany in the symptom complex of hypoparathyroidism is due to the low level of the calcium of the serum. The goal is to raise the level of calcium to normal without overdoing this and obtaining hypercalcemia. Thus, all that is needed is an agent to raise the serum calcium level readily to any desired degree and a simple method of gaging this level. Dihydrotachysterol fills the first need and the Sulkowitch test for calcium in the urine the second.

Dihydrotachysterol,[15] like vitamin D, is a photochemical derivative of ergosterol. It was developed by

13. Talbott, J. H.; Cobb, Stanley, Coombs, F. S.; Cohen, M. E., and Consolazio, W. V.: Acid-Base Balance of the Blood in a Patient with Hysterical Hyperventilation, Arch. Neurol. & Psychiat. **39**: 973 (May) 1938.

14. Albright, Fuller: Note on the Management of Hypoparathyroidism with Dihydrotachysterol, J. A. M. A. **112**: 2592 (June 24) 1939.

15. Dihydrotachysterol is distributed in the United States by the department of medical research of the Winthrop Chemical Company, Inc. It is marketed in an oily solution, each cubic centimeter containing 5 mg. of dihydrotachysterol. The preparation is administered by mouth.

Holtz in Berlin [16] for the treatment of hypoparathyroidism. One can easily raise the calcium of the blood to any desired or even undesired level with this substance. Its mode of action is beyond the scope of this article.[17]

The Sulkowitch reagent [18] is a solution containing oxalate radicals buffered at such a p_H that when an equal amount of the reagent is added to urine, the calcium will almost immediately come down as a fine white precipitate of calcium oxalate. If there is no precipitation there is no calcium, and the serum calcium level is probably from 5 to 7.5 mg. per hundred cubic centimeters. If there is a fine white cloud, there is a moderate amount of calcium, and the level of calcium in the serum is in the satisfactory range. If the precipitate looks like milk, the danger of hypercalcemia is present.

Once the diagnosis of parathyroid tetany has been made, dihydrotachysterol is administered until tests of the urine show moderate amounts of calcium. If large amounts of calcium appear in the urine, the dose is reduced and the danger of hypercalcemia is avoided. The patient makes his own tests and modifies the dose according to the results. I usually prescribe about 3 cc. of a dihydrotachysterol preparation a day until calcium appears in the urine; then the dose is dropped to a maintenance level—about 1 cc. three to five times a week.

This discussion has been slightly oversimplified. There are a few "ifs" and "buts" that must be added. Occasionally, a normal person who happens to be on a diet very low in calcium will show in a single specimen of urine practically no calcium. Almost any normal person shortly after drinking a large amount of milk will show an excess of calcium in the urine. Since one keeps patients with hypoparathyroidism on a diet high in calcium, one would expect them always to show hypercalciuria if their levels of serum calcium are normal. It thus turns out that if the dose is reduced when a large amount of calcium appears in the urine the blood calcium will be kept at a slightly subnormal level.

16. Holtz, F.: Die Behandlung der postoperativen Tetanie, Arch. f. klin. Chir. **177**: 32, 1933.

17. Albright, Fuller; Bloomberg, Esther; Drake, T. G., and Sulkowitch, H. W.: A Comparison of the Effects of A. T. 10 (Dihydrotachysterol) and Vitamin D on the Calcium and Phosphorus Metabolism in Hypoparathyroidism, J. Clin. Investigation **17**: 317 (May) 1938.

18. Two and five-tenths grams of oxalic acid, 2.5 Gm. of ammonium oxalate and 5 cc. of glacial acetic acid are dissolved in distilled water and made up to a volume of 150 cc.

This is probably all the better, since it further guards against hypercalcemia and since slight hypocalcemia is not deleterious.

A large number of measures other than the administration of dihydrotachysterol may be useful in the treatment of hypoparathyroidism.[19] To be sure, treatment with this drug is so satisfactory that one may be disinclined to bother with the other measures. A few of the salient points will be mentioned. There should be a high intake of calcium and a low intake of phosphorus. Milk, though high in calcium, is contraindicated because it is likewise high in phosphorus. The dietary conditions are sufficiently met if the patient omits milk as a beverage from the diet and takes a teaspoon of calcium gluconate or of calcium lactate dissolved in water three times a day. Just as alkalosis causes tetany, so acidosis tends to alleviate tetany. It is therefore helpful in some instances to make the patient slightly acidotic. Calcium chloride by mouth produces slight acidosis, since more chloride is absorbed than calcium. A favorite prescription in the past has been 10 cc. of a 30 per cent solution of calcium chloride diluted in water and taken three times daily after meals. Such a prescription produces slight acidosis and insures a high intake of calcium at the same time. It has been shown that thyroxin tends to raise the serum calcium level in hypoparathyroidism.[20] Since many patients with postoperative hypoparathyroidism are at the same time suffering from a slight thyroid lack, it is often wise to administer thyroid to the limit of tolerance. Although parathyroid extract has played a large part in the history of parathyroidology, it is not used routinely in the therapy of hypoparathyroidism. The drawbacks are that it is expensive, that it often causes a local reaction and that its effectiveness wears out, apparently because of the formation of an antibody.

In an acute emergency 10 cc. of calcium gluconate can be administered intravenously. It must be emphasized, however, that whereas the symptoms of tetany

19. Ellsworth, Read: Diagnosis and Treatment of Parathyroid Underfunction, Internat. Clin. **3**: 27 (Sept.) 1933.

20. Aub, J. C.; Albright, Fuller; Bauer, Walter, and Rossmeisl, Elsie: Studies of Calcium and Phosphorus Metabolism: VI. In Hypoparathyroidism and Chronic Steatorrhea with Tetany, with Special Consideration of the Therapeutic Effect of Thyroid, J. Clin. Investigation **11**: 211 (Jan.) 1932.

may be terrifying, the condition is seldom fatal. Laryngeal spasm is the one most dangerous manifestation.

If the blood calcium is kept normal in hypoparathyroidism, cataracts do not develop. Those, once formed, however, do not regress. The cause of the cataracts is apparently the hypocalcemia. Cataracts also occur in hypocalcemia without hypoparathyroidism (e. g., sprue).

PRIMARY HYPERPARATHYROIDISM

By "primary hyperparathyroidism" is meant a condition in which more parathyroid hormone is produced than is needed. By "secondary hyperparathyroidism" is meant a condition in which more parathyroid hormone is produced than is normal but this excess is needed for some compensatory purpose.

Causes.—Hyperparathyroidism may be due to a single adenoma of one of the four glands, to multiple adenomas or to hypertrophy of all the parathyroid tissue. In the first 50 cases of hyperparathyroidism proved to be such by operation or autopsy, at the Massachusetts General Hospital, the disease was due in 41 cases to a single adenoma, in 3 cases to two adenomas and in 6 cases to hypertrophy. By hypertrophy is meant a condition in which the amount of parathyroid tissue is about forty to one hundred times the normal. The histologic appearance of the tissue is entirely dissimilar from that seen in any other form of parathyroid disease.[21] The individual cells have about five times the radius or about one hundred and twenty-five times the volume of a normal cell ($V = 4/3\pi r^3 = 4/3\pi \times 5 \times 5 \times 5 = 4/3\pi \times 125$). The histologic appearance is not in the least suggestive of hyperplasia, in which one finds cells of almost normal radius. The cause of hypertrophy of parathyroid tissue remains entirely obscure.

Pathologic Physiology and Diagnostic Considerations.—As in other conditions of overactivity of an endocrine gland, in hyperparathyroidism one can observe every degree of the disease. The slighter the degree the less abnormal will be the chemical findings in the blood. It so happens that a very mild manifestation of the disease may lead to a fatal issue, hence it

21. Albright, Fuller; Sulkowitch, H. W., and Bloomberg, Esther: Hyperparathyroidism Due to Idiopathic Hypertrophy (Hyperplasia) of Parathyroid Tissue: Follow-Up Report of Six Cases, Arch. Int. Med. **62**: 199 (Aug.) 1938.

becomes important to recognize the mild forms. The serum calcium level is characteristically high (up to 18 mg. when the condition becomes severe) but may be within normal limits in the milder forms of the disease.[22] It is important that the determination of serum protein be done in conjunction with that of serum calcium, since a normal level of serum calcium in the presence of a low level of serum protein is really abnormal. Of the two fractions of serum calcium, calcium ions and calcium as proteinate, it is the former which is primarily high in hyperparathyroidism; if the serum protein should be low, owing to some complicating condition, it might be that the level of serum calcium would be normal in spite of the fact that the calcium ions in the serum were increased.[22] The serum phosphorus level in hyperparathyroidism is almost invariably low, 3.1 mg. or lower, unless renal damage is present. A normal person may have a low level of serum phosphorus on one determination, but it is characteristic of the patient with hyperparathyroidism that the level is consistently low. The calcium and phosphorus excreted in the urine are both increased. Again, however, there is an overlapping between normal and mildly hyperparathyroid states in the amount of calcium in the urine. If the hyperparathyroidism leads to bone disease, which it need not (see an earlier statement), the phosphatase level is increased, reaching 20 to 30 Bodansky units in severe forms.

There is a difference of opinion as to the cause of the bone disease in hyperparathyroidism. It is a fact that a patient may have severe hyperparathyroidism and still no clinical, roentgenologic or histologic evidence of bone disease.[22] Those who believe that the parathyroid hormone acts directly on bone tissue would probably argue either that the aforementioned condition was of short duration or that some evidence of bone change would have been found had one examined the right bone tissue (e. g., trabeculae on the inside of the bone shaft rather than the cortex). This is not my interpretation. In my opinion, hyperparathyroidism brings about a change in the blood chemical balance which results in an increased excretion of calcium in the urine. Other things being equal, this increases the

22. Albright, Fuller; Sulkowitch, H. W., and Bloomberg, Esther: Further Experience in the Diagnosis of Hyperparathyroidism, Including a Discussion of Cases with a Minimal Degree of Hyperparathyroidism, Am. J. M. Sc. **193**: 800 (June) 1937.

chances of the patient being in negative calcium balance. If the patient is in negative calcium balance, bone disease develops; if the patient happens to ingest as much calcium as is lost in the urine and feces, the calcium balance is not negative and bone disease does not develop. For all practical purposes, this usually comes down to whether the patient drinks milk. If he does, his calcium intake will be sufficient to keep him in positive calcium balance even if he has marked hyperparathyroidism.

Osteitis Fibrosa Cystica Generalisata.—Under normal conditions there is constant metaplasia of bone. There are trabecular surfaces where bone is being laid down and surfaces where bone is being absorbed. When the patient is in positive calcium balance, the former process outweighs the latter, and vice versa when the patient is in negative calcium balance. A decrease in bone tissue may result from increased absorption or from decreased formation of bone. The bone disease associated with hyperparathyroidism belongs in the former category. The histologic evidence of bone absorption is an increase in the number of osteoclasts. Since the bones become much weakened, and since there is no fundamental disorder of bone repair processes, one finds evidence of bone repair (increase in osteoblasts) in conjunction with bone destruction. In other words, there is a marked increase in the metaplasia of bone. There results an increase in serum phosphatase, which in the absence of hepatic disease is an index of osteoblastic activity. The bone matrix which is laid down by the osteoblasts is calcified. The stroma of the bone marrow is also increased, causing fibrosis. Sometimes one sees solid tissue tumors composed of osteoblasts and osteoclasts. These are designated as benign giant cell tumors, or osteoclastomas. In addition, one finds cysts filled with fluid and lined with fibrous capsules. These are undoubtedly due to secondary degenerative changes. Likewise, one finds fractures. Most of the tumors and the cysts look like cysts on the roentgenograms and are usually so designated by roentgenologists.

Symptoms.—The symptoms of hyperparathyroidism can be distributed under three subheadings: (*a*) those due to bone disease, (*b*) those due to renal disease and (*c*) those due to hypercalcemia per se.

The symptoms which are produced by the bone disease can readily be inferred from what has already been said. Any bone tumor which on biopsy turns out to be a benign giant cell tumor (osteoclastoma) may be evidence of underlying hyperparathyroidism. Such a tumor has a special predilection for the jaw; when it occurs there, it is called an epulis. Not every epulis, however, is due to hyperparathyroidism. Almost any skeletal manifestation — a spontaneous fracture, a decrease in height due to crushing of vertebrae, a pain in the back, tenderness in the shins—may be the first symptom of the disease.

Because of the increased amounts of calcium and phosphate excreted in the urine, the patient with hyperparathyroidism is predisposed to the formation of calcium phosphate or calcium oxalate urinary calculi.[23] Indeed, the symptoms associated with nephrolithiasis are the commonest first manifestation of the disease. Calcium salts may be deposited also in the pyramids (nephrocalcinosis) and lead to renal insufficiency. Such deposits give a characteristic roentgenographic appearance which is quite pathognomonic of this disease. Polyuria and polydipsia are rather constant and may become so severe in some patients as to lead to the diagnosis of diabetes insipidus. The cases in which polydipsia is most marked are of course the ones in which renal calculi are least apt to develop.

Just as hypocalcemia causes increased neuromuscular excitability, so hypercalcemia leads to decreased excitability. In patients with very high serum calcium there are certain symptoms apparently due to the high serum calcium per se. These patients feel tired, lose weight and are constipated. These symptoms are too indefinite to help in the diagnosis, but after the condition is corrected the relief from them is quite striking.

Parathyroid Poisoning.—By "parathyroid poisoning" is meant a sort of hyperhyperparathyroidism. If one administers parathyroid extract in large quantities to a dog, death will occur in two to three days, and at autopsy there will be calcium deposits in the alveoli of the lungs, the mucous membranes of the stomach and the kidneys.[24] The sequence of events apparently is

23. Albright, Fuller: Hyperparathyroidism: Its Diagnosis and Exclusion, New England J. Med. **209**: 476 (Sept. 7) 1933.

24. Heuper, Wilhelm: Metastatic Calcifications in the Organs of the Dog After Injections of Parathyroid Extract, Arch. Path. **3**: 14 (Jan.) 1927.

as follows: an increasingly high level of serum calcium, inspissation of the blood, an acute failure of the kidneys to excrete phosphates, a rapid rise in serum phosphorus, a combination at the same time of high serum calcium and high serum phosphorus, a precipitation of calcium phosphates into the tissues and chemical death. Patients with hyperparathyroidism seldom have such a degree of the disease that parathyroid poisoning develops. The condition, however, does occur occasionally.[25] It is sometimes precipitated by faulty therapy. If the serum calcium is near the critical level, where a slight increase in it would precipitate parathyroid poisoning, it may so happen that a diet high in calcium would make the difference. In my opinion, the patient with severe hyperparathyroidism should be kept on a low calcium intake until there is no danger of parathyroid poisoning. One's first impulse after seeing the decalcified bones is to insure that the patient's intake of calcium shall be high; this may be fatal.

Differential Diagnosis.—In this section will be discussed only those bone diseases which might be mistaken for that due to hyperparathyroidism.

Osteoporosis belongs in that category of bone diseases in which there is too little bone because the formation of bone is decreased. Furthermore, the lack of bone formation is due not to a failure of calcium salts to be deposited in the organic matrix (compare osteomalacia) but to a failure of the osteoblasts to lay down an organic matrix. The disturbance, therefore, is not really one of calcium metabolism but one of tissue metabolism. The serum calcium and phosphorus values are normal, and the serum phosphatase is not elevated. There is one important exception to this statement. Children have a more unstable calcium equilibrium than adults and apparently develop work hypercalcemia due to atrophy of disuse. One finds this condition in children in whom a large part of the skeleton has been isolated from stresses and strains by infantile paralysis (personal communication, Dr. William J. Orr) or by a plaster cast (unpublished). The calcium excretion in the urine may be increased in the early stages of the

25. Dawson, J. W., and Struthers, J. W.: Generalized Osteitis Fibrosa, with Parathyroid Tumor and Metastatic Calcification, Including a Critical Discussion of Pathological Process Underlying Osseous Dystrophies, Edinburgh M. J. **30**: 421 (Oct.) 1923. Hanes, F. M.: Hyperparathyroidism Due to Parathyroid Adenoma with Death from Parathormone Intoxication, Am. J. M. Sc. **197**: 85 (Jan.) 1939.

disease, when it may give rise to renal stones. The condition when not due to atrophy of disuse or advanced age is almost confined to women past the menopause and has a predilection for the bones of the spine and the pelvis. The skull is very seldom involved.

Osteomalacia, like osteoporosis, belongs in that category of bone diseases in which there is too little formation of bone. Here the lack of formation is not due to hypoplasia of the osteoblasts but to a failure of calcium salts to be deposited in the organic matrix. In this country the condition is extremely rare and found only in association with steatorrhea. The serum calcium is normal or low; the serum phosphorus is almost always low; the serum phosphatase is high. There is no increase in the calcium excreted in the urine; if anything, it is considerably decreased.

Paget's disease differs from osteitis fibrosa generalisata in that it is a circumscribed disease. Where the bone is normal, it is absolutely normal; where it is abnormal, it has many things in common with osteitis fibrosa, notably the marked increase in metaplasia of the bone. The roentgenographic appearance of the individual bone lesions in Paget's disease is almost always pathognomonic. To be emphasized are the coarse trabeculation, the marked tendency of the bones to be expanded and the sharp demarcation between normal bone and abnormal bone. The serum calcium and phosphorus levels are normal; the serum phosphatase level is higher for a given degree of bone disease than in any other condition.

There is a syndrome characterized by osteitis fibrosa disseminata (polyostotic fibrous dyspasia [26]), precocious puberty when it occurs in females, and areas of brown pigmentation.[27] Patients with this syndrome are frequently operated on in the belief that they have hyperparathyroidism. The diagnosis should offer no difficulty. The condition is not generalized but regional. This should immediately make one doubt the presence of an endocrine cause. The roentgenographic appear-

26. Lichtenstein, Louis: Polyostotic Fibrous Dysplasia, Arch. Surg. **36**: 874 (May) 1938.

27. Albright, Fuller; Butler, A. M.; Hampton, A. O., and Smith, P. H.: Syndrome Characterized by Osteitis Fibrosa Disseminata, Areas of Pigmentation, and Endocrine Dysfunction, with Precocious Puberty in Females, New England J. Med. **216**: 727 (April 29) 1937 Albright, Fuller; Scoville, W. B., and Sulkowitch, H. W.: Syndrome Characterized by Osteitis Fibrosa Disseminata, Areas of Brown Pigmentation, and a Gonadal Dysfunction, Endocrinology **22**: 411 (April) 1938.

ance of the bone is only superficially suggestive of that seen in hyperparathyroidism. There are areas of increased density as well as areas of decreased density. The serum calcium and phosphorus levels are normal; the serum phosphatase is high if there is a marked degree of the bone disease. The areas of brown pigmentation do not occur in all cases; the precocity mentioned occurs only in females.

Multiple myeloma can produce a clinical picture which may be most difficult to distinguish from that of hyperparathyroidism. The roentgenographic appearance of the bones can be quite similar, although in most instances of multiple myeloma the lesions are more sharply demarcated. For example, one expects punched-out areas in the skull rather than a diffusely "motheaten skull." The serum calcium can be high in myeloma; when it is, the calcium excreted in the urine is also high, and nephrolithiasis may be present. The high serum calcium is usually coupled with normal or high serum phosphorus. In some cases, however, the serum phosphorus is low, just as it is in hyperparathyroidism. The presence of large amounts of Bence Jones protein in the urine is strong evidence for myeloma; absence of this protein means little, as in only 15 of 30 cases of proved myeloma was it found.[28] Whether small amounts of this protein may be present with the bone disease of hyperparathyroidism is still questionable, as in those cases in which it has supposedly been found the most rigid criteria probably were not applied. Of course, the presence of plasma cells in the peripheral blood or a positive finding in a sternal biopsy or on sternal puncture are strong evidence for myeloma. The serum phosphatase is rarely if ever elevated in cases of multiple myeloma, an important differential point.[28]

Metastatic malignant neoplasm offers less difficulty in the differential diagnosis. The roentgenographic appearance is quite distinctive. The serum calcium may be high, and there may be hypercalciuria and renal stone formation. The serum phosphorus level is usually normal, very occasionally elevated and occasionally lowered. The phosphatase level may be elevated. A primary source should be looked for, and is apt to be in the breast, prostate, kidney (hypernephroma), bronchus or thyroid.

28. Jacobson, B.: Multiple Myeloma: Diagnosis and Treatment, to be published.

Other less common conditions which have been confused with hyperparathyroidism are: Boeck's sarcoid, xanthomatosis, Gaucher's disease, lymphoma, benign metastasizing hemangioma, osteogenesis imperfecta, chronic radium poisoning and renal osteitis fibrosa generalisata (see under "Secondary Hyperparathyroidism").

Treatment.—It will be impossible to discuss treatment here in any but the most superficial manner. It is important to remember that whereas the symptoms due to bone changes may be the most striking feature of the case, the ultimate prognosis will depend on the extent of the renal damage. Most of the changes in the bones, exclusive of the cysts, are reversible; however, once the kidneys are sufficiently damaged as a result of nephrocalcinosis, recovery will not take place. There is even evidence that sometimes the renal condition is progressive after its cause has been removed.[29] Therefore, when a patient with severe hyperparathyroidism is first seen, fluids should be forced, and milk should be withdrawn from the diet to avoid further nephrocalcinosis, not to mention the danger of parathyroid poisoning (already emphasized). Although the bone lesions can be made to regress with a high calcium diet,[30] this is not to be recommended.

Since no satisfactory medical treatment for the condition has yet been devised, the main therapeutic indication is to remove surgically the etiologic factor or part of it. This requires an especially trained surgeon, not just a "good thyroid surgeon." If the condition is due to one or even two adenomas, and if the serum phosphatase level is normal (i. e., no bone disease), the indication usually is to remove the tumor or tumors. If, however, the pathologic change under otherwise similar conditions is hypertrophy of all parathyroid tissue, the procedure is to remove three of the parathyroid glands and all except 200 to 300 mg.[19] of the fourth.

If in a patient with marked bone disease and high serum phosphatase (20 Bodansky units or over) one removes all of the parathyroid tumor, there will be immediate cessation of bone destruction while bone

29. Albright, Fuller: Unpublished data.

30. Albright, Fuller; Baird, P. C.; Cope, Oliver, and Bloomberg, Esther: Studies on the Physiology of the Parathyroid Glands: IV. Renal Complications of Hyperparathyroidism, Am. J. M. Sc. **187**: 49 (Jan.) 1934.

formation continues at its terrific speed. This process will result in the calcium and phosphorus being sucked into the bones from the blood serum, with an increase in the hypophosphatemia and with production of marked hypocalcemia and severe tetany.[22] The latter in the final analysis is not hypoparathyroid tetany, in which one has too little parathyroid tissue but normal bone. It is a much severer condition because of the "hungry bones." [31] This form of tetany, when it occurs, will not respond to any of the usual measures of treatment and requires constant intravenous administration of a solution of calcium gluconate. At the Massachusetts General Hospital, it has been the rule not to remove the entire tumor at the first operation in the patient with high serum phosphatase, unless there is some extenuating circumstance.

Another important consideration in deciding how much parathyroid tissue to leave behind is the degree of renal damage. In the presence of marked damage one leaves behind much more tissue than one otherwise would. The rationale is discussed under "Secondary Hyperparathyroidism."

The postoperative course should be an important subject of discussion in this section, but it will have to be omitted because of lack of space.

SECONDARY HYPERPARATHYROIDISM

There are at least two conditions in which more parathyroid hormone than is normal is needed, for compensatory reasons: rickets (or osteomalacia) and renal insufficiency.

With vitamin D insufficiency (rickets and osteomalacia) there is interference with the absorption of calcium from the gastrointestinal tract; this tends to cause a lowering of the serum calcium level, and this tendency, in my opinion, is met by hyperfunction of the parathyroid glands, the stimulus being the low level of serum calcium; the increased production of parathyroid hormone leads to a decrease in serum phosphorus; the net result is that the serum calcium level remains normal and the serum phosphorus level becomes low.[32] In

31. Cope, Oliver: Surgery of Hyperparathyroidism: The Occurrence of Parathyroids in the Anterior Mediastinum and the Division of the Operation into Two Sages, Ann. Surg. **114**: 4, 1941.

32. Albright, Fuller, and Sulkowitch, H. W.: The Effect of Vitamin D on Calcium and Phosphorus Metabolism: Studies on Four Patients, J. Clin. Investigation **17**: 305 (May) 1938.

patients with rickets or osteomalacia in whom serum calcium is low and serum phosphorus normal the compensatory parathyroid hyperfunction has not taken place. In patients in whom serum calcium and phosphorus are both low the hypocalcemia has occurred in spite of the hyperfunction of the parathyroid glands.[32]

Secondary Hyperparathyroidism in Renal Insufficiency.—In patients with renal insufficiency in whom there is retention of nonprotein nitrogen there is almost always retention of serum phosphorus. Such persons at post mortem have hyperplasia of all four parathyroid glands.[33] It is my opinion that the sequence of events is as follows: (*a*) renal insufficiency, (*b*) phosphate retention, (*c*) tendency toward a low level of serum calcium and (*d*) hyperplasia of the parathyroid glands to meet this tendency. In rare instances in which the condition is of long duration it may be accompanied by osteitis fibrosa generalisata[34] and may be confused with primary hyperparathyroidism. It is probably the secondary hyperparathyroidism which prevents most patients with renal insufficiency from having severe tetany.

There is an important corollary to the foregoing discussion which has considerable bearing on the treatment of some patients with hyperparathyroidism. It is theoretically possible that the following sequence of events might take place: (*a*) parathyroid adenoma producing primary hyperparathyroidism, (*b*) renal damage with phosphate retention and (*c*) a state of affairs in which the amount of secondary hyperparathyroidism needed because of the renal damage equals or exceeds the amount of primary hyperparathyroidism present. When this third stage has been reached, it is obvious that it would be harmful to remove the parathyroid tumor. The most important practical point to be gleaned from the foregoing discussion is that one should remove less parathyroid tissue than otherwise in patients with hyperparathyroidism and renal damage.

33. Pappenheimer, A. M., and Wilens, S. L.: Enlargement of the Parathyroid Glands in Renal Disease, Am. J. Path. **11**: 73 (Jan.) 1935.

34. Albright, Fuller; Drake, T. G., and Sulkowitch, H. W.: Renal Osteitis Fibrosa Cystica: Report of a Case with Discussion of Metabolic Aspects, Bull. Johns Hopkins Hosp. **60**: 377 (June) 1937.

CHAPTER XXVII

ACTIVATED STEROLS IN THE TREATMENT OF PARATHYROID INSUFFICIENCY

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It is now well established that certain products derived from activated ergosterol are highly effective in increasing the concentration of calcium in the blood and in relieving the symptoms of parathyroid insufficiency. It appears, from some years of accumulated clinical experience, that these products may be administered over a considerable time with reasonable safety, provided proper precautions are observed, and that their prolonged use is attended neither by injury to the patient nor by the development of tolerance.

The subject of the management of hypoparathyroidism with the activated sterols assumes especial importance because of the fact that substitution therapy, in the form of administration of solution of parathyroid, is virtually limited to rather acute conditions, in which the treatment needs to be continued for days or weeks rather than months. Not only is no tolerance to the activated sterols developed but they also have the decided advantage that the preferable method of administration is by mouth. On the other hand, the action of the sterols is somewhat delayed, so that for the relief of acute parathyroid tetany solution of parathyroid may be indispensable.

This review presents the principles underlying the use of the activated sterols in the treatment of parathyroid insufficiency, current knowledge concerning the mode of their action, a consideration of their toxicity and a brief summary of clinical experience with the preparations at present available for this purpose. Because of the unwarranted implication in the current literature that the beneficial effects of one of the derivatives of irradiated ergosterol (dihydrotachysterol) in hypoparathyroidism are specific for that substance, it will be necessary to emphasize the evidence to the con-

trary. The conclusion is reached that similar, if not identical, effects may be obtained from several of the activation products of ergosterol and cholesterol and that there is no satisfactory evidence that the therapeutic effects of dihydrotachysterol are superior to those of vitamin D when the latter is given in comparable doses. This review does not consider the use of vitamin D products for purposes other than to regulate the concentration of calcium in the plasma.

CHEMISTRY AND PREPARATIONS

The distinction between the antirachitic action of the irradiation products of ergosterol and those actions which are responsible for the toxicity of certain of these products was first clearly drawn by Holtz and Schreiber [1] in 1930. They were unable to make a preparation which was antirachitic but nontoxic but by appropriate chemical treatment could destroy most of the antirachitic action while retaining and actually increasing the toxic effects. As a reference to the dominating symptoms of the toxic actions of irradiated ergosterol—hypercalcemia and pathologic calcification in the soft tissues—they named the principle responsible for these effects the calcinosefaktor. Holtz and his co-workers were then responsible for the further study of this factor, here called the "calcemic principle," and eventually [2] for its introduction into clinical use in the form of dihydrotachysterol, originally known and frequently referred to in the literature as A. T. 10 (antitetanic preparation 10).

