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RECENT ADVANCES IN ENDOCRINOLOGY

By

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With 54 Figures, including Two Plates

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MY FRIENDS

THE MEDICAL PROFESSION

OF WINNIPEG

Printed in Great Britain

PREFACE

THIS volume, entrusted to me by Messrs. J. & A. Churchill, was commenced with some confidence, was continued with frequently some degree of bewilderment, and has been completed with full realization of many shortcomings.

For the bewilderment I do not apologize. Although it is a new branch of science, endocrinology fully holds its own, both for the multiplicity of writings upon its many phases, and for the complexity, confusion, and disagreements frequently found in its vast literature. This chaos has not yet given place to complete order, although order is emerging.

Endocrinology is essentially a biochemical subject to this extent : The precise truth of its teachings depends ultimately upon the isolation of the different endocrine principles in pure crystalline form, so that their physiological and pharmacological properties may be ascertained accurately. Physiology, biology, anatomy, pathology, and clinical medicine have done their share in indicating methods of test whereby these principles may be concentrated and finally isolated. The isolation and the determination of the chemical structure are in each case biochemical and chemical problems. The final problem, the elucidation of the precise mechanism of the actions of these principles, will require profound and prolonged biochemical and physiological study.

It would be impertinent of me, a biochemist, to stress or even to mention my own views in dealing with the clinical aspects of endocrinology. Yet these clinical aspects are perhaps the most important, and must be dealt with. I have ventured to criticize the frequently differing views found in the literature only by selection of what appear to be most reasonably logical and probable.

Marked advances in endocrinology have been made during the past decade. Texts on the subject written ten years ago are now not only very incomplete, but are, on many points, misleading.

The title of this volume suggests that some degree of selection of the material dealt with is permissible. I have nevertheless thought it desirable to deal to some extent with all the actual and supposed endocrine principles. The literature is too great to be adequately covered by one person, but I have attempted to mention all the important recent work on the phases of the subject that have been considered, to the end of 1932. I am aware of numerous gaps, but completer treatment would have enlarged the volume too greatly.

I wish to thank all those authors, editors, and publishers who have granted permission for the reproduction of the figures and photographs, and whose names, with the names of the journals concerned, are cited in the corresponding legends. My thanks are due particularly to my colleague, Professor William Boyd, for preparing for me the two photomicrographs on Plate I. and to Dr. Harry Medovy, for the photographs reproduced in Fig. 17.

Dr. A. T. Mathers, Dean of this Medical Faculty, has been kind enough to read through the whole manuscript, Professors William Boyd and Gordon Fahrni have read the chapter on the Thyroid, and Dr. Lennox G. Bell the chapters on the Adrenal and Pituitary Glands and the Gonads. To all of these my thanks are due for much helpful criticism. Miss Jean Guthrie has assisted with the proof-reading and verification of the references.

I wish finally to acknowledge my thanks and indebtedness to my former Chief, Professor Swale Vincent, who introduced to me the fascinating realms of this subject, and helped to develop whatever critical ability I may possess.

WINNIPEG, 1933. A. T. CAMERON.

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RECENT ADVANCES IN ENDOCRINOLOGY

CHAPTER I

INTRODUCTION

THE pre-history of endocrinology is the story of gradual failure of detoxication theories to explain accumulating facts, demonstrable by experiment, concerning certain "ductless glands." All such theories are not even yet universally rejected.

The history of endocrinology as an exact branch of science scarcely antedates the present century; the name itself is still younger. Until chemical studies progressed sufficiently to result in isolation of several of the "internal secretions," and to emphasise the fact that these are specific compounds, with specific physiological functions, endocrinology was nebulous, and necessarily inexact. Now that we know the chemical nature of some proportion of these internal secretions, and something of their physiological and pharmacological activities, it is possible to visualise endocrinology as an exact science, or branch of science, inseparably related to physiology, pharmacology, and biochemistry.

It seems desirable to stress at the outset two fundamental concepts, whose truth, though still unadmitted by numerous investigators, is becoming more apparent with each advance. *The normal function of an endocrine gland is not a detoxication, but the production of one or more specific chemical compounds essential to the normal life of the whole organism. In the different pathological states of such a gland it may produce*

too much or too little of these specific compounds, but it does not produce abnormal compounds.

The terminology of the subject is still so far from perfect that it frequently hampers, rather than helps, its progress. Numerous terms have been coined for the specific compounds as a class; some of these are based upon the presumed function of a single one of them. Typical of such names is *hormone* (from Gk. *hormōn*, rousing, or setting in motion). This was originally proposed for the class by Bayliss and Starling, after their discovery of secretin; they suggested the name because secretin aroused the pancreas to secrete its juice.

The term, however suitable for secretin, is not properly applicable to the majority of endocrine "secretions"; many of them are not "hormonal" in their action. With the idea that these secretions were either "excitants" or "depressants," the term *chalone* (Gk. *chalaō*, I relax) was suggested to designate the latter; it has never been widely used. Fuller knowledge of the varieties of action associated with different secretions indicates that many are not truly designated by either term. Their actions, by whatever intermediate process they are achieved, are related to specific biochemical changes, different for each "secretion" and incapable of correct designation by generic class names indicating one or two physiological or pharmacological effects.

By some writers the term "hormone" has been so widely extended that it has become almost meaningless; an extreme example of its indiscriminate use is the terming of such a normal catabolite as carbon dioxide a hormone because the concentration of carbonic acid in tissue controls the action of the respiratory centre (through its effect upon hydrogen ion concentration).

Certain terms have been invented which are less specific in their meaning. A typical example is *autacoid*, suggested by Sharpey-Schafer (Gk. *autos*, self; *akos*, a medicinal agent or remedy). *Autacoid* thus suggests an unusual agent, whereas the endocrine "secretions" are normal products, and in no sense

medicinal or remedial agents. The term autacoid does not appear appropriate for a number of chemical compounds concerned with the every-day normal physiological processes of the organism.

The term " secretion " (L. *secretus*, separated, or divided off), while its derivation does not exclude its application to a single specific compound, has come from long usage to connote an aqueous solution typified by the digestive juices and by sweat. Since this custom is fixed, the term is not most appropriate for the series of specific chemical compounds elaborated by the endocrine glands.

By a process of gradual selection the term " endocrinology " (Gk. *endon*, within ; *krinein*, to separate) has gradually become accepted as indicating the study of " internal secretion." It denotes the science, or the branch of science, concerned with the glands which separate within themselves *specific* compounds and secrete them into veins, or perhaps in one or two instances into their lymph vessels. These compounds effect, by reason of their specific chemical constitution, specific actions elsewhere within the organism. The glands concerned are *endocrine glands*. The specific compounds they form, frequently termed " internal secretions " or *endocrine secretions*, perhaps should more accurately be spoken of as *endocrine principles* or, even better, as *endocrine compounds*. The last term stresses the fact that we are dealing with specific chemical substances, and not nebulous uncertainties which never have been and perhaps never will be isolated.

The names which have been applied to the various endocrine compounds present some elegancies, some inaccuracies, and, not infrequently, some confusion. Where they can be derived from the name of the specific tissue concerned, they are beyond criticism ; of such *insulin* (from the " insulae " of Langerhans) is an excellent example. *Thyroxine* (thyro-oxy-indole) is an example of inaccurate description of the compound concerned. *Adrenaline*, a legitimate term to apply to the compound of the adrenal medulla, is rejected by many endocrinologists because patent laws confer upon it a specific meaning, and so *adrenine* and *epinephrine* and *adrenaline* in scientific papers have equal value. Of all the series the endocrine compound of the ovaries is perhaps

the best illustration of indiscriminate application of numerous unfelicitous terms.

No authoritative body decides such names. They are the choice, sometimes too fortuitous, of individual investigators. It is to be hoped that before long some sufficiently representative council of endocrinologists may pronounce upon these names, but, since impatient endocrinological research is world-wide, satisfactory and final agreement will not be attained unless the deciding authority is similarly widely representative. In the meantime, to the perplexities associated with the study of a number of these compounds is added the confusion of many names.

Endocrine compounds are definitely associated with the thyroid, parathyroid, pituitary, and adrenal glands, the islet tissue of the pancreas, the mucous membrane of the upper reaches of the intestine, and the organs of reproduction. All of these call for special attention.

The strength of evidence supporting the presumptive existence of other compounds varies for each presumptive compound; as long as such existence is problematical, obviously shorter treatment suffices in a volume dealing with definite advances.

By far the most perplexing problems in endocrinology are those concerned with the interrelationships of the actions of two or more endocrine compounds. Such interrelationships cannot be dealt with very systematically; they intrude into the majority of discussions of clinical cases exhibiting endocrine disturbances; they even intrude when normal functions are under consideration. They have suggested a multitude of syndromes, involving much unnecessary differentiation; the inaccurate conceptions underlying many of these suggested syndromes have led to much inaccurate therapy.

Therapeutic treatment is not stressed in this volume, although I endeavour throughout to indicate the logical treatment in light of present knowledge. If the assumption

be true, as I believe, that endocrine disorders are associated with either hypo- or hyperfunction of some one endocrine gland, then this logical treatment seems obviously to consist in the application of replacement therapy for hypofunction and application of some means of depression for hyperfunction.

Rational replacement therapy must always take into account the fact that only two or three endocrine principles have been definitely demonstrated to be effective when administered by mouth. Our knowledge of the actual nature and of the actions of the others creates a demand for properly standardised concentrates suitable for injection, and such a demand should before long be met for all of them. Only such properly standardised preparations should be employed.

Surgical treatment is an obviously correct procedure for the majority, if not all, conditions in which a hyperfunction exists. Claims for employment of X-ray therapy are frequent ; the relative benefit to be obtained from it and the types of case which will obtain most benefit have not yet been fully established.

CHAPTER II

THE THYROID GLAND AND IODINE METABOLISM

The normal structure of the thyroid gland 6
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The Normal Structure of the Thyroid Gland

THE general views concerning the macroscopic and microscopic structure of the thyroid gland are still in great part those expressed by Sharpey-Schafer in 1924 (229): "The thyroid consists of small closed vesicles of varying shape, but for the most part spheroidal. The largest are about 0·1 mm. in diameter, but many are much smaller than this. . . . Each vesicle is lined with epithelium, the cells of which are columnar, cubical or flattened in accordance with the state of distension of the vesicles. There is no definite basement membrane separating the epithelium from the connecting tissue and blood vessels. The vesicles are generally filled by the so-called *colloid*, a viscid fluid in the fresh organ, which is coagulated into a solid substance by fixative agents. The intervesicular substance is areolar tissue, containing in parts many small cells. Some of these are lymphocytes, which may be accumulated in considerable

masses, whilst others are like the epithelium of the vesicles, although the identity has not been established."

According to Williamson and Pearse (266) the thyroid unit consists of a system of closed tubules more or less suspended in a lymph sac. Marine (172) considers that the accuracy of this conception has not been established. Rienhoff (215), in a very accurate study and reconstruction of the thyroid gland, refers to " the rather bizarre conjectures of Williamson and Pearse," and goes on to show by accurate injection experiments that " The lymphatic system is . . .

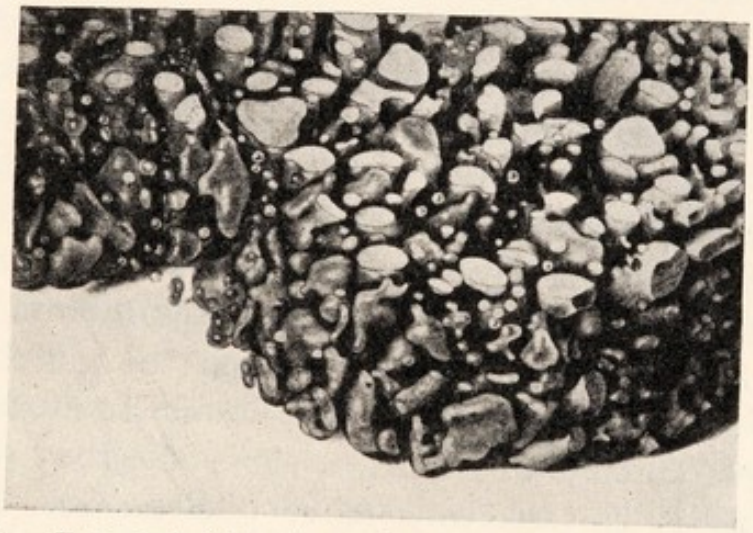


FIG. 1.—Top and side view of wax model of normal human thyroid gland. (From Rienhoff, *Medicine*, 1931, x, 293.)

a closed system and not an open one as suggested by Williamson and Pearse. . . . If one is allowed to speculate on the basis of minute structure, it would seem that the lymphatic system of the thyroid plays no *rôle* as a means of transmission of the specific secretion of the gland." A photograph of Rienhoff's reconstruction of normal thyroid tissue is shown in Fig. 1.

Jackson (123) has recently devoted special attention to the shape and size of the human thyroid follicle both in health and disease, using 75 per cent. hydrochloric acid as a special macerating fluid. Rather small follicles measuring in length from 0·05 to 0·12 mm. predominated in both

normal and pathological material. The length of the largest normal follicle measured was 1·294 mm. Each gland showed considerable variation in the size of its follicles. The average length was 0·163 mm. Cooper (51) has published a histological study of human thyroids at different periods of life. Her conclusions are : " During early intra-uterine life the thyroid gland is developing vesicles from solid epithelial cell masses through the intermediate stage of branching tubules. In the latter half of foetal life the epithelial cells become active, colloid is secreted, and stored in the vesicles. More and more colloid is secreted, and more vesicles are formed until birth. Then for the first few weeks the gland takes a rest and uses up the colloid previously secreted. Later its activity is renewed, the cells attaining their full size and secretive capacity. Throughout infancy and childhood secretory activity is marked, and so is absorption, but the gland always maintains a small reserve of colloid in its vesicles. At puberty the demand for this internal secretion is at its height, and the gland, though secreting to its greatest extent, is unable to store up any appreciable amount of colloid, as it is absorbed almost as quickly as it is secreted. After adolescence, when the requirements are diminishing, the gland continues secreting until a large amount of colloid is stored in its large vesicles. Then it is able to enjoy a period of comparative inactivity throughout adult life. Towards the fiftieth year, the thyroid seems again to attempt further activity, but this is not marked. In old age the necessity for thyroid secretion does not warrant great activity of the gland, so that it retrogresses."

At variance with earlier views that the thyroid of the aged has ceased to function or is atrophying, she stresses her findings that while anatomically the thyroid in later life is reduced in size and weight to a variable extent, yet histologically the individual secretory elements are still typical, although collectively their appearance is suggestive of

reduced activity. Such a conclusion is in agreement with the very slow decrease in basal heat production which is continuous after the age of forty or fifty. She draws attention particularly to the striking and significant " resemblance of the histological picture of the gland of the adolescent to that regarded as characteristic of exophthalmic goitre." [1] (Joll (126) has expressed doubt as to whether all her conclusions are fully justified from study of a limited amount of post-mortem material.)

It must always be remembered, in considering any description of the microscopic structure of the thyroid, that it is a labile organ, influenced by diet, by the secretions of other endocrine glands, by work, and by rest, and that its histological picture changes according to all such influences. Too definite a description—of the human thyroid especially—will lead to erroneous conclusions (cf. Boyd (24)).

This is the more important since so much stress is laid upon the histological appearance of the thyroid in pathological states.

Of the non-pathological factors influencing the gland it is known that diet can produce a slight but definite change. Although heat and cold produce no particular effect, marked differences have been observed between the thyroids of animals subjected for long periods to continued light and to continued darkness (60).

Again during pregnancy the thyroid follicles of the guinea-pig increase in size and number and show increased colloid and definite hyperaemia and karyokinesis. Towards the end of pregnancy the thyroid is rich in interfollicular epithelial islands ; after birth of the young these decrease. The results suggest a hyperplasia during pregnancy, and probably an increase of thyroid function (254). In female rabbits coitus causes a rapid and almost complete removal of colloid from the thyroid follicles, with parallel increased

[1] The thyroid gland in young laboratory animals normally appears hyperplastic (3A).

function of the follicular epithelium (coitus leads to ovulation in these animals). During pregnancy of these rabbits colloid is again stored (138).

The resemblance of the histological picture of the adolescent gland to that seen in the thyroid of Graves' disease has just been mentioned (cf. p. 9). The physiological changes in size of the gland, brought about by seasonal changes in temperature evoking increased or decreased heat production (cf. p. 31), are accompanied by histological changes. Somewhat similar alterations occur in the thyroids of women during the menstrual cycle.

Since under normal physiological conditions the thyroid can present such different pictures, it is obvious that too great a differentiation of thyroid histology in pathological states may lead to erroneous conclusions.

The blood supply of the thyroid is of considerable importance in studying its pathological changes. Besides the four main arteries (the paired superior and inferior thyroid arteries) and the occasional fifth (thyroidea ima) there are numerous unnamed irregular arteries, small in size under normal conditions, but capable of great enlargement in goitrous conditions ; they arise chiefly from the pharyngeal, oesophageal and tracheal arteries. Beneath the true capsule of the gland there is a rich arterial anastomosis. The veins commence as a perifollicular plexus and follow the small arteries to the periphery of the gland, there developing into a plexus covering the whole gland. The finer lymphatic radicles are present in intimate association with the follicular epithelium and a plexus exists around each follicle. By their union a coarser network is formed, with, ultimately, a close-meshed anastomosis enveloping the whole gland (cf. Joll (126)).

Iodine Distribution in Nature

Since it is now generally agreed that the function of the thyroid gland is bound up with the elaboration of a specific

compound containing 65 per cent. of the element iodine, and that insufficiency of iodine in the diet is one of the chief factors associated with simple goitre, knowledge of iodine distribution in nature and in different foods is indispensable to correct interpretation of studies of normal and pathological thyroid function.

Data concerning the distribution of iodine in plants and animals, based upon analytical methods then available, were summarised in 1914–15 as follows : " Iodine is an invariable constituent of all marine Algae. The limits observed in reliable analyses are 0·001 and 0·7 per cent. (dried material). . . .

" Land plants contain very much less iodine, although it is widely distributed in them. . . . The marked difference between fresh-water plants and vegetables on the one hand, and marine Algae on the other, is due to difference in iodine content of the environment, and therefore the diet of the plants.

" All sea species of animals contain iodine. As advances in evolution are made, there is more differentiation and probably less total iodine in the whole organism. . . .

" Of vertebrate tissue the thyroid alone is of importance in connection with the storage of iodine. The limits in the amount found in (desiccated) thyroid are 0·01 and 1·16 per cent. . . . Other tissues in mammals contain less than 0·001 per cent." (31).

For our present more exact and complete knowledge of iodine distribution we are largely indebted to the micro-analytical procedures perfected by von Fellenberg, and the similar procedures devised by McClendon and by Hercus and Roberts, and the results obtained with them by these investigators and others, of whom Lunde, a pupil of von Fellenberg, must especially be mentioned. Lunde has published an excellent comparative study of the different methods and their numerous modifications for analyses of different materials (155). Further modifications have recently been suggested (166).

The following table, summarising the most important of such results, is based chiefly on a review by McClendon (165) ; references to authorities cited are given in this review unless otherwise indicated. All values are in terms of " gamma " units (micro-grams, millionths of a gram) per 100 grams of

material, if solid, or per 100 c.c. if liquid; they refer to fresh material unless otherwise stated.

TABLE I

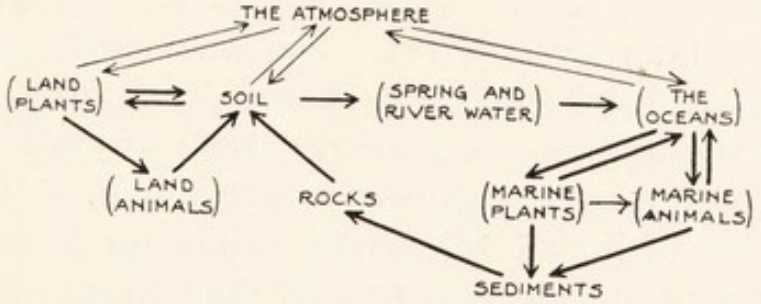
Distribution of Iodine in Nature

Material.	Iodine Content.	Authority.
	γ	
Rocks (Europe)—		
Tertiary	2·0–23·0	v. Fellenberg.
Chalk	1·7– 7·8	,,
Jura	3·8–92·0	,,
Trias	2·5–10·0	,,
Diluvium	9·7–13·8	,,
Sedimentary	2·3–88·5	,,
Granites, Shales, etc.	1·9– 8·1	,,
Soils (Switzerland)	6·2–119·0	,,
,, (New Zealand)	0·0–700·0	Hercus, Benson, Carter.
Sea-water, Mediterranean	0·17	v. Fellenberg.
,, English Channel	0·14	,,
,, Atlantic	0·23	,,
,, Pacific (off N.Z.)	0·18	Hercus, Benson, Carter.
,, ,, (off Calif.)	0·50	McClendon.
,, ,, (Str. Georgia)	0·25	Cameron.
Drinking waters (U.S.A.)	0·0001–1·85	McClendon *et al.*
,, ,, (N.Z.)	0·0–0·2	Hercus, Benson, Carter.
Mineral waters (Switzerland)	0·12–63·0	v. Fellenberg.
Atmosphere (*per cubic metre*)	0·0004–0·0254	,,
Rock salt (New Zealand)	14·0	Hercus, Benson, Carter.
,, (Switzerland)	0·1– 2·6	v. Fellenberg.
,, (France)	0·0– 0·18	,,
Sea salt	0·01– 0·10	,,
Land Plants—		
Vegetables	0·01– 0·64	,,
Lichens	1·4 – 5·0	,,
Fungi	0·06– 0·7	,,
Fresh water algae	3·4 –83·5	,,
Cereals	0·08– 0·60	,,
,,	0·01– 1·75	McClendon and Hathaway
Fruits	0·06– 1·2	v. Fellenberg.
Oils	0·30– 0·95	,,
Nuts	0·15– 2·0	,,
Marine algae (dried)	1,000–700,000	Cameron (31).
Marine animals—		
Molluscs (U.S.A. waters)	1·5–13·7	Tressler and Wells.
Crustaceans (U.S.A. waters)	0·9–13·8	,,
Bottom fauna (off Norway)	100–330	Lunde (153).
Fish (U.S.A. waters)	0·8–4·0	Tressler and Wells.
Teleosts (off Norway)	17–623	Lunde (153).
Anadromous fish (U.S.A.)	0·1–4·5	Tressler and Wells.
(Cod-liver oil, crude)	33·7	v. Fellenberg.
Fresh water fish (U.S.A.)	0·1–2·7	Tressler and Wells.
,, ,, ,, (Switzerland)	0·29–0·36	v. Fellenberg.
Land animal products—		
Milk (Switzerland)	0·05	,,
Butter	1·06	,,
Butterfat	0·4 –7·8	McClendon *et al.* (166).
Eggs	0·12–0·63	v. Fellenberg.
Veal	0·22	,,
Beef	0·05	,,
Ox liver	0·19	,,
Human blood	10–17	Kendall.
,, ,,	11–16	Lunde *et al.* (157).

According to v. Fellenberg the iodine content of soils is much higher than that of rocks which, by weathering, have produced these soils (165). He concludes that the soil receives iodine from water percolating through it, such (rain) water obtaining its iodine from the atmosphere. He has shown that the soil, and also sea-water, will give up iodine to the atmosphere (sea-water at the bottom of a desiccator loses 8 per cent. of its iodine in twenty-six days) (cf. also (16)).

The iodine content of plants is governed to some extent by that of the soil in which they are grown, although it has been shown that potatoes grown in the same area and in identical types of soil may exhibit large variations in iodine content (208). The immediate influence of the sea (through seaweed fertilisers and sea-sprays) does not extend beyond a very narrow coast-belt (208). (According to v. Fellenberg and Lunde (153) plants, such as lichens, with relatively high iodine content, " inhale " iodine from the atmosphere.)

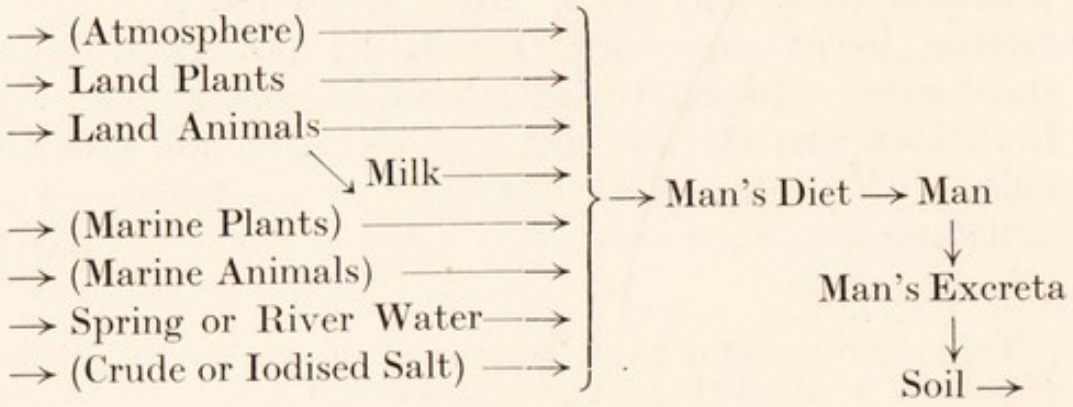
Lunde has dealt with the circulation of iodine in nature, and the following *schema* represents his considered views (153, 154). In it the main channels of iodine movement are represented by thicker lines.



Such a scheme undoubtedly represents the most important facts ; certain details may be inaccurate. For example, Remington has criticised the view that air-borne iodine plays any considerable *rôle* (206).

The cycle, as far as man and (to a lesser extent) the domestic animals are concerned, is frequently modified by man himself through utilisation of food material from wide sources, and recently, through deliberate selection of iodised

material. Modifying Lunde's scheme including man, it can be written :—



(Selected food material, or sources of negligible importance are shown in parentheses.)

Iodine is present in measurable amounts in all human and other mammalian tissues. Endocrine glands (with the exception of the testes and pancreas) contain relatively more than non-endocrine tissue. Of the total amount in the organism one-half to two-thirds is in muscular tissue, one-fifth to one-tenth in the thyroid (237). The average iodine content for certain tissues of six adult women was, in gamma per cent. : heart, 53 ; liver, 57 ; spleen, 61 ; adrenals, 112 ; ovaries, 741. The averages for tissues of a number of new-born infants were : heart, 12 ; liver, 17 ; spleen, 29 ; thymus, 46 ; ovaries, 138 ; thyroid, 250 (176).

The most accurate figures for *normal* human thyroids are still those of Zunz (275), whose values for fresh glands of adult men from nineteen to forty-four years of age were : extremes, 0·023 to 0·068 per cent. ; mean, 0·056 per cent. The corresponding figures for dried glands were : extremes, 0·119 to 0·281 per cent. ; mean, 0·229 per cent.

The Distribution of Iodine in the Thyroid Gland. The outstanding work determining the distribution of iodine in the gland is still that of Tatum and Van Dyke. Tatum devised the method (242) which consists in floating sections of the frozen thyroid on Ringer's solution, whereupon

the colloid material drops out of the acini and apparently dissolves in the solution. The cells are centrifuged off, dried, weighed, and analysed for iodine. The distribution of iodine between cells and whole gland is obtained by comparable analyses of control pieces of whole gland.

Tatum found that iodine is present both in the cells and colloid of beef, sheep, and pig thyroid glands, the ratio of percentage of iodine in cells to that in whole gland being relatively constant, in the majority of cases varying between 0·3 and 0·45. Van Dyke (252) found ratios for dog glands varying from 0·1 to 0·2, and for the majority of human glands (abnormal, from operative cases) from 0·1 to 0·4. Both agree that the ratio is relatively constant for any one species, despite great variations in morphology and iodine content.

The Iodine Compounds of the Thyroid Gland [1]

Three compounds containing iodine can be obtained by different chemical procedures from the thyroid gland ; these are iodothyroglobulin, diiodotyrosine, and thyroxine. The first exists as such in the gland ; the free existence of the others is doubtful.

Iodothyroglobulin was first isolated from thyroid tissue by Oswald in 1899. His method—extraction of fresh glandular material with normal saline, and precipitation of the globulin by half saturation with ammonium sulphate—is still a standard procedure, and little has been since added to the studies of its properties by Oswald himself, by Nürenberg (1909), and by others of that period (for the literature, see Kendall (129)). Iodothyroglobulin can be readily purified by dissolving it before it dries in normal saline, reprecipitating with ammonium sulphate (repeating these procedures once

[1] Blanchard, Pénau and Simonnet (14A), and Harington (99), have recently published monographs which give a full account of the chemical and pharmaco-dynamical properties of the thyroid principle.

or twice), and then dialysing free from salts. It can be precipitated by alcohol, and dried by washing with alcohol and ether ; this treatment denatures it ; it becomes insoluble in water.

Barnes (9) has devised a new procedure for its preparation. It is extracted from the gland by 0·1 *M* sodium acetate and is precipitated by addition of 0·1 *M* acetic acid to produce an optimum acidity (determined by trial). He claims that the procedure is more rapid, while no haemoglobin is precipitated (cf. also 39A).

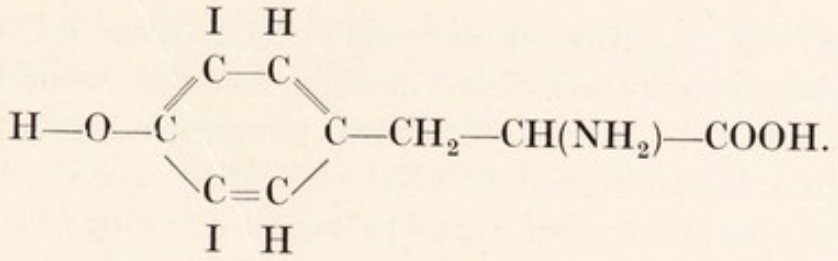
The properties of the globulin have been re-examined by Ingvaldsen (120). After dialysis it remains in solution in water, requiring addition of excess of alcohol for precipitation. It thus appears to be a pseudo, rather than a true, globulin. The dialysed solution is either neutral or just acid to litmus. It is not coagulated on boiling, but coagulation occurs at once on addition of a little acetic acid or sodium chloride solution to the boiling solution.

Its solutions give a positive test for tryptophane radicals, and markedly positive Millon's and Molisch's tests.

When dried, thyroglobulin is a white amorphous powder ; it hydrolyses to the usual amino-acids. Its composition appears to be constant, except as regards its iodine content, which, according to the earlier investigators, varies from 0 to 1·7 per cent. Successive extractions of the same thyroid material yield preparations with diminishing iodine content, suggesting that the thyroglobulin present is a mixture of molecules containing different amounts of iodine (120).

The amount present in the thyroid varies considerably. Wiener (1909) found that five dog's thyroids contained amounts (based on dry weight) varying from 14 to over 60 per cent. (It is doubtful, however, if the higher figure is correct.)

Diiodotyrosine was isolated by Drechsel in 1896 from the horny axial skeleton of a gorgonian coral. Its constitution was established by Wheeler as



It is a dextro-rotatory colourless crystalline compound, containing 58·7 per cent. of iodine. It is only very slightly soluble in cold water (1 part in 347 at 15° C.), but recrystallises from hot water in needles resembling crystalline tyrosine. It is easily soluble in dilute ammonia, alkalies and acids. It gives a positive xanthoproteic, but a negative Millon's test. Silver nitrate precipitates it, but does not split off iodine from it.

Oswald (1910–11) could only isolate it from gorgonin of coral and spongin of sponges to the extent of 7 and 15 per cent. of the total iodine content of these materials respectively.

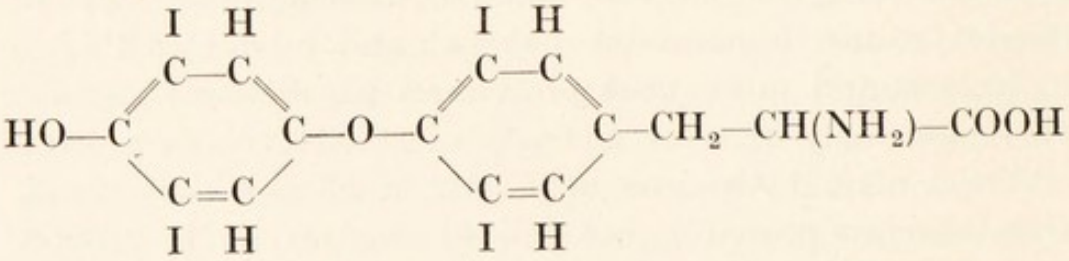
When iodine is allowed to react with solutions of proteins, such as egg-white or casein, it combines with the protein molecule. Oswald showed that subsequent hydrolyses of such iodised proteins liberated amounts of diiodotyrosine of the same order as those obtained from gorgonin and spongin. Ingvaldsen (120) added known quantities of diiodotyrosine to glandular material, and submitted this to hydrolysis and subsequent procedures paralleling Oswald's. He could only recover a trace of diiodotyrosine; even addition after hydrolysis only gave a 23 per cent. yield. It is therefore probable that Oswald's figures for the proportion of iodine held in such combination in naturally occurring or artificially produced iodised proteins do not at all indicate the total amount of such radicals present; it may well be that all the iodine is present in such organic combination. Such a conclusion bears also upon the iodine of the thyroid gland.

Various early attempts to isolate diiodotyrosine from the

products of hydrolysed thyroid gland material failed. Harington recently succeeded in doing so (101), while Foster has obtained it from hydrolysed thyroglobulin (76), and Harington has obtained it in the optically active dextro-form by enzymic hydrolysis of thyroglobulin (101).

Thyroxine was isolated in crystalline form by Kendall on December 25th, 1914 (129). He thus achieved a goal sought by many investigators ever since Baumann first showed the presence of iodine in thyroid tissue—the isolation of a crystalline iodine derivative from thyroid tissue. Subsequent work enabled him to isolate it in reasonable quantities. Misled by a slight analytical error, due apparently to some degree of volatility of the compound during fusion for iodine analysis, he regarded it as a partially oxidised tryptophane derivative containing an indole nucleus, and accordingly termed it " thyroxin " from " thyroid-oxy-indole." [1]

In 1926–28 Harington, by a brilliant series of researches, devised methods by which thyroxine could be obtained from thyroid material in much larger amounts, and completely established its constitution (98). The final proof of synthesis was furnished by Harington and Barger (100). Thyroxine, $C_{15}H_{11}O_4NI_4$, is a derivative of diiodotyrosine and an alpha-amino-acid containing 65·3 per cent. of iodine [2] :—



[1] The term " thyroxine " thus perpetuates the single error in a brilliant research, an error which has to be explained to every class of students of biochemistry and endocrinology by all teachers to whom the terminology of a subject has any significance. It would be of service if Kendall, or Harington, or both together, would suggest a name which truthfully indicates the nature or the derivation of this compound, before age has entirely wedded it to a term which always needs to be excused.

[2] Harington's chemical procedures have been outlined by Pryde in another volume of this series (200). In 1926 Dakin also proved that

Thyroxine, prepared from thyroid tissue, or synthetized, is optically inactive. Harington resolved it into its active dextro- and laevo-components (98); it was found that laevo-thyroxine possessed greater physiological activity. Proof that this laevo-thyroxine is the actual form of the compound elaborated in the thyroid was obtained by its isolation from the enzymic digest of iodothyroglobulin (102). Successive treatments by pepsin and trypsin followed by adequate chemical treatment gave a brown powder containing somewhat over 30 per cent. of iodine. This was not affected by erepsin; it proved to be a mixture of some free thyroxine and a larger amount in combination as a tri- or tetra-peptide. The latter is strongly resistant to enzymic hydrolysis.

Crystalline laevo-thyroxine melts at 235° C. with decomposition. Its specific rotation (5 per cent. concentration in 2 : 1 alcohol-*N* NaOH) is $[\alpha]_{5461} = -3{\cdot}8°$.

The Amounts of Iodothyroglobulin, Thyroxine, and Diiodotyrosine in Thyroid Tissue, compared with its Total Iodine Content. Kendall (129) states that no iodine is present in inorganic combination in normal thyroid tissue (although it may be present in that of patients to whom iodide or Lugol's solution has been recently administered). A definite separation of the organic iodine compounds is effected by acidifying the alkaline hydrolysate of thyroid tissue or iodothyroglobulin. Thyroxine is precipitated; diiodotyrosine remains in solution. Harington is of the opinion that no other organic compound of iodine is present.

If, as Harington and Barger logically deduce, thyroxine is formed in the thyroid from tyrosine through the stage of diiodotyrosine, then a varying ratio between the amounts of the two may be expected. Harington and Randall found them about equally distributed. In the thyroids of horses

thyroxine was a derivative of tyrosine and drew similar conclusions as to its constitution; on learning of Harington's work he withdrew his results which had already been submitted for publication (100).

the ratio of thyroxine-iodine to total-iodine varies from 28 to 60 per cent. (15). Leland and Foster (145), after alkaline hydrolysis of human thyroids (in which they estimated that the unavoidable destruction of thyroxine is not more than 15 per cent.), found, following butyl alcohol fractionation, that a series of fifty-two human thyroids gave a mean thyroxine content corresponding to 25 per cent. of the total iodine (extreme values 12 and 35 per cent.). Applying a 15 per cent. correction the average figure becomes 40 per cent., the extremes 27 and 50 per cent.

Fenger showed long ago that the iodine content of the domestic animals in the United States exhibits marked seasonal variations; such variations scarcely occur in British animals (129) but have been reported for Australian sheep (58). Kendall and Simonsen showed that a corresponding variation exists seasonally in the thyroxine content of hogs in the United States (129).

All these variations are comprehensible if one recollects that the iodine available is that provided by the diet, varying in different areas and at different times in the same area, that variable amounts of diiodotyrosine radicals must therefore result, and that it is improbable that any fixed proportion of these is transformed into thyroxine; further, that the thyroid is depleted in widely varying degrees of its principle (and therefore of thyroxine radicals) by the response of the organism to environmental changes.

The iodization of proteins with formation of diiodotyrosine radicals may normally be non-enzymic, even in the thyroid; the formation of thyroxine from diiodotyrosine, a reaction specific to thyroid tissue, suggests a specific enzymic action. No direct evidence of the existence of such a specific enzyme has as yet been obtained. (Attempts some years ago in my laboratory to obtain such evidence proved unsuccessful.)

The Essential Principle of the Thyroid Gland and its Path of Discharge

Qualitatively, thyroxine satisfies all the criteria we can apply to decide what is the active principle of the thyroid. Quantitatively, there is still doubt as to whether it does so. In order to understand completely what is the function of the thyroid, we must know what compound it elaborates and what compound it secretes. It is necessary to consider first the criteria of comparison that are available.

When desiccated thyroid tissue is fed to normal animals, or thyroidectomized animals, certain definite effects are produced. Since these include the restoration of thyroidectomized animals to normal condition, and their maintenance in that condition, it may reasonably be concluded that the essential principle of the gland withstands digestion and can thus be administered orally; effects following such administration can all be considered as directly or indirectly due to the action of this principle. Comparison of the effects following oral administration of thyroid derivatives and thyroid fractions with those produced by desiccated thyroid itself is therefore a legitimate method for ascertaining the relative physiological activity of such extracts in experiments designed to ascertain the nature of the active principle.

Kendall (129), in discussing the employment of such effects for the standardization of thyroid material, enumerates the following: "Relief of the symptoms associated with hypothyroidism, reduction in size of goitre, loss of body-weight, increased excretion of nitrogen, alteration in the viscosity and refractive index of the blood and of the reaction of the nerves to stimulation, increase in resistance of mice to poisoning by acetonitrile, increase in the metabolic rate, increase in susceptibility to lack of oxygen, denudation of glycogen from the liver, change in the plumage of birds, accelerated metamorphosis of amphibian larvae, and decrease in the rate of growth of

young rats, with coincident hypertrophy of most of the important organs." Regarding these as possible biological tests for thyroid activity, he continues: "To all except three . . . valid objections can be raised which prevent their use. . . . The three possible methods are: the influence of the thyroid on the basal metabolic rate of patients with hypothyroidism, or on thyroidectomized animals; the effect on the growth of small animals; and the effect on the resistance of the white mouse to acetonitrile."

The first method, from its nature, cannot be extensively utilized. The acetonitrile method of Reid Hunt (115) is more elegant and rapid, and requires less material than that based on growth-rate and organ hypertrophy (32). An additional method, easily applicable, is the determination of the oxygen consumption of rats (210). Amphibian metamorphosis has been frequently used for thyroid studies, but is non-specific, since many iodine compounds, inorganic and organic, hasten the rate of metamorphosis (129).

Results with the Acetonitrile Test. One of the most important conclusions from Hunt's work is that the activity of different thyroid preparations is closely proportional to their iodine content. The other methods of comparison fully confirm this conclusion, which presents a logical and easy basis of comparison. Based upon equal iodine dosage, the following results have been demonstrated:

Iodothyroglobulin injected intravenously into mice is inactive; administered by mouth its activity is approximately the same as that of desiccated thyroid. Diiodotyrosine only gives protective action against acetonitrile when injected into mice in relatively enormous doses; its physiological activity can be regarded as negligible (271, 255). Optically inactive (racemic) thyroxine exhibits on basis of iodine content about two-thirds the activity of desiccated thyroid. Intravenous injection confers about the same degree of protection on mice as oral administration (115, 181). The

relation between dosage and effect in such tests is probably logarithmic (34). This would accentuate the discrepancy. Thyroxine represents at most about half the iodine of the thyroid gland; the remaining half (in diiodotyrosine) is inactive. Hence thyroxine should actually exhibit *greater* activity than the corresponding amount of thyroid. (This applies to all methods used for comparison. On the other hand, racemic thyroxine is somewhat less active than laevo-thyroxine.)

Results with the Rat-growth Organ-hypertrophy Test. Thyroglobulin appears to contain the full activity of the thyroid from which it is prepared. Diiodotyrosine is inactive. The activity of thyroid tissue is not destroyed by the hydrolytic action of pepsin or trypsin. When thyroglobulin is hydrolysed by sodium hydroxide, and the hydrolysate acidified, the insoluble "thyroxine" fraction shows an activity of the same order as the original thyroglobulin, but the soluble "diiodotyrosine" fraction shows no activity. Racemic thyroxine shows definitely less activity than thyroid containing the same amount of iodine (32).

Results with the Oxygen-consumption Test. Laevo-thyroxine is about three times more potent than its dextro-isomer, so that it should be 50 per cent. more active physiologically than the ordinary optically inactive thyroxine; this does not fully account for the discrepancy in activity between desiccated thyroid material and thyroxine. Pure laevo-thyroxine is less active than the mixture of thyroxine and a thyroxine-peptide which constitutes the final product of enzymic digestion of thyroglobulin. Thyronine (thyroxine denuded of its iodine) and diiodotyrosine are inactive. Diiodothyroxine (with only half the iodine content of thyroxine) and tetrabromothyronine (bromothyroxine) only show slight physiological activity (82, 102, 101).

Results by Clinical and Other Procedures. The feeding of iodothyroglobulin increases the excretion of nitrogen and

produces a loss of body-weight in animals, and exercises the same beneficial influence on myxoedematous patients as does thyroid (191). Diiodotyrosine has no effect in cases of myxoedema and cretinism (235). Thyroxine has the same effect on such cases, qualitatively, as has thyroid itself (129), and Harington's thyroxine-peptide has at least as great a quantitative effect (102).[1]

Summary of Comparisons between the Activities of Thyroid Compounds. The physiological activity of diiodotyrosine, measured by any biological test of thyroid activity, is either *nil* or negligible. The activity of thyroglobulin is probably that of the thyroid itself. The full activity of thyroid tissue does not appear to be accounted for by that of laevo-thyroxine, and still less by that of racemic thyroxine. The activity of the thyroxine-peptide (mixed with thyroxine) isolated after enzymic digestion of thyroglobulin is probably equal to that of thyroid tissue. The resistance of this thyroxine-peptide to the action of digestive enzymes suggests that it is the chief product absorbed from the intestine after thyroid material is fed.

Certain evidence, to be quoted later, suggests that the thyroid may secrete into the circulation thyroglobulin itself. The active thyroid constituent is precipitated from blood with the blood proteins. Precipitin tests show traces of thyroglobulin in blood leaving the thyroid gland (36).

[1] Recent work somewhat confuses the issue. Thompson *et al.* (245) have compared the rates of utilization of racemic thyroxine and of desiccated thyroid in two patients with myxoedema, whose initial basal metabolic rates were respectively −33 and −41 per cent. Their results indicated that injection of 0·35 mg. of thyroxine daily had the same effect as feeding 1·5 grains of thyroid containing 0·23 per cent. of iodine ; in each case the iodine was 0·23 mg. They conclude that either all the iodine in desiccated thyroid is in combination physiologically equivalent to thyroxine, or else that there is a difference between thyroxine as it is separated from the hydrolysed gland and as it exists in the body. Somewhat similar results have been obtained by Salter, Lerman, and Means (223), using Harington and Salter's thyroxine-peptide (cf. p. 19), on myxoedematous patients. It is evident that such accurately controlled experiments with myxoedematous patients should be extended.

Nevertheless, compounds of the size of globulins, with molecular weights over 100,000, do not normally pass through the walls of the capillaries to the tissues. (Thyroglobulin, injected into mice, confers no resistance against acetonitrile, although it is active when fed.) The thyroid principle probably reaches every cell. Hence it would appear most probable that the thyroid principle is a tri- or tetra-peptide, one of whose radicals is thyroxine. That such peptide combination might confer greater activity upon thyroxine is suggested by comparison of the relative catalytic activities of glutathione (glutamyl-cysteyl-glycine) and the cysteine it contains ; the former is much more active.

The Path of Discharge of the Essential Principle. Earlier studies of the secretory process have been reviewed by Marine (172). The results given in some recent papers, while in general agreement with earlier theories, seem to throw clearer light on the process. Ludford and Cramer (151), studying the functional activity of the thyroid gland cytologically, find that droplets of secretion first appear in contact with the Golgi apparatus and pass out in the cytoplasm towards the lumen. The secretion is discharged into the lumen of a vesicle and then absorbed into the blood stream. Grant (91), using *Amblystoma Jeffersonium* and *opacum* as material, found that when the thyroid was repeatedly stimulated to marked activity by daily implants of anterior pituitary tissue the stored secretion could be seen to leave the follicle by passage through the cytoplasm of the epithelium ; the process continued until the follicles were almost completely emptied of colloid. During the larval, incipient metamorphic and metamorphic periods the same cytological pictures were seen. Transcellular export of stored colloid occurred, but more slowly, and less completely. Non-staining and staining material are probably different stages of the secretory product. During the process of release the stored secretion is absorbed through the apical membrane in non-staining form. During passage through

the cytoplasm it may become segregated into discrete chromophilic bodies. These pass out through the basal cell membrane in the non-staining state. During refilling the cell synthetizes a new secretory product which is probably non-staining when first formed. Within the cytoplasm it acquires an increased affinity for the stain. It leaves the apical end of the cell in non-staining condition, but acquires chromophilic properties when once free within the follicular lumen.

It would thus appear that the process of secretion of the thyroid principle may probably be summed up as follows : Iodine is absorbed (probably as iodide) from the blood by the cells lining the acini, then is converted into diiodotyrosine radicals in protein combination, and a proportion of these is changed to thyroxine radicals. The protein concerned is thyro-globulin. This passes through the membranes of these cells inwards into the acini and is stored. As demands of the organism require, the thyroglobulin is passed back into the acinar cells, and the thyroid principle is split off and excreted outwards into the capillaries within the gland, and so passes to all the cells of the organism.

The Normal Function of the Thyroid

It is generally considered that the normal function of the thyroid gland is causatively linked with the oxidative processes of the cell. In an animal which has been thyroid-ectomized oxidation and heat production are depressed to 60 per cent. of their normal value, while, on the other hand, when thyroid is fed to a normal animal, its oxidation and heat production are increased. Judging by the changes which ensue when, on the one hand, the gland is extirpated, or, on the other, desiccated thyroid or some active product is fed continuously, the thyroid principle is associated with many activities in the organism. Can these all be traced, directly or indirectly, to control of cell-oxidation, or is some

other direct action attributable to the principle? Some account of these varying activities must be set forth before an attempt can be made to answer this question.

The Effects of Experimental Thyroidectomy. Sharpey-Schafer has summarized these as follows (229): "In young animals the most striking effect is marked retardation of growth; ossification is delayed. . . . The generative organs remain relatively small, and the ova and spermatozoa may not come to maturity. The pituitary becomes enlarged; its anterior lobe contains many colloid-filled vesicles. . . . The temperature of the body is lower than normal. The abdomen is generally swollen. The skin is thickened; the hairy covering is usually imperfectly developed. . . . The involution of the thymus is delayed or may not occur; the cortex of the suprarenals is somewhat enlarged. The central nervous system—especially the brain—is involved in the general arrest of development. In most young animals deprived of their thyroids there is a marked lack of intelligence. . . . At all ages there is a marked diminution in basal and in general metabolism.

"After complete thyroidectomy in adults . . . the limit of assimilation of carbohydrates is raised, but there is no glycaemia or glycosuria. . . . The muscles lose tone and are weaker than in the normal animal. Muscular activity . . . is considerably diminished. Regeneration of tissues is retarded. Anaemia is generally present. . . . Heat production is diminished. The body temperature is low; the power of heat regulation lessened. There is diminished excretion of carbon dioxide . . . and also a diminished consumption of oxygen. . . . The sexual functions are depressed. The nervous system is markedly affected, dullness and apathy being prominent symptoms. The skin is dry . . . the hair often tends to fall out. . . . There is a delay in the healing of fractures, and in degeneration and regeneration of cut nerve."

The Effects of Feeding Thyroid to Mammals. When it is

fed to thyroidectomized animals (in whom the operation has not too greatly involved the parathyroids) normal metabolism is restored and maintained.

Fed to normal animals it causes a loss of body-weight, with an increased excretion of nitrogen. Provided the diet contains sufficient carbohydrate and fat, the minimum protein requirement for maintenance of nitrogen equilibrium is probably not altered; the initial increase in nitrogen is largely due to increased excretion of urea (129). There is some increase in excretion of creatine, but none in that of creatinine, uric acid, and other nitrogenous catabolites.

Oxygen consumption, carbon dioxide production and heat production are all increased. Susceptibility to diminished oxygen content of the inspired air is increased (in the thyroidectomized animal it is diminished) (129). Oxidation of carbohydrate is increased; one result is an almost complete depletion of glycogen from the liver (129). Muscle glycogen is similarly affected. It seems possible that some immunity to this process may be induced, for if after a normal period of some duration thyroid feeding is recommenced the denudation of glycogen is much less (62). A hyperglycaemia may or may not arise; in marked hyperthyroid conditions in man such a hyperglycaemia associated with glycosuria is by no means uncommon.

When thyroid is fed for two or three weeks to young laboratory animals (rats have been chiefly studied) there is a marked decrease in the growth rate, accompanied by hypertrophy of the heart, liver, kidneys, adrenals, pancreas, spleen and lymphatic tissue. There is an almost complete disappearance of body fat, while muscle tissue diminishes in bulk. The thyroid of the animal, distended with colloid, passes into a resting condition. The effect is transient, and when thyroid feeding is stopped most animals exhibit accelerated growth at first, and may even surpass controls; finally, the normal growth curve is re-established and the hypertrophies of the various organs disappear (129).

Rabbits behave similarly to rats. Mice exhibit the organ hypertrophy, but their growth rate is accelerated, not decreased (218).

The Acetonitrile Reaction. Thyroid feeding confers protection on mice against acetonitrile poisoning, but increases its toxicity to rats. Hunt considers the protective action as due to acceleration of the oxidation of acetonitrile to formic acid and thiocyanate. This explanation seems generally accepted, but does not account for the decreased resistance produced in rats; the precise mechanism of detoxication is probably more complicated.

The Metamorphosis of Amphibian Larvae. Administration of thyroid to tadpoles produces premature metamorphosis, but the action is not specifically due to thyroid (129). Thyroidectomized tadpoles never metamorphose unless they are given iodine preparations, and of these desiccated thyroid is most effective. Thyroxine is 100 times as effective as diiodotyrosine. Corresponding bromine compounds are ineffective (239, 220). The specificity of iodine, and the fact that metamorphosis can be produced in absence of the thyroid secretion, albeit less readily, obviously indicates that the thyroid principle itself is not essential to the metamorphosis, and that critical examination is necessary of all research in which estimations of thyroid activity have been based on metamorphosis.

The Colorado axolotl tends to metamorphose spontaneously when transformed from its native habitat, so that experiments with it are still more open to criticism. Ingram suggests that these, to be properly controlled, must be performed on individuals with both pituitary and thyroid removed, and has found that in such animals intraperitoneal implantation of powdered crystalline iodine rapidly induces metamorphosis (119, 251).

It would thus appear that the thyroid principle acts primarily, as regards metamorphosis, as a purveyor of iodine in a very effective but not specific form of combination.

Thyroxine retards the cleavage rate and differentiation of the eggs of the sea-urchin and the ascidian, and of *Paramoecium*. This effect is not due primarily to iodine. It suggests that thyroxine (and the thyroid principle) are depressants of cell division generally (248, 270). While it is not clear that such an action can definitely be regarded as due to enhanced oxidation, there is evidence that thyroxine prolongs, and so increases the maximum level of oxidation of sea-urchin spermatozoa (38).

The Effect on the Feathering of Birds. It is well recognized that the growth of hair is under the control of the thyroid

principle. Myxoedematous patients, suffering from a deficiency of that principle, tend to lose hair from the eyebrows and head, and in advanced cases show varying degrees of baldness. Thyroid feeding restores normal hair growth. Undoubtedly associated with this is the effect of feeding thyroid on the feathering of birds. Within recent years this has been accurately studied by several groups of investigators (249, 55, 44, 87, 117, 43, 272, 137).

The results seem to be as follows : the majority of races of fowls exhibit secondary sex differences in the feathers of the neck, wing-bow, and saddle, the male feathers having a marginal zone around the distal end, in which the barbs lack barbules and hooks. The male feathers appear pointed and silky in contrast to the rounded dull feathers of the female. The feeding of relatively small doses of desiccated thyroid to cockerels exhibiting this difference (if necessary, in adults, plucking a patch of feathers from the neck, wing-bow, and saddle regions) leads to a growth of new feathers which do not show the characteristic male plumage but tend to be darker. Capons do not yield this result, and Sebright Bantams, which do not normally exhibit the sex difference in feathering, are not affected by the treatment.

If larger amounts are fed, rapid moulting is produced, and the new feathers show depigmentation. The effect can be produced in cocks, capons and hens, but the dosage needs to be great enough to produce typical signs of marked hyperthyroidism. (However, if single large doses are given, hyperthyroidism does not ensue, although the moulting and depigmentation do.)

Similar results have been demonstrated with pheasants, and in lesser degree with the squabs of pigeons.

Such effects are probably produced through the gonads. This is supported by the fact that administration of thyroid in moderate dosage to aged cocks and hens in advanced senility results in distinct rejuvenation, the cocks being restored to sexual activity, and the hens showing increased egg production (54). The endocrine principles of the gonads control the secondary sex characters, including hair growth, and presumably feathering (cf. Chapter VII.).

That the effects are associated with an increased level of oxidation is not excluded. It has been suggested that the initial darkening is associated with an increased production of melanin, but that a still higher level of oxidative catabolism inhibits melanin formation, whence the observed depigmentation. The thyroid principle is specifically concerned. Diiodotyrosine produces no effect, crystalline iodine only a very slight effect.

Other Effects. The surviving hearts of thyroidectomized cats utilize less glucose than those from normal cats. Those from cats dosed with thyroxine use more and those of

thyroidectomized cats dosed with thyroxine use approximately the normal amount (6). Surviving strips of diaphragm-muscle from thyroidless rats show a diminution in oxygen consumption of 25 to 30 per cent. below normal (77). Tissue cells from cretin pups and lambs show a similar decrease (64).

In areas exhibiting moderate extremes of climate, such as the central United States, with the onset of cold weather there is usually a physiological enlargement of the thyroid, associated with decreased iodine content of the gland (through increased output of the thyroid principle). This is suggestive evidence of control of oxidation and heat production by the thyroid (129). A similar functional enlargement has been observed in pigeons (212).

The thyroid principle has not yet been shown to influence directly any specific chemical actions *in vitro* (129).

Summary. While the evidence is perhaps still too indirect to be final, and while we cannot yet trace an intermediate association with oxidation for all the known effects produced by the thyroid, there seems to be little doubt as to the general truth of Plummer's view that the thyroid principle exerts an influence on the oxidation proceeding in all the cells of the body, and thus produces its actions (199). Whether such action is catalytic, as Plummer suggests, is not certain (cf. 139, 64). It has been suggested that the essential action lies in facilitation of oxidation in the anaerobic stage, and in support of this it has been found that injection of thyroxine increases the lactic acid concentration in blood (66). The peculiar fixed limit of the effect on normal oxidation—40 per cent. under thyroid control—suggests control of specific reactions rather than an uncontrolled catalysis.

The Control of the Thyroid Secretion

It is frequently assumed that the thyroid gland is under the control of the sympathetic nervous system, and that in

abnormal thyroid states that system is definitely affected. (The nervous excitation of a patient with Graves' disease is a cardinal symptom ; the myxoedematous patient presents the opposite condition.)

There is no definite evidence supporting this view of thyroid control. Gley, after reviewing the known facts, wrote : " Il est actuellement bien difficile de considérer comme démontrée une influence directe du système nerveux sur la sécrétion thyroidiènne " (88, cf. 126).

It has been shown that after bilateral splanchnectomy, splanchico-vagotomy, and extirpation of the stellate ganglia in rabbits, there is no difference in the effect of thyroid feeding upon their gaseous metabolism, whence it was concluded that no metabolic centre of the central nervous system controls the action of the thyroid principle in the tissue cells (217).

There is definite evidence that the thyroid secretion is under the control of one of the principles secreted by the anterior lobe of the pituitary (cf. Chapter IX.).

The Utilization of the Basal Metabolic Rate in Evaluating Thyroid Function

The ever-increasing employment of determinations of the basal metabolic rate to confirm or disprove a diagnosis of thyroid disease, to control the pre-operative treatment of hyperthyroid patients, and to adjust the thyroid dosage of those exhibiting a hypothyroid condition, renders the precise evaluation of this test, and the recognition of its limitations, matters of considerable importance.

The determination is open to certain intrinsic errors, especially when the simpler portable forms of apparatus are used. Use of these involves the assumption of a " basal respiratory quotient " of 0·82. Unbalanced diabetics do not have this quotient. The normal heat production is usually calculated from a height-weight surface-area formula,

and the calculation of surface area from height and weight leads to a variable error necessitating an allowance of ± 15 per cent. for normal limits. The increased temperature associated with fever needs a large correction; although, curiously enough, sub-normal temperatures do not.

DuBois has dealt very fully with the subject of basal metabolism (63). Attention may be drawn to one or two phases dealt with in recently published papers.

Estimation from Pulse Rate and Pulse Pressure. Attempts have been made to determine the basal metabolic rate from formulae based upon pulse-rate and pulse-pressure (203, 83). While some degree of relationship exists, the potential error is too great to give that certainty of information required from a diagnostic test.

Standards. The normal standards of Aub and DuBois for heat production per square metre of body surface have been recently modified by Boothby and Sandiford (22), who have extended them to young children; their figures for children are undoubtedly more accurate than those previously in use. Further accurate studies of metabolism in children have been published by Nylin (189) and by Bierring (14).

Variations from Causes other than Disease. Various studies have been made contrasting basal metabolic rates of normal persons in tropical and subtropical climates, and of non-Aryan races with those of Aryans in temperate climates, (on which the Aub and DuBois standards are based). The results, though not in complete agreement, suggest that metabolism is somewhat less in warmer climates and that race exerts a distinct influence. The effect of climate seems to be shown by the fact that while the basal metabolism of Brazilian whites is 20 per cent. below the standards (5, 238, 95), that of students of South Carolina averages 10 per cent. below (207), and similar results have been obtained for students in Florida (247). (However, similar results were also obtained for those in the more temperate surroundings of Minneapolis (48)). Other findings for places in temperate

climates are in almost complete agreement with the standards (92, 33).

Studies on different races have proved interesting, although they evidently require to be extended before definite conclusions are permissible. The Chinese are stated to exhibit a lower metabolic rate than Western races (65). Results for Japanese are conflicting (250, 240, 169). Figures obtained for Armenians agree with the standards, but those for other Near Eastern peoples are lower. Syrian women in Beirut gave lower values than Anglo-Saxon women residing there (250). Eskimos in the Baffin Bay district gave values averaging 33 per cent. higher than the standards (104). The reported values for Jamaican Blacks are slightly low, but those for Mayans in the Yucatan are slightly higher than the values obtained for control whites there (10). Low values have been obtained for Australian aborigines (258).

While the precise causes of these racial differences cannot be stated, differences in diet are undoubtedly an important factor.

Under-nutrition markedly affects the basal rate. While moderate under-nutrition does not produce an appreciable effect an abnormally low diet can depress the rate more than 20 per cent. Since so many patients are under-nourished, this factor needs to be considered in the interpretation of results. In under-nourished children there is a tendency for the rate to be raised (63).

Diet, and especially the protein of the diet, produces an effect. It has been shown that a protein-free diet will produce a rapid fall in the basal metabolic rate of a normal individual within a few days. When such an individual is then given a high protein diet the rate not only returns to normal, but is definitely raised above normal (60).

During pregnancy there is a slow rise, perceptible during the second half and amounting at most to an increase of 20 to 25 per cent. above the values prior to the pregnancy (63).

Basal Metabolism in Disease. The relatively large correc-

tion of 7·2 per cent. per 1° F. above normal body temperature must be applied to results for all patients exhibiting a febrile condition. This not infrequently leads to a correction which is too large to permit stress to be laid on a moderate deviation from normal after the correction has been applied.

Excluding this temperature effect, probably over 90 per cent. of abnormal basal rates are directly attributable to abnormal thyroid function. The basal rate may be unduly elevated in cases of leukaemia, polycythaemia vera, and the leukaemic lymphoblastomata, in cases of pernicious anaemia, essential hypertension and acromegaly and in chronic encephalitis with Parkinsonism. In mild cases of diabetes there is no deviation from normal. Severer cases, generally under-nourished, may on this account show a decreased metabolism ; in extremely emaciated cases this may reach 30 or 40 per cent. below the average normal. Cases suggestive of hypofunction of the anterior pituitary may exhibit normal or slightly low rates (63). It is generally assumed that any change from normal in a patient with pituitary disease is due to a pituitary-thyroid interrelationship (cf. Chapter IX.).

Classification of Thyroid Diseases

The classification of thyroid diseases is a fruitful field of controversy. The most unsettled question at present is the unitary nature, or otherwise, of hyperthyroid conditions. The simplest classification for the present purpose is [1] :

1. Inflammatory conditions.
2. Simple (endemic) goitre.
3. Hypothyroidism.
4. Hyperthyroidism.
5. Malignant tumours of the thyroid.

[1] For an example of a fully differentiated classification, see Joll (126).

Even with these few divisions there is not complete mutual exclusion. Thus a small proportion of cases of Graves' disease (which is generally considered a hyperthyroid state) appear to exhibit no hyperthyroidism. Again, some malignant tumours of the thyroid are associated with hyperthyroidism.

From the endocrine standpoint the first and last of these divisions are of much less interest than the others, and will only be referred to very briefly.

Inflammatory Conditions of the Thyroid

In the rare instances when the thyroid is influenced by the toxins of acute infections the colloid may diminish or disappear, the cells lining the follicles may degenerate or desquamate, and there may develop increased vascularity and hyperplastic changes in the epithelium. The latter may become sufficiently conspicuous to resemble those seen in certain stages of Graves' disease. The basal metabolic rate may be increased. Administration of iodine lessens the effect (45, 72).

Chronic inflammatory conditions, also rare, may be due to tuberculosis, syphilis, actinomycosis, etc., and include Riedel's disease, and perhaps lymphadenoid goitre, and inflammatory conditions traceable to parasitic causes (Echinococcus disease and Chagas' disease, due to *Trypanosoma cruzi*).

Williamson and Pearse have endeavoured to show that there is a close connection between lymphadenoid goitre and Riedel's disease (woody thyroiditis). Joll has advanced a number of objections to their view, which he considers is erroneous (126).

Endemic Goitre

" Any enlargement of the thyroid gland which is neither inflammatory nor malignant and not associated with toxic features may be considered a simple goitre " (126).

The evidence associating undue lack of iodine in the diet

with prevalence of endemic goitre strongly suggests a causative relationship between the two, but does not afford final proof that lack of iodine can be the sole cause of this goitre. There is indisputable evidence, indeed, that such a goitre can arise from other causes. But there is overwhelming evidence that in communities where the diet contains a sufficiency of iodine such endemic goitre is extremely rare, and that if lack of iodine is not definitely the cause of development of such goitre, yet a sufficiency of iodine acts as a shield against its production.

The Nature of Simple Goitre. It seems possible that there are at least three types of simple goitre—one found in mountainous regions, the second common in non-mountainous countries, and the third traceable to a dietary deficiency, which is not of iodine, but which is perhaps the lack of vitamin A.

The goitre of mountainous regions has been variously termed parenchymatous goitre, adenoparenchymatous goitre, simple hyperplastic goitre and chronic hypertrophic goitre. According to McCarrison (163), the condition is " essentially a place disease," prevailing " with different degrees of intensity in different regions and in different parts of the same region." It exhibits distinct seasonal variations, and appears more commonly in the spring and early summer months. The children of goitrous mothers are prone to become goitrous, and consanguinity appears to favour the development of goitre. It is associated with cretinism, deaf-mutism and idiocy. The pathological picture indicates secretory activity; hyperplasia predominates. There is suggestion of formation of many new small vesicles. The gland is poor in colloid. Solid masses of cells—adenomata—are present.

In non-mountainous countries a diffuse colloid goitre predominates. Colloid accumulates, resulting in frequent distortion of the vesicles. The goitre gradually increases in size until puberty and then tends to disappear.

The differentiation of lymph-adenoid goitre as a separate entity is due to Williamson and Pearse, and McCarrison. The former describe the thyroid picture as exhibiting a preponderance of lymphocytic aggregates, a fibrosis, and a specific atrophy of the parenchyma (266). They consider, on probably insufficient evidence, that the ultimate atrophy is the cause of myxoedema in adults (cf. p. 58).

Joll has dealt very fully with the pathological histology of simple goitre (126).

The Relationship between Dietary Iodine and the Occurrence of Goitre. The wide distribution of iodine, and the marked variations in the amounts of the element present in rocks, soils, drinking waters and foods have already been referred to (p. 10). In many regions over the whole globe there is a striking inverse relationship between the content of iodine in the diet and the proportion of goitrous individuals among the total population.

Contrasts of the iodine content of the diet with the incidence of goitre have been made for goitrous and non-goitrous districts in Switzerland (73), New Zealand (107), Argentina (177), Hungary (18), and China (4A). There is complete agreement between these surveys; the inverse relationship definitely exists.

Maps have been published comparing the incidence of goitre in the United States with the relative content (low or high) of iodine in the drinking water; the resemblance is marked (165). The correlation is not quite so marked in Alberta, Canada (256).

Close proximity to the sea undoubtedly increases the intake of air-borne iodine. There is 20 per cent. less goitre at Lyttelton, the harbour of Christchurch, N.Z., than at Christchurch itself, two miles inland (106). Goitre is practically absent on San Juan Island, in the Puget Sound, while in the city of Seattle, stretching inland from the Sound, it is prevalent. McClendon considers that it is necessary to

live within three miles of the sea to derive perceptible benefit from air-borne iodine (165).

Since iodine is excreted almost entirely through the kidneys, measurement of the urine content of iodine per twenty-four hours' excretion gives a close clue to the daily iodine-intake. The results shown in Table II. fully confirm the inverse relationship between iodine-intake and incidence of goitre (73, 152).

TABLE II

Iodine Content of Human Urine in Non-Goitrous and Goitrous Districts [1]

Non-Goitrous Districts.					Goitrous Districts.				
District.	Goitrous Children.	No. of Urines N.G.	Urine-Iodine.		District.	Goitrous Children.	No. of Urines.	Urine-Iodine.	
			Extremes.	Mean.				Extremes.	Mean.
	Per cent.		γ	γ		Per cent.		γ	γ
witzerland					*Switzerland.*				
Effingen	1·0	7	28–108	64	Kaisten .	62	11 (10 G.)	4–29	19
taly.									
Ligurian Coast	0	3	94–140	112	Hunzenschwil	56	12 (10 G.)	5–28	17
'orway.					*Norway.*				
Vik i Sogn	0	6	107–240	173	Hostvedt .	60	4	26–69	40
					Vittingfoss .	55	8	14–105	48
					Ljöterud .	54	5	19–41	29
					Meheia. .	54	5	10–62	39
					Saggrenda .	43	4	11–101	64
					Komnes .	40	5	13–86	48
					Berg . .	39	4	6–128	56
					Eftelot. .	37	5	13–86	65
					Verp . .	36	5	55–140	87
					Ruud . .	30	5	49–79	61

[1] G.: from goitrous individuals; N.G.: from non-goitrous individuals.

The figures for Norwegian districts are particularly striking. It may not be without significance that in some of the goitrous districts in Norway the average iodine excretion from goitrous individuals is equal to that from non-goitrous individuals in a relatively non-goitrous district in Switzerland; there is a possible inference of some other factor beside lack of iodine (cf. 164, 160).

The Effects of Administration of Iodine Compounds in Preventing and Benefiting Endemic Goitre. Before dealing specifically with the subject named in the caption, it is perhaps desirable to stress the necessity of employing accurate terminology in references to iodine compounds. In clinical papers the term "iodine" is often somewhat loosely used. Iodine is administered very frequently as Lugol's solution (compound solution of iodine, a solution of iodine in potassium iodide, of which the strength varies in different countries), less frequently as (alcoholic) tincture of iodine, and very often as sodium or potassium iodide. Occasionally iodized fats are administered. Sodium and potassium iodide in solution circulate very rapidly throughout the body, the iodide ion penetrating all membranes with the greatest ease, so that within a few minutes of the introduction of an iodide into the stomach through a tube or within a capsule it can be detected in the saliva, and very shortly afterwards in the urine. Hydriodic acid is subsequently present in the gastric juice, and iodide is also secreted into the milk. Excretion is rapid ; the main channel of excretion is through the kidneys. When a single dose of iodide is given, 50 per cent. is excreted in the urine in twenty-four hours, and most of this within the first six hours. Practically all is excreted within ninety-six hours. Iodine is present in the tincture as elementary iodine, and in Lugol's solution probably as the active compound KI_3. In these forms it rapidly attacks protein material in neutral or alkaline medium. Whether given as the tincture or the compound solution, it is practically inconceivable that following oral administration any free iodine or KI_3 ever reaches the thyroid gland itself. The initial change is probably formation of iodide of starch (if the iodine is taken after a meal) from which the iodine is subsequently set free to attack other compounds. The final result of any reaction with protein is probably the setting free of diiodotyrosine through normal digestive processes. This can be absorbed,

and when it is fed directly it is in large part decomposed, iodine appearing in the urine at a somewhat slower rate than when the corresponding amount of iodide is given. Iodized fats, when given, are only very slowly decomposed, the fat being stored in the same way as other fats; iodide is very slowly excreted.

The effect of increasing the amount of iodine (in any one of these forms) in the circulating blood is to increase transiently the amount present in the various tissues. Only thyroid tissue is capable of storing any relatively large amount. This it does easily, no matter in what form of combination the iodine is supplied.

Although Coindet used iodine in the treatment of goitre in 1820, nine years after it was discovered, and Prévost advocated its employment as a preventive of goitre in 1849, so that during the following decade the treatment was used to some extent in Switzerland, Austria, and Italy, this treatment was criticized adversely in the Imperial Academy of Medicine in Paris in 1858, and gradually fell into disuse.

Marine remedied a serious condition of endemic goitre in the Fish Hatcheries at Shady Grove, Pennsylvania, by addition of a small amount of iodide to the water. This good result led Marine and Kimball to carry out the first systematic large-scale attempt to combat and prevent goitre in the schools of Akron, Ohio, in 1916. It was successful. Of those girls who were initially non-goitrous only 0·2 per cent. developed a goitre during four years of treatment, as compared with 27·6 per cent. amongst those whose parents refused to permit the treatment. Of those initially goitrous, 60 per cent. showed improvement during treatment, and only 14 per cent. amongst the untreated group (131).

Immediately following the publication of these results similar treatment was instituted elsewhere in schools and communities where goitre is prevalent. Good results have been reported from Switzerland (134), Norway (187A), Italy (184), Austria (125), New Zealand (127) and Canada (1).

In these wholesale experiments sodium or potassium iodide or iodized fat at stated intervals, or iodized salt continuously, were variously used. The incidence of goitre in new-born infants of goitrous mothers has markedly decreased following treatment of the expectant mothers with iodized salt throughout pregnancy (131, 195).

Keith (128) has reported the most interesting result of a natural experiment in iodine-deficiency and iodine-treatment, carried out in the Pemberton Valley, British Columbia, a valley situated in the Coast Range about ninety miles north of Vancouver, and watered by the upper Lilloet River, which rises from a not-distant glacial source. At the lower end of this valley is an Indian Reserve, peopled by about 150 Indians. In the Indian village no sanitation has ever been attempted, but salmon (an excellent purveyor of iodine) is eaten in quantity. No case of goitre has ever been recorded among these Indians. Only rarely has a litter of myxoedematous pigs been born on the Reserve.

Before the institution of iodine treatment it was noticeable that as one ascended the valley, the incidence of goitre increased both among the white population and all farm animals. By the end of 1917 settlers had suffered such severe losses in farm stock that they had almost decided to leave the valley. Litters of pigs were almost all born hairless and died within twenty-four hours. Those with some hair survived for a few days. Their thyroids were enlarged, dark red, and engorged with blood. All cows had at least a slight enlargement of the thyroid; all gave birth to goitrous calves. Ninety per cent. of the two-year-old heifers lost their calves within a few days of birth. Those that did not die usually became cretins. Subsequent calves from such cows were goitrous, but could be kept alive by personal care. Their goitres gradually diminished, and were not visible at two years. Many of the cows carried their offspring ten months, a month longer than usual.

Mares brought into the valley developed goitre; after

they had been in the valley three years their colts were goitrous. The colts were frequently carried twelve and sometimes thirteen months; such were weak, goitrous, and did not survive. About 85 per cent. of the colts died; the remainder were only carried eleven or eleven and a half months. All the farm animals were more subject to goitre in late autumn and early spring.

All hens, ducks, and turkeys brought into the valley appeared to thrive, but their eggs, though producing embryos, failed to hatch out. The apparent cause was a change in the white envelope within the shell, which became thick and rubbery, so that the young chick could not break its way through.

All the babies born in the district had goitre. Almost every woman coming into the valley would develop goitre within a few months or a year. Bachelors having no cows and using only condensed milk sometimes showed goitre within a few weeks, and always within a few months.

