Recent advances in endocrinology / by A.T. Cameron.

M. Watson.

RECENT ADVANCES IN ENDOCRINOLOGY

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By

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THIRD EDITION

With 65 Figures, including Three Plates

LONDON

J. & A. CHURCHILL LTD.

104 GLOUCESTER PLACE

PORTMAN SQUARE

1936

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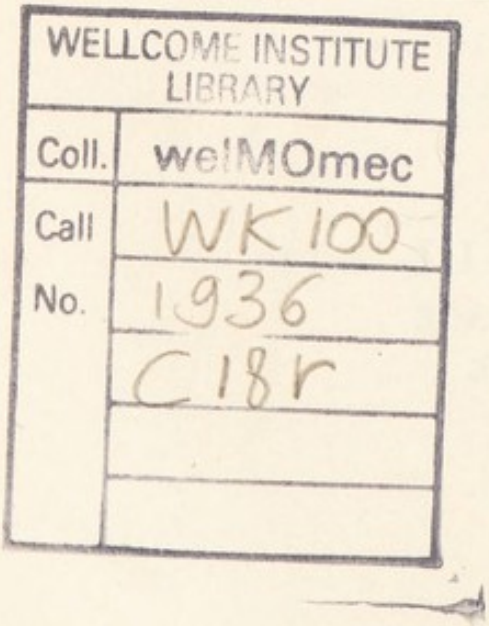
THE MEDICAL PROFESSION
OF WINNIPEG

First Edition . . . 1933
Second Edition . . . 1935
Third Edition . . . 1936



Printed in Great Britain

PREFACE TO THE THIRD EDITION

Although it is only just over a year and a half since the revision of the second edition was completed, yet new work of varying importance has appeared bearing upon almost every branch of the subject, and large parts of the text have had to be re-written. Much of the new work is concerned with the minutiae of facts which are slowly completing the complex mosaic of endocrine interrelationships whose pattern becomes dimly visible. In many cases these researches exemplifying so much patience and pertinacity cannot be fully evaluated until still further work will permit their incorporation into the framework of the whole story.

Some new work stands out more boldly. That of Rowntree and his associates on the thymus and the pineal at last definitely places these in the category of endocrine glands. The preparation and clinical use of protamine compounds of insulin is undoubtedly an important advance in the therapy of diabetes mellitus. Riddle's association of the function of prolactin of the pituitary with maternal behaviour is an important illustration of endocrine possibilities in connection with instincts. The recent publication of Knaus' monograph has concentrated attention upon his work and that of Ogino; their conclusions are steadily winning support and have facilitated comprehension of certain endocrine phases of reproduction.

Of the more purely chemical work attention may be drawn to Mansfeld's demonstration of the applicability of refined methods of chemical study to the elucidation of the mechanism of endocrine function. His results concerning the mode of thyroxine action constitute a distinct advance, and suggest that application of similar methods may be fruitfully applied to other endocrine compounds.

Accurate knowledge of the oestrogenic compounds accumulates. The very active compounds oestradiol (dihydro-oestrone) and testosterone are proved to be those actually associated with ovarian and testicular activity respectively. Progesterone of the corpus luteum has been crystallized, and emmenin appears to be the glycuronate of oestriol. A compound has been prepared with both " male " and " female " properties.

I have endeavoured to deal with most of the important literature to the end of 1935, and have included some of the earlier work of 1936. Some papers of importance have almost certainly been missed, or their importance insufficiently appreciated. I can only hope that these errors of omission and commission are few.

Several new figures are included. I wish to thank Dr. L. G. Rowntree and the Editor of the *Annals of Internal Medicine* for permission to reproduce Figs. 26, 27 and 28, and Dr. C. W. Dunn and the Editor of the *Journal of Nervous and Mental Disease* for like permission for Figs. 42 and 43.

My thanks are again due to Professor William Boyd, for permission to reproduce the unusually illustrative photomicrograph of the section of anterior pituitary shown in Fig. 40. I wish also to acknowledge the continued courtesy and co-operation of Messrs. J. & A. Churchill Ltd.

A. T. CAMERON.

WINNIPEG.