The action of the calcemic principle is nonspecific [3] and is shared by vitamin D_2, vitamin D_3, 22-dihydrovitamin D_2, dihydrovitamin D_2 II, tachysterol, dihydrotachysterol, 22-dihydrotachysterol and probably also toxisterol. Of these, only vitamin D_2, in the forms of crystalline calciferol and of viosterol, and dihydrotachysterol have received sufficient investigation and clinical trial to warrant a review of their usefulness in the treatment of hypoparathyroidism. Toxisterol

1. Holtz, F., and Schreiber, E.: Einige weitere physiologische Erfahrungen über das bestrahlte Ergosterin und seine Umwandlungsprodukte, Ztschr. f. physiol. Chem. **191**: 1-22, 1930.

2. Holtz, F.: Die Behandlung der postoperativen Tetanie, Arch. f. klin. Chir. **177**: 32, 1933.

3. von Werder, F.: Ueber Dihydro-tachysterin, Ztschr. f. physiol. Chem. **260**: 119-134, 1939.

appears to have toxic actions qualitatively different from those of the other substances considered.[4]

The chemistry of the activated sterols has been reviewed by Bills.[5] Irradiation of ergosterol leads to the following chain of reactions: ergosterol→lumisterol →tachysterol→calciferol (vitamin D_2). Further irradiation leads to formation of toxisterol and of the suprasterols I and II. More radiant energy is required to decompose calciferol than to produce it, so that when irradiation is not unduly prolonged calciferol is the chief product.

TABLE 1.—*Vitamin D Activity and Toxic Borderline Doses of Various Derivatives of Irradiated Sterols*

	Vitamin D Activity, I. U. per Mg.	Toxic Borderline Dose,* Mg.
Calciferol	40,000	0.05-0.07-0.125 (Morris and McLean,[36] Holtz, Gürsching and Kraut,[39] Shohl and Farber [43])
Dihydrotachysterol		
Crystalline, pure	200 [3]	0.010 [3]
Commercial, A. T. 10	80-100 [6]	0.025
Toxisterol	Little or no antirachitic activity	0.025 [9]
Tachysterol	Little or no antirachitic activity	0.200 [9]
Lumisterol	Little or no antirachitic activity	Not toxic

* All the data on toxic borderline doses are obtained from observations on mice, by the method described,[8] except for the observations of Morris and McLean [36] and of Shohl and Farbar,[43] which were obtained on rats by comparison with dihydrotachysterol.

Calciferol is vitamin D_2, to which the steroid formula shown in figure 1 is usually assigned,[5] and is available in pure crystalline form. The international unit (I. U.) of vitamin D, which is quantitatively the same as the U. S. P. unit, is equivalent to 0.000,025 mg., or 0.025 micrograms of crystalline calciferol. One milligram of calciferol is equivalent to 40,000 international or U. S. P. units of vitamin D. Vitamin D_3 is activated

4. Shohl, A. T.: Personal communication to the author.

5. Bills, C. E.: The Chemistry of Vitamin D, in the Vitamins, Chicago, American Medical Association, 1939, pp. 443-458; J. A. M. A. **110**: 2150-2155 (June 25) 1938.

6. Harnapp, G. O.: Monatschr. f. Kinderh. **63**: 262, 1935.[9] Shohl and Farber.[43]

7-dehydrocholesterol and is the chief form in which vitamin D is present in fish liver oils.

Viosterol is the nonproprietary name adopted by the Council on Pharmacy and Chemistry of the American Medical Association[7] for all acceptable preparations of irradiated ergosterol. Under the best conditions of preparation it is composed of approximately 50 per cent of calciferol, with lumisterol and tachysterol accounting for most of the remainder.[5] It is standardized in terms of its vitamin D activity, so that the content of lumisterol and tachysterol, which have little or no antirachitic effect, are not taken into account. In ordinary use, for the prevention or treatment of rickets, the amounts of these substances administered are negligible, but when

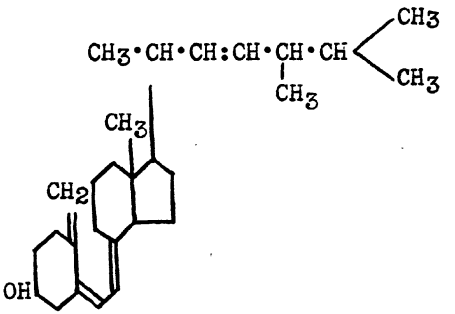

Fig. 1.—Usual or steroid formula of calciferol (vitamin D_2). (From Bills[5]).

doses sufficient to produce the calcemic effect of vitamin D are given tachysterol will contribute appreciably to this effect. Lumisterol has not been shown to have any toxic, or calcemic, effect.

The chemistry of dihydrotachysterol, including the method of preparation of a pure crystalline product, has been described in detail by von Werder.[8] Dihydrotachysterol differs from calciferol only in the saturation of a double bond (fig. 2), but this difference is sufficient for the disappearance of all but a small fraction of the antitrachitic action and for a decided enhancement of toxicity. Dihydrotachysterol is formed when tachysterol, one of the irradiation products of ergosterol, is treated with sodium and propyl alcohol. It is available only in the form of an oily solution, described as

7. New and Nonofficial Remedies, Chicago, American Medical Association, 1940.

containing 5 mg. of the basic substance per cubic centimeter, of which approximately 2 mg. is pure dihydrotachysterol.[3]

Holtz and his co-workers[8] introduced a biologic method by which the available preparation of dihydrotachysterol is standardized. A "toxic borderline dose" is the amount which when given in ten single daily doses over a period of thirteen days to a series of 8 male mice weighing from 16 to 18 Gm. will produce a loss in weight of more than 2.5 Gm. or death in at least 50 per cent of the animals. Table 1 summarizes the available information concerning antirachitic activity and toxicity of the preparations considered in this review.

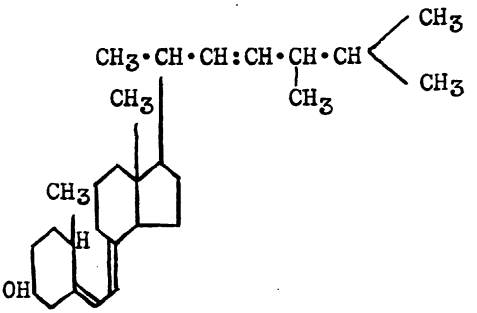

Fig. 2.—Probable formula of dihydrotachysterol (from von Werder[3]). Note saturation of double bond, with addition of two H atoms.

DOSAGE AND TOXICITY

It will be shown that much confusion over the use of vitamin D in the management of hypoparathyroidism has arisen from the fact that there are two distinct dosage ranges for this substance. The prophylactic and curative range for the antirachitic effect, with which this review is not concerned, is ordinarily 700 to 1,000 international units a day.[9] The dosage necessary for the elevation of a subnormal serum calcium and its maintenance at a normal level is commonly 60,000 to 200,000 international units daily and may be even higher over short periods. Administration of dihydrotachysterol has been completely divorced from the

8. Holtz, F.; Laquer, F.; Kreitmair, H., and Moll, T.: Beiträge zur Kenntnis des Vitamins D.: I. Mitteilung: Die Wertbestimmung im Tierversuch, Biochem. Ztschr. **237**: 247-275, 1931.

9. Park, E. A.: The Therapy of Rickets, J. A. M. A. **115**: 370-379 (Aug. 3) 1940.

system of units by which vitamin D preparations are standardized and, being standardized only in terms of its calcemic principle, is free from the confusion that exists in the case of vitamin D. Since the calcemic principle, common to both vitamin D and dihydrotachysterol, is identical with the factor responsible for the toxic manifestations of overdosage with both sterols, and since the desirable therapeutic effect depends on the administration of subtoxic doses of these substances, the question of dosage will here be considered in relation to toxicity.

The effectiveness of the calcemic principle is affected by the degree of parathyroid insufficiency, by the intake of calcium and phosphorus, by the female hormone, and perhaps by other physiologic variables. For this reason there are no absolute standards of dosages necessary to produce therapeutic or toxic effects. The available information, however, does not permit of any quantitative treatment of the subjects of dosage and toxicity in relation to the other variable factors concerned.

Initial Dosage.—Treatment of parathyroid insufficiency is commonly initiated with relatively large doses of the activated sterols, followed by smaller maintenance doses. Initial and maintenance doses will be considered separately.

Relatively large doses of dihydrotachysterol have been used and recommended for initiating treatment of acute attacks of hypoparathyroidism. More common practice, in chronic parathyroid insufficiency, is to initiate treatment with from 1 to 4 cc. daily of the oily solution (5 to 20 mg. of the basic substance). As it will be shown that at least 10 mg. of calciferol is required for a calcemic action equivalent to that of the 5 mg. of basic substance contained in 1 cc. of the solution of dihydrotachysterol, a corresponding daily dose of calciferol would be 10 to 40 mg., or 400,000 to 1,600,000 international units of vitamin D.

While these doses have the appearance of being enormous, owing to the system of units employed, there is little evidence of harmful effects of such doses, administered over relatively short periods. Spies and Hanzal[10] gave as much as 18,000,000 international units of vitamin D a day, in the form of viosterol, for

10. Spies, T. D., and Hanzal, R. F.: Experimental Production of Hypercalcemia in Human Beings by Means of Irradiated Ergosterol, Proc. Soc. Exper. Biol. & Med. **31**: 747-750 (March) 1934.

from nine to twenty-five days to patients about to die of disease. They observed no symptoms referable to the medication, except for a rise in the serum calcium level to as high as 16.6 mg. per hundred cubic centimeters, and at autopsy found no pathologic calcification or other changes which they could attribute to the vitamin D. Steck and his associates [11] administered as much as 3,000,000 international units of vitamin D a day for fifteen days to a normal subject without evidence of disturbance of any kind. Single doses of vitamin D, commonly about 600,000 international units, are now frequently administered to infants in the so-called Stosstherapie of rickets, the literature having been reviewed by Vollmer [12] and by Reed, Struck and Steck.[13] Cowdry and Scott [14] reported giving viosterol to monkeys, of less than 3 Kg. body weight, in doses amounting to 27,600,000 international units of vitamin D within two hundred hours. They made no mention of clinical symptoms. Goormaghtigh and Handovsky [15] reported lethal effects on 4 dogs following single doses of 13 to 20 mg. (520,000-800,000 international units) of calciferol per kilogram of body weight. Five other dogs, given from 2.9 to 8 mg. per kilogram, survived.

Much of the earlier literature on the toxicity of vitamin D preparations must be discarded, owing to the wide use of a preparation of irradiated ergosterol with low vitamin D and high toxisterol content.[16] In the literature of the past few years I have been able to find one report of serious toxic effects from large initial doses of dihydrotachysterol and only one case following large doses of vitamin D over a relatively short period of time. A case is reported [17] of an obese physician aged 74 with generalized arteriosclerosis who took by mistake 2,300,000 international units of

11. Steck, I. E.; Deutsch, H.; Reed, C. I., and Struck, H. C.: Further Studies on Intoxication with Vitamin D, Ann. Int. Med. **10**: 951-964 (Jan.) 1937.

12. Vollmer, H.: Treatment of Rickets and Tetany by Parenteral Administration of One Massive Dose of Vitamin D, J. Pediat. **16**: 419-432 (April) 1940.

13. Reed, C. I.; Struck, H. C., and Steck, I. E.: Vitamin D, Chicago, University of Chicago Press, 1939.

14. Cowdry, E. V., and Scott, G. H.: Effect on Monkeys of Small Doses of a Concentrated Preparation of Viosterol, Arch. Path. **22**: 1-23 (July) 1936.

15. Goormaghtigh, Norbert and Handovsky, Hans: Effect of Vitamin D_2 (Calciferol) on the Dog, Arch. Path. **26**: 1144-1182 (Dec.) 1938.

16. Bills, C. E.: Physiology of the Sterols, Including Vitamin D, Physiol. Rev. **15**: 1-97 (Jan.) 1935.

17. Kerr, W. J., in discussion of paper by Freyberg, Grant and Robb,[71] Steck, Deutsch, Reed and Struck,[11]

vitamin D, in the form of a concentrated solution of viosterol, daily for eighteen days, or a total of more than 40,000,000 international units. Nausea, anorexia, weakness, increased thirst and polyuria developed and finally he went into coma and died.

The largest doses of dihydrotachysterol reported in the literature appear to have been administered by Lever and Talbott,[18] in the treatment of chronic pemphigus, as much as 205 cubic centimeters of the oily solution (equivalent in calcemic action to 2 grams of calciferol, or 80,000,000 international units of vitamin D) having been given in 27 days, with no ill effects except for nausea and vomiting. On the other hand these authors report two deaths, one attributed by them to hypercalcemia and the other to "renal failure aggravated by the ingestion of dihydrotachysterol." The first patient, a woman of 80, received 10 cubic centimeters of solution of dihydrotachysterol daily for seven days, after which she became drowsy and comatose and died on the 11th day. The serum calcium rose to 14.8 mg. per hundred cubic centimeters. The other patient was under treatment with dihydrotachysterol for 18 days, and died on the 32nd day. The non-protein nitrogen content of the serum was 44 mg. per hundred cubic centimeters on admission, and the authors concluded that dihydrotachysterol should not have been administered to this patient.

Maintenance Doses.—The dosage of the activated sterols necessary to maintain the serum calcium within normal limits, and to avoid the symptoms and sequelae of hypoparathyroidism, is dependent on the subject and certain physiologic variables, more particularly the degree of parathyroid deficiency present and the intake of calcium and phosphorus, and varies within wide limits. The extreme limits for dihydrotachysterol are given as from 0.15 to 1.0 cc. (0.75 to 5 mg. of the basic substance) daily. More commonly 0.3 to 0.5 cc. a day is needed. An equivalent dose of calciferol would be from 1.5 to 10 mg. (60,000-400,000 international units) a day for the extreme limits, and 3 to 5 mg. (120,000-200,000 international units) for the more commonly prescribed dose. The same variability, but within

18. Lever, Walter F., and Talbott, John H.: Action of Dihydrotachysterol in Chronic Pemphigus, Arch. Derm. and Syphilol. **43**: 341-356 (Feb.) 1941.

approximately the same limits, in the amounts of calciferol actually found to be necessary for maintenance doses is reviewed later.

Reports of accidental poisoning with vitamin D preparations, even when administered over long periods, have been rare in late years, most of the literature on the toxicity of these preparations having come from the deliberate administration of large doses in conditions other than hypoparathyroidism. In this connection Steck, Deutsch, Reed and Struck [11] have made an exhaustive investigation of the toxicity of vitamin D. For the most part they used concentrated preparations of viosterol, but comparable results were obtained with crystalline calciferol. They conclude that both human subjects and dogs generally survive administration of 20,000 international units of vitamin D per kilogram of body weight daily for indefinite periods without intoxication. Out of 773 human subjects receiving more than 100,000 international units daily over variable periods, 63 showed toxic symptoms, but with no deaths and with no effects from which recovery did not occur. Steinberg [19] administered a preparation of vitamin D, usually in doses of 160,000 international units a day, for periods of several weeks to one and one-half years, to 40 patients and observed no untoward effects. He concludes that the "toxicity [of vitamin D] has been overemphasized." On the other hand, Vrtiak and Lang [20] administered from 150,000 to 250,000 international units a day to 20 patients and report that all became nauseated and in a few frequency of urination and nocturia developed. In one of Stacey's cases [21] the serum calcium rose to 15 mg. per hundred cubic centimeters after long administration of from 4 to 8 mg. (160,000-320,000 international units) of calciferol daily. In another of his cases 728 mg. of calciferol in five months, or an average of 4.8 mg. (192,000 international units) a day, led to loss of weight, anorexia and epigastric pains after meals. All the symptoms disappeared when the treatment was discontinued.

19. Steinberg, C. L.: Massive Doses of Vitamin D in Chronic Arthritis: Its Effect on Calcium Metabolism, J. Lab. & Clin. Med. **24**: 17-24 (Oct.) 1938.

20. Vrtiak, E. G., and Lang, R. S.: Observations on the Treatment of Chronic Arthritis with Vitamin D, J. A. M. A. **106**: 1162-1163 (April 4) 1936; correction, ibid. **106**: 1828 (May 23) 1936.

21. Stacey, R. S.: Treatment of Low-Calcium Tetany with Calciferol, Lancet **2**: 656-658 (Sept. 21) 1935.

Ross and Williams [22] reported 4 cases of intoxication, with 2 deaths, in infants at the ages of 8 to 14 months, who received 20,000 to 40,000 international units of vitamin D a day in the form of a concentrated preparation of viosterol over a period of several months. An autopsy was performed on one patient and extensive metastatic calcification was found. In one of the patients who survived the serum calcium reached a level of 18 mg. per hundred cubic centimeters. Thatcher [23] reported a case in which a diagnosis of hypervitaminosis D was made at autopsy on a baby aged 11½ months from the finding of pathologic calcification in the kidney. The baby had never received more than about 600 international units of vitamin D in the form of cod liver oil a day and had usually received less. Other similar cases, reported as poisoning with vitamin D, were noted by Reed, Struck and Steck.[13]

No reports of fatal chronic poisoning with dihydrotachysterol have been found. Rose and Sunderman [24] reported serious symptoms following 2 cc. a day for twenty-six days; on the other hand, Curtis [25] gave 3 cc. daily during the last four months of pregnancy, the latter dose being the equivalent, in calcemic action, of 30 mg. (1,200,000 international units) of calciferol a day.

Symptoms and Pathology of Intoxication.—I have emphasized in this review that the toxic actions of the activated sterols may be dissociated from the antirachitic action of these substances and that the "calcemic principle," which causes elevation of the serum calcium, is identical with the characteristics of the sterols responsible for their toxic actions. Consequently therapeutic use of the calcemic principle depends on the administration, usually over long periods, of subtoxic doses of certain of the activated sterols.

Reed [13] took a different point of view. He dissociated the toxic reactions from vitamin D from the hypercalcemia and based his belief that the two reactions are "distinct, or only incidentally related," on the

22. Ross, S. G., and Williams, W. E.: Vitamin D Intoxication in Infancy, Am. J. Dis. Child. **58**: 1142 (Nov.) 1939.

23. Thatcher, L.: Hypervitaminosis D, Lancet **1**: 20-22 (Jan. 4) 1936.

24. Rose, E., and Sunderman, F. W.: Effect of Dihydrotachysterol in Treatment of Parathyroid Deficiency, Arch. Int. Med. **64**: 217-227 (Aug.) 1939.

25. Curtis, J. K.: Parathyroid Insufficiency Treated with Dihydrotachysterol (A. T. 10), M. Clin. North America **24**: 833-840 (May) 1940. Schwartz, H. A., Curtis, J. K., and Lichtenstein, J. V.: Hypoparathyroidism in Pregnancy, Treated with Dihydrotachysterol, Am. J. Obst. & Gynec. **41**: 697 (April) 1941.

observation that "hypercalcemia of extreme degree may occur without intoxication and that severe intoxication to the lethal stage may occur without hypercalcemia." He reported that in his laboratory 5 dogs had died, with unquestionable symptoms of intoxication, in which hypercalcemia was never observed, and I have had a similar experience. Moreover, it is a matter of common observation, both in dogs and in man, that there is no absolute correlation between the calcium level and the severity of other toxic manifestations in hypercalcemia.

From a theoretic standpoint these two points of view may perhaps be reconciled by recognition of the fact that hypercalcemia is one symptom of the toxic action and that its occurrence depends on a combination of circumstances, of which one is mobilization of calcium salts from the bones. Reed [26] demonstrated in balance experiments that increased excretion of calcium of pronounced degree may occur without hypercalcemia under the influence of large doses of vitamin D. It may be assumed that mobilization of the bone salt is a more or less constant manifestation of toxicity and that as a rule, but not invariably, this mobilization leads to an increase in the calcium level in the blood.

From the practical standpoint the question is not so much that of the identity or nonidentity of the toxic and calcemic principles of the activated sterols as it is whether elevation of the serum calcium, when desired, may be uniformly produced and maintained, without the occurrence of serious acute or chronic toxic manifestations. A few cases, refractory to dihydrotachysterol, have been reported.[27] The symptoms and pathologic manifestations of intoxication, as observed in man and in animals, will be discussed here.

According to Reed and his collaborators,[18] the earliest symptoms of intoxication, more commonly seen in man, resulting from administration of excessive doses of vitamin D preparations, are anorexia, frequency of urination and loss of weight in excess of that expected from the lessened food intake. If administration of the sterol is continued, gastrointestinal discomfort, vomiting, persistent nausea, muscular atony, headache, lassitude, polyuria and diarrhea, sometimes with bloody stools, may follow. In the fatal case with the fatal

26. Reed, C. L.: Personal communication to the author.
27. Holtz, F.: Nebenschildrüseninsuffizienz, Deutsche med. Wchnschr. **65**: 750-752 (May 12) 1939.

outcome reported by Kerr [17] similar symptoms were followed by coma and death, as they also are in animals.[11] In general, and if the dosage has not been excessive, the symptoms of intoxication disappear rapidly on cessation of administration of the toxic agent, but Freeman and Farmer have reported [28] and we have confirmed, the persistence of hypercalcemia and of other symptoms in dogs for as long as a month after the last dose of vitamin D. Moreover, as a rule, once toxic symptoms appear they are not relieved unless administration of the substance responsible is discontinued.[13]

The pathologic changes following intoxication with the activated sterols have been reviewed by Reed, Struck and Steck.[13] In addition to the changes in the bones, discussed in another section of this review, the important finding is calcification in the soft tissues. The kidney is most frequently involved, but the lungs and the thyroid are also found to contain areas of calcification in many instances. Calcification of the aorta also occurs in dogs following long-continued administration of subtoxic doses of vitamin D.

Goormaghtigh and Handovsky [15] have investigated the effects of calciferol on the dog, with special reference to the arterial system. They found that doses of about 0.1 mg. (4,000 international units) per kilogram a day are harmless, but doses of from 0.6 mg. (24,000 international units) per kilogram a day cause regressive lesions in arterioles, leading to cell necrosis in from seventeen to forty-five days. This is in close agreement with the toxic threshold of 20,000 international units per kilogram a day, established by Steck, Deutsch, Reed and Struck.[11] According to Goormaghtigh and Handovsky, the smooth muscle cell of the artery wall can recover as long as the necrotic stage has not been reached.

I have found little evidence in the literature of chronic injury in man resulting from administration of dihydrotachysterol or of products of vitamin D, but the possibility of such injury is by no means excluded. Reed, Struck and Steck [13] stated that their extensive use of vitamin D in large doses has not indicated that

28. Freeman, S., and Farmer, C. J.: Correlated Studies of Calcium, Inorganic Phosphorus and Serum Phosphatase in Normal Animals and in Animals Influenced by Irradiated Ergosterol, Am. J. Physiol. **113**: 209-220 (Sept.) 1935.

any chronic injury is to be expected if toxicity has not occurred, and that even when toxic effects have been observed it seems improbable that any residual chronic effects will follow. This is in agreement also with the recent report of Holtz [27] on the prolonged use of dihydrotachysterol.

MODE OF ACTION

The activated sterols, like the parathyroid hormone, owe their usefulness in the treatment of parathyroid insufficiency primarily to their ability to control the mobilization of calcium salts from the bones and thereby regulate the concentration of the calcium ion in the blood. The property of the sterols which is responsible for this action, and which has been designated the "calcemic principle," is regarded in this review as identical with the property which, in sufficiently large doses, leads to toxic symptoms, and in fact the hypercalcemia produced by the activated sterols in normal animals is itself a manifestation of the toxic action.

The ordinary antirachitic doses of vitamin D are generally believed to produce their effects by increasing the net absorption of calcium, and perhaps also of phosphates, from the gastrointestinal tract. While infants with low calcium tetany associated with rickets usually respond to antirachitic doses of vitamin D by a rise in serum calcium, the effect of such doses on the absorption of calcium is not sufficient of itself to produce a rise of the serum calcium level either in normal individuals or in individuals with hypocalcemia resulting from parathyroid insufficiency. When calcemic doses of vitamin D are given, however, the calcemic effect is added to by increased absorption of calcium. Consequently, and although the calcemic effect of vitamin D may be produced without the feeding of calcium, smaller doses of the sterol may produce the same effect on the serum calcium level if an adequate intake of calcium is provided. The effect of dihydrotachysterol on the absorption of calcium [29] and the fact that this substance is also more effective when given in combina-

29. Albright, Fuller; Bloomberg, Esther; Drake, T., and Sulkowitch, H. W.: A Comparison of the Effects of A. T. 10 (Dihydrotachysterol) and Vitamin D on Calcium and Phosphorus Metabolism in Hypoparathyroidism, J. Clin. Investigation **17**: 317-329 (May) 1938. Albright, Fuller, and Sulkowitch, H. W.: The Effect of Vitamin D on Calcium and Phosphorus Metabolism; Studies on Four Patients, ibid. **17**: 305-315 (May) 1938.

tion with an increased intake of calcium may be due to its residual antirachitic activity. The effect on absorption of calcium is apparently lacking in the case of solution of parathyroid.[29]

Vitamin D (in calcemic doses), dihydrotachysterol and solution of parathyroid in ascending order,[29] lead to a decided increase in excretion of phosphate by the kidneys, the mechanism of which has recently been studied.[30] The lowered serum calcium in hypoparathyroidism is characteristically accompanied by an increase in the phosphate concentration in the serum. The increased excretion of phosphate through the kidneys, when any of the aforementioned agents is administered, permits a rise in serum calcium with a concomitant fall in serum phosphate while both calcium and phosphate are being added to the blood by liberation of these minerals from the bones. Albright[31] adhered to the view that the fall in serum phosphate actually brings about the mobilization of calcium from the bones, by causing the serum to be less saturated with respect to the bone salt. Von Brand, Holtz and Putschar[32] found the effect of the calcinosefaktor on the serum phosphate level in animals to be quite irregular, a rise in serum phosphate at times following the rise in serum calcium. Hoff[33] found the serum phosphate to rise, following administration of dihydrotachysterol, in contrast to the fall induced by solution of parathyroid.

There is now a considerable literature[34] on the effects of large doses of vitamin D on bone. That Holtz and his co-workers considered this action identical with that of the preparations studied by them is indicated by the fact that they did not study the bones of their animals

30. Harrison, H. E., and Harrison, H. C.: The Renal Excretion of Inorganic Phosphate in Relation to the Action of Vitamin D and Parathyroid Hormone, J. Clin. Investigation **20**: 47-55 (Jan.) 1941.

31. Albright, Fuller: Renal Osteitis Fibrosa Cystica, Tr. A. Am. Physicians **51**: 199-212, 1936.

32. von Brand, Theodor; Holtz, F., and Putschar, W.: Vergleichende pharmakologische Untersuchungen über Calcinosefaktor und Nebenschilddrüsenhormone, Arch. f. exper. Path. u. Pharmakol. **167**: 113-145, 1932.

33. Hoff, F.: Untersuchungen über die Wirkung des Präparates, A. T. 10 bei Parathyreopriver Tetanie, Arch. f. exper. Path. u. Pharmakol. **177**: 204-211, 1935.

34. Ham, A. W., and Lewis, M. D.: Hypervitaminosis D Rickets: Action of Vitamin D, Brit. J. Exper. Path. **15**: 228-234 (Aug.) 1934. Raab, H., and Cohn, B. N. E.: Knochenveränderungen bei Vigantol-Vergiftung, Frankfurt. Ztschr. f. Path. **47**: 152-158, 1935. Jones, J. H., and Robson, G. M.: The Effect of Overdoses of Irradiated Ergosterol, Administered for Approximately Two Months, on the Composition and Structure of the Bones of Rats, Am. J. Physiol. **103**: 338-350 (Feb.) 1933. Selye.[35]

but referred [32] to the studies of Hoff and Homann [35] on vitamin D as authority for the statement that the calcinosefaktor leads to demineralization of bone. Morris and McLean [36] believe that the effects of the activated sterols on bone result from a direct action on bone tissue. They have found that both calciferol and dihydrotachysterol produce resorption of bone and mobilization of bone mineral and that the latter effect can be visualized as described by McLean and Bloom [37] following administration of solution of parathyroid. These actions are followed by overproduction of new bone, as previously described for both vitamin D and solution of parathyroid,[38] but without the overproduction of fibrous tissue characteristic of hyperparathyroidism (osteitis fibrosa). It is my belief that the calcemic effect of the activated sterols results primarily from a continuous influence of these substances on the interchange of calcium between plasma and bone, resulting in an increased mobilization of bone salt sufficient to keep the serum calcium concentration at higher levels.

Comparative Studies.—It has not been shown by comparative physiologic studies or clinical trial that either calciferol or dihydrotachysterol is superior to the other in the management of parathyroid insufficiency. Throughout the experimental work leading to the introduction of dihydrotachysterol the calcinosefaktor was regarded as common to a variety of substances and of preparations, and it was stated [32] that the action of this factor was completely independent of whether the vitamin D activity in the preparations used was destroyed by heating or reduction or not. The nonspecificity of the calcemic effect has again been emphasized by von Werder,[3] and the method used for standardization of dihydrotachysterol, which depends on the toxicity in mice, is in no way capable of distinguishing between the qualitative actions of the various sterols.