These careful observations were made by Mr. John Ronayne, one of the chief farmers in the district. Seeking advice, he was referred to Marine; iodine, as tincture or iodide, was recommended. "The iodine acted in a miraculous way. All the goitres disappeared from the animals, and no further trouble in rearing stock or fowl has occurred." When Keith visited the valley in 1922 he could not find a goitre in animal or human being.

Some typical results of Ronayne's administration of iodine may be quoted. A cow, born in the valley but showing no apparent goitre, had already had four goitrous calves. She was given five drops of tincture of iodine twice a week, the treatment being initiated one month before the next calf was due. The result was a perfectly healthy and normal calf. The time when iodine administration was found to be most necessary was in winter and early spring. Mares in foal were given five drops of tincture once a week, beginning two months before foaling time. Pigs given skimmed milk

from cows receiving iodine obtained sufficient in that way. Administration of iodine to fowls in mash or in milk a few times in spring (with skimmed milk occasionally) was sufficient.

" It costs Ronayne for iodine to keep 100 cattle, 12 horses, 30 pigs and 200 chicken free from goitre, and their progeny free also, $2.00 worth of tincture annually. Any resident of the valley exhibiting a goitre takes a little iodine till it disappears. If the cows are getting iodine and the children and grown-ups are drinking milk from these cows it is not necessary for them to take iodine directly."

Ronayne " has never observed an enlarged thyroid in any wild animal killed in the district—deer, bear, squirrels, musk-rat, rabbit, and mink. He found that goitre in all animals tended to recede without treatment in summer and autumn. In fact, any animal born in the fall was able to live, before the days of iodine administration."

" In order to test out the idea that iodine in excess caused a lack of fecundity in animals, he gave a sow one drop of iodine tincture daily during June, July and August. During this time she never came into heat. The iodine was discontinued in September and she came into heat in October. . . . He gave ten drops of iodine for seven days to a sow he did not wish to breed from and she did not come round for three months."

Keith, thinking that probably the river water might contain the " contagium," fed rats and guinea-pigs upon bread soaked in this water for two months (they were given no other food, although they ate some of the hay of their bedding). Their thyroids did not perceptibly enlarge ; one rat littered and the young were normal.

Histologically the thyroids from goitrous calves a week old, and a seven months' old goitrous hen, showed marked hyperplasia, the picture, according to Keith, resembling typical exophthalmic goitre. Sections from the thyroids of imported mice who had developed goitre suggested a

colloid goitre with a secondary hyperplasia, active or receding.

Evvard (68) has given a comprehensive and excellent account of the effect of iodine deficiency on, and the beneficial action of iodine treatment of, farm animals, well illustrated with photographs of hairless pigs, and goitrous kids, foals, calves and lambs. He considers that, while the administration of iodine to breeding sheep prevents goitre in their newborn lambs, excessive doses have apparently a deleterious influence, especially in lessening resistance against haemorrhagic septicaemia. Good results have been reported following administration of iodine to goitrous animals in Finland (263).

Other Suggested Causes of Endemic Goitre. Numerous theories of the etiology of goitre have been put forward. Many of these do not deserve very serious consideration. In addition to the theory of iodine deficiency perhaps the most important are those concerned with a hypothetical water-borne infection, with incorrect diet, and with vitamin deficiency.

As a result of personal observations in the Himalayas, McCarrison (162) claims that the soil of non-goitrous regions may be rich or poor in iodine, while certain regions with an iodine-rich soil are goitrous. Drinking water relatively rich in iodine does not prevent the occurrence of endemic goitre in the presence of a high degree of bacterial impurity, while the substitution of a bacteriologically pure for an impure water has caused the rapid and complete disappearance of the disease from a place where it has been endemic for seventy years, although the new water supply contains less iodine than the old. He claims to have induced goitre in man by the administration of sediment from contaminated drinking water, and to have cured it by intestinal antiseptics. He admits, however, that iodine-containing salts appear to exert a beneficial prophylaxis, and that the disease is in general more prone to arise in iodine-poor than in iodine-rich localities.

Various other investigators have put forward observations supporting the theory of a water-borne infection (85, 46). Others have suggested factors which might prevent the absorption of iodine from the gastro-intestinal canal, such as its presence in an unassimilable form (128), or chronic digestive infections (193).

(It should always be remembered, in contrasting the incidence of goitre with the iodine content of drinking water, that, almost always, in the absence of definite addition of iodine, the greater part of the iodine we ingest is taken with the solid food constituents.)

Ineffectiveness of iodine in treatment of goitre has been reported by a few investigators (224, 132), who form but a small minority of those who have published results concerning the treatment.

Stott (234) has studied the distribution and cause of endemic goitre in the United Provinces (India). Graves' disease is absent, but subthyroidism and cretinism are common, and the association between deaf-mutism, cretinism and goitre is confirmed. In India as a whole congenital deaf-mutes, cretins and goitrous persons are located in a main endemic area in the Himalayas, and in districts bordering the Himalayan foothills, especially where the drainage water is carried from the Himalayas to the sea. Where the local distribution of this disease group has been investigated it is associated with a definite water supply, and in that water supply lime is usually present in excessive amounts. "Nowhere in India or Burma is the deaf-mute rate higher than among the Kachins of N. Burma. . . . These Kachins drink water from hill-streams which are no doubt impregnated with calcium, and moreover it is customary amongst the Kachins to eat calcium as a powder in large quantities." (With these observations may be associated Hellwig's experimental results quoted on p. 49.)

Several investigators have claimed to be able to isolate

various specific organisms from goitrous thyroid glands (221, 35, 56); it has been further claimed that dogs inoculated with such isolated organisms will develop goitre (56).

McCarrison (158), in a goitre survey of 2,651 albino rats at Coonoor, India (a non-goitrous district), found 84 slight goitres, 41 small goitres, and 23 large goitres. There were no insanitary conditions and no hereditary predisposition. None of 393 well-fed rats showed a goitre, but 6 per cent. of 2,168 rats on an ill-balanced diet exhibited the condition. Of the 148 goitrous thyroids 79 were studied histologically: 35 were lymph-adenoid (Williamson and Pearse type), 20 were hypertrophic, some of these merging into the lymph-adenoid condition, 16 showed only negligible changes, and 8 were colloid in type. Administration of iodine to rats on a deficient diet favoured the production of goitre, but had no effect on rats on a satisfactory diet. The urinary content of iodine bore no relation to the occurrence of goitre. McCarrison considers that such goitre is definitely due to a fat-soluble vitamin deficiency; probably vitamin A is concerned, and the effect of its lack is accentuated by diets low in animal protein; an infectious agency may be an additional factor.

Surveys of goitre in Winnipeg school children suggest strongly that there is a racial factor; the highest incidence is amongst children of Central European and Jewish parentage. It is suggested that an unbalanced diet may constitute the actual factor. Cabbage is frequently a predominant constituent of their diet (cf. p. 49). The diets of many Jewish children are poorly balanced and too rich in fat (1).

The Experimental Production of Goitre in Animals. The inverse relationship which exists between distribution of iodine in soils and foods and incidence of goitre, and the almost universal agreement that the prophylactic use of iodine in some one of its combinations lessens this incidence, suggest that deficiency of iodine may be in itself a cause of

goitre. On the whole, this view is substantiated by the results obtained when animals are fed on diets deficient in iodine.

The earlier work of McClendon and his collaborators (165) definitely suggested that rats fed on diets containing only 9 or 10 parts of iodine per 1,000 millions developed goitre (cf. also 241). Similar experiments by Hellwig gave negative results, but were less carefully controlled (105). Such criticism does not seem to apply to the careful work of Jackson and P'An, who also found that dietary lack of iodine caused no appreciable enlargement of the thyroid and no significant difference in histological structure (122). Krauss and Monroe obtained definitely positive results in similar experiments (136), and Levine (147), using a diet containing only 15 parts of iodine per 1,000 millions, obtained goitre in rats in thirty-five days. (In these experiments the calcium content of the ration was relatively high.) The (dark red) thyroids were four times the size of the (pale pink) glands in control animals on a sufficient ration of iodine. Histologically the goitrous glands showed marked hyperplasia, with lack of colloid. Chemically they showed a low iodine content. Remington (205), in the same laboratory, found that the size of the gland was inversely proportional to the iodine intake. A daily intake of between 1 and 2 micrograms was sufficient to prevent goitre in his rats.

Hellwig (105) has revived an old theory, which for a while was practically discarded, that excess of calcium salts in a diet may cause endemic goitre. In support of this view he quotes the statement of Kottmann (1920) that calcium decreases the dispersity of the blood serum and increases the viscosity of thyroid colloid, of Abelin (1928) that an excess of calcium lowers the metabolic action of thyroxine, and of Wilms (1910) and Répin (1911) that water from a goitrous district will induce goitre in rats, but that if its calcium salts are precipitated by boiling this goitrous effect is lost; he refers also to McCarrison's association of goitre

in Chitral and Gilghit in Northern India with waters from a limestone source (cf. p. 45).

Hellwig fed rats on barley and 2 per cent. calcium chloride solution (the latter being their only source of water) for ninety days. He states that they developed enlarged hyperaemic glands showing marked epithelial hyperplasia. On a " well-balanced " diet with plenty of green vegetables, but with the same calcium chloride solution as source of drinking water, the thyroids enlarged, but showed no hyperaemia, and histologically were small colloid goitres. He agrees that excess of iodine in the drinking water can exert an inhibitory influence on production of thyroid hyperplasia, in spite of a high content of calcium in the diet. His experiments are suggestive rather than convincing. His chemical control is open to criticism, and his administration of calcium salts is altogether too far removed from natural conditions. Hibbert has obtained somewhat similar results, but claims that they are also produced by excess of sodium chloride and are not produced by excess of calcium lactate, whence he believes that they are due to excess of the chloride ion in the diet (107A).

Krauss and Monroe (136) observed during their experiments on the result of an iodine deficiency that rats fed on a high calcium-low phosphorus diet invariably had larger thyroids than those fed on a normal mixed diet or on milk exclusively (cf. also Thompson (243)).

McCarrison (159) was able to produce " lymph-adenoid " goitres, three to four times normal size, in three of eighteen rats fed on a diet deficient in vitamin A. In later experiments cystic formation was found in six of fifteen glands. The results were not attributable to iodine deficiency; addition of iodine to the diet appeared to increase the incidence. Addition of manganese chloride to the diet prevented the development of a goitre.

The Goitrigenic Action of Cabbage. One of the most interesting recent developments in the experimental pro-

duction of goitre has been its definite production in rabbits by the feeding of cabbage. This work has been largely carried out between 1928 and the present time by Webster and Chesney in Baltimore and Marine in New York, and their respective co-workers, and the results have been recently reviewed by Webster (261).

The work originally started in the incidental observation that the average weight of the thyroids of rabbits that were being kept for studies in experimental syphilis was much greater than normal. In most instances the necks of these animals bulged, producing a dew-lapped appearance. The glands were soft and highly vascular, and pathologically were typical diffuse parenchymatous struma. Microscopically they showed a simple diffuse hyperplasia. The rabbits were of various common breeds, and their diet consisted of a daily ration of 250 grams of cabbage, and a weekly ration of 20 grams of hay and 50 grams of oats.

Controlled experiments showed that the cabbage in the diet was the goitrigenic factor. Possible insanitary conditions and the local water were ruled out. Addition of a small amount of iodine to the diet completely prevented the development of goitre.

A seasonal variation in the ease with which goitre was produced led to the observation that early in November some abrupt change took place in the growing cabbage, presumably involved with the maturation of the plants, which rendered them goitre-producing. Cabbage from different sources and from the same source at different times showed different goitrigenic powers. The iodine content of the cabbage was not a factor. (Brussel sprouts and cauliflower are also goitrigenic, while various other members of the Brassica group of vegetables are not.)

Cabbage dried *in vacuo* or in air, or extracted with ether or acetone loses its goitrigenic power. Cabbage steamed for thirty minutes has the property increased. If such steamed cabbage is pressed the residual cake contains all the

goitrigenic substance. This is not readily soluble in water, even at 100°. Mild acid hydrolysis does not destroy it, but alkaline hydrolysis destroys it to a slight extent. Irradiation of finely divided cabbage increases the goitrigenic power.

(McCarrison (161), working in India, confirmed the findings for both rabbits and rats as regards production of goitre and non-relationship to iodine content of the cabbage, and found independently that a seasonal variation exists at a corresponding period. The goitrous glands possess an iodine content inversely proportional to their size.)

The active material was believed to be glucosidic in character, but attempts to extract the glucoside were unsuccessful. It is known that cyanides are components of the glucosides in the Brassica group of vegetables. Hence Marine and his associates (174) injected various organic cyanides into young rabbits. Goitres were produced and even exophthalmos.

Marine suggests that the hyperplasia is a result of a compensatory attempt to overcome the depression of oxygen consumption caused by the cyanides, and that this mechanism may be an essential factor in the causation of simple goitre. He thinks that in most cases of naturally occurring goitre the cyanide must be of endogenous origin. One of the principles of the anterior pituitary is probably an intermediate agent (cf. Chapter IX.).

Early in the experiments it was found that the respiratory metabolism of the goitrous rabbits was 18 to 20 per cent. below that of normal controls. Addition of 7·5 mg. daily of potassium iodide (a rather large dose) to the diet of a group with large goitres caused an increase in heat production to two or three times the normal level, fall of body-weight, and death within forty-eight to seventy-two hours. Smaller doses of iodide to animals with smaller goitres gave more controllable and non-fatal effects of the same nature. Such effects are obviously due to increased output of the thyroid principle; the degree of increased output was

proportional to the amount of iodine fed. These experiments with such hyperplastic glands are believed to throw light upon the causation of so-called "iodine-Basedow" (see below, p. 73).

The Causes of Endemic Goitre. At present it is not possible to state the etiology of endemic goitre accurately and precisely, but it seems justifiable to conclude that it can arise from more than one cause. The possible causes include lack of iodine, too great a calcium content in the diet, an unbalanced diet (especially when associated with a definite deficiency of vitamin A), some goitrigenic factor (probably a glucoside liberating cyanide) in cabbage, and a water-borne infective agency. Some of these potential causes may be effective alone, others may only be contributory causes.

Joll stresses the desirability of considering sporadic goitre when discussing the etiology of goitre (126). He does not believe that it can be considered as the result of a temporary insufficiency of iodine caused by infection or improper diet. Nor does he think that sporadic goitre in reality merely represents a low endemicity. However, in McCarrison's dietary experiments only a small proportion of his rats developed goitre. Some rabbits exhibit much more resistance to the goitrigenic effect of cabbage than others. Remembering such individual variation in resistance, it seems probable that sporadic goitre may also be entirely accounted for by some one or other of the causes just listed.

Of more importance than the actual cause of goitre is the undoubted fact that, with the perhaps doubtful exception of "lymph-adenoid" goitre produced by lack of vitamin A, a sufficiency of iodine in the diet prevents the production of goitre. *Iodine acts as a shield against endemic goitre, and iodine-prophylaxis is the most important preventive measure.*

Methods of Administration of Iodine. The best method of prophylactic administration of iodine depends upon whether the incidence of goitre is so great and widespread

as to make the problem of its treatment a community one or not. In certain Cantons of Switzerland, in large areas of New Zealand, in a large part of the Central and North-Western United States, and in Canada from east of the Great Lakes to the Pacific coast, goitre menaces the community through the fact that the iodine intake of the average individual, unless fortified artificially, falls below the minimum protective level. It is immaterial whether the view be taken that the lack of iodine is causative of goitre or merely that presence of iodine protects against goitre. Wherever endemic goitre occurs or can occur the whole population can only be protected if the whole population be treated. The best means varies with the size and distribution of that population.

It has been suggested by different writers that iodine should be administered either as potassium or sodium iodide (weighed amounts in solution or in tablet form, chocolate-coated or otherwise rendered palatable to the young), or as an iodized fat (iodostarin) similarly presented, or as iodide added to the drinking water of the community, or as iodide added to table salt for bulk treatment of still larger communities or even whole nations, or by adding to the soil iodine-containing fertilizers in order to increase the iodine content of vegetables, or by feeding iodide to cattle to increase the iodine content of milk, or by encouraging the whole population to eat more marine foods, since these are rich sources of iodine.

All such methods are reasonably sound as far as the individual is concerned. They are not equally good when considering the welfare of large communities and the cost of administration. In treating large units of population it is essential, to prevent endemic goitre and associated cretinism, that the pregnant woman receive an adequate amount of iodine during her pregnancy and that the growing child be adequately supplied. The iodine supply of adult man cannot be merely taken as a matter of course.

Feeding iodine to cattle, suitable manuring of soil, and increased consumption of marine products are all uncontrollable methods, and unduly increase the cost of iodization of a community. The first two are wasteful procedures. Iodization of water supply means duplication for each community and does not reach a rural population. Tablet methods, used largely in schools, concern only the school population and have disadvantages even for it. " The body requires an adequate daily supply of iodine throughout life for normal thyroid activity. To recognize the deficiency and to supply it during the school period only, subject to the caprice of the parent, is unsound " (106). " The prophylaxis of a disease ought . . . to be removed as far as possible from any initiative on the part of the individual, for how difficult it is to carry out a hygienic measure, against the want of intelligence of mankind can be understood from the difficulties against which, for example, vaccination has at the present day to combat in many places " (125).

From such considerations the great majority of investigators seem to be agreed that the iodization of all salt used for table and culinary purposes is the ideal procedure for treating the *larger* community units. It is therefore pertinent to inquire what is the best dosage of iodine.

Correct Dosage of Iodine. At the present time iodized salt in different countries varies widely in its iodine content, as the following figures indicate :

Michigan, U.S.A..	1 part of KI or NaI	to	5,000 parts of table salt.
Canada . .	1 part of KI or NaI	to	10,000 parts of table salt.
Valtellina (Italy) .	1 part of KI	to	100,000 parts of table salt.
Switzerland. .	1 part of KI	to	200,000 parts of table salt.
New Zealand .	1 part of KI or NaI	to	250,000 parts of table salt.

Even assuming that the iodide is merely protective and that the goitrigenic causes vary considerably in intensity in different places, nevertheless such wide divergences seem unnecessary. Either the small amounts in Swiss and New Zealand salts are insufficient, or the much greater amounts

used in Canada and certain parts of the United States are unnecessarily large.

Data are available from several sources which enable us to ascertain the normal daily intake of iodine in healthy persons. Food analyses showed that the average difference in non-goitrous and goitrous districts in Switzerland was 18γ (73). This, spread over 10 grams of salt (an average daily intake), gives a ratio of 1 : 550,000. A similar comparison in New Zealand (107) gave a difference of 15γ and a ratio of 1 : 700,000. The content of iodine in urine, in a community on an established diet, gives an approximate clue to the dietary intake. Comparisons between non-goitrous and goitrous districts in Switzerland give a difference of 46γ (a ratio of nearly 1 : 200,000), and in Norway of 134γ (a ratio of 1 : 80,000) (cf. Table II.).

v. Fellenberg conducted an experiment of some duration on himself, and found that he could maintain iodine equilibrium on an intake of 14γ per day, while 50γ to 80γ per day led to a slight retention, easily lost on lowering the intake. The figure 50γ corresponds to a ratio of 1 : 200,000 (73).

The American ratio of 1 : 5,000 was based on a provision of 400 mg. per year, this being the amount a child would get by taking 10 mg. in tablet form once a week for forty weeks, with an estimated intake of 7·5 grams of table salt per day. Our knowledge of iodine excretion suggests that such a method of administration is very wasteful, the greater part of the weekly dose being rapidly excreted (cf. p. 40) ; small daily amounts are more likely to be utilized economically, and the necessary supply for the thyroid maintained satisfactorily with lessened intake.

The excellent reports of results of Swiss prophylactic measures, and the experimental evidence that has just been quoted, suggest that the proportions used in Switzerland and New Zealand are of the right order.

Potential Danger from Ingestion of Iodine. The problem

of widespread iodization of salt, and, still further, the question of the desirability of compulsory iodization, demand consideration of the possible existence of danger to any section of the community by such treatment.

Experimental results following dosage even greater than that involved in a 1 : 5000 ratio suggest beneficial effects to normal animals rather than the reverse. Rats and pigs grow faster (97, 69). When a little iodide is fed continuously to sows during pregnancy the litters are improved and grow more rapidly (262). Cattle and goats give an increased yield of milk (225). The increase in rate of growth is attributed to a slight depressant effect of small dosage on metabolic activity (108, 150).

It has been generally recognized, from the time when Coindet first used iodine in the treatment of goitre, until Kocher again stressed the point in 1910 (135), that *over-dosage* of iodine may induce a marked hyperthyroid condition in a goitre previously non-toxic. It by no means follows, although the assumption is frequently made, that the use of a properly iodized table salt will lead to such hyper-thyroidism. Much has been written on this subject, and much of what has been written is polemical. Probably the pertinent facts and justifiable conclusions are summed up by the two following quotations from Marañon (170) and von Jauregg (125).

"We have observed quite frequently the appearance of types of secondary hyperthyroidism in endemic goitre. Sometimes they appear spontaneously, especially during the critical period, *but almost always they are due to exaggerated cures with thyroidin, or iodine, or its derivatives*, which have been dispensed as a treatment of goitre itself, or for other reasons, principally against obesity. . . . *We have noticed symptoms* (of hyperthyroidism following administration of iodine) *only in the cases that had been treated with enormous quantities of iodine, and never in the cases where judicious doses were dispensed, or some preparation of iodized kitchen salt.*"

" It is a fact that individual authenticated iodine injuries have occurred through the use of complete salt (*i.e.*, iodized salt) only. . . . The Swiss official inquiries (show) . . . that they appear spontaneously only very slightly more frequently than such so-called thyrotoxicoses occur with a population that does not consume complete salt."

If it be admitted that the ingestion of iodized salt is a potential danger to persons with non-toxic goitres, even though that danger be negligibly slight, it follows that iodization should be reduced to that low optimum limit (1 : 200,000) which experience has shown is quite efficient in prophylaxis (30).

The Treatment of Endemic Goitre. As the results that have been quoted indicate, iodine administration leads in a large proportion of cases to the disappearance of an established goitre, but this beneficial result is not so consistently attained as prevention. Plummer and others have treated non-toxic goitre in adolescents and children with small doses of desiccated thyroid or of thyroxine, and have obtained good results indicating that these are more effective than iodine (199, 133, 71). There would seem to be some slight possibility of thyroid imbalance ultimately resulting from such treatment (32). Joll (126) reports his own disappointing results with desiccated thyroid and with thyroxine : " A few of the very small, soft goitres have diminished or disappeared, but in no instance has a large colloid, or nodular goitre shown any diminution which could be measured by calipers or detected by palpation." He found that large doses of intestinal disinfectants were of no value (cf. p. 45).

The Hypothyroid State

Little advance has been made in recent years in the study of hypothyroidism, beyond perhaps the differentiation of a hypothyroid state in adults which is distinct from myxoedema, and an interesting theory of the causation of

myxoedema itself. It can be regarded as well established that the syndrome of cretinism results from thyroid deficiency in the child and young animal, while that of myxoedema arises from such deficiency in the adult, whether that be caused by decrease in thyroid function through some pathology of the gland, or through too great a removal of thyroid tissue by thyroidectomy. It can also be regarded as established that administration of thyroid restores myxoedematous individuals to comparatively normal physical and mental health, so long as that administration is maintained, and improves and may completely restore the normal physical condition of cretins, although its effect on their mentality depends upon commencement of treatment at a very early age.

Myxoedema. Murray, who was the first to administer thyroid to myxoedematous patients (in 1891) made a final report on the first case in 1920 (185). She enjoyed excellent health until early in 1919, when she developed oedema of the legs and died in May of that year at the age of seventy-four from cardiac failure. A final report on a long-treated and spectacular case was made by H. M. Raven in 1924 (202); his father had published earlier reports on this case in 1894 and 1897. Mrs. S. developed myxoedema in 1870, at the age of forty-one; no treatment was instituted for over twenty years. At the end of this period she was bedridden, bald, and demented. Treatment with thyroid extract was commenced in 1893; within fifteen months she was practically normal, even to well-marked growth of hair. She continued a normal existence until 1924, living "to a ripe old age—happy, healthy, and mentally active," and finally dying of bronchitis. The photographs of this patient are particularly interesting, and are reproduced in Fig. 2.

Williamson and Pearse (266) believe that myxoedema is the end-result of lymph-adenoid goitre, a condition which can occur without marked thyroid enlargement, and which is not related to deficiency of iodine in the diet. If the con-

A.

B.

C.

D.

FIG. 2.—Thirty years' successful treatment of a case of myxoedema with thyroid gland. *A.* Condition before treatment; aged 65. *B.* After five weeks' treatment. *C.* After fifteen months' treatment. *D.* At age 94, after about thirty years' treatment. (From Raven, *Brit. Med. J.*, October 4th, 1924.)

clusions of McCarrison (cp. p. 49) and of Williamson and Pearse can be accepted, myxoedema is to be regarded as the final outcome of a process initiated by a faulty diet,

especially deficient in vitamin A. Further evidence is desirable.

When thyroid is administered to myxoedematous patients in non-toxic amounts it has no specific effect in reducing body-weight, except to the extent that it dissipates myxoedematous deposits and causes elimination of abnormal accumulation of fluid. By its effect on nutrition it may actually cause a gain in weight as basal metabolism becomes normal. Progressive and continued loss of weight following its administration indicates too great a dosage. It has no specific directional influence upon vascular tension, but through its influence upon nutrition it tends to bring either high or low blood pressure back to normal. In therapeutic doses thyroid has two effects on the heart—it increases its work promptly and rapidly, and improves its nutrition slowly. Therefore signs of cardiac insufficiency do not contraindicate its administration, but do emphasize the need for care in its use and adequate curtailment of the patient's activities (143). Anginal pain sometimes occurs following thyroid administration (71). The protein content of the cerebrospinal fluid is stated to be high in most cases of myxoedema, so that in rare instances this condition may be confused with brain tumour. Following administration of thyroid the concentration of protein usually drops to within normal limits (244).

Non-myxoedematous Hypothyroidism in Adults. Attention has been drawn to this syndrome by numerous recent writers (259, 167, 168, 233, 28, 141). The main features which seem to be agreed upon are a tired worn-out feeling, undue fatiguability, loss of strength, nervousness, and vague pains. Skin, hair and nail changes may be present; the patient may be sensitive to cold. Constipation and susceptibility to the slightest infection are frequently noted, and, in women, spare or profuse menstruation. A low basal metabolic rate is a constant factor. When it is not below —20 per cent. the gastric acidity is normal. With lower rates it is sub-

normal or achlorhydria is present. Rates as low as −30 per cent. have been reported, although Lahey has only seen one patient with a rate below −25 per cent. who did not exhibit frank myxoedema. He claims that in this group only those patients whose blood cholesterol exceeds 0·2 per cent. are benefited by thyroid treatment.[1]

To what extent such a group of cases should be considered as a separate syndrome, or as exhibiting conditions due to a thyroid failing in function so that ultimately frank myxoedema will result, or as merely exhibiting gastrointestinal disturbances leading to an undernutrition that can cause a lowered basal rate, remains to be determined. We have had, in the hospital to which I am attached, within the past few years a number of pupil nurses who exhibited a definitely low basal rate and various minor symptoms, resulting from voluntary undernutrition due to the current fashion of "reducing." Compulsory correct dieting has largely caused the disappearance of this type of case (52).

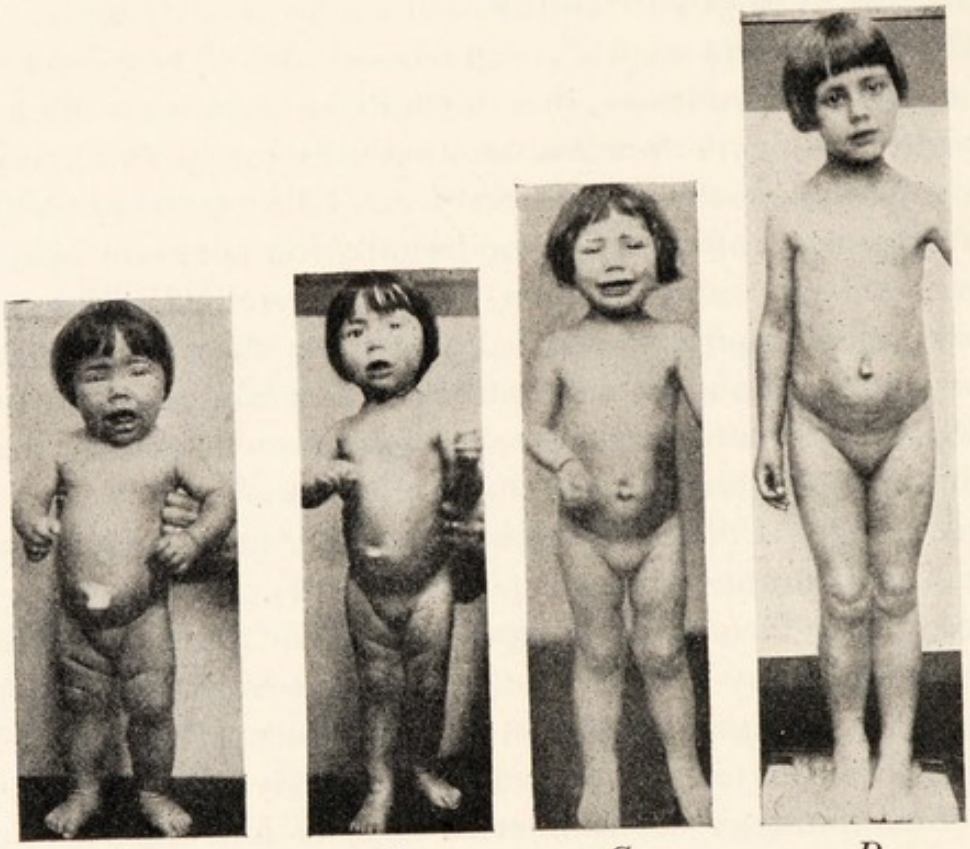
Atypical forms of myxoedema have been described, accompanied by rheumatoid pains, or severe menorrhagia or metrorrhagia, anaemia, or obesity (17). Thyroid insufficiency is sometimes most strikingly shown through malfunctioning of the brain cells. Depression, apprehension, slowness of thought, and slowness of bodily movement produce a condition which may be easily mistaken for a depressed psychosis. Irritability and excitement may be sufficient to suggest a disordered mentality; thought distortion, with hallucinations and delusions, may suggest dementia praecox (103).[2]

[1] Closely related are a group of cases in which somewhat similar symptoms are associated with nodular goitres (which produce pressure symptoms, in addition); the basal metabolic rate in these cases is normal or subnormal. The symptoms are not due to deficiency of thyroid secretion, since removal of the goitre causes their disappearance, while in cases in which the basal metabolic rate is subnormal it increases to normal following the operation (47, 71).

[2] It is perhaps not without significance in this connection that beneficial results have been reported following the treatment of cases diagnosed as dementia praecox with thyroid (114).

Cretinism. Thyroidectomy in very young animals is said to produce a condition strongly simulating rickets; this condition is not benefited by feeding cod liver oil (140).

A good example of both physical and mental benefit following administration of thyroid to a cretin from an early



A. *B.* *C.* *D.*

FIG. 3.—A case of cretinism successfully treated with thyroid. *A*. Eighteen months old. Large umbilical hernia. Just before treatment commenced. *B*. Twenty-three months old. The hernia has disappeared. *C*. Thirty-eight months old. *D*. Sixty-six months old. Weight, 46 lb. Height, 46 inches. Practically normal, both physically and mentally. (From Close, *Guy's Hospital Reports*, 1932, lxxxii, 155.)

age has recently been reported by Close (40). The excellent results are well shown by the photographs in Fig. 3.

Thyroid therapy in underdeveloped children is stated to produce a definite increase in height and in dental and bone development, even though the basal metabolic rate is not increased (247A).

Nephritic Conditions and Thyroid Hypofunction. That type of nephrosis in which marked oedema, albuminuria, and hypercholesterolaemia are accompanied by a lowered basal rate, an entity which Epstein termed " chronic nephrosis " and Munk " lipoid nephrosis," and in which at least a proportion of cases coming to autopsy exhibit degeneration of the kidney tubules, has sometimes been regarded as of thyroid origin, since it is markedly benefited by thyroid treatment (67). The condition is not one of myxoedema. Recent work suggests an etiology unrelated to the thyroid, and still another condition which can cause a lowered basal metabolism. Low basal metabolic rates are exhibited by patients in the second or chronic stage of glomerular nephritis (azotaemic nephritis) and in true nephrosis. The oedema in both conditions has been shown to be due to chronic hypoproteinaemia resulting from the albuminuria. Such chronic hypoproteinaemia experimentally produced in dogs results in marked oedema, and this is accompanied by a lowered basal rate (8).

The changed etiology does not necessarily exclude thyroid treatment, which has produced excellent palliative results for several years in such cases (cf. 57) ; Boothby, however, considers that it is not indicated unless there is an associated myxoedema (23).

The Hyperthyroid State

The question cannot yet be decisively answered as to whether, as Marine believes, there is only one hyperthyroid disease with numerous variations, or whether there are two distinct entities, as Plummer thinks. Nor can the etiology (or etiologies) be definitely stated.

The most important recent advance in the treatment of the hyperthyroid condition is the pre-operative use of iodine, generally as Lugol's solution. The mechanism of its beneficial action is still unsettled.

The Classification and Etiology of Hyperthyroid States. Marine has emphasized the importance of recurring physiological cycles as explanatory of the various forms of goitre. Thus :

Normal thyroid → hypertrophy → hyperplasia → exhaustion atrophy *or* involution to colloid state → hypertrophy → hyperplasia → atrophy *or* involution to colloid state, etc.

He considers that if, during such cycles, a sufficient degree of hyperplasia occurs, the picture of Graves' disease may ultimately be seen. He has summed up his general viewpoint of Graves' disease as follows (172) : " Exophthalmic goitre or Graves' disease . . . appears to be a disease of the nervous system in which the visceral nervous system is most prominently involved. It is characterized by a profound disturbance of the regulatory control and functional interactions of all organ activities, and the most prominent manifestations of the disease are increased metabolism of thyroid origin, general asthenia, tachycardia, and moderate enlargement of the thyroid. The thyroid plays an important *rôle* in the clinical manifestations of the disease, but we must look beyond the thyroid for the essential and primary lesions. The thymus, liver, pancreas and suprarenals are also involved in this disease in an important but still unknown way." Elsewhere he has written (171) : " It would be much simpler and more accurate in the present state of our knowledge to divide Graves' disease primarily into *acute* and *chronic* forms, and to divide each of these headings into *complete* and *incomplete* forms. This would eliminate such terms as toxic adenoma, adenomatous goitre with hyperthyroidism, thyrotoxicosis, etc." He considers the histological changes are constant, but not specific. Adverse to Plummer's views (see p. 66) is his comment : " Much has been made of the occurrence of islands of hyperplastic tissue in cases of Graves' disease with long standing goitre. This is due to the fact that, the thyroid being a

cystic organ, different groups of follicles stand in different relations to the blood supply, and those with the better blood supply regenerate earlier." The hyperplasia of the lymphoblastic tissue is prominent, and includes lymphoid hyperplasia in the thyroid itself, but is not of special importance in Graves' disease because it is not specific; it is also present in Addison's disease and in "status lymphaticus," and is probably an antagonistic reaction against an excessive thyroid secretion and a compensatory reaction against some adrenal-gonadal insufficiency. He is in disagreement with the views of Warthin and others who hold that the so-called Graves' constitution is always congenital; he thinks it can also be acquired. Cases occurring early in life may be associated with an inherited or congenital constitutional condition but cases occurring around the decline of sexual life in women are to be regarded as acquired.

Marine believes that Addison's disease, Graves' disease, and status lymphaticus are closely related states, all three intimately associated with insufficiency of some secretion of the adrenal cortex and gonads. He adduces in support of this view the production of a transient symptom complex in rabbits closely resembling Graves' disease, following sublethal injury to the adrenals, the involution of the adrenal cortex in infants associated with increased heat production, and the beneficial results which follow administration of adrenal cortical material to patients with Graves' disease (see below). He concludes: "I would like to point out that while the view that Graves' disease is essentially a thyroid disease is still the prevailing one, and while therapy should still be based on this assumption, I am convinced that a much more fundamental disturbance lies in a deficiency of some function of the suprarenal cortex and sex glands, which either provides another means of promoting tissue oxidations or has to do with the regulatory control of these oxidations. The most outstanding manifestation of Graves' disease is clearly a loss of control over these oxidative

processes, and as a result of this there occurs a physiologic attempt towards compensation by an increased production of the thyroid hormone."

Plummer first presented his views in 1913 before the Association of American Physicians (196). Basing his conclusions on over 4,000 operated cases and their histories, he said, at that time : " Correlating the statistical data, we may safely come to the conclusion that exophthalmic goitre is a definite clinical entity always associated with hyperplasia of the thyroid and that it should be sharply distinguished from the constitutional state or states that may develop from non-hyperplastic goitre. . . . The average length of time between the appearance of non-hyperplastic goitre and toxic symptoms is 14·3 years. That the patient comes under observation three years later indicates that the onset is usually insidious. Nervousness, tremor, loss of strength and weight, as a rule develop slowly, but may appear suddenly before definite evidence of myocardial damage. The administration of iodine may cause the sudden appearance of symptoms from myocardial insufficiency. . . . In some cases the clinical aspect . . . closely approaches that of exophthalmic goitre. However, the symptoms are less complex, less definitely associated, and except for a damaged heart, less intense. . . . The onset of exophthalmic goitre is, as a rule, relatively acute and the cause of the disease fairly definite. The clinical picture early in the history is that of a toxin action directly on the more vital organs, more notably the central nervous and vascular systems. Later it is more complex by the interaction of those organs whose functions have been directly disturbed by the toxin." He goes on to point out that the course of the disease generally shows fluctuations, remissions and exacerbations over several years.

In a later paper (1923) in which he introduces the use of Lugol's solution (197) he sets out his conception of an iodine-deficiency leading to the production of a toxic thyroid product : " There are two entities included in the term

hyperthyroidism : first, exophthalmic goitre ; second, hyperfunctioning adenomatous goitre. In the latter the basal metabolism is more nearly normal. In exophthalmic goitre there are . . . (1) nervous phenomena, (2) eye symptoms. . . . Anything that will overstimulate the thyroid can give the clinical picture of exophthalmic goitre. In such cases the normal hormone, thyroxine, is not completely iodized. This incompletely built up thyroxine, as it leaves the gland, can enter into catabolic reaction faster than the normal stable molecule, and raise the metabolic rate more rapidly. If, therefore, we can change the character of the molecule, we can change the basal metabolism. If there is intense metabolic stress for the lack of iodine, death occurs from lack of iodine. From this it follows that if we can change the production of abnormal substance to properly iodized substance we can cut down post-operative mortality, avoid crises, and change the picture of the nervous phenomena. Acting on this plan we administered 10 drops of compound solution of iodine for ten days following operation, with the result that we have found there is no such thing as post-operative deaths from hyperthyroidism if this dosage has been administered to the patient with regularity. In other words the patient is relatively short of iodine, and dies from lack of it. When we replace the iodine, we do away with post-operative death."

Still later (1928) (198) he further explains his theory. By " hyperplastic " he indicates diffuse hypertrophy, by " non-hyperplastic " a localized hypertrophy, or acinar hyperplasia —an adenoma. His theory is definitely associated with the idea that the thyroid gland can secrete more than one active agent. Mixed types of gland are possible. In approximately 20 per cent. of cases of exophthalmic goitre this is superimposed on old adenomatous goitres. He considers that the hyperthyroidism of exophthalmic goitre is caused by hyperfunction of the entire gland, but that probably the hyperthyroidism of adenomatous goitre is caused by localized reaction in the gland and is only an incident in the course of endemic goitre.

Patients operated on for adenomatous goitre scarcely ever have a recurrence of the condition; recurrences are much more frequent with exophthalmic goitre. Between 2,000 and 2,500 cases exhibited hyperthyroidism out of 9,362 with adenomatous goitre at the Mayo Clinic between 1912–1921. There were only three second resections. Of 4,992 cases of exophthalmic goitre 326 came to subsequent second operation. (Cf. also Boothby (21).)

Plummer's chemical views are unsound. They were based upon an incorrect theory of the chemical nature of thyroxine. Since Harington has shown that thyronine (thyroxine minus its iodine) is physiologically inactive, while tetrabromothyronine and diiodothyronine only exhibit negligible activity (but activity of the same type as that of thyroxine), we have every reason to be certain, even if the actual thyroid principle is a thyroxine-peptide and not thyroxine itself, that no abnormal and incorrect activity of the thyroid is traceable to a perverted thyroxine compound.

The incorrectness of Plummer's chemical views does not invalidate his main conception, which is based upon his far sounder clinical knowledge. Even Marine is forced to admit some type of differentiation of his single pathological entity. Plummer's theory of two hyperthyroid types is of great service in stressing the probability that hyperthyroidism can arise from more than one cause. The nomenclature probably requires revision.[1]

Joll's common-sense view seems to sum up the present situation (126): "It is convenient . . . to make a distinction

[1] Rienhoff (215) has written: "In the larger proportion of nodular goitre with hyperthyroidism the nodular element is certainly not due to adenomata in the true sense of a neoplasm. . . . If one examines the patient's thyroid and discovers a nodular enlargement one cannot tell clinically which group these nodules belong to; the greater chance is against the nodule or nodules being a neoplasm or an adenoma. The only logical and scientifically correct foundation for a clinical diagnosis is 'Nodular Goitre' with or without hyperthyroidism, as the signs and symptoms may suggest; or in case the enlargement be smooth and diffuse, the term 'Diffuse Goitre' with or without hyperthyroidism is equally correct. . . . The terms 'Toxic Adenoma' and 'Hyperfunctioning Adenomatous Goitre' are misleading and incorrect."

between exophthalmic goitre and other toxic goitres, because the former is generally an exceptionally well-defined disease, and is also, at any rate in my experience, far more common than are the other forms of thyrotoxicosis. Exophthalmic goitre is a disease which, whether due to causes intrinsic in the thyroid or of extra-thyroid origin, affects persons previously free from goitrous taint. It can therefore be designated *primary toxic goitre*, and since all other forms of thyrotoxicosis occur in persons bearing goitrous glands of different types, they may conveniently be classified as *secondary*."

Since many writers on hyperthyroid diseases do not accept the differentiation, some part of the literature is difficult of analysis. Some assistance in differentiation and in considering etiology may perhaps be obtained by comparing the results of thyroid administration to animals and man (pure hyperthyroidism) with the symptoms and signs in exophthalmic goitre (Graves' disease) and in toxic adenoma (adenomatous goitre with hyperthyroidism, secondary toxic goitre), as shown in Table III., based largely on Boothby (19), Joll (126), and Sharpey-Schafer (229).

TABLE III

Symptoms and Findings in Hyperthyroid Conditions

Experimental Hyperthyroidism.	Graves' Disease.	Toxic Adenoma.
—	Rapid onset of symptoms, which may even precede thyroid enlargement.	Slow insidious onset of symptoms following thyroid enlargement.
—	Not uncommon in young people.	Rare in the young.
Tachycardia.	Tachycardia.	Tachycardia.
—	Thrills and bruits.	No thrills or bruits.
Some nervous excitability.	Nervous phenomena prominent.	Nervous phenomena slight.
—	Diffusely enlarged thyroid.	Nodular enlarged thyroid.
Loss of weight.	Loss of weight.	Loss of weight.
Perspiration.	Perspiration.	Perspiration.
Tremor.	Tremor.	Tremor.
—	Dyspnoea.	Dyspnoea.
—	Fatigue.	Fatigue.
No exophthalmos.	Exophthalmos in most cases.	Exophthalmos rare.
—	Gastrointestinal crises.	No gastrointestinal crises.
—	No hypertension.	Tendency to hypertension.
Increased B.M.R.	Increased B.M.R. (which may exceed + 100 per cent.).	Increased B.M.R. (which rarely exceeds + 50 per cent.).

Graves' Disease was first described by Parry in 1786, then by Flajani (1802), Graves (1835) and Basedow (1840), all independently (229). It is termed most frequently, and least correctly, *exophthalmic goitre*, since it can occur without exophthalmos, and without perceptible enlargement of the thyroid. The contrast that has just been made suggests that certain symptoms are present which are not due to pure hyperthyroidism but indicate that the initial cause of the disease lies outside the thyroid gland itself. Numerous etiologies have been suggested. Of these Plummer's, that a perverted secretion is produced, is based upon incorrect chemical conceptions and must be rejected. Theories have been put forward that it is of bacterial origin, of nervous origin, of constitutional origin, and results from disturbances of the adrenals and ovaries. There is great probability that one of the principles of the anterior pituitary is also involved (cf. Chapter IX.).

Observations which support a bacterial origin, such as the reported isolation of specific organisms (112,142), or the production of hyperthyroid conditions following experimentally produced infections (269, 180), do not bear specifically upon Graves' disease, and indeed while infectious disease may have a definite effect upon the thyroid picture, the changes seem to be non-specific and may even suggest a hypofunction (144).

The idea that the disease may be of nervous origin is obviously suggested by the nervous phenomena associated with it. It has been supported by several recent writers (268, 274, 231, cf. also 118, 26). One of the difficulties of accepting a theory of nervous origin is the lack of evidence that the thyroid itself is under nervous control (cf. Gley, p. 32).

That a constitutional factor exists, as Warthin originally suggested (although perhaps merely as an inherited thyroid weakness as Cockayne thinks (42) rather than inheritance of the disease itself), is supported by the actual, though rare,

occurrence of the disease in very young children (cf., *e.g.* 84) and occasional histological appearances in foetal thyroids which suggest the disease (2), although the effect of infection is not ruled out in these cases.

Schereschewsky has recently made a careful clinical study of the disease in children (226), and believes that in them it develops most frequently following infections, especially of the naso-pharynx, and that the etiology in the child and in the adult tend to be different. In children the disease can evolve rapidly, can become established within a few days, and can disappear as rapidly. They seldom exhibit exophthalmos or tremor. Characteristic choreic movements may be present.

Bram (237) emphasizes the constitutional and neural aspects of the disease. He considers that in 85 per cent. of his adult cases the etiology was associated with psychic traumatism. (Thompson (244) considers that the peculiar nervous manifestations can be satisfactorily explained as exaggerations of the customary reactions of the patients, who are usually of the emotionally unstable type.) The theory of Marine that the etiology is associated with functional disturbances of the adrenals and gonads has already been referred to. Hill emphasizes the causal relationship of psychic trauma and sex epochs (109). Marine has shown that experimental cabbage goitre may even be associated with exophthalmos, and that, if iodine is present in sufficient amount, increased heat production may result (cf. p. 51).

Studies of the variable electrical excitability of the median nerve following operations for hyperthyroid conditions (96), and of the respective blood pictures in Graves' disease and in induced hyperthyroidism (114), both indicate that Graves' disease is not a pure hyperthyroidism.

It seems to be reasonable to conclude that Graves' disease has no single etiology but that it can arise from the influence of a number of different factors, which may but do not necessarily include a hereditary predisposition.

The disease can occur in absence of exophthalmos and of visible goitre, and even perhaps in absence of a measurably increased basal metabolic rate. Bram found (26), in a study of over 4,000 cases, exophthalmos absent in 12 per cent., thyroid enlargement absent in about 20 per cent., and both absent in 9 per cent. The basal metabolic rate prior to operation was stated to be low in about 0·5 per cent. The dissociation of exophthalmos from the hyperthyroidism of Graves' disease is exemplified by the appearance of the former *after* operation in a number of definitely authenticated cases (273). The interval of time between operation and this development varied from three to twelve months, and the condition, once developed, showed no subsequent improvement. Illustrative proof that this subsequent development cannot be ascribed to a recurrence of the Graves' disease is the finding that in several of these cases the basal metabolic rate was below normal, while one exhibited definite myxoedema.[1]

It has long been recognized that *achlorhydria* frequently accompanies hyperthyroid conditions. It has recently been shown that it is a true achlorhydria. Two-thirds of fifty

[1] According to Kunde (139) all the symptoms of Graves' disease can be induced in laboratory animals by artificial hyperthyroidism, but exophthalmos cannot be induced in the dog, although mild exophthalmos can result in the rabbit ; rabbits thyroidectomized at an early age develop marked proptosis. However, Whitnall (265) has recently published a critical review, from the anatomical point of view, of theories concerning the causation of exophthalmos, which suggests that, as far as this symptom is concerned, conclusions from experiments on animals are not applicable to man. Of the involuntary musculature of the orbit the Palpebral is responsible for the eye-lid signs, but only for these. The Orbitalis muscle cannot produce protrusion of the eyeball in man, since "it is a mere vestige tucked away deep in the infra-orbital fissure," but it can do so in the lower animals, where it "exists as a broad sheet of fibres filling the wide gap in the bony lateral orbital wall." In man "the Perivascular fibres suggest the possibility that dilatation of the vessels or paralysis of their walls favours increased exudate which may be the main factor in proptosis." Marine's views on the production of exophthalmos are quoted in Chapter IX. Naffziger (186) has studied histologically, and has described successful surgical treatment of cases of progressive exophthalmos following operation.

hyperthyroid patients remained achlorhydric after histamine; the incidence was the same in Graves' disease and in toxic adenoma. Of 42 cases examined six months after thyroidectomy 31 showed normal gastric acidity (12).

Toxic adenoma or **secondary toxic goitre** is a condition in which the symptoms are very similar to if not identical with those of the pure hyperthyroidism which follows undue thyroid administration. It does not follow that the condition is initiated from changes within the gland. At present our knowledge is still summed up by Boothby's statement in 1921 (19): "About middle age the adenomatous tissue after a considerable quiescent period begins to furnish an excessive amount of the apparently normal thyroid hormone . . . The underlying cause or stimulus that activates the thyroid to adenomatous growth and over-secretion is not known." Various etiologies are possible. A bacterial cause has been suggested (35) but seems unlikely. There is some evidence that in women adenomata increase with an increasing number of childbirths (227).

The "**Iodine - Basedow**" type of hyperthyroidism described by Kocher as resulting from the effect of treating goitres with iodine is possibly to be regarded as a toxic adenoma (20), but is more probably merely a transient hyperthyroidism; studies of the toxic action of iodine on hyperplastic thyroid glands produced by an excess diet of cabbage in rabbits (cf. p. 52) will probably throw further light on this condition. Children exhibiting the condition recover without operation following cessation of the iodine treatment (71).

The Hyperthyroid Heart. The consensus of recent opinion seems to be that hyperthyroidism, *per se*, has no toxic influence or direct pathological action on the heart, although, indirectly, it may accelerate the development and progress of pathological lesions arising from other causes. The heart in hyperthyroidism is suffering from its own accelerated metabolism and from the load thrown upon it by the

increased metabolism of the whole body. Relief of the hyperthyroidism relieves the heart (116, 148, 222, 7, 50).

The Administration of Iodine in the Treatment of Graves' Disease

Reference has already been made to the early employment of iodine in the treatment of goitrous patients, and its subsequent disuse following bad results from occasional overdosage (cf. pp. 41, 56). Trousseau, in 1863, accidentally employed tincture of iodine in a case of Graves' disease and got good results. Between 1920 and 1925 several papers were published recording definite clinical improvement and lowered basal metabolic rate in patients with Graves' disease, following small doses of potassium iodide administered several times daily (187, 150, 53). The introduction of Lugol's solution by Plummer led to the abandonment of treatment with potassium iodide.[1]

Plummer originally introduced use of Lugol's solution as part of the post-operative treatment in Graves' disease; the beneficial results were so striking that its use was extended to the pre-operative preparation of the patient; at once thyroidectomy by a skilful surgeon became an almost negligible operative risk. "When Lugol's solution is given in exophthalmic goitre, there may be a marked drop in the basal metabolic rate with coincident relief of excessive nervousness and nausea, and if the patient is in the critical condition which is sometimes seen in this disease, it is possible to bring about a remission of symptoms which permits surgical removal of the thyroid gland without undue risk" (129). The beneficial results have been extensively and completely confirmed.

Means, Thompson, and Thompson (179) write that the

[1] According to Joll (126), Waller (257), in 1914, anticipated Plummer in almost every detail. This conveys a wrong impression of the importance of Plummer's treatment, which essentially associated the use of Lugol's solution with operative treatment.

phenomenon "may be said to consist . . . in a striking decrease in intensity of the peculiar nervous and circulatory manifestations, a fall in pulse and basal metabolism, and a histological change in the thyroid gland in the direction of increase in colloid and decrease in vascularity and epithelial hypertrophy." The effect can be produced at any stage of

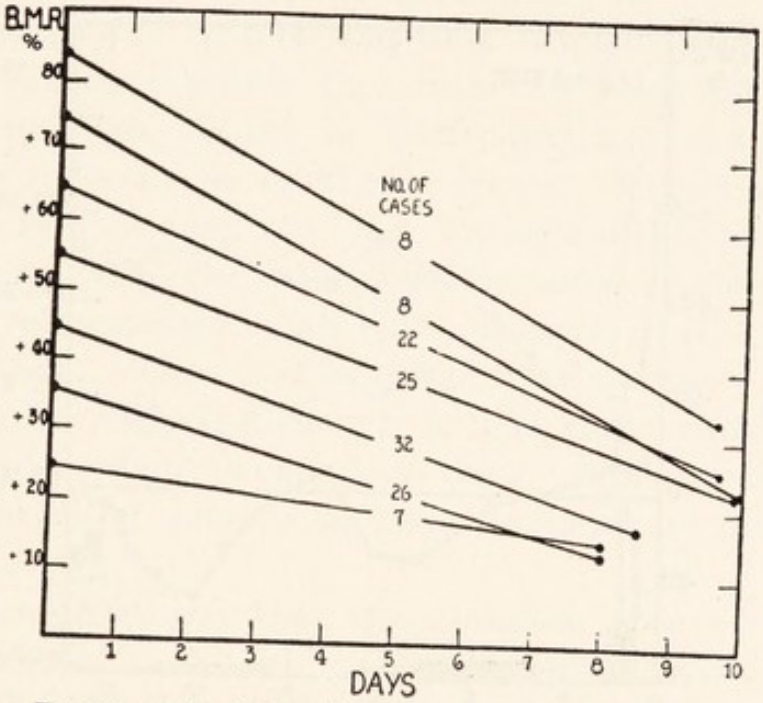


FIG. 4.—Response to iodine in Graves' disease. The average basal metabolism before is compared with that after the usual course of iodine in the form of Lugol's solution. The abscissae represent the average time required for the characteristic response. A total of 128 cases was divided into groups according to the pre-iodine metabolic rates, each ten-point rise defining a group. (From Means, Thompson and Thompson, *Trans. Assoc. Am. Physicians*, 1928, xliii, 146.)

the disease, provided the patient has not recently received iodine. The higher the initial rate, the greater is the resulting fall; this is well shown in Fig. 4. If the treatment is stopped, the basal metabolic rate rises abruptly. While, in the majority of cases of Graves' disease, thyroidectomy apparently effects a cure, yet "in certain cases the disease smoulders on even after this operative procedure, and certain residual phenomena yielding to iodine are not

infrequently encountered." An example of such a case is shown in Fig. 5. The administration of Lugol's solution for some months caused a drop in the basal metabolic rate, a fall in the pulse rate towards normal, and a steady rise in body weight, along with disappearance of the residual nervousness. It is not improbable that many cases of

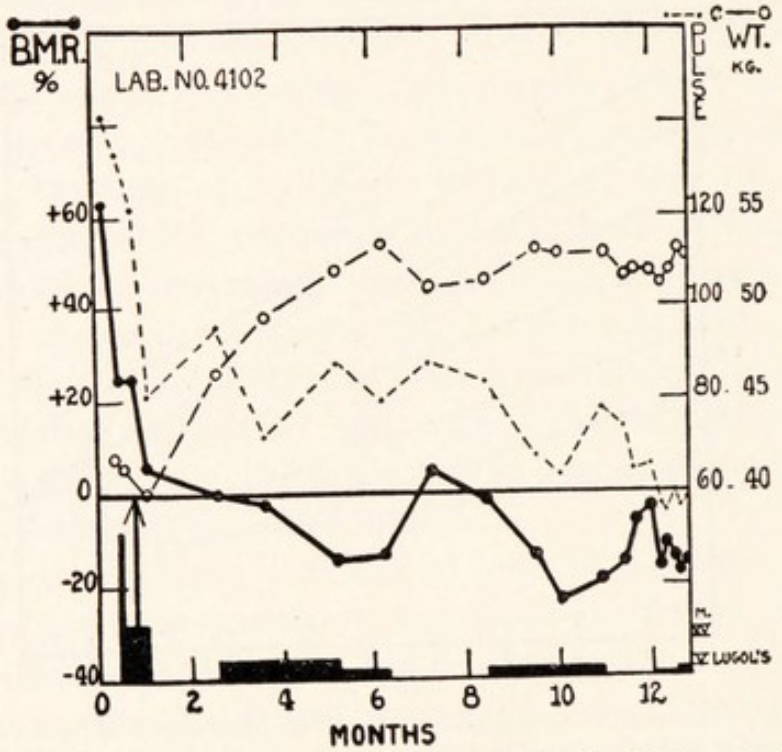


FIG. 5.—Iodine responses in a case of residual thyrotoxicosis following subtotal thyroidectomy (arrow) for exophthalmic goitre. The case was characterised by residual nervousness, which disappeared under the iodine treatment. (From Means, Thompson, and Thompson, *Trans. Assoc. Am. Physicians*, 1928, xliii, 146.)

recurrence might be prevented by judicious occasional use of iodine over a long period following the thyroidectomy (70).

In the great majority of cases *prolonged (pre-operative) treatment with Lugol's solution leads to development of a refractoriness to iodine.* Thompson has recently published a very complete study of this effect (244). After a period which generally does not exceed twenty days the beneficial effects gradually wear off, the basal metabolic rate increases, and the unfavourable symptoms return. If the adminis-

tration is still continued, the basal metabolic rate may exceed that before commencement of treatment, with more severe accompanying symptoms and more intense nervous manifestations. In two out of five patients thoroughly studied an exophthalmos was first noted while the basal rate was rising during such prolonged administration; in two other cases it became more prominent.

The majority of writers who have studied the action of Lugol's solution conclude that in severe cases of Graves' disease operation should be performed as soon as the maximum reduction in basal rate occurs. Should administration have continued too long, Thompson finds that it is necessary to cease the treatment for three or four weeks until the refractoriness shall have disappeared (the patient resting in bed). The exact length of time necessary has not yet been determined, although in one case refractoriness disappeared within twenty-four days. Subsequently, re-administration of Lugol's solution produces its full effect (cf. also 121).

Thompson considers that the optimum dose of Lugol's solution (U.S.A. standard) in Graves' disease is only 1 drop (6 mg. iodine) daily. A small percentage of cases do not respond to this or to larger dosage. Half a drop daily is insufficient. He thinks that it is doubtful if more than 5 drops daily is ever necessary. In the occasional case a very small dosage (one-quarter to one-half drop daily) appears to accentuate the symptoms. His ideas concerning optimal dosage seem to be at marked variance with general practice.

A number of investigators have studied the effects of prolonged treatment with Lugol's solution in lieu of operation. While there is a possibility of continued benefit in very mild cases, severer cases become worse under the treatment (244, 253, 246). It is doubtful if, in the majority of cases that appear benefited, such treatment does more than postpone operation.

The Effect of Other Iodine Compounds. Results, equally as good as those produced by Lugol's solution, have been obtained by a solution of iodine in hydriodic acid (75), iodized fat or sodium iodide given with a concentrated mixture of vitamins A and D (201, 3, 78), sajodin (calcium iodobehenate) (81), ethyl iodide (inhaled) and potassium iodide (146).

Lerman and Means (146) studied the effects of inhalation

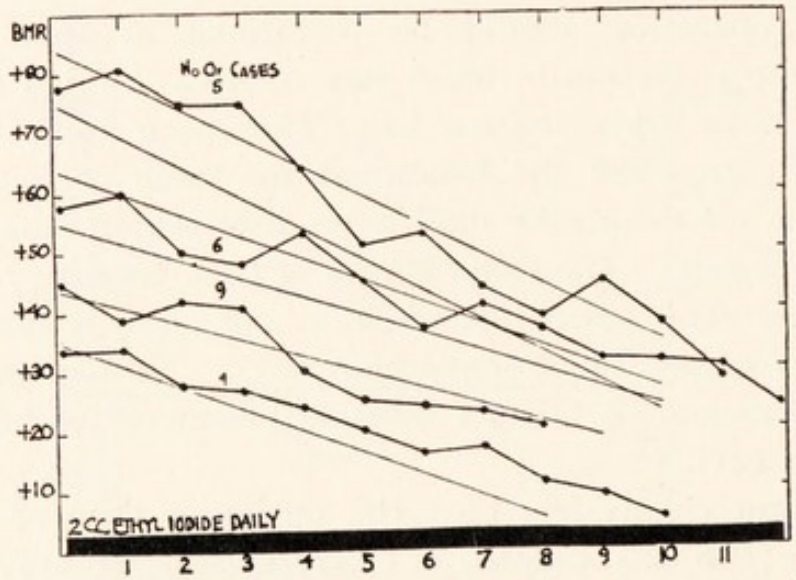


FIG. 6.—Comparison of the metabolic rate changes produced by Lugol's solution (cf. Fig. 4) and ethyl iodide in Graves' disease. The cases are grouped in accordance with the resting levels, each ten-point interval constituting a group. (From Lerman and Means, *Am. J. Med. Sci.*, 1931, clxxxi, 745.)

of ethyl iodide (4 grams inhaled in twenty minutes once a day) and of potassium iodide (0·36 gram, containing 0·275 gram of iodine, daily). Their results are shown in Figs. 6 and 7. They consider that potassium iodide is preferable to Lugol's solution for pre-operative treatment, since it is equally effective and more easily taken. (In all their measurements the initial basal metabolism was determined after a period of rest in bed; this is a very important precaution, since the occasional patient shows marked clinical improvement and fall in basal rate by this treatment alone.)

The Effect of Lugol's Solution in Toxic Adenomatous Goitre. The available evidence is conflicting. The Mayo school have expressed the opinion that no benefit is conferred (129). Since the condition is closely related to pure hyperthyroidism, artificially produced (cf. p. 69), it is pertinent to note that administration of Lugol's solution confers no protection against thyroxine dosage in animals or in man (236, 209, 139, 37). Yet there seems to be definite

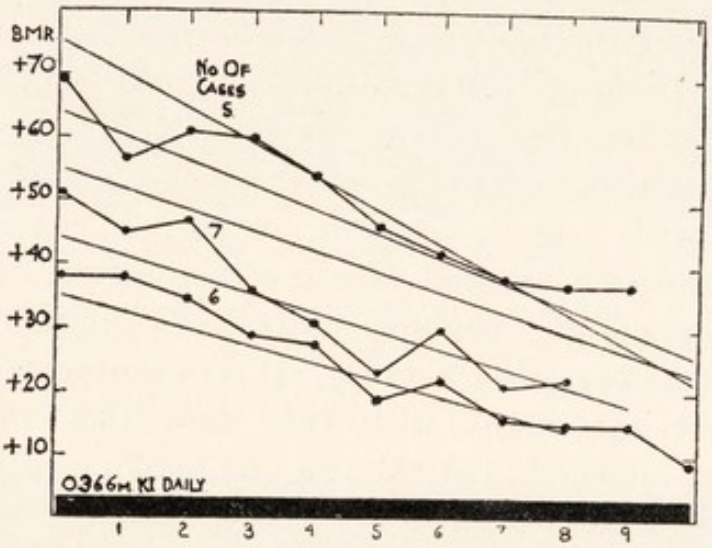


FIG. 7.—Comparison of the metabolic rate changes produced by Lugol's solution (cf. Fig. 4) and potassium iodide in Graves' disease. The cases treated by the latter are arranged in three groups, with initial rate less than + 45, between 45 and 59, and + 60 per cent. or over. (From Lerman and Means, *Am. J. Med. Sci.*, 1931, clxxxi, 745.)

evidence that it depresses metabolism in some cases in certain non-thyroid conditions, including pernicious anaemia (175) and lymphatic leukaemia (80), even though it is stated to have no appreciable effect on normal man (175, 204, 230, 149). Certain writers state definitely that it is just as effective in toxic adenoma as in Graves' disease (194, 29).

The Nature of the Action of Iodine in Graves' Disease. The precise action of iodine in Graves' disease will probably remain unknown until more is known of the nature of Graves' disease itself. A number of theories have been advanced. If the assumption be correct that Graves' disease is not

primarily but only secondarily a thyroid disease, then it is possible for the effect of iodine to be either directly upon the thyroid itself, or systemic, and at least in part extra-thyroid. Following Plummer's introduction of the treatment with Lugol's solution, the two theories which obtained most credence both assumed direct action upon the thyroid. Plummer postulated the correction of a condition in which an abnormal thyroxine was being produced in the gland; this view cannot be upheld (cf. p. 68). Marine (173) suggested that the beneficial action depends upon the rapid formation of colloid, which mechanically blocks the secretion of thyroxine into the general circulation.

The histological changes seen in the gland following treatment with Lugol's solution are varied, but are chiefly in the nature of a marked degree of involution, the general change in appearance being towards that seen in an ordinary colloid goitre (214, 216, 39, 264). (Certain observers are not in complete agreement with this view (105, 183).) The change is so marked, and the use of Lugol's solution is now so universal, that the appearances which used to be regarded as typifying Graves' disease are now seldom seen. Through the kindness of my colleague Professor William Boyd, a typical picture of a thyroid section from an untreated case of Graves' disease (old material) is contrasted with an average picture obtained after correct treatment with Lugol's solution (Figs. 8 and 9).

It is generally agreed that the untreated goitre of Graves' disease is iodine-poor, colloid-poor, and stains poorly with eosin. After treatment with Lugol's solution it tends to become iodine-rich, and rich in colloid, and stains well with eosin. Toxic adenomas show somewhat similar changes (111).

The numerous recent papers in the literature dealing with the presence and the nature of the iodine in the blood in normal and pathological conditions have been in large part summarized by Lunde (156), whose own studies are amongst the most important. The average iodine content of normal

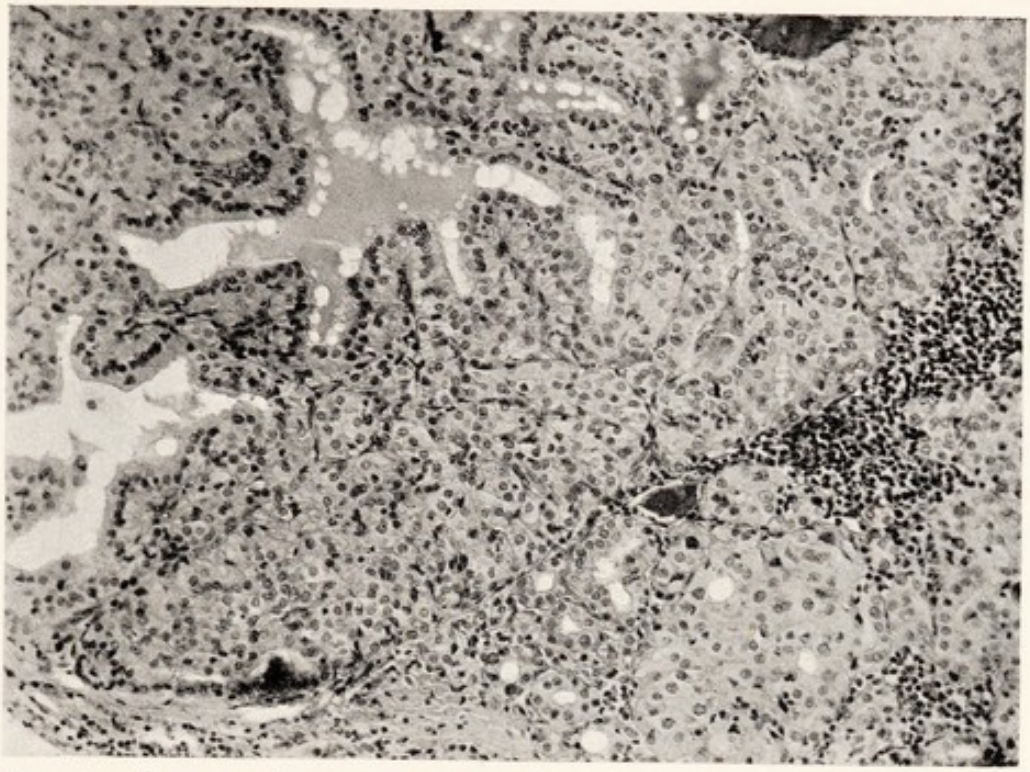


FIG. 8.—Graves' disease untreated with Lugol's solution. The acini are for the greater part filled with hyperplastic epithelium. Absorption of colloid, especially along the line of contact with the epithelium. To the right there is a small collection of lymphoid tissue. × 130. (Photo-micrograph and description by Professor William Boyd.)

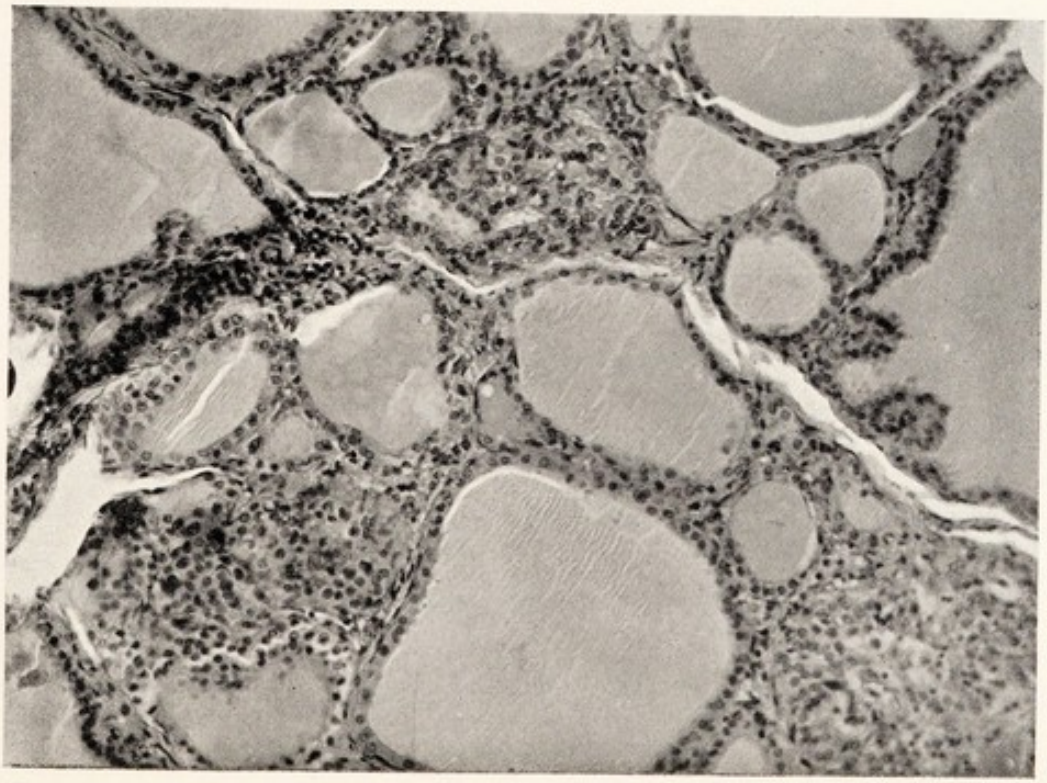


FIG. 9.—Graves' disease treated for a short time with Lugol's solution. A few epithelial buds are seen at the right, but most of the hyperplasia has disappeared, and the acini are filled again with colloid. × 130. (Photo-micrograph and description by Professor William Boyd.)

[*To face page* 80.

man varies from 9 to 13γ per 100 c.c. blood ; there are certain seasonal fluctuations. (Wider extremes have been reported, seldom exceeding 8 to 17γ.) Cretins show a lower figure, 6γ or less, while marked increases have been found in hyperthyroid states. Lunde separates the iodine fractions of the blood by adding one volume of blood to four volumes of alcohol, and then extracting the protein precipitate with more alcohol. Thus two fractions are obtained, one alcohol-soluble (considered the inorganic-iodine fraction), and the other alcohol-insoluble (considered organic-iodine). (Separate treatment has shown that the amount of lipoid-iodine present is negligible.) Normally, the inorganic iodine varies from 7 to 12γ, the organic iodine from 1 to 4γ.

In untreated patients with Graves' disease, while the inorganic fraction is not much affected, the organic fraction is markedly increased. Thus three patients showed, for inorganic and organic iodine respectively, the figures 15γ, 36γ ; 25γ, 18γ ; 9γ, 23γ. After treatment with Lugol's solution the inorganic fraction is of course vastly increased, but the organic fraction is *decreased* to normal or nearly normal limits, concurrently with the fall in basal metabolic rate and symptomatic improvement. A typical result is shown in Table IV. (D.H., female, aged twenty, with definite Graves' disease) (156).

TABLE IV

Effect of Lugol's Solution on the Distribution of Iodine in Blood in Graves' Disease

Date.	Lugol's Solution.	B.M.R.	Blood-Iodine.		Remarks.
	Daily Dosage.		In-organic.	Organic.	
7.2.1928	10 drops, 4 times	+ 80%	15γ	36γ	—
8.2.1928	,,	—	94γ	23γ	—
13.2.1928	,,	+ 40%	377γ	1γ	—
20.2.1928	,,	+ 7%	252γ	3·4γ	—
21.2.1928	,,	—	—	—	Sub-total thyroidectomy
23.2.1928	,,	+ 72%	243γ	6·7γ	—
7.3.1928	Iodine stopped	—	—	—	Symptom-free

Examination of the blood shortly after thyroidectomy in Graves' disease shows no increase in its iodine content, but usually a slight decrease in the organic fraction, so that there is no mechanical expression of the endocrine secretion by the operation (13). Marked post-operative shock can occur in patients who have had no pre-operative treatment (and in whom, therefore, a high organic-iodine content in their blood sinks rapidly after operation) and also in those who have been treated for a long time with small amounts of iodine, and this treatment stopped eight to ten days before operation. The shock is considered due to the acute sudden fall in blood content of the thyroid principle produced by operation and may explain the usefulness of post-operative iodine treatment (13).

In the thyroid of Graves' disease iodine treatment produces increase in both inorganic- and thyroglobulin-iodine, increase in the relative amount of thyroxine as contrasted with diiodotyrosine radicals, and absolute increase of both. These changes probably indicate a change towards the condition of the resting gland (94).