PREFACE TO THE FIRST EDITION

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. 18.

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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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 emphasize 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 visualize 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 con-

cerned 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 " oestrin " of the ovaries has provided 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. An excellent example of what can be done by agreement is the choice of the name " progesterone " for the corpus luteum principle by the leading investigators of that compound.

Endocrine compounds are definitely associated with the thyroid, parathyroid, pituitary, and adrenal glands, the islet tissue of the pancreas, the thymus and the pineal, 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 almost all endocrine disorders are

primarily associated with either hypo- or hyperfunction of only 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 of that gland.

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 standardized concentrates suitable for injection, and such a demand should before long be met for all of them. Only such properly standardized preparations should be employed.[1]

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.

The *correct therapeutic dosage of endocrine preparations* is not a subject for generalization, but rather for individualization. It is not possible in this volume to do much more than indicate some of the many potential errors which may arise in connection with dosage.

Where pure endocrine principles or active derivatives are available, such as thyroxine, oestrone (theelin), or crystalline insulin, then dosage can be based upon specific amounts of them. But if the treatment be in the nature of replacement therapy, each individual requirement must be different, for

[1] Replacement treatment by grafts has only proved of transient benefit, the duration of which appears to vary for different endocrine tissues. It is possible that the technique suggested by Stone, Owings and Gey (4) will give better results. Tissue cultures of thin sheets of cells are grown for two to four weeks in the blood serum of the future host, and are then implanted preferably in the region of the axilla or groin. They claim good results in cases of parathyroprivic tetany and of thyroid deficiency.

the amount of non-functioning endocrine tissue whose normal output has to be replaced differs in each patient. This is illustrated by Collip's *principle of inverse response* (1), which he defines: "The responsiveness of an individual to administered hormone varies inversely with the hormone content or production of the individual's own gland." This dictum aptly illustrates the impossibility of accurate dogmatism in endocrine therapy. Even when total replacement is necessary, as, for example, following total thyroidectomy, the requirements of individuals will be related to their body-volume or body-surface, or both, while sex and age will also modify them.

When such pure preparations are not available, not only is accurate standardization necessary, but a correct basis of standardization. The same weight of desiccated thyroid may give very variable results if different preparations are at different times given to the same patient. Thyroid should be standardized according to its iodine content—it now frequently is.

The numerous principles of the anterior pituitary gland afford a perfect example of this difficulty concerning precision of dosage. Not only are none of them yet commercially available in pure condition, but such commercial products as are available, suitable for injection (and the others seem valueless), are, it would appear, mixtures in uncertain proportions of two or more of these principles. Dosage for specific purposes must, in such cases, be a matter of trial; the invariability of action of the same firm's preparations may be doubtful until complete separation of different principles and accurate standardization of each are obtained. The standardization of most of the endocrine principles is by biological procedures, and these often yield only approximate results. No agreement has as yet been reached as to the most suitable biological procedure for many of these compounds.

Rynearson (3), speaking of the clinical use of pituitary preparations, perhaps a shade too pessimistically, said

recently "For the present little advance will be made by injecting into more and more patients bad to worse extracts. . . . Only a few worth while preparations of the anterior lobe of the pituitary gland are available from the better pharmaceutical and experimental laboratories, and even some of these have been known to produce disastrous results when injected into animals."

Endocrine interrelationships seem peculiarly liable to lead to therapeutic chaos.

The application of correct dosage is not rendered easier by the multitude of trade names. Too much nonsense is written about the non-utilization of certain scientific terms by scientists because commercial firms have employed them. It would be of much greater benefit to physician and patient if definite names and methods of standardization were generally agreed upon by the scientific workers in this field, and thereafter commercial firms were compelled by law to use these names and methods of standardization in labelling their products and the assay value of each. They could be permitted to add any special designation of their own that they desired.