35. Hoff, F., and Homann, E.: Zur Frage des Einflusses von Vitamin D und Epithelkörperhormon auf den Kalkhaushalt, Ztschr. f. d. ges. exper. Med. **74**: 258-273, 1930.

36. Morris, W. D., and McLean, F. C.: The Mode of Action of the Activated Sterols on Bone, A Comparative Study, in preparation.

37. McLean, F. C., and Bloom, W.: Mobilization of Bone Salt by Parathyroid Extract, Arch. Path. (Sept.) 1941.

38. Selye, Hans: On the Stimulation of New Bone Formation with Parathyroid Extract and Irradiated Ergosterol, Endocrinology **16**: 547-558 (Sept.-Oct.) 1932.

Holtz has published no experiments with a preparation identifiable with dihydrotachysterol as now distributed. However, von Brand, Holtz and Putschar [32] compared the actions of various preparations, standardized in terms of their toxicity in mice, and of solution of parathyroid, using a large number of animals of various species. They found numerous differences; in the nature of the pathologic calcification produced, in the curve of the rise and fall of the serum calcium and in the relative susceptibility of the various species to the two substances. They consequently assumed a difference in the mode of action of the sterols and of solution of parathyroid but found, in spite of these differences, that in experimental tetany in dogs, resulting from a deprivation of parathyroid tissue, the action of the parathyroid hormone could be completely substituted for by the calcinosefaktor. In a subsequent paper Holtz, Gürsching and Kraut [39] confirmed these results by observations in man.

Albright, Bloomberg, Drake and Sulkowich [29] have reported studies of the actions of dihydrotachysterol and of calciferol which have been much quoted. They made comparative studies of the actions of these two substances on the calcium and phosphorus metabolism of 3 patients with parathyroid insufficiency, and a similar study of the action of solution of parathyroid in 1 of these cases. They found that the two sterols have the same two actions of increasing calcium absorption from the gastrointestinal tract and of increasing phosphorus excretion in the urine, the ratio of the latter action to the former being greater with dihydrotachysterol. They noted also that the action of calciferol was slower in coming on and lasted longer than that of dihydrotachysterol. Albright, Sulkowitch and Bloomberg [40] reported similar results in a case of vitamin D-resistant rickets, with the exception that dihydrotachysterol did not cause as marked a rise in excretion of urinary phosphorus. In none of these studies was consideration given to the possibility of a direct effect of the preparations on bone. They gave calciferol at

39. Holtz, F.; Gürsching, J., and Kraut, H.: Vergleichende pharmakologische Untersuchungen über Calcinosefaktor und Nebenschilddrüsenhormon, Arch. f. exper. Path. u. Pharmakol. **174**: 51-62, 1933.

40. Albright, Fuller; Sulkowitch, H. W., and Bloomberg, Esther: A Comparison of the Effects of Vitamin D, Dihydrotachysterol (A. T. 10) and Parathyroid Extract on the Disordered Metabolism of Rickets, J. Clin. Investigation **18**: 165-169 (Jan.) 1939.

the most in amounts equal to those given of dihydrotachysterol, while for strictly comparable effects the ratio should have been at least 2 to 1.[36]

Himsworth and Maizels [41] treated a patient with congenital hypoparathyroidism for six years, mainly with calciferol. The dosage was maintained, during the greater part of the time, at 12.5 mg. (500,000 international units) a week, with excellent results. During an interim period of ten months dihydrotachysterol was substituted for calciferol. It was effective in controlling the tetany, but the impression of the authors was that "in this patient vitamin D_2 produced a more stable state than did A. T. 10." They used up to 6 cc. a week of the dihydrotachysterol preparation, which should have been equivalent to 60 mg. of calciferol, or nearly five times the dosage of the latter actually employed. Poer [42] has compared dihydrotachysterol and calciferol in the treatment of 11 cases of post-thyroidectomy hypocalcemic tetany. He found that the two substances are equally effective in controlling the symptoms of hypoparathyroidism and in maintaining the serum calcium level near the normal. His usual daily maintenance doses were 1.25 mg. (50,000 international units) of calciferol and 0.3 cc. (1.5 mg.) of dihydrotachysterol. It has not been found by others that this amount of calciferol is usually adequate for a maintenance dose.

Morris and McLean [36] have studied the actions of these two substances in rats. They compared the loss of weight, the mortality, the effects on the bones, as observed histologically, and the onset, distribution and intensity of pathologic calcification in the soft tissues. When given in a ratio of 2.24 mg. of calciferol to 1 mg. of dihydrotachysterol, the loss of weight of litter mates fed the two substances became indistinguishable, and the effects with reference to the other criteria enumerated were also comparable. Using the same dosage ratio, they administered the two substances to dogs. The serum calcium curves showed no significant differences, both substances exhibiting the same ability to raise the serum calcium. From these experiments it is concluded that calciferol must be administered in the ratio of at

41. Himsworth, H. P., and Maizels, M.: Vitamins D^2 and D^3 and A. T. 10 in Congenital Thyroid and Parathyroid Deficiency, Lancet **1**: 959-960 (May 25) 1940.

42. Poer, D. H.: Dihydrotachysterol, Parathormone and Vitamin D^2; Comparison of Their Values in the Treatment of Post-Thyroidectomy Hypocalcemic Tetany, South. M. J. **33**: 1174-1180 (Nov.) 1940.

least 2 mg. to 1 mg. of the basic substance of the commercially available solution of dihydrotachysterol to obtain the same calcemic effect. Others have reported a still higher ratio for the doses necessary to produce comparable toxic effects in mice [39] and in rats [43] (table 1), but McChesney and Kocher [44] found that the hypercalcemia produced in rats by a single dose of 2.5 mg. of calciferol was greater, both in height and duration, than that produced by 2.5 mg. of the basic substance of commercial dihydrotachysterol, but less than that produced by 5.0 mg. of this material. McChesney and Messer [45] have found that vitamin D_3, administered in single large doses to dogs, is followed by long persistence of a moderate degree of hypercalcemia, while dihydrotachysterol causes a rapid rise of serum calcium, followed by a rapid fall, and vitamin D_2, in comparable doses, fall between the two in its effects. In other respects the effects upon dogs were closely comparable.

Shohl [4] has made comparative studies of the pathologic changes induced in rats by toxic doses of calciferol and of dihydrotachysterol and has found that the two produce similar effects. Both caused striking lesions in the kidneys and blood vessels. Calcification was produced in the gastric mucosa. Both caused demineralization of the bones and hypercalcification at the zone of provisional calcification. Neither led to necrosis of the liver produced by an early preparation of irradiated ergosterol,[46] and now believed to be due to its high content of toxisterol.[16]

The comparative studies quoted indicate a qualitative difference between the actions of toxisterol on the one hand and vitamin D and dihydrotachysterol on the other. They fail, however, to disclose any difference in the actions of the two latter substances on which a preference for either one or the other may be based. Further carefully controlled comparative studies, in both clinical and experimental hypoparathyroidism, are required

43. Shohl, A. T., and Farber, S.: Effect of A. T. 10 (Dihydrotachysterol) on Rickets in Rats Produced by High Calcium-Low Phosphorus Diets, J. Nutrition **21**: 147-154 (Feb.) 1941.

44. McChesney, Evan W., and Kocher, Hugo: Comparison of Effects of Large Doses of Various Activated Sterols on Serum Calcium, Proc. Soc. Exper. Biol. & Med. **47**: 156-159 (May) 1941.

45. McChesney, Evan W., and Messer, Frederick: Personal communication to author.

46. Shohl, A. T.; Goldblatt, H., and Brown, H. B.: The Pathological Effects on Rats of Excess Irradiated Ergosterol, J. Clin. Investigation **8**: 505-531 (June) 1930.

before it can be finally said that calciferol and dihydrotachysterol are of equal value in the management of this condition or that either has advantages over the other.

CLINICAL EXPERIENCE WITH DIHYDROTACHYSTEROL

Of a large number of reports on the use of dihydrotachysterol in hypoparathyroidism, virtually all are unreservedly favorable. In nearly every report the possibility of overdosage is discussed and the necessary precautions are described, but there are few instances of actual toxic manifestations. In a discussion of the first eight years' experience with the preparation, Holtz [27] stated that long-continued administration is without harmful effects and without development of tolerance, that no lasting damage is done if a temporary hypercalcemia of about 15 mg. per hundred cubic centimeters is induced by overdosage and that the danger of toxic effects was overemphasized in the earlier publications. He reported that an occasional patient refractory to dihydrotachysterol was seen. This report of Holtz reflects the general tenor of the large German clinical literature, which will not be reviewed in detail here.

Dihydrotachysterol appears to have been first used in this country by Arnold and Blum,[47] who reported favorably on its effectiveness in 2 cases of postoperative and 1 of idiopathic tetany. Swinton [48] reported favorably on 6 cases of postoperative tetany. MacBryde,[49] from observation of its effects in 6 cases of chronic hypoparathyroidism and 1 of idiopathic tetany, stated that it was the only therapeutic measure in his experience to yield excellent results in treatment of chronic tetany. Pickhardt and Bernhard [50] reported 2 cases of postoperative tetany. Albright, Bloomberg, Drake and Sulkowitch [29] made extensive metabolic studies of 3 cases, concluding that dihydrotachysterol "is a most efficacious therapeutic agent in the treatment of hypo-

47. Arnold, C. H., and Blum, H.: The Control of Hypoparathyroidism, West. J. Surg., Obst. & Gynec. **44**: 56-65 (Sept.) 1936.

48. Swinton, N. W.: Postoperative Parathyroid Tetany, New England J. Med. **217**: 165-169 (July 29) 1937.

49. MacBryde, C. M.: The Treatment of Parathyroid Tetany with Dihydrotachysterol, J. A. M. A. **111**: 304-307 (July 23) 1938; South. M. J. **31**: 720-725 (July) 1938.

50. Pickhardt, O. C., and Bernhard, A.: The Treatment of Postoperative Tetany with Dihydrotachysterol, Ann. Surg. **108**: 362-373 (Sept.) 1938.

parathyroidism." Rose and Sunderman [24] reported 5 cases of hypoparathyroid deficiency, their observations including determinations of calcium balance, total and diffusible serum calcium, serum protein and inorganic serum phosphorus before and during administration of dihydrotachysterol. They concluded that this preparation is highly effective in increasing the concentration of calcium in the serum and in relieving the symptoms of parathyroid deficiency. In 1 of their cases, following 2 cc. of dihydrotachysterol daily for twenty-six days, severe symptoms of intoxication appeared, including vertigo, tinnitus, thirst, polyuria, nausea and abdominal cramps, with a rise of the serum calcium to 15.7 mg. per hundred cubic centimeters.

Numerous other reports have appeared,[51] the tone of all being favorable and all emphasizing the advantages of dihydrotachysterol over solution of parathyroid in the management of the hypocalcemia associated with parathyroid insufficiency. The reports are virtually unanimous with respect to the details of management. An initial dosage of 1 to 4 cc. daily is commonly con-

51. These include:
Greene, J. A., and Swanson, L. W.: Treatment of Hypoparathyroidism, with a Discussion of the Use and Action of Dihydrotachysterol (A. T. 10), J. Iowa M. Soc. **29**: 275-279 (July) 1939.
Hurxthal, L. M., and Claiborne, T. S.: Treatment of Tetany with Dihydrotachysterol (A. T. 10), New England J. Med. **220**: 911-916 (June 1) 1939.
Berk, J. E.: Clinical Experience with Dihydrotachysterol in the Management of Idiopathic Hypocalcemia, Endocrinology **25**: 984-990 (Dec.) 1939.
Blackford, J. M., and Hallenbeck, G. A.: Hypoparathyroidism, Treatment by Dihydrotachysterol, Clinics of the Virginia Mason Hospital, Seattle **19**: 25-29 (June) 1940.
Franco, S. C.: Parathyroid Tetany: Chronic Parathyroid Insufficiency of Ten Years' Duration Successfully Controlled with Dihydrotachysterol, Ann. Int., Med. **14**: 529-532 (Sept.) 1940.
Richards, C. G.: Postoperative Tetany, Rocky Mountain M. J. **37**: 436-440 (June) 1940.
Wilkinson, T. C.: Treatment of Tetany with Dihydrotachysterol, Pennsylvania M. J. **44**: 37-40 (Oct.) 1940.
Newman, H. F.: A Case of Hypoparathyroidism Treated with Dihydrotachysterol, J. Mount Sinai Hosp. **6**: 327-332 (March-April) 1940.
Weber, F. C., Jr., and Richardson, H. B.: Dihydrotachysterol (A. T. 10) and Mineral Metabolism: A Metabolic Study, J. Clin. Endocrinol. **1**: 32 (Jan.) 1941.
Margolis and Krause.[80]
Adams.[81]
Ryan.[80]
Cochrane, R. C.: Parathyroid Adenoma; Report of Three Cases, New England J. Med. **224**: 973 (June 5) 1941.
Emerson, K.; Walsh, F. B., and Howard, J. E.: Idiopathic Hypoparathyroidism; A Report of Two Cases, Ann. Int. Med. **14**: 1256 (Jan.) 1941.
Kowallis, G. F.: Spontaneous Parathyroid Insufficiency; Report of a Case, Proc. Staff Meet., Mayo Clin. **16**: 129-132 (Feb. 26) 1941.
Ryan, E. J., and McCullagh, E. P.: The Use of A. T. 10 in Chronic Tetany, Ohio State Med. J. **37**: 430-432 (May) 1941.

tinued until the serum calcium level rises to 9 to 10 mg. per hundred cubic centimeters and the symptoms of tetany subside. Following this, individualization of the maintenance dose becomes necessary, and this dose is determined by trial and error. The maintenance dose varies between 0.15 and 1 cc. a day and is more commonly 0.3 to 0.5 cc., but owing to the duration of the action of the substance the required dosage need not be given more often than twice a week. The rise of serum calcium may occur without any addition of calcium to the dietary intake, but the same effect can be obtained with considerably lower dosage of the sterol if the calcium intake is supplemented with from 12 to 20 Gm. daily of a calcium salt, commonly the lactate, chloride or gluconate. The desirability of a low intake of phosphorus is also emphasized.

The most acceptable criterion for regulation of the dosage is frequent observation of the serum calcium level, but the Sulkowitch test, introduced by Albright,[52] appears to be reliable, especially if checked occasionally with determinations of the serum calcium. The test gives a simple and rapid means of detecting or excluding excessive excretion of calcium in the urine, from which the presence or absence of hypercalcemia may be inferred. It has the great advantage that it may be applied daily, or even more frequently, by the patient himself, in much the same way that diabetic patients learn to perform rough quantitative tests for sugar in their urine. According to Goormaghtigh and Handovsky,[15] a serum calcium concentration of 13 mg. per hundred cubic centimeters should be cause for alarm, as it is the prelude to massive calcium excretion, tubular distention and subsequent glomerular regression.

Holtz and Rossmann[53] found that the dose of dihydrotachysterol had to be increased as much as six times during the latter half of pregnancy, and this has been confirmed by Curtis,[25] who gave 3 cc. daily during the last four months of pregnancy, or a dose equivalent to 30 mg. (1,200,000 international units) of calciferol

52. Albright, Fuller: Note on the Management of Hypoparathyroidism with Dihydrotachysterol, J. A. M. A. **112**: 2592-2593 (June 24) 1939.

53. Holtz, F., and Rossmann, E.: Schwangerschaft und Tetanie, Ztschr. f. Geburtsh. u. Gynäk. **116**: 187-199, 1938.

a day. Holtz and Rossmann [54] also found that the dosage of dihydrotachysterol may have to be increased during menstruation and that the effectiveness of a given dose is reduced by the administration of estradiol benzoate. On the other hand, the requirement for dihydrotachysterol is decreased after roentgen castration in women.[54]

Holtz and Kramer [55] have found that larger doses are required during periods of activity, nervous strain and menstruation, and Arnold and Blum [47] have also noted the increased requirement when the patient becomes very active. The variations that are required in dosage with pregnancy and with administration of estrogen are reminiscent of the early observations of the influence of estrus, pregnancy and lactation on the course of experimental parathyroid tetany.[56]

Hypercalcemia and other toxic manifestations of overdosage of dihydrotachysterol are usually managed by withdrawal of the medication, including the calcium added to the intake, by rest in bed and by increasing the intake of fluid. Holtz and Rossmann [54] suggested the administration of large doses of estrogens to combat toxicity, and Seiferth and Kolb [57] reported that the vitamin B complex and vitamin C have a far reaching inhibitory influence on the toxic effects of dihydrotachysterol. They reported that vitamin A increases the toxicity of this sterol, but Morgan, Shimotori and Hendricks [58] stated that a low intake of vitamin A leads to an increase in the damaging effects of toxic doses of vitamin D. None of these antagonistic actions appears to have been given adequate clinical trial in actual poisoning with the activated sterols, but Holtz [59] states that intramuscular injections of large doses of

54. Holtz, F., and Rossmann, E.: Ueber Beziehungen der Sexualhormone zum Kalkstoffwechsel und zu den Nebenschilddrüsen, Ztschr. f. Geburtsh. u. Gynäk. **116**: 199-212, 1938.

55. Holtz, F., and Kramer, F.: Ueber Nebenschilddrüsentetanie, Kalkhaushalt, elektrische Erregbarkeit und A. T. 10, Naturwissensch. **24**: 177-182 (March) 1936.

56. Dragstedt, L. R.: The Physiology of the Parathyroid Glands, Physiol. Rev. **7**: 499-530 (Oct.) 1927.

57. Seiferth, L. B., and Kolb, H.: Tierexperimentelle Untersuchungen über die Wirkung der Vitamine A, B and C bei A. T. 10-Vergiftung, Ztschr. f. d. ges. exper. Med. **106**: 167-180, 1939.

58. Morgan, A. F.; Shimotori, N., and Hendriks, J. B.: Progress of Hypervitaminoses D_2 and D_3 and Recovery in Rats, as Affected by Dietary Calcium and Phosphorus and Vitamin A, J. Biol. Chem. **134**: 761-779 (July) 1940.

59. Holtz, F.: Dihydrotachysterol; The Modern Treatment of Parathyroid Insufficiency, J. Clin. Endocrinol. **1**: 453-458 (May) 1941.

follicular hormone (about 100,000 international units) will hasten reduction of the serum calcium level when hypercalcemia has been inadvertently produced.

In judging the usefulness of an activated sterol over a long period, consideration should be given to its ability to prevent or to relieve the complications or sequelae of parathyroid insufficiency, as well as to its ability to raise the serum calcium level. Of these, the most troublesome is the occurrence of cataracts. Klemens [60] described the opacities in the lens characteristic of hypoparathyroidism, as seen with the aid of the slit lamp, and stated that a presumptive diagnosis of the nature of the condition can usually be made from the changes in the eye alone. He stated that administration of dihydrotachysterol prevents this condition and as a rule arrests its progress if it has already begun before the preparation is administered. Because there is a widespread impression to the contrary, he emphasizes his statement that no clearing up of already formed opacities of the lens can be expected from administration of dihydrotachysterol. Schmidt-La Baume [61] has reviewed the use of dihydrotachysterol in dermatologic conditions associated with hypocalcemia, more especially in impetigo herpetiformis. It is improbable that the improvement reported in pemphigus [18] or that in generalized scleroderma,[62] following administration of dihydrotachysterol, can be attributed to its effect upon the serum calcium level.

Certain cerebral and psychic symptoms are frequently associated with hypoparathyroidism. They include restlessness, irritability, depression, headache and occasional epileptiform convulsions, of central origin. These and other manifestations referable to the central nervous system are said to be relieved promptly by treatment with dihydrotachysterol. Barr, MacBryde and Sanders [63] reported a case of idiopathic hypocalcemic tetany, associated with increased intracranial

60. Klemens, F.: Auge und Epithelkörperchen-Unterfunktion, Deutsche med. Wchnschr. **65**: 753-754 (May 12) 1939.

61. Schmidt-La Baume, F.: Die Bedeutung des A. T. 10 für die Dermatologie als Substitutionstherapie bei Hypocalcinosen, Med. Klin. **33**: 1590-1595 (Nov. 26) 1937.

62. Perez, Willy M.; Soffer, Louis J., and Silbert, Samuel: Improvement in Two Cases of Diffuse Scleroderma by the Use of Dihydrotachysterol (A. T. 10), J. Mt. Sinai Hosp. **6**: 333-337 (March-April) 1940.

63. Barr, D. P.; MacBryde, C. M., and Sanders, T. E.: Tetany with Increased Intracranial Pressure and Papilledema: Results from Treatment with Dihydrotachysterol, Tr. A. Am. Physicians **53**: *227-232*, 1938.

pressure and papilledema, together with beginning opacities of the lens. The case responded promptly to treatment with dihydrotachysterol, and the authors suggested that edematous changes in the brain or meninges may occur in hypoparathyroidism more frequently than is recognized and that this factor may be of importance in producing cerebral and psychic manifestations of tetany. Eaton and Haines [64] reported 3 cases of parathyroid insufficiency with symmetrical cerebral calcification, in 1 of which the symptoms were relieved by administration of dihydrotachysterol. They regard symmetrical cerebral calcification, mental deterioration and convulsions as concomitant but not necessarily interdependent cerebral symptoms of hypoparathyroidism. Greene and Swanson [65] report five cases of psychosis in hypoparathyroidism. One case was treated with dihydrotachysterol, with cessation of convulsions and marked improvement of the psychosis, but convulsions returned and the psychosis became aggravated to its previous intensity while the serum calcium was still high.

Dihydrotachysterol has been little used in infantile tetany, and Chu and Sung [66] regarded it as of doubtful advantage in view of the common association of this condition with vitamin D deficiency and rickets. Bloxsom,[67] however, has reported that hypocalcemic tetany in an infant, believed to be of parathyroid origin, was controlled by this sterol, but, it will be noted, in doses large enough to have an appreciable vitamin D activity. Eisenstein [68] has reported a case of tetany in an adult, regarded as "secondary to prolonged intestinal dysfunction," relieved by the administration of dihydrotachysterol. The preparation has also been used in other conditions, even more remote from disorders of the parathyroids. Its use in the treatment of oto-

64. Eaton, L. McK., and Haines, S. F.: Parathyroid Insufficiency with Symmetrical Cerebral Calcification: Report of Three Cases, in One of Which the Patient Was Treated with Dihydrotachysterol, J. A. M. A. **113**: 749-753 (Aug. 26) 1939.

65. Greene, James A., and Swanson, L. W.: Psychosis in Hypoparathyroidism; with Report of Five Cases, Ann. Int. Med. **14**: 1233-1236 (Jan.) 1941.

66. Chu, T. F., and Sung, C.: Tetany in Infancy and Childhood: A Clinical Study of 45 Cases Seen in North China, with Special Reference to Etiology, J. Pediat. **16**: 607-623 (May) 1940.

67. Bloxsom, A.: Treatment of Tetany of the Newborn Infant with Dihydrotachysterol, J. Pediat. **16**: 344-346 (March) 1940.

68. Eisenstein, V. W.: Chronic Adult Tetany of Gastrointestinal Origin: Treatment with Dihydrotachysterol: Report of a Case, Pennsylvania M. J. **44**: 33-36 (Oct.) 1940.

sclerosis, introduced by Seiferth [69] on the basis of reported slight reduction of serum calcium values in this condition, is stated by Schütz [70] to be without theoretical justification or empirical usefulness.

Blum [71] has reported that dihydrotachysterol will control the symptoms of hypoparathyroidism over a period of years, without losing its effectiveness, but the American literature is still inadequate with respect to reports of the progress of patients kept under observation and under treatment with dihydrotachysterol over extended periods. It would be desirable to have further reports on some of the cases in which treatment was instituted some years ago.

VITAMIN D IN CLINICAL HYPOPARATHYROIDISM

It will be recalled, from the foregoing discussion of dosage and toxicity, that vitamin D ordinarily has no effect on the serum calcium level unless administered in a daily dosage range of 60,000 to 200,000 international units or even higher and that consequently no effect in the treatment of hypoparathyroidism, comparable to the beneficial effects reported from the administration of dihydrotachysterol, can be expected unless vitamin D is administered at these levels. Irradiated ergosterol was administered in these amounts in the treatment of clinical hypoparathyroidism as early as 1928 with good results,[72] but in most instances the dosage of vitamin D employed has been much below that which is necessary to produce the effects under discussion. The early literature has been reviewed by Boothby and Davis,[73] and many of the unfavorable or equivocal results quoted by them can be clearly traced to insufficient dosage. Except for purposes of comparison, this review will refer only to clinical experience with adequate doses of preparations of vitamin D.

Bauer, Marble and Claflin [74] reported in 1932 on 3 cases of hypoparathyroidism in which irradiated ergos-

69. Seiferth, L. B.: Ueber die Aetiology und Behandlung der Otosklerose, Arch. f. Ohren-, Nasen- u. Kehlkopfh. **143**: 429-455, 1937.

70. Schütz, in discussion on Holtz, F.: Deutsche med. Wchnschr. **65**: 781 (May 12) 1939.

71. Blum, Henry: The Further Use of Dihydrotachysterol (A. T. 10), West. J. Surg., Obst. & Gynec. **49**: 113 (Feb.) 1941.

72. Stern, A.: Zur Therapie der parathyreopriven Tetanie mid Vigantol, Deutsche med. Wchnschr. **54**: 1292 (Aug. 3) 1928.

73. Boothby, W. M., and Davis, A. C.: Treatment of Postoperative Parathyroid Insufficiency; An Interpretative Review of the Literature, Arch. Int. Med. **58**: 160-184 (July) 1936.

74. Bauer, Walter; Marble, Alexander, and Claflin, Dorothy: Studies of the Mode of Action of Irradiated Ergosterol, J. Clin. Investigation **11**: 47-62 (Jan.) 1932.

terol was administered in doses of 5 mg. a day. The preparation was that responsible for much of the confusion concerning toxicity of vitamin D. Beneficial results were observed in all 3 cases, but only when the calcium intake was adequate. Reed and Seed [75] reported in 1933 on the treatment of 10 cases of parathyroid tetany in which viosterol was administered in doses up to 920,000 international units a day over periods of several days. Reed, Struck and Steck [13] reported on the subsequent history of these patients, together with 5 more whose treatment was initiated after publication of the original report. In 1939 all but 5 of the 15 patients in the series had been able to discontinue vitamin D therapy without recurrence of tetany. Of these 5, 4 had continued the treatment for six or seven years but were able to secure freedom from tetany on approximately one tenth of the dosage required at first, and without recourse to calcium.

Freyberg, Grant and Robb [76] emphasized the necessity for large doses if satisfactory results from the administration of vitamin D in hypoparathyroidism are to be expected. They gave as much as 200,000 international units of vitamin D as viosterol a day, with definite benefits, but most of the time used from 20,000 to 40,000 units. Stacey [21] gave up to 8 mg. (320,000 international units) of calciferol a day, with favorable results. Himsworth and Maizels [41] reported a case in which calciferol was given for six years, with excellent results. For the greater part of this time the dosage was maintained at 12.5 mg. (500,000 international units) a week. During one period vitamin D_3 was substituted for calciferol (vitamin D_2), with identical results. Farquharson [77] undertook to find the smallest daily dose of irradiated ergosterol that would relieve the symptoms of 2 patients suffering from severe chronic postoperative tetany refractory to other forms of treatment. One of these patients became free from tetany and remained well for four and one-half years with a daily dose of 94,000 international units of vitamin D. In another

75. Reed, C. I., and Seed, L.: The Treatment of Clinical Tetany with Irradiated Ergosterol, Endocrinology **17**: 136-148 (March-April) 1933.

76. Freyberg, R. H.; Grant, R. L., and Robb, M. A.: Hypoparathyroidism: The Treatment of Chronic Cases, J. A. M. A. **107**: 1769-1775 (Nov. 28) 1936.

77. Farquharson, R. F.: Hypoparathyroidism and Tetany, Univ. Toronto M. J., November-December 1938.

patient, with the same dose, favorable results were obtained, the symptoms of tetany recurring when the medication was discontinued. Klatskin [78] made a careful study of 2 cases of chronic parathyroid tetany, giving calciferol in doses up to 7.5 mg. (300,000 international units) a day, without the occurrence of toxic manifestations. He recommended calciferol "as a valuable therapeutic agent in cases of chronic parathyroid tetany which do not respond to high calcium-low phosphorus diets and in whom satisfactory transplants of parathyroid gland are not possible." Poer [42] has used calciferol, in doses of 1.25 mg. (50,000 international units) a day, with effects comparable to those

TABLE 2.—*Summary of Reports Favorable to Dihydrotachysterol and Unfavorable to Vitamin D*

Authors	Dihydrotachysterol: Daily Dose Giving Favorable Effect, Cc.	Vitamin D: Equivalent to Dihydrotachysterol, Units	Vitamin D: Maximum Actually Given, Units
Margolis and Krause [75]	1.0	400,000	1,700
Ryan [75]	0.5	200,000	3,000
Curtis [26]	1.0	400,000	10,000
MacBryde [48]	0.25-1.0	100,000-400,000	40,000
Adams [76]	2.0	800,000	120,000

seen with dihydrotachysterol. Anderson and Lyall [79] found that the minimum dose of vitamin D necessary to raise the serum calcium to a normal level in parathyroid deficiency appears to lie between 30,000 and 40,000 international units a day.