These chemical studies, indicating as they do that the thyroid principle is present in the protein fraction of the blood, support the idea that it is thyroglobulin itself rather than a simple tri-peptide (cf. p. 25). They indicate that the output of the principle is gradually increased in Graves' disease. The effect of Lugol's solution during the period of beneficial action is to depress the output of the principle, which is stored in the gland (increased iodine ; increased colloid). While Marine's mechanical explanation is not disproved, it seems more rational, chemically, to suggest that, through perhaps a mass-action effect, the normal colloid-building process is restored until the thyroid acini are distended with colloid and mass-action in that direction is again equilibrated, whereupon, secretion of the principle continuing, it is once more secreted into the blood in excessive amounts.[1]

[1] Dodds (60A) confirms Lunde's work to the extent that he also finds two iodine fractions in blood, separable by alcohol extraction,

Summing up the effect of iodide (for all the iodine compounds actually supply iodide to the organism, and iodide is equal in effect to any of them), it has a direct effect upon the gland itself, temporarily depressing the output of the thyroid principle, but it almost certainly has an additional effect on the system, not produced through the thyroid. It cannot be considered to affect the cause of Graves' disease (179).

Other Methods of Treatment of Hyperthyroidism. Quinine has been advocated, apparently on the ground that hyperthyroid patients are relatively resistant to cinchonism. Enthusiastic claims for beneficial results have been made (25) and disputed (178, 27). Benefit has been stated by various clinicians to follow the use of gynergen—ergotamine tartrate (4, 232), physostigmine salicylate (25), potassium permanganate (188), and sodium or ammonium fluoride (89A). The rationale for most of such treatment is difficult to understand.

In support of Marine's views on the interrelationship of the thyroid and adrenal glands, good results have been claimed following administration of adrenal cortex in Graves' disease (219, 228) and of the concentrated principle (cf. p. 213). Good results have also been claimed following administration of insulin (246, 89).

Roentgen-ray and radium treatment are advocated with varying degrees of enthusiasm by different writers. Diathermy is stated to be of no great value (124). The most generally expressed opinion concerning X-ray treatment is that it is more suitable when the toxaemia is moderate than for severe cases (213, 86, 211, 192) (according to Joll (126) it may be used in early cases associated with great

and that the insoluble fraction is increased in amount in patients with toxic goitre, while oral treatment with iodine reduces the amount of this fraction. He does not consider that this decrease is necessarily associated with reduction in toxicity and basal metabolic rate. The main conclusion justifiable from his figures is that the decrease in the insoluble iodine fraction of the blood is not proportional to the fall in basal metabolic rate.

restlessness and irritability and a large goitre). Some insight into the success or failure of the treatment is given by such reports as those of Morley (182): forty out of 120 cases of Graves' disease coming to operation had previously been treated with X-ray without success. A few writers have claimed good results with radium emanation (93, 110).

Surgical Treatment of Hyperthyroidism. Since the introduction of routine pre-operative treatment with iodine, one of the chief interests in the surgical reports has come to be the ever-decreasing mortality. Clute (41) reports 0·65 per cent. of deaths in Graves' disease, and 1·87 per cent. in toxic adenoma for 3,389 cases at the Lahey clinic. De Courcy (59) considers the average mortality to be about 1 per cent. Joll (126) reports a mortality of 2·5 per cent. in his own cases. Hyman and Kessel (118), while admitting that specially trained teams obtain a very low mortality, consider that the general operative mortality over the whole population is considerably greater.

Boothby has drawn attention to the value of oxygen treatment following thyroidectomy (95A).

Don (61), discussing the late results of operative treatment, claims that the exophthalmos usually improves, but does not always disappear. Nervousness is invariably improved, but not always banished. Most, but not all, patients gain weight. Some percentage of patients are not improved by operation (cf. 182, 79).

Fenger (74), contrasting medical and surgical cases observed over many years, concludes that if 100 cases are submitted to medical, and an equal number of similar cases to surgical treatment, the latter will cure about twice as many as the former, nor will X-ray treatment materially affect the result.

At the present time there is no medical treatment which will re-establish thyroid balance to such a degree of stability that it will stand the strain of ordinary existence with the resistance exhibited by the thyroid of normal man. Sooner

or later, in the majority of cases, the hyperthyroid goitre is removed surgically.

Malignant Tumours of the Thyroid

From the point of view of the endocrinologist it is important to remember that carcinoma of the thyroid can give rise to functioning metastases. Thus Parkes Weber has reported a case of primary carcinoma of the thyroid with metastasis to bone. After thyroidectomy hyperthyroidism developed. Removal of the metastasis led to myxoedema (260).

Joll states that in areas where endemic goitre is prevalent malignant disease of the thyroid is relatively common (126).

Administration of Thyroid in Various Conditions

Good results have been reported in hebephrenic dementia praecox (114). A considerable number of gynaecological cases which exhibit as symptoms menorrhagia, sterility and abortion (separately or in combination) are apparently relieved by administration of thyroid and by no other treatment (49). Thyroid administration has proved of some service in the treatment of cataract (130) and, in slight dosage, in treatment of certain types of alopaecia (90); it has no beneficial effect when given to senile rats (113).

Certain of these results suggest interrelationships between the thyroid and other endocrine glands; these will be dealt with in the final chapter.

Unsolved Problems Related to the Thyroid Gland

After dealing with what we know of the thyroid, it is useful to point out, as Remington has done (206), what we do not yet know. While in the past twenty years considerable advance has been made, yet in some ways our

ignorance of essential facts has been brought out more prominently.

We know very little of the form of organic combination of iodine in animal food, and still less of that in plant food. We know practically nothing of the mechanisms by which thyroid tissue forms the thyroxine radical, can only guess at the processes of storage, and of secretion, and we are still uncertain as to the precise chemical nature of the principle that is secreted.

We do not know how this principle brings about its effects in the tissue cells throughout the organism, nor do we know what these effects are, except that they affect oxidation. We have no clue as to the reason for the marked delay in action exhibited by these tissue cells following injection of thyroxine or the feeding of thyroid, a delay whereby no action is perceptible for two or three days, and maximum action is not reached, even with single doses, for ten or fourteen days.

We do not know the precise nature of Graves' disease, nor the cause of the beneficial action of iodine (in various forms of combination) in pre-operative treatment. We do not know the initial factors which lead to manifestation of hyperthyroidism in any form. And until these are determined we shall probably not be in a position to find some rational medical therapy.

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CHAPTER III

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Introduction

LITTLE recent advance has been made in anatomical and histological interpretation of the parathyroid structure.

The glands in man are usually four in number, 3 to 15 mm. long and 2 to 3 mm. broad and thick, and yellowish-red to brown-red in colour. One occurs on the medial aspect of the dorsal surface of each lateral lobe of the thyroid gland; these form the "superior pair." The "inferior pair" also are found usually on the dorsal surfaces of these lobes, further caudad. Small accessory glands are by no means uncommon, especially near or embedded in the thymus (13).

The glands are relatively very vascular. They are each supplied by a special arteriole from the inferior thyroid artery; from it sinus-like capillaries come into close relation-

[1] Most of the recent work has been reviewed by Thomson and Collip (71), who have added a very complete bibliography. To prevent unnecessary duplication, this will be used wherever possible, italicized references in this text indicating references given in their review.

ship with the cells themselves. The nerve supply is probably sympathetic in origin (66). Some of the nerve fibres terminate in the vessel walls; many penetrate between epithelial cells, forming nodular endings (58). Transplantation experiments suggest that the glands can function adequately in absence of all nervous connections (58, 41).

The glands are composed of epithelial cells, which either form a compact mass, or are divided into lobules by strands of vascular connective tissue. The latter conveys the capillaries. Two forms of cells are described—ordinary or principal cells, small, and either clear or somewhat granular, and larger cells, containing oxyphil granules and staining with eosin (66). These probably represent a functional stage of the principal cells. Both types contain fatty granules or minute spherical globules, which increase in number with age (66, 12). Small colloid vesicles are sometimes found; the number of these also increases with age (cf. also 38, 48).

The definite association of those acute manifestations which we call tetany with experimental removal of the parathyroids is due to Gley (24). Vassale and Generali (74) produced tetany—death ensuing—in nine out of ten parathyroidectomized cats, and all of nine parathyroidectomized dogs; the majority of the animals died between the third and fifth days following operation. Such work has been frequently repeated, and the association of complete parathyroidectomy and tetany abundantly confirmed. Nicholas and Swingle (*375*) have dealt critically and satisfactorily with apparent exceptions (cf. also 71).

MacCallum and Voegtlin showed that the tetany following extirpation of the parathyroids was associated with a fall in the calcium content of the blood to about half its normal value (45). Intravenous injections of calcium salts temporarily banished the symptoms of tetany. It was consequently concluded that the glands regulate the calcium metabolism of the organism, and that the symptoms which

follow their extirpation are due to the resulting fall in blood calcium. It was subsequently demonstrated that the hyperexcitability of nerve, characteristic of tetany, can be induced by experimental production of a lowered blood calcium (44).

Parathyroid investigations were then confused for a while by the efforts that were made to prove that the function of the glands was essentially the detoxication of guanidine compounds.

Salvesen in 1923 confirmed MacCallum's theory and concluded from his own and previous work that the parathyroids control the calcium level of the blood. He showed that parathyroidectomized animals could be kept alive for long periods by including sufficient calcium in the diet (excess of milk and addition of calcium salts) (*440*). In such animals the plasma proteins remain unaffected, while in human nephritics exhibiting marked oedema both plasma proteins and plasma calcium are diminished, yet tetany does not result. Hence Salvesen concluded that the cause of this tetany is a decrease in that part of the blood calcium which is not combined with protein (*441*, *442*).

Thus the earlier work demonstrated clearly that parathyroid function is related to the prevention of tetany in the normal animal and the maintenance of a certain level of the blood calcium. It therefore seems desirable at this stage to discuss the nature of tetany, and to indicate what we know at present concerning the state of combination of calcium in the blood.

Tetany

Tetany results from many causes, and is exhibited in varying degrees. It is characterized by a hyperexcitability of the nervous system. If it be "manifest" there are spontaneous attacks of tonic spasm, which may be limited to groups of muscles, or which may involve the whole body. Usually in milder attacks groups of muscles associated with

certain nerves are affected, producing in man such characteristic phenomena as the "obstetrical hand," extension of the knee with supination of the foot, laryngospasm, facial spasm and trismus. Associated with these are pains in the muscles during spasms and paraesthesias, especially in the distal parts of the extremities. The phenomena vary somewhat in different species, but tremors, chorea-like jerky movements, and, in extreme tetany, convulsive fits of varying degrees of violence alternating with quiescent periods, are common to most animals after complete parathyroidectomy.

If the tetany be merely "latent," significant phenomena can be elicited by application of tests, such as Trousseau's and Chvostek's.[1]

Tetany is almost invariably produced following *complete* parathyroidectomy in all mammals, and in birds (71). When it is so produced, if blood is taken during an active seizure the serum calcium is found usually to be at some value between 7 and 4 mg. per 100 c.c., instead of the normal 10 or 11 mg. In latent tetany somewhat higher values may be found. As already stated, if the calcium level is raised by any treatment the tetany is relieved.

Some proportion of the clinical cases of tetany are associated with hypoparathyroidism; the majority probably are not. It develops following thyroidectomy in man, when insufficient parathyroid tissue has been left undamaged.

The condition of rickets in young children is not infrequently associated with tetany. In this combination the serum calcium is depressed to an extent comparable with that following parathyroidectomy. The tetanic manifestation can be temporarily relieved by administration of hydrochloric acid-milk, or of ammonium chloride (which tends to produce an acidosis in the organism) or of calcium

[1] For more complete descriptions of tetany in man and animals, and details of the various tests which can be used to demonstrate its presence in clinical cases, see Barker (3), Vincent (75), Sharpey-Schafer (66), and Salvesen (59).

salts. More permanent relief is conferred by continued administration of an active concentrate of vitamin D.

Many cases of infantile tetany do not exhibit a lowered plasma calcium. They are traceable to gastrointestinal disturbances, vomiting (causing loss of hydrochloric acid) and diarrhoea. In a recent study of idiopathic steatorrhoea it is stated that 14 out of 15 cases exhibited tetany, and 13 of these showed low serum calcium. The condition was associated with disturbance of gastrointestinal function (6). Severe vomiting, or continued gastric lavage, in adults, may lead to tetany.

In 1920 Collip and Backus (*100*) and Grant and Goldman (*173*) almost simultaneously showed that over-ventilation of the lungs could produce a tetany, through the deficit of carbon dioxide produced. In such tetany the blood calcium is either normal or slightly increased, while a definite alkalosis is present. These observations have been repeatedly confirmed (*192*, *239*, *480*, 27, 17).

A number of clinical cases have been reported in which such hyper-ventilation was the immediate cause, and generally the only conditioning factor. Such include tetany occurring during a paroxysm of hyperpnoea in a psychoneurotic patient convalescent from encephalitis lethargica (4), cases associated with continued pain from cholelithiasis and cholecystitis (26, 50), or from retention of urine (52), or from the prolonged discomfort of a pelvic condition (50). Even too violent exercise taken when in poor physical condition, or crying spells associated with a neurosis, have produced symptoms of tetany (26). McCance considers that certain individuals are peculiarly susceptible to hyper-ventilation, and that tetany may develop in them from a degree of over-breathing which is scarcely perceptible. He thinks that many cases of so-called " sporadic tetany " may come within this category (46). Prolonged immersion in hot baths can set up a hyperpnoea which may induce tetany (39).

In clinical, as in experimental hyper-ventilation tetany the

blood calcium is normal or very slightly elevated. The condition calls for treatment unrelated to calcium. Good results have been obtained by educating the patient as to the cause of the attack and the possibility of arresting it by control of breathing (50).

Tetany can be experimentally produced in animals by intravenous injections of sodium or potassium phosphate (*47*, *59*, *109*, *165*, *200*, *386*, *443*). The sodium or potassium concentration in the plasma is elevated and at the same time the calcium concentration is depressed, sometimes to 6 mg. per 100 c.c. (presumably through precipitation of calcium as phosphate or carbonate). Injection of phosphoric acid or of acid sodium phosphate, although it depresses blood calcium, does not induce tetany; instead of an increased sodium or potassium concentration there is an increased tendency to acidosis which offsets the effect on the calcium.

The literature contains references to some less usual forms of tetany.

The essential clinical manifestation in so-called " milk fever " of lactating cows is probably a tetany. It appears early in the course of the disease. It may be generalized and severe, accompanied by convulsive seizures, or of moderate degree, and then confined to isolated groups of muscles especially in the hind limbs (frequently evidenced merely by an extension of the hock joints with concomitant stiffness and " paddling " gait). It varies in duration, and is often so transient that it passes unnoticed or is masked by the lethargic or comatose stage which follows (and which precedes spontaneous recovery or death). It is accompanied by a hypocalcaemia of the degree usual in parathyroid tetany, and has been considered as due to a parathyroid deficiency (16). However, the blood phosphates are also depressed, whereas, following parathyroid extirpation, they are slightly increased (20). Many of the symptoms suggest dehydration and anhydraemia (29). In 90 per cent. of the cases udder inflation is sufficient to cure the animal and restore blood calcium to normal; hence parathyroid deficiency can be excluded. The actual tetany and any anhydraemia are probably traceable to undue drainage of calcium and of fluid from the blood at the height of a vigorous lactation.

" Lock-jaw " is a condition met with amongst Welsh mountain ponies. It has been observed in suckling mares soon after their being housed, and in ponies of either sex at the end of a railway

journey. There is marked hypocalcaemia (5 to 6 mg. per 100 c.c. serum), but a high blood phosphate and a high alkaline reserve. Subcutaneous, or, in the mare, intramammary injection of air restores these animals. They do not exhibit the characteristic secondary coma of milk fever ; where the tetany ends fatally tetanic spasms continue till death. The cause is still unexplained (51). A similar condition in cows and ewes following a period of close confinement has been described (15, 68). Dehydration may be a factor in all such cases (29).

Tetany is produced in a proportion of young white rats fed desiccated thyroid ; it often is apparent after a few days' treatment (8). It has been attributed to a combination of depression of the thyroid-parathyroid apparatus (from anaemia through diminished blood supply induced by the exogenous thyroid principle) and the added effect of an alkalosis due to sudden atmospheric changes, especially a fall of barometric pressure. However, there is some evidence that hyperthyroidism itself induces an increased excretion of calcium, and it has been suggested that this might lead at last to such a fall of blood calcium that tetany would result (71, cf. also 2).

The underlying disturbance in the production of tetany is an upset in the ratio of certain ions in blood and tissues. The work of Loeb and others has demonstrated that the degree of irritability of tissues depends upon the ratios between the ionic concentration of potassium, sodium and calcium in the fluids in contact with these tissues ; increase of either of the first two, or decrease of the third, increases irritability. The different methods of experimental production of tetany, and of causing relief from this tetany, suggest that the ionic ratio is somewhat more complicated, in so far as it is related to tetany. There seems to be a balance between sodium, potassium, and hydroxyl ionic concentrations, on the one hand, and calcium and hydrogen ionic concentrations on the other. Any increase in any one of the first three, or any decrease of either of the last two, conduces to tetany. Opposite changes tend to banish an established tetany. Whether or not a change in the hydrogen-ion concentration of the blood can in itself so affect the ionization of blood calcium as to cause or to banish tetany has not yet been proved. If this were the case, then the ionic ratio governing

tetany would be that governing tissue irritability in general.

While in the tetany following parathyroidectomy the excretion of phosphorus is definitely reduced (*181*, *184*), yet there is only slight increase in blood phosphate (*184*). Changes in hydrogen-ion concentration will undoubtedly change the equilibrium between the different phosphate ions (H_2PO_4', HPO_4'', and PO_4''') and thus may well alter the balance between unionized and ionized calcium (although we have no definite knowledge as to the nature of the unionized inorganic calcium compounds present). Equally, also, changes in calcium concentration may affect the other equilibria. Until we know more concerning the nature of calcium combination in the blood plasma it is easier to assume multiple rather than a single causative factor in tetany. (Thomson and Collip have reviewed this problem critically (71).)

Blood Calcium [1]

The calcium of the blood occurs wholly, or almost wholly, in the plasma. Results indicating its presence in the red cells in any but negligible amount are due to inaccuracy of technique (71). It seems unlikely that in normal blood the envelope of the red cells is at all permeable to calcium ions.

Calcium is present in the plasma in three distinct conditions, in organic combination, in unionized inorganic combination, and as calcium ions. It is usually estimated in the serum from clotted blood; reaction with ammonium oxalate, if sufficient time elapses and excess of oxalate is present, precipitates all the calcium of serum as calcium oxalate. It is uncertain whether all the plasma calcium passes into the serum unchanged in amount and in the nature of its combination. Most investigators seem to consider that this is the case. Since, however, calcium plays a definite *rôle* in clotting it seems quite possible that the equilibria between

[1] Thomson and Collip have dealt very thoroughly with the partition of calcium in the blood (71).

the different forms of combined calcium and calcium ions are not completely the same in plasma and in serum, and that investigations on serum do not necessarily yield results applicable to plasma.

Numerous experiments have been carried out to determine the partition of calcium between organic and inorganic combination. Such partition can be most properly considered as between "diffusible" and "non-diffusible" calcium. It is important, in considering all experiments involving dialysis (as many of these do), that the method of preparation of the membrane be carefully taken into account. It has been shown that collodion membranes can be constructed of all degrees of permeability, so that while some will permit passage of simple crystalloidal molecules only, others will even permit protein molecules to pass through (23). Collodion membranes should be standardized in all ultra-filtration experiments (37). Since this has only recently been realized it is not surprising that dialysis experiments have not led to very concordant results.

Compensation dialysis methods gave the following figures (serum was dialyzed against solutions of known calcium content): 60 to 70 per cent. dialyzable in normal men (*354*, *355*), and in dogs 69 to 70 per cent. (*354*, *355*), 55 to 70 per cent. (*118*), and 65 to 75 per cent. (*433*, *436*). By ultra-filtration under pressure the diffusible fraction has been found to be 62 to 70 per cent. (14) and 50 to 70 per cent. (*374*). By a combination of these procedures 68 per cent. has been found for the rabbit (*370*, *517*), and, for man, dogs, and cattle 45 to 55 per cent. (*517*), and 42 to 58 per cent. (42, *317*). Cantarow has discussed the validity of these results critically (*90*).

The majority of investigators who have considered the mechanism of formation of the cerebrospinal fluid seem now to be of the opinion that it can be regarded as produced by filtration through the membranes of the choroid plexus (22, 69, 51). Accepting this view and comparing the calcium

content of the cerebrospinal fluid and serum, the following values have been found for the diffusible calcium : 53 per cent. (*82*) and 46 to 53 per cent. (*369*) in dogs, 45 to 57 per cent. in syphilitic patients (5), 46 to 51 per cent. in dispensary patients (*369*), and 45 to 55 per cent. in normal men (*87*). (Cf. also (67).)

It would seem, therefore, to be a very close approximation to the truth to conclude that 50 per cent. of the 10 or 11 mg. of calcium in the blood plasma is held united in organic combination to molecules of such large size that they do not, under normal conditions, diffuse through capillary membranes. The most obvious conclusion is that it is held back by some protein union. Whether a specific protein is concerned, or merely a minute proportion of the plasma proteins in general, cannot be stated (71), although recent evidence suggests that the albumin fraction is involved (5A). The remaining 50 per cent. is present as ions or in unionized inorganic combination.

Within recent years much experimental work has been carried out to determine the state of inorganic combination of calcium in blood plasma. This has been fully reviewed by Peters and Van Slyke (53) and by Thomson and Collip (71). *In vitro* experiments suggest that blood serum and cerebrospinal fluid are approximately saturated with respect to neutral calcium phosphate $Ca_3(PO_4)_2$, because addition of this compound in solid form to either precipitates calcium—chiefly, however, as calcium carbonate. But, as Thomson and Collip point out (71) : " Even if pure solid $Ca_3(PO_4)_2$ were obtainable, which is by no means certain, it would decompose on shaking with aqueous media and form basic phosphates, so that experiments of this type are unreliable." The acid phosphate, $CaHPO_4$, dissolves in serum, which is therefore unsaturated with respect to it. There is as yet no definite evidence that appreciable quantities of any calcium phosphate or carbonate are present in plasma, and no definite statement can be made as to the form in which

calcium is present in unionized inorganic combination, although probably 3·5 mg. per 100 c.c. must be so accounted for. A suggestion that it is present in some citrate-like combination is no longer supported (71). It might seem permissible to conclude that plasma is in equilibrium with the solid constituent of bone, whatever that complex calcium-magnesium phosphate-carbonate compound be, although even that assumption probably requires modification (cf. p. 117).

From the standpoint of the production of tetany the amount of calcium present in ionized condition is the important fact; here also precise knowledge is lacking. Various estimates ranging from 1·2+ to 2 mg. per 100 c.c. have been made, based upon different experimental procedures (71, 53), and it is probable that the latter figure is of the right order.

Whatever are the precise compounds of calcium present, it is evident from the action of oxalate that calcium is easily split off even from its organic combination. It is therefore extremely probable that a series of equilibria exist, which may be written in some such form as :

$$\text{Organic Ca} \rightleftharpoons \text{Ca}^{++} \rightleftharpoons \text{Unionized inorganic Ca}$$

The Effect of Parathyroidectomy on the Blood Calcium

The change in blood calcium which follows removal of the parathyroid glands obviously suggests that these glands exercise direct or indirect control over its concentration. To ascertain the nature of this control it is important to find out whether the organic (non-diffusible) or the inorganic (diffusible) calcium is affected, or both. Early results (cf. p. 96) suggested that the diffusible fraction was affected. If this were the case, and if the cerebrospinal fluid accurately mirrors the level of the diffusible calcium, then the calcium content of this fluid should fall to a negligibly low level. This does not happen. It falls somewhat more slowly than the

serum calcium and finally the values are either equal (*e.g.*, about 4 mg. per 100 c.c. in an actual experiment) (*82*) or almost equal (*369*). Such results suggest that the non-diffusible fraction is mainly affected.

Experiments using ultra-filtration and compensation dialysis techniques permit no definite conclusion. For example, one series indicated that most rapid reduction occurred in the non-diffusible fraction (*118*), another the exact opposite (*370*), and others were indefinite (*355*, *411*).

Other Effects following Parathyroidectomy. The acute effects, tetany, a diminished blood calcium, and a retention of phosphorus, have been dealt with.

The effects of chronic hypoparathyroidism cannot easily be studied in most species of mammals, since on the one hand complete parathyroidectomy rapidly causes death, and on the other partial removal is followed rapidly by sufficient regeneration to restore a normal condition. In the rat, however, although accessory parathyroids are generally absent, extirpation of the glands is seldom fatal and chronic effects can be ascertained (71). The teeth become opaque, brittle, and distorted, with disorganized enamel and exostoses of alveolar bone. The bones become somewhat decalcified; analyses show them to be low in ash, calcium, and phosphorus, although relatively high in magnesium (71). These results are not easily explained, since in hyperparathyroidism the bones are also denuded of calcium.

The Preparation of an Active Parathyroid Extract

Unlike desiccated thyroid tissue desiccated parathyroid preparations are ineffective when administered by mouth, and beneficial results claimed for them in the past merely exemplify the danger of uncontrolled clinical optimism.

The earlier attempts to obtain active extracts of the gland have been reviewed by Collip (10). MacCallum, reviewing this earlier work, wrote in 1924 concerning the therapeutical

results (43) : " At best it is a slight and questionable effect and less satisfactory in experimental animals than in the tetany of adults, from which it may probably be assumed that the psychic effect of any treatment plays a part there." In the same year Hanson (28) prepared an extract of ox parathyroid glands by boiling them with weak hydrochloric acid, and claimed that it produced beneficial results in the treatment of human tetany. All such early work fell short of establishing beyond doubt the presence of an active principle in a concentrated extract. Collip achieved this in 1924.

Fresh glands were extracted with an equal volume of 5 per cent. hydrochloric acid (or glands preserved with acetone with 3 per cent. acid). After boiling for from thirty to sixty minutes the mixture was diluted with four times its volume of boiling water and allowed to cool slowly. Congealed fat was removed mechanically, and then sodium hydroxide added to a *p*H of 8·0 or 9·0. After practically all suspended material had dissolved hydrochloric acid was added to a *p*H of 5·5, and the resulting precipitate filtered off rapidly. The active principle remained in solution. It was salted out with sodium chloride after making the solution acid to congo red, then transferred to a filter, dissolved in weak sodium hydroxide, centrifuged, and the liquid adjusted to *p*H 4·8. An isoelectric precipitate containing the principle was thrown down, filtered off, dissolved in hydrochloric acid to *p*H 3, and the solution, after passing through a Berkefeld filter, was ready for use.

Various modifications of this procedure have been suggested, without material improvement (71). Allardyce (*9*), working in Collip's laboratory, has carefully re-examined a large part of the technique. He found that although acid hydrolysis freed the active principle, too prolonged hydrolysis destroyed it. The most potent extracts were obtained by hydrolyzing for forty-five minutes with 1·5 per cent. hydrochloric acid. Attempts to fractionate the various active

precipitates failed. Attempts to purify the final amorphous product, and to obtain it in crystalline form, by employment of pyridine and other agents that had proved successful in producing crystalline insulin, also failed. Extraction with butyl alcohol effected no concentration.

The chemical properties of the most highly purified preparation so far obtained are such as to indicate that it consists essentially of a protein. It gives the protein colour reactions, and is precipitated by picric and picrolonic acids. Tests for carbohydrates are negative. The dried product contains 15·5 per cent. of nitrogen, and traces of iron and sulphur. It is soluble in water and in 80 per cent. alcohol, but insoluble in ether, acetone, and pyridine. The desiccated product, and solutions in weak acid are stable. The physiological activity is completely destroyed by boiling for one hour with 10 per cent. hydrochloric acid or 5 per cent. sodium hydroxide, or by incubation with pepsin or trypsin. The latter facts explain why *the parathyroid principle is ineffective when administered orally.* Belief that it is a protein is supported by the fact that it does not dialyze through a collodion membrane. There is evidence that its activity is bound up with the presence of a primary amino-group (73A).

The method of standardization of the principle is dealt with later.

The Effects following Administration of an Active Extract

When a potent extract is injected, subcutaneously or intramuscularly, into a normal dog, the most striking and conspicuous effect is an increase in the concentration of the plasma calcium. This continues for from twelve to eighteen hours; the maximum attained, following a single dose, seldom exceeds 18 mg. per 100 c.c. serum. The calcium then slowly falls to normal value (71). Intravenous injections produce their maximum effect earlier—in four to eight hours—and this maximum is definitely less (*9*).

When continued injections are given, with only three- or four-hour intervals between injections, they produce within a relatively short period of time a very characteristic and striking train of events, which has been exhaustively studied and reported by Collip (10): "The symptoms manifested and the order of their occurrence are somewhat as follows: Some hours after the injections have been begun the animal has attacks of vomiting followed by diarrhoea; a certain uneasiness of manner may be manifested at this time but otherwise the animal is quite normal in its behaviour. During this period (approximately twenty-four hours) the blood calcium is steadily rising at a uniform rate. The peak point of the blood calcium curve is reached at about 20 mg. per 100 c.c. It is maintained at this level for some hours, and then the blood calcium starts to fall. The animal meanwhile may continue to have occasional attacks of vomiting and diarrhoea and is physically becoming more and more depressed. A certain degree of respiratory distress may also be noted. Coincident with the fall in the blood calcium curve, urgent symptoms become manifested. There is vomiting and passing of blood by bowel, and the animal passes into a state of collapse. Death follows as a rule within a few hours. In this period of urgent symptoms the blood phosphorus (inorganic) rises abruptly. The blood urea and non-protein nitrogen also increase several hundred per cent. There is a marked decrease in the blood volume, and a characteristic thickening of the blood. The coagulation time is decreased. The circulation gradually fails and blood samples are obtained from peripheral veins only with great difficulty. The carbon dioxide content and combining power of the blood serum are as a rule definitely and gradually increased during the first half of such experiments. The *p*H of the blood serum . . . may be coincidently increased very slightly. This would indicate that in this period there is a tendency towards alkalosis which is, however, well compensated. The increase in carbon dioxide content of the

blood serum is maintained for several hours, then this value gradually decreases and in the terminal state is greatly reduced. The *p*H on the other hand remains stationary until within a few hours of death, when it decreases very rapidly. The general effect, therefore, of parathyroid hormone overdosage upon the acid-base balance is to produce a condition of compensated alkalosis on the first day; this then passes over into a condition of compensated acidosis which in turn

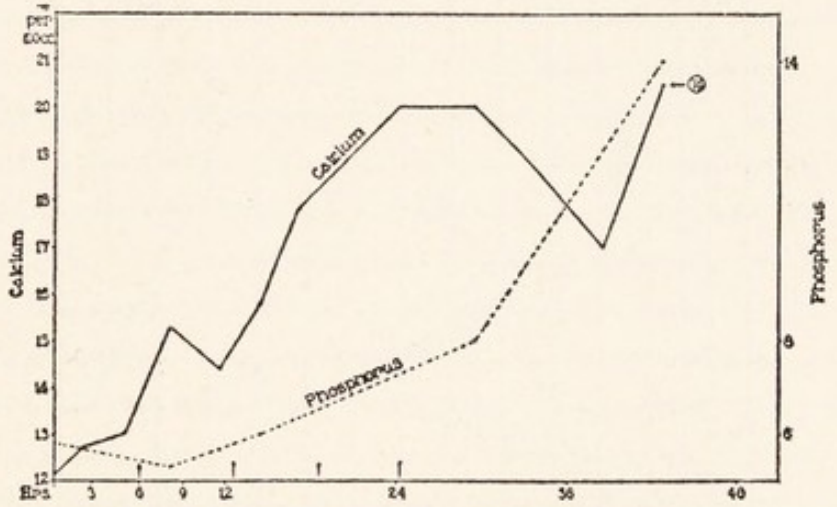

FIG. 10.—The blood serum calcium and whole blood inorganic phosphorus curves in continued parathyroid overdosage in the normal dog. (From Collip, *Medicine*, 1926, v, 22.)

is followed by an uncompensated acidosis just prior to death.

"The urinary findings . . . are also of interest. The kidney practically ceases to function very abruptly at about the time that the serum calcium curve has reached its peak point. There is a sudden decrease in the volume of urine produced, and, as a rule, both a relative and absolute decrease in the rate of excretion of phosphorus, ammonia, and titratable acid. Coincident with this abrupt decrease in kidney function the curves for whole blood phosphorus, urea, and non-protein nitrogen start to ascend. . . .

"Post-mortem examination of a dog dying from parathyroid hormone overdosage discloses marked congestion of

the alimentary canal and the presence of more or less blood in the stomach and intestine."

These changes are well shown in Figs. 10 and 11.

In later work, increased calcium content of the soft parts, and especially of the kidneys, has been noted. Histological examination reveals calcification in the space of Bowman's capsule and the lumina of the tubules, in the walls of the

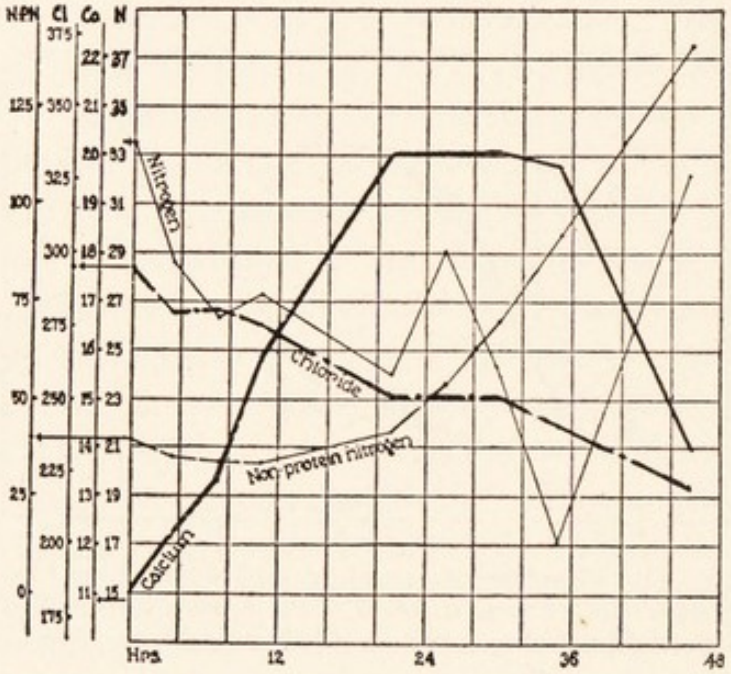

FIG. 11.—The blood serum calcium, whole blood chloride, nitrogen and non-protein nitrogen in the normal dog as affected by repeated injections of potent parathyroid extracts and frequent bleedings. (From Collip, *Medicine*, 1926, v, 23.)

lesser arteries, the Küppfer cells of the liver, and elsewhere. The kidney findings suggest that failing renal function is the cause of the pre-mortal rise in blood phosphate and urea (71).

This pathological picture of acute effects following over-dosage can be almost exactly paralleled by combined injection of calcium chloride and acid sodium phosphate (NaH_2PO_4), whence the actual symptoms may be ascribed to coincident hypercalcaemia and hyperphosphataemia (71).

The effects following prolonged treatment with sub-lethal

doses will be dealt with under the caption "hyperparathyroidism."

Different species of animals vary greatly in their response to injections of active extracts. Cats are much more refractory. Rats are almost immune, and rabbits seem immune to repeated injections (*9*, 71). The response of man is similar to that of the dog, although he seems more resistant to overdosage (71, *4*, *253*, *264*). Some of the contradictory results with animals that have been reported in the literature seem due to difference in the diets of these animals, others to differences in rate of excretion of calcium (71).

Conferred Immunity. The same dog does not give a constant response to the same dose. When repeated injections are given at intervals of several days (so that the blood calcium returns to normal before further injection is given) the second may produce a greater effect than the first, but later injections show a decrease in response—apparently tolerance to the principle is increased (*9*). Rats also appear to develop an immunity (55). The explanation appears to lie in the precise mechanism of action of the principle (see below).

The Parathyroidectomized Animal. Injection of the active extract into dogs produces results comparable to those obtained on the normal animal. The blood calcium rises as usual, but from a lower level. Repeated injections produce the same pyramided effect, and the same lethal result if continued sufficiently. Tetany is relieved, relief being coincident with increase of blood calcium to above the tetany level. The slight increase in blood phosphate produced by extirpation of the glands disappears (10). Collip has kept parathyroidectomized dogs alive for over a year by daily injections of potent extracts. Withdrawal of extract at any time led to early onset of tetany. The effects are shown clearly in Figs. 12 and 13. His results have been completely confirmed by numerous investigators.

According to Reiss (57) animals can be kept alive for long

periods following parathyroidectomy, provided the principle be administered at once. If marked deficiency effects have set in improvement is only transient. He believes that such marked deficiency produces irreversible metabolic changes.

Cats are similarly restored to normal. Rabbits, following removal of the glands, exhibit tetany rapidly, with a marked preterminal rise in blood phosphorus. The tetany can only be controlled by immediate injection of the principle (10). Man suffering from parathyroid deficiency following operative

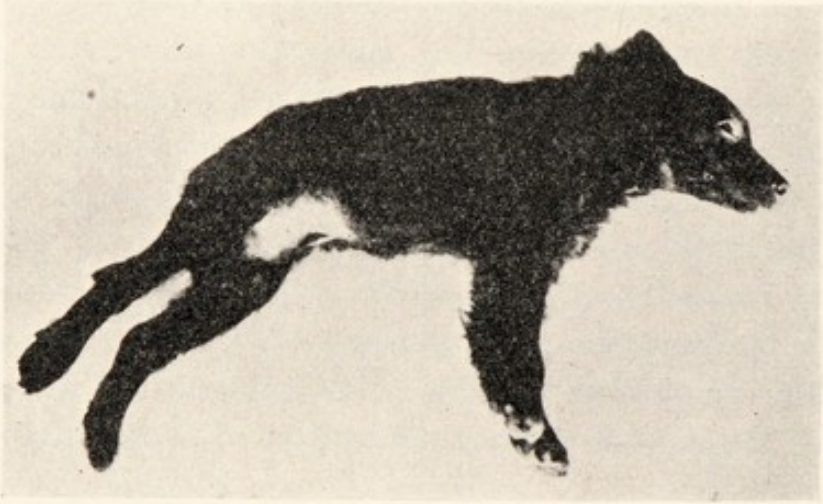

FIG. 12.—Dog in tetany, fifty-nine days after thyroparathyroidectomy. (From Collip, *J. Biol. Chem.*, 1925, lxiii, 400.)

procedures responds to treatment as satisfactorily as does the dog.

The Effects on the Calcium Distribution. The experimental data do not lead to any definite conclusion as to the relationship between the diffusible and non-diffusible fractions of calcium in the blood plasma. The methods of study used have been comparison of the calcium in cerebrospinal fluid and serum, ultra-filtration, and compensation dialysis.

Injection of a potent extract into parathyroidectomized and into normal animals causes a slight rise in the cerebrospinal fluid calcium, tending to be somewhat more delayed than that in plasma (*83*), as is to be expected from current

theories concerning the formation of the fluid (42, 5, 40). The ratio of diffusible calcium to non-diffusible calcium in plasma following such injections has been considered to be approximately the same as that in normal animals, suggesting control by the parathyroid principle (*83*), and this has been denied (*517*, *369*). Evidence has been furnished that the diffusible calcium is affected more than the non-diffusible (*370*, *420*, *317*); the reverse is also claimed (*214*). Cantarow (*90*) reports from study of injections of the extract into eleven individuals with no evidence of primary parathyroid dysfunction, that both calcium fractions are increased, the non-diffusible tending to be more greatly affected, but that they may vary independently, and that the level of diffusible calcium is not entirely dependent on the total serum-calcium level.

FIG. 13.—The same dog as in Fig. 12. Complete recovery three hours after subcutaneous injection of a potent parathyroid extract. (From Collip, *J. Biol. Chem.*, *loc. cit.*)

It has been suggested that the effect of the parathyroid principle is primarily upon the blood phosphate (*5*, *6*); the potential interrelationship between plasma calcium and phosphate has already been discussed (cf. p. 101).[1]

Thomson and Collip (71) have covered all the literature

[1] Following the administration of a potent parathyroid extract (parathormone) there is a slight increase in blood magnesium, antecedent to the rise in blood calcium (62, 25).

bearing upon the problem very fully, and have discussed it critically.

Methods of Assay. Up to the present time there is no biological procedure sufficiently precise to be generally acceptable.

Collip (*105*) originally defined the unit of potency of a parathyroid extract as one one-hundredth of the amount of extract which will produce in fifteen hours an average increase of 5 mg. per 100 c.c. serum in the blood calcium of normal dogs weighing about 20 kg., following subcutaneous or intramuscular injection. Individual dogs exhibit considerable variation in their response, and the response for any single dog may vary at different times. Hence the average for a fairly large number is necessary. They should be starved for twenty hours before the test, and young dogs are recommended (10). The actual response is roughly proportional to the dose. There is, however, no regular relationship between the result from a given dose, and the weight of the animal used for assay (71). The method can only be considered as roughly accurate.

Burn (*79*) has suggested that the rise produced in the serum calcium of cats in two hours, after intravenous injection, should be used as assay, but Allardyce (*9*) found no appreciable rise, so that the method seems unsound.

Hanson (*205*) has proposed a smaller unit—1 per cent. of the amount required to produce a 1 mg. rise in the serum calcium of 15 kg. dogs twenty-four hours after parathyroidectomy.

A somewhat tedious but possibly accurate procedure has been recently suggested based upon increase in urinary calcium when rats are injected with parathyroid extracts (18, 55).

The Relationship between the Parathyroids and Vitamin *D* and the Function of the Parathyroids

It should scarcely be necessary, with our present knowledge of the effects of the parathyroid principle, to refer in any detail to the guanidine theory of parathyroid function. This theory arose from the detoxication obsession which has not infrequently tended to retard the advance of endocrinology. The evidence against the theory has been fully summarized elsewhere (10, 71, *143*), and this evidence overwhelmingly disproves it.

Vitamin *D*, *calciferol*, $C_{27}H_{41}OH$, a steric modification of ergosterol, formed from it by irradiation with ultra-violet

light, is superficially so related to calcium metabolism as to suggest that there is an interrelationship between the vitamin and the parathyroid principle. When there is a deficiency of the vitamin through lack of exposure to the sun of the material of the diet or of the individual, or of both, the blood calcium may be lowered. Administration of the vitamin in such a condition (one form of rickets) restores the blood calcium to normal. Overdosage of the vitamin, if marked, leads to hypercalcaemia, and to deposition of calcium salts in various sites.

Hess and his co-workers found that when rachitic children were fed moderately large doses of a concentrate of vitamin *D*, a hypercalcaemia was sometimes produced. They concluded that the effect was due to stimulation of the parathyroids by the vitamin. If this were the case, parathyroidectomy should prevent the effect. They found no elevation of blood calcium following administration of the vitamin to parathyroidectomized monkeys and dogs (*216, 219;* cf. *186*). Other investigators have shown that when large doses are administered parathyroidectomized dogs can be maintained in good health at normal calcium level (*266, 131*), and that the treatment is beneficial when tetany follows human parathyroidectomy (*71, 479, 254*).

All such successes are open to the criticism that parathyroid tissue had not been completely removed, and that the residual traces had been stimulated to compensatory action by the vitamin. Taylor has investigated this point carefully, and has found that in animals in which all tissue liable to contain accessory parathyroids has been removed the resulting tetany is usually fatal and cannot be relieved by dosage of the vitamin, however excessive (*498*). Other investigators did not obtain such definite results (*476*).

The available evidence concerning the action of the vitamin strongly suggests that it controls the distribution of calcium (and, directly or indirectly, of phosphate) in blood and bony tissues, and that its presence either leads to increased

absorption of calcium from the intestine (9) or depresses the excretion of calcium into the intestine (70). There is no conclusive evidence that the parathyroid principle affects the absorption of calcium (71). The results ensuing from overdosage of the vitamin depend upon the availability of calcium. If the diet provides ample, hypercalcification follows. If the diet is deficient in calcium, bone is denuded of it, and this may or may not lead to metastatic calcification (9).

The Function of the Parathyroid Principle. It is evident, from what has just been stated, that it is still uncertain whether or not the active principle of the parathyroid is in any way controlled by vitamin *D*. There is no evidence suggesting any control in the reverse direction. That nervous control is at least unnecessary for correct function has been mentioned (cf. p. 95).

The most outstanding effects following injection of the principle suggest that it controls the height of blood calcium, and perhaps of some particular fraction of that calcium, but the evidence that has been cited permits no definite conclusion that there is any direct control.

There is very definite evidence that the principle acts directly on the solid material of bone. The complex mechanism of bone formation will not be dealt with here. Action of a specific enzyme, a phosphatase, is involved. The studies of Robison, Kay, and others, on the action of this bone phosphatase, have recently been summarized by Kay (*277*).

It is important in all studies of calcification and decalcification to remember that the solid material of bone is in a state of flux, liable to drain and repair according to other needs of the organism. This solid material not only functions as a supporting framework, but also as a storehouse for calcium and perhaps also for phosphate. This is well shown in the calcium exchanges during lactation, where frequently the drainage of calcium from the body during milk formation

is vastly greater than the total amount of calcium present in other than bony tissue (49). In many other less drastic events bone is denuded of some proportion of its store (71).

Thomson and Collip (71) believe that the parathyroid principle acts primarily on bone, causing liberation of calcium by some direct stimulating action. Such a theory possibly involves the assumption that the plasma is not normally saturated with respect to the bone solid. Equally possible is the assumption that such saturation only exists locally in bone, and is due to the action of bone phosphatase in increasing local concentration of inorganic phosphate. It seems, possibly, that one result of action of the parathyroid principle is depression of the action of the bone phosphatase (the data on this point are contradictory) (71).

Histologically the evidence is definite. Experiments in Collip's laboratory, carried out by Selye on rats, in which sub-lethal doses of a concentrated extract of the parathyroid principle were injected over long periods, showed that the effects can be divided into two stages. During the first stage fibrous transformation of the bone marrow and the formation of numerous osteoclasts can be seen. These osteoclasts bring about absorption of bone, and thereby denude the skeleton of calcium. During this first stage numerous calcium deposits appear in various organs. The bone picture is similar to that seen in osteitis fibrosa generalis (see p. 121).

When the injections are continued over a long period the rats pass into a stage of apparent immunity to the parathyroid principle, which is, however, actually a state of increased tolerance (cf. p. 111). In this stage the bone marrow again changes, osteoclasts disappear, and a large number of osteoblasts appear. These prevent further denudation of bone from the skeleton, and may even lead to increased deposition of solid in bone; the final pathological picture is suggestive of so-called "marble bone." The apposition of new bone tissue is most active in the

metaphysis of the long bones, just as in marble-bone disease ; the shaft remains practically normal (cf. 63 ; also 71, 25).

The experiments of Pugsley (55) are in chemical agreement with these findings. In such rats prolonged injections lead first to increased calcium excretion, but finally to decreased excretion.

Selye showed further that if only very small doses of the principle are administered there is no osteoclast formation, so that the first stage is omitted ; within a few days the osteoblasts become larger and more numerous and bone apposition is stimulated.

Vitamin *D* at first sight appears to produce comparable results. When it is given in large doses to very young animals it leads to bone resorption with spontaneous fractures (65, 11, 61). But when it is given in small amounts over long periods increased calcium deposition in bone results, the cortical tissue becoming denser and thicker (60).

Selye has shown (63) that while the macroscopical aspect of the bones after such treatment is extremely similar to that observed after chronic parathyroid overdosage, histologically the picture is very different. Osteoblasts and osteoclasts are present in normal quantities. The bone marrow is of the lymphoid type. The epiphyseal cartilage is extremely narrow and irregular. The zone of preliminary calcification is well developed in some parts and totally absent in others, in one and the same bone. The subepiphyseal zone is composed of small amounts of spongy tissue, while the rest of the metaphysis contains only compact bone. The enlargement of the shaft is less conspicuous, but is demonstrable. Both on the periosteal and on the inner wall of the original shaft thick layers of newly formed osteoid tissue are apposited. Many bone lacunae in the wall of the original shaft are empty, indicating death of bone cells under the influence of the vitamin. The new bone

formation in this vitamin intoxication may be merely of a compensatory nature.

Selye's observations seem to lessen the probability that parathyroid action is under vitamin control. Slight dosage of the principle, and, therefore, probably the normal action of the principle, facilitates bone deposition. Increased parathyroid action, if sufficiently prolonged, reverses the procedure. This seems to render unnecessary any assumption that there is direct action on blood calcium. It has also been shown recently (72) that the parathyroid principle does not increase the solvent power of blood plasma for the calcium compounds of bone.[1]

Present information thus permits a theory, very incomplete, to be enunciated concerning parathyroid action. Bone deposition or denudation depends upon the concentration of the principle. How that acts in controlling production of osteoblasts and osteoclasts, and why continued overdosage reverses this action, we do not know. The vitamin controls absorption or excretion of calcium through the intestinal wall. It affects bone structure when present in marked excess. Its effect, if any, on bone under normal conditions is not known. The rough constancy of blood calcium probably represents the result of rough equilibria depending on the rates of absorption and excretion from the gut and the degree of bone deposition or denudation which is taking place. Evidently undue drainage from the organism, as in lactation, causes bone denudation, and one might venture to suggest that the action of the parathyroid principle is to some extent governed by the calcium con-

[1] Ortenberg (51A) has recently reported a curious case of a man, aged fifty-nine, in whom X-ray examination showed a picture characteristic of osteitis fibrosa cystica, but whose blood calcium was normal. Traumatic fractures remained ununited for four months, despite rest, immobilization, and treatment with vitamin *D* preparations and calcium. Moderate dosage with "parathormone" (Collip's parathyroid extract) led to rapid formation of callus, to restoration of the bony architecture in varying degrees, and to rapid resorption of a pitting oedema.

centration of the blood circulating in bony tissues. When the glands are extirpated denudation does not occur and the blood calcium falls.[1]

Hypoparathyroidism

Hypoparathyroidism is seen most frequently as a clinical condition following surgical interference in thyroidectomies. Not rarely, following this operation, a transient state of latent tetany is found, accompanied by a slight fall in blood calcium (56). When such latent tetany persists, or open manifestations occur, they can usually be controlled by the oral administration of calcium lactate, or, still better, by injection of a potent parathyroid extract. Vitamin *D* is also serviceable (cf. p. 115). It but seldom happens that so much parathyroid tissue is irretrievably damaged that persistent tetany results. Even after a long interval hypertrophy of a trace of remaining tissue seems to be possible (*365*).

Boothby has recently outlined the most satisfactory treatment following post-operative parathyroid insufficiency (6A). Good results are stated to follow administration of lactose (47).

Lisser and Shepardson (*316*) have shown that when, through permanent and complete damage, continued administration of parathyroid extract is required, a gradual tolerance is set up, calling for increased dosage to control tetany, and finally even this becomes ineffective and death ensues. This acquired tolerance is in agreement with the effects of continued dosage noted with dogs and rats (cf. p. 111), and Selye's work indicates that it is due to a reversal of

[1] It has been claimed (36A) that when sufficient vitamin *D* is administered to parathyroidectomized pups to maintain normal blood calcium and phosphorus, normal bone development occurs, and, if such treatment is maintained, the parathyroidectomized animals can successfully survive an entire reproductive cycle. If such a statement is confirmed, then the conclusion reached by the investigators seems rational, and it would seem that the parathyroid glands do not perform a specific function in metabolism essential to life.

parathyroid effect (cf. p. 117). According to Reed and Seed (56A) large doses of irradiated ergosterol are equally effective and patients do not become refractory to this treatment.

There is some evidence that, especially in children, certain cases of tetany are associated with deficiency in parathyroid function due to haemorrhage into the glands (36, 3, 73). The administration of parathyroid extract has been found beneficial. Shannon (65A) has also found its administration is beneficial in certain children manifesting psychic disturbances (convulsions, irrationalism, acute maniac excitation, etc.) that he believed were due to hypoparathyroidism.

Hyperparathyroidism

One of the most important recent advances associated with the parathyroid gland is the recognition that generalized osteitis fibrosa (von Recklinghausen's disease of bone) is due to the hyperfunctioning of the gland.

In this disease the serum calcium is generally somewhat above normal, although normal values have been found (76). The hyperfunction is generally due to a tumour of some one of the glands, although the enlargement is seldom sufficient to cause a swelling of the neck. If the tumour is removed, marked improvement frequently results, and at the same time the blood calcium falls to normal or subnormal values. The operation was first performed by Mandl (*334*, *335*). The literature has been thoroughly reviewed by Hunter (30, 31), who has presented exact studies of two cases. The following description is largely taken from his review (31) :

Generalized Osteitis Fibrosa is progressive, with pain, fractures, and markedly disabling deformities, and usually proceeds to a fatal termination. All bones may show pathological decalcification with osteoclastomata. Multiple foci of osteitis fibrosa occur, with or without benign giant-celled tumours and cysts. The earlier cases were frequently

confused with osteomalacia. The condition has been found twice as frequently in adult women as in adult men. Renal calculi have been found in one-third of the cases. Metastatic calcification is not infrequent. Thirst and polyuria are less frequent symptoms.

The disease was differentiated from osteomalacia by von Recklinghausen in 1891. In 1904 Askenazy described a case associated with parathyroid tumour, the tumour appearing at autopsy almost like a second thyroid lobe. In 1925 Hoffheinz collected 45 cases in the literature in which definite enlargement of one or more parathyroid glands was reported. Of these cases 27 were associated with definite bone disease ; 17 were cases of generalized osteitis fibrosa, 8 of osteomalacia, and 2 of rickets. The enlarged glands either showed hyperplasia or an adenoma.

Histological examination of the bone in osteitis fibrosa shows lacunar resorption, apposition, fibrosis of marrow, and formation of osteoclastomata and cysts. There is a general osteoporosis (31).

Chemical study shows high blood calcium, low blood inorganic phosphate and markedly increased excretion of calcium and phosphorus, so that the metabolic changes resemble those in induced hyperparathyroidism in animals (*203, 418*).

By 1931, 32 definite cases of this clinical form of hyperparathyroidism had been reported. In 22 of these a parathyroid tumour was removed at operation, in 2 cases two parathyroid tumours, and in 5 no tumours were found. Two of the remaining three exhibited one tumour at autopsy, and the third two such tumours. A tumour was palpable in the neck in only 5 cases. The largest tumour so far recorded measured $7{\cdot}5 \times 5{\cdot}0 \times 1{\cdot}8$ cm. and weighed 26·2 grams. It was not palpable since it was situated behind the trachea.

The size of the tumour bears no relation to the severity of the bone lesions ; in a very severely crippled patient the tumour only weighed 1·3 grams. Radiographs show greatly

diminished density of bone shadow, and pictures comparable with those seen in osteomalacia and generalized carcino-

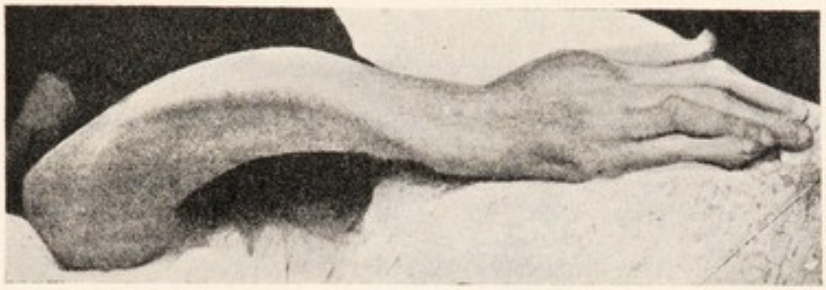

FIG. 14.—Antero-external curvature of forearm and large bony swelling on dorsum of right hand from a case of generalized osteitis fibrosa. (From Hunter, *Proc. Roy. Soc. Med.*, 1931, xxiv, 489 ; Clin. Sect.)

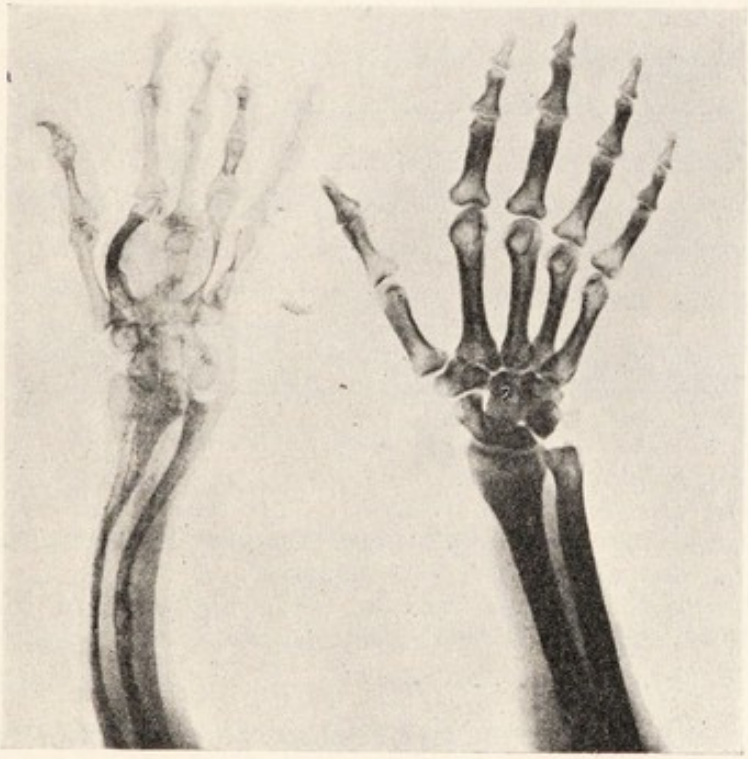

FIG. 15.—Controlled radiograph of right hand and forearm (cf. Fig. 14). (From Hunter, *Proc. Roy. Soc. Med.*, *loc. cit.*).

matosis. Figures recorded for serum calcium vary from 10·6 in one patient (actually on four successive days 11·5, 11·0, 10·6 and 11·0, showing thus a tendency to slight increase) (76) up to 23·6 mg. per 100 c.c., and for plasma

phosphorus from 1·0 to 3·0. In 9 cases in which the plasma phosphatase was estimated it was invariably high. Calcium excretion varied from slight increase up to eight times the normal amount.

Operation abolishes pain in almost all cases. Restoration of calcium metabolism to normal occurs with varying

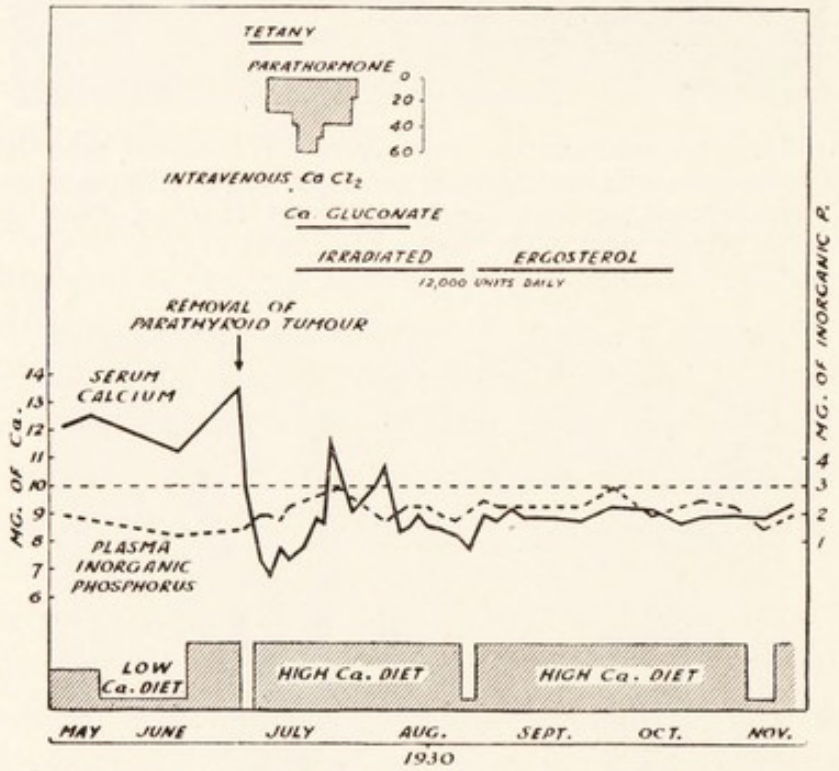

FIG. 16.—Chemistry of the blood of Hunter's case of generalized osteitis fibrosa. The blood was examined for two months before and for five months after operation. The low calcium diet was that given during the investigation of the calcium balance. The high calcium diet was not weighed. (From Hunter, *Proc. Roy. Soc. Med.*, *loc. cit.*)

rapidity. A hypocalcaemia frequently develops, and latent and even open tetany may occur. General symptomatic improvement takes place, and crippled patients may recover sufficiently to be able to walk without artificial aid.

Figs. 14 and 15 depict the typical bony curvature and diminished density of bone shadow seen in one of Hunter's cases. Fig. 16 shows the changes in blood calcium and phosphorus in the same case.

Experiments with animals confirm hyperparathyroidism as the cause of the disease. The typical picture has been produced in dogs, guinea-pigs and rats (*260*, 33, 35, 77, 34). In a normal human subject sufficient symptoms have been produced by somewhat less drastic overdosage to confirm the relationship (31, 35). Selye's studies (p. 117) afford final confirmation.

While, when the diagnosis is certain, surgery seems the most obvious method of treatment, good results have been claimed following administration of acid sodium phosphate (1).

Cases of Paget's osteitis deformans exhibit no evidence of hyperparathyroidism, although the bone picture is similar. Focal osteitis fibrosa, affecting one or more bones, and of slow progress with a tendency to arrest, is also not a condition associated with hyperparathyroidism. There seems to be a possibility that haematogenous myelomatosis may be associated with such hyperfunction (31).

Marble bone disease, a condition of extreme brittleness of the bones, can now be definitely associated with chronic hyperparathyroidism. A typical case has been described (54) in which enlargement of the parathyroids was found. Selye's experiments show that the histological picture of the bones in this disease is produced in rats following such prolonged overdosage of the parathyroid principle that a state of induced tolerance is produced (cf. p. 117).

Selye (64) has described a specific skin condition in very young rats following injection of parathyroid extract. Within two or three days the hair on the back, extending bilaterally from the head to the lower border of the ribs, begins to fall out, and the skin in this area becomes harder and thicker. Ulceration takes place in some parts, and, healing, leaves a bare, hairless, atrophic skin. The fibrous tissue in the skin hypertrophies, and amorphous deposits of calcium salts occur. The condition possesses striking points of similarity with human scleroderma and sclerodactylia,

and suggests that these may be related to hyperparathyroidism, since in most clinical cases the blood calcium is high.

Administration of Parathyroid Extract in Non-Parathyroid States

Lead is stored in the skeleton in a manner somewhat analogous to that by which calcium is laid down, and probably as a very insoluble tertiary phosphate (19). During the chronic stage of plumbism such storage prevents undue accumulation in other tissues to the point of toxicity. Absorption in large quantities, or liberation from bone in large quantities, leads to symptoms of acute poisoning. After exposure to lead poisoning with ensuing storage in the skeleton, lead is excreted in minute amounts over very long periods. Administration of potent parathyroid extracts to patients suffering from lead poisoning mobilizes a portion of the lead stored in bone, causing excretion of relatively large amounts. The effect lessens rapidly (32). Similar treatment has been employed in radium poisoning (21).

Since the parathyroid principle induces diuresis (*4*, *5*, *251*, *495*) it has been employed in nephrosis, and clinical improvement has been reported (*127*, *346*, *340*), oedema tending to disappear.

(Parathyroid extracts have been prepared which do not affect the level of blood calcium and which powerfully retard growth (*501*, *502*). Similar extracts may be obtained from other tissues, and the effect is not specific to the parathyroid glands, nor presumably concerned with their function (71).)

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CHAPTER IV

THE ISLETS OF LANGERHANS AND INSULIN

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Introduction

THE discovery of insulin by Banting and Best in 1921, working in Macleod's laboratory, and its preparation for clinical use, in which work Collip largely participated, led to rapid strides, not only in the treatment of diabetes mellitus, but also in the elucidation of many of the problems of carbohydrate metabolism. It seems desirable to set forth the main points, concerning which there is reasonable agreement, before considering in detail some of the more recent work (cf. 113, 155).

Insulin is an endocrine principle prepared by, and passed into the general circulation from the islets of Langerhans of the pancreas. When the islet function is disturbed definite symptoms follow. If the disturbance lessens the output of insulin below an essential minimum then a hyperglycaemia follows, and if the condition of *hypoinsulinism* persists, all the symptoms and findings associated with *diabetes mellitus* ensue. If, on the other hand, through generalized hyper-

plasia or a tumour of the islets, benign or malignant, the output of insulin is increased above a definite normal maximum, then this condition of *hyperinsulinism* produces a hypoglycaemia, which, if sufficiently pronounced, is accompanied by marked and characteristic symptoms, and if unrelieved, by coma and death.

The work of von Mering and Minkowski on the depancreatized dog, confirmed and extended by that of Allen, suggested most strongly the identity of its diabetes with human diabetes mellitus. The discovery of insulin permitted this identity to be finally established.

From studies of the diabetic dog, compared with the histories of diabetic patients, we know that as a result of diminution of islet function (through removal or through disease), there results first a loss of power to catabolize carbohydrate, shown by undue hyperglycaemia, and a glycosuria. This loss of power increases, and the increase is hastened if the diet continues to include the usual proportion of carbohydrate, but is slowed if that carbohydrate is largely replaced by protein and fat. When the amount of carbohydrate correctly catabolized falls below a certain definite level, fat catabolism is also affected, and instead of the fatty acids being completely oxidized to carbon dioxide and water through the stages of butyric acid, beta-hydroxy-butyric acid, and acetoacetic acid, a slower transformation to acetone gradually replaces this oxidation. This change is so slow that acetoacetic acid and its precursor accumulate in the tissues. They pass to the blood, which maintains its neutrality by combining them with blood base and excreting the neutral product through the kidneys. Consequently the blood base becomes diminished. As it gradually falls, so gradually, the symptoms of an acidosis become apparent. The untreated dog, or patient, finally passes into coma, in which "air-hunger" becomes a symptom through the incapacity of the diminished blood base to clear the organism of accumulating carbon dioxide. Finally death ensues.

Thus the depancreatized dog and the untreated diabetic patient show, in order, the development of hyperglycaemia, glycosuria, acetonuria (and acetone in breath), presence of acetoacetic acid in urine, and diminution of blood bases to low levels. These chemical changes are accompanied by the clinical symptoms of thirst (since more water is required to excrete unoxidized glucose), hunger (since much of the ingested carbohydrate cannot be profitably utilized), fatigue (since the carbohydrate that is utilizable is insufficient for muscular needs), loss of weight (again due to insufficiency of utilizable carbohydrate so that body fat and finally body protein are drawn upon), and the ultimate drowsiness and coma which accompany the acidosis.

Injection of insulin in sufficient quantity and at sufficient intervals reverses the order of these changes and ultimately restores normality. If a state of coma has supervened, insulin, with, if necessary, intravenous glucose solution, abolishes it; the ketonuria is banished, normal fat catabolism being restored. Glycosuria disappears; the hyperglycaemia lessens. With correct dosage of insulin (along with correct supervision of diet and control of exercise and work) the diabetic patient can be maintained for years in health. The depancreatized dog (fed raw pancreas to maintain pancreatic acinar function [1]) is also capable of living for a number of years. Hédon's dog, a classic example, was kept alive 57 months (cf. p. 154).

If the injection of insulin is too great for normal conditions, then the blood sugar is depressed below normal. The artificial hyperinsulinism leads to a hypoglycaemia, which is accompanied by striking symptoms. These were accurately described by Mann and Magath in the hepatectomized

[1] This has been the usual view concerning the beneficial action of raw pancreas in the diet of the depancreatized dog. Best, however, has quite recently shown that the pancreas supplies choline, a necessary dietary adjunct, and that lecithin, or choline itself, can be substituted for this raw pancreas. In absence of such material, fatty infiltration and degeneration of the liver sooner or later ensue (13A).

dog (116). The hypoglycaemia ultimately leads to a coma, but a coma in which the use of insulin may be (and has been) fatal.

Numerous texts have been written, dealing with the correct standardization and treatment of the diabetic patient. Such matter falls outside the scope of this volume, except in so far as the principles of treatment are concerned. The mechanism of insulin action is still in great part a riddle.

The Anatomy, Histology, and Physiology of the Islets

It has usually been considered, since the work of Macleod on the encapsulated islets in fishes (113), that the islets are tissue *sui generis*, whose function is not related to that of the acinar tissue of the pancreas, and which are concerned solely with the elaboration of insulin ; further, that insulin is not produced by other than islet tissue.

The question has been in part reopened by Bierry and Killman (14), who, while not denying that the islet tissue has a special function, believe it to be formed from acinar tissue, although they consider that it cannot revert to acinar tissue. They claim that even in fishes it is impossible to separate islet tissue completely from acinar tissue. Such a statement calls for further examination.

There is little new of importance concerning the histology of the islet cells. Bensley's work, showing the presence of two distinct types of cells, *A*, relatively large, with a large elliptical or spherical nucleus, and *B*, smaller, more numerous, with smaller nucleus and cytoplasm packed with granules, has been confirmed by various investigators and is generally accepted. He rejects the existence of transition types from acinous to islet cells. Opie has summarized the literature (130).

The islets appear to be under control of the vagus (113, 80, 181). La Barre (100) finds that the controlling centre is not in the cerebral hemispheres but is affected by separation

of the thalamic region from the remainder of the central nervous system.

The Chemical Nature of Insulin

Very powerful insulin preparations have been obtained by various procedures. Certain of these are probably 80 or 90 per cent. pure.[1]

Crystallization is recognized as a necessary step in the preparation of any compound in pure condition. Insulin was first crystallized by Abel in 1926 (1). His method depends on treating acetic acid solutions of commercial insulin preparations (of strength 10 to 20 clinical units per mg.) with excess of brucine acetate, and then with pyridine. At *p*H 4·2 to 5·3 pyridine precipitates various impurities. By addition of sufficient 0·65 per cent. ammonia the *p*H is raised to about 5·6. Insulin crystallizes out (2). It can be recrystallized (without the presence of brucine acetate) without loss of activity. It has been subsequently crystallized from crude preparations without brucine or ammonia (167). Harington and Scott (72) have devised a procedure whereby the use of saponins or of digitonin leads to crystallization.

Chemical and Physical Properties of Crystalline Insulin. The crystalline compound analyses to give the empirical formula $C_{45}H_{69}O_{14}N_{11}S$ (2, 72). It gives the Biuret, Pauly, Millon, and ninhydrin reactions, but tryptophane radicals appear to be absent. Accurate studies of the hydrolysis indicate the following distribution of amino-acid radicals: tyrosine 12 per cent., cystine 12 per cent., glutamic acid

[1] The present insulin standard, accepted by the Geneva Conference of 1925, is a particular preparation of insulin hydrochloride in dry powder form. The unit of insulin is the amount of the principle present in one-eighth of a milligram of this material. Insulin is assayed biologically by measuring the fall in blood sugar produced in rabbits under standard conditions of comparison. Its strength is expressed in the number of units per milligram of the material that is being assayed (cf. 26).

21 per cent., leucine 30 per cent., arginine 3 per cent., histidine 8 per cent., lysine 2 per cent. It is doubtful if any other amino-acid is present in large amount. Aspartic acid, hydroxy-glutamic acid, and glycine appear to be absent, nor has any constituent foreign to the ordinary protein molecule been detected. The presence of proline and valine is doubtful (167, 92, 90).

Insulin crystals are well defined; they seldom exceed 0·01 mm. in diameter (2). They are always uniform; seen under the low power, their most conspicuous feature is the hexagonal outline noted by Abel. Under high power they have the appearance of cubes, or of rhombohedra approximating to cubes, standing on one corner. They are weakly doubly refracting, and have a refractive index of approximately 1·58 (72).

Crystalline insulin is optically active and laevo-rotatory. The actual rotation varies markedly with the *p*H of the medium (2). Its molecular weight, determined by ultra-centrifuge methods, is 35,100, the molecules being spherical, with a radius of 2·18 mμ. Within the limits of error the molecular weight and size of insulin are identical with those of egg albumin and Bence Jones' protein (159).[1]

Abel (2) considered the question as to whether his crystals were really insulin, or whether insulin is in reality "an unknown substance of almost unbelievable potency adsorbed by the crystals." Since two recrystallizations did not affect the physiological activity he concluded that there was no adsorption of a still unknown compound.

Dingemanse (44, 22), has claimed that it is possible to prepare an insulin more active than crystalline insulin, with slightly higher sulphur and nitrogen content, and representing the prosthetic group of insulin, by adsorption on charcoal, elution with phenol, and precipitation from the

[1] Since its molecular size is only that of Bence Jones' protein and its weight half that of plasma albumin, it is not surprising that insulin is normally excreted in urine (132). The amount excreted does not exceed 1·5 clinical units per 100 c.c. of urine (23).

phenol solution by dilution with water. This work has not been confirmed (168, 87). Such views of active prosthetic groups, suggested by Willstätter's theory of enzymic activity, and supported by Waldschmidt-Leitz's claim to have decomposed crystalline urease by trypsin without destroying the enzymic activity (169), while of interest, cannot be considered, as far as endocrine compounds are concerned, to have any supporting evidence of sufficient strength to need serious consideration at present. Dingemanse's methods do not suggest sufficiently drastic interference with the protein molecule to cause separation of a prosthetic group.

Changes affecting the whole molecule support the view that the activity of insulin is inherent in the molecule itself. Insulin, acetylated by treatment with acetic anhydride and pyridine, and thereby inactivated, has one-third of its activity regenerated by addition of sodium hydroxide (58, 89). Solutions of crystalline insulin and also very active amorphous preparations coagulate on addition of hot, dilute hydrochloric acid; the coagulum retains almost all the activity. Addition of formaldehyde inactivates insulin; splitting off the formaldehyde in part regenerates the activity. Addition of dilute alkali to insulin solutions causes a loss of activity and a parallel loss of either ammonia or primary amine (58, 88). Crystalline insulin is inactivated by acid methyl alcohol, and partially reactivated by sodium hydroxide. When this reactivated material is recrystallized, the crystals show the same microscopic appearance, isoelectric point, and degree of physiological activity as the original crystals (34). A definite, small amount of iodine inactivates insulin immediately; this is probably due to oxidation of its disulphide linkages (91). Digestion experiments with different enzymes (trypsin, pepsin) indicate that the slightest degree of destruction of the protein molecule results in loss of activity (34).

The Identity and Clinical Value of Insulin from Different Sources. Crystalline insulin from fish islet tissue and from

beef pancreas is identical in shape, physiological activity (24 units per mg.) and sulphur content (93). The same beef material, assayed in four different laboratories, gave the respective values 23, 24, 24, and 23 to 28 units per mg. Three recrystallizations did not affect the strength (93). Four different batches of crystals prepared by two different methods and from different sources, and assayed by four different persons, gave strikingly uniform results, the average of all being $23{\cdot}3 \pm 0{\cdot}6$ units per mg. (41). Crystallized fish, hog, and sheep insulins have been compared with beef insulin recrystallized ten times, and found to have, within the limit of experimental error, the same physiological activity and sulphur content (153).