Pratt has recently published a thoughtful paper on this subject of dosage in endocrine disorders (2). He points out that the necessity of considering each individual separately is by no means limited to endocrine therapy, and that the ordinary dosages prescribed for such established drugs as digitalis, arsphenamine, the belladonna group and sedatives frequently produce very varying, and sometimes dangerous, consequences, so that it is not surprising that precision of dosage is still not possible for the much newer endocrine principles. He lays down the obvious but too often neglected dictum that *the reactions of the individual patient to any therapeutical agent should be the criteria for the final determination of the manner and amount to be administered.* It is equally obvious that error on the side of low dosage is the safest error.

Two of Zondek's fundamental hypotheses (5) may well be quoted here ; the first also has a bearing on the variation of dosage for different individuals, and for the same individual at different times. " Hormonal effect is not an absolute but a variable quantity, depending not least upon the momentary condition of the organ on which it acts—more especially, the physico-chemical condition of its cells. Functional and anatomical changes in endocrine glands should not always be regarded as the cause of disease, but in many cases the reaction of the glands to morbid processes located in certain other organs."

It is perhaps desirable to add a paragraph on *the order in which the endocrine glands have been dealt with* in this volume. It is possible that in one or two decades the logical order will be to commence with the pituitary, since, if the current trend of scientific discovery persists, proof seems likely that through its various principles the pituitary controls all the other endocrine glands. Such treatment, even when logical, will not be easy, since in order to understand the actions of these different pituitary principles it is necessary to know something of the other glands which they control, and the principles which these secrete. It is at present easier to deal with these other glands and their principles first, although, now and again, it will be necessary to anticipate their relationships with the pituitary, and even occasionally to duplicate pertinent matter. This method of treatment has been adopted.

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2. Pratt, *Endocrinology*, 1934, xviii, 211.
3. Rynearson, *Proc. Staff Meetings Mayo Clinic*, 1935, x, 385.
4. Stone, Owings and Gey, *Lancet*, 1934, i, 625 ; *Surgery. Gynecol., Obstetrics*, 1935, lx, 390.
5. Zondek, " The Diseases of the Endocrine Glands," 3rd edit., Arnold, London, 1935.

CHAPTER 11

THE THYROID GLAND AND IODINE METABOLISM

Introduction

RESULTS of active investigations in every phase related to the thyroid are continually being published. It is possible to present a connected picture of its mode of secretion, the nature of the compounds it elaborates, the action of its principle, and the diseased conditions associated with malfunction of the gland. All the details of the picture cannot yet be presented. A number of problems related to this gland continue to elude solution.

Thyroid function is so associated with the biochemistry of iodine that it has seemed desirable to introduce a section dealing with certain aspects of that subject.

The most important recent advance concerned with the thyroid links it very definitely to the pituitary, and an

account of that work must be deferred to the chapter on the pituitary.

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 (312): "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

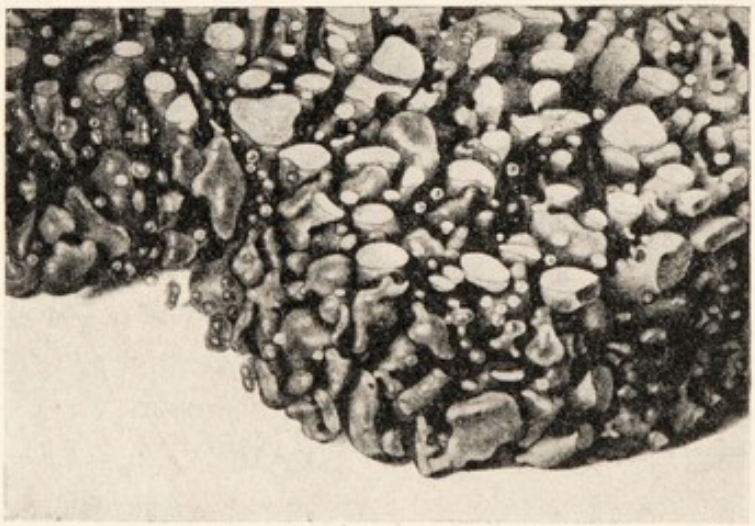


FIG. 1.—Top and side view of wax model of normal human thyroid gland. (From Rienhoff, *Medicine*, 1931, x, 293.)