Of especial interest, in view of the conclusions in this review, are the reports in which vitamin D preparations are compared unfavorably with dihydrotachysterol. Table 2 summarizes data derived from five such reports [80] and includes the dosage of dihydrotachysterol

78. Klatskin, G.: On the Actions of Crystalline Vitamin D^2 (Calciferol) in Chronic Parathyroid Tetany, J. Clin. Investigation **17**: 441-443 (July) 1938.

79. Anderson, I. A., and Lyall, A.: Treatment of Chronic Hypoparathyroidism, Quart. J. Med. **8**: 209-232 (July) 1939.

80. Margolis, H. M., and Krause, Gilbert: Postoperative Parathyroid Tetany, Complete Control of the Manifestations by Means of Dihydrotachysterol: Report of a Case, J. A. M. A. **112**: 1131-1133 (March 25) 1939. Ryan, E. J.: The Treatment of Postoperative Parathyroid Tetany; Use of Dihydrotachysterol (A. T. 10), M. Clin., North America **24**: 443-449 (March) 1940. Curtis.[26] MacBryde.[49] Adams.[81]

required to produce the desired effect and the maximum dosage of vitamin D which failed to produce this effect. The latter is compared with the dosage of vitamin D calculated to be equivalent in "calcemic principle" with the dosage of dihydrotachysterol.

It is at once apparent from table 2 that the chief source of the unfavorable comparisons made by the authors of these reports is a misapprehension as to what constitute doses of vitamin D comparable to those of dihydrotachysterol. In three of the five reports the doses of vitamin D are 10,000 international units a day or less, or within an entirely different dosage range from that required to raise the serum calcium level. MacBryde [49] used up to 40,000 international units a day, or doses on the borderline of those found by others to be effective in the treatment of parathyroid insufficiency, but gave no further details. In the single case reported by Adams [81] he gave 120,000 international units of vitamin D daily for two weeks during subacute tetany five weeks after thyroidectomy, or an amount which ordinarily should have been sufficient to produce the calcemic effect. After two weeks of this treatment the symptoms continued unabated and the serum calcium remained at about 5 mg. per hundred cubic centimeters. After two weeks of treatment with 2 cc. of dihydrotachysterol (equivalent in calcemic principle to 800,000 international units of vitamin D) daily, the serum calcium rose only to 6.8 mg. per hundred cubic centimeters, but the symptoms were relieved. Two and one-half months later the patient was discharged on a maintenance dose of 1 cc. of dihydrotachysterol twice a week. It appears that during the acute or subacute stage of her tetany this patient was resistant to both vitamin D and dihydrotachysterol and that sufficient dosage only of the latter was given.

It would appear that there is no reason to attribute a higher degree of specificity or a lesser likelihood of toxicity to dihydrotachysterol than to vitamin D. That doses of the latter sufficient to produce effects comparable to those of dihydrotachysterol can be given with at least the same degree of safety, provided the same precautions are observed, is established.

81. Adams, L. J.: Postoperative Tetany Treated with Dihydrotachysterol (A. T. 10), Canad. M. A. J. **42**: 373-375 (April) 1940.

There is needed, however, a larger volume of clinical experience with vitamin D comparable to that accumulated for dihydrotachysterol.

COMMENT

The present review emphasizes my point of view that the burden of proof is on those who would maintain that dihydrotachysterol is a better or safer agent in the treatment of hypoparathyroidism than is vitamin D. This statement does not in any way detract from the proved effectiveness, usefulness and comparative safety of dihydrotachysterol. It seeks merely, and on the basis of the available evidence, to put vitamin D in the same category with respect to the same physiologic actions and therapeutic usefulness.

The present favor in which dihydrotachysterol finds itself, in comparison with the disfavor or relative indifference to vitamin D, appears to have arisen from the following considerations: First, the earlier trials of vitamin D were mainly with inadequate dosage, and comparisons are still made between antirachitic doses of vitamin D and full calcemic doses of dihydrotachysterol. Second, the early experience with toxic preparations of irradiated ergosterol, resulting from their high content of toxisterol, led to a fear of poisoning with overdoses of vitamin D, a fear which has not been supported by the experience of the past few years. Third, the standardization of vitamin D products in terms of antirachitic units leads to the expression of dosages adequate for the management of hypoparathyroidism in terms of tens or hundreds of thousands or even of millions of units. With a background of fear of overdosage, the physician faces the psychologic barrier of having to prescribe what appear to him to be enormous, and frequently described as "massive," doses of a toxic preparation.

All these handicaps have been removed in the case of dihydrotachysterol. Although its toxic, and therapeutically effective, principle appears to be identical with that of vitamin D, it was introduced without the same fear of toxic effects. Its dosages are expressed in the innocuous terms of cubic centimeters, and even when "toxic borderline doses" are referred to the dosage is in hundreds rather than in millions of units.

Moreover, it was introduced for one purpose only and has been given publicity only for this purpose. Without any direct claim to this effect having been made, the impression has become widespread that its action is specific, and in the current literature [82] it is even referred to as "a new form of parathyroid hormone."

It is my belief, from the evidence available at present, that preparations of vitamin D are as effective and as free from danger as is dihydrotachysterol. More clinical experience is needed, and a system of standardization and of dosages free from the present handicaps to the use of vitamin D for the purposes here discussed is required. For pure vitamin D_2, or calciferol, dosage should be expressed in milligrams. Preparations of irradiated ergosterol should be standardized in terms of their content of the calcinosefaktor, which will include the activity of the tachysterol content. The method of Holtz, Laquer, Kreitmair and Moll [8] is probably suitable for this purpose, but standardization in terms of the ability to raise the serum calcium level, as suggested by McChesney and Kocher,[44] would seem to be more rational. The physiologic activity, in terms of the toxic or calcemic factor, should then be referred back to pure calciferol rather than to arbitrary "toxic borderline units." A rough calculation, assuming that irradiated ergosterol contains about 25 per cent tachysterol, indicates that average viosterol should have about 16 per cent more calcemic activity than is indicated by its content of vitamin D_2.

It now seems that the dangers of administration of both dihydrotachysterol and of vitamin D have been somewhat exaggerated. They must be administered with care and with the proper precautions, but the immediate dangers are apparently no greater than in the case of insulin. Frequent determinations of serum calcium are desirable not only during the initial stages but also after maintenance doses have been arrived at. The Sulkowitch test, 52 by which the excretion of calcium into the urine is observed by a simple and roughly quantitative method, may serve to avoid the necessity for frequent examinations of the blood.

82. Slaughter, E. C.: Experimental Hyperparathyroidism and Otosclerosis, Ann. Otol., Rhin. & Laryng. **49**: 130-140 (March) 1940.

That prolonged administration of these products will lead to chronic injury in man has not been shown, nor is it clear that the danger does not exist. The effects on the arteries and arterioles of dogs have been referred to. In any case the possibility of such danger must, in cases of hypocalcemia resulting from hypoparathyroidism, be weighed against the very probable occurrence of opacities of the lens, and perhaps of other sequelae of this condition. At present the evidence is that the risk of injury from the therapeutic agent is far less than that from the condition which it is intended to combat.

Finally, I wish to enter a plea for the abandonment of the term "hypervitaminosis D." Not only is the term misleading and nondescriptive but it is also a deterrent to the legitimate and desirable use of vitamin D products in the treatment of hypocalcemia. The term "vitamin D," without further qualification, refers solely to the antirachitic activity of the activated sterols, although "vitamin D_2" and "vitamin D_3" are now used to refer to specific chemical substances. Since the toxic effects from administration of vitamin D products are shared with other products having little or no vitamin D activity, it is clear that the characteristics of the sterol molecule responsible for its antirachitic activity are not those responsible for the toxic effects. There is no evidence that overdosage with the antirachitic factor per se leads to any undesirable effects. For these reasons the toxic actions of the activated sterols should be separated from their antirachitic activity in terminology as they are in fact.

SUMMARY

1. A number of substances derived from activation products of ergosterol and cholesterol have the effect of raising the serum calcium when administered in sufficiently large doses, this effect resulting from a combination of the mobilization of calcium from the bones and an increase in absorption from the gastrointestinal tract. Of these, only vitamin D_2 (calciferol) and dihydrotachysterol (also known as A. T. 10) have been extensively used clinically.

2. Because of this effect on the serum calcium, here called the "calcemic effect," both of these substances

are highly effective in increasing the concentration of calcium in the blood and in relieving the symptoms resulting from hypocalcemia in cases of insufficiency of the parathyroid glands. The substances may be administered by mouth over considerable periods of time and with reasonable safety, provided proper precautions are observed. Their prolonged use is attended neither by injury to the patient nor by the development of tolerance.

3. When the two substances are administered in comparable doses their effects are qualitatively and quantitatively similar, if not identical. It has not been shown that either is superior to the other in the management of hypoparathyroidism. Previous unsatisfactory experience with vitamin D products has been due either to insufficient dosage, in which case no beneficial results were obtained, or to the effects of a widely used preparation containing the toxic products of overirradiation.

4. The dangers of the toxic effects of these substances in the preparations now available, while real, have been somewhat overemphasized. Frequent determinations of serum calcium are desirable both to determine that the desired elevation has been obtained and to avoid the dangers of hypercalcemia. The Sulkowitch test, by which the excretion of calcium into the urine is observed and which the patient himself learns to perform, serves to permit close observation and at the same time to reduce the frequency of the necessary blood examinations.

5. While the possibility of chronic injury resulting from prolonged administration of these substances has not been excluded, such injury has not been demonstrated. At present the evidence is that the risk of injury from the therapeutic agent is far less than that from the condition it is intended to combat.

6. The "calcemic principle" responsible for the elevation of serum calcium and for the toxic actions of the activated sterols has been dissociated from the antirachitic activity of certain of these sterols. In the case of vitamin D the antirachitic activity and the calcemic action are apparent in widely separated dosage ranges. Confusion as to dosage and the expression of calcemic doses in terms of tens or

hundreds of thousands of units have been handicaps to the use of vitamin D in the management of hypocalcemia.

7. It is urged that these handicaps be removed by the expression of dosages for the calcemic effect in terms of weight of calciferol. In the case of viosterol, which contains other substances with the same action but not accounted for in the standardization of this preparation in terms of antirachitic activity, the calcemic effect should be determined by biologic standardization and referred back to pure calciferol.

8. The term "hypervitaminosis D" should be abandoned. The term is misleading and nondescriptive and is a deterrent to the legitimate and desirable use of vitamin D products in the treatment of hypocalcemia.

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CHAPTER XXVIII

THE PANCREAS AS AN ORGAN OF INTERNAL SECRETION

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Removal of a dog's pancreas produces in that animal a series of metabolic changes which are strikingly similar to those of severe diabetes mellitus in human subjects. No less definite is the speedy elimination of these abnormalities in both the depancreatized dog and the patient with diabetes following the injection of insulin, which is obtained from normal pancreatic tissue. The observations on the effects of pancreatectomy, made by von Mering and Minkowski,[1] and those on the effect of insulin on depancreatized dogs, made by the group in the department of physiology of the University of Toronto,[2] provided very good evidence for the association of the pancreas and its internal secretion with the condition of diabetes. These salient facts have been added to extensively during the last few years. Some of the additions have established older views more definitely, whereas some have led to entirely new conceptions of the cause of diabetes mellitus and the action of the antidiabetic hormone.

Thus, pancreatectomy in the dog is followed by glycosuria and ketonuria; the cat similarly treated shows these signs to an even greater extent, while in certain other animal species investigated recently (pig, monkey and goat) these symptoms are much milder. Depancreatized monkeys and goats and certain depancreatized birds have been maintained alive for long periods without administration of insulin. Again, it is now well known that hypophysectomy in the depancreatized dog or cat causes an appreciable reduction in the metabolic

1. von Mering, J., and Minkowski, O.: Diabetes mellitus nach Pankreasextirpation, Arch. f. exper. Path. u. Pharmakol. **26**: 371, 1889.
2. Banting, F. G., and Best, C. H.: The Internal Secretion of the Pancreas, J. Lab. & Clin. Med. **7**: 251 (Feb.) 1922.

rate of these animals, accompanied by a considerable decrease in the intensity of diabetes. It is probable that the mildness of the disease in the aforementioned species may be attributable to the lesser activity of their pituitary glands. Extracts of the anterior lobe of the pituitary gland injected into dogs and cats often produce severe, permanent diabetes.[3] So far there has been no clearcut indication that diabetes mellitus is due to overactivity of the pituitary gland, but the more frequent incidence of this disease among acromegalic persons constitutes circumstantial evidence in favor of this view. The diabetic state, therefore, may not be due primarily to subnormal secretion of antidiabetic hormone but to various other hormonal disturbances, especially of the pituitary and the adrenals.

If the pancreas is removed from a dog, the animal is unlikely to survive for two weeks, even with the greatest care, unless insulin is administered. With this hormone a rapid recovery from the operation is obtained and, provided the diet is satisfactory (see later comment on this), the animal soon appears to be normal and may be maintained so for several years. If insulin is withheld, however, certain characteristic symptoms soon become evident. The concentration of sugar in the blood increases progressively, even in the fasting animal, until it may be several times that of the normal value of 0.07 to 0.10 Gm. per hundred cubic centimeters. Consequently, dextrose may now be detected readily in the urine, often in considerable amounts. The qualitative test for sugar in the urine as ordinarily carried out with Benedict's reagent is often positive owing to the presence of "non-sugar reducing substances." To be sure that sugar is present, one of the several methods of estimating "true sugar" should be employed. Soon, too, "acetone bodies" may be detected, and these substances gradually increase in amount. The amount of nitrogenous substances in the urine increases. These changes depend on a number of factors, including the nature and quantity of the diet.

Two criteria which have been much in evidence in the metabolic study of the diabetic condition, and since the discovery of insulin, in the study of its action, are the D:N (i. e., dextrose:nitrogen) ratio and the R. Q. (respiratory quotient). The dextrose:nitrogen

3. Young, F. G.: The Pituitary Gland and Carbohydrate Metabolism, Endocrinology **26**: 345 (Feb.) 1940.

ratio characteristic of the completely depancreatized animal (i. e., depancreatized dog) receiving no food or insulin was considered to be 2.8:1. There is little reason in continuing to accept this fixed value for the ratio, for it varies considerably in different animals and in the same animal at different times. With respect to the respiratory quotient, it is conceded that in the patient with severe diabetes and in the depancreatized dog or cat it has a low value of about 0.7, while after the injection of insulin it may be appreciably raised. It provides a useful index for comparing or contrasting the state of animals under similar or different conditions. But the exact interpretation of the figures is generally appreciated to be uncertain, for they are composite figures, due to metabolic changes whose type and extent investigators have no way at present of determining accurately.

Much of present knowledge of the part played by insulin has been obtained from studies of the blood sugar and of quantitative variations in its concentration. It is therefore of prime importance to appreciate what is known about this essential metabolite. The concentration of sugar (dextrose) in the blood is dependent on the rate at which it is being added to the blood and the rate at which it is being removed. This perfectly obvious view has often been overlooked, more particularly by those who have made unwarranted interpretations of dextrose tolerance curves. In the postabsorptive state the sole source of the blood sugar is the liver. The sugar is obtained by hydrolysis (glycogenolysis) of the hepatic glycogen, which may be synthesized (glycogenesis) from ingested sugar, which usually is dextrose but which may also be fructose, usually from cane sugar, or galactose, from milk sugar. It was formerly believed that these enzymic changes which one may write glycogen $\rightleftarrows$ dextrose were mediated by an amylase. For long the close relationship between phosphate metabolism and carbohydrate metabolism has been recognized. As a result of recent work by Cori[4] and others, it now appears that the aforementioned reactions involve enzymes concerned with phosphorylation processes. The glycogen is also synthesized from dextrose derived (glyconeogenesis) from certain of the amino acids (the so-called glycogenic amino acids, aminoacetic acid,

4. Cori, C. F.: Glycogen Breakdown and Synthesis in Animal Tissues, Endocrinology **26**: 285 (Feb.) 1940.

alanine, cystine, proline, hydroxyproline, norleucine, serine, arginine and aspartic, glutamic and betahydroxyglutamic acids). Theoretically, dextrose might also be derived from fatty acids, but a satisfactory proof of hepatic conversion of fatty acids, except perhaps butyric acid, to sugar has not yet been given. One may regard all the extrahepatic tissues, especially the muscles and the brain, as withdrawing sugar from the blood continually. In the fasting animal at rest the liver is continuously secreting dextrose at a constant rate (studies on hepatectomized dogs indicate that in this species the rate of secretion is about 0.25 Gm. per kilogram of body weight per hour). Any extra activity of the animal calls forth an increased supply of sugar from the liver. If in spite of the increased secretion the rate of uptake increases more rapidly, hypoglycemia will result. This state may be observed in long distance runners or others engaged in prolonged physical exertion. When carbohydrate food is eaten, and the sugar is being absorbed from the intestine into the blood stream, the liver ceases to secrete sugar, the intestinal supply satisfies the energy demands of the tissues, while the excess of sugar is deposited as glycogen in the muscles as well as in the liver. The hepatic supply of glycogen is thus replenished, and when most of the sugar has been absorbed from the intestine and the blood sugar has returned to its normal value, the liver once again supplies sugar to the blood at a rate proportional, within limits, to the immediate necessities of the animal. Recently, strong support for this homeostatic mechanism of the liver has come from the researches of Soskin and co-workers.[5] In the diabetic state the amount of glycogen retained in the liver (and muscles) even immediately after a meal is small, but the secretion of sugar by the liver continues to be greater than usual, for the rate of glyconeogenesis is increased above the normal. This is reflected in the increased nitrogen excretion and also in the rapid loss of weight of the animal. In the diabetic condition the metabolic rate is raised.

Much debate has been made as to whether the diabetic condition is characterized by an overproduction or by an underutilization of sugar. There is now no doubt

5. Soskin, Samuel: The Liver and Carbohydrate Metabolism, Endocrinology **26**: 297 (Feb.) 1940.

that the completely diabetic patient is able to oxidize dextrose, and because of the increased concentration of sugar in the tissues, the amount of dextrose oxidized may not be much short of that which the animal would use under normal conditions, with a normal concentration of blood sugar. There is also no doubt that an appreciable overproduction of sugar by the liver occurs. We take the position that both overproduction and underutilization play a role in the diabetic state.

The disturbed metabolism of fat in the depancreatized animal is indicated by an increase of fatty substances in the blood: neutral fat, cholesterol esters and phospholipids. Presumably, these reflect an increased rate of mobilization of depot fat. The extent of these changes is dependent on the presence or absence of certain dietary factors (see later comments on this). Further evidence of the disturbance in fat metabolism is the development of ketosis.

Ketosis is a well recognized condition in the diabetic patient and in depancreatized animals of certain species. The condition is manifested by the presence in the blood and urine of abnormal amounts of "acetone bodies" (acetone, acetoacetic acid and betahydroxybutyric acid). Methods are available for the estimation of each of these constituents or for the combined estimation of all three (and this is the information usually sought). Although the amounts of these substances normally present in the blood are very small—so small as to be just detectable by sensitive methods—it seems justifiable to regard them as normal metabolites. They are produced in the liver. They may be formed from certain of the amino acids (the "ketolytic" amino acids, tyrosine, leucine, isoleucine and phenylalanine) and from fatty acids. We know that these "acetone bodies" are formed in greater amounts at times when there is reason to believe that oxidation of fat is providing much of the body's energy requirements. It was generally taught that their production at such times is due to the absence of oxidation of carbohydrate. Carbohydrates were thus regarded as antiketogenic substances, but there is now much to commend the teaching that carbohydrates are antiketolytic; that in the absence of carbohydrate, if fat is available, more fat is used, with a consequent increase in the production of "acetone bodies."

The ketosis in a depancreatized fat dog is greater than that in a lean one, but this species is characterized by its efficiency in metabolizing fats without ketosis. The loss of body fat is rapid, but the ketosis may be so severe even in this species that severe acidosis develops and the dog dies in coma before the fat reserves are depleted. The toxic enolic form of acetoacetic acid, $CH_3COH = CH.COOH$ (and other substances containing the enolic grouping $C.OH = CH$ are said to act likewise), is reported to stimulate the respiratory center and to depress the higher centers of the brain, so causing "air hunger" and the dimmed perception and loss of consciousness of diabetic coma. But the evidence does not justify the conclusion that acetoacetic acid is the sole cause of this syndrome.

CHEMISTRY OF INSULIN

The active material is a protein. It may be regarded as an albumin. By the Svedberg ultracentrifuge method it has a molecular weight similar to that of egg albumin, namely, 35,000. It now can be readily crystallized,[6] usually in the form of twin rhombohedra of microscopic size, a little above its isoelectric point, provided salts of zinc, nickel, cadmium or cobalt are present in the solution. These metals are evidently linked chemically with the protein in its crystalline state, for they occur in constant amounts and these amounts are proportional to their atomic weights. Zinc is present in zinc insulin crystals to the extent of nearly 0.5 per cent. The fact that normal pancreatic tissue is relatively rich in zinc may be of some significance in the storage of the hormone in the gland. The protein has a high sulfur content (3.2 per cent), all present in the form of cystine. The molecule contains no carbohydrate material, and apart from its low mineral content, appears to be wholly constituted of amino acids. The constituent amino acids and their percental distribution have been reported as leucine 30, glutamic acid 21, cystine 12, tyrosine 12, proline 10, histidine 4, arginine 3, lysine 2, phenylalanine 1. The figures should perhaps be regarded as indicating the relative amounts rather than the exact amounts of the different amino acids.

Slightly acidified insulin has been kept for long periods, but in dilute alkali insulin is relatively unstable.

6. Scott, D. A.: Crystalline Insulin, Endocrinology 25: 437 (Sept.) 1939.

It is hydrolyzed and so is rendered physiologically inactive by those enzymic preparations which attack proteins. Thus, trypsinogen is without effect on insulin, and a portion of pancreas may be incubated at 37 C. for some hours without any alteration in the amount of insulin which can be extracted from it. Various attempts have been made to ascertain if there is in the molecule a specific grouping of certain of the amino acids which is really responsible for its hormonal activity. It may be concluded that the physiologic activity of insulin may be slightly and sometimes reversibly decreased by certain minor chemical changes in the molecule, whereas appreciable chemical alteration gives a considerable diminution or complete absence of activity. These considerations are linked with the fate of insulin in the animal body after secretion from the pancreas or after parenteral injection. Proteases of the blood and other tissues may effect considerable destruction. Other changes have been suggested, such as the action of sulfhydryl groupings, as in glutathione, which may reduce the cystine disulfide linkage, a change which is known to be accompanied by inactivation.

INSULIN STANDARD

Zinc insulin crystals from all sources so far examined (man, cattle, hog, sheep, bison, fish) have the same potency. Very recently, preparations of zinc insulin crystals have been made of slightly greater potency than usual. This work may have considerable theoretic interest. The international standard, a preparation of zinc insulin crystals, is defined as containing 22 units per milligram. There are two well established methods of assaying the potency of an insulin preparation. The lowering of blood sugar in fasting rabbits and the production of convulsions in fasting mice furnish satisfactory effects of insulin for the comparison of unknown and standard products.

SOURCE OF INSULIN

The pancreas appears to be the only organ which makes and stores the antidiabetic hormone in detectable amounts. The hormone has also been demonstrated in blood, but this has been done, not by extraction procedures, but by passing the blood into another test animal.

The islet cells of the pancreas are of three types: α, β and a more ill defined type called D cells by Bloom. In dog pancreas the number of cells per islet varies greatly, as do the relative numbers of the three types of cells. One study gives the average number per islet as 30 and the average ratio of α, β and D cells as 20:75:5. The islet volume may be about one one-hundredth of the pancreas. The β cells occupy the periphery of the islets and are smaller than the others. It is these cells which are considered to be producers of the antidiabetic hormone; indeed, the granules of these cells may consist largely of this substance. Epithelial cells of the small ducts are considered to be the "mother cells" of the islet and acinar cells. New islet cells may therefore be produced from them. It may be mentioned here that in no frank uncomplicated case of diabetes mellitus has there appeared to be complete recovery of islet function. Although earlier claims of the demonstration of a pancreatropic principle in the pituitary have been questioned, recent research indicates the presence in anterior lobe extracts of a pancreatic stimulant. Thus the pancreases of dogs receiving these extracts show at certain times not only degenerative changes in islet cells but also obvious proliferation of new islet tissue. Certain strains of rats similarly treated show no degenerative changes but a definite increase in islet cells.

The main points of evidence which indicate that the hormone is produced in the islet cells are as follows: 1. Histologically, the islets are glandular structures, the obvious outlet for the secretion of which is through the blood stream. 2. There are relatively large amounts of the hormone in the principal islets of teleosteal fishes, in which few enzyme-producing cells are found. 3. The active substance is found in degenerated pancreas in which the loss of acinous tissue has proceeded more rapidly than that of the islet cells. Ligation of the pancreatic ducts eventually produces a decrease in the insulin content of the pancreas, but moderate amounts of insulin may still be extracted when very few enzyme-producing cells remain. 4. When most of the pancreas, approximately nine tenths, is removed from a dog, characteristic lesions (hydropic degeneration) are found in the β cells of the remnant. These changes can be accelerated by a high carbohydrate diet, and prevented or eliminated by administration of insulin or by fasting.

5. The clinical condition known as hyperinsulinism occurs when the pancreas liberates abnormally large amounts of antidiabetic hormone. In many of the cases there are definite tumors of the islet cells. After operative removal of these masses of islet cells the blood sugar is maintained at higher levels. 6. Metastases in other tissues arising from carcinoma of the islet cells have been shown to contain insulin. 7. The injection of anterior pituitary extracts leads to destructive changes in the islet cells, chiefly in the β cells, while there is little or no effect on the α cells. The pancreases from a number of dogs treated with these extracts have been assayed for their insulin content, and the values obtained were roughly proportional to the β cell concentration in histologic sections of these glands.

THE INSULIN CONTENT OF THE PANCREAS UNDER DIFFERENT CONDITIONS

The insulin content of the pancreas under widely different conditions and in various animal species has been extensively studied. The insulin is obtained from minced pancreases with an acid aqueous alcohol solution, from which certain contaminating material is removed before precipitating the active material, which is then redissolved and estimated by the mouse method of assay. In the dog, the insulin content of the free splenic end of the pancreas is greatest, that of the attached duodenal portion has an intermediate value, while that of the free duodenal end is lowest, the values being about 4, 3 and 2 units per gram, respectively. It might be mentioned here that the size of the pancreas in the dog, at least, bears no strict relationship to the weight of the animal. In partially depancreatized dogs, provided sufficient pancreas is left to prevent the onset of diabetes, the insulin content does not differ from that of the corresponding part in a normal dog, nor are any degenerative changes in the β cells noted, whereas, if diabetes supervenes, hydropic degeneration of these cells is observed, and the insulin content of the remnant of pancreas becomes extremely small. The daily injection into dogs of diabetogenic extracts from the anterior lobe of the pituitary gland produces a prompt and profound decrease in the insulin content of the pancreas (in seven days to 0.2 unit per gram). If administration of the extract is discontinued at this stage, the normal insulin content is restored

within four days, whereas continued administration of extract reduces the insulin content to negligible amounts, and no subsequent recovery occurs. Simultaneous administration of insulin prevents or greatly modifies the fall in the insulin stores. The latter fact strongly suggests that the β cells are permanently damaged by the extract through overwork and that the simultaneous administration of insulin relieves the cells of some of this excessive demand for the hormone.

The injection of anterior pituitary extracts into certain strains of rats does not cause a diabetic condition. Instead the insulin content is increased, as is also the islet count. Starvation (seven days) or a diet rich in fat produces a decrease in the insulin content of the rat pancreas to about half its normal value, which is about 2½ units per rat. These animals have their insulin stores speedily restored to normal (in six days) when they are returned to a balanced diet; carbohydrate alone effects a partial restoration. Daily injection of insulin into rats causes an even more marked decrease in the insulin content of the pancreas than does starvation. In this connection it is interesting to note the earlier report that injection of insulin inhibited the proliferative activity of the islet cells of young rats. Also feeding of a high fat diet to trout is said to lead to degenerative changes in the islets. No marked histologic changes were noted in the islets of the rats on the high fat diet.

The effect of age on the insulin content of the pancreas has been studied in the cow. In fetal calves under 5 months the pancreatic content was 34 units per gram; in calves 6 to 8 weeks old, 10 units per gram; in heifers 2 years old, 5 units per gram; in cows 9 years old and older, 2 units per gram. Pregnant cows 7 years old and older showed no change from the normal insulin content of 2 units per gram. Here it may be mentioned that the decrease in insulin requirements frequently noted at certain stages of pregnancy in the diabetic woman has not been clearly shown to be due to the passage of antidiabetic hormone from the fetus to the mother. What seems likely is that extra sugar may pass from the mother to the fetus as a result of excessive stimulation of the fetal pancreas. The changes may also be due directly to the considerable hormonal activity of pregnancy.