Such results suggest that there is but one insulin, and have, therefore, some bearing on the sensitivity reactions of certain diabetics to insulin.

Allergic and Other Toxic Reactions to Insulin. A very complete summary of the literature dealing with allergic manifestations following injections of insulin has recently been published by Allan and Scherer (5). They point out that while the first impure preparations of insulin caused local irritation of the skin and subcutaneous tissues at the site of injection, in a few cases there appeared general symptoms of an anaphylactic reaction. Such phenomena were observed less frequently as methods of extraction and purification improved. Possibilities of anaphylactic shock were recognized early, but it was found that in most cases sensitization effects were absent. Occasional sporadic cases of hypersensitiveness have been recorded. Summarizing the observations made at the Mayo Clinic during the past few years Allan and Scherer state that hypersensitiveness to insulin occurs in approximately one out of eight or ten cases. Of 100 consecutive cases manifesting such hypersensitiveness, four showed generalized symptoms of anaphylaxis, in eighty-four there was only a mild reaction at the site of injection, usually relieved by a change in the type of insulin or by

spontaneous desensitization, and in twelve cases there was a severe local reaction with less relief from change in insulin.

Such results appear to suggest, especially in those cases where benefit is obtained by change of the insulin material employed, that the allergic phenomena may be due to protein impurities and not to insulin itself. However the purest material can produce the effect. Campbell, Gardiner, and Scott (31) report that " one patient shows marked sensitivity to beef, hog, sheep, fish, and human insulin obtained from different sources. He is also sensitive to crystalline insulin though the reaction is less intense." It would therefore appear probable that insulin from different animals may possess slightly varying protein structure, the type of variation being comparable, but perhaps even less than that of the haemoglobins of different animals.

Other still more unusual toxic manifestations have been recorded as, for example, a transient haematuria (104); headache, dizziness, lack of muscular control (133), and transient hemiplegia.

The Mechanism of Insulin Action

The precise mechanism of insulin action has still to be elucidated. Following its subcutaneous injection the most striking phenomenon is the lowering of concentration of blood sugar. Glucose disappears from the blood. Yet *in vitro* experiments show no direct action of insulin on blood glucose. The tissues, under insulin stimulus, draw glucose from the blood more rapidly than in absence of insulin. Macleod terms this action the creation of a " vacuum for glucose " in the tissues (113). When a surviving heart preparation is perfused with a fluid containing glucose and insulin, the heart muscle tissue removes glucose at a faster rate than when the perfusion fluid contains glucose but no insulin.

Sugar tolerance curves in normal persons show a marked

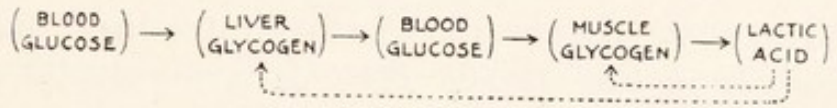
difference for venous and arterial blood. This at once suggests removal of glucose by the tissues at a fairly rapid rate during its passage through the capillaries, once its concentration has risen distinctly above the fasting level (53, 57, 65). This normal difference tends to disappear in the diabetic, and the severer the diabetes, the more closely the curves approximate (48, 65, 141), illustrating loss of power to utilize glucose by the tissues. This power is restored by the action of insulin (113, 101, 38). Mann and Magath (117) showed that the presence or absence of the liver in an animal had but little effect on the rate at which glucose is removed from blood under the influence of insulin ; muscle tissue is of greater importance. Macleod considers that the chief sites of insulin action are the cardiac and skeletal muscles (113).

It is claimed that insulin lessens the lactic acid content of muscle and increases the production of acetaldehyde in liver pulp ; it does not appear to affect the metabolism of fructose. Both the diabetic patient and the depancreatized dog seem able to form glycogen from fructose more easily than from glucose (113).

It is generally conceded that insulin facilitates and perhaps controls the formation of muscle glycogen from blood glucose. It is still disputed whether like control is exercised over formation of liver glycogen, and whether insulin facilitates disposal of glucose in any other way than by formation of glycogen. Thus Lawrence (103) writes " Insulin prepares and stores glycogen for burning but does not actually burn it." Joslin (95) says " To-day we know that the diabetic can burn sugar if the sugar first can be transformed into glycogen " ; since the diabetic suffers from hypoinsulinism the transformation is obviously attributed to action of insulin. Macleod (115) believes that its action is not limited to control of glycogen formation : " Taking all the effects which it produces, in diabetic as well as normal animals, we find that there is no known stage in carbohydrate metabolism,

up to that in which lactic acid is formed out of muscle glycogen, that this hormone does not affect. . . . We must assume that this hormone acts on some process which is dependent upon the structural integrity of the cells. Insulin must stimulate this process and lead, on the one hand, to a lowering of tension of glucose in the cell . . . so that sugar is removed from the blood, and, on the other hand, to accumulation of some intermediate substance which, perhaps in association with phosphate—since this decreases in the blood as markedly as sugar after insulin—is then either oxidized or polymerized to glycogen."

Of the numerous factors complicating studies of carbohydrate metabolism and insulin action one of the most important is the recently demonstrated secondary change of muscle glycogen to liver glycogen through intermediation of lactic acid, a transfer intensified by fatigue or the action of adrenine, and which completes a cycle of exchanges between liver and muscle (36) :



This cycle operates in the diabetic as well as in the normal animal (79). Thus to the extent that exercise, or any outpouring of adrenine, tends through increase of lactic acid in the blood (liberated from muscle) to effect transfer of glycogen from muscle to liver, the normal transfer in the reverse direction (liver to muscle) is lessened. If, as is perhaps the majority opinion, insulin acts by facilitating change of glucose to glycogen in both liver and muscle, in absence of such additional stimuli as adrenine or fatigue, the effect will be revealed chiefly by increase in muscle glycogen on account of the normal steady transfer of liver glycogen to muscle.

The experimental data still give no clear lead. A number of investigators find that insulin either has no action on

liver glycogen formation, or retards it, or even reverses the action (52, 17, 145, 107). Cori's experiments on rats suggest that under insulin action muscle tissue absorbs glucose so rapidly that none is available for liver glycogen formation, while blood sugar concentration also is a determining factor (37). Perhaps, however, the most important factor is the concentration of insulin itself. It has been shown that in starved rabbits, in which the liver glycogen had fallen to less than 0·2 per cent., injection of insulin in amounts insufficient to cause convulsions definitely increased liver glycogen (in eleven experiments the glycogen content varied from 0·6 to 3·8 per cent.). Similar results were obtained with dogs, using small doses of insulin. Larger (convulsive) doses caused disappearance of glycogen from the liver (54).

It seems reasonable to assume, in spite of the contradictory nature of much of the experimental evidence, that under physiological conditions one of the most important actions of insulin is the facilitation of glycogen formation from glucose in both liver and muscle tissue. Whether this is the sole action, or whether insulin also facilitates direct oxidation of glucose, cannot be stated. If the latter be not the case, it obviously follows that glucose, to be oxidized, must be first transformed to glycogen.

It has been conclusively demonstrated that when ordinary commercial preparations of insulin are injected intravenously into animals a distinct *hyperglycaemia* is produced within a few minutes, which subsequently gives place to the hypoglycaemia usually associated with insulin injection (24, 85). This anomalous effect is not produced by crystalline insulin, and must therefore be attributed to impurities in the commercial insulin preparations (60). Extracts of pancreas have been shown to produce hyperglycaemia when injected intravenously (114, 62), and the effect, when produced by insulin preparations, is probably due to traces of proteoses and peptones.

With this illustration in mind the following comment (85)

has, probably, wide application as bearing upon many of the contradictory statements in the literature dealing with endocrine principles and their reputed actions: "Many problems dealing with the physiological *rôle* of insulin in the body remain as yet unanswered; and we feel that investigators working in this field would be well advised to use the crystalline insulin rather than preparations containing variable and unknown amounts of impurities. It is only by using the pure principle that definite conclusions can be drawn as to its pharmacological action. . . . It seems particularly desirable to use as pure a preparation as possible when one does physiological experiments with hormones, since the usual impurities in them are tissue extracts, or protein split products. Both the latter as a rule are physiologically active substances which may even have a diametrically opposite effect to the active principle itself."

Terminology of Diseases Associated with the Islets of Langerhans

Following established usage, which employs such terms as hyperthyroidism, hypopituitarism and hyperparathyroidism, the diseases associated with underfunctioning or overfunctioning of islet tissue would presumably be termed hypoisletism and hyperisletism. Instead, terms have been coined from the name of the secretion rather than the gland.

In 1923 Harris (73) suggested the term *hypoinsulinism* as appropriate for *diabetes mellitus*. ("Diabetes mellitus," strictly speaking, only names a symptom, and a symptom which is not specific to the disease.) Recognizing in certain patients symptoms identical with those resulting from overdosage of insulin, he coined the term *hyperinsulinism* for their condition. The term *dysinsulinism* seems to be used with varied meanings by different writers.

While such cases of diabetes mellitus as are definitely

associated with decreased production of insulin are accurately described by the term *hypoinsulinism*, there seems to exist another type of the disease, in which the symptoms are associated, not with decreased output of insulin, but with increased output of some antagonistic principle, possibly a secretion of the pituitary, which counteracts the insulin action. Such a pituitary diabetes cannot accurately be termed a hypoinsulinism ; I have employed the term *pseudo-hypoinsulinism*.

Recent Developments in the Treatment of Diabetes Mellitus

Diet. In pre-insulin days the diabetic was kept alive by gradually decreasing the proportion of carbohydrate in his diet and replacing it by fat. Ultimately, very high fat diets were advocated, especially by Pétrén (134) and by Newburgh and Marsh (127). The limit was fixed almost solely by the necessity of avoiding ketonuria ; the ketogenic-antiketogenic ratio provided by the diet was made maximal.

Within the last few years views of diabetic specialists have been swinging more and more towards a rational normal diet, combined with the necessary insulin to control it. Such treatment is of course logical, and is parallel to that used with replacement therapy of other endocrine principles. The hypothyroid patient is kept normal by giving him such an amount of thyroid as will be equivalent to the amount of the principle which his own gland should supply, if it were normal. Under this treatment he becomes a normal person, requiring a normal diet.

Greater difficulties arise in applying such rational treatment in hypoinsulinism, since insulin is so intimately involved with the correct disposal of carbohydrate, while exercise is recognized as altering the insulin requirement. Correct treatment demands practical absence of glycosuria, and also, for at least some part of each day, of hyperglycaemia, while any dangerous degree of hypoglycaemia must be avoided.

The necessary balance is more delicate; its maintenance requires more care.

The three outstanding schools advocating a normal amount of carbohydrate in the diet are those of Porges and Adlersberg, in Vienna, of Sansum, in California, and of Rabinowitch, in Montreal.

Porges and Adlersberg have recently published a monograph dealing exhaustively with their experimental and clinical work (137). From numerous observations on non-diabetic patients they have shown that the sugar tolerance curve is affected by the type of diet on which such patients are subsisting. When curves are contrasted, obtained respectively during a period of ordinary mixed diet and a period on low carbohydrate diet, the curve for the latter tends to be higher and to return to fasting level more slowly, indicating *a relatively lower glucose tolerance*. Sometimes there is even an associated glycosuria, which is absent in tests during a period of normal diet. Excess of fat for a few days does not alter the form of the sugar tolerance curve, but an excessively fat diet continued for a long period causes as marked a lowering of sugar tolerance as does a diet poor in carbohydrate.

It is generally recognized that a slight stimulus to insulin-production—a "fore-meal" consisting of a little sugar—lessens the hyperglycaemia following a sugar meal (the Traugott-Staub effect). This effect is not abolished by feeding a fat or protein meal, but is lessened when the preparatory sugar meal is large (*e.g.*, 150 grams of glucose instead of 50 grams).

Since it is evident from such results that the islets respond to mild glucose stimulus by increased production of insulin, it seems reasonable to conclude, as Porges and Adlersberg do, that the lowered sugar tolerance curve following a *régime* poor in carbohydrate is due principally to diminished capacity of the islets for work, following diminished stimulus to them. Such results and conclusions, along with recognition of the

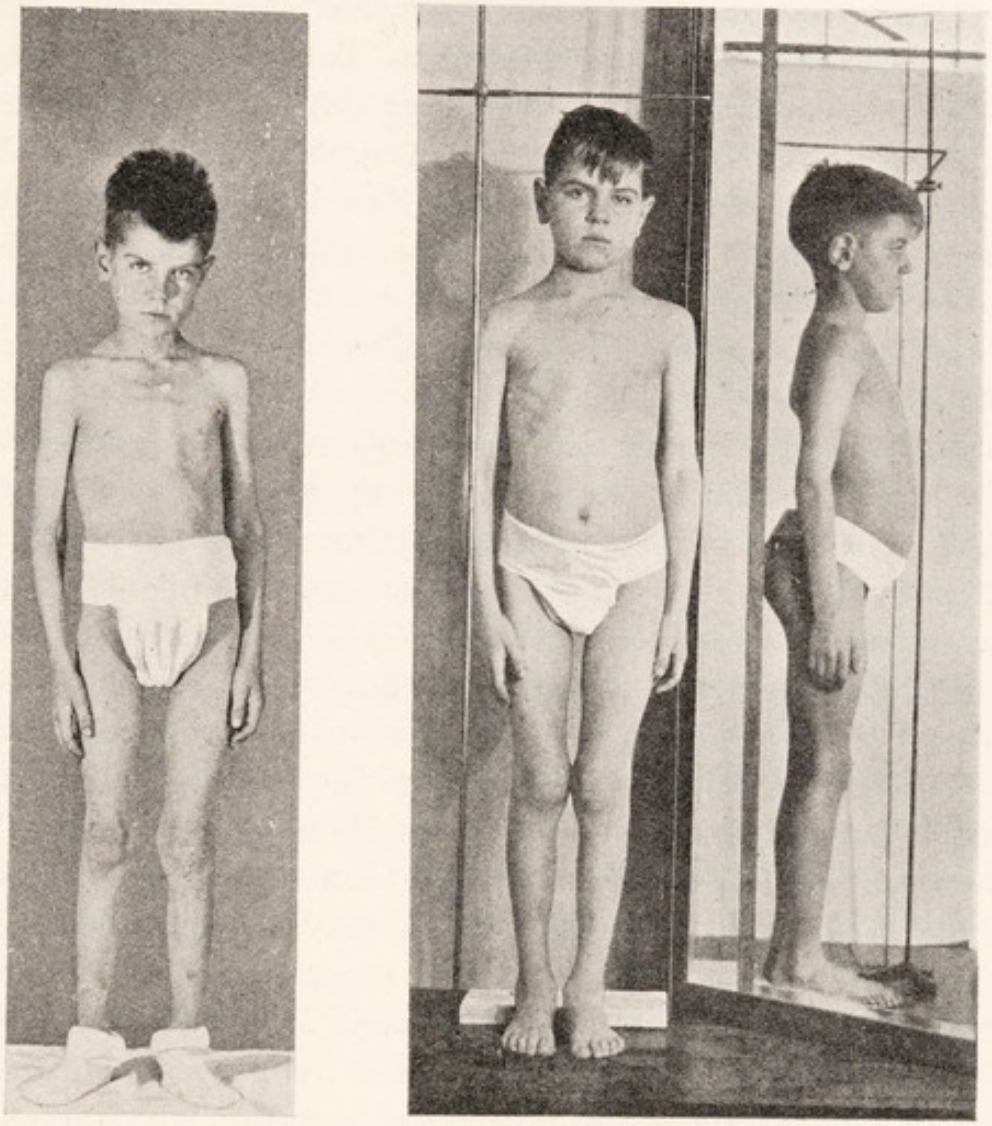
antagonistic *rôles* of glycogen-deposition and fat-deposition in liver metabolism led to the clinical treatment of severe diabetes which Porges and Adlersberg advocate, a treatment based upon what seem to be sound physiological principles.

Little fat is fed (even as little as 50 grams), in a diet yielding a total calorie value of 3,000–4,000 calories. The caloric value is mainly provided by carbohydrate, the usual amount of protein being given.

At first much insulin may be necessary, but tolerance for carbohydrate gradually increases and less insulin is required. Their diabetics put on weight under the treatment, as their tolerance increases; they like their diet better. (In mild cases no insulin is given, but a diet of 50 grams of carbohydrate, 50 grams of fat, and the rest protein.)

Rabinowitch (143) has been led to introduce a somewhat similar diet, based upon his clinical experience of the apparent benefit of slight undernutrition, combined with the potential danger of high fat, and the fact that liberal quantities of carbohydrate, approximating more closely to the diets of healthy people, seem more rational. His diet is low in fat (50 grams), normal in protein, and relatively high in carbohydrate, but so adjusted in total calorie value that the body-weight tends to be kept 5 to 10 per cent. below the normal optimum. He claims that in the majority of cases of all types of diabetes such diet leads to satisfactory results. In a few cases it has failed (16 out of 500), but in most of these the dietetic management was at fault. In many of his cases it was noteworthy that transference from relatively low to relatively high carbohydrate diet—with corresponding diminution in fat—does not require increased insulin dosage, and may even lessen the need for it.

Sansum (152) recommends a carbohydrate to fat ratio of 2 to 1, or even (especially with children) of 3 or 4 to 1, with adequate caloric requirement to maintain normal weight. He also claims to have obtained excellent results with such diets.



A. B.

FIG. 17.—**A.** September, 1931. Photograph of an eight-year-old boy after six months' treatment for severe diabetes, on a diet of 50 grams protein, 100 grams fat, and 50 grams carbohydrate, with initially 20 units of insulin daily, gradually increasing to 35 units. During this period he gained 4 lb. in weight, his urine was never completely sugar-free, and he frequently excreted acetone bodies. He could not be kept on the prescribed diet. At the period of the photograph he was tired, drowsy, and presented a pathetic figure. He was transferred to a diet of 65 grams protein, 50 grams fat, and 130 grams carbohydrate, with 30 units of insulin.
B. November, 1931. Appearance nine weeks later. During this interval he had gained 16 lb. His insulin requirement was now only 14 units daily. He appeared and was a happy, contented schoolboy. Since this time his course has been uneventful. (Reproduced by the kindness of Dr. H. Medovy.)

We have obtained, in Winnipeg, excellent results with normal carbohydrate and normal fat diets, fully bearing out the general principles just described (cf. 28). An excellent example of the beneficial effect of such a diet in diabetic children is shown in Fig. 17. For the data of the case and for permission to reproduce the photographs I am indebted to the kindness of Dr. H. Medovy.

Similarly good results have been obtained elsewhere (147, 63).[1]

Various explanations have been suggested for the common, although apparently paradoxical, experience, that replacement of fat in the diet by carbohydrate does not increase, and may decrease the exogenous insulin requirement. Thus Sansum and Gray write (151): "The mechanism underlying this experience is not clear, but may be in line with Adlersberg and Porges' conception of insulin secretion as the response of a complex reflex following stimulation of the buccal mucosa from contact with carbohydrate foods. The additional carbohydrate may have stimulated the secretion of additional insulin. Again there may be some beneficial influence upon the liver by loading it with glycogen. It has been suggested before (von Noorden, and Adlersberg and Porges) that the relative proportion of liver glycogen to liver fat may be a factor regulating the secretion of pancreatic insulin."

Rabinowitch (143) takes another viewpoint, and one which is not entirely in agreement with the idea that diabetes mellitus is truly a condition of hypoinsulinism. "In as yet some unknown manner, exposure to this diet appears to lead to an increase in the available supply of insulin. From Allen's classical experiments it was concluded that excess feeding of carbohydrates led to over-strain of pancreatic function in the partially depancreatized dog and loss of

[1] The successful employment of such diets explains the partial benefit obtained in pre-insulin days by the "oat" and similar diets of von Noorden and others (137).

carbohydrate tolerance. The question which therefore arises is : Why do not these high carbohydrate diets also lead to loss of carbohydrate tolerance ? Not only do they not lead to loss, but, apparently, to improvement. The view held generally at present is that, in diabetes, there is defective production of insulin. Much of our experimental data to date fails to support this view. Diabetes does not appear to be due to defective production of insulin but to interference with the action of a normal supply. Experiences with diabetes complicated by infections are suggestive. The loss of carbohydrate tolerance in these cases is generally attributed to defective insulin production due to the infection.[1] If this view is correct, how are we to explain the not infrequent experience that such patients are not able to make use of the insulin injected hypodermically. As is well known, such persons may fail to respond to huge doses."

The view-point of the average diabetic clinic, as expressed by various recent reports, indicates a slower but a steady change towards increased carbohydrate. Joslin (95), writing in 1928, said : " Fat forms the bulk of the diabetic patient's diet. Even with the most modern ideas of treatment the statement holds. Whereas in the normal diet it furnishes less than one-third of the total calories, that diabetic diet is exceptional which is not made up of one-half of fat, and there are few diabetic diets in which fat does not represent two-thirds," although he likewise expressed belief in a diet which approaches the normal as far as is possible. Writing in 1931 (94) he finds need for further reports from those clinics in which on the one hand high fat, and on the other hand normal carbohydrate, is advocated. He quoted five standard cases of his own in which the carbohydrate-fat ratio varied widely, yet with apparently equally good results. Such results compel him to avoid extremes. " Lack of

[1] Murray and Waters have shown recently (124) that there is a significant decrease in the insulin content of the pancreas of dogs suffering from an acute infection, when associated with fever and accompanied by suppurative processes.

knowledge, which time alone can rectify, compels the adoption of a middle course by those who are not in a position to carry on detailed investigations, or who, because of large numbers of patients, must avoid confusion in the minds of their clientele by rapid changes in type of treatment."

After Effects of High Fat Diet. Persistent use of a high fat diet in diabetic treatment has been supposed to lead to marked persistent lipaemia, mirrored by a high cholesterolaemia. Various writers stress the continuing lipaemia as a causative agency in the production of that form of arteriosclerosis which is assuming the position of the commonest immediate cause of death of the diabetic. (" The outstanding features of the diabetes of to-day are the prolongation of the lives of diabetic children and the replacement of coma by arteriosclerosis as the cause of death " (95).) Increased blood cholesterol has been suggested as one of the causes of arteriosclerosis (8) and as predisposing to diabetic gangrene and therefore of bad prognostic value (142, 176). Joslin (94) is not in complete agreement with these views : " No better illustration of the necessity for an open mind in the treatment of diabetes is afforded than in the consideration of the cholesterol content of the blood. Attempts to show the harm that results from its excess are still unsatisfactory. One would like to say that arteriosclerosis can be avoided, or at least postponed, if the cholesterol of the blood, as a representative of all the lipides, could be kept normal ; and this may be true, but the evidence is insufficient. Quite as indefinite are the methods by which the cholesterol can be controlled." [1]

[1] Joslin gives the following examples of cases which do not fit in with the views of Aschoff, Rabinowitch, and others. A man of sixty, with a diabetic history of fourteen years, showed one hour after a meal 0·2 per cent. of sugar in his urine, a blood sugar value of 0·26 per cent., and the practically normal blood cholesterol value of 0·223 per cent. Yet during the fourteen years he had been kept on a low calorie diet, with maximum carbohydrate content of 55 grams, and had never been given insulin.

On the other hand, a woman of twenty-three, with a diabetic history of five years, showed calcified arteries in the legs, and calcification of

The Application of Surgical Procedures in Juvenile Diabetes. Many investigators have found that ligation of the pancreatic duct will result in atrophy of the secreting parenchyma, with persistence and occasional hypertrophy of islet tissue. Mansfeld (118) conceived the idea of converting the tail of the pancreas into a purely ductless gland by interrupting its external secretion ; the head and body of the gland were left to secrete pancreatic juice. He showed that such a surgical procedure in dogs would produce a hyperinsulinism persistent for at least one and a half years.

de Takáts (163) ligatured or completely separated the tail of the pancreas in experimental animals, and showed by histological evidence that hypertrophy and hyperplasia of islet tissue resulted. The external secretion from this tail rapidly ceased, and the carbohydrate utilization of such animals increased three or four months after operation, and then, in the normal dog, gradually subsided.

From such results, and certain supporting clinical evidence of regenerative power of the pancreas following some degree of destruction by local processes, de Takáts was led, in conjunction with Wilder, to attempt to ascertain whether hypertrophy and increased islet function could be induced in children by corresponding surgical procedures. He has reported the results in two cases : A child with severe diabetes, whose previous history was well known, and who had had diabetes of increasing severity for six years, was finally stabilized for two years on a diet whose glucose value was 120 grams, with insulin 40 units (76 grams carbohydrate, 50 grams protein, 150 grams fat). He was operated on in January, 1929. A small hypoplastic pancreatic tail was divided with a high frequency cautery. After a stormy convalescence he was maintained on pre-operative diet and insulin dosage. Three

the pancreas. Her blood cholesterol was 0·102 per cent. When she was placed on a fuller diet, with 112 grams of carbohydrate, 73 grams of protein, and 120 grams of fat, at the age of twenty-seven, she had no pains in the legs, and was very healthy ; her blood cholesterol was only 0·067 per cent.

months subsequent to operation, insulin reactions began to occur, and insulin was reduced slightly. "A year and a half after the operation the patient is growing and gaining weight normally. While his insulin requirement is but slightly diminished, he is able to utilize an additional 80 grams of dextrose or its equivalent daily. Notwithstanding the small size of the isolated portion of the gland, and despite serious post-operative reactions, the patient's diabetes has not become any worse, and there is a moderate increase in carbohydrate utilization. This definite increase has occurred in a patient who for six years had gradually lost tolerance, and who for the last two years before the operation had not shown any change at all."

The second patient, a boy of sixteen, with a two years' history of diabetes, and a rapidly decreasing tolerance, was operated on in October, 1929. Ligation of the tail of the pancreas was performed; convalescence was rapid and smooth. Four months after operation insulin was reduced from 60 to 40 units a day, on account of the development of insulin reactions. A month later transference from high carbohydrate medium-fat diet to low carbohydrate high-fat permitted reduction of insulin to 18 units a day. Subsequently a severe chicken-pox infection aggravated the picture and, two months later the carbohydrate tolerance was about the same as before the operation.

Such results are suggestive, but little definite conclusion can yet be drawn from them. It is obvious that they do not lead to such strikingly rapid and definite improvement as to suggest that the operation should be widely employed.

Differentiation between Diabetes Mellitus and Renal Glycosuria

Within recent years attention has been drawn more and more to the occurrence of sugar in urine in conditions other than diabetes mellitus. With more precise methods and more accurate and extended observations the number of such cases detected is increasing steadily.

Of those cases in which a sugar other than glucose is present, only the lactosurias of nursing mothers are relatively common. Differentiation is easily possible by the yeast-fermentation and osazone tests. True fructosuria is rare. Three excellent studies have recently been published (10, 77, 7). Differentiation is not too easy. In cases of pentosuria the sugar seems to be either arabinose or xyloketose. Somewhat less than 100 such cases have been reported. Greenwald has recently summarized the literature critically (69). Bial's test serves to discriminate the sugar of the urine from glucose. All the cases of lactosuria, fructosuria, and pentosuria are relatively harmless anomalies, requiring no special treatment, and in no way associated with hypoinsulinism.

The commonest non-diabetic condition which exhibits a persistent (though not necessarily a continuous) glycosuria is that due to a lowered kidney permeability for glucose ; it is termed variously *renal diabetes*, *renal glycosuria*, *renal glycuresis*, *diabetes innocens*, and *benign* or *innocent glycosuria*. Of all such terms *negligible glycosuria*, suggested by Leyton (106), is most apt, since it describes the importance of the condition with precision. The condition is relatively common. It exhibits various grades of severity, with no sharp line of demarcation between them ; these are combinations of varying kidney thresholds with either normal sugar tolerance, or a somewhat diminished tolerance (66).

A sufficient number of cases of these renal glycosurias have been observed over long periods of time to warrant the conclusion that the duration of life of those so affected is not shortened by the condition. Cases have been reported with histories of 25, 29, 32, and even of 44 years (174). The importance of correct diagnosis in these cases is illustrated by the fact that many of them have quite unnecessarily been dieted for years as diabetics, and many others have been refused life insurance on the ground that they were diabetics.

Most cases of renal glycosuria can be diagnosed correctly, and diabetes mellitus ruled out, by a glucose tolerance test. The former usually exhibits a normal or slightly depressed curve, with glycosuria present through all or most of the test. The fasting value of the blood sugar is normal or low. (Diabetics exhibit a heightened curve, with slow return to normal, and usually a definitely increased fasting value.) In certain of the severer cases of renal glycosuria the tolerance curve simulates that of a mild diabetes, and sometimes only a long history of absence of diabetic symptoms with unchanged degree of glucose excretion, justifies exclusion of diabetes mellitus. An extreme example of such a case has been reported by Powelson and Wilder (135). The tolerance curve reached the value 0·28 per cent. at the end of the second hour of the test, and maintained it to the end of the third hour, although a history of thirteen years definitely

excluded diabetes. Faber has devoted attention to this severer type (48).

In an interesting recent analysis of 1,700 cases of diabetes mellitus and 224 cases of non-diabetic glycosuria, it was shown that while one-third of the latter were symptomless, only 2 per cent. of the true diabetics showed no symptoms (124A).

Cases of hyperthyroidism frequently exhibit a glycosuria, but the simultaneous occurrence of hyperthyroidism and diabetes mellitus is rare (96).

Diabetes mellitus of hepatic origin (hepatic diabetes) has been postulated by French authors (Glenard, Gilbert, Weil) as a condition occurring chiefly between the ages of forty and fifty, in persons eating and consuming alcohol somewhat too heartily. The liver is generally considerably enlarged, and often tender; it tends to become smaller during treatment. Glycosuria is mild, polydipsia and polyuria absent. Dietary treatment leads to good results; insulin is of slight, but only of slight, value. Motzfeldt (121) has reviewed the literature.

The Causes, Cure, and Complications of Diabetes Mellitus

The various possible causes of diabetes mellitus have been systematically discussed by Warren from the point of view of the pathologist (171). In autopsies on diabetics, degeneration and atrophy of the islets are the most common abnormalities found in that tissue. These represent the final picture and possibly give little clue to the initial lesion. Even then the islets are never completely destroyed. The autopsy picture always reveals some proportion still apparently capable of function.

Diabetes mellitus has no single cause. Although in adults many writers, and especially Joslin (95) stress obesity as a predisposing factor, the child diabetic is, as Joslin points out, seldom obese. While probably general bacterial infections are but seldom the direct cause, since they might be expected to precipitate suddenly the severest grade of diabetes, yet undoubtedly diabetes sometimes arises from such causes, both in the child and the adult. The extraordinary susceptibility of the diabetic to infections, with resulting complete upset of his insulin-diet equilibrium,

illustrates the important *rôle* which these infections can play in affecting the utilization of exogenous insulin (98) ; hence endogenous insulin may well be similarly affected. " Of all the conditions which tend to lower carbohydrate metabolism, infection stands at the head of the list with respect to frequency and capacity to do harm. Loss of carbohydrate tolerance is apparently not related to severity of infection ; according to the writer's experience, a small furuncle, or the ordinary ' cold ' has, at times, resulted in as much disturbance as was found in more severe infections (pneumonia, etc.). Most disturbing, at times, from the point of view of effective therapy, is the fact that in infection not only may the supply of insulin produced in the body (endogenous insulin) be reduced, but that which is administered hypodermically may also be ineffective " (144).

Acute pancreatitis leads to disturbances of islet function in a large proportion of cases ; only a few of these develop a true diabetes (166, 148, 12, 162).

There is still no cure for diabetes mellitus. Insulin bears only the same palliative relationship to this hypoinsulinism as desiccated thyroid or thyroxine does to the hypothyroid state.

Undoubtedly increased tolerance for carbohydrate follows correct treatment, through regeneration of islet tissue. However, except in rare cases resulting from infection (such as Schmitz's case, quoted by Joslin (95)) and in certain hyperpituitary cases, in which the diabetes may not be true hypoinsulinism, complete recovery has so far not been recorded.

While there seems no intrinsic reason why the diabetic, treated with insulin, should not by this replacement therapy proceed to the same old age as that recorded for certain myxoedematous patients (cf. p. 58) yet the history of Hédon's dog suggests that the final stage may be stormier. It must be remembered, however, that this dog was completely depancreatized, while the human diabetic has normal

external pancreatic function, and his islet tissue still functions to some degree.

Hédon's dog lived fifty-seven months after complete removal of the pancreas in January, 1924. It was kept alive by insulin injections, and the daily inclusion of 50 grams of raw pancreas in its diet (75) (cf. footnote, p. 131). For thirty months the animal remained in good health and in weight equilibrium—7 kg.—on two injections of insulin per day; the food ration was 500 calories. The only difference noted from the behaviour of normal animals was an insatiable hunger and some perversion of appetite, due probably to imperfect digestion. These symptoms persisted to the death of the animal. Its general health was good during 1927 and the first months of 1928, despite a severe enteritis with profuse internal haemorrhage, due to ingestion of some toxic food (in May, 1927), from which complete recovery was brought about by blood transfusion.

During the whole of the period there was no amelioration of the diabetic state. At any time complete omission of insulin always resulted in rapid and most severe diabetes, with acidosis, lowering of the alkaline reserve, and presence of fatty granular cylindroids in the urine.

In the latter months of 1928, *i.e.*, after the dog had remained for four and a half years in a practically normal state, it was no longer possible to maintain good nutrition. His weight varied. Insulin dosage had to be increased to keep glycosuria low. From July onwards emaciation progressed rapidly. Although even doubling the insulin dosage did not radically suppress the glycosuria, yet hyperinsulinism was produced easily, so that a few hours would transform an intense diabetic into a hypoglycaemic syndrome. (The efficacy of the insulin was tested on a dog recently depancreatized; it was maintained in excellently balanced condition on much less insulin.)

On October 16th the dog developed violent convulsions. Glucose injection produced no amelioration, although the

blood sugar was raised to 0·18 per cent. A further injection was ineffective. The alkaline reserve dropped to 46 per cent. of normal, the plasma urea increased to 0·92 per cent. Kidney secretion almost ceased. The small amount of urine that was excreted scarcely reduced Fehling's solution and contained no albumin. The animal died in hypoglycaemic coma, the blood sugar being only 0·03 per cent. in spite of previous glucose injections.

At post-mortem all the organs were apparently normal. The liver was not fatty either by macroscopic or by microscopic examination, but contained no trace of glycogen. Except for marked emaciation the dog showed no special difference from depancreatized dogs dying from the immediate diabetes. The only lesions found were in the kidneys, which exhibited an old interstitial sclerosis, plus a recent epithelial nephritis, the tubules especially exhibiting a fatty degeneration.

Hédon considered that the animal probably died from a combination of uraemic and insulin intoxications.

This end picture, emphasizing a final maladjustment of response to insulin, may perhaps have an application to the final stage of insulin treatment in man. The therapeutic use of insulin is still much too recent to decide this question.

The diabetic child affords the most interesting material for prolonged study of the effect of insulin. Priscilla White (177) has recently dealt with a number of interesting points concerning the etiology, treatment and prognosis of his condition. She considers that at the onset of his diabetes he shows a marked physical precocity, an overgrowth (eighteen months in advance of his chronological age) which corresponds to obesity in adults. There is a somewhat less degree of mental precocity. She gives a most hopeful prognosis.

As regards the general results of insulin therapy the remarks of Bowen have pertinence (21): "The adult diabetic who is treated with insulin compares quite

favourably with the normal individual with the exception that the majority have the subjective impression that they are not capable of normal physical effort without fatigue. Children apparently do not show this physical limitation." This mental effect is therefore probably capable of treatment by re-education, combined with the increased carbohydrate diet essential for muscular exercise, and sufficient insulin to control that carbohydrate.

Diabetic Complications. Hepburn and Graham (78) from heart studies on 123 cases of diabetes mellitus, fifty-six of which showed serious electrocardiographic abnormalities at the beginning of diabetic treatment, found that in a fairly large percentage the electrocardiograms returned to normal after the diabetic condition was controlled by treatment.

An atrophy of the subcutaneous fat at the sites of insulin injection has been reported in a number of cases (128). Avery (9), reviewing twenty-one of these cases, found no relation to insulin dosage, duration of treatment, or the original fat condition of the patient. He suggested that the effect was the result of undue local stimulation of carbohydrate metabolism, leading to local fat catabolism. No evidence affording an explanation has been found at autopsy (139). Similar effects were not produced by injection of insulin into fatty tissue in normal rats (146).

The relationship of pregnancy to diabetes has been subjected to frequent review. The general consensus of opinion seems to be that the diabetes is more menacing to the pregnancy than is the pregnancy to the diabetes. " The accidents of pregnancy occurred three times as frequently as the accidents of diabetes in sixty-nine cases " (95). Walker considers that although diabetes must be considered as a serious complication of pregnancy, if the patient is treated with insulin and properly dieted there seems to be no special incidence of puerperal complications and the pregnancy does not appear to have any ill-effects on the diabetic condition (170, cf. 111). Izquierdo (86) points out that it is

important to consider existence of a pre-diabetic state in pregnant women; he has observed repeatedly that when hyperglycaemia is present, with no glycosuria, abortion may follow. In pregnancy complicated by diabetes there is a considerable tendency to acidosis, due to a diminution of the glycogen reserve of the liver. Hence insulin plus increased carbohydrate in the diet are necessary.

Certain cases of diabetes seem unduly resistent to insulin treatment. Various explanations have been suggested, such as, for example, the existence of an " anti-insulin " (49), or the lack of a " co-enzyme " (103). Marked spontaneous resistance has been reported in two rabbits in whom thyroidectomy produced a much greater sensitivity to insulin than usual (180). It has been suggested that obesity lessens the response of the diabetic to insulin (11).

Little need be said here concerning such complications as coma due to acidosis, coma due to hyperinsulinism, infections, carbuncles, gangrene, and those associated with the diabetic surgical patient. No recent new treatment has been instituted and the general principles governing the onset and effect of these complications are reasonably well understood.

Insulin Administration and Insulin Substitutes

The chief objection to the employment of insulin in cases of mild diabetes (severe cases obviously need it) is the necessity for its hypodermic injection two or three times a day. Numerous efforts have been made to overcome this necessity, either by finding means of administering insulin orally, or by finding substitutes capable of producing an insulin effect when taken orally. None have yet achieved the desired effect, because, as far as insulin is concerned, they do not yield controllable effects, and, as far as insulin substitutes are concerned, those tested hitherto do not act in the same way as insulin, and, when effective, are also definitely toxic.

Oral Administration of Insulin. Since insulin is decomposed by pepsin and trypsin, all efforts to produce a preparation which can be used orally must be designed to protect the insulin against this digestive action. I am unaware of any method so far used clinically to which Lawrence's comment does not apply (102): "It has been known for years that very large doses of insulin administered by mouth in alcoholic solution or with saponin may occasionally have some slight hypoglycaemic action on the blood sugar of animals and diabetics. But this action is variable and uncertain, and depends on the absorption of some insulin before it is destroyed by the digestive enzymes, a factor over which we have no control."

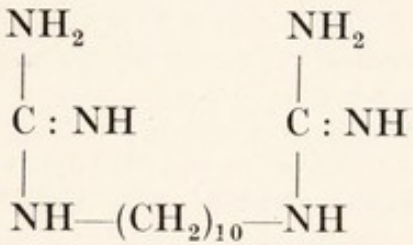
It has been claimed (123) that blood serum, administered with insulin, confers protection through its "anti-tryptic" activity. It is stated that blood sugar is definitely depressed in rabbits and also in diabetic patients, following oral administration of the precipitate obtained when commercial solutions of insulin are treated with phosphotungstic acid (122). The claim has not been substantiated, and there is obviously potential danger of toxic action on the kidneys from the phosphotungstate (102). The oral use of dry insulin preparations mixed with oily or fatty mixtures, or especially with desoxycholic acid (as "cholosulin") has been advocated and good clinical results claimed (161). The claim is not supported (13). Administration with liver extract is said to favour absorption from the stomach (13).

Endonasal application of insulin in the form of a snuff is said to be effective. The blood sugar falls, but no hypoglycaemia is produced. Carbohydrate tolerance is increased only in some cases (172, 82).

Oral Use of Insulin Substitutes. Of various preparations whose use as insulin substitutes has been suggested within the past few years the most promising, and therefore the most disappointing, was synthalin.

Watanabe (173) in studies relating to the supposed

connection between hypoglycaemia and tetany, and guanidine and parathyroid function, found that poisoning with guanidine produced a fall in blood sugar. Frank of Breslau confirmed this effect, and endeavoured to find a guanidine derivative in which the toxicity would be decreased, and the hypoglycaemic action increased. In order of efficacy in meeting this double requirement he found first agmatine, then the synthetically prepared aminopentylene guanidine, then diguanidino-octamethylene, and, best of all, diguanidino-decamethylene, or synthalin (55) :



Synthalin was tested orally on clinical cases of diabetes, and at first excellent results were claimed for it. The claims were subsequently modified. Further study indicated that its toxic action is too important to be neglected. Many patients showed such an idiosyncrasy to it that it could not be used for them ; for others its dosage had to be kept so small that it could at best be but an adjunct to insulin. Graham and Linder published a very just *résumé* of the earlier clinical tests of synthalin (67). More recent results lead to no more favourable conclusions, either regarding synthalin or Frank's later preparation, dodecamethylene diguanidine (synthalin B) (55), but throw some light on the reason for the inadequacy of these compounds.

Animal experiments showed that the action of synthalin in producing a hypoglycaemia is unlike that of insulin. It does not facilitate the oxidation of glucose. Muscle glycogen is diminished (17), and liver glycogen is caused to disappear (150). Not only is the respiratory quotient not increased (76) but oxidation seems actually to be inhibited and the formation of lactic acid increased (160).

There is a very definite toxic action on the kidneys, which affects the convoluted tubules more than the glomeruli; the non-protein nitrogen of the blood is increased, and albumin and casts appear in the urine. Given to dogs in doses corresponding to therapeutic doses for man, these toxic symptoms appear within a few weeks and death finally results. There is also severe hepatic poisoning (17, 99, 16). Comparison of various synthalin homologues shows that the toxicity and hypoglycaemic effects run parallel (15).

Glukhorment, which had a vogue for a short period, appears to have been a pancreatic preparation to which synthalin was added.

Myrtillin, on which Allen reported favourably, does not appear to possess any marked virtue.

Long and Bischoff (108), reporting on " uvursin," " reglykol," " pancrepatine," and " solanum sanitwongsei " berries, for which claims of usefulness in the treatment of diabetes have been made, find no evidence of insulin-like action; one infers from their report that these substances are valueless as far as diabetes is concerned.

Whereas it would appear that up to the present time attempts to synthetize a compound that can be used orally by the diabetic as a substitute for insulin have met with failure, the underlying idea is essentially sound. Whether or not the activity of insulin is associated with a prosthetic group, or with some specific portion of the protein molecule, it is almost certainly associated with some specific linkage or series of linkages, which sooner or later will be revealed by further investigation. If these can be synthetically reproduced in simpler compounds, some one of them may well be capable of oral administration and insulin action.

Pseudo-hypoinsulinism due to Antagonistic Secretions

After injection of insulin has depressed the blood sugar of an animal, injection of an extract of the posterior pituitary gland will elevate it (25, 43). Simultaneous injections of pituitrin and insulin can be so adjusted that the blood

sugar remains unaltered (43). The effect seems to be the result of a direct antagonism; apparently the so-called oxytocic principle of the pituitary is concerned (115A).

Cushing and Davidoff (43) found that twenty-five out of 100 cases of acromegaly exhibited glycosuria; in twelve of these there existed a true diabetes mellitus. Since acromegaly is associated with hypersecretion of the *anterior* pituitary, the relationship indicated by such observations is difficult to correlate with the antagonism just referred to. It seems also definitely established that certain cases which exhibit concurrent acromegaly and diabetes sometimes recover spontaneously from the latter (39). Ellis (47) found persistent hyperglycaemia and glycosuria in an acromegalic. After removal of a pituitary tumour the hyperglycaemia and glycosuria disappeared. Colwell (39) considers, however, that the pituitary has no direct control of carbohydrate metabolism, and that the apparent connection is only an indirect one.

Cammidge (30) believes that a group of cases exists in which hyperactivity of the posterior pituitary causes a persistent glycosuria, through too great a degree of neutralization of the effect of insulin. Such cases, if this theory be right, do not exhibit a true hypoinsulinism. The normal supply of insulin can be secreted, but is rendered useless. Acidosis and the usual sequelae of diabetes can ensue. Cammidge thinks that these cases can be differentiated from ordinary cases of hypoinsulinism by determining the existence (or non-existence) of abnormal changes in blood volume. "These patients, unlike ordinary people and other diabetics, increase the volume of fluid in the peripheral circulation as the sugar content of the blood rises after a meal, and reduce it again as the sugar falls."

That there exists some direct and important relationship between some one or more of the internal secretions of the pituitary and insulin seems well established by the work of Houssay and his school and of Geiling. Removal of the

pituitary in dogs enhances the hypoglycaemic action of insulin. When the anterior pituitary gland is removed in the toad, subsequent pancreatectomy does not produce diabetes, but pituitary implantation can then induce it. In the dog, after removal of the whole pituitary, pancreatectomy only causes a mild diabetes with long survival (up to four months); injection of anterior pituitary extract into normal dogs induces glycosuria, hyperglycaemia, and ketonuria (83, 61; cf. also 140, 165).

Hyperinsulinism

Harris observed in 1923 certain symptoms in non-diabetics which were identical with those resulting from overdosage of insulin, and so coined the term *hyperinsulinism*. One such patient, a physician aged sixty, had a blood sugar of 0·065 per cent. His symptoms were relieved by administration of sugar. He died after four years of such treatment. A second patient, a labourer aged fifty-two, with similar low blood sugar and symptoms controllable by food, could still be controlled in this way after eight years of treatment (73.)

Since Harris' early observations numerous cases have been described in the literature. These fall into three groups, associated respectively with hyperplasia, malignant growth and benign adenoma of the islet tissue.

In addition, hypoglycaemia, and symptoms associated with it, may arise as a transient or a permanent condition from causes in no way associated with excess of insulin in the organism.

The symptoms associated with hypoglycaemia, whether it is produced from removal of the liver, as in Mann and Magath's classic experiments (116), or from overdosage of insulin in diabetic patients, or from cases of hyperinsulinism, are in large part identical. The syndrome includes fatigue, anxiety, irritability, lassitude, gnawing hunger, twitchings of the muscles, tremors, diplopia, vasomotor changes, hot flushes, secretory irregularities, lacrimation, profuse per-

spiration, nausea and vomiting, vertigo, syncope, loss of emotional control, mental confusion, irrational action and phenomena resembling drunkenness, convulsions and coma. All these phenomena may not be exhibited in one patient (154, 59). "The convulsion is usually over in from a few minutes to half an hour, and even without food the patient, dripping with sweat and saliva, becomes conscious. Recovery is complete, without memory of the accident. . . . Complete abortion of the attack in any stage is produced by the administration of carbohydrate. The only exception is in certain terminal stages. . . . Recurrence of attacks with increasing frequency is characteristic . . . with the increasing frequency there is a tendency for the attack to become more severe" (59). The last remarks, of course, do not apply to insulin overdosage. Harrop has published a critical comparison of different degrees of insulin shock in diabetic patients (74). The symptoms are at least in part due to the fact that the central nervous system is peculiarly susceptible to glucose starvation, having no store of carbohydrate (81A).

Cases Associated with Tumour of the Islets. The first definite case was reported by Wilder, Allan, Power, and Robinson in 1927 (178). The patient exhibited marked hypoglycaemia. His condition was inoperable and became progressively worse until half-hourly doses of glucose were necessary to prevent convulsions. Blood sugar analyses included figures below 0·03 per cent. Post-mortem examination revealed a carcinoma of the islets with metastases in the liver. An extract of these carcinomatous metastatic nodules was made; injected into an animal, it produced insulin action. In 1929 Lemaire reported a very similar case with metastases in the liver (105; cf. also 164). Such cases demonstrate the fact, now becoming well recognized, that malignant tumours of an endocrine tissue function by producing the endocrine principle of that tissue, so that hyperactivity results.

Howland, Campbell, Maltby and Robinson (84) reported a case in which the patient exhibited convulsions and coma, associated with hypoglycaemia. They operated and removed a small carcinoma arising from islet tissue. The convulsive attacks and the hypoglycaemia disappeared.

McClendon and Norris (109) described similar symptoms in a negro ; the condition proceeded to a fatal termination ; at autopsy an adenoma was found originating in islet tissue. In a number of other cases operation has given complete relief (179, 42, 32, 56).

Cases not Definitely Associated with Tumours of the Islets. Several cases have been reported in which exploration did not reveal a tumour, but partial resection was of some benefit (51, 4). Such cases suggest the obvious possibility of a simple hyperplasia. Certain cases have been treated with varying success without operation, and treatment, where successful, also suggests a hyperinsulinism due to hyperplasia (59, 156, 110). Philipps (136) reported a case which clinically appeared to be uraemia associated with hypoglycaemia. At autopsy it proved to be a subacute glomerular nephritis, with hypertrophy of the islets of Langerhans. A premature child born of a diabetic mother died on the third day, at which time its blood sugar was 0·067 per cent. Autopsy showed hypertrophy and hyperplasia of the islets, apparently a compensatory effect (68). A curious case has been reported in which hyperinsulinism was associated with what appeared to be faulty digestion of starch (120).

The drop below normal fasting value frequently seen towards the end of a sugar tolerance test on a normal subject is undoubtedly due to a slight degree of physiological hyperinsulinism induced by the stimulus of the glucose meal.

Hypoglycaemia not Associated with Hyperinsulinism. The second most important cause of a recurrent hypoglycaemia is liver deficiency. The prime factor here is inability to store sufficient glycogen as a carbohydrate reserve.

In 1929 Nadler and Wolfer (125) reported a case exhibiting marked hypoglycaemia and convulsions; at subsequent autopsy the liver was found to be riddled with carcinoma. Crawford (40) reported a case of a negro with primary carcinoma of the liver; his blood sugar showed marked fluctuations; he frequently passed into coma with a blood sugar of 0·025 per cent. His sugar tolerance curve was normal in type but depressed; the maximum reached after ingestion of 100 grams of glucose was only 0·10 per cent.

In milder degree liver deficiency seems responsible for hypoglycaemia associated with "recurrent vomiting" in children, through some degree of fatty degeneration (97), or with phosphorus poisoning (112), or with acute yellow atrophy of the liver (50), or in chloroform poisoning in dogs (18). Lowered blood sugar also occurs along with parenchymatous changes in the liver caused by arsphenamine, by hydrazine, or by the fungus *Agaricus bulbosus* (136). The occasional cases of hypoglycaemia seen in pernicious vomiting of pregnancy (29) are also probably traceable to undue depletion of liver glycogen during the pregnant state.

Other causes of a hypoglycaemia sometimes sufficiently chronic or recurrent to produce persistent symptoms are severe burns (70), premature labour (135), adrenal insufficiency, including Addison's disease (6), menstruation (175), chronic infections, recurrent bilious attacks, neurasthenia, etc. (29).

Hypoglycaemic symptoms have been reported in a nursing mother; the symptoms ceased on weaning. There is a fall in blood sugar during the milking of cows, and during lactation of healthy women. The normal cause, occasionally leading to an abnormal result, seems obviously to be the extra drain upon the blood sugar during lactation. Certain symptoms exhibited by exhausted marathon runners and in other cases of extreme fatigue are probably due to a concurrent hypoglycaemia. (Cf. 28).

Association of Hypoglycaemia with Epileptoid Convulsions.

Shih-Hao and Hisao-Chien (157), in reporting a case with symptoms of insulin shock, suggested the desirability of determining blood sugar values in hysterical attacks, since some of these might possibly be due to hypoglycaemia.

Of a number of cases of chronic hypoglycaemia reported by Cammidge (29), seven exhibited convulsive attacks. Of these four had been believed to be mild epileptics, two had been diagnosed as cases of Ménière's disease, and one has been reported as a victim of secret alcoholic excess, although actually a total abstainer. Their inco-ordination was abolished by raising their blood sugar. Roth (149) has reported three cases exhibiting severe hysterical attacks ; one progressed to an epileptiform state, which proved to be due to hypoglycaemia. McGovern's case (110) showed frequent attacks of amnesia and coma, often accompanied by convulsions of epileptoid type. During the convulsions the blood sugar fell to 0·03 per cent. Treatment with carbohydrate every hour warded off attacks for a period of eighteen months.

Of particular significance is the psychiatrist's report in a case reported by Finney (51) : " If it were not for the fact that there is a very striking lowering of the blood sugar, and that the taking of carbohydrate aborts the attack, my feeling would be that these attacks were certainly hysterical." The possibility that hysterical conditions of varying degree may be due to hypoglycaemia (whatever the cause of that hypoglycaemia) cannot be lightly disregarded.

Differentiation and Treatment of the Causes of Hypoglycaemia. Harris (73) found 51 cases of hyperglycaemia and 67 of hypoglycaemia in a series of 1,497 blood sugar determinations on non-diabetics ; one may doubt his conclusion therefrom that hyperinsulinism is almost as common as diabetes, but his results suggest the importance of considering hypoglycaemia in both diagnosis and treatment.

When a constant or recurrent hypoglycaemia is revealed by analysis, accompanied by definite symptoms and not

explicable by any simple cause, some pathological state of the liver, or hyperinsulinism, should be suspected. Of these the latter is by far the more likely. Unless other symptoms strongly suggest malignancy, it seems most rational to attempt to combat the condition first by diet adjustment. In the earlier reports increased carbohydrate, and especially increased frequency of taking carbohydrate, gave satisfactory results in a number of cases (73, 59, 110, 29).

More recently Harris and others have advocated diets relatively low in carbohydrate, with moderate protein content and high fat, and the taking of food every two or three hours, and at night if necessary; the underlying theory is that excessive ingestion of glucose-forming foods helps to overstimulate the islets. Good results have been claimed with such treatment (73, 156).

If dietary control is insufficient, or gradually becomes insufficient, surgical interference seems warranted. If tumours are found the outlook is even better for complete recovery than if hyperinsulinism is due simply to a hyperplasia of the islets.

The Use of Insulin in Non-diabetic Conditions

Within recent years insulin therapy, usually combined with administration of glucose, has been attempted in various non-diabetic conditions, and the results, for the most part, have been good. Until many more reports have been made, however, many of the claims presented in the literature will require cautious and critical consideration.

" Insulin plus glucose " therapy is undoubtedly of value in many cases of malnutrition (158, 119, 20, 33), and wherever improvement of appetite is a requisite (especially in mental cases). The combination is of value in combating acidosis, recurrent vomiting, and acute intestinal intoxication in children (64), and to induce fattening in tuberculous patients (35, 129, 45).

Several writers claim that it is useful in certain dermatoses, especially those associated with a disturbance in carbohydrate metabolism (20, 3, 126). A somewhat curious statement has been made that the treatment causes quick disappearance of the painful phenomena in cases of gastro-duodenal ulcer although no absolute cure is claimed (27). Benefit has been claimed in cases of hyperthyroidism, arteritis (20), typhus, chronic uraemia, heart-insufficiency (131, 159A), cholelithiasis, and melanosarcoma with metastases (46). Claims that this therapy is useful in pernicious vomiting of pregnancy are denied (71).

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CHAPTER V

THE ADRENAL GLANDS

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Introduction

CONCERNING the normal function of the paired glands whose close anatomical juxtaposition to the kidneys has led to the name *suprarenal* or *adrenal* glands, there are three generally recognized series of facts ; until recently there were only these three.

These glands are composed of two separate types of tissue, which in mammals constitute their *cortex* and *medulla ;* in other vertebrates these tissues exhibit no form of union. We have no evidence to prove that the approximation in mammals is not fortuitous, although that seems unlikely.

Removal of both whole glands from an animal is fatal within a period of days. Destruction of both medullas, with a reasonably large proportion of cortical tissue left capable of functioning, is not fatal, and indeed seems to have no particular effect upon the animal. Hence the adrenal cortex is essential to life, while apparently the medulla is not.

The tissue of the medulla contains a compound, *adrenaline*, or *epinephrine*, or *adrenine*, which following intravenous injection produces a series of pharmacological effects all of

which can be induced by stimulation of some one or other nerve of the autonomic system, hence this compound, and others which behave in the same way, have been termed "sympathomimetic." Of these effects the most striking are the increase of blood pressure, and of glucose concentration in the blood; the latter is caused through mobilization of liver glycogen.[1]

Additional, and most important for study of adrenal function, is the recognition that Addison's disease is associated with a pathological condition of the adrenal cortex.

Comparative Anatomy. This has been fully dealt with by Vincent (92) and others. The following facts will suffice here. The adrenal cortex corresponds to the interrenal body of elasmobranch fishes, and Giacomini's "anterior interrenal body" of teleostean fishes.[2]

"Accessory cortical bodies" are found in varying numbers and positions. Their total mass in mammals is relatively small when contrasted with that of the adrenal cortex itself.

"Chromophil bodies" [3] are found in close relationship to the ganglia of the sympathetic nervous system in elasmobranch fishes. In mammals the relative total amount of chromophil tissue seems to increase. Some part of the

[1] For an account of the accepted facts concerning the adrenal glands, see Vincent (92), Sharpey-Schafer (77), Goldzieher (33), or the articles in Barker's "Endocrinology and Metabolism" (5).

[2] Vincent suggests the name "cortical adrenal body" in place of Giacomini's term, and has confirmed Ramalho that this, and not the "corpuscles of Stannius," represents the adrenal cortex in teleosts (93).

[3] The staining reaction of the cells of the medulla with chromic acid and its salts was discovered by Henle in 1865. Stilling discovered the cells having the same reaction along the sympathetic ganglia and in the carotid gland, and called them, and the corpuscles which they formed, and the medulla of the adrenal, *chromophil*. Vincent, following a suggestion of Sharpey-Schafer, modified this term to *chromaphil*. Kohn used the term "chromaffin," and called the bodies *paraganglia*. Poll, more recently, invented the term "phaeochrome." Obviously terms based merely upon staining reactions should at least be consistent, and the term "chromophil" will be used here in line with the similar terminology used for the cells of the anterior pituitary body, even though the precise significance and the derivation differ.

carotid body,[1] and the whole of the abdominal chromophil body, are made up of chromophil tissue. The largest mass of all is the adrenal medulla, but the proportion of chromophil cells in the two adrenal glands to total chromophil tissue is relatively less than that of cortical cells in the glands to total cortical tissue.

Development, and Macroscopic, and Microscopic Structure of the Adrenal Bodies. Cortical tissue is of mesoblastic origin, but chromophil tissue originates from a certain section of the sympathetic structure and thus may be considered to be of nervous origin. Gross section of the human adrenal shows three chief layers, a grayish-white or silvery-gray medulla, surrounded by an intermediary yellow- or dark-brown zone, which is again surrounded by a yellowish-gray peripheral layer, the cortex. The widths of these three zones show wide variations, especially in different age groups. Microscopically the cortex exhibits three strata, the glomerular (external) zone, the fasciculate, and the reticulate (adjacent to the medulla). There is no sharp demarcation between them. The specific cells of the cortex have been described as "clear" and "dark," according to their appearance after staining with iron-haematoxylin. This may not reveal more than a difference in functional activity. They contain typical mitochondria and are characterized by presence of lipoid granules. In the reticulate zone pigment granules are responsible for its brownish-yellow colour (92, 33).

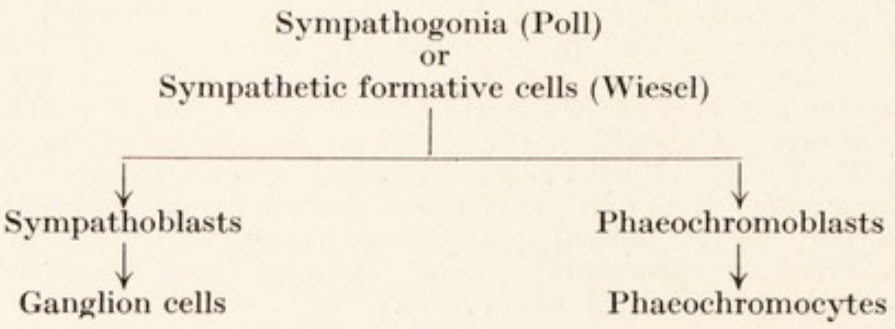
The medulla consists of "a solid cell-mass permeated by sinus-like blood vessels. . . . There can be little doubt that

[1] Christianna Smith (80), in a study of the origin and development of the carotid body, found that chromophil cells were abundant in that of the cow, were present in the same structure of the cat, but were absent in that of the rat, and concluded that there is no evidence to warrant the inclusion of the carotid body in the endocrine system.

Recent work has suggested a particular association of the carotid body with the carotid sinus in the regulation of blood pressure through a nervous reflex. One may nevertheless venture to maintain that when chromophil cells are present in the carotid body, they function as do other cells of the chromophil system, especially those associated with sympathetic ganglia. (Cf. also p. 343.)

the materials (secreted by the cells) find their way directly into the blood within the blood spaces. The cell-protoplasm contains chromaphil granules which vary in size and amount in different cells. These cells are stained brown with chromic acid and its salts " (77).

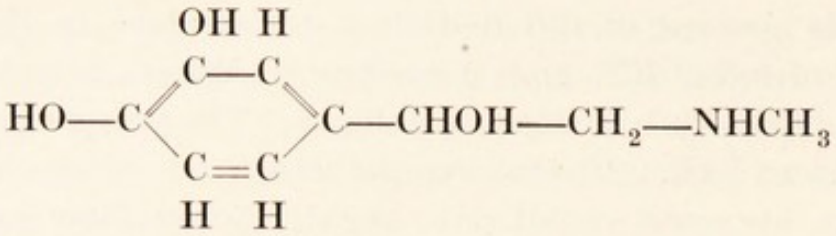
Rabin (67) has summarized the embryonic development succinctly. " The suprarenal cortex develops from the splanchnic mesoderm. Developmentally, and in most respects functionally, also, it may be considered a separate organ. . . . The immediate anlage of the suprarenal medulla, and the anlages of the remainder of the chromaffin organs, lie in the sympathetic ganglions, which, in turn, are derived from the cells of the neural crest. In the human 17 mm. embryo may be seen the beginning of the migration of the primitive cells of these ganglions, the sympathogonia, or the sympathetic formative cells, laterally. This migration continues during early foetal life. In mammals, the sympathogonia migrate until they reach the cortical anlage of the suprarenal gland. In the selachians the migration ends just lateral to the aorta. Migration is complete in the 85 mm. embryo ; at this time they have taken up the position of the medulla. During the migration, portions of the embryonic tissue may become split off ; these develop as separate organs at varying distances from the aorta in the region of the renal arteries or the inferior mesenteric artery to form the organs of Zuckerkandl. The further development of the suprarenal medulla consists in the process of differentiation from sympathogonia to the mature elements. This may best be diagrammatically plotted :



"This process of differentiation takes place in the last month of foetal life, and, according to Wiesel, is not completed until about the time of puberty. The sympathogonia, the parent cells of the sympathetic and phaeochromic systems, are small round cells, slightly larger than lymphocytes, with each a scanty cytoplasm and a round, large nucleus containing a densely staining chromatin network. These differentiate on the phaeochromic side into larger cells, also round, with each a larger area of clear cytoplasm and a more vesicular nucleus. The final stage of differentiation brings forth the mature phaeochromocytes, which are large, irregular, or polyhedral cells, with each a round or ovoid vesicular nucleus, containing a loose chromatin network, and a well formed nucleolus. The cytoplasm is abundant and usually finely granular. In contradistinction to the phaeochromoblasts, the phaeochromocytes have the peculiar property of staining brown with chromic salts."

The Adrenal Medulla and its Normal Function

The series of observations which step by step led irresistibly to the isolation of adrenine have been well described by Vincent (92). That a powerfully reducing substance present in the adrenal medulla gave certain colour reactions was noted much earlier than the discovery that extract of the medulla produced a powerful pressor effect when injected intravenously into animals. From the investigations of Vulpian (1856), Krukenberg, Moore, Fränkel, and v. Fürth it became evident that this reducing chromogen was a derivative of pyrocatechol, extremely unstable, and easily oxidized. (An emerald green or blue colour is given with ferric chloride, a rose red with chlorine or bromine water.) Further work by v. Fürth and Abel finally led to the isolation of crystalline adrenine, $C_9H_{13}NO_3$, by Aldrich and Takamine independently in 1901. The researches of v. Fürth, Jowett, and Pauly established its constitution as :



and comparisons with extracts of adrenal medulla demonstrated that it was responsible for all their activities. Adrenine prepared from the gland is laevo-rotatory; that prepared by synthesis is of course racemic. The dextro-rotatory isomer of the natural product is, according to Schiltz, one-third as physiologically active as the laevo-compound.

Various names have been suggested for this derivative of tyrosine. The obvious *adrenaline*, from its source and basic nature, has been criticized through its use for a pharmaceutical preparation of the compound, and *epinephrine* and *adrenine* are as often employed. The last term, due to Sharpey-Schafer, will be used in this text. "Suprarenin" was applied by v. Fürth to an impure but potent preparation, and the term is still sometimes used.

The Actions of Adrenine. Of the sympathomimetic actions of adrenine the most striking are the constriction of arterioles leading to increased blood pressure, and its effects on carbohydrate metabolism. But little has recently been added to our knowledge of the first effect; important advances have been made concerning the second.

The actual seat of action is still not decided, and is variously considered to be smooth muscle fibre, some receptive substance in muscle fibre, or at the myoneural junction.

The action of adrenine on carbohydrate metabolism has recently been reviewed by Cori (20) and the additions that have been made to our knowledge during the past few years are in large part due to the investigations of Cori and his co-workers.

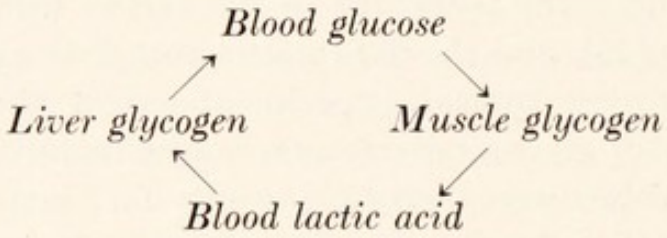
In earlier work, perfusion experiments with the livers of such cold-blooded animals as the frog and turtle demonstrated that adrenine increases glycogenolysis with resulting formation of glucose. The effect is not so marked or so regular when mammalian livers are used. It has been assumed that the same action takes place in the intact animal, but definite proof of this has only recently become available, since the technique of the earlier work was open to criticism (22), and the data not in complete agreement.

The following typical experiments yield the necessary proof for liver glycogenolysis as a normal result of adrenine action. Rabbits were fasted for twenty-four hours, and then injected with adrenine in dosage of 0·5 mg. per kg. subcutaneously. Hyperglycaemia and glycosuria resulted. The liver glycogen content diminished for one-and-a-half hours, then slowly rose, until, at the third hour, it had risen above the original basal level, although a hyperglycaemia was still present. It continued to rise until the eighteenth hour (74). When rats were injected with adrenine in amount sufficient to produce hyperglycaemia but not glycosuria, there was a definite fall in liver glycogen during the first fifteen minutes, then a slow rise, until the original value was surpassed in just over an hour (22).

These experiments not only demonstrate change of glycogen to glucose as an effect of adrenine action, but suggest a synthesis of glycogen from some other source. Synthesis and hydrolysis of glycogen can apparently proceed simultaneously in the liver during adrenine action, since hourly determinations show that the amount of glycogen formed in the liver of rats is at least as great in animals which receive an adrenine injection as in controls (22).

We know, from Schöndorff's work, that, excluding the liver, by far the greater proportion of the body glycogen is in muscle. Following adrenine injection in rats, even in physiological dosage, this non-liver (and chiefly muscle) glycogen definitely and markedly diminishes (22), while

liver glycogen at first decreases and then increases. Obviously a transfer of glycogen from muscle to liver is suggested. Since muscle glycogen is known to hydrolyze to lactic acid, and since the liver can transform lactic acid to glycogen, this appears to be the intermediate agent of transfer, permitting muscle glycogen to become available as blood glucose through the cycle (20) :



(The results of certain other investigations are not in complete agreement with these conclusions (23, 73, 28).)

Cori (20) concludes from all such work that " the acceleration of glycogenolysis in muscle is a physiological effect of epinephrine. The basic action of this hormone in liver and in muscle is therefore the same, except that the end product of glycogenolysis is mainly glucose in the liver, while it is lactic acid in muscle." He inclines to the view that the " glycogenase " in the liver cell is usually in large part rendered inactive by adsorption on to some surface ; adrenine lessens the adsorption through surface activity action and so favours glycogenolysis ; insulin favours adsorption thus decreasing glycogenolysis. This view is difficult to accept completely, since it suggests different enzymes for glycogen hydrolysis and synthesis in the liver, an unnecessarily complex and therefore unlikely procedure.

The necessary final proof of the cycle of glycogen just dealt with is afforded by the observation, repeatedly confirmed, that adrenine injection produces a marked increase in the lactic acid content of blood (20). When the injection is of physiological magnitude the effect on blood sugar and blood lactic acid passes off more slowly than that on pulse rate, respiration, blood pressure, and basal metabolic rate.

A temporary rise in the respiratory quotient is produced, due to the increased production of lactic acid causing a hyperventilation resulting in increased elimination of carbon dioxide. There is no increased oxidation of carbohydrate ; it may even be decreased (20).

That the same series of changes can follow secretion of adrenine from the adrenal glands is suggested by the fact that effective puncture of the floor of the fourth ventricle produces not only rise in blood sugar and blood lactic acid, but increases secretion of adrenine from the glands, which presumably is the causative mechanism of the other changes (20).

The Calorigenic Action of Adrenine. The term was introduced by Boothby and Sandiford (9) to describe the increase of oxygen consumption which occurs after subcutaneous injection of adrenine. Dogs, injected intravenously at rates varying from 0·0006 to 0·0025 mg. per kg. per minute, showed, during periods of six to thirteen minutes, increased caloric outputs of from 12 to 33 per cent. In man it has been shown that injection of 0·0005 mg. per kg. per minute raises heat production 8 to 17 per cent., although half that dosage is without effect (21). The effect does not seem to be due to muscular activity, and is not prevented by hepatectomy. It seems due to extra expenditure of oxidative energy required for reconversion of lactic acid into glycogen (20). It is produced rapidly and ceases rapidly, following cessation of injection.

The Formation of Adrenine in the Adrenal Medulla. While there is no evidence available to demonstrate that the precursor of adrenine in the gland which forms it is laevo-tyrosine, yet their close relationship is so obvious that any suggestion of another precursor will require overwhelming evidence to support it. We have no precise knowledge of any precursor. Probably intermediate products between tyrosine and adrenine have such a transient existence that they are and will remain undetectable.

Mouriquand (61) states that when adrenal glands, dissected from an animal immediately after death, are left *in vacuo* over sulphuric acid for twenty-four hours, the adrenine content appears to increase, and suggests that the gland contains an "adrénaline virtuelle," a pro-adrenine. His chemical technique of measurement is open to criticism and more accurate work does not support his conclusions (60). Szent-Györgyi (87A) has furnished more convincing evidence of the existence of a compound in fresh adrenal medullary tissue, which is much more active physiologically than is adrenine, and which he has termed, provisionally, "novadrenine."

Stefl (82) states that "oxidized adrenine," physiologically inactive, is reduced to a compound giving the chemical and physiological actions of adrenine in the presence of adrenal cortical lipoid preparations. He suggests that, since adrenine is rapidly converted in the blood stream into a physiologically inert substance, there is a circulation of adrenine in the organism; it is secreted into the circulation from the medulla, oxidized, taken up by the cortex, and reactivated and passed to the medulla for further secretion.

The Normal Function of the Adrenal Medulla. While adrenine can be shown to produce very definite effects when injected, it does not automatically follow that these results are physiological in nature and not merely pharmacological. The lack of finality ten years ago in theories concerning the function of the medulla is well exemplified by the presence of two articles by two different investigators in Barker's "Endocrinology and Metabolism." Much of the somewhat controversial character of these articles was due to differences in the critical evaluation of mechanisms for measuring the output of adrenine through the adrenal veins (for which the original articles must be consulted (84, 15)).

Stewart (84) considered it to be established that a measurable and fairly constant amount of adrenine is constantly being discharged into the circulating blood under control of

the nervous system, suggesting that it has a definite function, but that even when the glands are strongly stimulated, as by electrical stimulation of the splanchnic nerves or by strychnine, the increased output of adrenine is merely subordinate in its effect on blood pressure to that of the nervous system. " All the best evidence is to the effect that the blood pressure remains practically unaltered for a time when the suprarenal veins are carefully clipped." He believed that adrenine is not indispensable for life or health.

Cannon (15) stressed the subjection of the adrenal medulla to central nervous influences through the splanchnics ; emotional excitement, pain, asphyxia, and similar phenomena causing nervous discharges through the sympathetic system, stimulated the adrenals so that there was prompt discharge of adrenine into the circulation—hence his " emergency theory " of adrenine action.

According to the " tonus theory " (originally supported by Elliott and Biedl) the function of adrenine is to maintain the sympathetic nerve endings in a state of responsiveness, of moderate activity, of tone. Since small doses of adrenine induce relaxation of the blood vessels and lower blood pressure, Cannon found it difficult to understand how its function could be to maintain a state of tonic contraction.

He regarded the secretion as discontinuous and summed up : " Suprarenal secretion is not a necessity, at least in times of serene existence. There is evidence, however, that epinephrine is secreted in times of great emotional stress and under circumstances which cause pain or asphyxia. The function of the suprarenal medulla is to be looked for under conditions which rouse it to action. Excitement, pain, and asphyxia are, in natural existence, commonly associated with violent struggle for self-preservation. Under such circumstances . . . the operation of the sympathetic division of the autonomic nervous system, together with the aid which epinephrine affords, will muster the resources of the organism in such a way as to be of greatest service to such organs as

are absolutely essential for combat, flight, or pursuit. The cessation of activities of the alimentary canal ; the shifting of the blood from the less insistent abdominal viscera to the organs immediately essential to life itself, such as the lungs, the heart, the central nervous system, and, at critical moments, the skeletal muscles as well ; the increased cardiac vigour ; the quick abolition of the effects of muscular fatigue ; the mobilization of energy-giving sugar in the circulation—these are the changes which occur when fear or rage or pain causes the suprarenal glands to pour forth an excessive secretion. . . . The organism which with the co-operation of increased suprarenal secretion can best muster its energy, can best call forth sugar to supply the labouring muscles, can best lessen fatigue, and can best send blood to the parts essential in the run or the fight for life, is most likely to survive."