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 (362), the thyroid unit consists of a system of closed tubules more or less suspended in a lymph sac. Marine (239) considers that the accuracy of this conception has not been established. Rienhoff (294), 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 . . . 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 (170) 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 (76), from a histological study of human thyroids at different periods of life, draws a number of definite conclusions. During early foetal life the gland is developing vesicles from solid epithelial cell masses through the intermediate stage of branching tubules. In later foetal life the epithelial cells become active and colloid is secreted and stored in the vesicles. Vesicle formation and colloid storage increase until birth. Then the gland rests for some weeks, using up the colloid already stored. It then exhibits renewed activity. Secretory activity is marked throughout infancy and childhood, and so is absorption ; a small reserve of colloid is always present. At puberty the gland exhibits its greatest activity, and

colloid storage is minimal. Subsequently the colloid store gradually increases and the gland is comparatively inactive throughout adult life, with perhaps a slight increase in activity towards the fiftieth year. In old age the gland gradually retrogresses, yet the thyroid of the aged, though reduced in size and weight, still shows typical individual secretory elements, although collectively their appearance suggests 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. Joll (174) has expressed doubt as to whether all Cooper's conclusions are fully justified from study of a limited amount of post-mortem material. Dogliotti and Nuti (89), from a careful study of thyroids of patients dying after sixty-five years of age, conclude that there are profound structural modifications in the thyroid of old age (seventy to eighty years)—diminution of colloid, and hypertrophy of epithelium, indicating an augmented thyroid secretion of a compensatory character.

Cooper stresses the striking resemblance, histologically, of the gland of the human adolescent to that regarded as characteristic of Graves' disease. Abbott (3) has reported that in young laboratory animals the gland normally appears hyperplastic. He has recently completed an exhaustive comparative study of the thyroids of domestic and wild animals in Western Canada (4). His findings for normal glands are in general agreement with those of Cooper. In the younger animals hyperplastic active glands are the rule, and are physiological, not pathological. The characteristic picture shows small acini and little colloid. As the animal grows older, the acini tend to become larger and colloid increases in amount. In old animals there is still more colloid, the cells tend to become flattened, interacinar fibrous tissue is increased, and " the generalized picture is one of a gland past its prime, sluggish, and gradually declining to decay and death."

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 (39)).

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. 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 (345, cf. 4A). In female rabbits coitus causes a rapid and almost complete removal of colloid from the thyroid follicles, with parallel increased function of the follicular epithelium (coitus leads to ovulation in these animals). During pregnancy of these rabbits colloid is again stored (190).

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. 12). The physiological changes in size of the gland, brought about by seasonal changes in temperature evoking increased or decreased heat production (cf. p. 35), 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 error.

The blood supply of the thyroid is of considerable import-

ance 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 (174)).

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 a high percentage 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 summarized 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

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	(52, 149, 226.)
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 (210).
Fish (U.S.A. waters)	0·8–4·0	Tressler and Wells.
Teleosts (off Norway)	17–623	Lunde (210).
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.* (227).
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.* (214).

found in (desiccated) thyroid are 0·01 and 1·16 per cent. . . . Other tissues in mammals contain less than 0·001 per cent." (52).

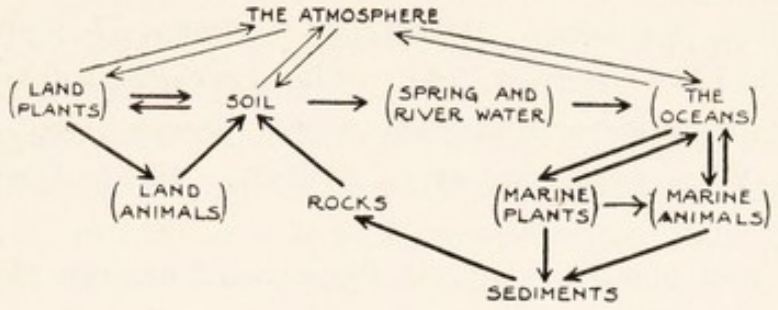
For our present more exact and complete knowledge of iodine distribution we are largely indebted to the micro-analytical procedures perfected by von Fellenberg, and the similar procedures devised by McClendon and by Hercus and Roberts, and the results obtained with them by these investigators and others, 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 (212). Further modifications (227) and simpler procedures (230) have recently been suggested.