Pancreases obtained from nondiabetic persons post mortem have an average insulin content of about 2 units per gram; those of diabetic persons show wide variation, the average content being 0.4 units per gram. The insulin content of a tumor of islet tissue surgically removed from a patient suffering from hyperinsulinism was as high as 85 units per gram.

It is, of course, apparent that these "insulin contents" indicate the balance between the rate of production of the hormone in the islets and the rate of liberation. There is no reason to doubt that under different conditions these rates may vary considerably and in either direction. The tentative conclusion has been drawn from some of these results that the islet cells are "rested" by administration of insulin, by starvation and by a high fat diet; that in these conditions less insulin is excreted by the pancreas than under normal conditions.[7] Partial pancreatectomy, sufficiently extensive to result in diabetes, and administration of diabetogenic extracts cause marked stimulation of the islets and, because of this "overwork," they degenerate.

SECRETION OF INSULIN

Nerve endings of the vagus have been located in the β cells of the islets. There is reason to believe that the nervous control plays a minor role in secretion, and certainly secretion of insulin has been demonstrated in denervated pancreatic transplants. It is generally believed that the rate of secretion of the antidiabetic hormone is regulated by the concentration of dextrose in the blood passing through the pancreas. Other substances may play a part in this regulation, possibly the antidiabetic hormone itself; at certain concentrations in the blood it may inhibit further secretion by the β cells. Houssay[8] obtained a lowering to normal of the blood sugar of a depancreatized dog by means of a pancreatic transplant; the transplantation of three more pancreases produced no further effect. When sugar is fed to an animal, the resulting hyperglycemia evokes increased secretion of insulin, which prevents the continued increase of the blood sugar and ultimately is responsible for the return of the sugar to the normal value, at which time the secretion of insulin returns to its former rate.

7. Haist, R. E.; Campbell, J., and Best, C. H.: The Prevention of Diabetes, New England J. Med. **223**: 607 (Oct.) 1940.

8. Houssay, B. A.: Diabetes as a Disturbance of Endocrine Regulation, Am. J. M. Sc. **193**: 581 (May) 1937.

It has been maintained that the pancreas secretes the hormone continuously at a constant rate, and that the dextrose tolerance curve is determined by a change in activity of the liver as already discussed. A more satisfactory interpretation of known facts can be made if these two views are amalgamated: the concentration of blood sugar is controlled by supply or withdrawal of sugar on the part of the liver and by changes in the rate of secretion of insulin on the part of the pancreas.

It has been claimed by a number of investigators that the duodenal mucosa possesses a hormone (incretin or duodenin) which lowers the blood sugar by its supposed stimulation of the islet cells to secrete insulin. The presence of such a hormone has been rendered extremely doubtful by the recent work of Ivy and co-workers,[9] who further offered a critical analysis of the earlier experimental findings.

Interesting studies have been made of the insulin requirements of depancreatized dogs under different conditions. Thus the blood sugar has been kept at a normal level by simultaneous and continuous intravenous injection of insulin and dextrose solutions. The insulin required was between 0.06 and 0.4 units per kilogram per hour, while the corresponding requirement of dextrose was 0.2 to 0.6 Gm. per kilogram per hour. The higher values for insulin and dextrose were those required by unanesthetized dogs; the others, by anesthetized dogs. In another study the amount of insulin necessary to keep the blood sugar at a normal value in depancreatized dogs under basal conditions was between 0.005 and 0.035 unit per kilogram per hour, with an average value of 0.017 unit per kilogram per hour. The duration of action of insulin is not proportional to the size of the dose injected but is a simple function of the logarithm of the dose; i. e., insulin is inactivated in the body at a rate proportional to the amount in the body at the time. Thus if 1 unit lasts four hours, 10 units would last eight hours.

ADMINISTRATION OF INSULIN

Overdosage of insulin or spontaneous secretion of the hormone in excessive amounts is followed by a series of subjective sensations which have been frequently

9. Loew, E. R.; Gray, J. S., and Ivy, A. C.: The Effect of Duodenal Instillation of Hydrochloric Acid upon the Fasting Blood Sugar of Dogs. Am. J. Physiol. **126**: 270 (June) 1939.

described. Objective signs are also evident in man and in animals, becoming progressively more alarming and finally giving way to convulsions or coma. This train of symptoms runs parallel to the progressive decrease in the concentration of the blood sugar. With certain species, e. g., dogs and rabbits, the convulsions may be severe and prolonged before coma ensues, while with other species, e. g., man and rats, the convulsions may be relatively mild, the comatose state quickly supervening. The physiologic mechanisms involved in these changes have yet to be elucidated; certainly the central nervous system is involved, for convulsions are annulled by general anesthesia, and they do not appear in spinal preparations. There is no good evidence to support older views that these symptoms depend on an accumulation of certain substances, toxic or otherwise. At present it may be said that these symptoms are associated with, and induced by, the low concentration of dextrose. It may be mentioned that under most circumstances the extreme condition of convulsions or coma does not occur until the concentration of blood sugar is extremely low or even zero. It will be remembered that many of the methods used to estimate blood sugar are not specific, and the values obtained are too high owing to the presence of "non-sugar reducing substances." In the diabetic patient and the depancreatized animal the convulsive level of the blood dextrose is frequently much higher than in the normal subject. But the onset of convulsions, with respect to the concentration of blood dextrose, depends on a number of factors. If the blood sugar is lowered rapidly, the convulsive symptoms may appear at a somewhat higher concentration of dextrose, while a very gradual lowering may be made to what ordinarily would be the convulsive level without convulsions or coma. If the patient or animal remains in this state for some time and convulsions finally occur (and they may be precipitated by relatively slight stimuli, such as a noise or a touch), they are often very severe, and restoration by administration of dextrose is much slower than when convulsions result from a speedy lowering of the blood sugar. This fact is of some consequence in the treatment of diabetic patients with protamine zinc insulin. It has been reported that rats are much more sensitive to insulin (as judged by the speedy production and

severity of the hypoglycemic reactions) under reduced barometric pressure (460 mm. of mercury) than under normal pressures.

The term "hyperinsulinism" should be reserved for those cases in which there is excessive secretion of insulin, usually the result of a tumor of the islet cells, though in some instances it is due possibly to general hyperplasia of the islet tissue. It is by no means easy to prove that a given condition is one of hyperinsulinism. Even alleviation of the condition by surgical removal of a large part of the pancreas does not necessarily prove the point. The same result would have been secured if the hypoglycemia had been due, for example, to diminished secretion of the anterior lobe of the pituitary, for it has been shown that the hypoglycemia produced experimentally by extirpation of the anterior lobe of the pituitary may be alleviated by complete removal of the normal pancreas.

Very large doses of insulin can be given without apparent damage to animals of different species, provided they eat to the capacity of a normal hungry animal, otherwise frequent parenteral injection of small doses of dextrose are necessary and sufficient.[10] There appears, however, to be a limit to the tolerance for insulin even when hypoglycemia is prevented, but for most persons this is extremely high. Some have withstood the injection of very large doses, up to 1,000 or more units of insulin. In the treatment of schizophrenia the use of insulin in sufficient amounts to produce coma appears to depend on the prolonged marked hypoglycemia, which results in a curtailment of the energy supply of the brain. The use of insulin in other nondiabetic persons (those with tuberculosis, digestive disorders or malnutrition) depends on its stimulation of the appetite. Many persons seriously underweight before treatment have been brought relatively quickly to their normal weights. The increase in gastric motility and secretion following the administration of insulin was considered to be wholly due to the induced hypoglycemia, but there is now reason to believe that this is only part of the mechanism, for these effects of insulin are abolished by vagotomy. It has been well established that the administration of insulin to nondiabetic persons is followed for some time by a lowered

10. Allen, F. M.: Diabetic Experiments, Tr. A. Am. Physicians **53**: 320, 1938.

tolerance for dextrose. This is probably due to suppression of the secretion of insulin by the subject's pancreas.

MODE OF ADMINISTRATION

A considerable advance in insulin therapy was made by the combination of insulin with protamine. The method was further perfected by the discovery that the addition of a small amount of zinc salt not only prolonged the effectiveness of the insulin but also stabilized what was otherwise a troublesome mixture to administer. Whereas solutions of zinc insulin crystals are rapidly absorbed from the subcutaneous tissues, preparations of protamine zinc insulin, having but slight solubility in the tissue fluids, are absorbed from the site of injection much more slowly, thereby simulating more closely the secretion of the normal pancreas. The following substances among others have also been added to insulin, and a delayed action of the hormone noted: zinc salts, spermine, arginine, thymus extract, alum, globin and hexamethylenetetramine.

Studies continue to be made on the problem of the administration of insulin other than by parenteral injection. In the main, efforts have been made to combine insulin with various materials, dyes, phenolic substances, tannic acid and others, which will protect the protein molecule from destruction by the intestinal enzymes when the hormone is given by mouth. The difficulties involved are obvious, and it is therefore not surprising that, while some success has attended these efforts in the laboratory, no satisfactory application to the treatment of diabetic patients has yet been made.

ALLERGY

Mild local reactions at the site of injection of insulin are not uncommon, while several local reactions and even generalized anaphylactic reactions have been reported occasionally. With the progressive purification of insulin preparations, allergic reactions have become much less frequent. Often the use of insulin from another animal source (insulin is available from cow, pig and sheep) eliminates or greatly modifies the reactions, though occasionally even preparations of zinc insulin crystals from a different source are not without local effect. Troublesome local reactions are said to have been observed with protamine zinc insulin in patients unaffected by the commercial brands of unmodi-

fied insulin. It might be mentioned that protamine is regarded as one of the nonantigenic proteins.

Recently claims have been made of successful active sensitization of guinea pigs with insulin. Zinc insulin crystals were used in some of these studies.[11] These findings raise a number of interesting points which may lead to results of fundamental importance. It has been supposed that hormones are identical in different species. Of the possible explanations of the apparent antigenicity of insulin preparations, the most obvious, perhaps, is that in spite of crystallization there may be present a minute amount of contaminating material which is actually responsible for the sensitivity reactions, or that in the process of isolation insulin has been altered in some way so as to render it a "foreign protein," unless one accepts the view that insulins from different biologic sources have certain differences in chemical structure, revealed by the delicate test of anaphylaxis.

THE MODE OF ACTION OF INSULIN

Of recent years several investigations have been published demonstrating in vitro actions of insulin in various minced or sliced animal tissues or cell-free extracts of them. Various interpretations have been placed on some of the findings.

Some have supposed, though on no very secure basis, that the carbohydrate substance which is oxidized in the animal body is glycogen rather than dextrose. Certainly insulin is concerned with the supply of glycogen, for, as already mentioned, in the depancreatized animal there is marked depletion of glycogen in the muscles (except the cardiac muscle, which shows an increase over the normal) and especially in the liver. A certain limited synthesis of glycogen in the liver and muscles has been shown in depancreatized dogs deprived of insulin for a few days. But, for a restoration to normal values, insulin is necessary. It has been debated whether insulin stimulates glycogenesis or inhibits glycogenolysis. It may well be that insulin exerts both of these effects under different conditions. When insulin is injected into a normal animal, there is a decrease in the concentration of blood sugar. Presumably some of the sugar which is removed is converted to muscle

11. Harten, M., and Walzer, M.: Allergy to Insulin, Liver, Pituitary, Pancreas, Estrogens, Enzymes and Similar Substances, J. Allergy 12: 72 (Nov.) 1940.

glycogen. The synthesis of muscle glycogen is easily demonstrated if sugar is also given. While insulin increases the deposition of hepatic glycogen in the diabetic animal, when it is given to a normal animal receiving sugar at the same time, the amount of glycogen stored in the liver is less, and may be considerably less, than when sugar is given without insulin. This well established fact should be borne in mind when for any reason it is necessary to build up rapidly the hepatic glycogen stores in the nondiabetic subject. There is no reason to doubt that considerable amounts of sugar (either as dextrose or as glycogen) are oxidized in the body in the absence of insulin. Insulin in conjunction with other substances appears to fulfil the role of arbitrator as to the relative amounts of the different sources of energy—carbohydrate, fat and protein—which shall be oxidized, and also as to the extent of the interconversion of these materials. If one were obliged to name the organ in which insulin exerts the most potent influence, there would be little hesitation in selecting the liver. In this connection the anatomic position of the liver with respect to the pancreas may be of considerable significance. It is true that so far no one has been able to demonstrate any difference in effect when insulin is injected into the portal system and when it is injected elsewhere in the body, but the relatively big doses used in comparison with the slow secretion of insulin by the pancreas may have masked the effect of the normal distribution of insulin. It is suggested, then, that the liver in the normal animal obtains a disproportionately large share of the secreted insulin. Not only do many chemical changes take place in the liver, but the extent of some of these changes is very great, which is more fully appreciated when one recalls that the liver alone is said to account for about one third of the total metabolic rate of the body. When the insulin supply is deficient, the rate of glyconeogenesis in the liver is increased. Administration of insulin decreases the production of sugar from amino acids, presumably by its effect on the deamination system. As has been already indicated, the production of sugar from fat is still hotly debated. Insulin exerts its effect also on other tissues. Thus the normal usage of sugar by the muscle is dependent on the correct supply of insulin. Mirsky has suggested that insulin has another effect on muscle metabolism in that it

appears to increase the rate of the utilization of amino acids by the muscles for the synthesis of proteins.

The injection of insulin into a diabetic animal decreases the lipemia and cholesteremia. It would be unwarranted to presume that therefore insulin directly controls the mobilization of lipids and cholesterol. Again, an injection of insulin into a normal or a diabetic animal is followed by changes in the concentration of various other blood constituents. Perhaps the best known changes are the decreases in the inorganic phosphate and different nitrogenous substances, such as amino acids, urea, creatine and creatinine. In part these changes are regarded as secondary to hypoglycemia and due to the consequent liberation of epinephrine. This is so, but insulin itself can bring about these changes. Various changes in the concentration of the metallic constituents of the blood have been reported. It would appear that some of these changes, especially that of the concentration of potassium, point to a fundamental relationship between electrolytes and carbohydrate metabolism.[12]

To summarize this discussion on the action of insulin it may be stated that the hormone (1) decreases glyconeogenesis from protein, (2) encourages the formation of glycogen, (3) increases the combustion of carbohydrate, (4) decreases the insulin content of the pancreas in the fed or the fasting animal and (5) protects the islet cells, probably by preventing overstrain.

INSULIN RESISTANCE AND SENSITIVITY

These terms are used to describe vastly different conditions. Thus the term "insulin resistant" is applied to the so-called adult type of diabetes, as opposed to the juvenile type of diabetes referred to as "insulin sensitive." It is also applied to the alteration in the diabetic state due to infection or to diabetic coma. It is obvious that the terms bear no rigid connotation. Again, the administration of thyroxin is said to increase "insulin resistance." This is only partially true, for continued administration of thyroxin until the hepatic stores of glycogen become very meager brings on a state of "insulin sensitivity." In comparing the effect of insulin under different conditions—even in the same animal—different results may be obtained, depending

12. Fenn, W. O.: The Rôle of Potassium in Physiological Processes, Physiol. Rev. **20**: 377 (July) 1940.

on the particular criterion employed in the comparison. A dog that has been starved for a long time will deposit much less hepatic glycogen after an injection of dextrose and insulin than a dog that has fasted for a short period. One might therefore conclude that the animal is insulin resistant in its starved condition. However, if the change in blood sugar is used as the criterion, this is not necessarily the conclusion. Chambers has shown that while the absolute decrease in the concentration of blood sugar due to the injection of insulin is less in the starved than in the normal condition, the percental decrease based on the initial concentration during fasting is the same. The following tabulation lists the conditions and substances which are considered to influence the response to insulin and should be liberally interpreted in the light of the aforementioned qualifications.

Resistance to Insulin	Sensitivity to Insulin
Diet	Diet (e. g., addition of vitamin B_1, addition of sodium chloride)
Infections	von Gierke's disease
Allergic reactions	Sympathectomy
Coma accompanied by acidosis	Hypothalamic lesions (occasionally)
Hyperthyroidism (thyrotoxicosis)	Hypopituitarism (hypophysectomy)
Hyperpituitarism (acromegaly)	Adrenal insufficiency
Glycotropic principle of anterior pituitary extracts	Adrenal denervation
Diabetogenic principle of anterior pituitary extracts	
Posterior pituitary extracts	
Epinephrine	
Steroids of the adrenal cortex	

INSULIN SUBSTITUTES

Much experimental work continues to be done in efforts to obtain a substitute for insulin, preferably one which will be active when given by mouth. Numerous plant extracts have been tried, and with some a lowering of the blood sugar has been noted. Several derivatives of guanidine have also been tried, perhaps the best known being decamethylene diguanidine. This substance does not increase deposition of muscle glycogen and its effect on hepatic glyconeogenesis is accomplished in a highly unphysiologic manner—by damaging the liver. Other substances besides this substance interfere with sugar formation in this manner. It is preferable for the diabetic organism to excrete the large quantities

of dextrose made by a relatively healthy liver than to be made "sugar free" by damaging the liver so that less dextrose is formed.

THE QUESTION OF A SECOND INTERNAL SECRETION OF THE PANCREAS

It has been claimed by various research workers that there is evidence for the production of an internal secretion by the α cells of the islands of Langerhans. Extracts purported to consist largely of substances obtained from the α cells are believed to exert an effect on fat metabolism. These results raise an interesting point but cannot at present be regarded as convincing.

Dragstedt and collaborators have published in a series of articles results which they believe demonstrate the existence of a new pancreatic hormone which they have named lipocaic. This substance prevents deposition of fat in the livers of depancreatized dogs under certain conditions. They have shown that the active substance of this extract is not choline, which had been previously proved, by the Toronto group, to exert similar effects. Some investigators have secured evidence which supports Dragstedt's conclusions, and others have challenged his interpretations.[13]

New light has been thrown on this problem by our colleagues McHenry and Gavin, who have obtained definite evidence that the pancreatic extract lipocaic and other materials, such as rice polish concentrate and yeast, do contain a *dietary factor* which is quite distinct from choline. This active material prevents the fatty change of the liver produced in rats by certain liver extracts (Blatherwick and co-workers) under conditions in which choline is ineffective.

The situation with regard to the pancreas at present is, therefore, that it has been shown to contain a dietary factor or factors other than choline and that the one of these which affects liver fat in rats is present also in rice polishings and yeast. This unidentified dietary factor may or may not prove to be identical with the pancreatic factor other than choline which affects liver fat in depancreatized dogs. Until these points are settled the active substance in lipocaic cannot be accepted as an internal secretion of the pancreas.

13. Best, C. H., and Ridout, J. H.: Choline as a Dietary Factor, in Luck, J. M., and Smith, J. H. C.: Annual Review of Biochemistry. Stanford University, Calif., Stanford University Press, 1939, vol. 8, p. 349.

CHAPTER XXIX

THERAPY WITH PREPARATIONS OF PANCREAS

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Pharmaceutic preparations of internal secretions of the pancreas are limited to insulin, modified insulins and lipocaic. Insulin is a secretory product of the beta cells of the islands of Langerhans; lipocaic may represent a secretion of the alpha cells of these islands. Evidence supporting this assumption has been presented by Dragstedt [1] and by Bensley and Woerner.[2]

INSULIN

The principal use for insulin is in the control of diabetes. For this purpose it is indispensable. It has found employment also in the treatment of schizophrenia and in the management of malnutrition in certain cases. Claims have been made that the addition of insulin to the dextrose commonly given to patients with disease of the liver and to others following surgical operations is advantageous.

Preparations of Insulin.—Insulin is a protein. Its molecular weight approximates that of egg albumin. It is made available commercially as a buffered solution of insulin hydrochloride in water. For many years this was the only form in which it was dispensed, and therefore at present this preparation is spoken of as "old insulin," "regular insulin" or "unmodified insulin." A more satisfactory term would be "solution of insulin hydrochloride."

Neither the free protein nor the hydrochloride has been crystallized, but salts of insulin with zinc, cadmium or other metals have been prepared in crystalline form (first by Abel), and a solution of zinc insulin crystals was made available commercially early in 1939. It was

1. Dragstedt, L. R.: The Present Status of Lipocaic, J. A. M. A. **114**: 29-32 (Jan. 6) 1940.
2. Bensley, S. H., and Woerner, C. A., cited by Woerner, C. A.: The Effects of Continuous Intravenous Injection of Dextrose in Increasing Amounts on the Blood Sugar Level, Pancreatic Islands and Liver of Guinea Pigs, Anat. Rec. **75**: 91-105 (Sept.) 1939.

claimed originally that the action of this preparation was significantly retarded as compared with the action of solution of insulin hydrochloride. These claims, however, have not been substantiated.[3] Solution of crystalline zinc insulin represents an insulin of high purity, and that it should give rise to fewer dermal allergic reactions is probable.[3b]

Retard Insulin: A number of means have been employed in attempts to retard the speed of action of insulin and thereby to prolong the duration of activity of a given dose. The only one of the "retard preparations" available commercially in America is protamine zinc insulin. This substance is insoluble at the p_H of the tissue, and thus absorption from the site of injection proceeds slowly. The amount of zinc contained is 1 mg. per 500 units of insulin, which is harmless. Hagedorn,[4] of Copenhagen, introduced protamine insulin in 1937. Protamines are basic polypeptides. They have been obtained from the sperm of trout, mackerel and salmon. Scott and Fisher,[5] of Toronto, added zinc, after noting that protamine had little effect on the rate of absorption of purified amorphous insulin. The original Danish preparation presumably contained metallic impurities.

Strength of Preparations of Insulin.—The strength of insulin is expressed in units. A unit as now defined is a twenty-second part of a milligram of a preparation of zinc insulin crystals in the possession of the National Institute for Medical Research, London, England. All forms of commercial insulin are dispensed in 5 or 10 cc. rubber-capped vials in various concentrations designated "U-20," "U-40," "U-80" and so forth, to indicate the number of units contained in 1 cc.

Administration of Insulin.—Insulin is inactivated by the gastric enzymes and is not absorbed by the rectum or colon. Parenteral administration is therefore obligatory. Soluble preparations of insulin, but not protamine zinc insulin, may be given by vein. Resort to intra-

3. (*a*) Ricketts, H. T., and Wilder, R. M.: Solutions of Amorphous Insulin and Solutions of Zinc Insulin Crystals: Clinical Studies on the Comparative Speed and Duration of Action, J. A. M. A. **113**: 1310-1312 (Sept. 30) 1939. (*b*) Marble, Alexander, and Vartiainen, Ilmari: Crystalline Insulin, ibid. **113**: 1303-1309 (Sept. 30) 1939. (*c*) Jackson, R. L., and Boyd, J. D.: Relative Efficiency of Commercial Forms of Insulin, Proc. Soc. Exper. Biol. & Med. **41**: 15-16 (May) 1939.

4. Hagedorn, H. C.: Fortschritte in der Insulin-Therapie, Schweiz. med. Wchnschr. **68**: 37-41 (Jan. 8) 1938.

5. Scott, D. A., and Fisher, A. M.: Studies on Insulin with Protamine, J. Pharmacol. & Exper. Therap. **58**: 78-92 (Sept.) 1936.

venous injection, however, is limited to emergencies; the usual method of administration is by subcutaneous injection.

None of the so-called antidiabetic pancreatic preparations reputed to be effective when taken by mouth have withstood rigid clinical testing. Claims that any such preparations exert a rejuvenating or stimulating action on the diseased pancreas have not been established.

Control of Diabetes with Insulin.—The theory which underlies the administration of insulin in diabetes mellitus is that of substitution therapy. Insulin is injected to provide for utilization of the dextrose which enters the circulation from the liver or is absorbed from the intestines; the dosage depends on the degree of pancreatic efficiency, on the intensity of activity of hormonal and nervous mechanisms opposed to the action of insulin and on the character of the diet. In the milder form of diabetes glycosuria usually can be controlled by limiting the intake of carbohydrate. In such cases insulin is not indicated. When the degree of insular (pancreatic) insufficiency is greater or when abnormal contrainsular activity, such as is encountered with hyperthyroidism, infection and other complications, is present, insulin must be used. It is unwise to sacrifice the nutrition of a patient simply to avoid the necessity of giving insulin.

It rarely is possible to obtain satisfactory control of severe diabetes with protamine zinc insulin alone. When the total dose of insulin required exceeds 20 or 30 units, injections of supplementary unmodified insulin are desirable in most cases to prevent gross glycosuria after meals. The ideal use of insulin would be to imitate the action of the normal pancreas. This, as Lawrence and Archer [6] suggested, probably involves (1) a continuous secretion of small amounts of insulin and (2) an increase in the secretion of insulin after meals to deal with ingested carbohydrate. No one type of insulin therapy can imitate both aspects satisfactorily. Protamine zinc insulin provides well for the small continuous supply required during the night, but if given in doses large enough also to control the glycosuria which follows meals, it provokes hypoglycemia in the night. Unmodified insulin acts quickly and is best adapted to meet the requirement for more insulin activity at meal

times. These considerations led Lawrence and Archer [6] and Graham [7] to attempt the simultaneous injection from one syringe of both protamine zinc insulin and unmodified insulin. In the mixture some of the soluble insulin may be bound by the excess of protamine present in the protamine zinc insulin, but even so irregularities of action from day to day are little more conspicuous than when the two insulins are injected into separate sites. This also has been the experience of Warvel and Shafer [8] and of Himsworth.[9] The latter stated:

> It is only in mild cases that the new preparations may legitimately be expected to control the disease during the whole twenty-four hours. . . . In cases of any severity their action should be reinforced by the administration of ordinary insulin at those times when a sudden influx of sugar from the intestine is found to overwhelm their mild action. An analogy may be drawn between the use of the new insulin and a modern technique in anaesthesia. The protamine insulins are comparable to the basal anaesthetics whose effect is both mild and prolonged; ordinary insulin is comparable to the volatile anaesthetic which is superimposed at times when a stronger control is required.

I have adopted the technic of mixing unmodified and protamine zinc insulin in one syringe, with satisfactory results.[10] In most cases adequate control both of postabsorptive and postprandial glycosuria can be obtained by this means with only one injection a day, the injection being made before breakfast. It is important to draw the dose of unmodified insulin into the syringe first, to avoid introducing any alkaline protamine zinc insulin into the bottle of unmodified insulin. The patient is taught to adjust the components of the mixture of insulins so that the urine passed before breakfast and before the evening meal will contain small traces of sugar. This degree of glycosuria is compatible with freedom from ketosis, stability of the nitrogen balance, normal growth of children and maintenance of normal body and mental

6. Lawrence, R. D., and Archer, Nora: Zinc Protamine Insulin: A Clinical Trial of the New Preparation, Brit. M. J. **1**: 487-491 (March 6) 1937.

7. Graham, George: The Use of a Mixture of Ordinary and Protamine Insulin, Acta med. Scandinav., 1938, supp. 90, pp. 54-63.

8. Warvel, J. H., and Shafer, M. R.: Protamine Insulin in the Treatment of Diabetes Mellitus, J. Indiana State M. A. **30**: 325-332 (July) 1937.

9. Himsworth, H. P.: Protamine Insulin and Zinc Protamine Insulin in the Treatment of Diabetes Mellitus, Brit. M. J. **1**: 541-546 (March 13) 1937.

10. Wilder, R. M.: Clinical Diabetes Mellitus and Hyperinsulinism, Philadelphia, W. B. Saunders Company, 1940, pp. 91-95.

vigor, whereas by avoiding more rigid control of glycosuria the danger from insulin reactions is minimized.

Insulin Reactions.—The hypoglycemia which results from overdoses of insulin entails more serious danger of provoking injury than is commonly appreciated. If an insulin reaction can be combated early, serious consequences almost always are avoided, but otherwise lasting danger may be done to the central nervous system. The lesions observed in the brain in fatal cases consist of hemorrhages, perivascular infiltration of round cells, atrophy of the cortex and swelling of the glia and axis-cylinders.[11] The danger is greater when protamine zinc insulin is used. This probably is explained by the fact that the blood sugar after administration of protamine zinc insulin is lowered so gradually that early symptoms of insulin reaction may be unobserved. Much chronic debility is now encountered among patients who are misusing protamine zinc insulin. It is characterized usually by headaches and asthenia, but in some cases sensory and motor neuritis are observed, and in others severe psychopathic conditions. Permanent idiocy has been recorded as a sequel of induced hypoglycemia by two groups of observers,[12] and the number of fatalities attributable to overdosage of insulin and to induction of insulin collapse (shock) for the treatment of schizophrenia is not inconsiderable. This accumulated experience has led me to the opinion that the control of diabetes with insulin should be less rigid than heretofore has been demanded.