Sharpey-Schafer, writing in 1924 (77), considers " On the whole, the evidence is in favour of the view that there is not only a normal and fairly constant passage of adrenaline into the blood, but that this secretion is under the influence of the nervous system through the splanchnics. Although usually insufficient to produce any externally appreciable action, it may be temporarily increased under conditions of nervous excitement, whether caused by drugs, . . . by emotions, or by reflex stimulation, and may then produce definite reactions, similar to those obtainable by intravenous injections of the autacoid." Vincent, also writing in 1924 (92), says : " It would appear that the evidence, physiological and histological, that the adrenal medulla is, in fact, an internally secreting gland, and that adrenine is the product of its secretion, is not very convincing."

Recent work tends to harmonize the views of Stewart and Cannon, and to suggest that the true view is somewhat akin to that set forth by Sharpey-Schafer.

One of the most damaging pieces of evidence against the view that adrenine normally helped to control blood pressure

was the claim that clamping the adrenal veins did not lead to fall of blood pressure. Recent work does not support this claim, and explains the cause of it.

Bazett (6) showed that following adrenalectomy in cats the blood pressure commenced to fall after about an hour, and continued to fall until death ensued in about six hours. His work has recently been confirmed by Vincent and Thompson (94). They showed that Cow (24) was correct in claiming that there is a collateral circulation in the neighbourhood of the adrenal glands, there being "one or more small veins draining the adrenal vein in its course across the gland, into the renal vein, and also a more complicated plexiform group of vessels situated posteriorly." They point out that in consequence of this complex arrangement the older experiments in which only the adrenal veins were clamped or ligatured led to fallacious conclusions, since the adrenine could still leak out through the collateral circulation. They have shown, in experiments on anaesthetized and decerebrate cats, in which both the adrenal veins and the collateral circulation were tied off, that a fall of blood pressure always follows such ligation. This is not permanent. There is slow recovery, probably dependent on vaso-motor control of the splanchnic area. They conclude "the adrenal glands should not be considered as essential to the maintenance of blood pressure . . . but should be described as a normally functioning accessory-mechanism, the removal of which causes a transient fall of pressure."

Prolonged subjection of animals to fatigue, or to cold, markedly depletes the adrenal medulla of adrenine (91, 26, 27). Emotional hyperglycaemia evoked in caged cats by an aggressive dog is but little modified when the splanchnic branches to the liver are cut, but is profoundly affected following removal of both adrenal medullas. Blood sugar is significantly depressed, and liver glycogen remains within normal limits, suggesting a failure to mobilize liver glycogen

through lack of adrenine, and supporting Cannon's emergency theory (11).

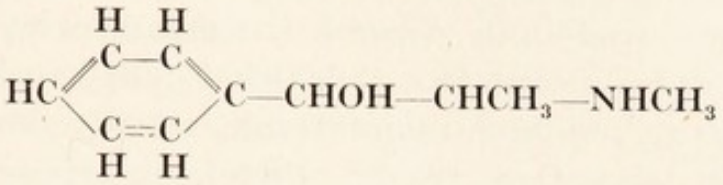
Cannon (14) has recently summarized the evidence in favour of discontinuity of adrenal secretion, but admits that " there is no logical antagonism between the ' tonus ' theory and the ' emergency ' theory." Since even such minor exercise as walking has been shown to call forth a definite secretion of adrenine (16), obviously the difference of viewpoint is of little more than theoretical interest ; under the ordinary conditions of existence sufficient adrenine must be available in at least regularly intermittent intervals to affect both blood pressure and carbohydrate metabolism almost continuously.

Systemic Effects Intermediated through the Adrenal Medulla. The essence of the emergency theory relates emotional glycosuria to increased action of adrenine. Nicotine poisoning leads to glycosuria and increased secretion of adrenine, and the slight hyperglycaemia which follows the smoking of tobacco is attributable to the same intermediation (52). In certain states of emotional tension in mental patients sugar tolerance curves show a delayed return to normal fasting values, and this effect is also traceable to adrenine action (53). In hyperthyroidism the emotional instability generally present is probably in part responsible —through adrenine action—for the hyperglycaemia and glycosuria so often present.

Histological Demonstration of Adrenine Secretion. Cramer (25) treats the resting adrenal gland with osmic acid vapour and states that adrenine becomes visible as granules in the medullary cells. When the gland is stimulated to activity these adrenine granules are seen to be expelled into the veins of the gland, giving a clear visual demonstration of " internal secretion." By this procedure he has demonstrated that exposure to cold is a powerful stimulus to the medulla, while asphyxia and ether anaesthesia also stimulate secretion.

Ephedrine. Since adrenine is without action when administered by mouth, it is interesting to contrast with it ephedrine, the recently discovered principle of the ancient Chinese drug Ma Huang. The literature concerned with it has been reviewed by Chen and Schmidt (18).

It is laevo-rotatory, with the formula :



It is the chief active principle of the Asiatic species of *Ephedra* plants.

It produces its pharmacological effects when given by mouth or by injection. Its toxicity is low. Individuals who do not have a vago-sympathetic equilibrium may experience untoward symptoms.

It produces certain sympathomimetic actions. It raises the blood pressure, increases cardiac activity, dilates the pupil, relieves bronchospasm, and contracts the uterus. It produces hyperglycaemia, and slightly increases the basal metabolic rate and oxygen consumption.

Its action, compared with that of adrenine, is less intense, but more prolonged.

It has been used clinically with success in the treatment of bronchial asthma, hay fever, whooping cough, bronchitis, postural hypotension, etc.

Abnormal Conditions of the Adrenal Medulla

Hypofunction and hyperfunction of the adrenal medulla are theoretically possible. It is sometimes supposed that the former is the cause of the lowered blood pressure in Addison's disease, although that is primarily a disease of the cortex (cf. p. 211), while, as has been seen, there is no sound evidence that depression of adrenine function permanently depresses the blood pressure. There is as yet little sound evidence of disease-entities in which the symptoms are definitely attributable to hypoplasia of chromophil tissue. Goldzieher has reviewed the favourable evidence (33).

It is at present only useful to examine the indications for a disease-entity including hyper-medullary-adrenalism. From

the known effects of injections of adrenine in amounts greater than the physiological output of the glands, a pathological hyperfunctional state of the medulla may well be accompanied by a persistently heightened blood pressure and some degree of hyperglycaemia, with low-level muscle glycogen. Such effects would only occur if the organism were unable to effect a readjustment. Goldzieher (33) considers that while severe symptoms might result, usually death would not ensue, and they would, therefore, probably evade interpretation.

Within the past few years a number of cases of tumours of chromophil tissue have been reported, some authenticated at autopsy, and others actually removed surgically with beneficial results. These permit definite characterization of the disease syndrome which such tumours produce. These tumours consist of abnormal proliferations of mature phaeochromocytes, in Rabin's terminology (67), and are variously termed " chromaffin tumours," " paraganglioma," and " phaeochromocytoma." (Other groups of tumours arising from the adrenal medulla, neuroblastomata and ganglioneuromata, are derived from cells comprising the nervous or non-specific elements of the adrenal glands (67).)

Rabin (67), Labbe (50), and Goldzieher (31) have summarized the literature of such cases. About forty have been reported. Some are much better authenticated than others. Typical authenticated cases are :

Labbe's cases (50). A young woman developed, rather suddenly, attacks of vasomotor disturbances, nausea, vomiting, and crises of hypertension. The attacks set in with shivering and pallor, followed by palpitation, rapid pulse, perspiration, and cyanosis of the extremities. During the attacks her temperature rose. Within a few weeks a mild albuminuria and some degree of nitrogen retention developed. She died as a result of lung oedema, following one of the attacks. Autopsy showed an adenoma (or a paraganglioma)

of the left adrenal medulla, and no other change except slight kidney lesions.

A man aged twenty-nine had had attacks of paroxysmal hypertension, with malaise, tachycardia, and profuse sweating, during several years. In these attacks his blood pressure rose from 160/100 to 250 or 300, or even more. The attacks started with a feeling of anxiety, pallor, eyes looking haggard, pupils dilated, violent palpitation followed by tachycardia and profuse sweating. They lasted about an hour, and were frequent, sometimes several in twenty-four hours. Finally a right-sided hemiplegia developed, then permanent albuminuria, acetonuria, and cholesterolaemia. He died in an acute attack. Autopsy showed chronic nephritic lesions. The left adrenal was normal and weighed 7 grams. The right adrenal was about the size of a small orange, and weighed 120 grams, the tumour, histologically, being a paraganglioma.

Oberling and Jung's case (78). A woman, close to term in her second pregnancy, was found to have a blood pressure of 250/190, with marked albuminuria and severe headaches. The pressure subsequently varied considerably. Two hours after delivery she went into shock, dying some hours later. At autopsy a paraganglioma was found.

Rabin's case (67). A woman, aged forty-five, had had palpitation and dyspnoea on slight exertion, and a tremor of the hands for ten years. She had been treated—as a case of Graves' disease—by Roentgen rays for suspected substernal goitre ; she had been operated on several times for sterility. For several years she vomited about once a week ; this increased until she vomited almost nightly. She exhibited moderate exophthalmos, a marked tremor, and hypertrichosis of the chin. Her blood pressure varied from 226/108 to 177/122. The clinical diagnosis was Graves' disease and chronic nephritis with hypertension.

Autopsy showed marked hypertrophy of the heart, generalized arteriosclerosis, chronic passive congestion of the viscera, infarcts of the lungs, and a phaeochromocytoma of

the medulla of the right adrenal. The thyroid was small and granular and, microscopically, showed no evidence of hyperplasia. The tumour was examined chemically twenty-four hours after death and found to contain 60 mg. of adrenine, 1·5 mg. per gram. The tumour occupied almost the whole volume of the right adrenal; at the upper pole there was a cap of cortex and medulla, normal in appearance. Surrounding the tumour was a fibrous capsule, which blended with the yellow cortex. The tumour tissue was rather soft, homogeneous, and reddish brown; it showed areas of haemorrhage. Microscopically, the tumour consisted of anastomosing cords and islands of large polyhedral cells, with marked staining capacity. Many showed the chromic reaction.

Reviewing the reported cases, Rabin remarks: "Usually the tumour is benign. It is perfectly encapsulated, does not give rise to metastases, and does not cause a cachectic state. . . . It is evident that the tumour is actively secretory. . . . The cellular structure is similar to that of the normal suprarenal medulla." [1] He points out that outstanding features of these cases are the frequency of hypertension and signs of vasomotor or autonomic instability, the inability of many of the patients to withstand minor operations, sudden death ensuing without demonstrable cause, and the occurrence of unexplained glycosuria.

[1] Concerning the terminology, Rabin remarks: "It is perhaps advisable to offer some justification for the term phaeochromocytoma. The tumour has been known variously by the names angiosarcoma, perithelioma, struma medullaricystica suprarenalis, paraganglioma, and chromaffin cell tumour. The first three names may be excluded for obvious reasons. The term paraganglioma was originated by Alezais and Peyron in 1907 in describing a tumour of the sacro-coccygeal region. It was derived from the name paraganglion, which was applied by Kohn to the chromaffin system, appropriate since it described the embryonic origin of the system. Pick, however, suggested the advisability of naming the tumour from the predominating type of cell—in this case the phaeochromocyte, the name of which, originated by Poll, is generally accepted. It appears especially advisable to use the name of the mature chromaffin cell, because of the parallelism between this tumour and the ganglioneuroma, which was named after the mature sympathetic cell, which is developed from the same anlage."

Goldzieher's four cases showed at autopsy nodular and diffuse hyperplasia of the adrenal medulla. In two of these cases there had been definite hypertension.

Mayo, in 1927 (57), reported the first case which came to operation. A woman, aged thirty, suffered from intermittent attacks of paroxysmal hypertension, which were attended by generalized vasoconstriction or spasm, as evidenced by pallor of the skin, and complete obliteration of the capillaries of the nail folds during the attacks. Presence of abdominal distress, together with the belief that the abdominal sympathetics might be involved, led to exploration. A tumour was found, situated over the left adrenal gland beneath the tail of the pancreas. In addition, the left adrenal was twice normal size, and the right gland slightly enlarged. Permanent relief resulted from removal of the tumour, suggesting that this was the sole cause of the illness. The tumour was diagnosed as a retroperitoneal malignant blastoma.

Shipley, in 1929 (78), reported the case of a woman, aged twenty-six, who suffered from paroxysmal attacks of hypertension of increasing frequency, while severe occipital headaches became an increasingly troublesome symptom. Between attacks her blood pressure was 120/90; during attacks it rose to 219/110, and even higher. Diagnosis of adrenal tumour was made; there was no clue to indicate which gland was affected. Exploration showed the right adrenal involved, and this was removed at subsequent operation. Convalescence was stormy. Ten months later she was entirely free from symptoms, with normal blood pressure. The tumour weighed 115 grams, measured $9 \times 7 \times 3{\cdot}5$ cm., and was completely encapsulated. Macroscopically, it was a tumour of the medulla, microscopically a paraganglioma.

Porter (66) has reported another case, diagnosed before operation. The patient, male, aged thirty-nine, presented slightly different symptoms. Peculiar attacks had occurred

for some time, usually while he was in bed, apparently without cause or warning, and accompanied by an unpleasant sensation in the epigastrium, similar to, but not exactly, nausea. It was found that these effects could be induced posturally, if he was slightly inclined forward and to the left. During the attacks the systolic pressure rose from 110 to 200 in 90 seconds, while the heart slowed to about 55, but with an unusually forcible beat, sufficient to shake the chair or bed he was occupying. The attacks lasted three or four minutes; the pressure then dropped rapidly, and in 10 or 15 minutes his condition was normal. At operation, a tumour of the right adrenal was removed. Recovery was uneventful, and the paroxysmal attacks did not recur. The tumour was spherical, with a perfect capsule. Ewing diagnosed it as an adrenal adenocarcinoma, and considered it as probably a cortical tumour.

Volhard (95) has described a very interesting case of a man, aged thirty-eight, who showed marked paroxysmal hypertension, the blood pressure rising from 180/130 to over 300; the attacks were frequent, 3 or 4 a day, and were accompanied by severe headache. A medullary tumour was diagnosed, and a laparotomy performed. No tumour of the right adrenal could be felt, and it was decided that the left adrenal must be affected. Subsequently, at operation, the left adrenal was found to be three times the normal size. It was removed, and found to consist chiefly of medulla. The patient died during the following night. At autopsy an encapsulated egg-sized tumour of the right medulla was found. Only a trace of cortex tissue was present. It would thus appear that the enlargement of the left adrenal was compensatory, and that the patient died of cortical insufficiency.

Vaquez, Donzelot and Gerardel (90) have reported the case of a thirty-seven year old man, in whom similar changes of blood pressure occurred during the paroxysmal attacks. X-ray treatment was applied to the lumbar region and led

to temporary improvement, but the attacks returned after some months, accompanied by a permanent hypertension (210–230). Operation was advised and refused. The patient died in coma. Autopsy disclosed a paraganglioma of the right adrenal. A case of Volhard's also showed gradual development of a permanent hypertension, on which the increase due to the paroxysmal attacks was superimposed. X-ray treatment in this case was ineffectual, and, on other grounds, operation could not be advised.

It is evident that in the majority of these cases the tumours are definitely functioning tumours of the adrenal medulla, while the most characteristic abnormality during life is paroxysmal hypertension, although subjective symptoms vary considerably in different patients. It can therefore be concluded that at least some proportion of the cases of paroxysmal hypertension are due to hypersecretion of adrenine through an increased volume of functioning medullary tissue.

There is reasonable ground for belief that the accessory chromophil bodies also secrete adrenine. It is not surprising, therefore, that similar tumour masses should be found associated with some one or other of these bodies. Rabin (67) has reviewed the literature of such tumours, and considers that Mayo's case should perhaps be included among them.

If it be admitted, and there is good ground for so doing, that marked hyperproduction of adrenine from tumour masses can lead to a definite pathological syndrome, then there must occur intermediate stages with less definite symptoms. Obviously some degree of hypertension—probably intermittent—is to be expected. It by no means follows that hypersecretion of adrenine is to be considered a common cause of hypertension or of arteriosclerosis. The evidence in favour of its being a possible cause has been set out by Goldzieher (33).

Shapiro has suggested that a syndrome exists whose outstanding symptoms are increased basal metabolic rate and vascular hypertension (systolic and diastolic), and which bears some resemblance to Graves' disease (76). Oppel (63) thinks that Raynaud's disease may be a hyperadreninaemia, and claimed an improvement lasting sixteen months, following removal of one adrenal in one case.

The Adrenal Cortex

The fact that extirpation of the adrenals leads rapidly to death, while destruction of both medullas does not do so, is in itself no proof that the adrenal cortex secretes an endocrine compound, even though the adrenals are ductless glands.

One of the most characteristic phenomena following removal of both adrenals in an animal is the delayed but rapidly increasing asthenia. Vincent describes the results in Hultgren and Andersson's early experiments : " After the operation the animal recovers in a few hours, and in the first few days shows no ill effects from the operation, except some loss of appetite. During the last twenty-four hours before death, or earlier, the animal becomes stupid and quiet, and shows (especially is this the case with cats) weakness and uncertainty of movement in the hinder extremities. During this period too, the temperature begins to fall, and the apathy and weakness increase. Then the hind limbs become stiff, the animals tire on the slightest exertion, and show extreme prostration. Finally, with increasing asthenia, there is dyspnoea, heart weakness, and death. In rabbits, convulsions are common, but do not occur in cats and dogs " (92, cf. also 4).

The blood urea rises and the blood sugar falls, but does not usually drop to convulsive level. The inorganic phosphate of the plasma gradually increases, while the carbon dioxide capacity decreases (69, 98).

Biedl showed, in 1910, that removal of the " interrenal body " (cf. p. 174) in elasmobranch fishes produced a very similar series of symptoms. His results have recently been fully confirmed by Kisch (45). The train of events is : a persistent balling of the pigment of the skin chromatophores, so that the animals take on a dirty-gray colour, slowing of respiration, muscle weakness, shortening of the body musculature, hypersensitivity to oxygen-scarcity, and death. Injection of acid extracts of interrenal tissue may delay death for hours. Injection of sea-water, adrenine, or liver extract is without effect. Death appears due to respiratory failure.

Kühl (48) utilized the effect of muscle weakness in adrenalectomized animals as a test for potency of adrenal extracts. His experimental technique seems open to criticism, so that his conclusions cannot be stressed (30, 59).

Vincent and Thompson (94) found that, while decerebrated cats continue to breathe for several hours, after extirpation of both adrenals they invariably die within thirty to sixty minutes. Ligaturing the total blood supply to the glands is as rapidly fatal as extirpation, but ligaturing the veins is not. Interference with the flow of lymph from the glands by removal of the adjacent posteriorly-situated lymph node, or similar procedure, produces rapid death. Death is delayed by injection of fresh saline extracts of adrenal glands, but not by injection of adrenine. Hence they concluded that the adrenal cortex discharges an endocrine principle through lymph channels which is essential for normal respiration in decerebrate cats (and for this they suggested the name " pneumin "). Their work has been repeated by several groups of investigators ; the results are not concordant, but are on the whole adverse to Vincent's conclusions (68, 30, 96, 65).

Preparation of Active Extracts of the Cortex

For the evidence that the adrenal cortex produces an internal secretion, and for the successful preparation of concentrated extracts of that secretion, we are indebted to three groups of investigators—Stewart and Rogoff, Hartman and his co-workers, and Swingle and Pfiffner.

Stewart and Rogoff recognized that the only successful biological test for the principle was the prolongation of the life of an adrenalectomized animal by injections of a potent extract. They showed first that with fine surgical technique it was possible to prolong the lives of adrenalectomized dogs to a moderately constant span. The adrenals were removed in a two-stage operation. An interval of a week gave as good results as a longer period. In their first series the average length of life following the second operation was seven days; two out of seventy-four animals lived to the fifteenth day. In a later series with still better technique, the average duration of life was eight or nine days; the maximum was practically unaltered. Cats survived an average period of eleven days; one lived thirty-two and a half days. (They noted, incidentally, that such adrenalectomized animals frequently develop ulcers in the stomach or duodenum.)

Extracts of fresh adrenal cortex in 0·9 per cent. saline or glycerol, injected intravenously into adrenalectomized animals on alternate days, frequently prolonged their lives beyond the maximum span observed with controls. (Injections of Ringer solution containing glucose also had beneficial effect.) They considered that their cortical extracts contained an active principle, and for this they suggested the name "interrenalin" (69, 85, 83).

Hartman (34, 35) employs adrenalectomized cats as test animals. He removes the adrenals through the lumbar path in two stages, with an interval between operations of from two to seven days. After the second operation the animals are not fed for forty-eight hours. Their diet consists of canned salmon, with occasional meals of beef. They are kept at a room temperature of 27° to 28° C. Such cats, without treatment, live five or six days after complete adrenalectomy.

He has prepared extracts by two procedures. In the first beef cortex was extracted with excess of water, 0·1 *N* acetic

acid added to *p*H 4·9, the precipitate that resulted centrifuged off, and the liquid saturated with sodium chloride. The salted-out precipitate was dissolved in water, the *p*H adjusted to 7·35 and the sodium chloride content to 0·9 per cent. The solution was sterilized by passage through a Seitz filter. He claimed that this extract prolonged the lives of his test animals to an average of twenty-seven days. Stewart and Rogoff (85) could not confirm his results. This extract probably contained some slight amount of the active principle, but such amount was insufficient to restore animals who had reached the pre-mortal stage of prostration ; it only delayed death.

The second procedure has given better results. Cortex tissue is extracted with ethyl ether, which removes very little adrenine. The ether is distilled off *in vacuo*, the residue extracted with warm 80 per cent. ethyl alcohol, and the solution chilled ; inert material separates and is filtered off, and then the alcohol distilled off *in vacuo*. The residue is dissolved in water and sterilized. It is almost free from adrenine.

Hartman terms the active principle *cortin*.

Adrenalectomized cats can be kept alive indefinitely by continued injections of this second extract. It restores animals from the last stages of adrenal insufficiency. Young rats, whose adrenals have been extirpated, have been maintained in normal condition to the adult stage and have reared normal litters. Adrenalectomized rats exposed to cold show a marked fall of body temperature and may die ; following injection of the extract they react to cold like normal rats.

The high blood urea of the adrenalectomized animal disappears following injection. The threshold for fatigue is increased. Fatigue is one of the earliest symptoms to appear in adrenal insufficiency, and is among the last to disappear following successful treatment. Hartman claims that the nerve reflex, the myoneural junction, and the muscle

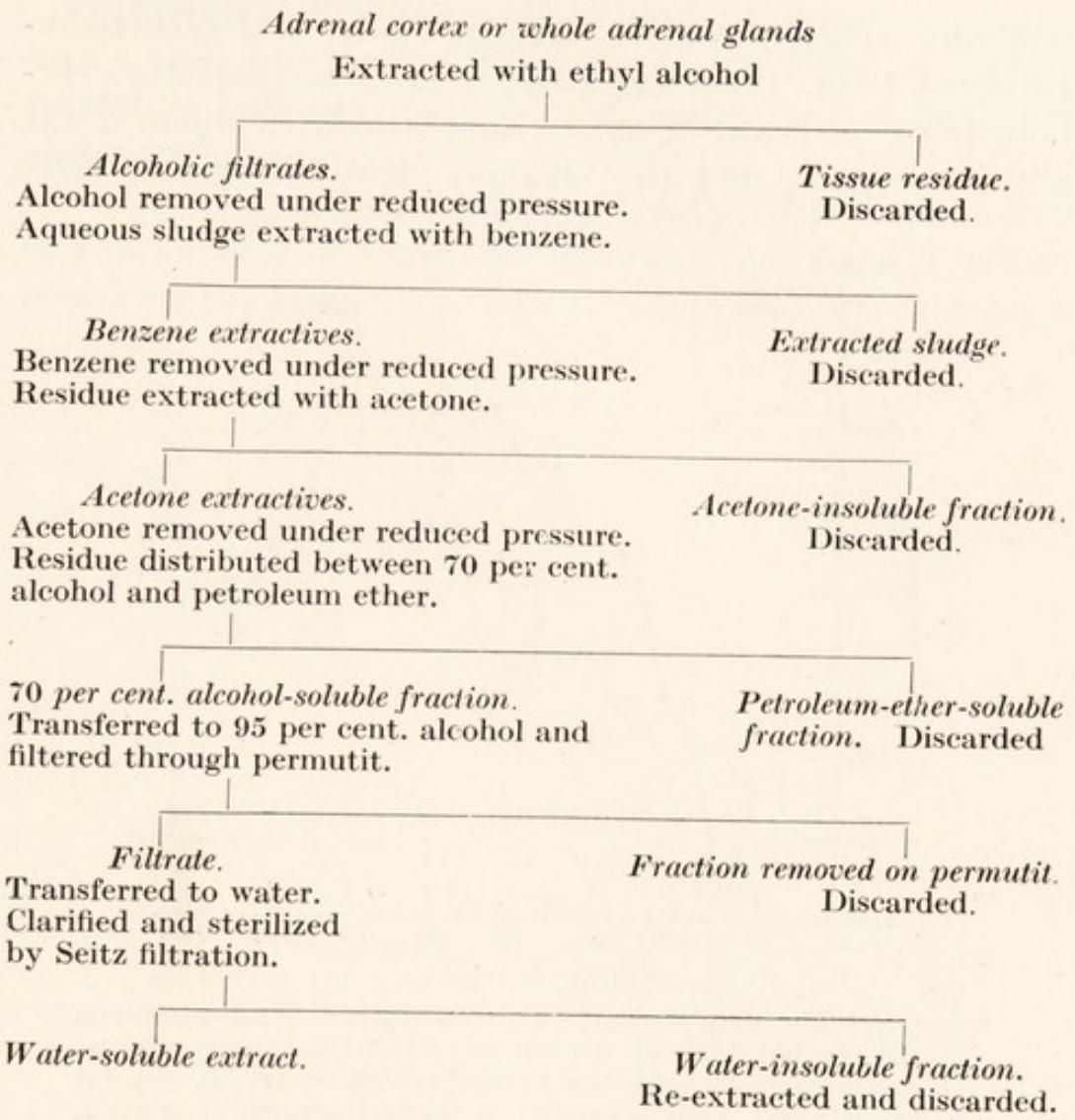
itself are involved in the production of this fatigue and that his cortical extract restores all three of these to normal condition.

His extract is said to be ineffective by mouth. When given subcutaneously its effect can be detected in a few hours.

Swingle and Pfiffner commenced to publish their results in 1929. A complete *résumé* of their work has recently appeared (86). They have, for the most part, used cats, in whom they find that only about 3 per cent. have accessory cortical bodies. The animals are kept in thermostatically heat-regulated rooms, fed identical diets, and adrenalectomized in two operations; there is an interval of at least seven days between removal of the right and left adrenal. Such cats, untreated, die on the average 8·6 days after the second operation. The maximum period of survival (one cat out of 100 animals) was twenty-five days.

Their original extracts were potent, but were prepared for injection in oil, and repeated injections gave rise to painful abscesses. Their present method is shown in the following schema [1]:

[1] It has been recognized for some time that, presumably because of the common circulation, cortical tissue always contains a small amount of adrenine. One of the difficulties associated with preparation of cortical extracts is the removal of this adrenine; this is necessary especially when it is desired to use such extracts for intravenous use clinically. Swingle and Pfiffner have observed that the solubility of adrenine in lipoid solvents is markedly increased by the presence of lipoid material, such as occurs in the adrenal cortex.



Due to the action of permutit, the final product is practically free from adrenine. The volume of the final solution is adjusted so that 1 c.c. is equivalent to 30 grams of fresh beef adrenal cortex.

On the ground that the principle is still insufficiently characterized to be named, Swingle and Pfiffner prefer to refer to it at present simply as the "adrenal cortical hormone." Their method appears to give a purer preparation than other procedures and they are steadily obtaining more concentrated preparations (64A).

Chemical Properties of the Cortical Principle. The purest preparations are protein-free. They give negative biuret,

ninhydrin, glyoxylic acid, Molisch, Pauly, and Lieberman-Birchard tests, excluding polypeptides, tryptophane, carbohydrate, cholesterol, etc. Xanthoproteic, Millon's, the alkaline copper, and the alkaline phosphotungstate tests

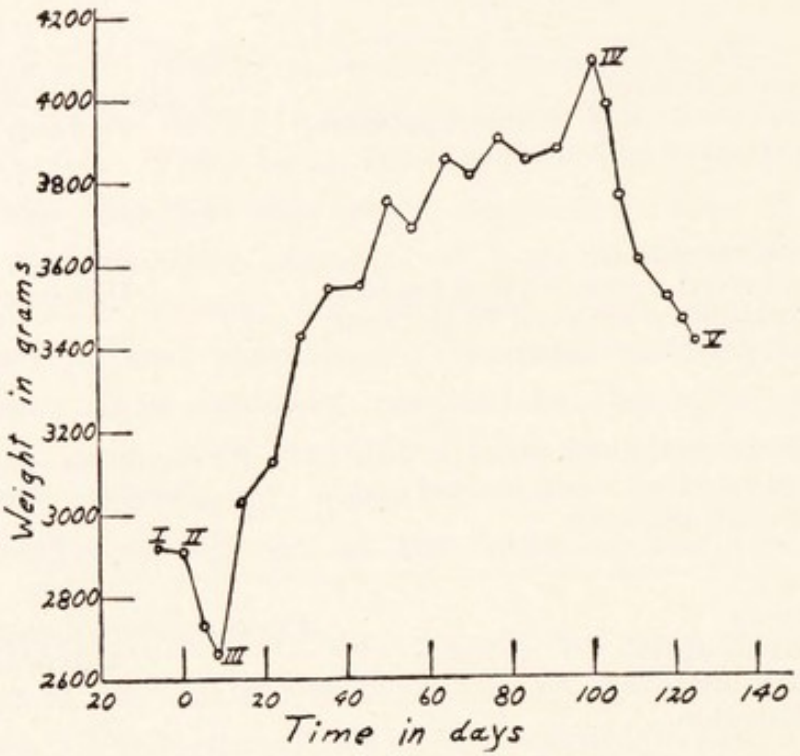


FIG. 18.—The weight chart of a bilaterally adrenalectomized cat treated with an active extract of the cortico-adrenal principle, following the exhibition of severe symptoms of adrenal insufficiency. I. Right adrenal removed. II. Left adrenal removed. III. Animal prostrate; treatment begun. IV. Treatment discontinued. V. Death from adrenal insufficiency. (From Pfiffner and Swingle, *Endocrinology*, 1931, xv, 338.)

are positive; this is attributable to traces of phenolic decomposition products of adrenine.

The method of separation of the compound suggests that it is to some extent lipoidal in nature. It is thermolabile, is decomposed by dilute alkali, but is apparently stable to dilute acid.

A claim has recently been made that an active preparation has been obtained which appears crystalline under the microscope (33A).

Physiological Properties of the Cortical Principle. Hartman's and Swingle and Pfiffner's observations with their respective extracts are in good agreement. The latter observers have proceeded farther.

Adrenalectomized cats, injected daily with a small dose (0·5 to 1·0 c.c.) of active extract, have been kept in perfect health for 100 days, when they were sacrificed to demonstrate

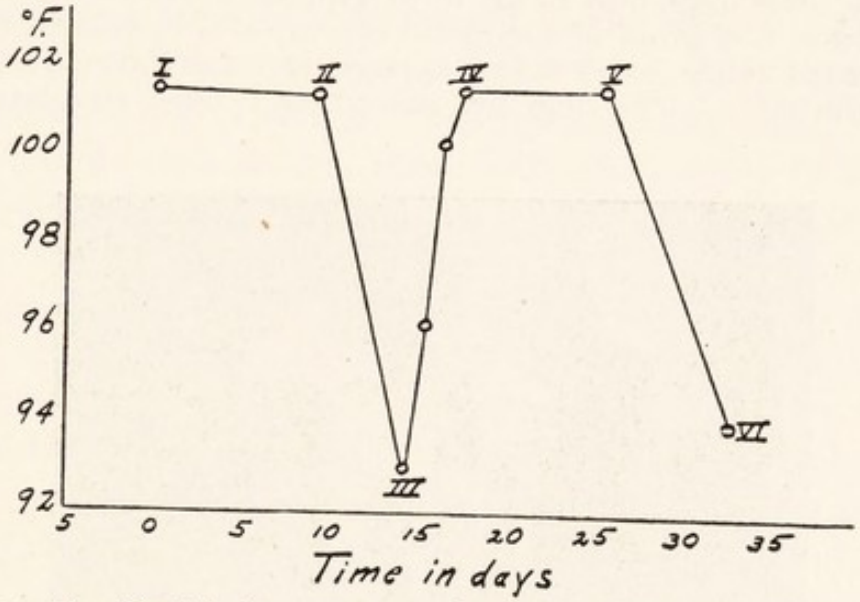


FIG. 19.—Rectal temperature chart of a bilaterally adrenalectomized cat treated intraperitoneally with an active extract of the cortico-adrenal principle, following the exhibition of severe symptoms of adrenal insufficiency. I. Second adrenal removed. II. No symptoms. III. Prostrate; treatment begun. IV. No symptoms. V. Treatment discontinued. VI. Animal prostrate (died of adrenal insufficiency). (From Pfiffner and Swingle, *Endocrinology*, *loc. cit.*)

absence of cortical tissue. In such experiments the cats ate, played, fought, and kept themselves sleek and clean; in other words, their behaviour was perfectly normal. If at any time injections were stopped such animals developed adrenal insufficiency in usual fashion and died within ten days. At any time before death recommencement of injections in larger and more frequent dosage brings complete restoration. Similar results have been obtained with dogs.

The effects of deficiency and administration of the principle are well shown in Fig. 18 (showing changes in body-weight)

and Fig. 19 (showing changes in body temperature). The perfect condition of adrenalectomized animals following prolonged replacement therapy is demonstrated in Fig. 20.

Assay of the Principle. Harrop and Weinstein, working with Swingle and Pfiffner, have developed a principle of assay based largely upon the change in blood non-protein nitrogen and urea which follows complete adrenalectomy.

Adult male dogs, 6 to 10 kg. in weight, are adrenalectomized in two stages, and proof of successful operation is demonstrated by withdrawal of the extract and appearance of definite symptoms of insufficiency. The dogs are placed on a fixed standard diet

FIG. 20.—Bilaterally adrenalectomized female and male dogs about one year after operation. Both animals had been repeatedly brought into a condition of adrenal insufficiency by temporary discontinuance of the injections of adrenal cortex principle. (From Swingle and Pfiffner, *Medicine*, 1932, xi, 389.)

and the amount of extract is determined, necessary to keep them in normal condition.

" A dog unit (D.U.) is defined as the minimum daily kilogram dose of cortical hormone necessary to maintain normal physiological conditions in the adrenalectomized dog for a period of seven to ten days; the two criteria of normal physiological condition being maintenance of body weight and blood level of non-protein nitrogen (or urea) " (86).

Britton and his co-workers (12), using extracts made in accordance with Swingle and Pfiffner's procedure, have made a number of valuable observations. Intraperitoneal injections into cats in extreme pre-terminal prostration

produce evident benefit in from fifteen to thirty minutes. Convulsions are abolished; the animals show an interest in their surroundings and attempt to sit up. Within an hour they may walk about and appear practically normal; two hours after injection they may take food. The amount necessary to produce complete restoration is 5 to 10 c.c. of an extract (of which 1 c.c. is equivalent to 40 grams of fresh whole gland) per kilo body weight in the course of twenty-four hours. This dose represents at least 2,000 times the amount actually present in a normal cat's adrenals.

They note that 20 c.c. given intraperitoneally to a 2-kg. cat produced no ill-effects. The extract is non-toxic when given subcutaneously, intramuscularly, intravenously or intracardially. Normal dogs are stimulated to carry on two or three times as much work as usual under its influence. It produces sexual precocity in rats, through precocious maturation of the sex glands. Marked effects were observed in the ovaries (presence of corpora lutea) and uterus (hypertrophy) in female rats at twenty-eight days of age after two weeks' injection. Appearance of early maturity in the testes was not so striking and occurred later. Hypertrophy of the anterior lobe of the pituitary gland was observed in twenty-day-old rats after injection, and Britton considered it possible that the effects on the gonads are secondary to enhanced pituitary secretion. No significant changes were found in the thyroid or adrenals of such animals.

The extract is effective when administered by mouth; the dosage, to be effective, must be three to five times as great as by the intraperitoneal route.

Wilson (96) and Houssay (42) have confirmed the activity of extracts prepared following Swingle and Pfiffner's directions. Kutz (49) has devised a somewhat simpler method of preparation and suggested a method of assay with rats.[1]

[1] Attempts, in no way comparable with those that have just been described, to prepare active cortical extracts have been made by Goldzieher (32), Koehler (47), Schmitz ("cortisupren") (75), Reiss (68), and Frankl ("cortigen") (29). de Mira (58) compared extracts made

Hoskins (40) has prepared a potent extract very simply by extraction with glycerol. This is effective orally, and has been tested on schizophrenic patients, whom he believes to be in a condition of chronic hypoadrenalism, on account of secondary anaemia, low blood pressure, reduced body temperature and subnormal oxygen consumption. After ten weeks' treatment the average systolic blood pressure had increased from 106 to 133, and the diastolic from 69 to 84. The patients also showed some increase in body weight and red cell count.

From their general results on animals Swingle and Pfiffner conclude that the function of the adrenal cortical principle is the regulation and maintenance of a normal circulating volume of fluid within the vascular system. They consider that the adrenalectomized animal eventually dies from circulatory collapse due to insufficiency of circulating fluid, there being a progressive fall of blood pressure, paralleled by a steady decline in blood volume; the fluid is lost, presumably, by transudation.

They point out that, in general, the events bear a striking resemblance to traumatic or secondary shock in man, and therefore they believe that injections of the principle should be of value in shock (86A). Britton disagrees with their views and has put forward a theory of carbohydrate control (11A).

Hypo-Cortico-Adrenalism. Addison's Disease

Two classical studies of Addison's disease have been presented, that of Thomas Addison himself in 1855, and the recent monograph of Rowntree and Snell (72). It is significant that the latter not only reproduce Addison's original paper in their monograph, but, in agreement with

by a number of procedures including that of Swingle and Pfiffner; using the adrenalectomized guinea-pig. He found none active. As none were tested on the adrenalectomized cat, it is not possible to comment on the accuracy of his chemical or biological technique.

all other recent writers on this subject, confess their inability to better his description materially. Any discussion of Addison's disease in the near future must largely refer to their monograph. Its clinical conclusions are based on a study of 115 cases in which a positive diagnosis of the disease was made. In thirty-three of these the diagnosis was confirmed at necropsy.

Signs and Symptoms. Addison wrote : " The leading and characteristic features of the morbid state to which I would direct attention are anaemia, general languor and debility, remarkable feebleness of the heart's action, irritability of the stomach, and a peculiar change in the colour of the skin." Rowntree and Snell write : " Little of importance has been added in the years that have intervened, except recognition and appreciation of loss of weight and decrease in blood pressure." The onset is usually, but not invariably, insidious ; this is especially true of tuberculous patients. A respiratory infection, often diagnosed as influenza, may mark the beginning of the illness. Rowntree and Snell suggest that this may not be influenza at all, but an acute initial phase of Addison's disease.

The duration of the disease is usually between six months and two years. Lippmann has recorded a case with symptoms lasting only eighteen days. Chronic cases may persist several years. The patients are invalids throughout the course of the disease ; few can be rehabilitated to 50 per cent. of their former working capacity. No definite cure has been produced.

Subjective and objective asthenia, mental as well as physical, is a cardinal symptom, and often the first to appear. It fluctuates, being worse after periods of physical or mental activity. There is marked lack of resistance to infection, exposure, stress, and drugs. Many of the symptoms and complications of the disease are secondary to that " remarkable feebleness of the heart's action " which Addison stresses, with the resultant hypotension and poor circulation.

Anorexia, nausea and vomiting, gaseous distension, and occasional periods of intense diarrhoea (although there is a greater tendency to constipation) are among the gastro-intestinal manifestations. Stomach and intestinal ulceration is often found, just as in adrenalectomized animals (cf. p. 196). There is occasionally frank haemorrhage. Hypochlorhydria is common, achylia frequent. These digestive disturbances are responsible for the marked loss of weight, which averages 30 lb. There is no noticeable emaciation. Muscular tissue atrophies.

The *acquisition* of skin pigmentation is the most striking visible sign, although not constant. The colour varies from negroid to amber and blue-gray ; the depth of colour varies still more. The hands and arms, face and neck, and areas subjected to pressure or friction are especially affected. Areas normally pigmented have the pigmentation accentuated. The colour of the hair often darkens. The lips are usually dark, and dark patches are seen in the mucous membranes of the mouth. Jet black freckles are common. (Racial pigmentation must be excluded.)

Course of the Disease. It usually progresses steadily, but striking remissions and exacerbations may occur, and even sudden death. Hypotension and gastrointestinal symptoms are pronounced in crises. In such crises the blood volume is often markedly decreased, the blood is thick and viscid, and there are clinical evidences of dehydration. Death may occur in such crises.

Failure may be gradual, with increasing asthenia to complete exhaustion, or termination may be characterized by persistent nausea and vomiting and cerebral symptoms, or there may be sudden collapse after exercise or during a mild infection. "The manner of death is not greatly different in many cases from that seen in the experimental animal after suprarenalectomy."

Etiology and Related Factors. The disease is commoner in men, and commonest between the ages of thirty and fifty

years. Tuberculosis and atrophy of the adrenals are responsible for the majority of cases. Syphilis, according to Warthin, is a frequent cause of the atrophy. Carcinoma does not seem to be a cause. Marañon (56) thinks that there may be a racial factor, and that the disease is relatively commoner in Spain.

Laboratory and Clinical Data. Rowntree and Snell's study presents the most accurate and complete series of data. In uncomplicated cases the body temperature is usually decreased (97° to 98° F.) in keeping with the lowered rate of metabolism; it does not fluctuate markedly. In presence of active tuberculosis the temperature may be above normal. There may be a considerable rise in temperature two or three days before death. Respiration is usually normal, but becomes markedly irregular in crises and in the advanced stages of the disease. Air hunger may be complained of, and sighing respiration sometimes develops.

The urine volume remains at low normal except in advanced stages, when it is markedly diminished. Its specific gravity tends to be low, and, in late stages, to be fixed between 1·008 and 1·012. Albuminuria in traces or larger amounts is frequent, but glycosuria is not found in uncomplicated cases. Hyaline and granular casts are common, but pus cells and erythrocytes, when present, are usually due to concomitant tuberculous lesions in the kidney or urethra. Creatinuria is not uncommon, but since it is usually present in conditions involving muscular atrophy, it is of no special significance.

Renal insufficiency, partly due to circulatory asthenia, is often present in crises and in the terminal stages. Nephrosis and tubular atrophy are frequently seen at autopsy. Of the blood constituents sulphates increase in crises. Blood sugar tends to low normal values. Achlorhydria is frequent; hypochlorhydria the rule. The basal metabolic rate falls when there are marked nausea and vomiting, and in crises; such decrease is probably due to partial starvation. The rate is usually within normal limits.

Treatment of the Disease. The history of this treatment falls naturally into two parts—before, and after the preparation of active extracts of the adrenal cortex. During the earlier period treatment could only be palliative, somewhat postponing death. During the second, the present period, it is possible to aim higher, and we may well believe that cortical replacement therapy may become as completely successful as insulin therapy has in diabetes mellitus. Since, however, adrenal material is much more difficult to obtain, success will depend ultimately upon synthesis of the active principle. It is fortunate that, whereas insulin, a protein, will probably never be synthetized, the cortical principle seems to be a relatively simple lipoidal compound, and its synthesis in the early future is a strong possibility.

Where the underlying cause of the adrenal lesion is known (tuberculosis or syphilis) its own special treatment should, if possible, be instituted. For the general care of the patient Rowntree and Snell's monograph should be consulted. They stress the value of rest, relaxation, and freedom from work during the early and progressive stages. In crises adrenine is given to the point of tolerance, and 10 per cent. glucose and 0·9 per cent. saline intravenously (presumably as palliative to the dehydration and hypotension).

The Muirhead *régime* was commenced by Dr. A. L. Muirhead on himself in 1920. The results were so beneficial that it has been used in fifty-seven of the cases of Rowntree and Snell. Of these thirty-two were benefited, and in twenty the immediate results were excellent. Some were rehabilitated for many months, and ten for periods of from three to seven years.

Adrenal gland was taken by mouth and adrenine injected to the limit of tolerance. It has only recently been demonstrated (cf. p. 203) that the adrenal cortical principle produces its effect when taken orally.

" When improvement is definite it is just as striking as that seen in cases of exophthalmic goitre under the influence

of compound solution of iodine " (72). Pigmentation lessens. Adrenine and the related ephedrine are not effective when given alone in Addison's disease.

Effect of Cortical Extracts. Rogoff and Stewart (70) administered gelatine-coated capsules of their preparation " interrenalin " in several cases of Addison's disease, and obtained slight, but only very slight, improvement.

Hartman's preparation of " cortin " has been tested by himself and others on a number of patients (35, 38, 36, 2, 7). Several were revived from crises by increasing the dosage sufficiently. The requisite dosage for treatment seems to be relatively larger than that of Swingle and Pfiffner's preparation, and there is evidence that the procedure of the latter gives more potent concentrates (54).

Hartman's first case died 238 days after commencement of " cortin " treatment, after being successfully carried through several crises. The autopsy report on this case presents several points of interest (88). The adrenal cortices were almost completely absent, but the two medullas were almost normal. Staining reactions indicated that there was no deficiency of adrenine, *so that the patient's low blood pressure* during his illness (90 to 95 mm.) *was probably due to deficiency, not of adrenine, but of the cortical principle.*

Swingle and Pfiffner's extract has been subjected to thorough trial by Rowntree and Greene (86, 72, 71), who have reported results with twenty patients. These results show that, provided treatment be instituted before a moribund condition is reached, and sufficient extract is available for massive dosage when necessary and for continued treatment, favourable results are always to be expected. The response of the majority to such treatment is striking. Nausea and vomiting stop. Appetite reappears. There is gain in weight and strength. The pigmentation decreases. The patient regains a sense of both physical and mental vigour and well-being. The blood pressure may increase slightly, but this for the most part is a response to

increased activity and is not a specific effect of the cortical extract.

A course of treatment usually has consisted of the administration of 40 to 60 c.c. over four to ten days. One patient received 500 c.c. in two months; 20 c.c. have been given intravenously at one time and 40 c.c. in one day, without untoward effects.

One c.c. of the extract represents 30 grams of adrenal cortex (cf. p. 199), or the entire supply from two steers. Hence such clinical dosages appear to represent enormous amounts of the principle. Yet, as Rowntree points out, "It must be remembered that the amount of active material present in the excised suprarenal gland bears no definite relationship to the total normal daily output of the actively secreting gland."

The effects of treatment are usually apparent within twenty-four to seventy-two hours. The maximum effect is usually seen during a period of from five to seven days after stopping the treatment. "In the more severe cases, in which patients are unable to maintain strength and activity on the Muirhead treatment, a falling off of appetite, strength and weight is noted from seven to fourteen days after stopping the injection of the cortical hormone. Severe symptoms of suprarenal insufficiency, however, may not appear until several days or weeks later. Those patients who are so seriously ill as to need continuous treatment with the cortical hormone vary in their requirement, but usually have been given from 3 to 10 c.c. a day. On the other hand, patients who are able to maintain partially reduced or normal activity with the aid of the Muirhead *régime* require treatment with the cortical hormone only at intervals to combat the effects of intercurrent infection, excessive fatigue, or similar deleterious influences. . . ."

Intravenous injection is recommended. Intramuscular injection is well borne by some proportion of patients, but subcutaneous injection is too irritating.

The patients are, subjectively, often so improved that they wish to return to work. They are more resistant to infection, the effects of drugs, etc., and it may well be expected, when adequate quantities of the principle are available (although that probably will not be until it has been synthetized), that patients whose treatment is commenced sufficiently early may be maintained for many years in normal health and working capacity.

Favourable results have been reported by other clinicians with the Swingle-Pfiffner preparation (89, 79, 3, 17). A case of the disease has been successfully carried through pregnancy by combined oral and injection administration of the extract (64).

The Relation of Addison's Disease to Hypo-cortico-adrenalism. That deficiency of the adrenal cortical principle is primarily responsible for most of the symptoms of Addison's disease is obvious when the conditions exhibited in the disease are compared with those following double adrenalectomy in animals, and when it is remembered that destruction of the medullas has no significant sequence. It would seem to be logical to conclude that administration of adrenine in Addison's disease is unnecessary therapy.

The asthenia and the lowering of blood pressure in Addison's disease are due to deficiency of the cortical principle (cf. pp. 194, 204). The pigmentation is not invariably present and is not paralleled in adrenalectomized animals. It possibly bears the same relationship to this disease as melanin formation does in melanotic sarcoma. It is a side-product, of no intrinsic significance in itself. The pigment is probably a melanin (81), and is almost certainly derived from tyrosine. The fact that adrenine is also a tyrosine derivative is probably without significance in this connection. It seems rather probable that the increased power of melanin formation seen in Addison's disease may be related to decreased content of that peculiar "ascorbic acid," a compound which inhibits formation of pigment in biological

oxidative systems (87), is present in relatively large amount in normal adrenal cortical tissue (87), and seems to be identical with vitamin *C* (cf. 13). Adrenal cortex probably merely stores it ; for what purpose we do not know.[1]

Other Conditions possibly Associated with Hypofunction of the Adrenal Cortex. These may be sufficiently dealt with at present by a quotation from Lawrence and Rowe (51) : " Contrary to the relative frequency with which pituitary, thyroid, and ovarian disorders are encountered, demonstrable adrenal disease seems to be of rare occurrence. . . . The intrinsic association of lowered adrenal activity with the Addisonian syndrome may be regarded as definitely established. A similar authority does not obtain for that other type of adrenal failure which is assumed to result from a lowered functional activity, and to be unassociated with gross anatomical changes in the gland. This syndrome, possessing many of the characteristics of Addison's disease, such as asthenia, hypotension, and usually emaciation, has been in large measure developed by the work of the French clinicians . . . (it) more nearly equates with the picture of adrenal insufficiency as produced in numberless animal experiments involving interference but not complete extirpation. . . . A third type of failure, chiefly associated with suprarenal haemorrhage, is an acute condition usually terminating fatally in a few days."

Use of Active Extracts of the Cortex in Other Conditions

Hartman (36) has tested the effect of " cortin " on six normal men and six normal women. He believes that a definite pharmacological effect was demonstrated, manifested by a capacity for increased effort, a certain composure of the nervous system, and a sense of well-being. Psychical

[1] Ascorbic acid (at first termed by Szent-Györgyi " hexuronic acid "), $C_6H_8O_6$, readily disappears from the adrenal glands of guinea-pigs kept on a vitamin-free diet. The maximum content of the glands on a normal diet is about 0·1 per cent. (86B, 33B).

effects were ruled out by occasional control doses of saline or brain tissue extract. In four of the six women 3 to 5 c.c. doses given daily for four or five days brought on menstruation some three to five days earlier than usual.

He has reported improvement in 10 out of 16 cases of nervous asthenia, following use of his extract, and in 3 out of 4 cases of toxic goitre, but practically no improvement in cases of myasthenia gravis and muscular atrophy and dystrophy.

The Swingle-Pfiffner extract has produced benefit in two of four cases of Graves' disease, in 2 of 6 cases of anorexia nervosa, and no benefit in myxoedema nor in asthenia associated with psychoneuroses (71).

On the ground that Paget's disease is an entity representing disturbance of bone metabolism through imbalance between the parathyroid glands and adrenal cortex (there being excessive function of the former), Berman administered a crude extract of the cortex along with high calcium diet to 18 patients and claims benefit in 16 of them (8). Still more empirical is the use of desiccated adrenal cortex orally in 6 cases of vomiting of pregnancy; good results were claimed (44).

Hyper-Cortico-Adrenalism

Some clue to the nature of the disease-syndrome which will result from hyperfunction of the adrenal cortex can be obtained by careful studies of the effects following administration of heavy and continuous doses of active cortical preparations to normal animals. Since it has been demonstrated that the principle produces its effect when given orally (cf. p 203), experiments in which adrenal cortex or even whole adrenal gland has been fed may provide results if dosage has been sufficiently large and over a sufficiently long period.

Swingle and Pfiffner have been unable to detect any toxic reactions or overdosage phenomena following administration

of huge doses of active extract to cats and dogs (86), but the possibility of insufficient period of treatment cannot be excluded. Britton's results (cf. p. 203) suggest a definite stimulation on the gonads, perhaps through intermediation of the pituitary. These results confirm the early work of Hoskins in which desiccated adrenal was fed to young rats (47, cf. 55).

The results of Müller (62) and Klein (46) in Asher's laboratory are not in complete agreement. They find that development of the female gonads is depressed, and that of the male gonads promoted (cf. also 1).

(Changes in sexual function are inconstant in Addison's disease. To the extent to which they occur they represent depression (72). Wyman has found that adrenal insufficiency in rats, produced by extirpation, results in partial or complete inhibition of oestrus (97).)

These results, although not concordant, do suggest that some degree of control is exercised by the cortical principle, and that when it hyperfunctions some corresponding gonadal development may be expected.

The chief source of information, concerning the clinical conditions associated with hyperfunction of the adrenal cortex, is in studies of patients with tumours of that tissue. It is to be remembered that such tumours of endocrine tissue are potentially active even when malignant.

Adrenal cortical tumours, either benign or malignant, are frequently found at autopsies of cases of virilism and of pubertas praecox. Hoskins has reclassified such conditions associated with cortical hyperplasias (39) into three groups :

(i.) Cortical hyperplasia occurring during foetal life, and producing pseudo-hermaphroditism. The external genitalia do not correspond to the true sex of the child.

(ii.) Early post-natal cortical hyperplasia leading to pubertas praecox, the clinical picture differing according to the sex of the patient, but emphasizing " maleness," so that in the male there is an accentuation of male characteristics,

and in the female there is a tendency to change towards the male type, accompanied by secondary sex characters simulating the latter (such as hypertrichosis, masculine voice, enlarged clitoris, and absence of menstruation).

(iii.) Cortical hyperfunction in women between fifteen and twenty-five years of age, thus occurring after puberty, and producing virilism or hirsutism, sex characters tending towards the male type.

Numerous cases have been reported in the literature. Goldzieher (33) discusses them with reasonable criticism. He considers that the *rôle* of the adrenal in the genesis of pseudo-hermaphroditism is still uncertain, although frequently hyperplasia of cortical tissue is found at autopsy. Such hyperplasia as a source of excessive sexual development is more certain, although sexual precocity can arise from other causes. Sexual precocity, when associated with cortical hyperfunction, is almost exclusively found in females (and is thus exactly opposite to that of pineal origin). In hirsutism (virilism), seen only in women, the growth of hair is accompanied by a change of larynx and a more masculine voice. The genitalia usually do not change, although the clitoris is sometimes enlarged. Menstruation ceases. In all the cases which have been autopsied there has been found either a tumour or hyperplasia of the adrenal cortex.

Cases have been described, in both young children and adult women, in which, after removal of a tumour of the adrenal cortex, hirsutism and other masculine characteristics largely disappeared, and menstruation was re-established in adults, and in the children signs of premature development subsided (*e.g.*, 19, 37).

There is a somewhat close parallelism between the syndrome that has just been described, in adult women, and that which occurs in both sexes, which has been recently differentiated by Cushing and traced to tumours of the basophile cells of the anterior pituitary (secondary tumours

of the adrenals being also found) (cf. p. 260). It is therefore possible that some proportion of the cases hitherto associated with adrenal cortical tumour in reality have had as the initial pathological lesion a pituitary basophile tumour.

It may therefore be of interest to record that the typical case of hirsutism described by Hunter (43), in which there was a malignant tumour of the right adrenal cortex with metastases to the lungs and pelvis, was in reality of adrenal and not of pituitary origin, since serial sections of the anterior pituitary disclosed no tumour (10).

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CHAPTER VI

THE PITUITARY GLAND

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Introduction

THE pituitary body, like the adrenals, is built up of two unrelated parts, composed of different types of tissue, of which one is typically glandular, the other related to nervous tissue. The two parts come together in foetal life. The embryology and histology have frequently been fully described (62, 79, 22). The following brief statements are taken chiefly from Bailey's description (6).

The human pituitary (hypophysis cerebri) is a small organ, averaging about 0·57 gram in weight, and tending towards an ovoid shape. Rasmussen gives its average dimensions as 10 mm. (antero-posteriorly) × 6 mm. (dorsi-ventrally) × 13 mm. (side to side). It is situated beneath the brain in the sella turcica of the sphenoid bone. "No other single structure in the body is so doubly protected, so centrally placed, so well hidden" (24).

The customary division into two lobes, anterior and posterior, separated by a cleft lined with epithelium, is

merely gross. When the posterior lobe is examined microscopically it is itself seen to be composed of two distinct parts, the inner core or *pars nervosa*, an extension from the hypothalamic region of the brain, and an outer lining of epithelium, the *pars intermedia*. This intermediate part is continuous at the stalk which unites the gland with the brain, and at the posterior extremity, with similar cells of the anterior lobe.

The anterior lobe, or *pars distalis*, is more homogeneous. From it a thin layer of cells, the *pars tuberalis*, spreads out over a small adjacent area of the base of the brain.

In the foetal stages of development these various parts show a fair degree of parallelism in different mammals ; the glands of adults show greater differences. In adult man the epithelial lined cleft between the two lobes is either obliterated, or persists as isolated cystic cavities. Rasmussen (56) states that the pars intermedia is practically absent in the adult human pituitary (cf. p. 232, footnote).

The anterior portion rises from the ectoderm of the stomodeum just in front of the bucco-pharyngeal membrane as a long evagination (*Rathke's pouch*) which grows upwards to meet the nervous portion ; the apex, applying itself to the surface of the nervous tissue, becomes the pars intermedia. The nervous portion arises as a downward evagination from the floor of the diencephalon, in the region of the tuber cinereum, and becomes almost completely enveloped by the anterior portion. The cavity of this evagination disappears (except in the cat), leaving a funnel-shaped extension of the third ventricle (the infundibulum). The attachment of the epithelial portion to the buccal epithelium becomes attenuated, and is finally broken. (Islands of such " anterior-pituitary " cells may occur separately in the pharyngeal wall, or enclosed in the sphenoid bone.)[1]

[1] Engelbach (31) has summarized the divergences in different mammals : " The three mammalian types of hypophysis are exemplified in the cat, the dog, and man. In the cat, the posterior lobe is hollow and its cavity is in free communication with the third ventricle of the brain. The epithelium of the anterior lobe almost completely surrounds

The anterior portion is richly supplied with blood from a number of small vessels arising from the circle of Willis, and descending along the stalk in the pia mater of the infundibulum. The less abundant vascular supply of the nervous portion enters mainly at its posterior inferior extremity, where it is not covered by epithelium of the anterior lobe. The intermediate portion is poorly supplied.

Fine amyelinated nerve fibres follow the vascular supply to the anterior lobe, branches leaving at intervals to traverse the cellular columns and end between the glandular cells. An important tract of nerve fibres originates in the nucleus supraopticus, and descends the anterior wall of the infundibulum, to spread out in the nervous portion, ending " in tangled masses around the blood vessels and among the cells " (6).

It is stated that lymphatic vessels have not been demonstrated in the pituitary.

Microscopically, the anterior portion consists of columns of cells separated from one another by large vascular sinuses and some connective tissue. Two groups are differentiated as *chromophile* and *chromophobe* by the different intensities of their staining reactions. The deeper staining properties of the former are due to granules in their cytoplasm. These granules are of two types. From the presumption that their staining reactions are restricted to acid and basic dyes respectively, they are usually termed *acidophile* and *basophile*. Since they do not show such restricted staining properties, Bailey prefers to term them *alpha* and *beta* cells respectively. It is generally considered that no cell contains more than one type of granule.

the posterior lobe. In the dog, the body of the posterior lobe is solid, but the neck is hollow and communicates with the third ventricle. As in the first type, the posterior lobe is almost completely surrounded by epithelium. In the third type (man, monkey, ox, pig, and rabbit), the body and neck of the posterior lobe are solid, although traces of a cavity are occasionally found in the neck. The epithelium of the anterior lobe does not spread so far around the neck and spreads over and into the adjacent surface of the brain (Herring)."

According to Rasmussen (54), the distribution of these three types of cell in the anterior pituitary is :

	Extreme Values.	Mean Values.
	Per cent.	Per cent.
Chromophile (Acidophile (Alpha))	23 –59	37
(Basophile (Beta)) .	4·5–27	11
Chromophobe (Neutrophile) . .	32 –66	52

The alpha granules are large and spherical, and usually so close-packed as to obscure all other structural details of the cell. They appear during the third foetal month ; the beta cells appear a little later. The chromophobe cells, for the most part, contain but little cytoplasm. Cushing (24) has written : " These tinctorially distinguishable cells are distributed somewhat indiscriminately throughout the gland, and cytologists have been at a loss to know whether they merely represent differing stages of activity of one and the same cell, or whether they have gained morphological and functional independence. It is safe to assume that they have." [1]

The nervous portion contains three different cellular elements, typical ependymal cells, mossy neuroglial cells, and larger pyramidal or spindle-shaped cells. The last are peculiar to this tissue, and have been termed pituicytes by Bucy (15). In the human gland these pituicytes compose the bulk of the tissue of the pars nervosa. They give off fragile processes and often contain greenish-brown granules of pigment, readily stained by neutral red or methyl green.

While the anterior portion resembles a typical secreting gland, so that a theory that it produces an endocrine secretion

[1] Biedl has suggested that the chromophobe cells are " mother cells " from which both basophile and acidophile cells are derived. These, becoming granuled, assume independent secretory action. Certain so-called *cells of pregnancy* and *cells of castration* have been described as occurring specifically in these respective conditions (cf. 31).

seems rational, the resemblance of the cells of the nervous portion to those of nervous tissue so closely allied to it in origin has presented difficulties in formulating reasonable theories as to its secretory function. Even at the present time, Bailey (6) is able to write: "Pituitrin, an active substance extracted from the pars nervosa, has not been conclusively demonstrated in either blood or cerebrospinal fluid."

It has been suggested by Houssay (40), and widely accepted, that the internal secretion of the posterior pituitary is elaborated in the intermediate part and transferred to the nervous portion for secretion. This view is, of course, scarcely in agreement with Rasmussen's statement, already quoted, that the intermediate part is practically absent in the adult human pituitary. Moreover, if comparison with the adrenal medulla is legitimate, true secretion by the pituicytes themselves is not definitely excluded, since such secretion is generally admitted as a function of the corresponding adrenal chromophile cells.[1]

Our knowledge of the principles and functions of the pituitary is, more than that of any other of the endocrine glands, due to study of diseases associated with pathological conditions of the pituitary, and to the application of surgery to these diseased conditions. We are particularly indebted to one surgeon, Cushing, for such studies and applications, in which he has exemplified that combination of savant and surgeon whose rarity he has himself deplored (24).

The first important observation bearing upon the function of the posterior pituitary was that of Oliver and Schafer in 1895. They showed that extract of the gland, when injected intravenously into animals, produced a marked and prolonged rise of blood pressure. Shortly afterwards Howell proved that this effect is due to extract of the posterior lobe, while Dale found that this extract caused contraction of uterine

[1] Atwell has studied the interrelationship between the pars intermedia and nervosa, and possible specific action of the pars tuberalis (5).

muscle. The results following extirpation experiments strongly suggested that the condition of diabetes insipidus is due to depression of the function of the posterior pituitary. This view seemed supported, when it was found that injection of extract of the posterior lobe controlled the polyuria of the condition in most patients, even if only transiently. It seemed less probably accurate when Camus and Roussy (21) demonstrated that damage to the adjacent region of the hypothalamus was equally productive of a persistent polyuria. The involved interrelationship between the posterior pituitary and the hypothalamus is only slowly becoming understood.

Knowledge of the function of the anterior lobe began when the condition of acromegaly was shown to be accompanied by a pituitary tumour, and when it became recognized that pathological gigantism was an allied condition. Knowledge of such function has become much more precise with the recognition that each type of cell of the anterior pituitary can, tumefied, provoke its own disease-syndrome. Tumours of the acidophile (alpha) cells are associated with acromegaly and gigantism; tumours of the basophile (beta) cells are associated with certain pathological gonadal syndromes; tumours of the chromophobe cells lead, through obliteration by compression of most of the chromophile cells, to disease syndromes such as those of Fröhlich and of Lorain.

The Chemistry and Pharmacology of the Posterior Pituitary Gland

The early studies of extracts of the posterior lobe showed that there are three outstanding effects: (i.) ability to raise the blood pressure (pressor activity), (ii.) stimulation of uterine contraction (oxytocic activity), and (iii.) stimulation of diuresis or anti-diuresis under differing conditions (renal activity). Additional effects are the contraction of the melanophores in the skin of the frog, a galactogogue action, an effect on the coagulability of blood, an effect on intestinal

peristalsis, inhibition of gastric secretion, mydriasis, and control of capillary tone. "Pituitrin" is a typical preparation.

Earlier work suggested and then disproved the theories that these actions were due to an adrenine-like compound or to histamine. Later work was largely concerned with ascertaining whether these varying actions were due to one or more principles.

Abel (1), one of the leading exponents of the unitary theory, obtained from posterior lobe tissue a very potent preparation in tartrate combination. This he considered to be almost pure. It produced maximal uterine contraction in dilution of 1 in 15,000 million. In high dilution it produced all the typical effects of a good extract of posterior lobe. Abel noted that the different types of activity of different preparations all vary in parallel fashion with the degree of purification. He and other investigators stressed the fact that all these activities were destroyed in equal degree by such varying treatment as exposure to heat, to tryptic digestion, or to the action of hydrochloric acid or of alkali. They thought it extremely improbable that three or four different principles should be simultaneously destroyed to equal extent.

Dudley, in 1919, put forward evidence which strongly indicated that extract of the posterior lobe contained more than one active principle (29). Support of this view has gradually accumulated (61, 28). In 1928 an important paper was published by Kamm and his associates; they gave almost indisputable evidence of the separation of the pressor and oxytocic principles (41, 16).

Fresh beef pituitaries were carefully dissected, so that the posterior lobe was sharply separated from the anterior lobe. The posterior lobe material was desiccated with acetone, and the dry product extracted with 0·25 per cent. acetic acid (a recognized procedure in preparing pituitary extracts) and the extract then concentrated at low temperature. The

solution was salted out with either sodium chloride or ammonium sulphate ; the active principles were precipitated with proteins. The precipitate was treated with anhydrous acetic acid, which (in absence of water) dissolved very little protein, but extracted the active principles fairly readily. The acid extract was fractionated by successive treatments with acetone, ether, and petroleum ether, none of which decompose the active principles. Although the physiological effects produced by the two active principles are so widely different (see below), yet the compounds are so alike, chemically and physically, that twenty fractionations were found necessary to effect a satisfactory separation of them. (The ether filtrate contained the oxytocic principle, which could be thrown out of solution by adding water to the limit of its solubility, and then excess of petroleum ether.)

By such procedures, Kamm and his associates obtained from 200 beef pituitaries weighing 550 grams 50 grams of posterior lobe material. This, desiccated, weighed 8 grams, and yielded 0·05 gram of purified pressor principle, and 0·015 gram of purified oxytocic principle.

It was found that if amounts of the two fractions were combined in these proportions, dissolved in acidified water, and diluted to a volume corresponding to the original volume, they gave a solution indistinguishable in physiological properties from the original extract of the gland, from which it seems legitimate to conclude that in the processes employed during separation none of the activity had been destroyed, and one principle had not been converted into the other.

Both the final fractions are white, stable powders, water-soluble, basic, and probably amines. Both are considered to be substantially pure. The pressor principle is, as regards its pressor effect, eighty times as powerful as the international standard preparation of powdered pituitary. It has been named *beta-hypophamine* and, pharmaceutically, *vasopressin* and *pitressin*. The oxytocic principle is more than 150 times as powerful as the international standard, and is also believed

to be substantially pure. It is termed *alpha-hypophamine*, and, pharmaceutically, *oxytocin* and *pitocin*.[1]

The pressor principle is responsible for the diuretic-antidiuretic action of pituitary extracts (41, 37). In normal animals, not under anaesthesia, the predominant effect is suppression of flow of urine (17). The beneficial effects produced on patients with diabetes insipidus are due to this principle, oxytocin being without effect (37). There is also evidence that the stimulating effects on the smooth muscle of the intestine (37), and on the melanophores of the frog's skin (60), are due to pitressin.

It is stated that pitocin has been tested in obstetrical practice, and appears to produce the full effect of pituitary extract (16).

The effect on blood coagulation may not be due to any specific principle, since purification lessens or abolishes it (43).

It may be concluded that posterior pituitary tissue contains two compounds capable of producing marked but very different pharmacological effects, and that these are so similar in chemical and physical properties that they are probably closely chemically related.

Standardization of extracts of the posterior lobe has been based upon the effect on uterine muscle. The newer discoveries obviously suggested the need of redefinition, and the international unit has now been defined in terms of both pressor and oxytocic activity (60).

A Relationship with Fat Metabolism. The effect of posterior pituitary extract (" pituitrin ") on fat metabolism has been extensively studied by Raab (52). He has found that large subcutaneous or smaller intraventricular doses of pituitrin decrease the neutral fat content of the blood. This effect is abolished by mechanical obstruction or by pharmacological paralysis of the centres in the tuber cinereum, by

[1] Pitressin is much richer in cystine radicals than pitocin. Both are rich in phenolic (presumably tyrosine) radicals (78A).

transection of the spinal cord, by paralysis (by ergotamine), or by section of the abdominal splanchnics, or by phosphorus poisoning. Raab concludes that pituitrin promotes the absorption and destruction of circulating fat by the liver through a nervous pathway starting in the tuber cinereum and running through the cervical spinal cord and the abdominal splanchnic to the liver. If this conclusion is correct, then any disturbance of the co-operative pituitary-mesencephalic system would lead to a retention of excess fat in the body, and thus to an obesity. There is some evidence associating the lipoid-phosphorus of the blood with these pituitrin effects (13, 52).

The Posterior Pituitary as an Endocrine Gland

The effects described in the previous section are unquestionably pharmacological effects produced by extracts of the posterior lobe of the pituitary, and such extracts undoubtedly contain two principles which are responsible for the effects. Further evidence is necessary before it can be assumed that these are endocrine principles, secreted from the gland under physiological conditions.

The presence of adrenine in blood has been reasonably demonstrated; that "pituitrin" has been demonstrated in blood or cerebrospinal fluid is denied (77). Bailey has presented a histologist's viewpoint concerning the mechanism of its secretion (6): "The colloid and hyaline material which collects in the pars tuberalis, the pars intermedia, and the pars nervosa has been considered to represent a secretory product (Herring, Collin, Cushing), which passes up the stalk into the third ventricle. This material varies considerably in its staining reaction, and has no constant characteristics which enable it to be proved that it has a unique origin and chemical constitution. In the intermediate lobe it is formed by degeneration of the cells (de Beer), while the work of Tello indicates that the hyaline material of the pars nervosa

has a quite different origin, arising by degeneration of nervous fibres."

Of course, no secretion in the organism leaves its secretory gland as a pure chemical compound, or even in a solution of constant composition. Cushing's views on the subject seem more appropriate (24): "In the fully formed adult gland under certain experimental or pathological conditions, one may see viable pars intermedia cells streaming into the posterior lobe in great numbers. Even under normal circumstances, as first described by Herring (1908), the epithelial cells of the pars intermedia appear to invade the pars nervosa, becoming transformed into hyaline bodies which stream upward towards the ventricle between what appear to be loosely textured, long drawn-out tails of ependymal glia. It has been shown, moreover, that if the hypophyseal stalk is mechanically obstructed the secretory product becomes dammed back in the lobe which becomes turgid with hyaline. There is strong evidence, therefore, to indicate that these Herring bodies represent the secretory principle which acts either directly on the nervous centres of the tuber, as some assume, or actually passes between the ependymal cells into the infundibular cavity as others have believed. As a matter of fact, under either assumption it might well enough affect the nerve centres, one of the more important of which lies directly under the ependyma, and . . . posterior lobe extracts are far more potent when injected into the cerebral ventricles than by any other method of administration."

Pathological states sometimes virtually ablate the posterior pituitary. (Cf. Fig. 21, p. 239.) "By the time the sella turcica has become widely distended by a large adenoma, the posterior lobe will have disappeared without recognizable trace" (24). The results of such natural human experiments are interesting. Cushing continues: "Most patients with craniopharyngiomas which have served to compress the pituitary stalk, as these tumours usually do, have an

unaccountably low blood pressure. Even in adults a systolic pressure in the 90's is common. . . . Still more to the point is the fact that patients with large chromophobe adenomas, which remain confined within the sella and compress the posterior lobe, but leave the hypothalamus unaffected, usually have a relatively low systolic blood pressure (below 100 in 11 per cent. and below 110 in 46 per cent. of the cases). Beyond this we have nothing much to go on. What is more, confession must be made that two out of our series of 243 patients with verified chromophobe adenomas had coincidental vascular hypertension. In want of a satisfactory explanation of these exceptions to the general rule, it must be admitted that from a clinical point of view the posterior lobe has no such definite influence upon blood pressure as the laboratory experience with its extracts would have led us to expect."

Maddock, working in Cushing's laboratory, applied silver " clips " at various levels of the hypophyseal stalk in experimental animals, and found that a marked and enduring polyuria can be produced, with no tendency to adiposity or to other recognizable symptoms. Such results again suggest the damming back of some principle which normally passes to the ventricles by this channel from the posterior lobe, and which controls water metabolism, while at the same time, as Cushing points out, in such experiments the nerve impulses to the posterior lobe are interrupted.

Raab (52), in his studies on fat metabolism, showed that extract of the posterior pituitary lobe is particularly effective when injected into the ventricle. Cushing was thereby led to inject 1 c.c. of " surgical pituitrin " intraventricularly into patients with pronounced hypopituitary states, who had all been previously operated upon for pituitary adenoma. He anticipated a rise in basal metabolic rate, a rise in body temperature, a rise in blood pressure, and possibly a temporary hyperglycaemia. Quite the reverse happened. " The reaction has not been marked in all instances, but when

marked, almost immediately, and certainly before the extract could reach the blood stream, the patient flushes, breaks out into a drenching sweat, the blood pressure sinks, and the temperature falls." In one patient the rectal temperature fell during two hours to 93·8°, stayed at this value for two hours, and slowly returned to normal. During this period the basal metabolic rate changed from — 27 to — 50 per cent., and at the end of the fourth hour had only risen to — 37. Subcutaneous injections of pituitrin produced no appreciable effect in this patient except an anti-diuresis. Injected intravenously, the pituitrin caused immediate pallor but no sweating and acted as a prompt purge ; the antipyretic response and effect on the basal rate were negligible. Corresponding, but less marked, results were obtained with other patients. Cushing remarks : " However these effects are to be interpreted, they at least indicate that we cannot safely ignore the possibility of some posterior lobe participation in the vasomotor and other activities of the interbrain. Whether there are other drugs that would act by way of the ventricle in this same vigorous way with the same promptitude is not known, but certainly the extract when so administered must act on the hypothalamic centres by direct absorption rather than by finding its way first into the blood stream, else, when injected intravenously, it would be expected to produce the same reactions with equal or greater promptitude." Similar results have been obtained when the lateral cerebral ventricles of monkeys were injected with pituitrin, or with purified vasopressin (45A).