Treatment of Schizophrenia with Insulin.—The resort to insulin collapse (shock) in the treatment of schizophrenia was introduced by Sakel.[13] The insulin shock has been employed extensively in psychopathic hospitals. It is a dangerous procedure with a relatively high mortality and should be undertaken only by physicians who are thoroughly familiar with the method. Other convulsants unrelated to insulin also bring about an improved condition in patients with mental disorders.

11. Malamud, N., and Grosh, L. C., Jr.: Hyperinsulinism and Cerebral Changes: Report of a Case Due to an Islet Cell Adenoma of the Pancreas, Arch. Int. Med. **61**: 579-599 (April) 1938. Moersch, F. P., and Kernohan, J. W.: Hypoglycemia: Neurologic and Neuropathologic Studies, Arch. Neurol. & Psychiat. **39**: 242-257 (Feb.) 1938.

12. Klein, F., and Ligterink, J. A.: Insulin and Cerebral Damage, Arch. Int Med. **65**: 1085-1096 (June) 1940. Layne, J. A., and Baker, A. B.: Hypoglycemic Cerebral Damage in Diabetic Patients, Minnesota Med. **22**: 771-776 (Nov.) 1939.

13. Sakel, Manfred: Zur Methodik der Hypoglykämiebehandlung von Psychosen, Wien. klin. Wchnschr. **49**: 1278-1282 (Oct. 16) 1936.

Insulin Treatment for Addiction to Morphine.—Insulin therapy for symptoms of morphine withdrawal, as proposed by Sakel,[14] has not met with critical approval.[15]

Insulin Treatment for Anorexia.—Insulin is much used to induce appetite and hunger and thereby to increase consumption of food. The results reported are variable. The greatest effectiveness is observed when the use of insulin is combined with strong suggestion. Heinz and Palmer[16] could demonstrate no consistent effect on the contractions of the empty stomach or the hunger pangs.

Insulin Combined with Dextrose for Nondiabetic Abnormalities.—After an operation the patient may exhibit diminished ability to utilize dextrose from several causes, none of which involves any permanent derangement of the endocrine function of the pancreas. Among such causes are starvation, anesthesia and surgical trauma. The use of insulin in the treatment of the glycosuria and occasional acidosis which may follow operations on nondiabetic patients was frequently recommended after the discovery of insulin, but more recently it has been largely abandoned. Experimental evidence for increased rates of utilization of dextrose by normal subjects given insulin is not striking, and the temporary depression of tolerance for dextrose as well as the accompanying ketosis which results from fasting can be overcome rapidly by administration of dextrose alone.[17]

LIPOCAIC *

The name "lipocaic" was given by Dragstedt, Van Prohaska and Harms[18] to an extract of beef pancreas which prevented the fatty infiltration and degeneration that commonly develop in the livers of depancreatized dogs when such dogs are maintained alive with insulin and do not receive fresh pancreas, lecithin or choline.

14. Sakel, Manfred: Neue Behandlung der Morphinsucht, Deutsche med. Wchnschr. **56**: 1777-1778 (Oct. 17) 1930.

15. Ossenfort, W. F.: Drug Addictions, in Barr, D. P.: Modern Medical Therapy in General Practice, Baltimore, Williams & Wilkins Company, 1940, vol. 1, p. 1160.

16. Palmer, W. L.: Diseases of the Digestive System, in Barr, D. P.: Modern Medical Therapy in General Practice, Baltimore, Williams & Wilkins Company, 1940, vol. 2, p. 2236.

17. Sprague, R. G.: Dextrose and Insulin in Nondiabetic Cases, editorial, Minnesota Med. **22**: 649-650 (Sept.) 1939.

* This section was prepared with the assistance of Dr. J. L. Bollman.

18. Dragstedt, L. R.; Van Prohaska, John, and Harms, H. P.: Observations on a Substance in Pancreas (a Fat Metabolizing Hormone) Which Permits Survival and Prevents Liver Changes in Depancreatized Dogs, Am. J. Physiol. **117**: 175-181 (Sept.) 1936.

The existence of a condition exactly similar to this has not frequently been recognized in diabetes of human beings. There have been, however, two reports of cases [19] in which hepatomegaly did not respond to insulin or diet but receded after administration of lipocaic. Also of interest from the clinical standpoint is the observation of Dragstedt and his associates [20] of arteriosclerosis in 6 depancreatized dogs in which for a period of from six to nine months the diabetes was well controlled with insulin, while fatty liver was intermittently permitted to develop by intermittently withholding treatment with lipocaic. Spontaneous arteriosclerosis of the degree observed is exceedingly rare in the domestic dog, so that the observation seems to be significant. It may have a bearing on the question of frequent occurrence of arteriosclerosis among diabetic patients.

The lowered blood lipids of depancreatized dogs are elevated to normal values by the administration of lipocaic. Lipocaic has been used by Clark et al.[21,22] to lower the elevated blood lipids of certain patients with psoriasis and others with xanthoma. Clinical improvement was noted in all patients with xanthoma concomitant with the reduction of the blood lipids. Improvement was also noted in the patients with psoriasis when the blood lipids were lowered but several failed to show any alteration due to the administration of lipocaic. There are a few reports of single instances in which lipocaic appears to have influenced lipemias of undetermined origin. The clinical use of lipocaic in disorders of lipid metabolism appears to offer promise, but should still be considered as being in the experimental stage.

Some of the uncertainties of the action of lipocaic may be due to the lack of a quantitative method for its assay. The use of depancreatized dogs in its assay is both time consuming and expensive and most assays have been qualitative rather than quantitative. McHenry

19. Grayzel, H. G., and Radwin, L. S.: Hepatomegaly in Juvenile Diabetes Mellitus Treated with Pancreatic Extract, Am. J. Dis. Child. **56**: 22-32 (July) 1938. Rosenberg, D. H.: Proved Case of Recovery from Fatty Metamorphosis of the Liver After Treatment with Lipocaic, Am. J. Digest. Dis. **5**: 607-613 (Nov.) 1938.

20. Dragstedt, L. R.; Goodpasture, W. C.; Vermeulen, C., and Clark, D. E.: Arteriosclerosis in Depancreatized Dogs, Am. J. Physiol. **126**: P479-P480 (July) 1939.

21. Clark, D. E.; Walsh, E.; Julian, D. C., and Dragstedt, L. R.: Experimental Use of Lipocaic in the Treatment of Psoriasis, Am. J. Physiol. **129**: 334 (May) 1940.

22. Clark, D. E.; Julian, O. G.; Vermeulen, C. W.; Allen, J. G., and Dragstedt, L. R.: The Effect of Lipocaic on Essential Xanthomatosis, Am. J. Physiol. **133**: 239 (June) 1941.

and Gavin [23] have suggested a method for assay of lipocaic. They found that young rats, fed liver extract for one week following three weeks deprivation of vitamin B developed fatty livers which could be prevented by the feeding of lipocaic with the liver extract. The administration of the crude liver fraction to rats deprived of vitamin B causes marked synthesis of fat and the development of fatty livers highly resistant to the lipotropic action of choline. The increase of fat and cholestrol in the liver can be prevented by using lipocaic, rice polishings, or brewers yeast. The effect of lipocaic is not due to its choline or protein content. The difference in action of lipocaic and choline is further indicated by the fact that choline will prevent the formation of fatty livers in rats fed cholesterol, and also the fatty livers of vitamin B deficient rats fed thiamine, while lipocaic is ineffective in both instances. Bollman [24] has been successful with a modification of McHenry's method for assays of lipocaic. In this method young rats are maintained on a vitamin B-free, fat-free diet for three weeks; they are then given a vitamin B-free, fat containing diet for one week and graduated dilutions of the lipocaic to be tested are fed with liver extract during the last week. The rats receiving insufficient lipocaic develop fatty livers clearly recognizable by chemical analysis or by fat stains of sections of liver. The rats receiving adequate lipocaic have livers of normal fat content which does not stain with the usual fat stains. Various preparations of lipocaic show considerable variation of potency by this test. It is possible that the action of lipocaic on blood lipids and its action in preventing fatty livers in vitamin deficient rats may be due to substances in lipocaic other than that which is effective in depancreatized dogs. Further work with standardized lipocaic is necessary.

23. McHenry, E. W., and Gavin, G.: The Effects of Liver and Pancreas Extract on Fat Synthesis and Metabolism, J. Biol. Chem. **134**: 653 (July) 1940.
24. Bollman, J. L.: Unpublished data.

CHAPTER XXX

INTERNAL SECRETIONS OF THE GASTROINTESTINAL TRACT

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GASTRIC SECRETION—GASTRIN

The secretory response of the gastric glands to a meal is due in part at least to a humoral mechanism. This has been amply demonstrated by means of transplanted pouches of the stomach.[1a] Just what proportion of the total secretory response is mediated humorally is difficult to determine. There is now direct evidence to indicate what areas of the gastrointestinal tract are the site of action for the stimuli which give rise to the humoral phase. It has recently been shown,[2] contrary to earlier views, that the corpus is sensitive to chemical stimuli, although less so than the pyloric region. The application of meat or liver extract to a pouch of the entire stomach will cause a transplanted pouch to secrete. After the pyloric portion has been removed from the "pouch of the entire stomach," application of liver extract to the remaining corpus still causes the transplant to secrete, but not as much as before. The application of the extract to the intestine likewise causes the transplant to secrete. These facts demonstrate that a humoral mechanism is concerned in exciting gastric secretion when secretagogues (liver extract, meat juice) are in contact with the mucosa of the corpus and pylorus of the stomach and of the intestine.[1b] Whether a humoral mechanism is elicited from either area is not known.

1. (*a*) Ivy, A. C., and Farrell, J. I.: Contributions to the Physiology of Gastric Secretion: VIII. The Proof of a Humoral Mechanism, Am. J. Physiol. **74**: 639 (Nov.) 1925. Lim, R. K. S.; Loo, C. T., and Liu, A. C.: Observations on the Secretion of the Transplanted Stomach, Chinese J. Physiol. **1**: 51 (Jan.) 1927. Klein, E.: Gastric Secretion: II. Studies in a Transplanted Pouch Without Auerbach's Plexus, Arch. Surg. **25**: 442 (Sept.) 1932. (*b*) Gregory, R. A., and Ivy, A. C.: The Humoral Stimulation of Gastric Secretion, Quart. J. Exper. Physiol., in press. Ivy, A. C.: The Mechanisms of Gastric Secretion, Surgery, in press.

2. Kim, M. S.: Effect of Secretagogues on Gastric Secretion; Role of the Pylorus in Gastric Secretion, Mitt. a. d. med. Akad. zu Kioto **12**: 1015, 1934. Wilhelmj, C. M.; O'Brien, F. T., and Hill, F. C.: The Influence of the Pylorus on the Secretion of Acid by the Fundus, Am. J. Physiol. **116**: 685 (Aug.) 1936.

The weak point in the gastrin theory has been the difficulty in demonstrating that the humoral secretion is due to a hormone rather than to the absorption of secretagogues or to vascular or circulatory changes. This demonstration is particularly important in the case of gastrin, since most gastric secretagogues are active on parenteral administration. Several types of evidence have been advanced in favor of the hormone theory. For example, histamine-free liver extracts perfused through the lumen of a pouch of the entire stomach will elicit gastric secretion, in doses much smaller than are required to produce a response on parenteral injection.[3] During the period of perfusion the extract is not appreciably absorbed. Although this evidence is suggestive of a hormone mechanism, it is not conclusive. Recently, an older observation [4] has been confirmed, namely, that procainization of the stomach prevents the stomach from secreting in response to the application of secretagogues.[1b] However, the procainization of the mucosa of a transplanted pouch does not prevent the pouch from secreting when secretagogues are applied to the main stomach pouch. This shows that the procaine does not intoxicate the parietal cells. Since procainization of the main stomach does not prevent stimulation of the transplant when histamine is applied to the main stomach, it is difficult to believe that procaine acts by preventing absorption or diffusion of secretagogues. Accordingly, it is logical to conclude that the humoral agent is not the same substance as the secretagogues in the meat extract, and that procaine paralyzes the local mechanism which is normally responsible for the local stimulation of the gastric glands by secretagogues and for the production of a hormone.

It has been shown that the feeding of broken bones or of pieces of rubber to dogs will produce pathologic hypersecretion eighteen hours later in dogs with completely denervated pouches.[5] Since the bones or rubber could not act by being absorbed, it has been suggested that they elicit a hormone response. However, the

3. Kim, M. S., and Ivy, A. C.: On the Mode of Action of Secretagogues (Liver Extract) in Promoting Gastric Secretion, Am. J. Physiol. **105**: 220 (July) 1933.

4. Sawitch, V. V.: The Mechanism of the Second Phase of Gastric Secretion, Russ. J. Physiol. **4**: 155, 1922; abstracted, Physiol. Abstr. **7**: 431, 1922.

5. Lim, R. K. S.; Hou, H. C.; Chang, H. C., and Feng, T. P.: The Basal Secretion of the Stomach: III. The Influence of Feeding Bone and Other Hard Objects, Chinese J. Physiol. **4**: 1 (Feb.) 1930.

bones were fed with a meal, so that delayed gastric evacuation or obstruction may have permitted the absorption of secretagogues for many hours; since the pouches used retained their original vascular supply, circulatory changes are not ruled out.

The third type of evidence which has been used to support the hormone theory is that extracts of pyloric mucosa may be prepared which stimulate gastric secretion on parenteral injection. The active constituent of such extracts has been shown to be histamine, which has been isolated in the form of the crystalline picrate.[6] Careful assays of the histamine content of pyloric and fundic mucosa have revealed that the fundus contains the largest share of histamine as well as of gastric excitant,[7] yet, as previously mentioned, it is not the area most sensitive to chemical stimuli. The fact is now well known that histamine may be extracted from practically all tissues of the body, so that this evidence is not conclusive in favor of the theory that histamine is gastrin. Attempts to show that the histamine titer of the blood rises during digestion of a meal have been unsuccessful,[8] but this is not unexpected, since the quantity of histamine required to stimulate gastric secretion would be so diluted by the body fluids as to become unmeasurable. Furthermore, if it is maintained that histamine is the gastric hormone, one must conclude that atropine prevents the formation of this hormone, since investigators have repeatedly shown that atropine can abolish the gastric secretory response to a meal but not that to histamine.[9]

Recently it has been claimed that pyloric extracts free of histamine stimulate gastric secretion and that the action of such extracts is not affected by atropine.[10] Attempts to confirm this report in my laboratory and elsewhere have been unsuccessful.

6. Sacks, J.; Ivy, A. C.; Burgess, J. P., and Vandolah, J. E.: Histamine as a Hormone for Gastric Secretion, Am. J. Physiol. **101**: 331 (July) 1932.

7. Gavin, G.; McHenry, E. W., and Wilson, M. J.: Histamine in Canine Gastric Tissues, J. Physiol. **79**: 234 (Sept.) 1933.

8. MacIntosh, F. C.: Histamine as Normal Stimulant of Gastric Secretion, Quart. J. Exper. Physiol. **28**: 87 (June) 1938.

9. Gray, J. S.: The Effect of Atropine on Gastric Secretion and Its Relation to the Gastrin Theory, Am. J. Physiol. **120**: 657 (Dec.) 1937.

10. Komarov, S. A.: Gastrin, Proc. Soc. Exper. Biol. & Med. **38**: 514 (May) 1938; Further Studies on Gastrin, Am. J. Physiol. **126**: 559 (July) 1939.

PANCREATIC SECRETION—SECRETIN

Bayliss and Starling demonstrated clearly that pancreatic secretion is regulated by a hormone mechanism. This demonstration consisted in showing that a specific substance, hydrochloric acid, when introduced into a specific region of the digestive tract, the small intestine, stimulates the flow of pancreatic juice, that this action occurs independently of the nervous system and that the humoral agent is not absorbed hydrochloric acid but a specific substance which they were able to extract from the intestinal mucosa.[11] This substance was given the name "secretin." Since this time the humoral mechanism for pancreatic secretion has been conclusively demonstrated by the use of dogs with transplanted pancreas and transplanted intestinal loops.[12]

It is well known that many substances besides hydrochloric acid, including fats and the products of protein digestion, are capable of stimulating pancreatic secretion when admitted to the duodenum. It has been shown that they do not act by being absorbed from the intestine.[13]

A large number of investigators have prepared potent secretin, and within recent years two laboratories have obtained this substance in crystalline form.[14] However, its chemical structure has not yet been elucidated.

In addition to being a stimulant to the flow of pancreatic juice, secretin has been shown to be a true cholagogue, although the biliary response is considerably less than the pancreatic.[15] It is interesting in this regard that secretin is reported to stimulate the primitive hepatopancreas of the octopus.[16] Crystalline

11. Bayliss, W. M., and Starling, E. H.: The Mechanism of Pancreatic Secretion, J. Physiol. **28**: 325 (Sept.) 1902.

12. Ivy, A. C.; Farrell, J. I., and Lueth, H. C.: Contributions to the Physiology of the Pancreas: III. A Hormone for External Pancreatic Secretion, Am. J. Physiol. **82**: 27 (Sept.) 1927.

13. Gray, J. S.; Kim, M. S., and Ivy, A. C.: Is a Portion of the Pancreatic Secretory Response to a Meal Due to the Absorption of Digested Food Products? Am. J. Physiol. **116**: 210 (June) 1936. Thomas, J. E., and Crider, J. O.: The Pancreatic Secretagogue Action of Products of Protein Digestion, Am. J. Physiol. **134**: 656 (Oct.) 1941.

14. Hammarsten, E.; Jorpes, E., and Ågren, G.: Versuche zur Reinigung von Sekretin, Biochem. Ztschr. **264**: 272, 1933. Greengard, H., and Ivy, A. C.: The Isolation of Secretin, Am. J. Physiol. **124**: 427 (Nov.) 1938.

15. Tanturi, C. A.; Ivy, A. C., and Greengard, H.: Secretin Is a True Cholagogue, Am. J. Physiol. **120**: 336 (Oct.) 1937. La Barre, J., and Goffin, R.: À Propos de l'hypersécrétion biliaire consécutive à l'administration de sécrétine ou à l'injection intraduodénale de l'acide chlorhydrique dilué, Arch. internat. de physiol. **44**: 444, 1937.

16. Ledrut, J., and Ungar, G.: Action de la sécrétine chez l'octopus vulgaris, Arch. internat. de physiol. **44**: 205, 1936.

secretin has also been reported to stimulate the flow of intestinal juices.[17] Secretin, which is active on intravenous administration, produces little or no action when administered in any other way.

Within the last few years, a number of reports have been concerned with the use of secretin as a clinical test for pancreatic function.[18] In some of these investigations a commercial product which has recently become available has been employed. These investigations reveal that secretin produces the same effects in man as it does in animals; the volume of pancreatic juice and the concentration and output of bicarbonate are augmented, the outputs of the various enzymes are increased, although their concentrations are diminished, and the secretion of bile is promoted. The latter is not reflected in the duodenal drainage unless the gallbladder is incompetent or absent, or the sphincter of Oddi is relaxed. Attempts have been made to establish normal values for the test, and it is claimed that the procedure is a valuable aid in the diagnosis of pancreatic disease.

GALLBLADDER EVACUATION—CHOLECYSTOKININ

The ingestion of a meal has been repeatedly observed to cause the gallbladder to contract and evacuate.[19] Most effective in this regard are fatty substances and acids acting from the small intestine. The evacuation of the gallbladder is not appreciably affected by denervation, nor does electrical stimulation of the nerves of the organ produce more than slight and temporary effects in most animals. Further evidence for a humoral mechanism has been provided by cross circulation experiments in which the presence of hydrochloric acid in the duodenum of a donor dog causes evacuation of the gallbladder in a recipient dog.[20] Furthermore, a

17. Ågren, G.: Ueber die pharmacodynamischen Wirkungen und chemische Eigenschaften des Secretins, Skandinav. Arch. f. Physiol. **70**: 10, 1934.

18. Chiray, M., and Bolgert, M.: Le diagnostic des affections pancréatiques par l'épreuve à la sécrétine purifiée, Presse méd. **44**: 428 (March 14) 1936. Voegtlin, W. L.; Greengard, H., and Ivy, A. C.: The Response of the Canine and Human Pancreas to Secretin, Am. J. Physiol. **110**: 198 (Nov.) 1934. Lagerlöf, H.: The Secretin Test of Pancreatic Function, Quart. J. Med. **8**: 115 (April) 1939. Diamond, J. S.; Siegel, S. A.; Gall, M. B., and Karlen, S.: The Use of Secretin as a Clinical Test of Pancreatic Function, Am. J. Digest. Dis. **6**: 366 (Aug.) 1939. Comfort, M. W., and Osterberg, A. E.: Pancreatic Secretion in Man After Administration of Different Substances: A Comparative Study, Am. J. Digest. Dis. **8**: 337 (Sept.) 1941.

19. Ivy, A. C.: The Physiology of the Gall Bladder, Physiol. Rev. **14**: 1 (Jan.) 1934.

20. Ivy, A. C., and Oldberg, E.: A Hormone Mechanism for Gall Bladder Contraction and Evacuation, Am. J. Physiol. **86**: 599 (Oct.) 1928.

gallbladder transplanted into the neck of a dog will respond to the presence of acid in the duodenum.[21] Blood transfusion experiments, though equivocal, indicate the humoral nature of gallbladder evacuation in man.[22] It has been repeatedly shown that the humoral agent is not fat or other substances which might be absorbed from the intestine.[23] The implication that a hormone is involved has been substantiated by the successful preparation of intestinal extracts which activate the gallbladder in man as well as in experimental animals.[24] The hormone has been given the name "cholecystokinin." The evidence indicates that this hormonal mechanism is the chief stimulus for evacuation of the gallbladder.

Cholecystokinin, which has been separated from secretin, has not yet been obtained in pure crystalline form, nor has it been rendered available for clinical investigation. It is not active on oral or rectal administration.[25] The available evidence suggests that it relaxes the sphincter of Oddi.[26] The tone and degree of distention of the gallbladder have been shown to be important factors in determining the response of the organ to cholecystokinin.[27] The gallbladder in pregnancy does not evacuate normally in response to cholecystokinin.[27b]

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21. Houssay, B. A., and Rubio, H. H.: L'hormone duodéno-cholécystokinétique, Compt. rend. Soc. de biol. **111**: 455 (Sept.) 1932.

22. Sandblom, P.: The Function of the Human Gall Bladder Studied in Connection with Blood Transfusion and After Stomach Operations, Acta radiol. **14**: 249, 1933. Bettman, R. B., and Tannenbaum, W. J.: Gall Bladder Contractility After Blood Transfusion, J. A. M. A. **106**: 1376 (April 18) 1936.

23. Higgins, G. M., and Wilhelmj, C. M.: The Effect of Intravenous Injection of Various Emulsions of Fat on the Emptying of the Gall Bladder, Am. J. M. Sc. **178**: 805 (Dec.) 1929. Voegtlin, W. L.; McEwen, E. G., and Ivy, A. C.: On the Humoral Agents Concerned in the Causation of Gall Bladder Contraction, Am. J. Physiol. **103**: 121 (Jan.) 1933.

24. Ivy, A. C.; Kloster, G.; Lueth, H. C., and Drewyer, G. E.: On the Preparation of "Cholecystokinin," Am. J. Physiol. **91**: 336 (Dec.) 1929. Ågren, G.: On the Preparation of Cholecystokinin, Skandinav. Arch. f. Physiol. **81**: 234, 1939. Ivy, A. C.; Drewyer, G. E., and Orndorff, B. H.: The Effect of Cholecystokinin on the Human Gall Bladder, Endocrinology **14**: 343 (Sept.) 1930.

25. Doubilet, H., and Ivy, A. C.: Absorption of Cholecystokinin and Secretin from the Colon and Rectum, Proc. Soc. Exper. Biol. & Med. **39**: 129 (Oct.) 1938.

26. Sandblom, P.; Voegtlin, W. L., and Ivy, A. C.: The Effect of Cholecystokinin on the Choledochoduodenal Mechanism (Sphincter of Oddi), Am. J. Physiol. **113**: 175 (Sept.) 1935.

27. (*a*) Doubilet, H., and Ivy, A. C.: The Response of the Smooth Muscle of the Gall Bladder at Various Intravesicular Pressures to Cholecystokinin, Am. J. Physiol. **124**: 379 (Nov.) 1938. (*b*) Smith, J. J.; Pomaranc, M. M., and Ivy, A. C.: The Influence of Pregnancy and Sex Hormones on Gall Bladder Motility, Am. J. Physiol. **132**: 129 (Feb.) 1941.

GASTRIC INHIBITION—ENTEROGASTRONE

That the ingestion of neutral fat inhibits gastric secretion and motility has been known since the last century. It has been repeatedly confirmed that this effect is elicited from the small intestine and not from the stomach.[28] Experiments with transplanted pouches of the stomach have shown that the inhibition is humoral in nature.[29] The possibility that absorbed products of digestion constitute the humoral agent has been excluded.[30] The substance concerned, which can be prepared from intestinal mucosa, has been shown to inhibit gastric motility of both the fasting and the digestive type and to inhibit gastric secretion stimulated by a meal, sham feeding or the injection of insulin or histamine.[31] The active principle of these extracts has been given the name "enterogastrone." This principle has not yet been obtained in pure crystalline form.

Recently several independent laboratories have reported that urine contains a gastric inhibitory substance.[32] This principle has been demonstrated in the urine of normal men and women, pregnant women, patients with various gastric disorders and normal,

28. (*a*) Sokalov, A.: Zur Analyse der Abscheidungsarbeit des Magens bei Hunden, Jahresb. ü. d. Fortschr. d. Tier-Chem. **34**: 469, 1905. (*b*) Lonnqvist, B.: Beiträge zur Kenntnis der Magensoftabsonderung, Skandinav. Arch. f. Physiol. **18**: 194, 1906. (*c*) Waugh, J. M.: Effect of Fat Introduced into the Jejunum by Fistula on Motility and Emptying Time of the Stomach, Arch. Int. Med. **33**: 451 (Sept.) 1936. (*d*) Quigley, J. P.; Zettleman, H. J., and Ivy, A. C.: Analysis of the Factors Involved in Gastric Motor Inhibition by Fats, Am. J. Physiol. **108**: 643 (June) 1934.

29. (*a*) Farrell, J. I., and Ivy, A. C.: Studies on the Motility of the Transplanted Gastric Pouch, Am. J. Physiol. **76**: 227 (March) 1926. (*b*) Feng, T. P.; Hou, H. C., and Lim, R. K. S.: Mechanism of Inhibition of Gastric Secretin by Fat, Chinese J. Physiol. **3**: 371 (Oct.) 1929. Quigley, Zettleman and Ivy.[28d]

30. Kosaka, T., and Lim, R. K. S.: Mechanism of Inhibition of Gastric Secretion by Fat: Role of Bile and Cystokinin, Chinese J. Physiol. **4**: 213 (May) 1930. Quigley, Zettleman and Ivy.[28d] Feng, Hou and Lim.[29b]

31. Lim, R. K. S.: Observations on Mechanism of Inhibition of Gastric Function by Fat, Quart. J. Exper. Physiol. **23**: 263 (Aug.) 1933. Gray, J. S.; Bradley, W. B., and Ivy, A. C.: On the Preparation and Biological Assay of Enterogastrone, Am. J. Physiol. **118**: 463 (March) 1937.

32. Necheles, H.; Hanke, M. E., and Fautl, E.: Preparation and Assay of Inhibitor of Gastric Secretion and Motility from Normal Human Urine, Proc. Soc. Exper. Biol. & Med. **42**: 618 (Nov.) 1939. Friedman, M. H. F.; Recknagel, R. O.; Sandweiss, D. J., and Patterson, T. L.: Inhibitory Effect of Urine Extracts on Gastric Secretion, ibid. **41**: 509 (June) 1939. Friedman, M. H. F.; Sandweiss, D. J.; Recknagel, R. O., and Patterson, T. L.: Effects of Extracts of Urine from Pernicious Anemia and Gastric Cancer Patients on Gastric Secretion, Anat. Rec. **75** (supp.): 53 (Dec.) 1939. Friedman, M. H. F.; Saltstein, N. C., and Forbman, A.: Effect of Urine from Gastrectomized and Duodenectomized Dogs on Gastric Secretion, Proc. Soc. Exper. Biol. & Med. **43**: 181 (Jan.) 1940. Culmer, C. U.; Atkinson, A. J., and Ivy, A. C.: Depression of Gastric Secretion by Anterior Pituitary-like Fraction of Pregnancy Urine, Endocrinology **24**: 631 (May) 1939. Gray, J. S.; Wieczorowski, E., and Ivy, A. C.: Inhibition of Gastric Secretion by Extracts of Normal Male Urine, Science **89**: 489 (May 26) 1939.

duodenectomized or gastrectomized dogs. The earlier extracts contained toxic impurities (pyrogenic substances) which exerted an inhibitory action on gastric secretion, but potent preparations free of these contaminants have now been obtained.[33] Such extracts have been shown to inhibit the secretory response of the human stomach to histamine.[34] Since the gastric inhibitory principle of urine has been differentiated from a number of other suspected substances, it has been given the name "urogastrone." That it may possibly represent excreted enterogastrone is suggested by the finding that it is excreted in increased quantities in the urine of fat-fed dogs and is decreased in the urine of dogs from which the entire small intestine has been removed.[35] The evidence does not support the view that enterogastrone and urogastrone are identical.[35a] The gastric inhibitory principles, enterogastrone and urogastrone, probably offer greater therapeutic possibilities than the other gastrointestinal hormones. The parenteral administration of enterogastrone three times daily prevents the development of experimental post-operative jejunal ulcer.[35b]

INTESTINAL SECRETIONS—ENTEROCRININ

As long ago as 1902 it was shown that Brunner's glands in an isolated segment of duodenum will respond slightly to the ingestion of certain types of food, although locally acting stimuli are probably more effective.[36] More recently it has been reported that goats, pigs, cats and dogs with isolated duodenal segments exhibit in these segments an increase in the rate of secretion from Brunner's glands in response to feeding.[37] This has been confirmed in the case of the dog.[38]

33. Gray, J. S.; Culmer, C. U.; Wieczorowski, E., and Adkison, J. L.: Preparation of Pyrogen-Free Urogastrone, Proc. Soc. Exper. Biol. & Med. **43**: 225 (Feb.) 1940.