There is thus strongly suggestive, but not yet final evidence that the posterior pituitary secretes two principles, and that the secretion is, at least in part, by way of the third ventricle.[1]

[1] Brander claims that a blood space of variable size almost completely envelops the pituitary, communicating at several points with the blood supply of both lobes, and the marrow of the sphenoid. He thinks that colloid is secreted into the interglandular cleft, whose lower end remains patent and in connection with the blood space (14). Rasmussen can find no evidence in support of such views (55).

Popa and Fielding (49 B) describe a system of veins taking origin

Diseases associated with the Posterior Pituitary Gland

From what we know of the actions of the principles extractable from the posterior pituitary, hyperfunction or hypofunction of that lobe should lead to symptoms associated with blood pressure, altered degree of contractility of smooth muscle and abnormality of renal function.

It was pointed out in the previous section that tumours of the anterior pituitary may damage the posterior lobe, even to the extent of almost complete obliteration. There is some evidence of a resulting decrease in blood pressure. Nevertheless, as Cushing has pointed out, lesions of the posterior pituitary, whether of human occurrence or experimentally produced in animals, frequently do not lead to perceptible symptoms.

The most outstanding abnormal condition which is presumably associated with hypofunction of the posterior lobe is *diabetes insipidus*. This disease is characterized by the continued excretion of large volumes of a pale urine of low specific gravity, free from sugar and other abnormal constituents. In many patients the only symptoms present are this polyuria and a proportional polydipsia. Others may exhibit weakness and emaciation. At autopsy of such patients lesions of the pituitary gland have been found. Further, in many cases normal kidney secretion could be restored by continued injections of "pituitrin." Hence it

from the sinusoids of the buccal portion of the pituitary and from the capillaries of the neural portion, which ascend through the stalk to the region of the floor of the infundibular recess of the third ventricle, and there break up into a secondary capillary net. They contain colloid. Pietsch (49A) has made similar observations.

Brander also states that the pars intermedia in the human adult is extremely variable in extent and arrangement, a finding more in accordance with current views of the mechanism of secretion of the posterior pituitary than that of Rasmussen (cf. p. 220).

A theory has been put forward that the oxytocic principle is physiologically associated with uterine contractions towards the term of pregnancy. The available experimental evidence is somewhat adverse but not final. The literature has recently been reviewed by Smith (66). (Cf. also Parkes (49), Allan and Dodds (2), and Marshall (47).)

seemed reasonable to conclude that some pituitary lesion caused the condition.

The results of earlier extirpation experiments lent support to this view. Intense polyuria was produced (Cushing; Houssay). The issue became confused in two ways. Injection of " pituitrin " into an experimental animal sometimes produced diuresis. Damage to brain structures adjacent to the pituitary also caused polyuria. Camus and Roussy were the leading workers in experiments of the latter type.

They summarized the results of their observations in 1920 (21). They found that ablation of the dog's pituitary produced marked polyuria, but that this was only transient if the base of the brain was uninjured during the operation. Experiments in which the base of the brain bordering on the pituitary was damaged by a heated needle (through a previously perforated sphenoid), while the pituitary was not damaged, resulted in marked polyuria, lasting in some animals for several months. This could not be controlled by injections of pituitary extracts. Such damage must be within the opto-peduncular region at the level of the grey substance of the tuber cinereum. A few of their animals developed genital atrophy, and this also they attributed to damage of brain tissue rather than to a pituitary lesion. (Cf. also Bailey and Bremer (7).)

Cushing (24) has given the clearest and most harmonious account of this problem which is so far available in the literature. He admits that " most of the earlier experimental hypophysectomies on dogs now unquestionably lie open to the just criticism first raised by Aschner (1912) that a coincidental damage of the adjacent tuberal nerve centres probably accounted for the post-operative polyurias, glycosurias, as well as the more tardy adiposity and genital atrophy that not infrequently supervened. Whether the canine gland was approached from below through the pharynx, or from the side by elevating the temporal lobe,

these secondary symptoms were apt to ensue." He points out that in the rat and in man " the gland is overlain by a dural diaphragm merely perforated for the passage of the stalk, so that either the nervous tissues and pars tuberalis above or the body of the gland below can be separately subjected to experimental lesions." Smith (67), experimenting on the rat, showed that a sub-diaphragmatic removal of the pituitary leads only to inhibition of growth and sexual activity. Supra-diaphragmatic injury to the tuber produces an adiposity, sometimes of extraordinary extent, but may produce little or no effect on growth and sex functions. Richter (59) has produced persistent and marked polyuria by puncture through the base of the rat's skull, just in front of the pituitary, without any ensuing adiposity. " By these experiments a strictly hypothalamic syndrome, as opposed to a strictly pituitary one, has seemingly been produced, there being no apparent overlap in the symptoms " (24).

However, Cushing stresses the abundant nerve supply and circulatory apparatus to the posterior lobe, and considers that in interpreting the effect of tuberal injuries insufficient consideration has been given to the fact that they are likely to interfere with the blood supply to the gland, the usual consequence of stalk separation being a certain amount of cerebral necrosis in the pars anterior. The production of polyurias by various tuberal lesions, if ascribed solely to hypothalamic injury, leaves unexplained the counteraction of such polyurias by injection of extract of the posterior lobe. Further, " the large intrasellar adenomas which are almost certain to obliterate the posterior lobe are not accompanied by polyuria unless in the process of removing one of them the ventricular wall adjacent to the chiasm should happen to be injured. This would speak strongly in favour of the independent hypothalamic origin of diabetes insipidus, were it not for the abundant nerve-fibres that sweep around both sides of the chiasm and descend in the

walls of the infundibulum to their destination in the posterior lobe."

The simplest conclusion is that diabetes insipidus cannot properly be attributed to any single specific lesion. Experimental injury of the diencephalic part of the nucleus supraopticus appears to give the most pronounced and enduring polyuria, yet posterior lobe injury in the rat in absence of tuberal injury will also produce a definite, although less striking, diuresis. Possibly adjustment takes place to some extent through the smaller masses of tissue of the pars tuberalis and tuber cinereum, for it has been shown in Trendelenburg's laboratory that after extirpation of the posterior lobe these tissues contain abundance of the two posterior principles (normally only present in them in trifling amounts), and persistent polyuria is only produced by subsequent destruction of the tuber.

Cushing (24) sums up our present knowledge of the causation of diabetes insipidus as follows: "The evidence at hand seems reasonably convincing that the disorder can be produced by nuclear degeneration from disease, by surgical injuries of the supraoptic region in operations about the chiasm, by the interruption of the nerve tracts in course, whether from tuberal tumours, or punctures, by the experimental placement of a compressing clip on the infundibulum, and probably also (could this be accomplished) by complete removal of the epithelial investment which apparently elaborates the posterior lobe secretion—all of which indicates a diencephalo-hypophyseal mechanism which can be broken at any one of three principal points—nucleus, fibre tract, and pars intermedia et tuberalis. We, nevertheless, in regard to this most carefully studied of all diencephalo-hypophyseal reactions, are left in doubt as to whether the phenomenon is stimulatory or paralytic, and the key to the problem may possibly lie in the neglect of the physiologist's contention that posterior lobe extracts have diuretic as well as antidiuretic effects, the former conceivably being the

property of the epithelial investment, pars tuberalis in particular." [1]

The adiposity frequently associated with tumours of the anterior pituitary (cf. p. 244) is probably associated with interference in secretory function of discharge of the posterior lobe. Raab's work has been referred to (cf. p. 227). Although Smith obtained extreme degrees of obesity in rats by damage to the pars tuberalis (cf. p. 234), yet one of Maddock's dogs with tuberal clip became extremely obese within some months, and Cushing considers that blockage leading to retention of secretion may at least play some part in causing obesity. (In the hypophysectomized tadpole a persistent fat organ is one of the striking abnormalities; injection of "pituitrin" causes its early absorption.)

If suppression of the secretion of the posterior lobe leads to obesity, accentuation of that secretion should tend to produce emaciation. Cushing has found that laboratory animals given prolonged treatment with posterior lobe extract seem to lose weight. Raab's experiments are in agreement.

Among conditions involving neuro-pituitary disturbances should perhaps be mentioned the so-called "pituitary headache." This, according to Engelbach, is a descriptive term of the most constant chief complaint of pituitary disorder, and should not be regarded as a clinical entity. A disorder of the pituitary without tumour is a frequently unrecognized cause of severe headache, migrainous in character (31).

It has recently been suggested that undue secretion of the

[1] Zondek (81) claims to have prepared an aqueous extract of a principle "intermedin," which he believes is secreted by the pars intermedia, and to be extractable from the whole pituitary gland, the infundibular stalk, and the walls of the third ventricle. He states that it produces none of the effects of known pituitary principles in warm-blooded animals, but has a specific action on the pigment-forming cells of certain fishes. Sulzberger (71A) has tested this extract on two cases of diabetes insipidus, and claims that the beneficial effects surpass those of posterior pituitary extracts.

posterior pituitary may be a factor in the causation of gastro-intestinal ulcers. Since experimental lesions anywhere in the intracranial course of the fibre tracts from anterior hypothalamus to vagal centre are prone to cause gastric erosions, perforations, or ulcers, while intracranial injuries and diseases affecting these basilar regions of the brain are known to be accompanied by ulcerative lesions of the upper alimentary canal, and since intraventricular injections of " pituitrin " cause in man (presumably through stimulation of a " parasympathetic centre ") an increase in gastric motility, hypertonus, and hypersecretion, leading to retching and vomiting (the vomit ultimately containing occult blood), Cushing considers that it is possible to reconcile Rokitansky's neurogenic theory of ulceration with Virchow's theory of a primary local cause, whether the lesions concerned are simple erosions, acute perforations, autodigestive softening, or chronic ulcers, and whether they chiefly involve the oesophagus, stomach, or duodenum. He thinks that while all ulcerative processes, under all conditions, cannot be so accounted for, yet the majority can (25A).

Diseases associated with the Anterior Pituitary Lobe

The anterior pituitary, with its three types of cells, can show various types of hyperfunction and hypofunction, and also, through compression and neighbourhood effects, mixtures of hyper- and hypo-function. At the present time our knowledge of the anterior pituitary is based very largely upon clinical studies of diseases associated with its abnormal states, and on implantation and injection experiments in animals. Biochemical knowledge of the endocrine principles lags behind. It is therefore convenient at this stage to give some account of the diseases associated with the anterior lobe, and of the corresponding experimental pathological results which have helped to elucidate their nature.

These diseases, or at least the most important of them, are :

(A) Hypofunctional conditions (and mixed syndromes)—
  (i.) Simmonds' Disease. General hypofunction of the anterior lobe, usually due to actual destruction of the glandular elements.
  (ii.) Pure anterior lobe deficiency, possibly a true hypoplasia, but probably merely one form of —
  (iii.) The Lorain-Levi, Fröhlich, and Lawrence-Moon-Biedl syndromes, associated with hypofunction of the beta cells.

(B) Hyperfunctional conditions (and mixed syndromes)—
  (i.) Gigantism, a functional disturbance in childhood and adolescence, associated with hyperplasia or tumours of the alpha cells.
  (ii.) Acromegaly, associated with tumours of the alpha cells.
  (iii.) Cushing's pituitary basophilism, associated with tumours of the beta cells.
  (iv.) Amenorrhoea and disturbances of vision, associated with tumours of the chromophobe cells, which cause pressure effects.

It is perhaps of service at this point to anticipate some of the results of experiments and clinical studies. These suggest that the alpha (acidophile, eosinophile) cells elaborate a principle which promotes body growth in general and skeletal growth in particular, so that deficiency of the principle tends to produce dwarfism, and overproduction to produce gigantism.

The beta (basophile) cells elaborate one principle (and probably only one, although many investigators believe that two are produced) which stimulates the ovaries and testes to maturity and the production of their own endocrine secretions, and thus through these controls the development of the secondary sex glands and secondary sex characters.

Early deficiency of this principle tends to produce sex infantilism. Later deficiency causes amenorrhoea in the female and impotence in the male.

The function of the chromophobe cells is unknown.

Principles elaborated by the anterior pituitary definitely control the functioning of the thyroid and the adrenal cortex,

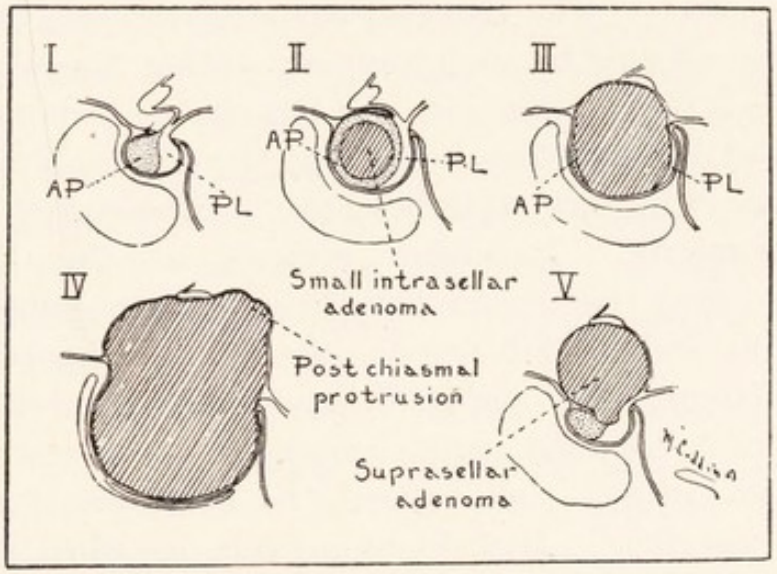

FIG. 21.—A series of drawings to illustrate the mechanical effects of an expanding pituitary adenoma. I. The normal pituitary gland and optic chiasm. II. A small intrasellar adenoma with only a slight expansion of the sellar. III. A larger adenoma beginning to stretch the chiasm—a little anterior lobe still remains. IV. A widely expanded sellar and greatly stretched chiasm. V. A suprasellar adenoma which has implicated the chiasm without compressing the anterior lobe. (From Henderson, *Endocrinology*, 1931, xv, 120.)

and exercise some degree of control of the development and secretion of the mammary glands. It still remains to be determined to what extent, if at all, these endocrine compounds are identical with those controlling general growth and gonadal development. Most investigators in this field seem to consider that at least three principles, and possibly more than three are concerned.

Tumours of the alpha and beta cells lead to hypersecretion of their respective principles. Tumours of the chromophobe

cells lead to hyposecretion of these principles through neighbourhood pressure effects tending to obliterate the chromophile cells. Such pressure effects may also affect the posterior lobe and its secretion, and even neighbouring structures in the hypothalamus, if the tumours are of sufficient size. Tumours of the alpha cells (and to a less extent of the beta cells) can also exert such neighbourhood pressure effects. The size and shape of the sella turcica is frequently affected by such tumours, so that X-ray examination reveals them. Some idea of the changes accompanying tumours of different sizes is given by the diagrams in Fig. 21.

Anterior Pituitary Insufficiency; Simmonds' Disease; Splanchnomicria. Excellent reviews of the literature concerned with this disease have been recently published by Calder (19) and by Silver (63). Paulesco demonstrated in 1907 that removal of the pituitary in dogs was followed by a train of symptoms characterized by weakness, loss of weight, and death, and termed the syndrome "cachexia hypophyseopriva." In 1914 Simmonds described a clinical case exhibiting the same syndrome. The patient at the age of thirty-eight developed puerperal sepsis following the birth of her fifth child. During the next eight years she developed amenorrhoea, muscular weakness, anaemia, loss of weight, attacks of giddiness and unconsciousness, and the general appearance of premature senility. She was admitted to hospital in coma and died without regaining consciousness. Autopsy disclosed atrophy of the kidneys, ovaries, pancreas, and liver, with necrosis and scar-tissue replacement of the anterior lobe of the pituitary. Simmonds insisted that the primary etiological factor in this case, and in two somewhat similar cases which he subsequently reported, was destruction of the anterior pituitary.

From analysis of seventy cases in the literature (eighteen males, forty-seven females, and five of unrecorded sex), Calder presents the following conclusions: Emaciation develops sooner or later, and is a striking and characteristic

feature. Falling of the teeth and hair, particularly that of the axillary and pubic regions, trophic changes in the nails, and thickening and loss of lustre of the skin combine to give the patient the appearance of premature senility. General muscular weakness is accompanied by corresponding atony of the gastrointestinal tract, with marked constipation, vomiting, and a consequent distaste for food. There may be subnormal temperature, with a subjective feeling of chilliness. In those cases in which the basal metabolism has been measured it was subnormal; the blood pressure was invariably low. In women menstruation ceases and sterility ensues. In men there results sexual weakness which may amount to complete impotence. In both sexes desire ceases. Many patients display peculiar forms of pathological sleep; coma frequently precedes death.

Without exception autopsy reveals destruction of the anterior lobe of the pituitary. In about half of the cases examined the glandular elements were replaced by scar tissue, indicating healed injury. Various causes have been suggested for such injury. Calder considers that probably no one pathological process has given rise to all the cases observed.

Many of the symptoms resemble those in Addison's disease, the chief differentiation being the pigmentation of the skin generally present in the latter. Autopsy shows adrenal involvement, so that, as Calder points out, the asthenia, low blood pressure, and subnormal temperature may be due to secondary involvement of the adrenal cortex.

In a few instances beneficial results have been reported following injection of extracts of anterior pituitary (31, 58, 20, 46).

Pure Anterior Lobe Deficiency; a Form of Pituitary Infantilism. Whether pituitary infantilism can be truly differentiated into cases with a pure hypoplasia of the anterior lobe, and others in which the hypoplasia is acquired from tumour pressure, cannot yet be stated. Engelbach (30)

defines the condition as a general arrest of growth and development of all organs and systems of the body because of hypofunctioning of both growth and sex principles of the anterior lobe of the pituitary. He considers that the condition is inherited, and not acquired. It is rare. Few patients die from this endocrine defect, and few established cases have been autopsied.

FIG. 22.—Comparison of a pituitary dwarf girl at the age of 9½ years with a normal boy of the same age. (From Engelbach, *Endocrinology*, 1932, xvi, 11.)

If Engelbach's view is correct, the pure case of pituitary infantilism, in which a tendency to hypopituitarism may perhaps have been accentuated by some slight intercurrent infection, bears a relationship to the anterior pituitary corresponding to that which cretinism bears to the thyroid, while Simmonds' disease corresponds to myxoedema.

The following appears to be a classical case of this condition, as put forward by Engelbach himself (37). It possibly should merely be considered as the childhood form of the Lorain-Levi syndrome.

A girl, aged nine-and-a-half years, exhibited marked physical underdevelopment and diminished appetite, conditions present from birth. During the first two years she was overweight. She could sit alone at six months. She did not walk until four years of age. Growth-rate was retarded from the first year, and growth ceased after the sixth year. Her mentality was good, and she was physically active. She had suffered practically no illnesses.

Her height when examined was 35½ inches, her weight 27½ lb. Her stature was miniature, being that of an average

three-and-a-half years' old child. The body measurements were typically those of hypopituitarism (cf. Figs. 22 and 23). The head was large in proportion to the body. The sella turcica was normal for the size of the head.

She was placed under treatment with a purified extract of the pituitary growth principle (cf. p. 273). Intradermal injections were given in gradually increasing dosage until she was getting 9 c.c. daily for six days of the week. In eight-and-a-half months she grew 2·7 inches in height and gained 7·5 lb. in weight, with concomitant increases in other measurements. Her appearance became somewhat more mature, but no indication of primary or secondary sex development had appeared. (The purified extract had been separated from the pituitary sex principle.) Equally good results have been reported in a number of other cases exhibiting retarded growth due to pituitary deficiency (31A).

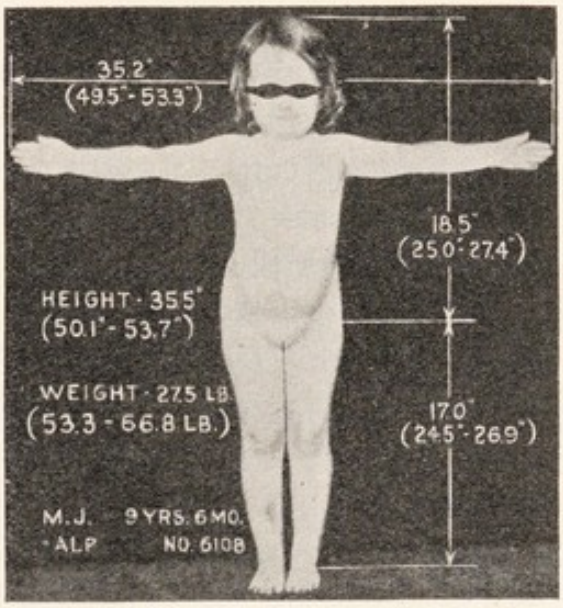

FIG. 23.—Comparison of the actual body measurements of the pituitary dwarf of Fig. 22 with the normal maximum and minimum measurements for her age. (From Engelbach, *Endocrinology*, *loc. cit.*)

The Lorain-Levi, Fröhlich, and Laurence-Moon-Biedl Syndromes. In all of these the functions of the anterior pituitary concerned with growth and sex development are depressed. Hence (depending on the age of onset) growth tends to be stunted, and sex-infantilism is a dominant characteristic. In the two latter syndromes obesity is superimposed.

Theoretically the abnormal state of pituitary function can

arise from a pathological hypoplasia, or from neighbourhood pressure effects of a tumour.

In patients with the Lorain-Levi syndrome there is seen a diminution of all parts of the body with retention of infantile proportions. This is accompanied by genital underdevelopment with absence of primary and secondary sex characters. Mental activity is not retarded. In women menstruation is not established or is irregular.

Engelbach considers that heredity is the prime causative factor of the Lorain-Levi syndrome, with infections and intoxications playing a secondary, excitory rôle. Early recognition is very desirable in order that treatment may be instituted while the retarded osseous development is still capable of modification.

Biedl (10) has examined many cases of pituitary dwarfism clinically and by X-ray. While some showed clinical symptoms of brain pressure, and X-ray evidence of sella turcica destruction, others gave no evidence of a tumour.

Fröhlich's syndrome can become established in childhood and in adult life. Juvenile cases exhibit marked adiposity—"juvenile obesity." Most of them are overweight during infancy. When the condition arises before adolescence, varying degrees of dwarfism and osseous retardation occur, according to the age of onset; infantilism persists.

In such early cases the adiposity usually precedes the genital non-development by several years. It usually begins as a more or less generalized obesity, which later on localizes about the mammae, mons, and girdle region. In the female genital hypoplasia is not conspicuous, and consequently abnormalities of this system are not recognized until attention is attracted by delayed and disordered menstruation. In the male underdevelopment of the genitalia is usually noticeable before adolescence (30). The typical picture of skeletal and sexual infantilism combined with a specific type of obesity led to the term *degeneratio* or

dystrophia adiposo-genitalis, originally employed by Bartels to describe the syndrome.

In those cases in which onset occurs after the genital and osseous systems have been developed, functional gonadal symptoms may be the only positive pituitary sign accompanying the obesity (30).

Engelbach holds the same views concerning the etiology of all these hypopituitary conditions, believing that a tumour is present in only a small proportion of cases. Such a view is mainly valuable in stressing the probable multiple origin of these syndromes.

From what has been written in the previous section it seems most probable that the adiposity is due either to hypofunction of the posterior pituitary, or to some damage to the hypothalamic region from tumour pressure. Obviously chromophobe tumours within the sella turcica or extra-sellar tumours such as cranio-pharyngiomas can provide the pressure effects necessary, both to depress the functions of the alpha and beta cells, and to interfere with the function of the posterior pituitary or cause damage to the adjacent hypothalamic region.

If the condition arises without tumour growth, then hypoplasia of both parts of the pituitary must be assumed.

Patients with Fröhlich's disease have an increased assimilatory power for carbohydrate, in agreement with their increased power to lay down fat. Their basal metabolism tends to be somewhat low (down to — 20 per cent.), and their temperature subnormal.

The Laurence-Moon-Biedl Syndrome exhibits, in addition to the syndrome of Fröhlich's disease, retinitis pigmentosa, polydactylia, and retarded mentality. The disease usually affects several children in one family. The two sexes are equally affected. It does not necessarily lead to early death, since a case aged fifty-one has been reported. A recent article has listed seventy-three cases in the literature (57).

Treatment of these conditions, to be correct, must obviously

depend on recognition of the true cause. When this is a tumour, removal, or perhaps in some cases X-ray treatment, may be beneficial. When the cause is a simple hypoplasia, replacement therapy seems the obvious treatment. Fortunately potent extracts of the posterior pituitary principles are available, and—as will be seen later—potent extracts of the anterior lobe principles should soon be generally available. The conditions present in the Laurence-Moon-Biedl syndrome obviously require more than pituitary correction.

Gigantism. Since somatic development is largely influenced by the growth principle of the pituitary, and since the pituitary appears to function completely from birth, it is to be expected that, if alpha (growth) cells can hyperfunction without adenomatous growth, gigantism can arise in infancy and early childhood. Many of the cases reported in the literature give a history of early accelerated growth.

Gigantism becomes most marked during adolescence. Growth may continue far beyond the normal period, even to the age of thirty years (11). The majority of cases are males. Engelbach's description seems complete, although it is doubtful if tumours can be so summarily dismissed in all cases: "Anterior lobe hyperpituitarism is defined as abnormal overgrowth of the entire body caused by excessive function of the anterior lobe of the hypophysis, unrelated to tumour. This somatic overgrowth is due to a proportionate overdevelopment of all the regional parts and organs. It is unaccompanied by adiposity. . . . The overdevelopment of the osseous system is due to hyperosseogenesis of both the epiphyses and the periosteum. . . . The skeletal overgrowth attained during adolescence remains permanent throughout the adult age, although in many cases the hyperactivity later changes to inactivity. In such event, the early virility and normal menses are transformed into genital hypofunction, as expressed in frigidity and sterility, with amenorrhoea in the female, and in loss of libido, impotency, and aspermatism

in the male. Concomitantly, the muscular hypertonicity and capacity and increased mental activity are changed to muscular weakness, fatiguability, and mental inertness."

One of the most interesting and completely documented cases of hyperpituitarism in the literature has recently been recorded by Behrens and Barr (9), whose observations extended over eighteen months. Somewhat against Engelbach's views, the family history of this boy suggests no marked tallness in his ancestors, and no endocrine disorders. The father's height is 5 feet 11 inches, the mother is of medium height and weighed 140 lb. There are two sisters and one brother of normal size. The paper of Behrens and Barr seems worth quoting in some detail :

" At birth he weighed only 9 lb., but began almost immediately to grow at an abnormal rate. At six months he weighed 30 lb. . . . He started to walk at the age of twelve months. At a year and a half he weighed 62 lb., and by the time he was two years old his extraordinary size attracted general attention. At six he entered school in a suit which was the largest his father could buy for a boy, and which was labelled size 17. When he was nine he measured 6 feet 1 inch, weighed 178 lb., and was able to pick his father up and carry him about. . . .

" He suffered from headaches whenever he read or studied. Examination of his eyes showed a moderate myopia, but the headaches disappeared when he wore his glasses. He had always drunk large quantities of water, and had to get up occasionally at night to urinate. This never was, however, a prominent symptom, and did not seem to indicate any degree of diabetes insipidus. His appetite was vigorous. . . . His record in school had been excellent. . . .

Examination at the time of the first visit was accomplished with considerable difficulty. The boy was so shy as to appear depressed and almost stupid. He was extremely modest, and would allow only partial exposure of his body. He became sulky, and finally wept when X-ray pictures were suggested.

FIG. 24.—A case of pituitary gigantism. Front view and profile of the patient at the age of 11, showing characteristic facies of preadolescent hyperpituitarism and complete absence of mandibular prognathism. (From Behrens and Barr, *Endocrinology*, 1932, xvi, 121.)

His interest, however, was easily excited and sustained. He was greatly diverted by a pocket flashlight which one of the doctors carried, and he displayed genuine amusement when he was encouraged to perform feats of strength. While in the photographic studio he picked up without any effort the somewhat astonished photographer, who weighed over 150 lb.

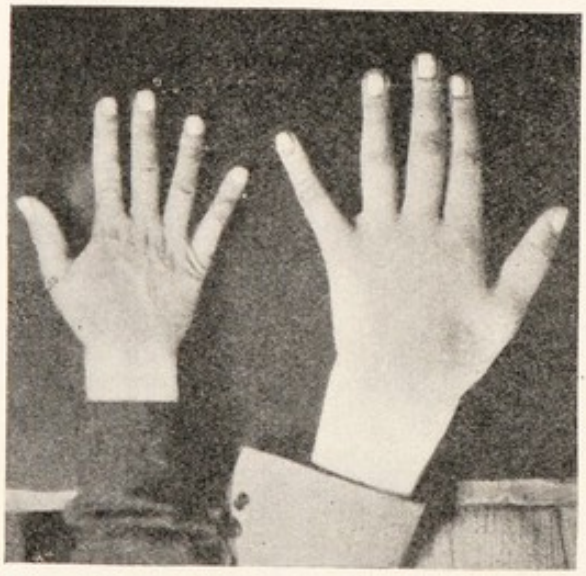

FIG. 25.—Hand of patient (Fig. 24) compared with that of a man 6 feet in height. Noteworthy are the long, lightly tapering fingers and the delicate, fine skin. (From Behrens and Barr, *ibid.*, p. 124.)

"His expression and appearance are best shown by the photographs. Notable is the wide spacing between the eyes and the complete absence of mandibular prognathism. There is some spreading

of the upper teeth. The skin was moist, delicate, and of fine texture, but the hands and feet tended to be cold and slightly cyanotic. He had no hair on his face, and the hair on his body was scanty. His father reported that he had a small amount of pubic hair, and the genitalia might be considered small for an 11-year old boy."

The visual field was practically normal. The heart, lungs, and abdomen were normal. Hands and feet were beautifully

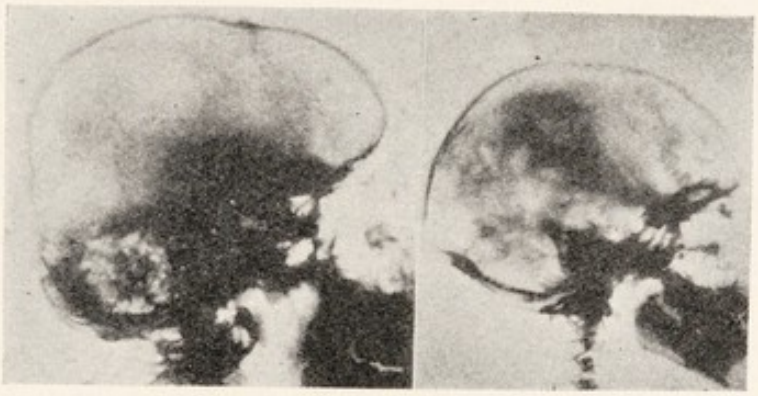

FIG. 26.—X-ray photograph of skull of patient (Fig. 24) at age of eleven, compared with that of a normal boy of the same age. There is an extraordinary development of the mastoid air cells. The sella turcica measured 2·5 cm. anterior-posteriorly; it has been outlined with dots to indicate its extent. (From Behrens and Barr, *ibid.*, p. 122.)

shaped in spite of their size. Many of the essential points of the description are illustrated in Figs. 24 to 27.

"The X-ray examination revealed in the bones of the face and maxilla a moderate tendency to prognathic development. The mastoids showed extraordinary development of pneumatic structure. . . . The sella was of extreme size, measuring 2·5 cm. in its anterior-posterior diameter. The floor of the sella showed a loss of continuity, being broken by a tubular structure which extended downward and forward from the sella and reached almost to the posterior wall of the pharynx, where there was an indefinite soft tissue shadow encroaching upon the lumen of the pharynx itself." It was thought that there was evidence of a persistent

Rathke's pouch. X-ray photographs of the hands showed no abnormality in the state of the epiphyses or degree of calcification, as compared with a normal boy of the same age.

He was seen again at the time of his thirteenth birthday. Measurements at the two examinations were :—

Age.	11 yrs. 11 months.	13 yrs.
Weight	112·3 kg.	126·4 kg.
Height (bare feet)	208·0 cm.	219·0 cm.
Sitting height	103·5 ,,	—
Arm spread	203·5 ,,	215·0 ,,
Head circumference	65·5 ,,	—
Chest circumference	104·5 ,,	107·5 ,,
Length of hand	22·0 ,,	23·5 ,,
Length of foot	37·0 ,,	38·5 ,,

During the interval between these examinations he had shown good progress at school, had lost much of his bashfulness, and displayed general interest and co-operation. His physical strength had been maintained. "The external genitalia had increased slightly in size. There was a greater growth of pubic hair, but no history of erections. . . . X-ray examination of the skull showed a progression in the growth of all bones with continued overgrowth of the pneumatized structures." The eyes showed myopic astigmatism, but the fundi were practically normal.

Fig. 27 pictures the boy at thirteen and a half, with a height of 221·5 cm. At this time blood and urine examinations gave normal results, a partial sugar tolerance test was normal, and oxygen consumption was low. "Except for the enormous size of the sella turcica, local signs of pituitary involvement are almost entirely absent. . . . It is extremely difficult to judge whether there is in this patient any retardation of sexual development."

Engelbach (30) has reported a case in which there was definite hyperfunction of the alpha (growth) cells, and also

possible hyperfunction of the beta (gonad-controlling) cells. The man, aged twenty-five at examination, weighed 11 lb. at birth. Subsequent to a febrile attack at seven months he commenced to grow rapidly, with corresponding strength. At seven years of age his height was that of an adult man. His mentality was normal. Puberty occurred between the

FIG. 27.—The patient at the age of 13½, shown standing with his 19-year-old brother, and his father, whose height is 5 feet 11 inches. (From Behrens and Barr, *ibid.*, p. 125.)

ages of nine and ten, at which period he associated with young men of nineteen and twenty and could do a man's work at manual labour. At thirteen he was known as the strongest man in Holland; his muscular development was supernormal, and he could support a 175-lb. man on each outstretched arm. He continued to grow larger with increasing vigour until the age of nineteen, and an extreme libido began to be manifested. At twenty-three he weighed 312 lb.

During the following two and a half years his weight dropped to 243 lb. His height was then 92·2 inches. With the loss of weight he exhibited a progressive loss of strength and diminution in size of the muscles. Occasional frontal headaches occurred, and he began to exhibit a slight pigmentation. Libido decreased, without impotency.

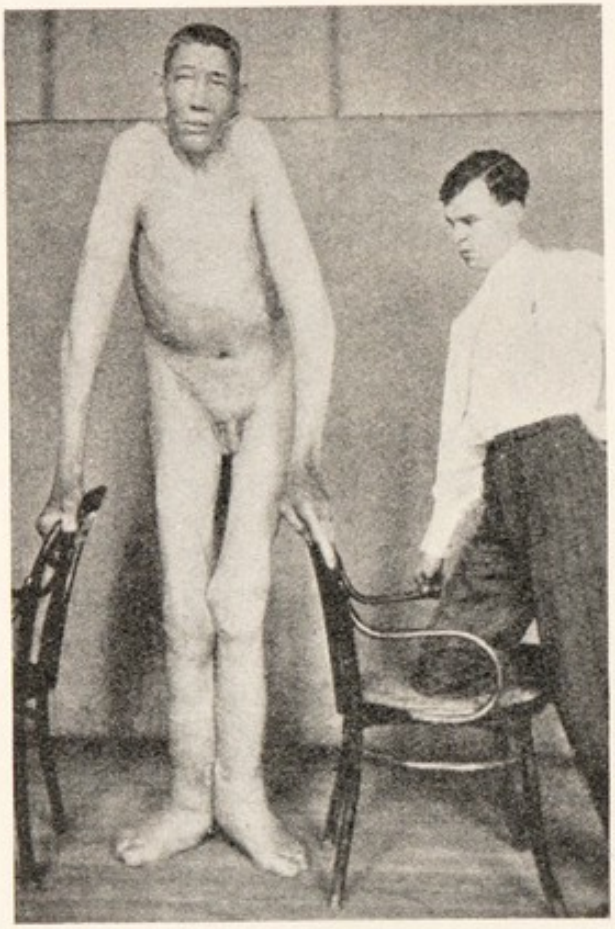

FIG. 28.—A case of gigantism. Final stage. Note the narrow chest, large joints, hypotrichosis, and the large size of the hands compared with that of the normal person of height 68 inches. (From Cushing, "The Pituitary Body and its Disorders," Lippincott, 1912, case XXXII.)

The sella turcica showed no evidence of proliferation or erosion, measuring 13 × 12 mm. The urine showed a faint trace of albumin. The blood cell count and basal metabolic rate were normal, the Wassermann test 4 +. Engelbach considered that the change from hyper- to hypo-activity might be associated with acquired syphilis.

With these two cases may well be contrasted the classical example described by Cushing (25), a man aged thirty-six, "an extraordinary prototype of the folk-lore giant—overcome by his own size." His appearance is shown in Figs. 28 and 29. His family and personal history reveal little of importance except that his overgrowth dated from childhood, when his size was such an embarrassment to him that he

played truant from school and never learned to read or write. His growth became rapid at fifteen years of age, at which time frontal headaches were frequent. His health began to fail when he was twenty-six. His weight at examination was 275 lb., his height 8 feet 3 inches. His complexion at that time was a peculiar greyish-white.

There was no definite polyuria, but a slight albuminuria. Temperature and pulse tended to be subnormal. The eyes were normal.

Though without education, he was shrewd, competent and

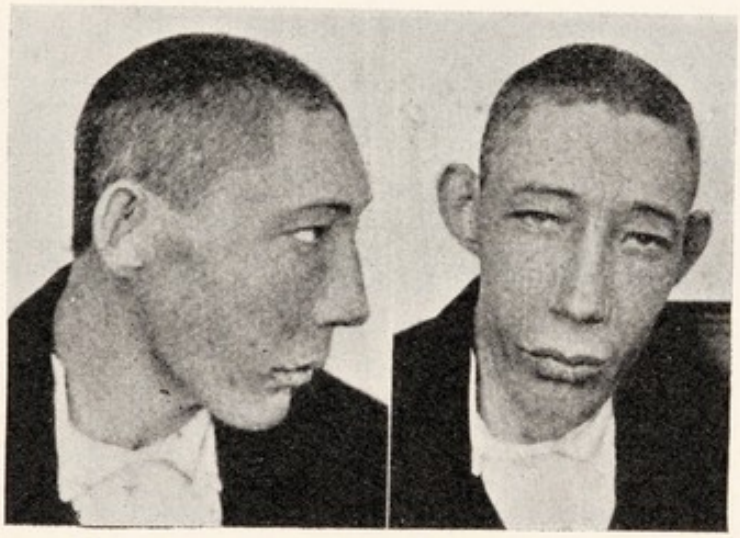

FIG. 29.—The same patient as in Fig. 28. Exhibiting a maxillary, rather than the mandibular prognathism of the acromegalic. (From Cushing, *loc. cit.*)

independent. There were no motor or sensory changes, but extreme muscular enfeeblement. His skin was soft and pliable, with marked hypotrichosis. He had practically no beard, absolutely no axillary hair, and very scant pubic hair. There was considerable pigmentation.

The lower extremities gave the appearance of elephantiasis. There was no disproportionate hypertrophy of the tongue as in acromegaly. The genitalia were small, and the testes atrophic. There had never been any temptation to sexual indulgence.

The skeletal framework was enormous. Bony deformation

about the joints caused bending at the knees and hips (cf. Fig. 28). His gait was feeble and he required the use of two heavy canes.

The overgrowth of the skull was restricted for the most part to the facial bones. The mastoids were huge ; the malar bones projected. The facial prognathism involved the maxillary rather than the mandibular jaw (cf. Fig. 29). X-ray of the skull showed a relatively shallow sella turcica, 2·7 × 1·7 cm. (anterior-posterior × depth measurements). There were huge maxillary and frontal sinuses.

He exhibited a high carbohydrate tolerance.

He died six months later. Autopsy showed diminutive adrenals, fibrosed testes with almost complete disappearance of spermatogenous cells, and a small and fibrosed pancreas. The pituitary gland was largely represented by a cyst. Cushing commented on the pituitary condition : " As regards the hypophysis itself, it is fair to assume that there was originally an extreme functional hyperplasia of the pars anterior with subsequent cystic degeneration. These hyperplasias are capable of various transformations—here a degenerative one."

These giants are usually believed to die young and childless. However, they occasionally reach middle age. The giant Chang is said to have died at fifty-one, and Palozzi, reported by Levi and Franchini in 1909, at sixty-six (25).

It seems to be inaccurate to represent gigantism and acromegaly as linked too closely. Some proportion, perhaps a large proportion, of cases of the former condition do not exhibit an adenoma, but only a generalized hyperplasia of the anterior pituitary.

Acromegaly. The condition of acromegaly has been often described, is easily recognized, and never forgotten when once seen. It is of slow onset, characterized by gradual enlargement of the limbs and head. The face, hands, and feet slowly hypertrophy. The gradual onset of the facial hypertrophy is beautifully shown in the photograph of Cushing's case

XXX. (25), reproduced in Fig. 30. The enlargement affects the skeleton generally, as far as that can be enlarged; the connective tissues become thickened and hypertrophied. The lower jaw becomes prominent, the face lengthens and broadens and the features coarsen; the tongue enlarges. Some initial degree of hypertrichosis is gradually transformed to a hypotrichosis. As the disease progresses, amenorrhoea in the female and impotence in the male become distinctive features. Deep-seated headache is a frequent early symptom. The organs enlarge, especially the heart.

I. II. III. IV.

FIG. 30.—A case of acromegaly. I. Photograph at the age of 24, before onset of the disease. II. Aged 29, at time of onset. III. Aged 37. IV. Aged 42, with pronounced acromegalic changes. (From Cushing, "The Pituitary Body and its Disorders," Lippincott, 1912, Case XXX.)

X-ray examination indicates an enlargement of the sella turcica.

There may be some degree of gigantism, depending on the age of onset. If onset does not take place until after adolescence, when the epiphyseal cartilages are ossified, the long bones cannot grow longer and height is but little affected.

The acromegalic frequently exhibits glycosuria, through a lowered carbohydrate tolerance. The combination of acromegaly and diabetes mellitus is not uncommon. The basal metabolism tends to be raised (26).

At autopsy the acromegalic usually presents an adenoma of the alpha cells of the anterior pituitary—frequently of the size of an orange. Such a pathology completely accounts

for his condition. This functioning adenoma provides that excess of growth principle necessary to produce such degree of over-development as was possible at the time of commencement of the adenomatous growth. Pressure of this tumour on the basophile cells of the pituitary causes that depression of stimuli to the gonads which results in amenorrhoea, impotence, and depression of secondary sex characters. Pressure effects may also well account for impaired carbohydrate metabolism, through depression or blockage of the posterior pituitary secretion.

When tumour is definitely recognized as the cause, removal of the tumour (or perhaps X-ray treatment) seems the obvious procedure of treatment. Cushing's work illustrates the frequent beneficial effects following surgical removal, including even apparent subsidence in size of extremities.

Rare instances of acromegaly have been reported in which the condition was associated solely with functional hyperplasia, tumour being absent (45, 42).[1]

Cushing's Pituitary Basophilism. Cushing (23) has recently suggested that " a polyglandular syndrome, hitherto supposed to be of cortico-adrenal origin, characterized in its full-blown state by acute plethoric adiposity, by genital dystrophy, by osteoporosis, by vascular hypertension, and so on," is due to an adenoma of the basophile (beta) elements of the anterior pituitary, since this syndrome was found " at autopsy in six out of eight cases to be associated with a pituitary adenoma, which in the most carefully studied cases has been definitely shown to be composed of basophilic elements, the lesion in one instance having been clinically predicted before its postmortal verification."

The history of the case thus clinically predicted (73) has been summarized by Cushing : " An exceedingly obese and

[1] " Fugitive acromegaly," in which symptoms of acromegaly and of the hypopituitary syndrome develop synchronously, and which is associated with an adenoma with distinctive type of foetal cells, has been described by Bailey and Cushing (8).

abundantly hirsute young woman, twenty years of age, admitted to hospital in a comatose condition due to a meningococcal meningitis, was under clinical observation for only three days before she died. Owing to her physical condition, a personal history was not obtainable, but it was learned that at the age of nine she had a continuous menstrual flow lasting four months. Subsequently, at the age of fourteen, she was said to have attained a normal adolescence, but her periods were subsequently most irregular. From the age of fifteen she had grown excessively stout, her maximum weight of 206 lb. (93·4 kg.) having been recorded seven months before her hospital admission. Because of excessive fatiguability she had consulted a physician about that time, and when he found she had a basal metabolic rate of + 33, her enlarged thyroid was roentgenologically radiated. This was said to have caused little or no symptomatic improvement.

"At autopsy, a suppurative meningococcic leptomeningitis was found to be the obvious cause of death. The pituitary body appeared to be of normal size, but suspecting from the patient's general appearance what might be found, Dr. Teel had the gland serially sectioned, and a small though unmistakable basophile adenoma measuring 2·5 mm. in diameter was disclosed. There was a persistent thymus, a slight enlargement of the thyroid, questionable enlargement of the pancreatic islets, and a definite enlargement (20 grams) of the suprarenals with no histological change of structure, no definite secondary adenomata being present in any of these organs. The ovaries were enlarged, apparently from increase in stroma; there was a single large corpus luteum with a small central haemorrhagic area and several smaller ones in various stages of organization. The only true neoplastic growth was the small anterior-pituitary adenoma to which the other endocrine changes were regarded as purely secondary."

According to Cushing, the syndrome appears to be

commoner in women than in men, although that is possibly due to the unusual combination of amenorrhoea, adiposity, and heterosexual hirsuties, which arrests attention.

A typical case in man was reported by Raab in 1924 in Biedl's clinic (53); Cushing has reproduced the case report. The outstanding features were: A man aged thirty-one, 192 cm. high, with well-developed external genitalia and normal masculine distribution of hair, marked obesity of face and abdomen, but no adiposity of the long slender extremities or the buttocks, was admitted to hospital complaining of headaches and marked gain in weight. The abdomen was very prominent and showed flame-shaped striae of dark red colour, which were in part more than 2 cm. broad. The hips showed the same feature. He weighed 211 lb. X-ray examination suggested some enlargement of the intrasellar space. A few weeks later he developed severe pains in the lumbar vertebral column, and he died shortly afterwards from acute sepsis, following a streptococcal infection of the hand. At autopsy the pituitary was found to be scarcely enlarged. A small basophile adenoma had almost entirely replaced the posterior lobe, and had destroyed about two-thirds of the substance of the anterior lobe. An osteoporosis of extreme degree, involving the vertebral column and long bones, accounted for the vertebral pain.

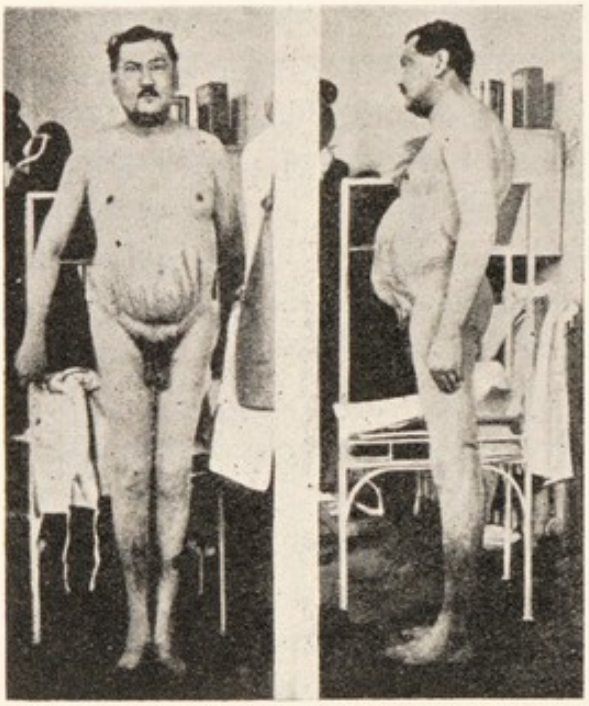

FIG. 31.—Dr. Raab's patient with verified basophilic adenoma of the pituitary. (From Cushing, *Bull. Johns Hopkins Hosp.*, 1932, l, 137.)

The essential symptomatic and pathological data are, according to Cushing: Males may be tall; females tend to be short. All cases show: (i) a rapidly acquired, peculiarly disposed, and usually painful adiposity, confined to the face, neck, and trunk; (ii) a tendency to become round-shouldered (kyphotic), even to measurable loss of height; (iii) a sexual dystrophy, shown by early amenorrhoea in females and ultimate functional impotence in males; (iv) an alteration in

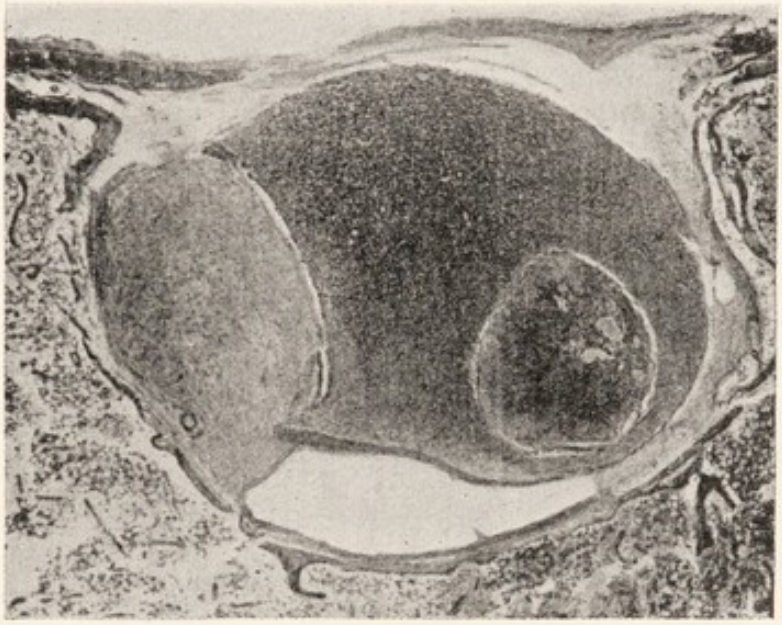

FIG. 32.—Cross section of the pituitary from a case of pituitary basophilism. (From Bishop and Close, *Guy's Hospital Reports*, 1932, lxxxii, 143.)

normal hirsuties shown by a tendency to hypertrichosis of face and trunk in the females and in pre-adolescent males; (v) a dusky or plethoric appearance of the skin, with purplish *liniae atrophiae;* (vi) vascular hypertension; (vii) a tendency to erythaema; and (viii) variable backaches, abdominal pains, fatiguability and ultimate extreme weakness.

Secondary adrenal involvement is strongly suggested by the hypertension, pigmentation and terminal weakness.

Other typical cases have recently been described (12, 48). The essential features of the condition are shown in Figs. 31 and 32.

The differentiation of this syndrome from that associated with a primary tumour of the adrenal cortex is not absolutely clear. Cushing believes that the secondary involvement of the adrenals may even proceed to actual development of adenomata. If such contention is correct, then virilism, hirsutism, etc., are only primarily ascribable to cortical adrenal tumours when serial section of the anterior pituitary at autopsy fails to reveal a basophile tumour (cf. p. 215).[1]

It is difficult to believe that the obesity of " pituitary basophilism " is ascribable to neighbourhood pressure on non-pituitary tissue. It must either be due to direct influence on the posterior pituitary (depression or occlusion of the secretion) or to secondary involvement of other endocrine glands.

Chromophobe Adenomas of the Anterior Pituitary. According to Bailey and Cushing, adult hypopituitarism (presumably both of the Lorain-Levi and Fröhlich type) is commonly associated with an adenoma of purely chromophobe type (8). Ophthalmologists and gynaecologists first drew attention to a syndrome in which X-ray examination showed an expanded sella in absence of acromegaly.

Women with unaccountable amenorrhoea not infrequently complained of disturbance of vision ; examination often gave indication of pressure against the optic chiasm. Men showed, along with the visual disturbance, some degree of gonadal involvement. Cushing has termed the condition " pituitary goitre." Unless it were relieved, blindness might ensue. The tumours were found to be of chromophobe tissue of the anterior pituitary. Their symptomatic effects were produced by pressure. Pressure within the sella inhibited the basophile elements and gonadal disturbances resulted. Pressure on the

[1] Moehlig and Bates (48) have described a case in which pituitary basophilism was accompanied by polycythaemia. They believe that, following adrenal cortex destruction, the proportion of basophile cells in the anterior pituitary increases rapidly, and that the increase is accompanied by a polycythaemia. They claim that such events follow bilateral adrenalectomy in dogs.

optic chiasm, if the tumour was of sufficient size, affected vision. Successful surgical intervention restored both sight and sexual function to normal (24, 38).

The ocular signs involved through such pressure include perimeter defects and optic disc changes, diplopia and strabismus. The general intercranial pressure signs include deep-seated headache, projectile vomiting, choked disc and photophobia.

Patients with chromophobe adenomas usually exhibit a lowered basal metabolic rate (26).

Experimental Investigations of the Function of the Anterior Lobe

Animal experiments designed to ascertain the function of the anterior lobe of the pituitary have consisted of extirpation, of implantation, and of the injection of extracts.

Extirpation. Paulesco's early work has been referred to (cf. p. 240). Most of the earlier workers, including Cushing, Biedl, Houssay, Bell, and Dott, concluded that sooner or later the result of extirpation was fatal, and that therefore the pituitary (more exactly the anterior pituitary) was essential to life. Horsley, Benedict and Homans, Camus and Roussy, Engelbach, and others hold the contrary view (62, 31).

The usual results of complete extirpation, following an initial latent period, are fall in body-temperature, slow respiration and pulse, limp musculature, coma, and death. Houssay noted polyuria in young pups and oliguria in adult dogs, effects due to posterior pituitary ablation (cf. p. 233). He further noted that animals which survived for some time showed retardation in general and sexual development, development of adiposity, and an increase l tolerance for sugar.

Partial extirpation of the anterior lobe leads to characteristic symptoms of hypopituitarism. Young animals remain small, their milk teeth and their juvenile fat are retained.

Their epiphyses do not ankylose. The thyroid enlarges, the thymus persists and the adrenal cortex thickens. Sexual maturity is markedly retarded. A subnormal temperature is shown and basal metabolism is diminished. Carbohydrate tolerance is increased. Adult animals also show a tendency to gonadal atrophy and obesity (62).

Section of the stalk leads to somewhat parallel changes which are probably traceable to interference with the blood supply of the anterior pituitary (27). Smith (68) produced Fröhlich's syndrome in rats by injecting Chinese ink into the pituitary gland and so destroying it.

Any lack of agreement in the general results is largely due to the degree of disturbance of surrounding structures. Some clear-cut results have been obtained with amphibia.

Smith (69) and Allen (4) showed independently in 1916 that the hypophyseal pit can be located and the minute portion of pituitary tissue removed in frog tadpoles which are only 3 or 4 mm. in length (and are therefore at a stage at which but little surface development has taken place). A remarkable change in development is produced by this operation. The tadpole acquires a silvery appearance, remains dwarfed, and does not metamorphose. Its thyroid remains reduced in size, also its adrenal cortex, but the medulla is unaffected. Thyroid feeding will not bring about complete metamorphosis of such hypophysectomized tadpoles.

Selye has developed a rapid and accurate technique for extirpating the pituitary of rats. Collip, Selye and Thomson (21A) report that following such operation the testes of male rats, whether immature or adult, undergo atrophy, with reduction both of germinal epithelium and of interstitial tissue. The epididymis, prostate and seminal vesicles in such rats are also reduced in size. In adult females, during lactation, hypophysectomy leads rapidly to retrogression of the mammary glands and failure of milk secretion (cf. p. 353).

Transplantation Experiments. Numerous investigators have shown that feeding pituitary preparations to young animals does not hasten sexual maturity. Single transplants also fail to produce any demonstrable effect on the gonads. Daily transplants over some period are necessary. The effects of such transplants on growth do not appear to have been thoroughly studied.

Zondek and Aschheim (80) and Smith and Engle (70, 64) showed independently and almost simultaneously late in 1926 that the continued implantation of anterior pituitary transplants into young female animals markedly accelerates sexual maturity. The results of such work are very definite.

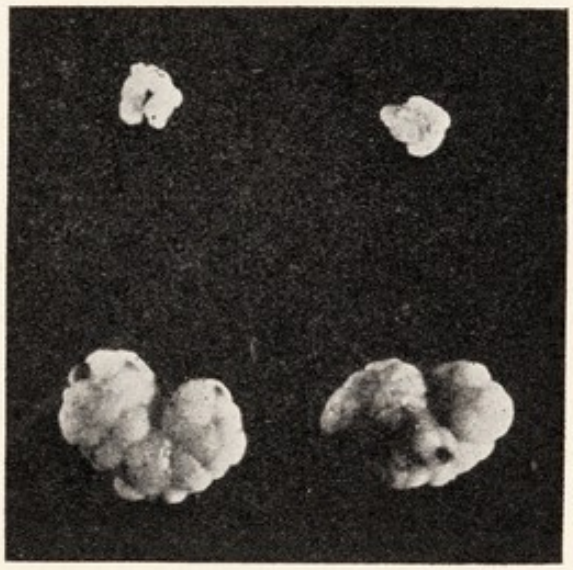

FIG. 33.—Above. Ovaries of littermate control rat. Weight, 21 mg. Below. Ovaries of experimental rat after thirty implantations of fresh rat pituitary gland over eighteen days. Weight, 340 mg. (From Collip, *Proc. California Acad. Med.*, 1930.)

Daily transplants of anterior pituitary tissue from mice, rats, cats, rabbits, and guinea-pigs into sexually immature mice and rats produce precocious sexual maturity, as shown by development and by mating—in mice at the age of fifteen days after five transplantations and in rats at the age of twenty-two days after eight transplantations. In older animals the effect is produced more rapidly. When the considerable degree of variability in the age of maturity of normal female animals is remembered, the uniformity of response of the treated animals is the more striking.

The weights of the ovaries of precociously matured animals are vastly greater than those of controls of the same age, and

are even greater than those of controls which have reached normal maturity. The increased weight is due to the formation of an increased number of normal follicles or of corpora. Superovulation, the liberation of an unusually large number of ova, invariably occurs. Such results are illustrated in Figs. 33 and 34. The uterus corresponds in weight to that of normal animals maturing at normal time, and structurally the uterus and vagina are typical of the adult animal.

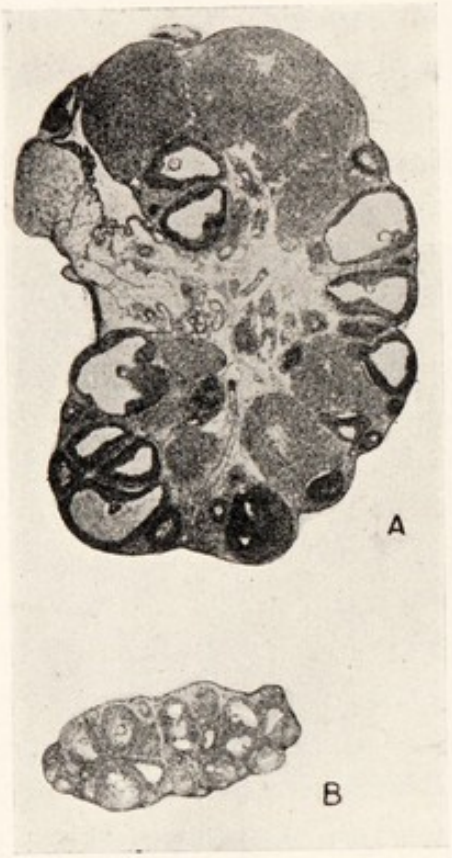

FIG. 34.—A. Cross section of ovary of rat. Sexual maturity induced on the twenty-seventh day of life, following four daily transplantations of anterior pituitary lobe of the rabbit. B. Ovary of untreated, litter-mate control. (From Smith and Engle, *Am. J. Anat.*, 1927–28, xl, 188.)

The genital system of the immature male is not so definitely affected. The testes show a more variable response; the secondary sex organs are increased in weight and in physiological activity.

Similar treatment applied to the adult female rat leads to ovarian hypertrophy and superovulation. The male exhibits no demonstrable response.

The secondary sex responses are not shown in spayed and castrated animals. The gonadal degradation following extirpation of the pituitary ceases following pituitary implantation and the gonads are restored to normal condition.

Pituitary implants from both immature and senile animals are active.

Small implants into pregnant mice produce no untoward effect on the pregnancy. Moderate-sized implants lead to

ovulation during pregnancy. Large implants produce toxic effects and may lead to abortion.

While the precocious sexual development leads to a complete oestrus with ovulation in some proportion of female mice, and the majority of the animals will mate, the second oestrus is delayed to a period later than that of normal first

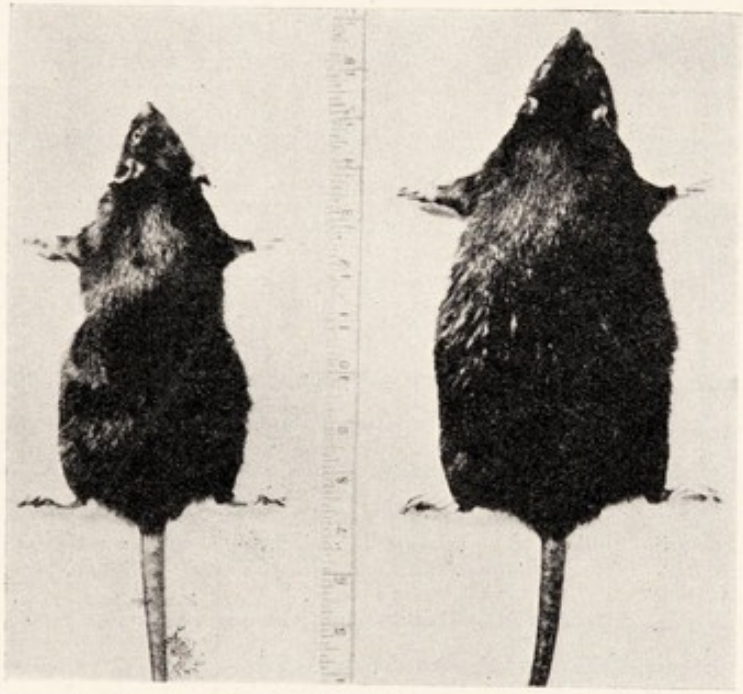

FIG. 35.—Photograph at time of autopsy (at somewhat over 400 days of age) of two female rats whose growth curves are shown in Fig. 36. The rat on the right received daily intraperitoneal injections of anterior lobe extract for over a year. The rat on the left is the untreated litter-mate control. (From Evans, *Harvey Lectures*, 1923–24, p. 212.)

oestrus; the first pregnancy is similarly delayed. This is possibly due to reciprocal action between the ovarian and pituitary secretion concerned (32).

Implants from male or female rats which had been castrated two months earlier produced in female rats heavier ovaries than implants from normal rats. The result is thought to be due to storage of the endocrine principle concerned in the so-called "castration cells" of the pituitary of the castrated animal. No experimental evidence could

be obtained to support the view of similar storage in the so-called " pregnancy cells " (34).

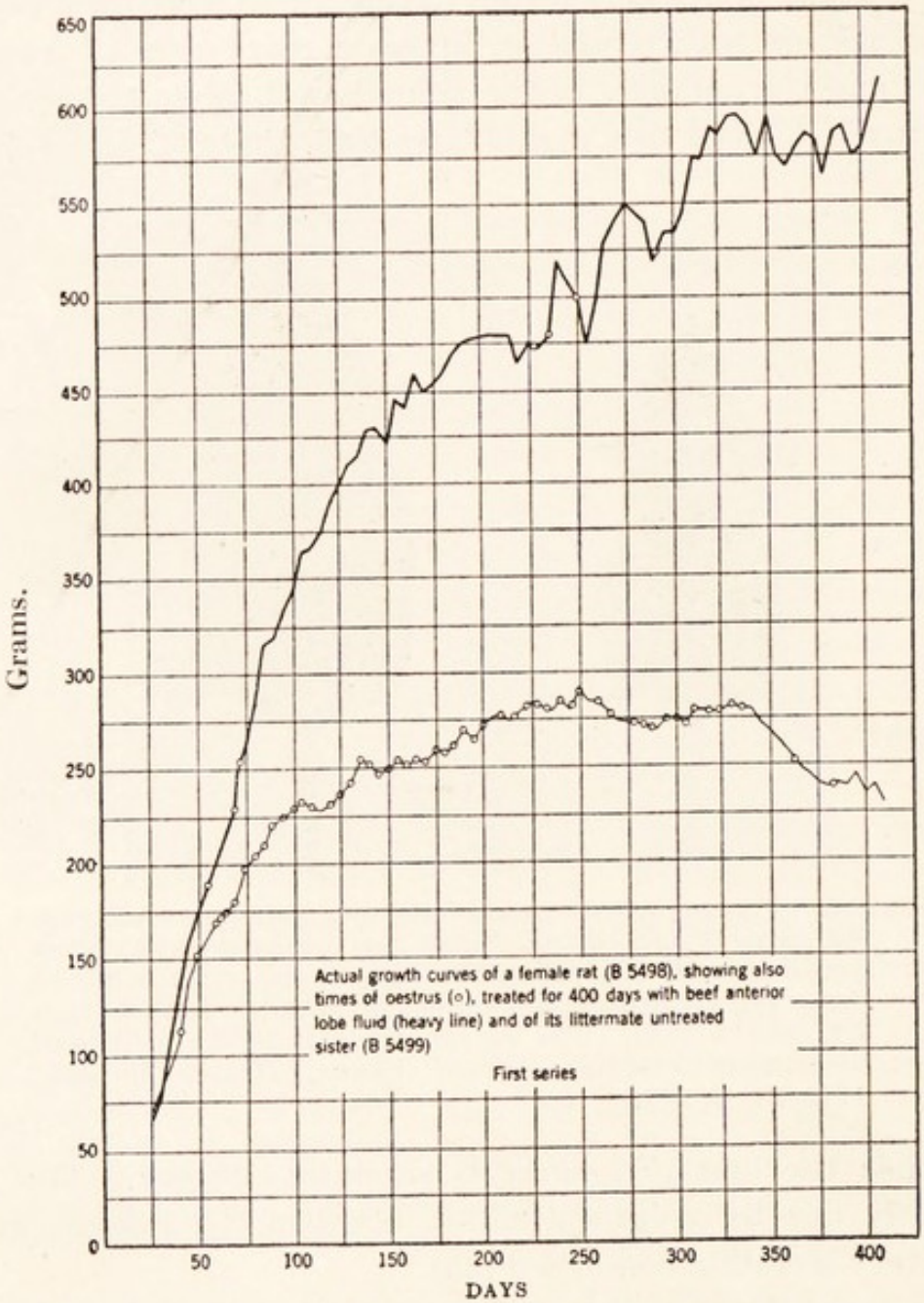

FIG. 36.—Actual growth curves of the two female rats shown in Fig. 35. That of the treated rat is given in heavy line. (From Evans, *loc. cit.*)

Restoration of normal growth has been demonstrated following pituitary implants into hypophysectomized (dwarf) tadpoles (3), and into a strain of dwarf mice (71).

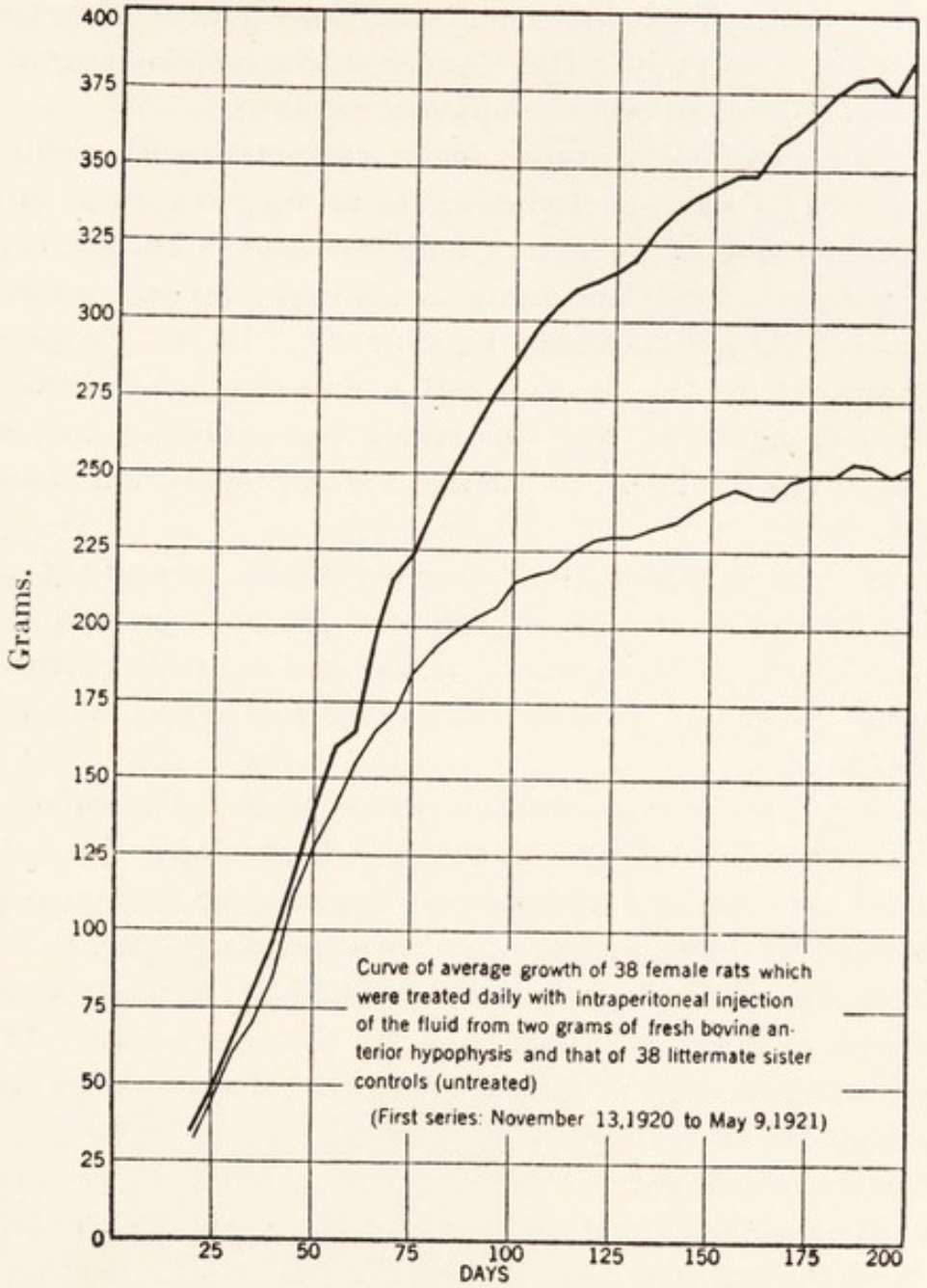

FIG. 37.—Curve (thick line) of average growth of thirty-eight female rats which were treated daily with intraperitoneal injections of the fluid from 2 grams of fresh bovine anterior pituitary, contrasted with the curve (thin line) of thirty-eight untreated litter-mate sister controls. (From Evans, *loc. cit.*)

Injection Experiments. Evans and his co-workers showed, in a series of publications commencing in 1921, that injections of potent pituitary extracts into rats produced

gigantism (33). Their first results were accidental, observed in an attempt to modify the vaginal smear response in rats (cf. p. 284) by injections of endocrine extracts.

Anterior bovine pituitaries were extracted with saline, and the extract was injected daily for prolonged periods into rats, commencing at the age of fourteen days. The animals so treated grew faster and more steadily and became giants. Typical results are shown in Figs. 35–37. In these experiments growth of the ovaries and maturation of ova were impaired or inhibited, but this result was probably due, as later work has disclosed, to the method of preparation of the extract.

Evans and Simpson have shown more recently that alkaline-aqueous extracts of pituitary promote growth but have no effect on the gonads, while acid-aqueous extracts have no effect on growth but a marked effect on the gonads (35).

Cushing, Teel, and co-workers have published a series of important studies on rats and dogs. Daily injections into pregnant rats delayed gestation by a period of from two to six days ; this delay was due to a corresponding delay in the implantation of the ova. Foetuses died at term *in utero*, and were expelled still-born one to two days later (72). Experiments on dogs produced a growth effect very similar to that obtained in those on rats.

An active sterile extract was prepared, capable of preservation. Beef glands, fresh or kept frozen, were split lengthwise and the anterior lobe shelled out ; it was then ground up as finely as possible. To every 100 grams of this " mash " were added 5 grams of sodium benzoate (as preservative) and 50 c.c. of 1 per cent. sodium hydroxide, and the whole was made up to 1 litre with tap-water. The mixture was allowed to stand twelve hours or longer in a cold place until the *débris* settled. The pink turbid solution was decanted through glass wool, and brought to *p*H 7·8 by addition of dilute hydrochloric acid. The colloid precipitate which

formed was filtered off (a matter of some difficulty), using the centrifuge, paper filtration, and the Sieck filter. A dose of 1 to 2 c.c. per kg. was used for dogs, and 4 c.c. per kg. for rats (51).

FIG. 38.—Effect of continued injections of the growth principle of the anterior pituitary. Litter-mate bull-dogs, three months after the beginning of the experiment. The treated animal (on the right) was already slightly larger than the control. Note the enlargement of tongue and paws. (From Putnam, Benedict, and Teel, *Arch. Surgery*, 1929, xviii, 1709.)

It was found that this extract accelerated growth in rats and dogs, and restored growth in hypophysectomized dogs. It brought on oestrus in the immature rat (51). Nitrogen-retention, and prolonged diminution of blood non-protein nitrogen was produced in dogs (76).

Some improvement was obtained in the condition of a female pituitary dwarf (51).

An experiment on bull-dogs was carried through to the death of the experimental animal, and the details have been published in full (50, 75). It shows perfectly the gigantism, ultimately an enfeebled gigantism, produced by prolonged and marked hyperpituitarism.

FIG. 39.—The same two animals eight months later. Treated animal on right. (From Putnam, Benedict, and Teel, *ibid.*, p. 1710.)