34. Wieczorowski, E.; Gray, J. S., and Ivy, A. C.: The Effect of Urogastrone on Gastric Secretion in Man, Am. J. Physiol. **129**: 496, 1940.

35. (*a*) Culmer, C. U.; Gray, J. S.; Adkison, J. L., and Ivy, A. C.: On the Origin of Urogastrone, Science **91**: 148 (Feb. 9) 1940. Gray, J. S.; Culmer, C. U.; Wells, J. A., and Wieczorowski, E.: Factors Influencing the Excretion of Urogastrone, Am. J. Physiol. **134**: 623 (Oct.) 1941. (*b*) Hands, A. P.; Fauley, G. B.; Greengard, H.; Preston, F. W., and Ivy, A. C.: Prevention of Experimental Gastrojejunal Ulcer by Enterogastrone, Am. J. Physiol. **133**: 314 (June) 1941.

36. Ponomarew, cited by Babkin.[42]

37. Florey, H. W., and Harding, H. E.: Further Observations on the Secretion of Brunner's Glands, J. Path. & Bact. **39**: 255 (Sept.) 1934.

38. Fogelson, S. J., and Bachrach, W. H.: Response of Brunner's Glands to Secretin, Am. J. Physiol. **128**: 121 (Dec.) 1939.

It was later claimed that this response to feeding could be obtained after cutting the vagi and splanchnic nerves and could be obtained from transplanted duodenal loops.[39] It has not been shown that the humoral agent is not some absorbed product of digestion. In support of the view that a hormone is concerned are the reports that impure preparations of secretin stimulate the secretion of Brunner's glands.[40] It has not been possible to establish that the accompanying increase of duodenal motility does not force out accumulated secretions from the duodenal loop.[38]

In the case of jejunal and ileal loops of intestine, which secrete succus entericus, the evidence is not so clear. Both chemical and mechanical stimuli are effective when applied directly to the loop. If the loop is spared all mechanical influences, usually no secretion results whether the animal is fasted or is fed. A rubber collecting tube in the lumen is sufficient to evoke continuous secretions, which, however, is scarcely affected by feeding the animal.[41] If local stimuli produce an obvious response and distant stimuli only a scarcely perceptible one, it is difficult to escape the conclusion that the latter are of relatively little physiologic significance.

Recently the secretion of succus entericus has been studied in greater detail.[42] In these experiments intestinal loops were transplanted beneath the skin. Following this the secretory response to a meal was studied before and after complete denervation, accomplished by severing the pedicle. The succus entericus was collected by means of a rubber tube in the lumen of the fistula. In a large series of experiments it was found that neither the volume nor the output of enzymes was augmented by feeding before denervation. Following denervation a 60 per cent increase in the volume of juice was observed in response to a meal, and a ques-

39. Florey, H. W., and Harding, H. E.: A Humoral Control of Secretion of Brunner's Glands, Proc. Roy. Soc., London, s. B **117**: 68 (Feb.) 1935. Wright, R. D.; Jennings, M. A.; Florey, H. W., and Lium, R.: The Influence of Nerves and Drugs on Secretion by the Small Intestine, Quart. J. Exper. Physiol. **30**: 73 (Jan.) 1940.

40. Florey, H. W., and Harding, H. E.: The Nature of the Hormone Controlling Brunner's Glands, Quart. J. Exper. Physiol. **25**: 329 (Dec.) 1935. Fogelson and Bachrach.[38]

41. Babkin, B. P.: Die aussere Sekretion der Verdauungsdrusen, Berlin, Julius Springer, 1928.

42. Nasset, E. S.; Pierce, H. B., and Murlin, J. R.: Proof of a Humoral Control of Intestinal Secretion, Am. J. Physiol. **111**: 145 (Feb.) 1935.

tionable enzyme response was obtained. A later study [43] of a similar nature showed a more clear-cut increase in the production of enzymes in the denervated fistula. These results have been interpreted to indicate that the secretory response of the intestine is augmented by removing the influence of inhibitory nerves and that a humoral mechanism is involved. Attempts by earlier workers to detect a response of the denervated or transplanted intestinal loop to feeding were unsuccessful.[44]

That the possible humoral agent may be absorbed products of digestion is suggested by the finding that peptones are active on parenteral and on oral administration.[45] On the other hand, evidence that a hormone is concerned has been obtained by preparing extracts of intestinal mucosa which stimulate the flow of succus entericus.[46] The active principle of these extracts, which has been given the name "enterocrinin," has been shown to be distinguishable from vasodilator substances, secretin and enterogastrone.

INTESTINAL VILLI—VILLIKININ

It has been reported that the ingestion of a meal greatly augments the movements of the intestinal villi. The early investigations emphasized the importance of local chemical and mechanical stimuli for evoking these movements, since the movements occurred only in intestinal segments to which chyme had descended.[47] However, a humoral mechanism was proposed as a result of the discovery that the introduction of hydrochloric acid into the duodenum would activate the movements of the villi of a jejunal segment which had been removed from the abdominal cavity and viviperfused by anastomosis with the external jugular vein and the carotid artery.[48] This was subsequently repeated successfully in experiments with cross circulation between two

43. Schiffrin, M. J., and Nasset, E. S.: The Response of the Jejunum and Ileum to Food and Endocrinin, Am. J. Physiol. **128**: 70 (Dec.) 1939.

44. (*a*) Puestow, C. B.: The Activity of Isolated Intestinal Segments. Arch. Surg. **24**: 565 (April) 1932. (*b*) Ivy, Farrell and Lueth.[12]

45. Nasset, E. S., and Pierce, H. B.: On the Influence of Peptones and Certain Extracts of Small Intestine upon the Secretion of Succus Entericus, Am. J. Physiol. **113**: 568 (Nov.) 1935.

46. Nasset, E. S.: Enterocrinin: A Hormone Which Excites the Glands of the Small Intestine, Am. J. Physiol. **121**: 481 (Feb.) 1938.

47. von Kokas, E., and von Ludany, G.: Weitere Untersuchungen über die Bewegung der Darmzotten, Arch. f. d. ges. Physiol. **225**: 421, 1930.

48. von Kokas, E., and von Ludany, G.: Die hormonale Regelung der Darmzottenbewegung: I. Arch. f. d. ges. Physiol. **232**: 293, 1933.

dogs.[49] An indication that the humoral agent is a hormone was provided by the claim that crude preparations of secretin on parenteral injection stimulated movements of the villi.[50] The active principle, which was shown to be distinguishable from histamine, choline, adenosine, secretin and cholecystokinin, has been given the name "villikinin."[51] Evidence for the physiologic significance of villikinin consists in the report that augmentation of the movements of the villi promotes intestinal absorption and that this action can be demonstrated in cross circulation experiments.[52]

None of the work on villikinin has as yet been confirmed. It has been reported that the introduction of hydrochloric acid into the duodenum failed to promote the absorption of dextrose from isolated intestinal loops in unanesthetized dogs.[53]

CARBOHYDRATE METABOLISM—DUODENIN—ISLET-STIMULATING HORMONE

During the past ten years or more there has accumulated a rather extensive literature in which claims are made that extracts of intestinal mucosa on parenteral or oral administration will lower the fasting blood sugar level of normal or of depancreatized dogs and of rabbits and will reduce the dextrose tolerance curve. These claims have been widely held to indicate the existence of a duodenal hormone which regulates the blood sugar level. It has also been claimed that the introduction of hydrochloric acid into the duodenum will likewise lower the fasting blood sugar level, although some investigators have maintained that this can be demonstrated only in the absence of the adrenal glands.[54]

Little effort has been made to establish the necessary requirement that the effect of the acid is mediated humorally or to provide evidence that the phenomenon would operate in normal animals as the result of the

49. von Kokas, E., and von Ludany, G.: Nouvelle recherches sur la régulation hormonale des mouvements des villosités intestinales, Compt. rend. Soc. de biol. **117**: 972, 1934.

50. von Kokas, E., and von Ludany, G.: Die hormonale Regelung der Darmzottenbewegung: II. Das Villikinin, Arch. f. d. ges. Physiol. **234**: 182, 1934. von Kokas and von Ludany.[48]

51. von Kokas, E., and von Ludany, G.: Ueber das Villikinin, Arch. f. d. ges. Physiol. **234**: 589, 1934.

52. von Kokas, E., and von Ludany, G.: Relation Between "Villikinin" and the Absorption of Glucose from the Intestine, Quart. J. Exper. Physiol. **28**: 15 (June) 1938.

53. Loew, E. R.; Gray, J. S., and Ivy, A. C.: The Effect of Acid Stimulation of the Duodenum upon Experimental Hyperglycemia and the Utilization of Glucose, Am. J. Physiol. **128**: 298 (Jan.) 1940.

54. La Barre, J.: La sécrétine, Paris, Masson & Cie, 1936.

entrance of food into the intestine. With the intention of providing these essential links in the chain of evidence for the proposed hormone, attempts were first made to repeat the original experiments. It was found, however, that the presence of hydrochloric acid in the duodenum failed to lower the fasting blood sugar level of normal anesthetized or unanesthetized dogs or dogs with ligated adrenal glands.[55] Negative results were also obtained in attempts to improve intravenous dextrose tolerance or to reduce the hyperglycemic response to the absorption of dextrose, to the injection of epinephrine or to removal of the pancreas.[53] Finally, extracts of intestinal mucosa were prepared by a variety of methods which have been reported to yield the hypoglycemic substance, but none lowered the fasting blood sugar level of trained, unanesthetized dogs.[56] Throughout these investigations, critical examination of the earlier literature disclosed apparently adequate explanations for the contrary results reported by the previous investigators. The possibility, however, has not been excluded that the gastrointestinal tract may elaborate an "insulin synergist" which affects carbohydrate metabolism.

INTESTINAL MOTILITY

It has been repeatedly observed that segments of the small intestine still in normal continuity with the remainder of the intestine exhibit an increase in motility when the animal is fed.[57] This augmented activity may appear within several minutes after feeding. It occurs when food is admitted to the stomach through a gastrostomy, and it is not abolished by bilateral vagotomy. However, no one has been able to demonstrate this phenomenon in a loop of intestine removed from continuity with the rest of the intestine, whether the loop retains its nerve supply or is denervated, or

55. Loew, E. R.; Gray, J. S., and Ivy, A. C.: The Effect of Duodenal Instillation of Hydrochloric Acid upon the Fasting Blood Sugar of Dogs, Am. J. Physiol. **126**: 270 (June) 1939.

56. Loew, E. R.; Gray, J. S., and Ivy, A. C.: Is a Duodenal Hormone Involved in Carbohydrate Metabolism? Am. J. Physiol. **129**: 659 (June) 1940.

57. (*a*) Barcroft, J., and Robinson, C. S.: A Study of Some Factors Influencing Intestinal Movements, J. Physiol. **67**: 211 (March) 1929. (*b*) Castleton, K. B.: An Experimental Study of the Movements of the Small Intestine, Am. J. Physiol. **107**: 641 (March) 1934. (*c*) Hukuhara, T.; Kinose, S., and Masuda, K.: Beiträge zur Physiologie der Bewegung des Duodenums, Arch. f. d. ges. Physiol. **238**: 124, 1936. (*d*) Douglas, D. M., and Mann, F. C.: The Activity of the Lower Part of the Ileum of the Dog in Relation to the Ingestion of Food, Am. J. Digest. Dis. **6**: 434 (Sept.) 1939.

is transplanted.[58] These observations practically rule out a hormonal mechanism and favor the view that local stimuli are of the greatest importance. It has been shown that local chemical and mechanical stimuli are very effective in evoking intestinal movements and that their action is abolished by local anesthetization of the mucosa.[59]

Certain intestinal extracts contain a substance which is neither histamine nor choline but which augments intestinal motility.[26] My co-workers and I have occasionally obtained enterogastrone preparations that have produced gastric motor inhibition followed by stimulation. With one such extract we were successful in isolating two fractions, one which produced inhibition only and one which produced stimulation only. However, until such time as some motor activity of the gastrointestinal tract has been shown to involve a humoral mechanism, such substances are of pharmacologic interest only. To attach physiologic significance to them at the present time is to advance an explanation for an unknown phenomenon.

SALIVARY GLANDS

There is no evidence indicating that an internal secretion is specifically concerned in the secretion of saliva.[60]

THERAPY

Although the internal secretions of the gastrointestinal tract are of great physiologic interest, none of them have been demonstrated to possess therapeutic value. Histamine, if it is gastrin, and secretin possess diagnostic value. Cholecystokinin is of potential diagnostic value. Enterogastrone and urogastrone possess therapeutic promise.

58. Ivy, A. C., and Vloedman: The Small Intestine in Hunger, Am. J. Physiol. **72**: 99 (March) 1925. Quigley, J. P.; Highstone, W. H., and Ivy, A. C.: A Study of the Propulsive Activity of a Thiry-Vella Loop of Intestine, ibid. **108**: 151 (April) 1934. Hukuhara, T., and Kinose, S.: Existiert das Hormon der Darmbewegung, welches sich durch die Einwirkung der Zalzäure auf die Darmschleimhaut bildet? Jap. J. M. Sc., III, Biophysics **5**: 45 (March) 1938. Ivy, Farrell and Lueth.[12] Puestow.[44a] Douglas and Mann.[57d]

59. Borchardt, W.: Gibt es nervöse Chemoreceptoren in der Dünndarmschleimhaut? Arch. f. d. ges. Physiol. **215**: 402, 1926.

60. Ivy, A. C.: Gastrointestinal Principles, in Glandular Physiology and Therapy, Chicago, American Medical Association, 1935, p. 439.

CHAPTER XXXI

PRESENT STATUS OF COMMERCIAL ENDOCRINE PREPARATIONS

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In the past few years physicians have had clinical experience with numerous potent endocrine preparations, many of which are pure chemical compounds. The chemical structure of most of these compounds has been identified and their physiologic activities thoroughly studied in the laboratory. With the advent of these, physicians have become well aware that preparations of desiccated ovary, testicle, thymus, pineal gland, prostate, mammary gland and other organs or simple extracts of these tissues, which have been on the market many decades are unscientific and that their physiologic activities are insignificant. Evidence supporting claims of therapeutic value for such products is either completely lacking or based on fragmentary reports or testimonials. In view of the fact that there are at present on the market pure potent endocrine principles whose effects are well known and whose properties have been investigated scientifically, there is no justification for the use of preparations whose activity is obscure and whose therapeutic effectiveness remains, after many years, unestablished. The wastefulness of prescribing these "gland" products is so apparent that the Food and Drug Administration issued a trade notice against the marketing of one type of such preparations, i. e., ovarian extracts in which neither estrogenic nor progestational activity can be satisfactorily demonstrated. A recent editorial in THE JOURNAL called the attention of physicians to this action as follows:[1a]

> . . . Except for the thyroid and perhaps posterior pituitary, products derived from a few grains of desiccated glandular material have never been proved to be effective in endocrine therapy.

1. (*a*) Ovarian Preparations, editorial, J. A. M. A. **114**: 415 (Feb. 3) 1940. (*b*) Estrone (Theelin) in New and Nonofficial Remedies, Chicago, American Medical Association Press, 1941, p. 376.

The Council on Pharmacy and Chemistry has repeatedly exposed such obsolete preparations as worthless and has warned the medical profession against the insidious advertising and sales policies of those firms which promote them. Now it is gratifying to learn that the Food and Drug Administration has ruled against one type of these unscientific remedies:

"NOTICE TO MANUFACTURERS OF PREPARATIONS OF OVARY:

"There are on the market drug products in liquid form designated as 'Ovarian Extract' or by some similar title. In some instances these products have been found not to contain the known therapeutically and physiologically active constituents of ovary, namely, those having estrogenic and progestational activities. The Food and Drug Administration is of the opinion that such inert or essentially inert preparations when sold as 'Ovarian Extract,' or under any other designation or under labeling which states or implies that such active principles are present, are both adulterated and misbranded as those terms are defined in the Federal Food, Drug and Cosmetic Act."

Numerous other "gland" products, relics from the ancient endocrine armamentarium, deserve similar condemnation.

ESTROGENS

There are more preparations of estrogenic substances on the market than of any other endocrine product. This popularity is probably due to the fact that estrogen therapy has proved reliable and effective under the proper conditions. The Council on Pharmacy and Chemistry has up to the present time recognized the value of estrogens in the following conditions:[1b]

. . . the treatment of symptoms of menopause, natural or artificial, of certain other conditions related to deficiency of estrogen including senile vaginitis, kraurosis vulvae and pruritus vulvae and of gonorrheal vaginitis of children.

Although there have been numerous reports on the use of estrogens in a variety of other conditions, both endocrine and nonendocrine, it has yet to be adequately demonstrated that such therapy is of proved value in routine practice.

The estrogens marketed at present are of several varieties. Many are relatively simple extracts of urine obtained from pregnant women or mares. Others are crystalline estrogens obtained from natural sources or are natural estrogens modified by chemical procedures. Recently completely synthetic estrogens have become available, and one of these is now marketed in this country.

The choice of an estrogen for clinical use is still somewhat of a problem to the physician. The flood of advertising material constantly washing up on his desk with arguments in favor of one particular estrogen and claims for superiority based on laboratory experimentation is most confusing. Then, too, the prices of the various products differ considerably and it is frequently difficult for the physician to select what he considers an effective, reliable estrogen at an economical cost. This unfortunate situation is in part due to the lack of a satisfactory nomenclature and concise therapeutic standards.

In 1936, on the recommendation of the Advisory Committee on Nomenclature, the Council adopted the terms "estrone," with its synonym "theelin," and "estriol," with its synonym "theelol," for crystalline ketohydroxyestrin and trihydroxyestrin, respectively, and "estradiol" for dihydroxyestrin. A more difficult problem has arisen in the establishment of the nomenclature for preparations of mixed estrogens. These noncrystalline estrogens are usually derived from the urine of pregnant mares and the urine or placentas of pregnant women. Until recently there was no official term for these preparations, which contain mainly estrone and varying amounts of phenolic compounds, some of which are estrogenic. Numbers of these preparations are on the market, and each is known by a proprietary name which undoubtedly causes considerable confusion as to the nature of the preparation. The Council has recently adopted the terms "Solution of Estrogens" and "Solution of Estrogenic Substances" to signify such preparations in solution. The Council has also advised that the source of each preparation should be stated on the label. Estradiol and its compounds are known only by their proprietary names. A simplification of this nomenclature would undoubtedly be appreciated by the medical profession.

The question of standardization of the estrogens is still a major problem in therapy. The establishment of the international standard for estrone has helped somewhat; 0.0001 mg. of estrone equals 1 international unit (1 I. U.). This international standard is not to be confused with the international standard for estradiol benzoate, 0.0001 mg. of estradiol benzoate being 1 international benzoate unit (1 I. B. U.). This standardiza-

tion was attempted in order to avoid the variations inherent in bioassays in different laboratories. Some of the factors which influence the results of bioassays are (1) difference in the responses of animal colonies, (2) variation in technics, (3) species difference and (4) lack of uniformity in determining the end point of activity as observed in vaginal smears. The wide fluctuation in bioassays compared with weighed amounts of estrone is indicated in table 1, showing the number of international units of estrone equivalent to 1 biologic unit as obtained by different workers.

TABLE 1.—*A Comparison of One Biologic Unit of Each of a Number of Oil Solutions of Estrone with the International Standard (0.0001 Mg. Equals 1 International Unit)*

Authors	1 Mouse Unit	1 Rat Unit
Hain and Robson	0.9 I. U.	33 I. U.
Schoeller, Dohrn and Hohlweg	5.0 I. U.	25 1. U.
Pedersen-Bjergaard	3.5 I. U.	28 I. U.
Emmens [2b]	1.5 I. U.	.....
Doisy	0.5 I. U.	
D'Amour & Gustavson	.....	13 I. U.
Hinglais and Hinglais	.....	25 I. U.
Burn	.....	9.6 I. U.
Mazer and Israel	.....	10.0 I. U.
Gerard	.....	41 I. U.
Rowe & Simond	.....	3.0 I. U.

The standardization of noncrystalline estrogens in terms of international units may be misleading, since the international unit refers only to crystalline estrone. Emmens [2b] has pointed out significant differences in assays when impure estrogen preparations are used. He has concluded that "we cannot in practice arrive at a trustworthy estimate of the nature or amounts of the estrogens present in an impure extract." With highly purified estrogen preparations, containing traces only of contaminants, assays in terms of international units may be practical for clinical purposes if the necessary controls are included in such a study. Standardization of the experimental animals with weighed amounts of estrogens increases the reliability of bioassays.[2a]

2. (*a*) Palmer, A.: The Effect in Ovariectomized Mice of Past Estrogenic Stimulation on the Subsequent Responses to Estrogen, with Special Reference to Method of Bio-Assay, Univ. California Publ., Pharmacol. 1: 375 (Feb. 7) 1941. (*b*) Emmens, C. W.: Reports on Biological Standards—V. Variables Affecting the Estimation of Androgenic and Estrogenic Activity, Medical Research Council, Special Report Series, no. 234, London, His Majesty's Stationery Office, 1939.

A more complex problem arises with assays of estradiol compounds. It has been repeatedly shown that in the rat estradiol itself is more potent than estrone. In the mouse, however, there is some evidence which indicates that estrone is more nearly equal to estradiol in activity. The ratios of activity between estradiol and estrone in mice and in rats as determined by different investigators are given in table 2. The wide variation in the results indicate that any exact conclusion regarding the relative activity of estrone and estradiol in laboratory animals is unsafe.

Some of the manufacturers of estradiol and its compounds have of late advertised that their preparations

TABLE 2.—*The Ratios of the Potencies of Oil Solutions of Estrone and Estradiol in Rats and Mice Obtained by Various Investigators*

Authors	Estrone-Estradiol Ratio in Mice	Estrone-Estradiol Ratio in Rats
Schoeller, Dohrn & Hohlweg.....	1: 0.8	1: 6
Sondern & Sealey.....	1: 6	1: 2
David, de Jongh & Laqueur.....	1: 2	1: 3
Dirscherl	1: 5 to 10	.....
Whitman, Wintersteiner & Schwenk....	.....	1: 12
Pedersen-Bjergaard	1: 0.08	1: 0.5
MacCorquodale and associates.....	.....	1: 6

of estradiol are many times more potent than estrone for clinical purposes. There is no information which indicates conclusively that the relatively greater potency of estradiol over estrone in the rat is obtained in man. This situation is further complicated by the fact that compounds of estradiol such as estradiol benzoate or estradiol dipropionate cannot be compared directly with estrone by animal assays, since the rates of absorption of these substances differ considerably. Substances like estrone or estradiol in oil solution are absorbed relatively rapidly and produce a short estrus in rats, while estradiol dipropionate, which is slowly absorbed, produces a prolonged estrus. In view of the differences in persistence of estrous changes between estrone and estradiol dipropionate, a comparison of these in biologic units such as "rat units" is meaningless. Similarly, a comparison of estrone and estradiol benzoate on the basis of rat units is unjustified.

It would seem that compounds of estradiol such as the benzoate and the dipropionate would be more efficient therapeutically than estrone because the slower absorption of these compounds would result in a more protracted action resulting in less loss by destruction of the material administered, thus necessitating fewer injections. Surprisingly, there are few conclusive data available comparing the therapeutic efficiency of weighed amounts of these estrogens. There is some evidence which indicates that with administration of estrone and estradiol benzoate to human subjects the rates of urinary

TABLE 3.—*The Ratios of the Potencies of Parenterally and Orally Administered Estrone in Various Animals Obtained by Different Investigators*

	Parenteral-Oral Ratio in			
Authors	Mouse	Guinea Pig	Rat	Monkey
Sondern and Sealey...	105:1	.....	75:1	.....
Pedersen-Bjergaard ...	62:1	19:1	198:1	5:1
Rowe and Simon.....	.....	.....	15:1	.....

The Ratios of the Potencies of Parenterally and Orally Administered Estradiol in Various Animals Obtained by Different Investigators

Sondern and Sealey...	500:1	.....	97:1	.....
Pedersen-Bjergaard ...	75:1	1.8:1	620:1	20:1
Rowe and Simon.....	.....	.....	15:1	.....

excretion of estrogens are equal.[3] Since estrogens are used in human subjects, it stands to reason that assays employing human subjects will give a greater measure of reliability over laboratory procedures employing animals. Such a study should be based on weighed amounts of the estrogens tested rather than on biologic units. Until such data are made available, the physician is forced to rely to a great extent on his own clinical experience as to the comparative effectiveness of the various estrogens.

In the rat or the mouse estrogens are active to only a slight extent when administered by mouth. In such

3. Mazer, C., and Israel, S. L.: Studies on Optimal Dosage of Estrogens: Experimental and Clinical Evaluation, J. A. M. A. **108**: 163 (Jan. 16) 1937.

laboratory animals only a small portion of the administered estrone or estradiol escapes destruction; in other animals, including the human, however, there is evidence which indicates that these estrogens administered orally are relatively more effective. Table 3 indicates the relative efficiency of orally administered natural estrogens in several animal species. This table shows once more the difficulty of interpreting the therapeutic effectiveness of estrogens on the basis of laboratory data.

Estrogens are also effective by absorption following the application of ointments or tinctures to the skin or the insertion of vaginal or rectal suppositories. The efficiency of such methods of treatment is not as great as that by injection of oil solutions when a systemic effect is desired. Topical application is more efficient than parenteral administration in the treatment of such conditions as gonorrheal vaginitis, atrophic vaginitis and kraurosis. The following are the Council-accepted preparations of crystalline estrogens:

Estrone (Crystalline)

Source: Urine of stallions or pregnant mares.
Assay: By the international standard (0.0001 mg. equals 1 international unit).
License issued by: St. Louis University.

Product	Firm
Estrone-Abbott	Abbott Laboratories
Estrone-Lilly	Eli Lilly & Company
Theelin-P. D. & Co.	Parke, Davis & Company

Estriol (Crystalline)

Source: Urine of pregnant women.
Assay: By weight.
License issued by: St. Louis University.

Product	Firm
Estriol-Abbott	Abbott Laboratories
Estriol-Lilly	Eli Lilly & Company
Theelol-P. D. & Co.	Parke, Davis & Company

Crystalline alpha estradiol and its compounds estradiol benzoate, estradiol propionate and estradiol dipropionate are available. These compounds are prepared from the estrone derived from the urine of

stallions or pregnant mares. They are assayed either by weight or in terms of rat units. Estradiol is usually provided in the form of tablets for oral administration and in ointments and suppositories for absorptive administration. The other estradiol preparations are dissolved in oil for injection purposes. While there is an abundance of evidence in the scientific literature to indicate that estradiol and its compounds are apparently potent and effective estrogens, the Council on Pharmacy and Chemistry has not as yet accepted any brand for inclusion in New and Nonofficial Remedies. Only one firm marketing such preparations has submitted its products to the Council, and these are still in the process of examination. Physicians are advised that many of the advertising claims frequently made for estradiol and its compounds are considered by the Council to be exaggerated and without adequate proof.

There are a number of noncrystalline estrogen preparations, which as a rule are refined extracts of urine from stallions, pregnant women and pregnant mares. They contain principally estrone together with varying amounts of other urinary phenols. There is no systematic study on the reliability of the assays presented by the firms which market these products. The Council has accepted the following preparation of noncrystalline estrogen:

Estrogenic Substances (Noncrystalline)

Source: Urine of pregnant mares.

Assay: Biologic—transposed to equivalents of international units

Product	Firm
Amniotin	E. R. Squibb & Sons

Another type of noncrystalline estrogen preparation, containing principally estriol glycuronide, is also on the market. This material, originally obtained from human placenta, is now derived from human pregnancy urine. Its use is confined to oral administration.

Recently a number of synthetic preparations have been developed which possess sufficiently high estrogenic potency to suggest their use in estrogen therapy. Stilbene compounds have been intensely investigated in this respect, and it appears that the most promising of

these synthetic compounds at the present time is 4,4′-dihydroxy-α,β-diethylstilbene.

In accordance with the writings of Dodds and associates,[4] who introduced the stilbene compounds for their estrogenic activity, the Council on Pharmacy and Chemistry has adopted the term "diethylstilbestrol" as the official designation of 4,4′-dihydroxy-α,β-diethylstilbene, reserving the term "stilbestrol" for the compound 4,4′-dihydroxystilbene, the mother substance, which is considerably less active than the diethyl compound. Unfortunately, the name stilbestrol has been used to a considerable extent to designate the active diethyl compound. In view of this situation, the term "stilbestrol" should not be confused with the official designation of the substance 4,4′-dihydroxy-α,β-diethylstilbene, which is diethylstilbestrol. In spite of the common usage of the terms at present, the official terminology was adopted in order to make allowance in the future for the development of additional compounds of the 4,4′dihydroxystilbene series which might promise to be efficient estrogens. There is already evidence that some of these compounds may be desirable estrogens. It is obvious, therefore, that there might be considerable confusion in naming such compounds if the diethyl compound were known as stilbestrol. With the mother substance, 4,4′-dihydroxystilbene, known as stilbestrol, compounds belonging to this series may be more appropriately named.

Diethylstilbestrol is formed from common laboratory reagents. It is not a steroid like the natural estrogens, but on hypodermic injection it is one to three times as potent as estrone when its activity is determined in the rat, and it possesses practically all the properties of the natural estrogens.[4] In the rodent, oral administration of diethylstilbestrol is usually considered to be one-half to one-third as effective as injections of estrone in oil solution. In the human subject diethylstilbestrol is also quite potent by oral administration, with

4. Dodds, E. C.; Lawson, W., and Noble, R. L.: Biological Effects of the Synthetic Oestrogenic Substance 4: 4′-Dihydroxy-α: β-Diethylstilbene, Lancet **1**: 1389 (June 18) 1938. Kellar, R. J., and Sutherland, J. K.: Clinical Experiences with New Synthetic Oestrogen-'Stilboestrol' (Diethyl-Stilboestrol): Report to Therapeutic Trials Committee of Medical Research Council, J. Obst. & Gynaec. Brit. Emp. **46**: 1 (Feb.) 1939. Stilbestrol, report of the Council on Pharmacy and Chemistry, J. A. M. A. **113**: 2312 (Dec. 23) 1939.

some evidence indicating that the ratio of the potency of intramuscular to that of oral administration is from 2 to 5 : 1.[5] The convenience of oral administration of diethylstilbestrol gives this substance certain advantages over the estrogens which require injections at frequent intervals. The chief disadvantage of the therapeutic use of diethylstilbestrol is the development of unpleasant symptoms. There is considerable difference of opinion as to the incidence of these symptoms but on the average it is considered to be about 15 to 20 per cent.[6] The symptoms most commonly complained of are nausea and vomiting, dizziness and headaches. The possibility that these symptoms are the result of tissue damage has been investigated and as yet there has been no conclusive evidence that in the human subject therapeutic doses produce any lesions.[7] The incidence of unpleasant symptoms in postpartum patients is practically nil.[8] It has been considered by some investigators that these symptoms are the result of systemic reactions following the sudden introduction into the body of estrogens, which produce a state similar to that of early pregnancy.[9] Diethylstilbestrol has recently been released by the Food and Drug Administration for sale on physicians' prescriptions. The Council has not had the opportunity to consider diethylstilbestrol for acceptance.

Dihydrodiethylstilbestrol or, as it has been termed, hexestrol, is likewise effective on oral administration. The reports from England indicate that it is about equal in therapeutic efficiency to diethylstilbestrol but

5. (*a*) Mazer, C.; Israel, S. L., and Ravetz, E.: The Synthetic Estrogen Stilbestrol: An Experimental and Clinical Evaluation, J. A. M. A. **116**: 675 (Feb. 22) 1941. (*b*) Shorr, E.; Robinson, F. H., and Papanicolaou, G. N.: A Clinical Study of the Synthetic Estrogen Stilbestrol, ibid. **113**: 2312 (Dec. 23) 1939.

6. Morrell, J. A.: Stilbestrol: Summary of Some Clinical Reports on Stilbestrol, J. Clin. Endocrinol. **1**: 418 (May) 1941.

7. (*a*) von Haam, E.; Hammel, M. A.; Rardin, T. E., and Schoene, R. H.: Clinical Studies on Stilbestrol, J. A. M. A. **115**: 2266 (Dec. 28) 1940. (*b*) Karnaky, K. J.: Endocrines in Gynecology and Obstetrics with Special Reference to Stilbestrol in Treatment of Uterine Bleeding—Original Research on Menstruation, Texas State J. Med. **36**: 379 (Sept.) 1940. (*c*) Freed, S. C.; Rosenbaum, E. E., and Soskin, S.: Alleged Hepatotoxic Action of Stilbestrol, J. A. M. A. **115**: 2264 (Dec. 28) 1940. (*d*) Shorr, Robinson and Papanicolaou.[6b]

8. Connally, H. F., Jr.; Dann, D. I.; Reese, J. M., and Douglass, L. H.: A Clinical Study of the Effects of Diethylstilbestrol on Puerperal Women, Am. J. Obst. & Gynec. **40**: 445 (Sept.) 1940.

9. Freed, S. C.: Recent Progress in Estrogen Therapy, Illinois M. J. **80**: 139 (Aug.) 1941. Karnaky.[7b]

less liable to cause untoward reactions.[10] The only available data on the use of hexestrol in this country indicate that it is about one-fifth as effective as diethylstilbestrol following oral administration and that in therapeutically effective doses it may be less liable to produce unpleasant symptoms.[11] This compound is not available commercially.

Ethynil estradiol, a derivative of estradiol, is claimed to be relatively active by mouth. The comparatively high incidence of unpleasant symptoms, chiefly nausea and vomiting, has, up to the present time, prevented establishment of this compound as a desirable estrogen.

Implanting the natural or the synthetic estrogens in the form of crystals or pellets directly into the tissues of patients is an effective procedure for long-continued substitution therapy.[12] Such dosage forms are not on the market as yet. Recent investigations reveal that suspensions of crystalline estrone in aqueous mediums are more effective therapeutically than oil solutions of estrone.[13] An aqueous suspension of estrone behaves like an implant of crystals, since the crystals remain at the site of injection after absorption of the aqueous medium. Such suspensions are not yet available commercially.

PROGESTERONE

Preparations containing progesterone, the active principle of the corpus luteum, have been recommended for a large number of conditions, including menorrhagia, dysmenorrhea, preeclampsia, habitual and threatened abortion and menstrual disturbances of various sorts. The early reports in the literature on clinical investigations appear to be overenthusiastic and unreliable inasmuch as insignificant doses, as little as $\frac{1}{25}$ rabbit unit, were claimed to be effective. More recent reports on progesterone therapy concern the use of far larger quantities of the substance, chiefly in combating abortion, either habitual or threatened. The

10. Bishop, P. M. F.; Bowes, R. K.; Boycott, M.; Kellar, R.; MacGregor, T. N., and Murless, B. C.: Oestrogenic Properties of Stilboestrol Dipropionate and Hexoestrol, Lancet **1**: 629 (April 6) 1940.

11. Freed, S. C.; Greenhill, J. P., and Dahlberg, A.: Therapeutic Efficiency of Hexestrol, to be published.

12. Salmon, U. J.; Walter, R. I., and Geist, S. H.: Prolonged Therapeutic Effect of Subcutaneously Implanted Crystals of Ovarian Hormone in Women, Science **90**: 162 (Aug. 18) 1939.

13. (*a*) Freed, S. C., and Greenhill, J. P.: The Prolonged Therapeutic Effect of Suspensions of Estrone in Water, to be published. (*b*) Freed, S. C.

findings of Venning and Browne [14] that pregnandiol is an excretion product of progesterone offers the possibility of a method for assaying any deficiency of progesterone in human beings. It has been calculated that a daily dose of at least 5 to 10 mg. of progesterone is necessary to replace the progesterone normally secreted by the placenta. While there are indications that progesterone may be of value in the treatment of functional abortion, more evidence should be forthcoming along the lines laid down by Venning, Browne and associates before physicians accept any claim relating to this and other conditions.

The preparations of progesterone on the market are in two categories, synthetic progesterone and extracts of animal ovaries. The latter preparations contain in addition to progesterone small amounts of other lipids which are found in ovaries. Crystalline progesterone is prepared for commercial purposes by synthesis from certain steroids. While crystalline progesterone may be obtained from ovarian extracts, the procedure is too expensive for practical purposes. A synthetic preparation of progesterone mixed with other steroids having little physiologic activity is also available. There is no apparent disadvantage in administering such a preparation as compared with crystalline preparations. The international unit of progesterone has been used extensively in Europe and in this country. By definition, it is 1 mg. of crystalline progesterone; this is approximately 1 Corner-Allen rabbit unit. The Council has deferred consideration of either type of preparation owing to the fact that the evidence available in the literature does not indicate that progesterone or extracts containing progesterone have been adequately demonstrated to be of value for therapeutic purposes.[15]

Pregneninolone is a derivative of testosterone or of progesterone and possesses the unique ability of inducing a progestational-like condition of the endometrium in animals when administered by mouth; it differs from progesterone, however, in certain of its other physio-

14. Venning, E. H., and Browne, J. S. L.: Studies on Corpus Luteum Function: The Urinary Excretion of Sodium Pregnanediol Glucuronidate in Human Menstrual Cycle, Endocrinology **21**: 711 (Nov.) 1937. Browne, J. S. L.; Henry, J. S., and Venning, E. H.: The Significance of Endocrine Assays in Threatened and Habitual Abortion, Am. J. Obst. & Gynec. **38**: 927 (Dec.) 1939.

15. Progesterone, Report of the Council on Pharmacy and Chemistry, J. A. M. A. **116**: 1523 (April 5) 1941.

logic activities. When administered by mouth it must be given in an amount about four to six times that of injected progesterone to induce similar progestational changes.[16] Recent reports indicate the possibility that it may be of value in the treatment of habitual abortion, dysmenorrhea and other conditions in which progesterone has received trials. The evidence at present is inadequate and inconclusive, and further experimentation is required before the therapeutic worth of pregneninolone can be evaluated. The Council on Pharmacy and Chemistry has recently declared the substance not acceptable.[17]

ANDROGENS

After considerable experimental trials with various compounds and extracts having androgenic activity, it appears at the present time that the most useful preparation from a clinical standpoint is testosterone propionate. Preparations containing material from a few grains or grams of testicular material are definitely considered worthless. They are part of the ancient endocrine armamentarium which is rapidly being discarded. Testosterone propionate has been declared unacceptable by the Council on Pharmacy and Chemistry because androgen therapy is considered to be still in the experimental stage. The advertising claims for preparations of this compound have been largely exaggerated and unwarranted.[18] In the Council report it was indicated that the treatment of eunuchoidism and castration in men with testosterone propionate is effective if carried out with the proper dosage. This therapy is purely substitutional, and all beneficial effects recede soon after cessation of the therapy. Testosterone propionate is not recommended for the treatment of cryptorchism by Hamilton.[19] The "male climacteric," the period at which vasomotor and mental changes resembling menopausal symptoms occur, is a relatively rare condition,

16. Emmens, C. W., and Parkes, A. S.: Some Biological Properties of Anhydro-Hydroxy-Progesterone (Ethinyl Testosterone), J. Endocrinol. **1**: 332 (Nov.) 1939. Hamblen, E. C.; Cuyler, W. K.; Pattee, C. J., and Axelson, G. J.: Endocrine Therapy of Functional Meno-Metrorrhagia and Ovarian Sterility: Oral Use of Anhydro-Hydroxy-Progesterone and Estrogens, J. Clin. Endocrinol. **1**: 221 (March) 1941.

17. Pregneninolone, Report of the Council on Pharmacy and Chemistry, J. A. M. A. **116**: 1054 (March 15) 1941.

18. Testosterone Propionate, Report of the Council on Pharmacy and Chemistry, J. A. M. A. **112**: 1949 (May 13) 1939.

19. Hamilton, J. B., and Hubert, G.: Effect of Synthetic Male Hormone Substance on Descent of Testicles in Human Cryptorchidism, Proc. Soc. Exper. Biol. & Med. **39**: 4 (Oct.) 1938.

but it is stated to have responded favorably to androgen therapy. Androgen therapy of prostatism, while at first promising, has been discounted by recent work.[20] Histologic studies have shown that therapeutic doses of testosterone propionate have little or no effect on the prostate.[21]

Androgens have received therapeutic trials in the treatment of gynecologic conditions, and while there are indications that they may have some measure of usefulness in the treatment of dysmenorrhea, metrorrhagia, painful breasts and premenstrual tension, the therapy in these conditions should be left to specialists until more definite conclusions may be drawn as to the reliability of androgens in such treatment.

Testosterone propionate is available in oil solutions containing up to 25 mg. per cubic centimeter. Testosterone and testosterone propionate are incorporated in ointments for inunction purposes. Although androgens have a significant physiologic effect on percutaneous administration, the amounts present in most commercial ointments, namely, about 2 mg. of androgen per gram of ointment, would have little therapeutic effect unless relatively enormous amounts of ointment were applied.[22] Therefore the ointments on the market are considered ineffectual and their application impracticable for therapeutic purposes. Effective replacement therapy has been obtained with the implantation of pellets of testosterone and testosterone propionate. Such dosage forms are not available at present.

There has recently been prepared a derivative of testosterone which has significant activity when administered by mouth. This oral androgen, methyl testosterone, is being marketed at the time of publication. Investigations reveal that it is about one-fourth as active when given by mouth as when injected, and the clinical evidence available indicates that substitution therapy in castrate men or eunuchs requires about four times as much methyl testosterone for administration by mouth as testosterone propionate for injection. The

20. Heckel, N. J.: Influence of Testosterone-Propionate upon Benign Prostatic Hypertrophy and Spermatogenesis: Clinical and Pathological Study in the Human, J. Urol. **43**: 286 (Feb.) 1940.

21. Sharpey-Schafer, E. P.; Schrire, I., and Shackman, R.: Testosterone Propionate: Its Effect on Histology of Prostatic Enlargement in Man, Lancet **1**: 1254 (June 3) 1939.

22. Foss, G. L.: Percutaneous Absorption of Male Hormone: Its Practical Application to Human Therapy, Lancet **2**: 1284 (Dec. 3) 1938.

practicability of such therapy is therefore open to question, since the average dosage for replacement of testicular function would have to be about 40 to 100 mg. of methyl testosterone a day.[23] Other androgenic preparations have little therapeutic efficacy. Such preparations are extracts of urine containing androsterone or similar steroids whose activity in the mammal is significantly less than that of testosterone.

PITUITARY PREPARATIONS

Extracts of the posterior lobe of the pituitary gland containing the oxytocic and pressor factors are well known to clinicians. Several recent reports indicate that a combination of pitressin with tannic acid results in a preparation which is absorbed slowly from the tissues, thereby giving a prolonged physiologic effect. This product, pitressin tannate, has been recommended in the treatment of diabetes insipidus. It has not been accepted by the Council. A number of preparations containing both factors have been accepted by the Council on Pharmacy and Chemistry, as well as preparations containing the individual factors:

Solution of Posterior Pituitary

Source: Animal Pituitary glands.
Assay: U. S. P. units.

Product	Firm
Ampoules of Pitocin	Parke, Davis & Company
Ampoules of Pitressin	
Pituitary Extract–Lilly	Eli Lilly & Company
Pituitary Extract–Merrell	The Wm. S. Merrell Company
Pituitary Liquid–Armour	The Armour Laboratories
Pituitary Solution–U. S. P.	U. S. Standard Products Co.
Pituitrin	Parke, Davis & Company
Posterior Pituitary Solution–Abbott	Abbott Laboratories
Posterior Pituitary Solution–Squibb	E. R. Squibb & Sons
Solution Pituitary Extract U. S. P. (Upjohn)	The Upjohn Company
Solution Posterior Pituitary (U. S. P.)–Wilson	Wilson Laboratories

23. Foss, G. L.: Oral Application of Methyl Testosterone and Its Simplification of Androgen Therapy, Brit. M. J. 2: 11 (July 1) 1939.

There are a large number of preparations of the anterior lobe of the pituitary gland on the market. The Council on Pharmacy and Chemistry has accepted no preparation of the anterior lobe because there is no suitable evidence that such preparations are of value in therapy. Although the commercial preparations are unsatisfactory, experimental preparations have been developed in which the active principles have been isolated in a relatively pure form. It has been claimed that the lactogenic factor, for example, has been crystallized. Confirmation of this has not been made, although almost chemically pure preparations of the lactogenic factor have been developed by several groups of workers. In addition, preparations of the growth principle have been made which exert definite growth-promoting effects in hypophysectomized rats though administered in doses of fractions of a microgram. Preparations of the gonadotropic factors have also been produced which have high potency and contain only traces of contaminating substances. Other factors which have been isolated in relatively pure form are the thyrotropic and the adrenotropic. None of the other active substances of the anterior lobe have been prepared to similar degrees of purity. The commercially available preparations are relatively crude in comparison with those developed experimentally. They usually contain significant amounts of several other factors and inert proteins. Furthermore, it has been demonstrated that the active substances of many of these preparations deteriorate on standing in solution. Thus, D'Amour and his co-workers [24] have demonstrated that anterior lobe preparations purchased in drug stores have little demonstrable activity. This is probably due to a characteristic of anterior lobe preparations in aqueous solution, namely, loss of potency on standing. It is quite possible that in the future more satisfactory preparations will be available for clinical use.

The League of Nations has recently set up an international standard for the lactogenic factor by providing an international powder as the basis for bioassays. Other factors of the anterior lobe of the hypophysis have not been so standardized.

24. D'Amour, F. E.: The Potency of Certain Commercial Hormone Preparations, Endocrinology **26**: 88 (Jan.) 1940.

CHORIONIC GONADOTROPIN

"Chorionic gonadotropin" is the official term for the gonadotropic substance present in the urine of pregnant women. Its properties differ from those of the gonadotropic substances of the anterior lobe of the pituitary gland in a number of respects as described in the chapter on gonadotropic hormones of the anterior lobe and chorionic tissue. Almost chemically pure preparations have been obtained experimentally. It is indicated that this substance is a glycoprotein.[25] The Council has recently accepted chorionic gonadotropin (follutein) for inclusion in "New and Nonofficial Remedies" but recognizes that the only condition in which the therapeutic response is of value is cryptorchism unassociated with any anatomic abnormalities that would prevent testicular descent.[26] The Council considered its use in the treatment of functional uterine bleeding in women and also in that of hypogonadism in men to be still in the experimental stage and that its use in the treatment of other conditions is unsatisfactory at the present time. Chorionic gonadotropin is standardized according to the international standard as adopted by the League of Nations. Therefore, these products may be found assayed in international units. Biologic units may differ significantly, since there is considerable variation in the types of assays performed by the different firms. Preparations in aqueous solution are subject to loss of potency on standing for several months at room temperature. The following preparation has been accepted by the Council on Pharmacy and Chemistry:

Chorionic Gonadotropin

Source: Urine or placenta of pregnant women.

Assay: International units (0.1 mg. of the international standard equals 1 international unit).

Product	Firm
Follutein *	E. R. Squibb & Sons

* The active substance is carried in glycerin. This preparation is diluted with saline solution for injection.

25. Gurin, S.; Bachman, C., and Wilson, D. W.: Gonadotropic Hormone of Urine of Pregnancy: Chemical Studies of Preparations Having High Biological Activity, J. Biol. Chem. **133**: 467 (April) 1940.

26. Chorionic Gonadotropin, Report of the Council on Pharmacy and Chemistry, J. A. M. A. **113**: 756 (Aug. 26) 1939.

EQUINE GONADOTROPIN

The serum of pregnant mares during a certain stage of gestation contains large amounts of a gonadotropic substance. This substance is a chorionic gonadotropin but differs in its physiologic effect from the substance found in human pregnancy urine. It resembles more closely the gonadotropic complex of the anterior lobe of the pituitary gland. The Council recently published a report on the status of therapy with equine gonadotropin.[27] The Council stated that although this gonadotropin may be available in a relatively potent form, its use in the treatment of various gonadal dysfunctions is still unestablished. Claims have been made that it is capable of inducing ovulation in women. There is little evidence, however, that ovulation can be induced in those who do not ovulate ordinarily. Therefore, its use in the treatment of sterility in women is open to considerable question. There is also a lack of evidence that it is of any value in the therapy of testicular disorders, such as cryptorchism and abnormal spermatogenesis. Preparations of equine gonadotropin are assayed according to an international standard provided by the League of Nations. Formerly the various firms used biologic units which were considerably different, so that a rapid comparison of potencies of the preparations was difficult.

THYROID

Desiccated thyroid U. S. P. is still the most widely used preparation of thyroid gland. Some claims have been made that certain types of extracts of the thyroid gland contain the metabolic factor apart from a component which gives rise to nervous and vascular responses. There is no conclusive evidence that animal thyroid gland contains an active compound that evokes responses qualitatively different from those to thyroxin. Inasmuch as thyroxin produces effects identical with those of desiccated thyroid, there does not seem to be an adequate basis for postulating at the present time the presence of a thyroid hormone which has a metabolic effect separate from a factor which gives rise to nervous and vascular hyperactivity. The Council has accepted thyroxin, the active principle of the thyroid gland. Thyroxin may be injected intravenously. Its use is

27. The Present Status of the Gonadotropic Hormone from the Serum of Pregnant Mares, Report of the Council on Pharmacy and Chemistry, J. A. M. A. 115: 1998 (Dec. 7) 1940.

usually limited to the occasional case where it might not be practical to administer thyroid substance by mouth.

Thyroxin

Source: Animal thyroid gland.
Assay: U. S. P. units.

Product	Firm
Synthetic Thyroxin–Roche	F. Hoffman-La Roche & Company
Thyroxin Fraction	E. R. Squibb & Sons
Thyroxin (Squibb)	

PARATHYROID EXTRACTS AND OTHER CALCIUM-REGULATING COMPOUNDS

Commercial extracts containing the parathyroid hormone have been accepted by the Council on Pharmacy and Chemistry. These preparations are standardized according to parathyroid units as determined by the United States Pharmacopeia for solution of parathyroid. Their use is restricted to the treatment of parathyroid tetany. The administration of solution of parathyroid is not recommended for chronic hypoparathyroidism, owing to the fact that a resistance is effected after a number of injections. The following preparations of solution of parathyroid have been accepted by the Council on Pharmacy and Chemistry:

Solution of Parathyroid

Source: Animal parathyroid gland.
Assay: U. S. P. units.

Product	Firm
Parathyroid Extract–Lilly	Eli Lilly & Company
Parathyroid Hormone–Squibb	E. R. Squibb & Sons
Paroidin	Parke, Davis & Company

Certain steroids have a strong influence on the calcium content of the blood and on the elimination of calcium from the body.

For continuous treatment over long periods, activated sterols are being used with success. "Viosterol" is the official N. N. R. term for activated or irradiated ergosterol. It contains calciferol, lumisterol and tachysterol. Dihydrotachysterol is obtained from tachysterol; it has been found of considerable value in the maintenance of the normal blood level of calcium in patients with hypoparathyroidism. No preparation of this sub-

stance has been submitted to the Council on Pharmacy and Chemistry up to the time of publication. The following preparations of activated sterols have been accepted by the Council:

Activated Sterols

Source: Ergosterol.

Product	Firm
I. V. C. Viosterol (A. R. P. I. Process) in Oil	International Vitamin Corp., Inc.
Mead's Viosterol in Oil	Mead Johnson & Company
Parke, Davis & Co.'s Viosterol in Oil	Parke, Davis & Company
Stearns Viosterol (A. R. P. I. Process) in Oil	Frederick Stearns & Co.
Viosterol (A. R. P. I. Process) in Oil–Hospital Liquids, Inc.	Hospital Liquids, Inc.
Viosterol in Oil–Abbott	Abbott Laboratories
Viosterol in Oil–Merrell	The Wm. S. Merrell Company
Viosterol in Oil–Squibb	E. R. Squibb & Sons
Winthrop Viosterol in Oil	Winthrop Chemical Co., Inc.

ADRENAL CORTEX

The adrenal cortex secretes numerous steroids. Many of these steroids have no known physiologic activity; others possess properties which differ qualitatively and quantitatively. These compounds have roles in the regulation of salt and water metabolism, carbohydrate, fat and protein metabolism and other activities essential for the maintenance of health. In addition to steroids with these activities, the adrenal cortex contains small amounts of estrogens, androgens, progesterone and related compounds.

Desoxycorticosterone or its acetate is the only pure steroid available for therapeutic purposes. This compound has a powerful effect on salt and water metabolism, especially in adrenal insufficiency, but lacks other properties pertaining to the function of the adrenal cortex. It has been used to a considerable extent in the treatment of Addison's disease and in that of various types of peripheral circulatory failure. This substance is usually administered in oil solution by intramuscular injection. It has also been used dissolved in propylene glycol, to be placed under the tongue for absorption.

This method of administration is about one-third to one-half as effective as injections of similar amounts in oil solution. The most efficient method of administering desoxycorticosterone acetate is the implantation of pellets where the slow continuous absorption results in economical and effective therapeutic action. This method, however, is best suited for use by specialists and may be dangerous in the hands of those who are not aware of the possibility of overdosage. When there is excessive dosage this substance is harmful; in patients with Addison's disease hypertension, edema and heart failure may develop. Fatalities have been reported from overtreatment of patients with Addison's disease with desoxycorticosterone acetate.[28] In experimental animals overdosage produces overexcretion of potassium, resulting in asthenia. In the treatment of patients with Addison's disease with desoxycorticosterone acetate, it is advisable to maintain a moderate potassium intake and a relatively low sodium intake because of the possibility of undesirable shifts in electrolyte balance. As to the treatment of conditions other than adrenal insufficiency, there is not available sufficient evidence to justify the use of this substance in other than experimental trials. It has been claimed that circulatory failure due to infectious diseases, such as pneumonia, and that observed in such conditions as surgical shock and anesthesia may be combated by desoxycorticosterone acetate. There is a lack of sufficient objective clinical data to substantiate these claims. In the treatment of an addisonian crisis, it is stated, desoxycorticosterone acetate may not be successful despite the use of large doses. Apparently other factors of the adrenal cortex may be essential in treating such states successfully. Pellets or crystals for implantation and propylene glycol solutions are not available commercially at the present time.

Adrenal cortex extract has been accepted by the Council on Pharmacy and Chemistry for the treatment of Addison's disease and for prophylactic use in preparation for operations about the adrenal glands. Adrenal cortex extract is used in conjunction with a high sodium and a low potassium intake. There have been no reports of overdosage with it. Although there is definite activity when adrenal cortex extract is administered orally, the effectiveness of oral administration is still

28. Desoxycorticosterone, Report of the Council on Pharmacy and Chemistry, J. A. M. A. 114: 2549 (June 29) 1940.

subject to some debate. Oral therapy should not be depended on in an addisonian crisis. The treatment of conditions other than adrenal insufficiency with adrenal cortex extract is being studied in the laboratory and clinic. Conditions on the borderline of adrenal insufficiency are not considered an indication for such therapy, nor has its use in a variety of other conditions, including asthenia, infectious diseases, allergic conditions, toxemia and pregnancy, been recognized by the Council. Adrenal cortex extract accepted by the Council on Pharmacy and Chemistry is standardized according to dog units, although biologic assays of preparations of adrenal cortex extract are not entirely satisfactory. The following preparation has been accepted by the Council on Pharmacy and Chemistry:

Adrenal Cortex Extract

Source: Adrenal glands of animals.

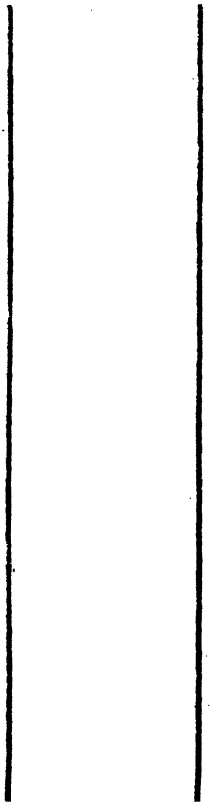
Assay: Biologic units (1 cc. of extract is derived from approximately 50 Gm. of fresh gland).

Product	Firm
Adrenal Cortex Extract	The Upjohn Company

MISCELLANEOUS GLANDULAR PREPARATIONS

Commercial preparations of thymus gland, pineal gland and pancreas, excluding insulin, either alone or in various combinations, are available. There is no reliable evidence that any of these preparations possesses endocrine activity. Lipocaic, according to Dragstedt and associates, is an internal secretion of the animal pancreas which prevents deposition of fat in the liver following pancreatectomy. There is considerable controversy regarding the nature of this substance and whether or not it is an endocrine secretion. The clinical value of the substance has not as yet been demonstrated. The endocrine factors from the gastrointestinal tract as described in the chapter on gastrointestinal hormones are not available commercially. Insulin and epinephrine are described in New and Nonofficial Remedies.

INDEX



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