At seven weeks of age two female bull-dogs weighed 4·87 and 5·0 kg. Daily intraperitoneal injections of the sterile extract were given to the smaller

FIG. 40.—As in Fig. 39. The experimental animal on the right shows pendulous abdomen, enlarged teats, thick extremities, and weakness of hind limbs. (From Putnam, Benedict, and Teel, *ibid.*, p. 1710.)

dog from this time, for fourteen months. The initial dose was 10 c.c. After one month it was raised to 20 c.c. (this caused some vomiting, but tolerance was established). Six weeks later the dose was increased to 30 c.c. daily. Finally the animal was receiving 75 c.c. daily. The changes in appearances are shown in Figs. 38–41.

After three and a half months' treatment the lower jaw and skull were perceptibly larger than those of the control, the tongue was larger and the animal stood higher. After four months the animal became weak and languid. Muscular movements were poorly controlled. The appetite increased. After six months sluggishness had increased. Movements were plantigrade rather than digitigrade. Owing to muscular laxity the spine sank beneath the scapulas and the experimental animal, although much heavier, stood less high than its control. The abdomen was large and pendulous. There was prolapse of the vagina. The animal suffered from stubborn diarrhoea. Blood analyses revealed no striking changes. Sugar calcium, and total phosphorus were slightly high.

FIG. 41.—The experimental animal, two and a half months later, showing plantigrade gait, sunken head and protruding tongue. (From Putnam, Benedict, and Teel, *ibid.*, p. 1712.)

After eleven months

the udders were abnormally large and colostrum could be squeezed from them. The animal never went into heat; its control sister did so at thirteen months.

After developing polyphagia, asthenia, sialorrhoea, and spontaneous lactation, the animal died at the end of fourteen and a quarter months' treatment, on a very hot day; the actual cause of death was myocardial failure and oedema of the lungs. At death the dog weighed 44 kg., the control 23·5 kg. The control was killed and the animals autopsied.

Comparison with the control showed absence of fat, dis-

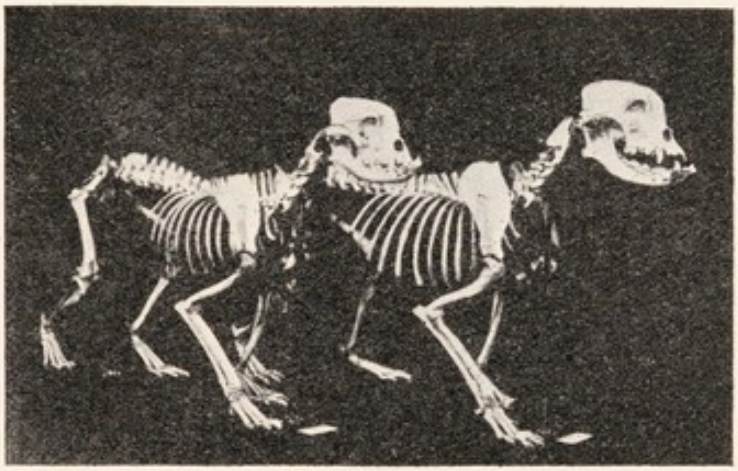

FIG. 42.—Skeletons of the treated and untreated animals at the end of the fourteen months' experiment. Treated animal on right. (From Teel and Cushing, *Endocrinology*, 1930, xiv, 158.)

proportionately small and soft musculature, and a generalized splanchnomegaly. The heart and kidneys were enlarged, the liver enormous; it showed passive congestion and central necrosis with disappearance of liver cells. The thyroid was much enlarged and microscopic examination showed an abnormally dense and cellular structure, with small acini and paucity of colloid. The adrenals were not disproportionate but the cortex was relatively enlarged and showed numerous small adenomas, measuring up to 1 mm. in diameter.

The skeletal changes are well shown in Fig. 42. The ovaries were large and contained ripe but unruptured

follicles. "The uterus and vagina showed the most striking changes in the entire body. The uterine horns were long, 13 cm. in the injected animal as compared with 5 cm. in the control, and stretched well up into the hypochondrium. They were approximately twice the diameter of those of the control. The vagina was greatly elongated, and the tissue deep and thickly furrowed." The changes are shown in Fig. 43.

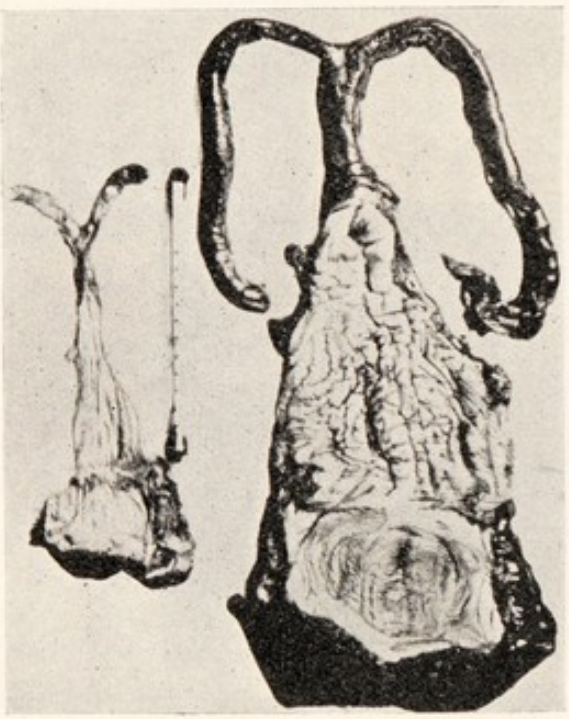

FIG. 43.—Vaginae, uteri and left ovaries of the two animals. The vaginae have been laid open by an incision along the anterior wall. Note the rugosity and thickness of the specimen from the treated animal (right). (From Putnam, Benedict and Teel, *loc. cit.*, p. 1719.)

The pituitary was the same weight as that of the control.

These experimental results of Smith and Engle, of Evans, of Cushing, and of others, are in full agreement with the conclusions drawn from clinical studies that the anterior pituitary elaborates at least two principles, one concerned with growth, and the other with control of genital development.

Further work has been somewhat confused in two ways. Claims, so far insufficiently substantiated, have been made that there is a third principle which is specifically concerned with luteinization of the ovaries. These claims are closely bound up with the work of Zondek and Aschheim, who showed that the urine of pregnant women contained one or more principles which produced gonadal effects, and who have developed therefrom a very specific test for pregnancy. It is usually assumed, but is

far from being established, that this principle (or these principles) is produced by the anterior pituitary (and presumably by the basophile cells). It seems possible that the actual elaboration takes place in the placenta.

It is simpler, in this chapter, to deal only with extracts prepared from the pituitary itself, and to discuss the results obtained with material from the urine of pregnant women in the next chapter.

As already stated, there is indisputable evidence that the anterior pituitary controls the thyroid and adrenal glands, and (to some degree) mammary secretion. Nevertheless it will be convenient to deal with this evidence in Chapter IX.

The Preparation of Anterior Pituitary Extracts

Van Dyke and Wallen-Lawrence (78) have published procedures for the preparation of separate concentrated extracts of both growth-promoting and gonad-stimulating principles. The first they propose to call *phyone* (Gk. *Phyo*, I cause to grow), and the second *hebin* (Gk. *Hebe*, puberty). In their papers they have reviewed all the earlier work bearing upon preparation and purification.

The growth principle is prepared by a procedure somewhat similar to that employed by Cushing (cf. p. 268). A fine hash of anterior lobe material is extracted with very dilute sodium hydroxide solution. The extract is brought to *p*H 7·2 and the precipitate so produced is discarded. The active material is precipitated by addition of sodium sulphate and purified by solution at *p*H 7·5 and reprecipitation with sodium sulphate, dialysis against distilled water in collodion sacs impermeable to congo red, and adjustment to *p*H 4·75. A heavy precipitate forms which is centrifuged off and the filtrate brought back to *p*H 7·5 and passed through a sterile Berkefeld filter and sealed in sterile ampoules for clinical use. It produces no untoward effect on the blood pressure and respiration when injected intravenously, and no

local reaction when injected subcutaneously. It is stated to be free from any significant amount of the gonad-stimulating principle.

The solution is of such strength that 0·35 c.c. per kilogram injected into an adult or a hypophysectomized rat daily for three days produces a 3 per cent. increase in weight.

The method of preparation indicates that " phyone " is of protein-like nature, is soluble in dilute alkali, and will not pass through collodion membranes which are impermeable to congo red.

A somewhat similar method has been described by Bugbee (18, cf. also 39).

While it seems to be agreed that Van Dyke's " phyone " preparation is strongly growth-promoting, yet the subsequent results obtained by a number of investigators suggest that his procedure does not free it from the gonad-stimulating principle (76A). Collip, Selye and Thomson (21B) have very recently obtained a much purer preparation.

Anterior lobe tissue is treated with several volumes of dilute alkali (0·5 to 1 per cent. sodium hydroxide, or 1 per cent. ammonium hydroxide). The mixture is acidified with acetic acid and filtered, and the residue is again suspended in dilute alkali and reprecipitated with acetic acid and filtered. This may be repeated five times. Then ammonia is added to the combined filtrates to give approximately a 1 per cent. concentration, and, by the addition of appropriate amounts of calcium chloride and sodium phosphate a suspension of calcium phosphate is produced in the solution and the whole is concentrated at low temperature and pressure until the ammonia is practically all removed. The calcium phosphate is then collected on a Buchner funnel and is extracted repeatedly with 0·5 per cent. sodium hydroxide. The alkaline solution thus obtained is acidified with acetic acid to *p*H 6·5, is then made alkaline with ammonia, and concentrated at low temperature and pressure to remove ammonia slowly. At *p*H 7·5 to 8 a semi-crystalline material separates out. It is removed and extracted with dilute sodium hydroxide. The alkaline solution is almost neutralized. The volume is so adjusted that 1 c.c. represents approximately 2 gm. of original gland tissue. The total organic solids are between 1 and 2 mg. per gm. of original tissue. 0·25 c.c. administered twice daily to completely hypophysectomized rats produces marked growth.

Van Dyke's method for "hebin" is as follows: Whole sheep-glands are dried in acetone, the acetone is removed, and the dry glands powdered. Ten volumes of M/7·5 sodium acetate-acetic acid buffer of *p*H 4·5 are added, and the mixture is allowed to stand overnight at 0° C. The fluid is filtered off and alcohol added to 90 per cent. concentration. A white flocculent precipitate separates and is centrifuged off (the fluid is inactive). This precipitate is washed with absolute alcohol, and then with anhydrous ether. The yield is about 5 per cent. of the dried lobe.

Hebin is soluble in water. It does not dialyse through collodion membranes. Boiling its solution does not destroy it.

Bugbee extracts ground sheep glands with four volumes of 0·05 *N* sodium hydroxide containing 0·4 per cent. tricresol. Sulphuric acid is added to *p*H 7·2 and the mixture centrifuged. The cloudy solution contains the active extract.

It would appear that "hebin" is also of protein-like nature, is also not dialysable through collodion, and is soluble in dilute acid.

Clinical trials of the concentrated extracts now available have given some good results (cf. pp. 241, 243, 269).

It must be stressed that, in spite of some apparent clinical support, due probably to a mixed therapy, there is no convincing evidence to indicate that any effect is produced by oral administration of anterior pituitary preparations (51, 44, 45, 36).

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CHAPTER VII

THE ENDOCRINE SECRETIONS OF THE ORGANS CONCERNED WITH REPRODUCTION

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Introduction

The internal secretions of the ovaries and testes are closely related, chemically, and since their actions have a superficial degree of resemblance, it is desirable to bring together, rather than to separate, all that pertains to our knowledge of their nature and functions.

Of the various organs producing an endocrine secretion, the ovaries exhibit the greatest degree of regular cyclical change ; this must be understood before their secretion (or secretions) can be adequately discussed.

In the earliest stage of gonadal development of the human embryo no histological sex differentiation is as yet possible ; the tubular system is bisexual. Sex is first distinguishable

in the third week of embryonic life (13 mm.) by appearance of the " testis cords " in the male (35). In embryos of both sexes Wolffian (male) and Müllerian (female) ducts develop equally for a while ; subsequently the development of one predominates, and that of the other lags, and finally is only represented by vestigial remains (35). Sexual differentiation in mammals is exemplified by (i) the gonad itself—ovary in the female, testis in the male, (ii) the accessory reproductive organs—(corpus luteum), uterus, vagina, clitoris, and mammary glands in the female, seminal vesicles, prostate, penis, in the male, and (iii) the secondary sex characters, markedly diverse in different species, and characterized in human beings by distribution of hair, by voice, and by a relative enlargement of the capacity of the pelvis in women.

The time relationship between the cyclical changes of the ovary, on the one hand, and of the uterus and vagina, on the other, varies in different species, and frequently is by no means clearly established.

The ovary can be considered as divisible into a superficial or cortical, and a deep or medullary layer. The latter consists of a highly vascular, highly cellular stroma of connective tissue, in which follicles are embedded along with the results from their degeneration or maturation. In addition, in many species, blocks or groups of epithelial cells are present, the " interstitial tissue " of the ovaries. In the human ovary the presence of this interstitial tissue has not been clearly demonstrated. The external layer of the cortex, single low cylindrical germinal epithelium continuous with the peritoneal epithelium, covers the tunica albuginea, an ill-defined layer of connective tissue containing some unstriped muscle fibres. From the epithelium epithelial cords grow into the substance of the ovary, and subsequently break up into small nests of " primordial follicles," some of them subsequently becoming enlarged to form the primitive ova. Each follicle consists, from without inwards, of the

theca externa, the theca interna, and the follicular epithelium which carries the ovum. As the follicle matures it extends inwards until it reaches some size, but eventually also projects outwards, bulging the surface of the ovary. The cavity within this mature Graafian follicle is filled with a viscous liquid, the liquor folliculi. At birth the human ovary contains some thousands of primordial follicles and a few growing Graafian follicles.

From birth to puberty the ovaries slowly increase in size. With the approach of puberty the Graafian follicles become greatly enlarged, and eventually rupture, discharging their ova. Following such rupture the point of rupture closes, the cavity fills with blood, and is subsequently invaded by connective tissue. The follicular epithelium multiplies and its cells enlarge. They acquire more and more lipoid material, which, in bovine and human ovaries, is coloured orange or yellow from the presence of a trace of carotene (with some xanthophyll), whence the name *corpus luteum*. In many other species, including the rat and mouse, the corpora lutea are not yellow in colour.

If the discharged ovum is unfertilized, after a short period involution and obliteration of the corpus luteum set in. If, however, the ovum is impregnated and becomes embedded in the uterus (or abnormally elsewhere) the corresponding corpus luteum enlarges still further, to involve about a third of the ovary, and persists throughout pregnancy.

The majority of the Graafian follicles fail to reach complete maturity and rupture, but undergo atresia at some stage short of this. Such follicles are finally entirely absorbed or else are metamorphosed into corpora lutea atretica and finally small corpora albicantes. The atresia seems to be associated with definite stages of the oestrous cycle.

Abruptly, with the first ovulation, occurs the first sexual cycle, the first *oestrus*. Characteristic changes occur in the uterus, and in many animals (mouse, rat, guinea-pig, ferret) in the vagina. Primates and other mammals exhibit some

differences in the cycle. In the lower mammals it can be divided, using the nomenclature of Heaps, into :

1. *Anoestrus*, the quiescent or resting stage (absent of course from the first cycle) ;

2. *Prooestrus*, the coming on of " heat," in which occur turgescence of the uterus and vagina, together with certain endometrial changes ;

3. *Oestrus*, the period of heat and of desire ;

4. Either *pregnancy*, or a return to anoestrus.

In polyoestrus animals, in which the cycle is repeated several times during the breeding season, oestrus is followed by periods of recuperation and growth, *metoestrus* and *dioestrus*, and these again by prooestrus.

In the immature female rat and mouse the external orifice of the vagina is closed by a " plate," a thin wall of cells, which is ruptured during the first cycle by enlargement of the vagina. In the guinea-pig a corresponding membrane is regenerated after each period of oestrus.

In primates, if pregnancy does not take place, *menstruation* occurs. In the turgescent uterus a rapid necrosis of its functional layers is accompanied by haemorrhage.

A comparison of the time relationships gives some such table as the following (65) [1] :

Phase.	State of the Ovary.	State of the Uterus (and Vagina).
Anoestrus .	Rest.	Rest.
Prooestrus.	Maturation of follicles.	Growth.
Oestrus .	Ovulation.	Degeneration (Copulation)
Metoestrus.	Formation of corpus luteum.	Recuperation.
Dioestrus .	Transitory development of corpus luteum.	Transitory development or no change.

[1] For further details, see Frank (35), Parkes (65), or Sharpey Schafer (73), from which sources the above is drawn.

The testes of mammals show no such cycle of changes, nor do their functions call forth any cyclical change in the secondary sex glands of the male. The internal secretion of the testis is generally believed to be associated with the *interstitial cells* or *cells of Leydig*, epithelium-like cells associated with the intertubular connective tissue, and forming conspicuous isolated groups of cells in man.

Prior to the intensive biochemical investigation of the gonadal secretions, which is rapidly leading to complete elucidation of the nature of their endocrine compounds, much information was gained concerning the functions of these principles by study of the effects of extirpation and of grafting. Such experiments afforded information of great value concerning the control of the secondary sex organs and characters by these principles.

The experiments of Nussbaum on the frog in 1912 produced reasonable evidence that the sex characters of the male are controlled by a specific endocrine principle of the testis. In the breeding season of these amphibians a thickened pad of skin develops on the first digit of each forelimb of the male, associated with increased muscular development of the limb ; This development is preparatory to his prolonged copulatory embrace of the female. Nussbaum showed that if the male is castrated the thickened pad and the increased muscular development do not occur, but that if a piece of testis is introduced into the dorsal sac of such a castrate, these mating changes ensue normally. The absence of nervous connections from such a graft indicated an effect due to an endocrine principle of the testicular tissue (79).

In the young male rat, four to six weeks old, the penis is short and thin, with undeveloped corpora cavernosa, the prostate is scarcely visible, and the seminal vesicles are very small. In the adult rat the penis is relatively long and wide, and can be easily protruded, the corpora cavernosa form its proximal part, the prostate is a relatively large, lobular organ, and the vesicles are similarly large and filled with a

coagulable secretion. If castration is performed at the age of four to six weeks the adult castrate shows scarcely any change in the sex apparatus from the period of castration. The effect of castration on male mice is very similar. Corresponding changes have been observed in the guinea-pig, rabbit, and dog (47).

The precise effect of castration on man, practised throughout the centuries, has only within recent years received exact study from the physiological standpoint. Much information has been gained by studies of the Skopecs, a Russian religious sect who practise castration in the first decade of life. Following such early castration the adult castrate has small and underdeveloped penis, prostate, and seminal vesicles. Masculine distribution of hair does not develop. The beard is absent. The limitation of hair in the pubic region is feminine. Obesity may or may not be present. The larynx is an enlarged infantile larynx, and the voice of the prepuberal boy persists throughout life. The skeleton shows some characteristic changes. Growth of the long bones persists beyond the usual time; the castrate tends to be tall through disproportionate length of leg. The general intelligence is not specially influenced, but apathy is a characteristic feature. Post-puberal castration produces less marked effects (47).

Observations on the results following castration in different species of mammals indicate that, wherever specific structures are associated with sex, castration affects their growth. Castration in young stags leads to non-development or arrest of development of antlers, according to the age at castration. But in eland and in horned cattle, where both sexes possess horns so that these are not related to sex differentiation, their growth and development is not affected by castration (79).

Ovariectomy in the female leads to corresponding changes. In young rats, mice, guinea-pigs, and rabbits, the uterus and vagina remain infantile. The mammae remain undeveloped. The sex-cycle does not occur.

In women observations are available almost exclusively following post-puberal castration, and are less accurate and uniform. In all cases, however, atrophy of the uterus and vagina takes place, and menstruation ceases. Such castrated women usually gain weight through deposition of fat. Certain of these changes are comparable with those observed at the climacteric.

The effects of gonadal implants will be dealt with later. Generally speaking they tend to restore the secondary sex organs to normal function.

Extirpation and implantation experiments in birds are of some importance in the present connection, since certain of the results have been employed as biological tests, especially for the testicular principle. Fowls have been chiefly used. Different races show considerable variation in results. Much of our present accurate knowledge is due to Pézard and Goodale.

Castration of young cockerels at the age of three months leads to a characteristic development of comb, wattles, and barbles, which remain small, bloodless, and thin, infantile rather than feminine. The spurs are not influenced. The plumage is not greatly changed. The capon becomes somewhat larger and heavier than the normal bird, but the increase in weight is mainly due to the laying down of more fat (whence the ancient practice of castrating fowls). Castration in the hen leads to the development of a comb similar to that of the capon, and the acquiring of " male " plumage, which, however, more closely resembles that of a capon than of a normal cock. Thus removal of either testes or ovaries results in production of a neutral bird (47).

The Vaginal Smear Test

The earlier work on the endocrine secretion of the ovaries was handicapped through the lack of a simple biological test which could be used for extracts. Such a test, the

vaginal smear test, became available from the work of Stockard and Papanicolaou.

Studies by Moran and Lataste, Heaps, L. Loeb and others had suggested that distinct cyclical changes occur in the vaginal walls of animals, but no definite knowledge was available until Stockard and Papanicolaou published in 1917 a complete account of the changing types of cells found in vaginal smears of the guinea-pig during the course of oestrus. In 1920–22 Long and Evans showed that the same series of changes take place in the rat, and Allen and Doisy were thereby led to employ these changes to measure the potency of ovarian endocrine preparations. Stockard has recently reviewed the subject (77).

In the guinea-pig the period of oestrus lasts about twenty-four hours and occurs very regularly every fifteen to seventeen days. Throughout the twenty-four-hour period fluid is abundant in the vagina. For the first six to twelve hours (during which period the female will accept the male), the fluid is a fairly clear, frothy mucus. It gradually increases in quantity until it fills the lumen of the vagina. During the second stage, two to four hours, the fluid presents a cheesy appearance, and during the third stage, five to ten hours, it slowly becomes more liquid and serous. A fourth stage is also differentiated, in which there may be slight bleeding. Following this period of sexual activity the " vaginal closure membrane " grows over the vaginal opening (a change specific to the guinea-pig). If this membrane be broken during the dioestral period the vagina is found to contain only a scanty amount of slimy fluid, poor in cells.

Smears prepared from the vaginal fluid at the different stages show such characteristic differences in appearance as to be diagnostic of the exact sexual state of the animal.

In the first stage the mucous fluid contains an abundant mass of cells, of a squamous type and showing considerable plasmolysis with bent and wrinkled cell-membranes. Their nuclei are very small and pycnotic ; the protoplasm has

degenerated and does not stain well; it exhibits a reticular structure. These cells, derived from the wall of the vagina, predominate over all others at this stage.

Towards the end of the first stage and at the beginning of the second there are also present some elongate, cornified cells, without nuclei, which are desquamated from the more external portions of the vagina. They stain decidedly red with haematoxylin and eosin, while the commoner type appear merely gray.

During the second period the enormously increasing

FIG. 44.—Dioestrous smear: leucocytes in stringy mass. × 40.

FIG. 45.—Pro-oestrous smear: chiefly nucleated epithelial cells with an occasional leucocyte. × 40. Present thirty-five to forty hours after first injection.

FIG. 46.—Oestrous smear: non-nucleated cornified epithelial scales. × 40. This type usually appears within forty-eight hours after the first injection and is a certain criterion of the positive action of an extract.

FIG. 47.—Flat, cornified elements of the oestrous smear stage. × 250. Eosin stains these cells a brilliant red. Although the site of the former nucleus is apparent, all basophilic staining reaction has been lost.

FIG. 48.—Early stage of leucocytic infiltration (metoestrum). × 40. Few nucleated epithelial cells have appeared as yet.

FIG. 49.—Late stage of the metoestrum. × 40. Enormous numbers of leucocytes, some cornified scales (in the centre of the field), and many nucleated epithelial cells.

number of cells in the fluid causes its cheese-like consistency. These cells are derived mainly from the vaginal wall, and are healthy epithelial cells, as contrasted with the plasmolyzed cells of the first stage. The nuclei show only slight signs of degeneration. The protoplasm stains well. The second stage corresponds in time with the rupturing of the Graafian follicles and discharge of the ova.

While leucocytes are rare in smears of the first and second stages, in the third stage they predominate to such an extent that the epithelial cells become isolated from each other and each is surrounded by a number of leucocytes. These

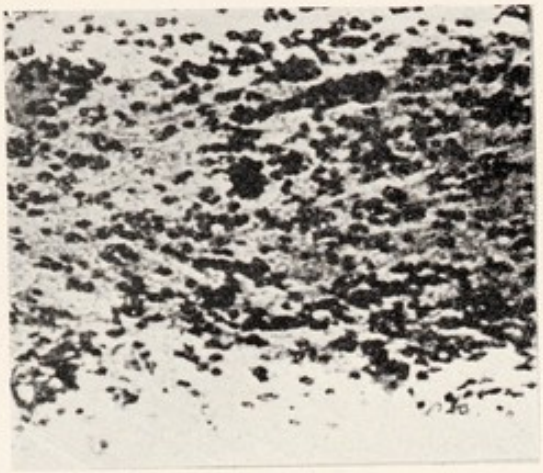


FIG. 44.

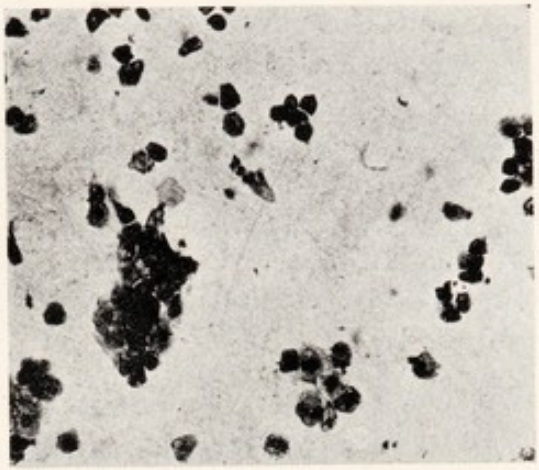


FIG. 45.

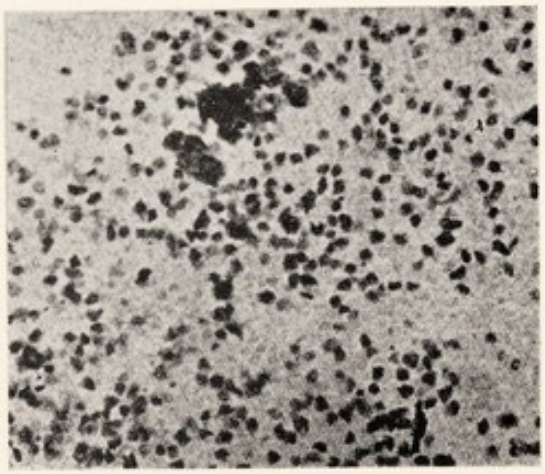


FIG. 46.

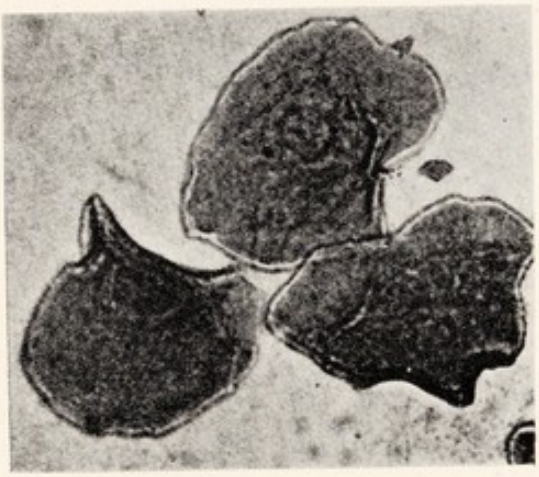


FIG. 47.

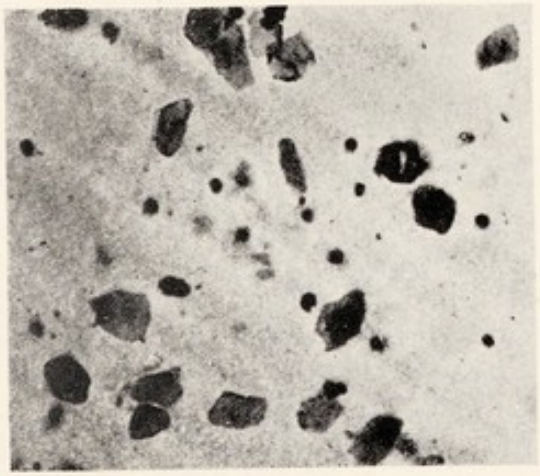


FIG. 48.

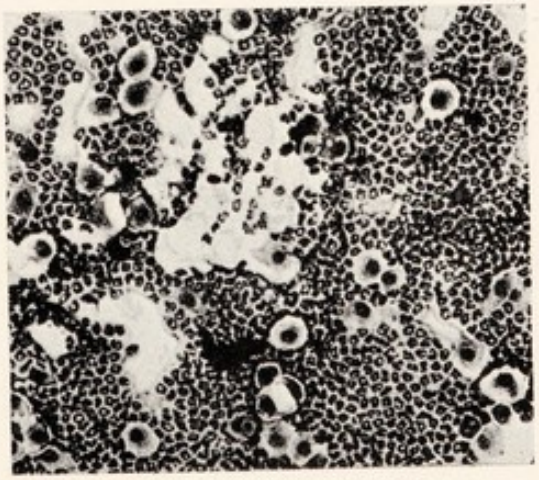


FIG. 49.

Vaginal smears of artificially induced oestrus in spayed rat. (From Allen, Doisy, *et al.*, *Am. J. Anat.*, 1924–25, xxxiv, 169–171.) (For legends see p. 286.)

[*To face page* 286.

appear to dissolve or digest the epithelial cells. The fluid thus becomes more serous.

The fourth stage presents a similar appearance ; sometimes red blood cells are present from a slight haemorrhage.

Fluid obtained during the dioestral period shows gradual changes from the fourth to—just before new oestrus—the first stage.

Different investigators have examined the vaginal discharge in the mouse, rat, monkey, opossum, cow, and rabbit, and all have found a strikingly uniform correlation between the particular cellular composition of the vaginal smear, and the several stages in the process of follicular growth and ovulation.

The importance of the vaginal smear test lies in this correlation. Immature animals and castrates do not exhibit the vaginal cycle. Its induction by injection of ovarian extracts constitutes a positive test for the efficiency of those extracts. The test has the additional advantage that the castrated animal need not be sacrificed but can be used repeatedly. The cycle in the mouse and rat is only of four to six days' duration, so that these animals are particularly suitable for the test.

The vaginal smears of an artificially induced oestrus in the spayed rat (Allen and Doisy's procedure) are shown in Plate II., Figs. 44–49.

Keto-Hydroxy-Oestrin (Theelin)

Early efforts to obtain extracts from ovarian tissue which would exhibit properties ascribed to the endocrine principle were suggestive rather than successful (35, 65). The experiments of Allen and Doisy placed such investigations on a firm foundation (2, 31).

They aspirated *fresh* follicular liquor from hog ovaries, added it to two volumes of 95 per cent. alcohol, filtered off the proteins, and concentrated the liquid to dryness in a

current of warm air. The residue was emulsified with a little water and somewhat more alcohol-acid, boiled, and two volumes of acetone added, the mixture cooled and filtered. The filtrate was distilled to dryness, and the residue extracted several times with small volumes of boiling 95 per cent. alcohol. The extract was cooled and filtered, the solution evaporated to dryness, and the residue extracted with anhydrous ether. The ethereal extract contained the active principle ; it was evaporated, and the principle taken up with corn oil.

This extract induced oestrus in spayed rats. The *rat unit* was defined as the quantity of material necessary to induce oestrus—as judged by the smear test—in an ovariectomized, sexually-mature rat, weighing 120 to 160 grams, when three injections were given at four-hour intervals (the sum of the three constituting the unit). Similar tests on rabbits, in which the animals were sacrificed and the uteri compared with controls, fully confirmed the correctness of the results.

This early work definitely demonstrated that the principle was lipoid-soluble. Allen and Doisy also found that it could be extracted by the same procedure from whole ovaries or from placenta.

Nomenclature. The results of Allen and Doisy were amply confirmed by investigators in many countries. Many names have been used for the principle, both by scientific investigators, and by commercial drug houses. Such names include *oestrin*, *folliculin*, *feminin*, *ovarin*, *oophorin*, *thelykinin*, *menformin*, and *progynon*. Of these *oestrin* is widely used, and is very suitable " since the substance in question has not adequately been shown to produce any features other than those characteristic of oestrus " (65). Doisy, having obtained the compound in crystalline form (cf. p. 291), has named it *theelin*.

The name *oestrin* leads to a little confusion, since, as will be seen, the compound is frequently accompanied by a closely related " hydrate " containing three hydroxy groups (" tri-

hydroxy-oestrin "). Since it contains a keto and a hydroxy group, it has been termed *keto-hydroxy-oestrin* (55), and this name will be used here.

Other Methods of Preparation. Zondek and Brahn (86), by the use of acid, separated the principle from unsaponifiable lipoid material, and obtained a protein-free dialyzable product which gave a clear solution in water and was highly active. Laqueur (44) prepared it from placenta by initial treatment with boiling benzene, treatment of the extract with alcohol, and of the alcohol extract with dilute hydrochloric acid; the final clear solution was stated to be highly active. Dodds (26) saponified placenta with baryta, extracted the filtrate with butyl alcohol, and concentrated the extract by similar procedures.

These various methods of preparation seem to demonstrate that the principle is fairly stable.

Following the initial discovery the next most important advance was the extraction of the compound from urine. The method is due to Zondek and Aschheim (86), who showed that the compound is present in *relatively* enormous amounts in the urine of pregnant women and of pregnant mares. They developed different methods of extraction, involving saponification, adsorption and precipitation with heavy metals. The first may be quoted as indicating the type of procedure.

Saponification Method. One litre of urine is evaporated to half volume, acidified (if necessary) with acetic acid until it reacts weakly acid to litmus, and filtered (with Kieselguhr). The filtrate is warmed, and extracted two or three times with four volumes of ether or benzene. The ether or benzene is evaporated. A yellowish-white mass is left. This is warmed with 50 c.c. of 2 per cent. sodium hydroxide at 60° C. for twenty-four hours, then cooled, and extracted with considerable excess of ether. The ethereal extract is concentrated to dryness, the residue taken up in about 50 c.c. of

0·1 *N* acetic acid, filtered, and neutralized. The last two stages can be repeated for further purification. Finally the preparation is dissolved in 50 c.c. of water; the solution is colourless and odourless. (Mare's urine, alkaline, requires strong acidification with hydrochloric acid and boiling for some minutes, before extraction with the lipoid solvent.)

The Site of Formation of Keto-hydroxy-oestrin, and its Distribution in Tissues. As Allen and Doisy showed, the compound is present in the follicular liquid.

Using an implantation method, Zondek and Aschheim (86) claim to have shown that the principle is present in the theca interna cells, and especially in atretic follicles, but is absent in the follicular granulosa, the ovarian stroma, and the germinal epithelium. It does occur, however, in residual ovarian tissue, after removal of large follicles and corpora lutea, in much larger amounts than the remaining follicles could account for. It has been found in ovarian cysts.

Various claims have been made that the principle is present in the corpus luteum; there has been marked disagreement on this point. Parkes (65) attributes this to the fact that, in the cow especially, many corpora lutea contain a fluid centre derived from the remains of the liquor folliculi; such fluid may well contain the principle. As Parkes points out: "It is unlikely that the corpus luteum itself, an organ whose development is always associated with the absence of oestrus, would produce the oestrous hormone." He sums up the distribution in the ovary thus: "The liquor folliculi of all mammals which have been examined contains this hormone, as does the stromal tissue, . . . the corpus luteum contains it incidently or doubtfully."

The placenta contains large amounts of the principle. It has been found in the foetal membranes, the amniotic fluid and the umbilical cord, but is absent from extracts of the

foetus itself. It is absent from tissue unrelated to the sex organs.

It is present in the blood of the non-pregnant woman and of the oestrous sow. According to Frank (35), it is present in greatest amount in human blood about the first day of menstruation, after which it rapidly decreases. It is present in menstrual blood in larger amounts. Zondek and Aschheim found very considerable amounts in human blood during pregnancy. In the later months of pregnancy sufficient is present to permit a positive reaction with 2 c.c. of blood.

The urine of non-pregnant women contains small amounts, varying with the stage of the menstrual period. The very large amounts during pregnancy have been referred to. It rapidly decreases in amount after parturition.

Zondek's figures gives some idea of the relative amounts present in various material. They are expressed in " mouse units," which have a similar significance to the rat unit, which has already been defined.

Follicular liquid . .	4,000 mouse units per litre.
(Corpus luteum . . .	4,000–5,000 mouse units per kg.)
Placenta	10,000 mouse units per kg.
Urine from pregnant cows .	500–800 mouse units per litre.
Urine from women late in pregnancy . . .	12,000 mouse units per litre.
Urine from pregnant mares.	100,000 mouse units per litre.

The Preparation of Crystalline Keto-hydroxy-oestrin (Theelin). The compound was obtained independently in pure crystalline form by several groups of investigators. It is but just that Doisy had slight priority in publication. His original communication was made to the Thirteenth International Physiological Congress in August 1929, and the detailed account of the work was published in the following year (30). Butenandt announced his success in October 1929 (7), and Laqueur in 1930 (45).

Doisy's method is shown in the following scheme :

Urine extracted by butyl alcohol in a continuous extractor. Butyl alcohol distilled off ; residue leached with benzene.

- Solution distilled and residue transferred to solution in dilute NaOH
  - Alkaline solution extracted with ethyl ether. Ether distilled ; residue steam-distilled, and residue from this leached with hot 0·25 *N* NaOH
    - Solution extracted with ethyl ether ; ether distilled and residue leached with cold 0·25 per cent. *N* NaOH
      - Solution extracted with ether ; ether distilled. Residue crystallized from hot 25 per cent. alcohol or butyl alcohol.
      - Insoluble residue discarded ; 9 per cent. loss.
    - Residue discarded ; 1 per cent. loss.
  - Insoluble tar discarded ; 4·2 per cent. loss.
- Insoluble residue discarded ; 1·2 per cent. loss.

By this procedure Doisy obtained 32 mg. of crystals from four litres of butyl alcohol extract.

Butenandt (9) started with a commercial "crude oil" preparation from pregnant urine, a dark red-brown syrup averaging 30,000 mouse units per gram. His method is shown in the scheme on p. 293. The crude crystal stage represented 64 per cent. of the original content of the principle in the crude oil.

Chemical and Physical Properties of Keto-hydroxy-oestrin (Theelin). The compound has the composition $C_{18}H_{21}O(OH)$, one oxygen atom being in a keto grouping. It contains one double bond. It melts at 254° to 257° (corr.) (29), or 250° to 251° (corr.) (9), as determined by different investigators. It is dextro-rotatory, the specific rotation being $(\alpha)_D^{18°} = +156°$ in chloroform solution (9).

It is easily soluble in alcohol, acetone, chloroform, and benzene, more difficultly in ether and acetic ether, and very difficultly in petroleum ether. In pure (neutral) water the

"Crude oil," 30,000 M.U. per gm.

Partitioned between 50 per cent. methyl alcohol and petroleum ether.

Alcohol-phase. Excess water added and mixture extracted with ether. Ether evaporated leaving oil. 100,000 — 200,000 M.U. per gm. Partitioned between 70 per cent. methyl alcohol and benzene.

Petroleum ether-phase. (Benzoic acid, cholesterol).

Benzene-phase. Washed with 60 per cent. methyl alcohol, dried with sodium sulphate, and evaporated *in vacuo*. Oil, 300,000–500,000 M.U. per gm. Dissolved in ethyl alcohol, treated with dilute HCl, and extracted with ether. Solution treated with sodium carbonate solution.

Alcohol-phase.

Part soluble in carbonate.

Residual ethereal solution. Treated with *N* NaOH.

Alkaline solution. Acidified with HCl. Extracted with ether. Ethereal extract washed, dried, and distilled. Oil, 1·5–2 million M.U. per gm. Fractionally distilled in high vacuum (0·02–0·03 mm. Hg.).

Alkali-insoluble portion. From this was obtained crystalline *pregnandiol*, $C_{21}H_{36}O_2$, an alcohol.

Portion distilling between 100–115° C. (20–25 per cent.) rejected.

Portion distilling between 130–220° the raw crystallizate, crystals with a little raw yellow oil. Oil removed with cold ether. Crystals recrystallized from acetone, 7–8 million units per gm.

Dissolved in a hot mixture of acetic ether and petroleum ether, filtered and concentrated, then recrystallized from acetic ether, giving pure crystalline keto-hydroxy-oestrin, 8–10 million M.U. per gm.

pure crystals are only very slightly soluble; 1 c.c. of a saturated solution contains about 150 mouse units, and at most 1 litre dissolves 15 mg. It shows a characteristic

absorption band in the ultraviolet, the maximum absorption being at 283 to 285 mμ.

It gives no characteristic colour reactions (9). It is fairly easily oxidized. In corked vessels it remains unchanged for months, but in alcoholic solution it is slowly changed to a non-crystalline brown resin.

The crystals vary in appearance according to the method of preparation: needles, rhombic plates, flower-like leafy

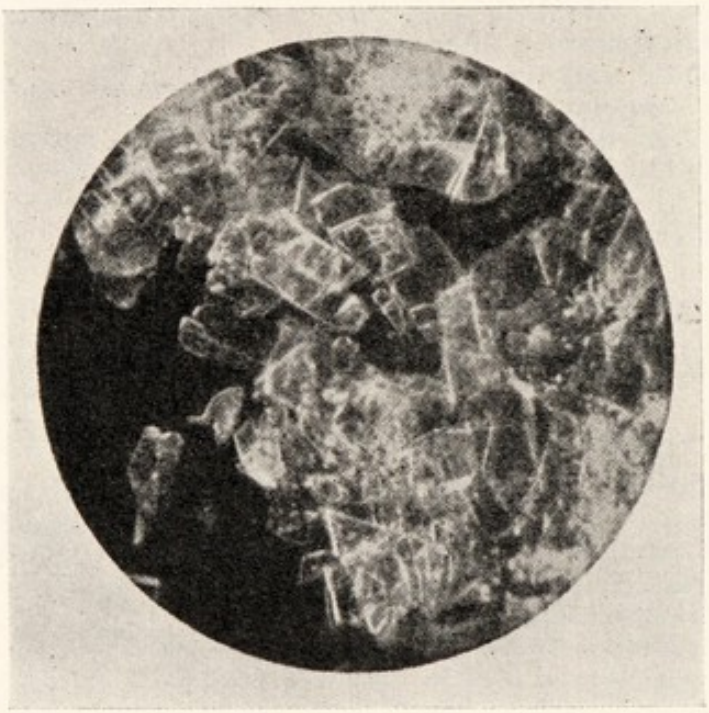


FIG. 50.—Crystallized keto-hydroxy-oestrin from acetic ether. (From Butenandt, *Zeitschr. physiol. Chem.*, 1930, cxci, 127.)

branching crystals. A preparation of Doisy's, which had been recrystallized twenty times, has been described by Slawson (74). The crystals, very small, are monoclinic with a tabular development, and have a rhomboid outline. They possess a pronounced basal cleavage. A photomicrograph of Butenandt's crystals from acetic ether is shown in Fig. 50.

Tri-hydroxy-oestrin

In 1930 one compound, termed by different names, and possessing the physiological properties of the ovarian

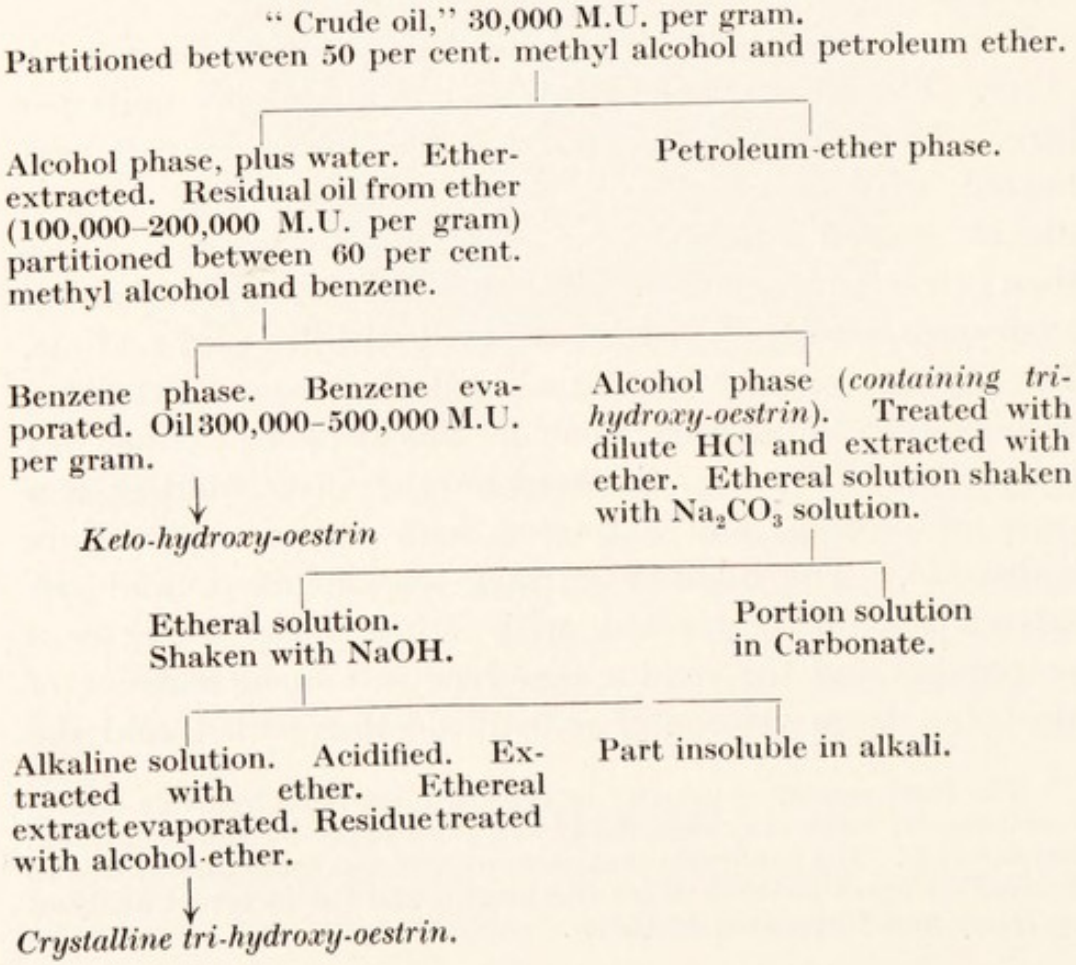
principle, had been isolated in crystalline condition from urine. To this " oestrin " Doisy *at that time* ascribed the formula $C_{18}H_{23}O_2$ (one atom of hydrogen too much), and Butenandt the formula $C_{23}H_{28}O_3$ or $C_{24}H_{32}O_3$. Marrian, by a different procedure, obtained a crystalline compound with similar physiological activity, and obtained its correct formula, $C_{18}H_{24}O_2$ (54). It was natural to consider that it was also " oestrin." When the true formula of keto-hydroxy-oestrin was found to be $C_{18}H_{22}O_2$, it was seen that the two compounds only differed by the elements of a molecule of water. Butenandt suggested that Marrian's compound was the " hydrate " of the other (9). It proved to have three hydroxy groups, whence the term " tri-hydroxy-oestrin " was naturally suggested. Marrian has suggested the differentiation in terminology which is used here (55). Doisy isolated Marrian's compound somewhat later, and has termed it " theelol " (32).[1]

Marrian extracted the urine of pregnant women with ether. The ether extract contained 18,800 mouse units per litre. The ether was evaporated, the dried extract was heated with aqueous potassium hydroxide and carbon dioxide passed into the mixture for twelve hours. It was then extracted thoroughly with ether, the extract evaporated to dryness, and the residue extracted with ice-cold acetone. The acetone extract was dried and the residue extracted with ice-cold 50 per cent. alcohol. The alcoholic extract was evaporated to dryness, dissolved in ether after addition of a trace of alcohol, and extracted with aqueous potassium hydroxide. The alkaline extract was acidified, and the active principle extracted with ether. The ether was evaporated and the residue dissolved in a small amount of alcohol, a large volume of redistilled ether added, and the

[1] The facts regarding priority in the discovery of tri-hydroxy-oestrin have been set forth very reasonably in a joint paper by Butenandt and Marrian (12). The confusion that arose at first was undoubtedly due to Marrian's correct analysis of his compound, and the incorrect analyses by Doisy and Butenandt of theirs.

mixture cooled to −15° C. A large amount of solid material separated and was filtered off. This solid was boiled with charcoal in alcoholic solution, filtered, and evaporated to dryness. The residue was crystallized from ethyl acetate. The white crystals melted at 264° to 266° (uncorr.). The potency was between seven and eight million mouse units per gram, and corresponded to 32 per cent. of that of the original ether extract. The formula has been stated.

Butenandt showed that the method employed by Marrian does not affect keto-hydroxy-oestrin, so that the tri-hydroxy compound is not formed during this preparation from urine (5). He has since cleared up the whole confusion between the two compounds and has succeeded in converting one into the other (11). From the crude oil obtained from urine he succeeded in obtaining *both* compounds in crystalline form by the procedure outlined in the following scheme.



Tri-hydroxy-oestrin is only slightly soluble in ether, somewhat more soluble in methyl and ethyl alcohol, chloroform and acetone, and easily soluble in pyridine. It dissolves slowly but completely in 5 per cent. aqueous potash, and from this solution is immediately precipitated by carbon dioxide. It is quite insoluble in aqueous sodium carbonate. It gives an orange colour with green fluorescence on warming with concentrated sulphuric acid, the colour being indistinguishable from that given by crude bile acids under the same conditions. Millon's reaction and the xanthoproteic test are both positive, indicating the presence of a hydroxyphenyl group. Acetylation gives an acetate whose composition indicates the presence of three hydroxy groups, whence the compound is $C_{18}H_{21}(OH)_3$. One of the hydroxy groups has acidic properties. The compound is not appreciably volatile at 165° and 0·001 mm. mercury. Its optical rotation is $(\alpha)_{5461} = +38°$.

When tri-hydroxy-oestrin is heated with potassium bisulphate at 0·02 mm. mercury pressure and 180° to 200° for some hours, keto-hydroxy-oestrin is produced as a distillate (5); the conversion has also been effected by heating with fused potassium bisulphate, suspending the product in excess of 0·01 *N* sodium hydroxide, and repeatedly extracting with ether. Keto-hydroxy-oestrin was obtained from the ethereal extract (55).

The Physiological Properties of the Oestrin Compounds

Replacement therapy with spayed animals and injection experiments with immature animals have demonstrated conclusively that keto-hydroxy-oestrin is the ovarian principle. The following is largely taken from a recent review by Allen (1).

Subcutaneous injection is probably most effective. Direct intravenous injection is possible with aqueous solutions of crystalline products, but the slower absorption from extra-

vascular sites probably produces results more efficiently. Soon after injection the principle can be detected in the cerebrospinal fluid, indicating its rapid distribution throughout the organism. It is usually stated that oral administration is not efficient, and that the oral dose required to produce oestrus in rats and mice is 20 to 100 times the minimal effective subcutaneous dose.[1] The loss of efficiency is not due to destruction by digestive enzymes. The principle is readily absorbed through the walls of the vagina, and through the nasal mucous membrane.

Within four to six days following ovariectomy in the mouse or rat the castrate condition of the epithelial wall of the vagina is well advanced. A series of injections of keto-hydroxy-oestrin leads to a considerable amount of growth of the vaginal wall within twenty-four hours. Within the same period following ovariectomy in the rat the uterus becomes small and anaemic and its lumen slit-shaped in cross-section. After several injections of the principle, rapid recovery is apparent. There is active mitotic division in the surface epithelium and glands followed by an extensive increase in the number of cells ; then a clear fluid is secreted into the lumen of the uterus, which becomes distended, the whole organ finally becoming hyperaemic. Uterine contractions increase in amplitude. Following ovariectomy the uterine tubes atrophy ; injection of the principle brings about repair. Ovariectomy induces atresia of the mammary glands, which involves the ducts, the alveoli, and the epithelium of the nipples. These degenerations can also be repaired by injection of keto-hydroxy-oestrin.

It would thus appear that the principal action of the ovarian principle is to induce growth in the tissues of the accessory genital organs (1).

Injections cause corresponding changes in the immature

[1] Doisy (32) has stated recently that when the establishment of the vaginal orifice in immature rats is used as test, " theelin " is as effective orally as subcutaneously. Its oral effect clearly requires further study.

young animal, leading to premature oestrus, accompanied, in the rat and mouse, by premature opening of the vagina.

Precisely the same effects are produced on the secondary sex organs of the immature animal by injections of keto-hydroxy-oestrin and by pituitary implants (cf. p. 263). If such animals are spayed, pituitary implants do not produce these effects, indicating that the pituitary principle must act through the ovary, by stimulating the production of keto-hydroxy-oestrin.

It is generally considered that while in the pre-puberal stage the principle ("hebin") of the anterior pituitary gradually stimulates the production of keto-hydroxy-oestrin, this, so produced, causes the gradual growth of the secondary sex organs, their rapid growth changes at the first oestrus, and also the corresponding development of secondary sex characters (in the female, differential skeletal development, fat deposition, etc.).

The heightened voluntary activity of female rats just before and during oestrus is usually attributed to an increased secretion of the ovarian principle, since it is not shown by ovariectomized animals, but is restored in them by ovarian transplants. Injections of keto-hydroxy-oestrin have been only partially successful in reproducing this effect.

Effects on Normal Animals. These are of importance in suggesting what may result from pathological endocrine hyperactivity of the ovaries. Since, however, such very large amounts of keto-hydroxy-oestrin are produced and excreted during pregnancy, it seems unlikely that there can be any pathological condition in women due merely to hyperoestrinism.

The ovarian principle produces no effect on the heart, blood pressure, and general metabolism. It has no stimulating effect on the ovaries. There is some evidence that it can inhibit follicular development.

When it is injected into a normal adult female, it accentuates and maintains growth in the accessory sex organs,

and continued administration—if in large enough doses—may completely eliminate the degenerative phase of the cycle in the vagina. The uterine mucus is maintained on a high functional level. Oestrus can be produced in the dog during anoestrus, and even in the hibernating hedgehog and ground squirrel.

If it is injected into pregnant animals in sufficient dosage, pregnancy may be terminated by abortion or by resorption of the embryos in utero. The dosage necessary to produce this effect increases as gestation progresses (Smith, 1926 ; Parkes, 1926. Doisy, 1931, is not in agreement).

During lactation, when the cyclic changes in ovaries and genital tract are suspended for some period, injection of the ovarian principle induces oestrus. Injections into old animals after sexual function has ceased lead to oestrus, but the effect is only transient.

Injections into adult males perhaps produce testicular degeneration. Injections into immature males definitely inhibit normal gonadal growth : the testes remain infantile, no sperm being developed, and the descent into the scrotum being inhibited. Moore considers that this is due to a depressing effect on the anterior pituitary. (Cf. also 76, 40.)

The precise mechanism of the action of the ovarian principle on the genital tissues has not yet been elucidated. Allen (1) presents the alternatives : " Do the sex hormones act directly upon the cells of the accessory genital organs to induce cell division and heightened functions, and thus increase their nutritive needs to react secondarily upon the blood supply, or do these hormones directly stimulate the vascular control mechanism ? " The available evidence suggests that vascular control, leading to hyperaemia, is an important factor.

Tri-hydroxy-oestrin. The effect on immature female rats and on spayed adults is qualitatively the same as that of keto-hydroxy-oestrin, as judged by the vaginal smear test, and (in the former) the premature opening of the vagina (32).

While keto-hydroxy-oestrin seems to be only slightly active by oral route, Curtis and Doisy find that tri-hydroxy-oestrin is one-half to one-third as active orally as it is subcutaneously (32).

The most important physiological difference between these two oestrins lies in their quantitative effects. Comparisons are at present a little difficult, since different investigators not only use different animals (mice *or* rats), but also different techniques of injection. Furthermore, it has been shown that the response of individual animals varies widely (23, 25). Such factors have led to variation in estimates of the ratio between the rat and mouse units from 1 : 1 to 1 : 12 (29). Many of the earlier figures with concentrates must have been for the summed effects of the two compounds.

Very varied figures for the physiological activity of tri-hydroxy-oestrin appear in the literature. Different preparations exhibited different activities. Thus Butenandt (13) has found the following variations :—

Preparation	(i)	m.p. 279°	Activity	75,000 M.U.	per gm.
,,	(ii)	,, 273–6°	,,	100,000	,,
,,	(iii)	,, 266–8°	,,	250,000	,,
,,	(iv)	,, 263°	,,	800,000	,,
,,	(v)	,, 268·5°	,,	1,250,000	,,

He believed that these variations could be accounted for by admixture with a slight trace of keto-hydroxy-oestrin, and purified these preparations by treating them with semicarbazide. The keto-compound gives a semicarbazone, but the triol-compound does not. From the mother liquor following such treatment crystalline preparations of tri-hydroxy-oestrin were obtained in each case which uniformly melted at 279–280° C. (uncorr.), had an optical rotation of $(\alpha)_D = 30°$, and a physiological activity of 75,000 mouse units per gram.

Butenandt explains the slight activity of such purified preparations as due to the power possessed by the organism to reconvert the " triol " into the " keto " compound. (He

has suggested that the "triol" is normally formed in the organism to aid in excretion, since it is more soluble than the "keto" compound.)

Yet this suggestive result does not completely elucidate the problem. The "triol" is undoubtedly very active orally. Furthermore, Marrian (55) has recently obtained a preparation with a melting point of 279·5–280·5° (equal to Butenandt's purified product), with the high activity of 7·6 million mouse units per gram. Admixture seems excluded.

Confusion (but perhaps ultimate clarification) arises from the existence of other oestrin-derivatives. Butenandt (10) has shown that by distilling tri-hydroxy-oestrin at 140–165° at 0·02 mm. mercury pressure, ordinary keto-hydroxy-oestrin is produced (which, in this connection, he terms the "α-follicular hormone"). If this distillation is carried out rapidly at 200°, mixed crystals are obtained of this and a second, "β-hormone," which he believes he has purified by fractional crystallization. It melts 2° higher, and has a different optical rotation from the "α-hormone." It is somewhat less soluble in alcohol and chloroform. Its physiological activity is about 1·25 million mouse units per gram, so that it is much less active than ordinary keto-hydroxy-oestrin. Doisy stated recently that he had never encountered such an isomeric form (28).

The Endocrine Activity of the Corpus Luteum

The various theories of the function of the corpus luteum that have been enunciated during the past thirty years have been admirably set forth by Hisaw (39). Here a very brief recapitulation suffices.

Evidence from operative procedures (removal of corpora lutea) suggested that the presence of the corpus luteum inhibits ovulation during pregnancy. Removal, at any rate early in pregnancy, leads to abortion or resorption (in mice, rats, opossums, guinea-pigs). If such removal takes

place late in pregnancy normal birth of living young may occur in guinea-pigs and rabbits. Such evidence indicates a function of the corpus luteum in sensitizing the uterus for implantation. Loeb has shown that the corpus luteum secretes a substance which sensitizes the uterus, so that it responds to mechanical stimuli by formation of decidual tissue. The effect has been demonstrated in the guinea-pig, rabbit and bitch. Conversely, in the guinea-pig, the uterus influences the life of the corpus luteum, since hysterectomy prolongs it (Loeb). There is also evidence associating the persistence of corpora lutea with lactation.

[Ovulation in the rabbit only follows copulation. " Pseudo-pregnancy " can be induced by sterile mating with a vasectomized male. Ovulation is then followed by development of normal corpora lutea, modification of the uterine mucosa, and hypertrophy of the mammary glands (conditions typical of the early stages of actual pregnancy). Pseudo-pregnancy is probably due to a nervous reflex set up through copulation, and acting through the anterior pituitary to produce ovarian development and formation of corpora lutea. When pseudo-pregnancy is produced by similar procedure in the rat and mouse, the lives of the corpora lutea are prolonged and the next oestrus delayed.]

Parkes (65) considers that the experimental evidence shows that four functions are performed by the corpora lutea of pregnancy, pseudo-pregnancy, or lactation—the inhibition of ovulation and of oestrous changes in the accessory organs ; the sensitization of the uterus for the implantation of fertilized ova ; the development of the mammary glands from the condition in which they are found at oestrus to that characteristic of the end of the luteal phase ; and the maintenance of pregnancy.

Extracts of the Corpus Luteum. The earlier experiments led to conflicting results, many of which were probably due to oestrin, and not to any principle specific to the corpus luteum. While numerous investigators have shown that

inhibition of the oestrous cycle and of ovulation can be induced in various species by injections of extracts of luteal tissue, Hisaw (39) concludes " the fact that similar results may be had with extracts of corpus luteum tissue representing entirely different fractions and that non-specific substances also inhibit the cycle seem to indicate that this method of determining corpus luteum activity is not reliable." Mucification of the vaginal mucosa during pregnancy and pseudo-pregnancy seems attributable to oestrin.

During pregnancy of the guinea-pig there is a separation of the pelvic bones (" a separation at the ileo-sacral union and symphysis pubis so that the two ossa innominata may be moved freely and independently of each other "). This *relaxed condition* reaches its maximum extent during the last half of pregnancy ; after parturition there is gradual but usually not complete regression to the original condition. Hisaw has found that these pelvic changes are under endocrine control. He states that the blood serum of the pregnant guinea-pig, dog, cat, sow, mare, and rabbit (but not of cows and women), when injected into virgin guinea-pigs during oestrus produces marked relaxation of the pelvic ligaments within eight to twelve hours. The essential principle responsible for this action appears to be especially abundant in the blood of pregnant rabbits—its presence is demonstrable by the seventh or eighth day of pregnancy in these animals, and is maximal by the twentieth. It cannot be detected twelve to eighteen hours after parturition. Extracts from the maternal and foetal parts of the placenta and from the corpora lutea of the sow are rich in the principle. Hisaw claims that he has obtained it in a relatively pure state from the corpora lutea of the sow, and has named it *relaxin* (39).

Continued injection of extracts of " relaxin " does not prevent the return to the original rigid condition of the pelvic bones. Furthermore, in many, and probably in the majority of mammals in which specific pelvic modifications

facilitate parturition, it can be shown that these changes are not under endocrine control. It seems, therefore, rather peculiar that relaxin should be widely distributed in female mammals, unless it possesses some important function quite unassociated with the pelvic changes for which Hisaw has named it.

Hisaw's acid extract of sow corpora lutea, in addition to relaxing the guinea-pig pelvis and inhibiting the oestrous cycle of rats, also produced modifications characteristic of pregnancy in the rat endometrium, and promoted the growth of deciduomata in the uterus of castrate rats and guinea-pigs. Corner and Allen (22) demonstrated in a series of very excellent experiments that lipoid extracts of sow corpora lutea would produce the changes typical of early pregnancy and pseudo-pregnancy in the uterus of castrate rabbits, and would also cause continuance of life and normal development of embryos in rabbits castrated during pregnancy.

From the work of the two laboratories it would seem that two endocrine principles are involved : *relaxin*, responsible for pelvic relaxation, and a second, responsible for the progestational developments, and termed *progestin* by Allen (3), *corporin* by Hisaw (and the *beta-factor* by Wiesner, and *lutin* by Clauberg).

Chemical Properties of the Endocrine Principles of the Corpus Luteum. The distribution of relaxin has been indicated. So far only the corpora lutea of sows and cows have been studied for progestin. It has not been detectable in foetal blood or the blood and urine of pregnant women. It is difficult to detect its action in presence of oestrin.

Hisaw sums up our present knowledge of the two presumed principles as follows :

Relaxin is soluble in acid and alkaline aqueous solutions and in water. It is insoluble in acetone, ethyl ether, petroleum ether, absolute alcohol, and benzine, but is somewhat soluble in aqueous alcohol solutions. It is stable to non-oxidizing acids, but unstable to alkalies. Temperatures

above 50° destroy it in dry form, and above 80° in solution. It is fairly resistant to mild oxidizing agents but is decomposed by formaldehyde and by proteolytic enzymes. It is probably a peptide, since such enzymes and formaldehyde destroy it, since it contains 11 per cent. of nitrogen, and since it has a definite isoelectric point (*p*H 5·4) and possesses both acidic and basic properties.

Progestin (corporin) is slightly soluble in water, more soluble in alkaline solution (which rapidly decomposes it), soluble in acetone, methyl and ethyl alcohol, pyridine, benzine, ether, petroleum ether, and chloroform. It is stable in non-oxidizing acids, and is not destroyed by heating its solutions, in the absence of oxidizing agents. It is easily oxidized. Its alcoholic solution is stable. It seems to be a fat-soluble compound somewhat resembling keto-hydroxy-oestrin, but differing in its instability in alkaline solution.

Much further work is necessary on the corpus luteum. It seems unwise to characterize an endocrine principle by a reaction unique to one species. The close resemblance of "progestin" to oestrin-compounds suggests that it may actually be one of these.[1]

The Presence of the Ovarian Principle in Plants

Using the vaginal smear test, various investigators have claimed to demonstrate the presence of traces of an oestrin-like substance in plants. Extracts of willow catkins are said to be relatively rich in this compound, the stigmata of willow blooms, and the stalks and flowers of the water-lily to contain smaller amounts (65). It has been found that a concentrated extract of keto-hydroxy-oestrin and also Butenandt's crystalline β-compound markedly stimulate the development of plant buds (72). Butenandt (6) has obtained crystalline keto-hydroxy-oestrin from palm kernels and has shown that it is identical with that from mammals.

These results not only suggest that the ovarian principle has a specific *rôle* in the plant kingdom comparable with that in

[1] Hisaw has recently claimed to have separated a third principle, whose specific action is stated to be the mucification of the vagina during oestrus (41). Fevold and Hisaw claim to have obtained "corporin" in crystalline condition (34).

mammals, but also give a partial explanation of various claims in the literature that this principle is present in male urine (although such claims are more probably explicable by use of non-specific tests).

The Endocrine Principle of the Testes

The biological tests employed for the concentration of the endocrine principle have been the prevention of atrophy of the prostate and seminal vesicles in castrated rats and mice, and the production of comb-growth in capons. Several independent groups of investigators have demonstrated effective methods of initial concentration of the principle (43, 48, 58, 38). Frattini and Maino (36) have obtained nitrogen-free crystals, lipoid soluble, which are physiologically very active. Butenandt (6) has also obtained a crystalline product, and has partly determined the constitution of the compound and its relationship to the oestrin-compounds. Koch has summarized the earlier work (42).

McGee, working under Koch's direction, first conclusively demonstrated, in 1927, that an extract can be prepared from testes which causes comb-growth when injected into capons. He found that the benzene-soluble fraction of bull testicles was active in producing comb-growth, and also in preventing atrophy of the seminal vesicles in castrated guinea-pigs. Histological technique for the latter test has been devised, and it has been shown that a properly prepared lipoid fraction from testicular tissue causes regeneration of the comb, wattles, and ear-lobes in the capon, and of the normal histological structure of the seminal vesicle, prostate, Cowper's gland, and vas deferens in the castrated rat.

The active principle is absent from cow's ovaries, bull's pancreas, prostate, thyroid, adrenals, seminal vesicles, liver, and calves' thymus, but is present in the epididymis of bulls. It is present in the testes of the hog, ram, calf, and of the calf foetus, and in traces in bull's blood. The quantity present is of such an order that the equivalent of 50 to 75 grams of bulls' testicular tissue is required per day to

produce comb-growth in a Brown Leghorn capon in five days. The urine of men contains an appreciable quantity of a principle (48, 38) which there is good ground for believing is identical with that from the testes (51).

Gallagher and Koch's method of standardization consists in determining the minimum dose which, given in five daily injections, causes on the sixth day a total increase of 3 to 7 mm. in the length and height of the comb of Brown Leghorn capons. They have defined a " bird unit " as the amount of the principle which yields an average of 5 mm. increase in length plus height of the combs of at least five out of ten birds. The German workers (including Butenandt) determine the increase in total area of the comb by photographing it and making planimeter measurements of the photographic shadows. Their unit is considered to be the amount causing a 15 to 20 per cent. average increase of comb area in three White Leghorn capons. Koch states that at present the two units cannot be related to each other.

The seminal vesicle test, as used by Loewe and Voss, is defined as the minimum amount which, when injected in three doses in thirty-six hours into a four-weeks castrated mouse, restores histological normality in 100 hours after the first injection. Other biological tests, such as spermatozoon motility in the castrated guinea-pig's epididymis, ejaculation etc., have been employed.[1]

Koch's method for preparation from testicular tissue is as follows : The tissue is extracted at room temperature with four volumes of 95 per cent. alcohol, the extract is concentrated to an aqueous emulsion, and this is extracted with

[1] Korenchevsky (43A) uses rats castrated before the thirtieth day of age, and defines the rat unit as the minimum daily dose which, when injected twice a day in half doses during seven consecutive days into at least three litters of rats, will produce on the average an increase of 40 per cent. in the weight of the prostate with seminal vesicles, as compared with the average weight of these organs in uninjected control litter-mates. Rats should be used 30 to 50 days after castration. He states that the rat unit, so defined, is nearly equal to the capon unit as assayed in the Schering laboratories.

benzene. The benzene extract is evaporated *in vacuo*, and the residue dissolved in acetone and cooled for some hours at −10° C. The portion still soluble is evaporated and the residue is dissolved in 70 per cent. alcohol and freed from cholesterol and other inactive material by shaking with hexane. The solution is evaporated to a heavy oil or paste which is dissolved in ether and shaken with 10 per cent. aqueous sodium hydroxide. The ether layer contains the principle and the residue from it has an activity of about 0·1 mg. per bird unit.

Butenandt (6) prepared the crystalline principle from a very active crude oil obtained from male human urine by Funk's method (43). This oil was subjected to hydrolysis and fractionation with organic solvents. An aqueous-alcohol soluble fraction treated with hydroxylamine gave a crystalline product, which was purified by fractional sublimation in high vacuum. The final product melted at 173° to 175° C.; its activity was 0·0005 to 0·001 mg. per bird unit. Its formula is either $C_{19}H_{30}O_2$ or $C_{18}H_{28}O_2$. It is resistant to hydrolysis by acid and alkali, its solubility properties are similar to those of keto-hydroxy-oestrin, and it also possesses a keto-group, but it is neutral instead of weakly acid. Its constitution is discussed in a later section. The amount present in male urine is extremely small. Butenandt (6) estimates that about two million litres contain 1 gram. It is accompanied by several related compounds, which are physiologically inactive.

Names such as *androkynin* and *androtin* have been suggested for this compound.

The Endocrine Activity of the Placenta

The placenta stores or elaborates three different principles: keto-hydroxy-oestrin, emmenin (Collip), and the anterior-pituitary-like principle (Collip).

Keto-hydroxy-oestrin is present in large amounts. When

it is remembered that it is excreted in the urine in large amounts during pregnancy, the question at once arises: Can the follicular tissue of the ovary, the presumed source of its principle, prepare this largely increased output for placental storage and urinary secretion, or does the placenta itself take over this function ?

Emmenin appears to be an isomer or at least closely chemically related to tri-hydroxy-oestrin. One naturally asks, are they both prepared from keto-hydroxy-oestrin, and by what tissue ? If the keto-compound is itself produced in the placenta, it may well also be in part transformed there.

The anterior-pituitary-like (A-P-L) principle behaves very similarly to the gonad-controlling principle (" hebin ") of the anterior pituitary itself. Its possible identity with this, and with Zondek's pituitary principle from urine, requires discussion.

It is evident that there is a possibility, if not a probability, that the placenta is a gland of internal secretion. Hence its histological structure needs some consideration. Frank (35) considers that of the two portions of the (definitely formed) placenta the maternal decidual portion, which develops differently in each species, has no endocrine function ; the foetal portion, the chorion ectoderm, is the active endocrine organ.

Butenandt (5), from the literature and his own experiments, advanced strong evidence that the oestrins from the ovary and the placenta were identical.

Hirose demonstrated in 1920 that intraperitoneal injections of a suspension of placenta produced marked changes in the ovaries of rabbits (the appearance of numerous corpora lutea) accompanied by enlargement of the uterus. The observation was confirmed by Murata and Adachi (63), who found that the effect on the uterus was not produced in spayed rabbits. Such actions of course strongly suggest those of anterior pituitary implants (cf. p. 263).

Wiesner (84) prepared potent extracts of an ovary-

stimulating principle from human placenta by extracting fresh material or its press-juice with sulphosalicylic acid. Collip has extended this work and has obtained active concentrates of two principles (19). His method of separation is shown in the following scheme :

Fresh human placenta + 1½ volumes of acetone.

- Residue, discarded.
- Filtrate + 0·5 c.c. of acetic acid per litre, concentrated at low temperature and pressure until 1 volume equals 1½ volumes of placenta. Ten grams of Lloyd's reagent added per litre.
  - Residue, discarded.
  - Filtrate concentrated at low temperature and pressure until 1 volume equals 10 volumes of placenta. Then 7 volumes of 95 per cent. alcohol added.
    - Precipitate extracted with water. Filtrate concentrated to small volume at low temperature and pressure and 7 volumes alcohol added.
      - Filtrate discarded
      - Precipitate extracted with water : 7 volumes alcohol added. This procedure repeated four or five times. Acidified aqueous solution is then extracted with ether, and the aqueous phase adjusted to *p*H 5·6 and 0·9 per cent. NaCl concentration and sterilized → A-P-L principle.
    - Filtrate concentrated at low temperature and pressure until all alcohol removed and 1 volume equals 15 volumes of placenta, then acidified with 0·5 c.c. of acetic acid per litre, and extracted repeatedly with ether.
      - Ethereal extracts
      - Aqueous phase. Ether distilled off. Contains *emmenin* in form suitable for feeding or injection experiments in animals and for oral administration in clinical work.

The emmenin can be purified further either by repeatedly concentrating to a syrup and extracting with absolute alcohol, or else by an initial saturation with ammonium

sulphate and extraction of the precipitate with absolute alcohol, concentration, and repetition.

The A-P-L principle can be purified further by extracting the final alcohol precipitate with water, saturating the solution with ammonium sulphate, acidification of the dissolved precipitate and subsequent fractionation; saturation of the slightly acid solution by sodium chloride, the watery solution of the precipitate with 5 per cent. potassium acid phthalate, and addition of 3 volumes of acetone to the filtrate. The precipitate thus obtained is finally extracted with 30 per cent. acetone and the acetone removed.

Emmenin produces its physiological effect when administered orally or by injection. The A-P-L principle is apparently of protein nature and is not effective following oral administration.

Physiological Actions of Emmenin and the A-P-L Principle. Oral administration of an active extract of emmenin to immature rats nineteen to twenty-one days of age for three days produces oestrus in from three to five days. Collip terms the minimum amount which consistently produces this effect the *oral day unit.* The ovaries are not definitely affected by the treatment. Continued daily administration of 1 to 3 units produces the vaginal smears of oestrus at slightly irregular intervals. Continuous daily administration of 10 units results usually in a continuous oestrus vaginal reaction until the normal age of puberty, when normal cycles are established.

The cycles of normal adult female rats are not affected by the continuous daily administration of ten or more units. No interference with the normal processes of pregnancy or lactation has been observed. In no case has the production of corpora lutea nor true hypertrophy of the ovaries been seen.

There is no action on adult castrates unless very large doses are given, when the effect is possibly attributable

to traces of oestrin, or to conversion of emmenin into oestrin.

The time of appearance of normal puberty and maturity is not shortened.

It will be seen that the action differs from keto-hydroxy-oestrin in two ways : lack of effect on the adult castrate, and

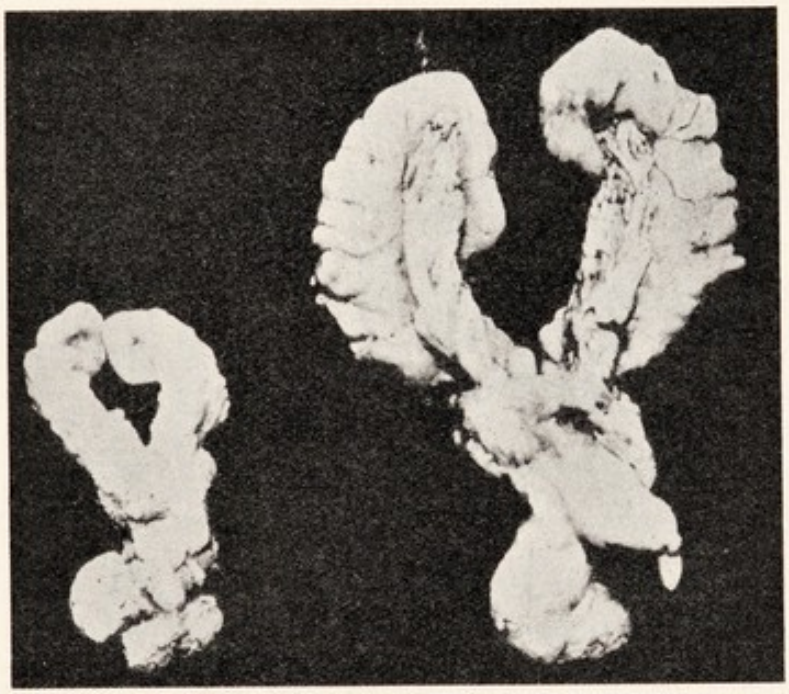


FIG. 51.—Seminal vesicles and prostate of control (left) and experimental adult rat (right) after injection of the anterior - pituitary - like principle (the equivalent of 15 grams of placenta) administered daily except Sundays for forty-two days. (From Collip *et al.*, *Can. Med. Assoc. J.*, 1931, xxiv, 201.)

exhibition of effect on the immature animal by oral route. Differentiation from tri-hydroxy-oestrin is less clear-cut.

When immature rats nineteen to twenty-one days of age are *injected* subcutaneously daily for three days with the A-P-L principle in sufficient dosage, oestrus is manifested on the third to fifth day. The minimum amount which consistently produces this effect is termed the *day unit* by Collip.

Dosage just sufficient to induce the positive vaginal smear of oestrus has but little effect on the ovaries. When the

dosage is doubled or trebled, the ovaries on the fifth or sixth day correspond very closely in appearance with those of unmated animals shortly after the appearance of spontaneous maturity. Normal young corpora lutea are present and also healthy maturating follicles. Increased and prolonged dosage rapidly results in ovaries similar to those of the normal adult, but normal adult size is not exceeded. A succession of normal four- or five-day cycles is produced.

Adult females given daily injections of from 5 to 20 units are practically unaffected. They mate successfully and rear normal litters.

Continued daily injections into normal adult male rats lead uniformly to marked enlargement of the accessory genital tract structures, especially the seminal vesicles and prostate gland. These results are illustrated in Fig. 51. Similar effects are produced in immature males. The weight of the testes is not much affected. Collip concludes that the A-P-L principle stimulates the testes to work rather than to grow.

Combined Effect of Emmenin and the A-P-L Principle. When 10-unit doses are given young female rats orally every day, and combined with simultaneous daily injections of the A-P-L principle, the continuous oestrus vaginal reaction is broken, and a prolonged period of dioestrus results. After three weeks of such treatment the ovaries of these animals are greatly enlarged and extensively luteinized, the effect being exactly similar to that produced by repeated implantation of anterior pituitary tissue (cf. Fig. 34). Such an effect is never produced when either principle is given alone.

Further Work on the Placental Principles. Collip, using his method for separating the principles from placenta, has prepared from the urine of pregnancy two concentrates which appear to correspond with emmenin and the A-P-L principle. From the " emmenin " fraction he has obtained crystals which seem identical with those of tri-hydroxy-oestrin, while, judged by their physiological behaviour, they

are crystals of emmenin itself, and not of Marrian's compound (16).

Browne, working in Collip's laboratory, prepared from the *ether-soluble* fraction of placental extracts (cf. the scheme on p. 311) a crystalline product which is also chemically identical with Marrian's compound, but shows the same marked difference in effective dosage for the immature normal and the adult castrated female rat as does emmenin (16, 4c). It has also been shown that the ether-insoluble (emmenin) fraction can be rendered soluble in ether by autoclaving it in 1 per cent. acetic acid solution at 150 lb. pressure for two hours (18).[1]

The potency of such crystalline preparations when tested on the immature rat is equal (by direct comparison) to that of Doisy's " theelol " crystals. Tested on the adult castrate it is only 60,000 day units per gram.

It is now desirable to consider Zondek's preparations from the urine of pregnancy. In point of time his work was prior to that of Collip.

The Presumed Pituitary Principle from Urine

Zondek and Aschheim's earlier work (from 1925) showed that the effect of anterior pituitary implants on ovarian development was produced by glands from many species, including man, and from both sexes, and all ages, and was not produced by implants of other tissues. They put forward the theory that the anterior pituitary secretes two gonad-stimulating principles, one controlling and stimulating the ripening of the follicles and oestrus, and the other, the

[1] Collip and Thomson (personal communication) suggest that the term " emmenin " be restricted to that ester of tri-hydroxy-oestrin in placenta which is insoluble in ether. Butenandt and Brown (10A) have confirmed the identity of " theelol " (Doisy's preparation) and Marrian's compound, and consider that the crystalline derivative from " emmenin " is almost certainly also this compound. They stress the importance of such biological comparisons being carried out in one, and not in different laboratories.

"luteinizing principle," stimulating the change of follicles into corpora lutea. These they termed *prolan A* and *prolan B* (86). In 1927 they gave up attempts to prepare prolan compounds from the pituitary, believing that the urine of pregnant women was a much better source, since such urine gave some thousand mouse units per litre, while a cow's pituitary only yielded 100 units. Alcohol precipitated both prolan compounds from urine. They used the following procedure :

One litre of urine was acidified (if necessary) with acetic acid to weak litmus reaction, and filtered. Four litres of 96 per cent. alcohol were added, the whole shaken, and allowed to stand twenty-four hours. The precipitate (containing prolan) was centrifuged off and extracted with ether. The ether extract was discarded, and the precipitate shaken with water and centrifuged. Prolan dissolved ; the residue was discarded. It was further purified by renewed alcoholic precipitation, treatment with ether, and solution in water. Finally, after removal of water, a fine white-yellow powder was obtained, which dissolved easily in water to a yellow solution.

The same treatment was applied to the urine of non-pregnant castrates, and castrated animals, and it is claimed that only prolan *A* was obtained. It is evident that both *A* and *B* have very similar properties. Zondek considers that the compounds from urine are identical with those from the pituitary, since they produce the same effects on the genital apparatus of infantile animals as do pituitary implants, and appear to have similar physical and chemical properties—reaction to solvents, heat, acid, and alkali.

Lejwa (46) has obtained a crystalline preparation of prolan of such activity that 0·001 mg. suffices to cause maturation and luteinization of the ovaries of an infantile mouse. Zondek (88) has obtained preparations of similar potency which occasionally exhibited crystalline appearances.

Wiesner and Crew (85) also hold the view that the pitui-

tary produces two gonad-stimulating principles, which they term *rho* 1 and *rho* 2, and which they also prepare from urine.

The Present Position concerning the Pituitary-Gonad Principles

There is no conclusive evidence that there are two separate " prolans " (or " rhos "). Many of the statements in the literature are confusing, such as the different effects on the genital system following injection of pituitary extracts and of pituitary implants (cf. pp. 263, 267), the first, according to Evans, leading to hyperluteinization but no ovulation. Yet all such discrepancies may equally well be attributed to treatment of extracts before injection, or, as Van Dyke believes, to difference in dosage : " The minimal effective doses cause relatively more follicular maturation than luteinization " (81), or, following Collip's ideas, to the combined effects of the pituitary and ovarian principles. Until much more definite experimental evidence is available only one prolan (one rho) need be considered (cf. also Collip (17)).

What is the relationship between *prolan* or *rho* from urine, the *anterior-pituitary-like principle* from placenta, and *hebin* from the anterior pituitary basophile cells ? It is very doubtful if they are all three identical.

The anterior pituitary compound (or those resembling it) is absent from the blood and urine of all pregnant animals so far tested below the higher apes (except the mare). As Collip points out (17), it is difficult to believe that that found in the blood, placenta, and urine of pregnant women is furnished by the anterior pituitary body only in the higher primates. Varied evidence has been put forward that the material of urinary origin is not the same as the pituitary secretion. Thus Van Dyke says (81) : " Fairly pure extracts containing urinary hebin stimulate the testes just as effec-

tually as the ovaries. Preparations of pituitary hebin, however, stimulate the testes only in not less than ten times the dose which stimulates the ovaries of immature rats." Evans finds that implants stimulate a greater number of follicles than does the extract (33). Collip has discussed the subject fully (17).

The curious difference in the results of the A-P-L principle alone, and combined with emmenin, in stimulating the ovaries, has been referred to (cf. p. 314).

If one ventures to hazard an opinion until more work has been done and these various principles have been obtained in state of greater purity for comparison, it is that the placental and urinary principles are identical, and produced in the placenta, and differ slightly chemically from the principle elaborated by the basophile cells of the pituitary.[1]

The apparent confusion now existing concerning the oestrin compounds is probably preliminary to a complete clarification, which may have been made before this matter appears in print. At the present time one can only put forward a tentative scheme which seems to fit most of the facts. The parent oestrin-compound, keto-hydroxy-oestrin, is closely related to cholic acid and cholesterol (see next section) and to the male-principle. It is prepared in the ovary and controls the secondary sex organs and characters apparently by specifically stimulating their growth. It is prepared in excessive amount during pregnancy—in such amount that it seems almost certain that it is in great part secreted by placental tissue.[2] This excessive production

[1] Accumulating evidence strengthens the view that the A-P-L principle differs from the corresponding pituitary compound. Evans inclines to the view that the pituitary growth principle is activated to the gonad-stimulator and that prolan may be the activator (33). Collip considers that, although the A-P-L principle induces luteinization in very young rats, nevertheless a pituitary factor (probably *sui generis*) is necessary for follicular maturation and corpus luteum formation (19A, 20). The relationship between the two compounds concerned is as yet in no way clear.

[2] v. Probstner (70) has published the report of a case which demonstrates that placenta actually secretes its endocrine principles. At the

suggests a function specific to that period and calling for such an excess. Inhibition of the secretion of the basophile cells of the anterior pituitary seems probable, especially if slightly different function be admitted for the principle secreted by these cells and for the A-P-L principle.

The excessive production leads to excessive excretion. Whether, in order to aid secretion, or for some specific function, keto-hydroxy-oestrin is converted, in the placental tissue or elsewhere, we do not know, but a large proportion is so converted into the tri-hydroxy-oestrin of Marrian (Doisy's theelol), and into emmenin (tri-hydroxy-oestrin in ester combination), and probably also in smaller amounts into other oestrin variants.

The Chemical Interrelationships of the Sex Principles

The following are closely related :

(i.) Keto-hydroxy-oestrin (theelin),
(ii.) Tri-hydroxy-oestrin (theelol),
(iii.) The male principle of the testes,
(iv.) Emmenin (a tri-hydroxy-oestrin), and
(v.) The plant sex principle.[1]

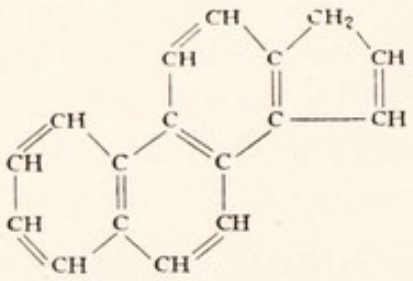
commencement of pregnancy in a woman, aged thirty, bilateral ovarian cysts were diagnosed, and at operation dermoid cysts were removed ; no trace of ovarian tissue was found. The subsequent history of the pregnancy was normal, a normal child was born and lactation was normal. The urine throughout the pregnancy contained Zondek's pituitary principle, and also keto-hydroxy-oestrin, although following the operation there was a lag in the usual marked increase of the latter. It was also found in normal amount in the blood at the end of the seventh month and at term. The placenta contained a normal amount of it. v. Probstner mentions two somewhat similar cases in the literature. Any conclusion other than that the ovarian principle during such unusual pregnancies is produced in the placenta would infer gross error of observation concerning the lack of ovarian tissue.

[1] Keto-hydroxy-oestrin occurs in two or more isomeric forms (cf. p. 302). According to Girard, urine of the pregnant mare contains, along with keto-hydroxy-oestrin, traces of the closely related " equilin " and " hippalin," both $C_{18}H_{20}O_2$. To this series should probably also be added Marrian's " equol," $C_{15}H_{12}O(OH)_2$, from mare's urine (55).

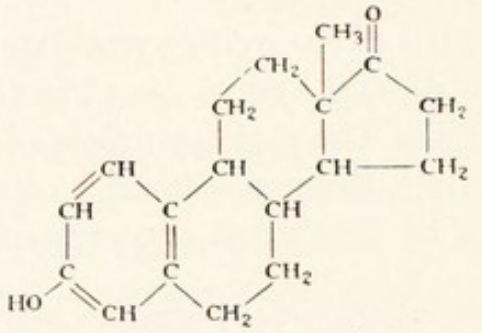
It is not unlikely that progestin (corporin) of the corpus luteum, if it has a separate existence, is also one of this chemical group.

While the elucidation of their relationships is not yet complete, Butenandt, to whom much of the recent advance in their study has been due, has put forward a scheme of interrelationship which seems to agree well with present knowledge (6, 8) (Cf. also (89).)

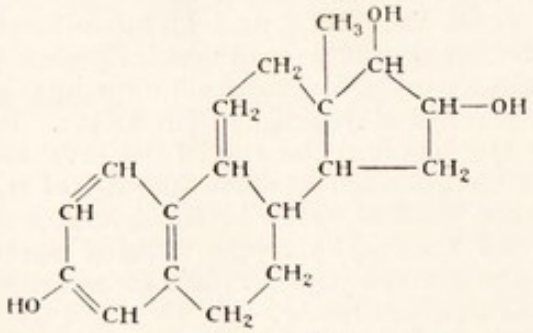
Keto-hydroxy-oestrin (theelin) $C_{18}H_{22}O_2$, tri-hydroxy-oestrin (theelol), $C_{18}H_{24}O_3$, and the testicular principle, which is probably $C_{19}H_{30}O_2$, are all methyl derivatives of a four-ringed hydrocarbon, $C_{17}H_{12}$, as is also pregnandiol,[1] $C_{21}H_{36}O_2$, and are closely related to cholic acid, if Rosenheim and King's structure for it, as based on Bernal's X-ray studies, and modified by Wieland and Windaus, be accepted. These relationships are exhibited in the following formulae :



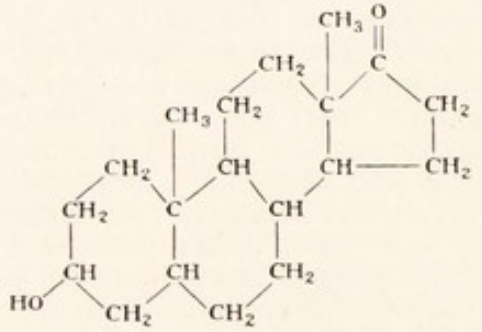
PARENT HYDROCARBON, $C_{17}H_{12}$



KETOHYDROXY-OESTRIN, (THEELIN), $C_{18}H_{22}O_2$

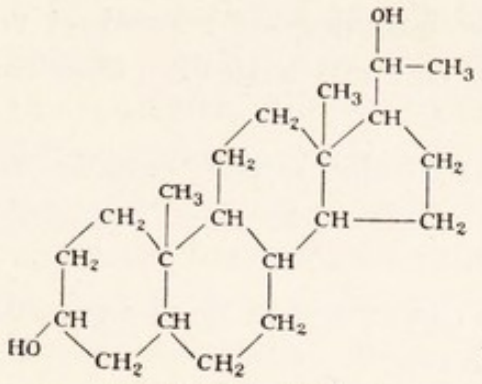


TRIHYDROXY-OESTRIN (THEELOL), $C_{18}H_{24}O_3$

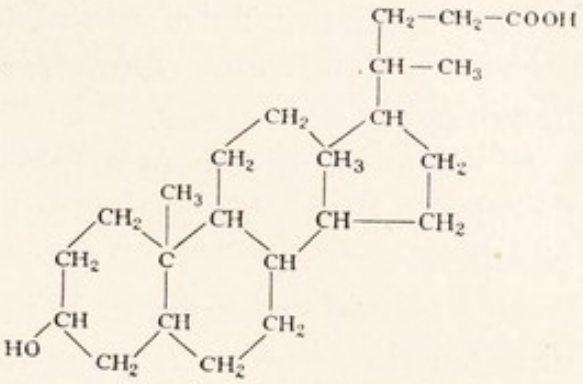


TESTICULAR PRINCIPLE, $C_{19}H_{30}O_2$

[1] Cf. the scheme on p. 293. Pregnandiol was also isolated by Marrian (53).

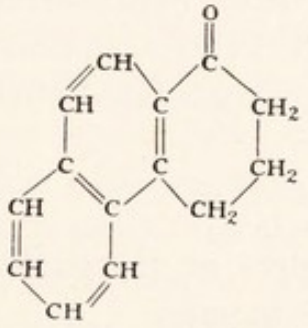


PREGNANDIOL, $C_{21}H_{36}O_2$

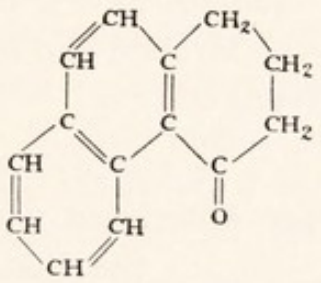


A CHOLIC ACID, $C_{23}H_{37}O_3$

(In this connection cf. also Marrian and Hazlewood (56).) Such a theory concerning the structure of these compounds is strongly supported by the recent observation of Cook, Dodds and Hewitt (21) that 1-keto : 1 : 2 : 3 : 4 :-tetrahydrophenanthrene is weakly but definitely oestrus-producing in the ovariectomized rat. The corresponding compound 4-keto : 1 : 2 : 3 : 4 :-tetrahydrophenanthrene is inactive.



1-KETO DERIVATIVE OF 1:2:3:4-TETRAHYDROPHENANTHRENE



4-KETO COMPOUND

Intersexuality

The earlier work of Steinach and others, suggesting that there was an antagonistic action between the gonad principles of the two sexes led to much research which became intensified when concentrated extracts of these principles became available. The results that have been obtained do not accord with the theory of such antagonism. Moore (62) considers that four basic laws govern these interrelationships.

(i.) Gonad principles stimulate homologous reproductive accessory glands and characters, but are without effect on heterologous accessories.

(ii.) The pituitary principle stimulates the gonads to function, both in germ cell and endocrine principle production.

(iii.) Gonad principles have no direct action on the gonads of either the same or the opposite sex.

(iv.) Gonad principles of either sex exert a depressing effect on the pituitary which results in a diminished amount of the sex-stimulating principle being available to the organism.

These assumptions explain most of the observed facts, such as the injurious effect of injections of keto-hydroxy-oestrin on the male reproductive system, and the proved diminution of the gonad-stimulating principle in the pituitaries of female animals following injections of keto-hydroxy-oestrin. They scarcely give an easy explanation of such apparent changes in sex characteristics as accompany, for example, the virilism and hirsutism associated with tumours of the adrenal cortex.

Diseases Associated with Hyper- or Hypoactivity of the Gonadal Principles

The possible effects of hyper- and hyposecretion of these principles are perhaps deducible from their normal functions.

The ovarian principle controls the normal development of the secondary sex organs and secondary sex characteristics in the female. It is believed that the principles of the corpus luteum control certain uterine changes leading to implantation of the ovum, prevent further ovulation, and maintain pregnancy, and have, in addition, some control over the development of the mammary glands. The testicular principle controls the normal development of the secondary sex characters of the male.

It is doubtful whether hypersecretion of keto-hydroxy-oestrin produces any unusual effect in adult women, since the condition uniformly accompanies pregnancy ; the results following such excess are probably limited to depression of the action of the basophile pituitary cells (cf. p. 319). Hypo-secretion should lead to changes comparable in kind but less in degree than those following castration and occurring at the climacterium. Such changes should include disturbances of menstrual function, and finally amenorrhœa, and atrophy of the uterus and vagina. If the functional significance of the corpus luteum is accurately understood, an over-production of its secretion should lead to prolonged inhibition of ovulation, and an undersecretion to abortion, especially at the earlier stages of gestation. From oversecretion of the testicular principle we might expect a " hypermasculinization," and from undersecretion persistence of infantile sex characters and some degree of obesity (resembling that following castration).

Evaluation of the relationship between endocrine function of the gonads and disease in human beings is less easy than for disease associated with other endocrine glands, since comparison with animals lower in the scale than primates may lead to error. In the female, length of cycle, and some of the cyclical manifestations are different. In the lower animals all the important events of the cycle—ovulation, mating, greatest growth of the genital organs—occur at the height of oestrus. In primates menstruation follows the end of the period of greatest growth of the secondary organs, but ovulation occurs (usually) about midway during the intermenstruum, and mating is not confined to a specific time. Even as between man and monkeys the menstrual cycle exhibits differences, whilst " probably the greatest obstacle to satisfactory comparison of reaction to similar stimuli is the psyche which holds minimum importance in lower animals, but maximal in man " (68).

Clinical evidence is open to some error, since the subjective

symptoms described by the patient are frequently inaccurate, through inexperience, inaccurate observation, and sometimes even intentional suppression of fact. Regularity of menstrual flow exists much more rarely than patients state, yet the most frequently useful symptom is the rhythm and amount of menstrual flow (68). The changes in the vaginal epithelium during the cycle, so useful and so definitely related to ovarian function in the lower mammals, have not yet been definitely determined in woman, although there is some evidence that changes do occur, and may be utilizable (24). Objective criteria which can be employed are the condition of the secondary sex organs and sex characteristics.

While prepuberal castrates are rare among women, such evidence as is available is in agreement with that following experimental castration in young animals—arrest of sexual development, and even some degree of regression. Surgical removal of the ovaries during the reproductive period through cancerous or other lesions leads to gradual regression involving all the other sex organs and sex characteristics. Certain subjective changes are prominent—nervousness, hot flashes, irritability, and fatigue. The earlier this artificial menopause is produced in the reproductive period, the severer may be the resulting symptoms. At the natural menopause the same changes occur, more gradually, and at least 50 per cent. of women exhibit the same subjective symptoms (68).

The rarely-occurring ovarian tumours in female children are accompanied by precocious sexual development, as evidenced by menstruation. Removal of the tumours tends to a return of the normal infantile condition. Hyper-secretion of keto-hydroxy-oestrin from a tumour of endocrine tissue (usually, in these ovarian cases, described as a sarcoma) seems the evident explanation, since the results correspond with those following injections of keto-hydroxy-oestrin into immature rats and mice. An illustrative case was published recently by Southam (75).

The objective indications of normal or abnormal secretory activity of the human testes are the condition of the accessory sex organs (prostate, seminal vesicles, vasa deferentia, Cowper's glands), and the sex characters (body habitus, distribution of hair, and pitch of voice). The subjective symptoms, interest in the opposite sex and potency, are particularly open to criticism, especially in considering the possibility of hyperfunction, so that, for example, Pratt (68) and Rowe (71) believe that such hyperfunction does not occur. The effect of the psyche can be a pre-eminent source of error in uncritical examination.

Pratt considers that conditions such as precocious puberty and increased sex urge can be explained better on other grounds than hyperfunction of the testicular endocrine principle. Adrenal tumours, for example, through increased production of the cortical principle, may stimulate *premature* endocrine activity of the testes, but not an overactivity.

The effect of castration in the male, as in the female, leads to persistence of infantile characteristics or some degree of regression, according to the age of castration (cf. p. 283). A recent study by McCartney (49) of twenty Chinese eunuchs and three Skopecs illustrates the mental tendency of such castrates. He found in them typical dementia praecox or schizoid characters. They exhibited good intelligence and orientation, but were introspective and apathetic. They could talk intelligently but appeared stupid, were methodical, but usually not purposeful, and were cold, passive, and moody. Some retained sexual function, but without libido. (McCartney has found that a large proportion of schizophrenic patients have abnormal gonadal endocrine function.)

Rowe's studies (71) are in agreement. "The male castrate is the victim of a profound mental depression. In his mutilation he sees the loss of all the virile qualities that made him male, and in this loss resides an unhappiness that

tinges all the events of life with a sombre hue." He has quoted some cases exhibiting a striking psychological effect following demonstration of a partial masculinity; these illustrate the fact that, at least in adult man, successful coitus is more largely due to psychic than to endocrine control.

Treatment. Marrian and Parkes (57) have calculated that if 200 mouse units of the ovarian principle are required to produce complete oestrus in the mouse, 400,000 units would be necessary to produce the corresponding changes in woman. It would seem obvious, even if these figures are widely incorrect, that very large doses are essential for effectual therapeutic treatment. Numerous claims have been made that such treatment is effectual. Those claimed for desiccated products used before Allen and Doisy's work can be neglected (35).

Menstruation has been induced in a woman who had been castrated two years previously (87) and in castrated monkeys (69). Good results have been obtained in secondary amenorrhoea (68, 67, 27, 50) and, less certainly, in primary amenorrhoea. In all such cases the principle was injected. Pratt at present draws very conservative conclusions (68): "The subjective symptoms of the menopause as well as dysmenorrhoea have frequently been reported to have been relieved by ovarian hormone. This action as a psychotherapeutic agent need not be discouraged, but the manner of action should be admitted. A considerable number of other conditions, such as eczema, mental depression, etc., have been reported as improved by ovarian hormone therapy, but the results are inconclusive. There is no conclusive evidence that theelin does harm."

However, this conservative attitude is perhaps needlessly gloomy. A much more promising picture is given by a recent report of Werner and Collier (82). They have studied the results following prolonged injections of "theelin" into four women with complete bilateral

ovariectomies but intact uteri and a fifth whose uterus had also been removed. These all exhibited complete amenorrhoea, and atrophy of the breasts and had had no noticeable vaginal mucous discharge since operation. "All complained bitterly of the symptoms that accompany ovarian hypofunction." The injections leasted 89 to 93 days in the different cases, commencing with 4 c.c. (200 rat units) daily for twenty-eight days, then 6 c.c. for twenty-eight days, and finally 8 c.c. (400 units). There was no discomfort, except with the largest dose. The results were accurately controlled histologically by curettage.

It was found that theelin restored the breasts and genital tract to, apparently, the normal sexual state, and produced changes in the atrophied endometrium that approximated or equalled those occurring in normal women at the time of ovulation. The bleeding of the uterus, which occurred in all of the first four cases, was from an endometrium of similar development to that in normal menstruation, and such bleeding was qualitatively indistinguishable from that of normal menstruation, and was accompanied by the usual subjective symptoms. The subjective symptoms following castration were relieved. Mazer and Ziserman (59) have found somewhat similar dosage is effective.

Suggestions that, on account of its method of transmission, haemophilia might respond to treatment with the ovarian principle, have been tested by Birch, who has claimed excellent results in nine cases and good results in a number of others (68, 64B).

Schroeder, and also Novak (64), claim to be able to control severe functional bleeding by administration of Zondek's pituitary principle obtained from urine. It is not at all unlikely that these actions are really due to the A-P-L principle of Collip (cf. p. 312).

Campbell and Collip (15, 14) have described beneficial effects from administration of the placental principles, which, while they still lack confirmation by independent

observers, merit careful attention. The following table summarizes their published results with emmenin:

Condition.	No. of Cases Treated.	No. Improved.	Comment.
Primary amenorrhoea .	8	(1)	No effect.
Secondary amenorrhoea :			
Oligomenorrhoea .	20	18	Individuals with a diminished or scanty flow, and with periods at irregular intervals of 5 to 8 weeks.
Oligomenorrhoea (with lapses).	14	12	As above, but having intervals of amenorrhoea of duration of 4 months or more.
Regular (with lapses).	19	11	Normal cycles, but periods of amenorrhoea of upwards of 4 months' duration. (No results were obtained when the amenorrhoea had lasted more than 2½ years. The apparent response, when obtained, occurred after an average period of 23 days.)
Dysmenorrhoea . .	36	26	—
Polymenorrhoea . .	8	7	Intervals of 18 to 24 days. In the 7 cases which benefited the intermenstrual period was increased by from 4 to 8 days. There was a favourable effect on intermenstrual pain.
Menopausal symptoms.	18	14	No relief was obtained in cases of over a year's duration.
Menorrhagia . .	—	—	No effect.
Metrorrhagia . .	—	—	No effect.

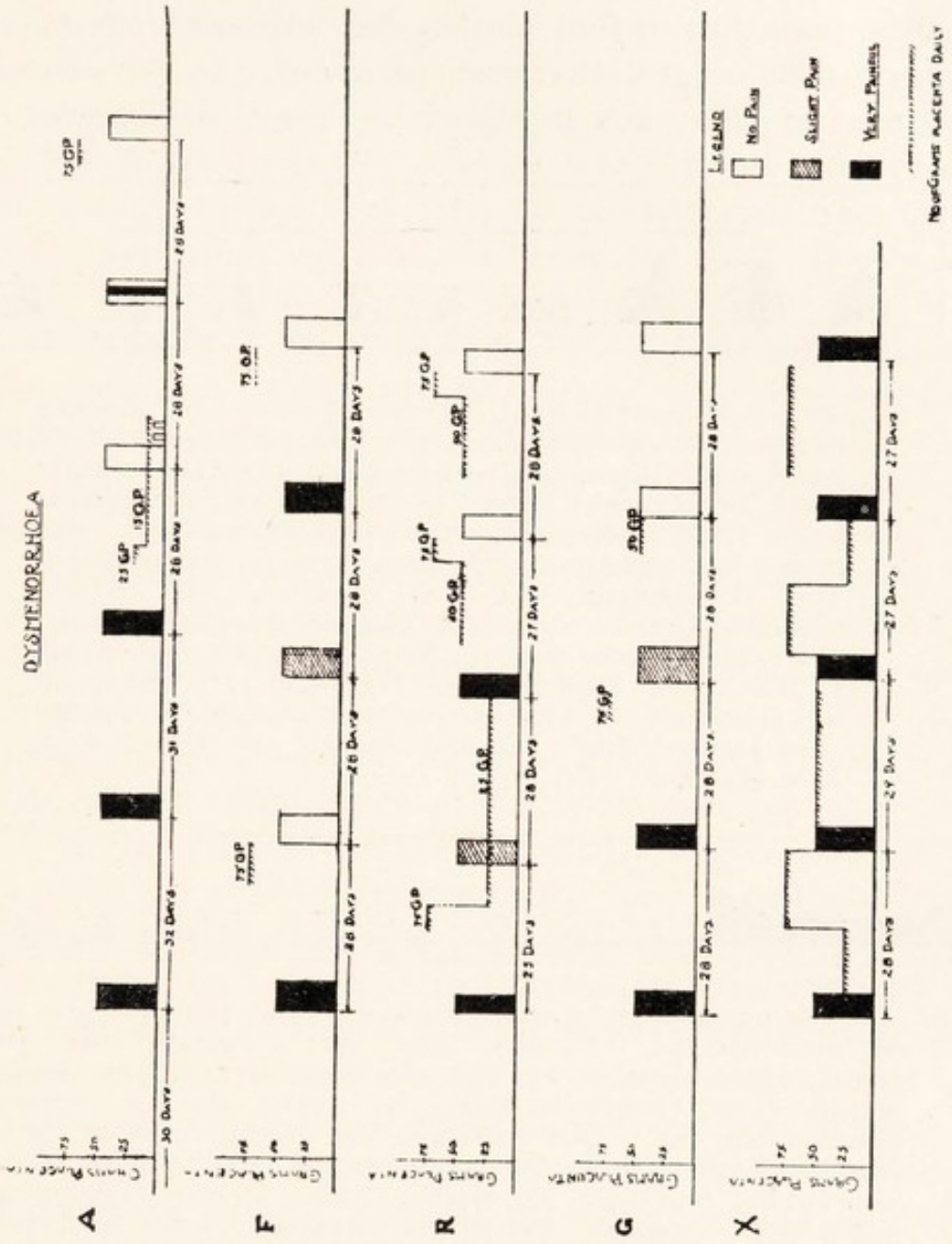


FIG. 52.—Five characteristic cases of dysmenorrhoea treated with emmenin. In case A there is a decreasing intermenstrual period, and in case R a slight lengthening of interval and period, the cycle being established in both cases in twenty-eight days. Case G stopped her extract two days before the period on one occasion and had only partial relief. With proper administration complete relief was obtained. Case X received no benefit. (From Campbell and Collip, *Can. Med. Assoc. J.*, 1930, xxiii, 633.)

The dosage in such cases was usually equivalent to 75 grams of placenta daily, and was given in divided doses in water or

orange juice just before meals. No untoward effects on impregnation or gestation were produced. In ten normal controls no effect was produced on length of interval or

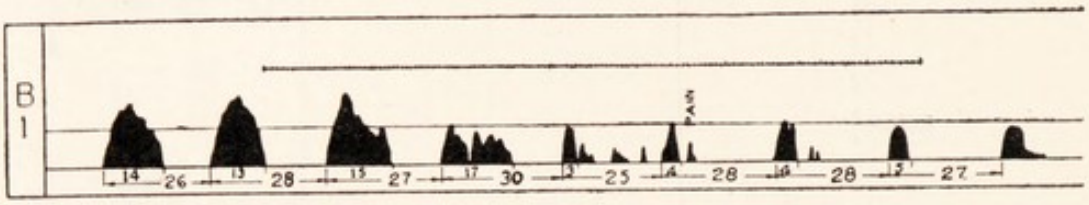


FIG. 53.—Administration of anterior-pituitary-like principle in a case of menorrhagia. A woman aged 35, married six years, no children. Menstruation began at 14; regular every twenty-eight days. In March, 1930, the periods began to be prolonged to thirteen to fifteen days. She complained of feeling giddy from excessive loss of blood. The Wassermann test was negative. All systems normal. Uterus normal in position, shape and size; other pelvic viscera normal. Treated from November 18th to 28th, 1930. Recommenced December 17th, 1930, and has continued. Period of treatment in figure shown by cross-hatched line. (From Campbell and Collip, *Can. Med. Assoc. J.*, 1931, xxv, 9.)

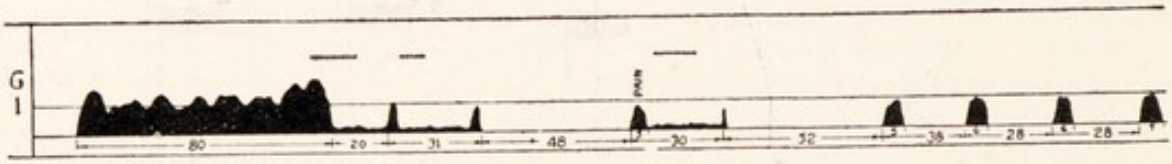


FIG. 54.—Administration of anterior-pituitary-like principle in a case of metrorrhagia. Woman aged 36; married one year. Menstruation began at 17, and was irregular and very painful; periods every twenty to thirty-five days; amount excessive, duration one day. Two years ago had uterine bleeding for one month. Took ergot and strychnine, and was well for a time afterwards. Suddenly in March, 1930, uterine haemorrhage began and continued. Pelvic examination; vagina and cervix normal. Uterus long, movable; appendages negative. Treatment (shown by cross-hatched lines) began May, 1930. (From Campbell and Collip, *Can. Med. Assoc. J.*, 1931, xxv, 9.)

duration of epoch. The dosage given in dysmenorrhoea was equivalent to 25 grams of placenta daily for seventeen days, beginning with the cessation of period, then raised to the equivalent of 75 grams until onset, and then stopped.

Some of the results following oral use of emmenin are shown in Fig. 52. Campbell has reported that fifteen

childless patients became pregnant when disturbed ovarian function was corrected with emmenin (14).

In menorrhagia of the simpler type treatment by injections of the A-P-L principle for one week before the epoch materially reduces the flow. Menorrhagia of the more severe type may require treatment for three months or longer before normal periods are re-established. A typical good result is shown in Fig. 53. In cases of metrorrhagia the continuous uterine bleeding has been in some measure controlled, but a tendency to establishment of normal cycles has not been observed. Intervals of amenorrhoea may appear. Successful treatment is illustrated in Fig. 54.

The logical use of the testicular principle would seem to be for replacement therapy in senility. This assumes that the active function of the principle continues throughout adult life and slowly decreases. Such an assumption is accepted neither by Moore nor by Rowe. The former states (61): "To my knowledge there is no single criterion or set of criteria that clearly indicates in man a hypogonadal state of the testicle. When objective means have been found that bridge this difficulty, the way will be opened for sane procedures. It cannot be too strongly emphasized that in this field of investigation subjective indices are misleading and independable." Rowe writes (71): "The testicle . . . gives but scant evidence of any endocrine activity in adult years."

The means that have to greatest extent been introduced to overcome senility, particularly sexual senility in man, are open to grave criticism. These means are testicular implants, and ligation of the vas deferens. It is well established that the effects from single pituitary implants into animals are negligibly small. Continuous daily implants are necessary to produce both growth and gonadal effects. By analogy, testicular implants may well be expected to have but a short period of functional life and activity. Voronoff has adduced some evidence to the contrary (80), yet Pratt's summing up of work such as Voronoff's is at least very sound

corrective criticism : " It would seem, therefore, that all the effects claimed to have been produced by transplantation could be explained as a re-eroterization, which can be equally well accomplished by other and simpler forms of psychotherapy." [1] Steinach's operation, ligation of the vas deferens, a procedure which has been supposed to increase the production of the testicular endocrine principle, " is to be considered with the same degree of pessimism as testicular transplantation for the same reasons. Up to the present time there has been no indication that such an operation in any way modifies the rate of hormone secretion, or that it is advantageous in any other respect aside from a sterilizing operation " (68).

It is possible to over-emphasize the psychic influence and to lay too great stress upon the sexual urge, rather than the effect of the testicular principle upon the organism as a whole. Experiments with animals have given definite results, and man cannot be entirely placed in a class apart and excluded from those results.

The preparation of definitely highly concentrated extracts of the testicular principle permits more precise and scientific treatment to be adopted, although claims for benefit following such treatment must still be open to severe criticism unless definite objective improvement as well as indefinite subjective improvement can be demonstrated.

The minute amount of the principle present in normal male human urine, and the huge amounts of such urine which must be worked up to provide quantities of the principle sufficiently large for therapeutic treatment, will limit progress in this field until the principle has been synthetized. However, such synthesis is only a matter of time, and probably of a short time.

[1] Thorek (78) has summed up the literature concerning *ovarian* transplants, and makes the more modest claim that they improve waning physical and psychic conditions and retard onset of symptoms of senility. No claim for rejuvenation is made. The transplants are said to persist for several years.

One or two reports have appeared in the literature, concerning the results following injections of potent extracts. Benjamin (4) has claimed good results, based mainly on subjective improvement. McCullagh (52) has determined the amount of testicular principle in the blood and urine of normal men, by preparing concentrated extracts and finding the minimal amount capable of producing the comb-growth effect in capons. In this way he has differentiated a group of male patients who really seem to exhibit a measurable hypogonadism (cf. p. 331), and has treated them with large doses of the principle : " Some degree of improvement has been noted in all cases in which there were suggestive signs and symptoms together with a measurable deficiency of the hormone." In other cases, in which no gonadal deficiency was demonstrable by the bird test, no benefit resulted from such treatment. The results seem to have been as reasonably well controlled as present methods permit, and are promising.

The Zondek and Aschheim Test for Pregnancy

Aschheim and Zondek suggested in 1928 a technique for determining pregnancy in women, based upon a sound principle, the detection of their " pituitary hormone " in the urine of such women. It has been pointed out that during pregnancy the amount present is tremendously increased. It is immaterial, as far as this test is concerned, whether this protein-compound originates in the anterior pituitary, or in the placenta.

Their method has been subjected by them to various modifications. As at present employed by them, the technique is as follows (86) : The urine sample, a morning specimen, preserved if necessary by addition of tricresol in ratio of 1 drop to 25 or 30 c.c. of urine, is filtered, and then 30 to 40 c.c. are extracted with three times the volume of ether, and the phases separated in an extraction funnel.

The ether is allowed to evaporate off from the aqueous phase by exposing it to an air current for one hour. Toxic compounds, and tri-hydroxy-oestrin are removed by the ether. The urine is injected in six 0.3 c.c. doses into five mice, three to four weeks old, and not less than 6 nor more than 8 grams in weight, over a forty-eight-hour period. The animals are killed 100 hours after the first injection. Positive results (which must be exhibited by at least two animals) are " blood-points " in the ovaries, presence of one or more corpora lutea, and a uterus filled with fluid.

Various modifications have been suggested, including that by Friedman and Lapham (37) in which rabbits are used as test animals. White and Severance (83) have compared these various procedures with each other, and with certain other tests supposed to be diagnostic of pregnancy, and consider that Friedman's procedure is most satisfactory (cf. also (66) and (4A)).

Summarizing the results obtained with his procedure to 1931 (86) Zondek claims an error of only 1 to 2 per cent., in 5,515 tests by thirty different groups of observers.

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CHAPTER VIII

SOME ACTUAL AND PRESUMPTIVE ENDOCRINE PRINCIPLES

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Introduction

THE literature contains a large number of suggestions that certain phenomena indicate the existence of new endocrine principles. There is no appearance of any falling off in the number of such suggestions through the development of more critical tendencies.

Of all the principles dealt with in this chapter only the existence of secretin can be considered as definitely established. The others will therefore be very briefly dealt with, although a few of them almost certainly have a real existence.

Actual and Presumptive Principles of the Gastrointestinal Tract

A Possible Endocrine Principle of the Submaxillary Gland. Babkin (6) records the fact that after administration of a small dose of physostigmine to a cat, stimulation of the chorda tympani on one side activated or increased the secretion and accelerated the blood flow in the denervated submaxillary gland on the other side, while at the same time the systemic blood pressure was lowered. Reflex influences, including an increased output of adrenine, and the actual lowering of the blood pressure, were excluded as causative factors. Atropine abolished the effect.

The whole phenomenon was abolished or greatly diminished by clamping the vein of the submaxillary gland, while the acceleration

of the secretion of the control gland was always delayed in comparison with that of the stimulated gland. Babkin suggests that a special chemical substance is formed in the submaxillary gland, is preserved from inactivation by physostigmine, and is transferred in the blood stream, causing secretion and vasodilation in the opposite gland, and a fall in the systemic blood pressure.

Somewhat similar conclusions were drawn by von Beznák, who used the method of cross-circulation in two dogs. Stimulation of the chorda tympani in one dog activated the secretion in the other (7).

Babkin summarizes the literature concerning the humoral transmission of nerve stimulation from one organ to another in mammals, and considers that his results firmly establish the existence of such phenomena. Whether, as he points out, such local effects, produced by compounds which are probably of the type of acetyl-choline and which are rapidly decomposed in the tissues, are to be regarded as truly endocrine or not, is a problem which may well await further knowledge of the phenomena.

Gastrin. The discovery of secretin in the duodenal mucosa led, perhaps too suggestively, to claims that a similarly-functioning compound, gastrin, existed in the gastric mucosa (9). Subsequently such claims did not seem to be justifiably established, and endocrinologists have tended to disbelieve in the existence of gastrin. Murlin (27) has recently reviewed the work on gastrin, and his views present the gastrin theory in its most favourable aspect. Ivy (19), in 1925, transplanted a small "stomach-bag" from the fundus of that organ, along with its blood supply, into the mammary gland of a dog which had recently suckled a litter of pups. After a new blood supply had become established, he severed the original supply, and along with it any extrinsic nerves which happened to be present. A fistulous opening into this pouch enabled its secretory activity to be studied. Whenever the dog was fed the pouch secreted gastric juice. Since the only possible connection between the normally functioning stomach and the pouch was by way of the circulation, Murlin considers that an endocrine control of the stomach has been established by this experiment, and that normally gastrin is formed by the gastric mucosa in the pyloric portion whenever food reaches this region, is then absorbed into the blood, and so ultimately reaches the glands of the fundus. There is thus a provision for continuous secretion of gastric juice after the initial (psychological) central nervous control ceases.

Ivy has, more recently, isolated histamine from acid extracts of the pyloric mucosa, and considers that there is strong, if not conclusive evidence that it is the sole secretory excitant present in such extracts, so that "*gastrin*" *is probably histamine* (30). Whether histamine should therefore be classed as an endocrine principle is still an open question.

Secretin. The classical work of Bayliss and Starling in 1902, demonstrating the existence of secretin and its action in stimulating the outflow of pancreatic juice and bile, was confirmed at that time by numerous investigators. Little further work of importance on this compound was accomplished, until in 1928 J. Mellanby isolated what appears to be almost a pure preparation of it (25). Pig's duodenal mucosa was extracted with absolute alcohol, bile salts added to the extract, and then dilute acetic acid. The bile acids were precipitated and carried down secretin along with them. The wet precipitate was treated with absolute alcohol; secretin passed into solution. It was precipitated by addition of excess of acetone, then dissolved in water, and finally precipitated by dilute acetic acid.

Mellanby thus obtained an amorphous pale brown powder, which was of polypeptide and perhaps of protein nature. It was slightly soluble in water, and more readily soluble in dilute alkali, but insoluble in acetone, ether, and absolute alcohol (though soluble in aqueous alcohol). It was rapidly decomposed by pepsin, trypsin, and the tissue proteases. Injection into dogs of an amount which gave a concentration in the blood of only one part in five millions gave a maximum flow of pancreatic juice.

Bayliss and Starling put forward the theory that when acid reached the duodenum from the stomach an unknown prosecretin in the gastric mucosa was changed to secretin which passed inwards to the capillaries of the mucosa and so to the general circulation. Mellanby believes the mechanism to be otherwise. Under normal digestive conditions the presence of food in the intestine leads to an outpouring of bile. Absorption of bile salts commences immediately and these carry with them preformed secretin to the general circulation. This evokes the secretion of pancreatic juice and of more bile, and so more secretin is absorbed. Such a theory seems more rational, and requires no explanation (as the earlier theory did) for the continued functioning of the pancreas in

cases of achlorhydria. Ivy has repeated Mellanby's work, but did not get uniformly good results with it (26). (Cf. also Cunningham (11).)

Mellanby has recently (1932) modified his procedure, omitting the use of bile salts. One kg. of fresh mucus membrane gave 20 mg. of a white amorphous powder, with solubility properties similar to those of the less pure preparation. The material appeared to be a polypeptide, containing sulphur but no phosphorus. Its activity was rapidly destroyed by trypsin. It did not dialyse through collodion.

Ågren and Wilander (2) have still more recently obtained a white amorphous preparation easily soluble in water and 95 per cent. alcohol, but insoluble in absolute alcohol. It is active when injected into cats in dosage of 0·005 mg. per kg. It contains 2·3 per cent. of nitrogen and 0·1 per cent. of sulphur. Phosphorus is absent. It appears to contain no cyclic amino-acid radicals, and to behave as a base. They consider that its molecular weight is less than 1,800.

Nothing is known of any condition associated with hyper- or hypofunction of secretin.

Cholecystokinin. Ivy found by cross-circulation experiments that when acid is injected into the duodenum, something passes into the blood which causes the gall-bladder to contract. He claims (20) to have prepared an extract from the upper intestinal mucosa free from secretin, which when injected into dogs, cats, or man (but not rabbits), causes contraction and evacuation of the gall-bladder. He considers that an endocrine principle is involved, which he terms *cholecystokinin*. Still has obtained similar results (32).

A Possible Principle Stimulating the Production of Insulin. Heller (17) showed that when extracts of duodenal mucosa were injected into normal rabbits just prior to injection of a definite amount of glucose solution, the degree of hyperglycaemia was less than would be produced by the glucose alone. This could not be attributed to secretin, which possesses no hypoglycaemic action (33). Laughton and Macallum prepared an extract from the duodenal mucosa freed from protein and peptone, and still showing the activity described by Heller (22). This extract, when injected into depancreatized dogs, was inactive. This suggested that the effect is produced through increased output

of insulin. Should the work be confirmed it possesses distinct clinical significance, since, as Laughton and Macallum point out : " Insulin failure may result from excessive stimulation of the islets by the duodenal hormone produced as a result of excessive sugar intake over long periods. Secondarily, inflammatory conditions in the duodenum may lead to a deficiency in the hormone, followed by a diminished activity in the islets themselves resulting in a hyperglycaemia."

Choline as an Endocrine Stimulant of Intestinal Peristalsis. Although choline is a radical of lecithin, and lecithin is widely distributed throughout the tissues of the body, we do not know the origin or the fate of choline in the body. The compound is powerfully toxic, and produces, when injected intravenously in sufficient dosage, salivation, intestinal cramps, a fall of blood pressure followed by a rise, and, in still larger dosage, death from heart standstill. Its action is produced through stimulation of the sympathetic nerve endings in glands and muscles, and is abolished by atropine.

Le Heux found, some years ago, that choline has a stimulating effect on the surviving intestine of the rabbit. By a complicated procedure he isolated choline from the intestinal mucus, and suggested that it is a " hormone " producing intestinal peristalsis. His theory is that through local reflex action it is set free in the cells of the mucus, is absorbed into the circulation, and stimulates contractions further down the gut. He has shown, using the bismuth meal and X-ray examination, that choline administered intravenously to cats in sub-toxic doses, reduces to one-half the time of stay of food in the stomach and small intestine, peristaltic waves and the passage of food from stomach to intestine being more frequent (23). Much more work is necessary to establish choline as an endocrine principle.

The Pineal Principle

Little recent work has been done on the pineal gland. The earlier work—extirpation and feeding experiments—led to confusing results. Only two outstanding facts seem definite.

A rare syndrome is found in young children, usually boys, who exhibit abnormal growth, associated with some degree of premature genital development. They die at an early age, following symptoms suggestive of brain tumour. At autopsy there is frequently found a teratoma of the pineal gland, suggesting hypofunction of the organ (34).

If ox-pineal is fed, along with plant food, to tadpoles from the beginning of larval life, about half an hour after each feeding they become translucent (the heart-beats are quite visible). This translucency persists for about three hours. The phenomenon lasts until metamorphosis (24, 18, 1). Its significance is not known.

The Thymus Principle, Thymocrescin

The general consensus of opinion amongst those who have especially studied the thymus is that its function is associated with growth (15). However, the histological resemblance of thymus to lymphoid tissue has suggested to many endocrinologists that the former merely functions as a large mass of such tissue (34).

Asher and his colleagues have recently obtained, by aqueous extraction of thymus tissue, a concentrated product free from protein and lipoid material, which seems to accelerate growth in rats. Asher believes that this concentrate contains a specific endocrine principle, which he has termed *thymocrescin* (4, 5).

The purest preparation, active in daily dosage of 1 mg., is obtained as follows: Calves' thymus is extracted with 3 volumes of acetone, and then with ether. The residue is extracted with water, and the water extract is precipitated with 90 per cent. alcohol. This precipitate is extracted with 70 per cent. alcohol and the extract evaporated at low temperature and pressure. The residue is dissolved in the smallest amount of water possible, and ammonium sulphate added almost to saturation. The precipitate is extracted with 70 per cent. alcohol, and the extract evaporated as before.

The powder so obtained is free from protein and lipoid, and contains no tryptophane radicals. It gives strong biuret and ninhydrin reactions and appears to be a sulphur-containing polypeptide.

Tested on rats it increases general growth, growth of the skeleton, and growth of the gonads. Similarly prepared extracts of lymph glands are inactive.

Other Suggested Principles

Haberlandt's "Heart-hormone." Haberlandt has published numerous papers (16), in which he claims that a specific "heart-hormone" exists, which will stimulate the non-beating (frog's) heart to movement. Oppenheimer (28) finds that the active substance in such experiments is not specific.

Sympathin. Cannon (9) has found that the rate of the denervated heart of the cat is increased by struggling, in animals that have been deprived of all chromophile tissue, and after removal of testes, thyroids, parathyroids, and pituitary, and denervation of the liver, pancreas, stomach, and small intestine. The effect is abolished by complete removal of the chains of sympathetic ganglia. Stimulation of the peripheral end of the sympathetic chain accelerates the denervated heart and stimulates the denervated submaxillary gland.

From such experiments Cannon concludes that an adrenine-like substance is passed into the circulation when the smooth muscle of the abdominal viscera receives sympathetic impulses. Cocaine sensitizes the vascular system to this substance, just as it does to adrenine (29), but not to tyramine and ephedrine (8). Cannon terms the substance *sympathin*.

A Blood-pressure Depressant. Various groups of workers have prepared extracts from the pancreas, which are stated to be free from insulin and to have a definite effect on the circulation, lowering the blood pressure. Beneficial results have been claimed from the use of such extracts in cases of hypertension.

It would seem probable that the same substance is responsible for these effects, although neither its specificity nor its endocrine nature can be regarded as established. Gley and Kisthinios made an acidified-alcoholic extract and termed it *angioxyl* (10, 14). Kraut and Frey's extract is termed by them *kallikrein* (21), while Santenoise has termed his preparation *vagotonine* (31), and claims that when it is administered with insulin, the effect of the latter is increased. *Carotidin*, from the carotid gland, may be similar (9A).

A Controller of Fat Metabolism. Claims have been made that such a principle can be extracted from normal urine (2, 13).

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CHAPTER IX

ENDOCRINE INTERRELATIONSHIPS

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Introduction

CUSHING has written (6) "Endocrinology lends itself to two glaring faults, one the popularization of writing on the subject, and the other a tendency of clinical observers to draw upon their fancy in a symptomatology which does not lend itself to precision." Nowhere is this statement more true than in discussions of the actual, and the far more numerous imaginative, interrelationships between the endocrine glands. Writers on the subject have shown varying degrees of fertility in differentiations which frequently are, at the very least, unnecessary. A sterility of ideas is probably safer in these considerations.

In this volume certain intrinsic interrelationships have already been discussed. In this chapter a brief *résumé* of these will be given, and some others will be dealt with at short length. There will be no attempt at complete treatment.

These interrelationships must be carefully differentiated from the simultaneous presence of two or more unrelated endocrine disorders in the same patient, the true pluriglandular syndromes. These are rare, and when they do occur, each disorder requires its own treatment. But the

importance of an accurate knowledge of interrelationships lies in the fact that such knowledge frequently permits recognition of the endocrine organ primarily involved in disease, and such recognition permits accurate treatment, limited to that primary malfunction. Other treatment of the secondary disorders is at least wasteful and unnecessary, and is certainly unscientific.

Pituitary Interrelationships

These are of outstanding importance. "All pituitary syndromes are essentially polyglandular" (Cushing (6)) and in consequence most of these interrelationships were dealt with in Chapters VI. and VII., and need but brief mention here.

The Basophile Cells of the Anterior Lobe and the Gonadal Secretions. The relationship has been dealt with thoroughly (cf. pp. 256, 263, 317). It will be recalled that the compound elaborated by the basophile cells, for which the name "hebin" has been suggested, and which appears to be a protein, stimulates the ovaries to maturation and formation of corpora lutea, and the concomitant production of keto-hydroxy-oestrin (theelin), and, in the male, stimulates the testes to mature growth, and elaboration of their endocrine principle. It has also been pointed out that excess production of the ovarian principle appears to depress the secretion of hebin, and that this view is supported by such experiments as the injection of keto-hydroxy-oestrin into immature male animals, when, presumably through depressed pituitary function, the male genitalia remain infantile (cf. p. 322).

The Anterior Pituitary and the Adrenal Cortex Principle. Evidence has been quoted in Chapters V. and VI., based especially upon the clinical observations in cases of pituitary basophilism, that overproduction of "hebin," through the occurrence of a basophilic adenoma, tends to set up overproduction of the cortical principle, either through hyperplasia (cf. p. 215) or through secondary adenomatous

growths (cf. p. 260). Some suggestive evidence, clinical and experimental (cf. p. 260, footnote), exists that marked depression of cortical function leads to increase in number of basophile cells, and, therefore, presumably to increased production of hebin. The two theories are not necessarily contradictory, but seem a little difficult to harmonize. Nor, of course, is it certain that the same principle acts both as a gonad-stimulator and a controller of the adrenal cortex.

The Anterior Pituitary and Growth. It has been shown that the acidophile cells of the anterior pituitary elaborate a principle, " phyone," which promotes growth. If Cushing's dictum is true, one may infer that stimulation to growth is produced through the effects of phyone on one or more of the other endocrine glands. This may well be, but the path of action cannot yet be indicated with precision.

The Anterior Pituitary and the Thyroid. In Chapter VI. mention has been made, more or less casually, of the effect of the anterior pituitary secretion upon the thyroid gland, as evidenced by its condition following pituitary implants and injections, following extirpation of the pituitary, and in diseases associated with the pituitary. This relationship calls for some detailed consideration. Much of the recent work has been reviewed by Thomson and Collip (27).

When the pituitary is extirpated in frog tadpoles development of the thyroid ceases (p. 262). Its removal in adult toads leads to flattening of the thyroid epithelium, and accumulation of colloid. Conversely, injections or implants of anterior pituitary into salamander larvae (28) or adult toads (12) provoke hyperfunction of their thyroids. The thyroid atrophies following hypophysectomy in young rats (5).

Schockaert has carried out very accurate studies on the duck (23, 24). Following daily injections of potent extracts of bovine anterior pituitary into young male ducks, they show a notable and rapid regression of the thymus, a very marked hypertrophy of the testes, and a definite increase in the size of the thyroid. The effect on the thyroid is apparent within

twenty-four hours, and after three weeks the gland may reach more than thirty times the size of the thyroid in normal controls. It shows progressive structural changes. At first there is a complete excretion of all colloid material, and increase in height of the epithelium, with pycnosis, desquamation, and mitosis. Later, there is some formation of colloid, and the epithelium becomes of high columnar type, forming hyperplastic folds and papillae. From the third week of treatment the vesicles become large and are filled with a pale granular colloid ; the hyperplasia and height of the epithelium decrease.

At the end of the first week's treatment the total iodine content of the gland has fallen to between one-tenth and one-twentieth of the original amount. Due to the increasing hypertrophy the percentage content continues to decrease, but the total content is not much further affected.

When the treatment is continued for more than three weeks there is a definite exophthalmos, a loss of down, and an increased weight of the heart. If the treatment is stopped the exophthalmos disappears in about a week.

Schockaert considers that the effect is not due to the secretion of the basophile cells, nor to prolan, but is produced by the growth principle (phyone). However, the " phyone " preparations used cannot be regarded as containing only one active compound (cf. p. 274).

Schockaert's work shows definitely that, as far as the young duck is concerned, some principle of the pituitary controls the thyroid and causes discharge of its secretion, and that excess of this particular principle induces a hyperthyroid condition. The exophthalmos is due also to a pituitary principle.

Houssay and his co-workers have studied the relationship in dogs. Pituitary extirpation tends to produce decrease in the weight of the thyroid, with a tendency to atrophy. The histological picture indicates hypoactivity. The iodine

content of the whole gland is not affected, but the percentage increases, due to the shrinkage of the gland.

On the other hand, injections of alkaline extracts of the anterior lobe of the pituitary cause a marked augmentation in the size of the thyroid, even in hypophysectomized animals, with colloid resorption, hypertrophy, hyperplasia, lowering of the iodine percentage, and a corresponding increase in the iodine content of the blood (13).

The autopsy on the giant bulldog bitch, whose gigantism was produced by prolonged injections of an anterior pituitary preparation, showed, amongst other findings, an enlarged thyroid, with a dense and cellular structure, small acini, and paucity of colloid (cf. p. 271).

The findings in pituitary diseases are in harmony. Acromegaly is often accompanied by a palpably enlarged thyroid, and by symptoms suggesting thyrotoxicosis. When the thyroid gland has been removed, colloid changes of adenomatous type have been found, but no evidence of toxicity (7).

The determinations of basal metabolic rate in experimental pituitary conditions and in diseases associated with the anterior lobe are also in harmony with the above findings. Thus Foster and Smith (10) found that the basal metabolic rates of seven totally hypophysectomized rats showed an average drop of — 35 per cent., as compared with forty-four normals. This lowered rate was restored to normal by either daily homotransplants of anterior pituitary, or daily injections of thyroid extract, but not by daily injections of posterior lobe extract.

In human pituitary insufficiency the basal rate tends to be low. In 107 cases, in which this insufficiency was due to neighbourhood pressure from chromophobe adenomas, the rates found varied from + 10 to — 36 per cent.; in most of the cases the figures were below — 10 per cent. (7). (Cf. also (2).)

In acromegaly, on the other hand, the rates are either normal or high (2). Cushing and Davidoff (7) found that

almost half of seventy-two cases of acromegaly had rates above + 10 per cent. The maximum found was + 61 per cent. In cases in which the basal rate was high, removal of a pituitary chromophilic adenoma was followed by a fall in the rate almost as uniform and striking as that following thyroidectomy in Graves' disease (and this even in cases in which there was no palpably enlarged thyroid).

The acromegalic frequently exhibits a glycosuria attributable to a lowered carbohydrate tolerance. This may or may not be produced through thyroid intermediation.

These results and observations are all in agreement with the view that a principle of the anterior pituitary controls (in some way still undetermined) the output of the thyroid principle. Any increased pituitary function (as far as the anterior lobe is concerned) leads to increased output of the thyroid secretion and may even cause hypertrophy of the gland. Any decreased pituitary function of this kind leads to decreased thyroid output and even to atrophy. Whether pituitary hyperfunction can in any way be regarded as a prime factor in the production of Graves' disease or of other clinical hyperthyroid conditions cannot be yet stated.

Marine has been able to produce marked thyroid hyperplasia, accompanied by exophthalmos, in immature rabbits by daily intramuscular injections of 0·05 to 0·1 c.c. of methyl cyanide. Even thyroidectomized rabbits develop exophthalmos following this treatment (17). It has been shown by a number of investigators (26, 28, 16) that acetic acid extracts of anterior pituitary contain the thyrotropic principle. Such extracts produce exophthalmos in both normal and thyroidectomized guinea-pigs, indicating that *exophthalmos is not dependent on a normal or an abnormal thyroid secretion.*

Marine (17) has put forward the following hypothesis of the action whereby cyanide (exogenous or endogenous) affects the thyroid gland, and simultaneously produces exophthalmos.

Cyanide inhibits tissue oxidations. Amongst other tissues the hypothalamic centres are affected. These stimulate the anterior pituitary, so that discharge of its thyrotropic factor is increased, and the thyroid subsequently exhibits hypertrophy and hyperplasia. At the same time the sympathetic system is stimulated, either directly or through the pituitary and a hypothalamic centre, and thereby the pupillo-dilator and Müller's muscles are affected, and exophthalmos results.

Paal (18) claims to have prepared an extract of whole anterior pituitary which he terms " hormothyrin," and which is stated to increase the resistance of mice to acetonitrile (cf. p. 29). This effect is not exhibited after thyroidectomy.

Anderson and Collip (1) have prepared a highly purified extract of the thyrotropic factor from the residues of anterior pituitary tissue *after removal of the growth principle*. It has been freed from prolactin (see below) by isoelectric precipitation of that fraction, and further purified by salt precipitation and fractionation by alcohol and acetone. Daily injections of their preparation into guinea-pigs markedly increase the basal metabolic rate, and the thyroids of such animals show at autopsy definite hyperplasia. When the extract is administered to hypophysectomized rats it prevents the atrophy of the thyroid which invariably occurs in untreated animals. Collip believes that this principle is distinct from all other pituitary principles.

To what extent there is any specific countercontrol of the pituitary by the thyroid cannot be stated. Thyroid extirpation in rabbits is followed by a definite enlargement of the pituitary which affects mainly the posterior and intermediate parts. Certain histological changes, including increase of colloid, have been noted. Some degree of pituitary hypertrophy has been observed in thyroidectomized lambs. There is no evidence of pituitary hyperfunction in such experiments (29). It is very doubtful if observations of this nature are sufficient to justify certain differentiations such

as Engelbach (8), for example, has suggested. It is perhaps desirable to allow him to speak for himself :

" Interhormonic action exists between the thyroid and pituitary glands resulting in the clinical entities of their combined disorders, thyropituitarism and pituitarothyroidism. At the time of observation it can usually be determined from the history that one or the other of these glandular disorders was initial and preceded the other. This is indicated by the order in which glandular terms are expressed. The natural interaction of these two glands results in the clinical complex in which, at the time of observation, both are concurrently involved to the extent that neither can be regarded as the primary cause of the hormonic symptomatology. Since this biglandular disorder is usually mistakenly diagnosed as uniglandular disorder of either the thyroid or hypophysis, more accurate interpretation of it is considered pertinent. Furthermore, it is associated with the secondary hormonic signs of the gonads, which adds more diagnostic confusion. The fact that all the genital symptomatology is relieved by correction of the function of the thyroid and hypophysis, as is true of the reactions occurring in the non-endocrine systems, would strongly indicate that the gonadal defects are purely secondary.

" The justification for the independent grouping of these biglandular disorders . . . lies in (i) their frequent occurrence as compared with the uncomplicated thyroidisms and pituitarisms ; (ii) the constant cytologic and hormonal interaction of these two endocrine glands ; (iii) the well-defined clinical picture differentiating them from these uniglandular disorders, as well as other endocrinopathies and non-endocrine diseases ; (iv) the reaction in the majority to *combined* thyroid and pituitary treatment ; and (v) the failure to relieve the hormonic symptomatology by either thyroid or pituitary replacement treatment when given alone."

The majority of the cases which he considered infantile

thyropituitarism amongst his material had been diagnosed as cretins, while most of the adult cases that he termed the obese varieties of thyropituitary disease had been diagnosed and treated as cases of hypothyroidism or myxoedema, and those considered by him to be pituitarothyroidism had been considered as exhibiting uncomplicated pituitary disorder. "This incomplete diagnosis, as a basis for treatment, accounts for the poor therapeutic response and unfavourable prognosis prevailing in these two groups."

Since his patients appear to have received most or all of their pituitary-replacement therapy orally, a useless procedure (cf. p. 275), only a few receiving intramuscular injections of "antuitrin" and also of "pituitrin," it is doubtful if pituitary treatment alone had a fair trial in such cases.

With our present knowledge, it seems safest to regard all such cases as due primarily to pituitary insufficiency, and as requiring pituitary-replacement therapy, which must be by injection of potent extracts of the correct principle or principles and not by oral administration. At best, concomitant thyroid administration can only be considered justified when used to accelerate restoration of a subthyroid condition, induced as a secondary consequence of the pituitary disorder.

The Pituitary and the Islets of Langerhans. That an interrelationship exists between the pituitary and the islets of Langerhans is certain. It is exemplified by the co-existence of diabetes mellitus in some proportion of cases of acromegaly, and the disappearance of the former after successful operation for the latter. Whether the apparent antagonism between insulin and "pituitrin" is involved in this interrelationship, or constitutes another, we do not yet know. Nor can the precise nature of the relation be stated. Some details of pertinent observations were given in Chapter IV. (p. 160).

The Pituitary and the Mammary Glands. In some way or other, whether through a special principle or one or more

of the others, the anterior pituitary controls lactation. The majority of investigators appear to consider that a special principle is involved, a principle which Riddle has termed "prolactin" (19). He has obtained, by isoelectric precipitation of an acid extract of anterior pituitary tissue, a fraction which stimulates development of the crop-gland in male, female, or castrate pigeons. The effect is not produced by growth-controlling or by gonad-controlling preparations of the pituitary (19).

When the pituitary is removed from lactating rats there is prompt cessation of milk secretion (5). When the operation is performed on pregnant rats ten days before term they exhibit milk secretion for a few hours after parturition, but this may be associated with the foetal pituitaries, since, if at the time of hypophysectomy the foetuses are also removed by Caesarian section, there is no subsequent secretion of milk (4).

When the A-P-L principle of pregnancy urine is injected into virgin rats marked development of the mammary glands follows, but no secretion of milk (9, 3, 4). However, removal of the intensely luteinized ovaries of these rats is followed by abundant milk secretion within thirty-six hours. If pituitary and ovaries are simultaneously removed, milk secretion does not ensue (4).

Adrenal Cortex Interrelationships

The relation between the pituitary and adrenal cortex has been dealt with.

The Adrenal Cortex and the Gonads. That the secretion of the adrenal cortex exercises control over the gonads is exemplified by the "virilism" and "hirsutism" which accompany functioning tumours of the cortex (cf. p. 214). The adrenal effect on the gonads is also exemplified in pituitary basophilism (cf. p. 256).

The Adrenal Cortex and the Thyroid. As has been pointed out in Chapter II. (p. 65), Marine believes that one potential

cause of Graves' disease lies in an initial disturbance of the adrenal cortex, presumably leading to decreased function. In agreement with this theory Shapiro (p. 83) obtained moderately good results from administration of adrenal cortex to patients with Graves' disease, while use of potent adrenal cortical extracts has also proved to be of benefit in some cases (cf. p. 213).

Thyroid Interrelationships

Thyroid relationships with the pituitary and the adrenal cortex have been dealt with.

The Thyroid and the Islets of Langerhans. Since glycosuria is a not uncommon accompaniment of hyperthyroidism, the idea that there may be some association between the thyroid secretion and insulin naturally arises. Many sugar tolerance curves of patients in hyperthyroid states are indistinguishable in type from those of patients with mild diabetes. Yet the decreased tolerance is almost certainly due to depletion of the liver glycogen reserve which occurs in hyperthyroidism and an apparent inability to form glycogen which is probably in actuality such an increased demand for glucose by the tissues that no great reserve of carbohydrate material can be built up.

Nevertheless, John (14), who has studied the sugar tolerance of many hyperthyroid patients, appears to be of the opinion that the lowered tolerance is provoked by the hyperthyroid condition through the islet apparatus, and that hyperthyroidism, if prolonged, may lead to a true diabetes mellitus. Such a combination is extremely rare (see below). Hyperthyroidism cannot be definitely accepted, with our present knowledge, as amongst the potential causes of diabetes mellitus, although such a possibility cannot be entirely excluded.

The Thyroid and the Gonads. Various phenomena indicate that a relationship of some kind exists between the thyroid

and the organs of reproduction. In women, at puberty, during the menstrual periods, and during pregnancy, the thyroid becomes enlarged. Thyroidectomy in young animals results in some degree of sexual infantilism. Myxoedema is accompanied by depression of sexual function in both sexes (25). Menstrual disturbances are frequent accompaniments of thyroid disorders in women; an uncontrolled rhythm is often recorded and seems especially characteristic (21).

It is doubtful if the relationship can be regarded as a direct one. The thyroid hypertrophies in many conditions where there is an increased demand for its secretion (cf. p. 9). Many of the other phenomena can be regarded as incidental developments following changes in the degree of the thyroid control of general oxidative processes throughout the organism (cf. p. 26).

Pluriglandular Disorders

An excellent example of the simultaneous occurrence of two unrelated endocrine disorders in the same individual is the combination of hyperthyroidism and diabetes mellitus. The incidence of this condition has been studied by Wilder (30) and by Joslin and Lahey (15).

Wilder found 15 true diebetics amongst 2,340 cases of Graves' disease, and 23 amongst 1,131 cases of toxic adenoma. Joslin and Lahey found only 75 cases of the combination amongst 5,790 diabetics and 5,908 hyperthyroid cases. In the majority of cases the hyperthyroidism preceded the diabetes. The possibility that diabetes can result from hyperthyroidism has already been discussed (p. 355). Such possibility can only be admitted through an indirect action, through the strain of a constant hyperglycaemia upon the islets of Langerhans. The incidence of the combination is scarcely more than might be expected from the laws of chance.

In rare instances hypothyroidism and diabetes mellitus are associated (30, 21).

Rowe and Lawrence (22) published in 1928 a pleasingly critical account of pluriglandular syndromes. Among many hundreds of patients exhibiting endocrine disorders they found only twenty-two in whom they considered that two unrelated endocrine glands were involved. Since of these eighteen exhibited a functional error in one gland, with results from surgical interference with another, while of the remaining four all exhibited a combined pituitary-thyroid dysfunction, in which in light of present knowledge, interrelationship cannot be considered as excluded, their results illustrate the great rarity of true pluriglandular conditions.

Rowe has summed up the matter still more recently (21): "The so-called 'pluriglandular' group . . . is made up almost without exception of cases in which surgical intervention in one endocrine gland is superimposed upon functional aberration in another. In a series of over 5,000 cases the writer has seen but two or three in which there has been apparently a coexistent primary disturbance in more than one endocrine gland."

General Considerations

The interrelationships revealed by experiment and by disease, both between two or more of the endocrine glands and between such glands and non-endocrine tissues, illustrate not only the many repercussions which malfunction of one gland can set up throughout the organism, but also how, during normal existence, there must be vast interlocking of functional action of the numerous compounds which these endocrine glands secrete.

Of them all the pituitary can be regarded as of prime importance. Through some one or other of the several principles it secretes it controls (i) the thyroid, and thereby the oxidative processes throughout the organism; (ii) the

adrenal cortex, and thereby, in some still undetermined fashion, normal muscle contractility and perhaps also the degree of dilution of the blood; (iii) the gradual development of the gonads, and, when these are sufficiently matured to secrete enough of their own specific compounds, through them the development of the secondary sex organs and secondary sex characters; (iv) to some degree, fat metabolism; and (v) the water exchanges of the body.

Thus it is easy to imagine not only the many effects which marked abnormality of pituitary functions can cause, but also how even slight pituitary changes within normal range of variation can be reflected in so many ways as to result in marked variations in the physiological behaviour of the organism.

The imagination may be tempted by such facts to belief that racial differences and even differences of personality may be traceable to endocrine variations within physiological bounds. Such fancies can be carried too far; the present state of our knowledge does not now justify them. As this knowledge extends, however, we shall be justified in careful examination even of these fanciful possibilities, and may perhaps find some trace, although probably not more than a trace, of truth in them. Hoskins (11) has presented a conservative statement of possibilities in this direction.

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