The table on p. 15, summarizing the most important of such results, is based chiefly on a review by McClendon (224) ; 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.

According to v. Fellenberg the iodine content of soils is much higher than that of rocks which, by weathering, have produced these soils (224). 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 (29)).

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 (288). The immediate influence of the sea (through seaweed fertilizers and sea-sprays) does not extend beyond a very narrow coast-belt (288). (According to v. Fellenberg and Lunde (210) 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 (210, 211). 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 criticized the view that air-borne iodine plays any considerable *rôle* (286).

The cycle, as far as man and (to a lesser extent) the domestic animals are concerned, is frequently modified by man himself through utilization of food material from wide sources, and recently, through deliberate selection of iodized material. Modifying Lunde's scheme including man, it can be written :—

→ (Atmosphere) ——→
→ Land Plants ——→
→ Land Animals ——→
↘ Milk ——→
→ (Marine Plants) ——→
→ (Marine Animals) ——→
→ Spring or River Water ——→
→ (Crude or Iodized Salt) ——→
} → Man's Diet → Man
↓
Man's Excreta
↓
Soil →

(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 (325). 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 (185).

The most accurate figures for *normal* human thyroids are still those of Zunz (371), 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 (332) 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 (343) 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. (Cf. also Behrens (22).)

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 (179)). Iodothyroglobulin can be readily purified by dissolving it before it dries in normal saline, reprecipitating with ammonium sulphate (repeating these procedures once 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.

Slight modifications have recently been suggested, permitting greater rapidity of purification and possibly greater purity and less denaturation (18, 63, 144A). The thyroglobulin of thyroid desiccated at a sufficiently low temperature remains largely undenatured, and can be extracted by cold water (142).

After dialysis thyroglobulin 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

[1] Blanchard, Pénau and Simonnet (27), and Harington (140), have published monographs which give a full account of the chemical and pharmaco-dynamical properties of the thyroid principle.

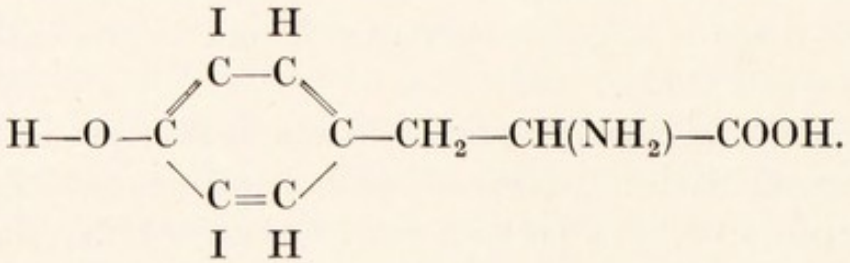
solution to the boiling solution. Its solutions give a positive test for tryptophane radicals, and markedly positive Millon's and Molisch's tests (167).

Pure thyroglobulin is a white amorphous powder. Its composition appears to be constant, except for an iodine content varying 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 (167).

White and Gordon (360) have recently analysed a pure specimen containing 0·75 per cent. iodine, 1·46 per cent. sulphur, and 15·58 per cent. total nitrogen ; the hydrolysate contained in per cent. histidine, 0·62, arginine 8·22, lysine, 1·93, glutamic acid 6·56, aspartic acid 1·59, tyrosine 3·17, tryptophane 1·80, cystine 2·05, and proline 4·47.

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 recrystallizes 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 iodized proteins liberated amounts of diiodotyrosine of the same order as those obtained from gorgonin and spongin. Ingvaldsen (167) 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 possible that Oswald's figures for the proportion of iodine held in such combination in naturally occurring or artificially produced iodized proteins do not at all indicate the total amount of such radicals present; it may well be that all the iodine is present in this organic combination. Claims, however, have been made that hydrolysis of such iodized proteins yields fractions exhibiting thyroid effect, and therefore presumably containing some thyroxine-like compound (5).

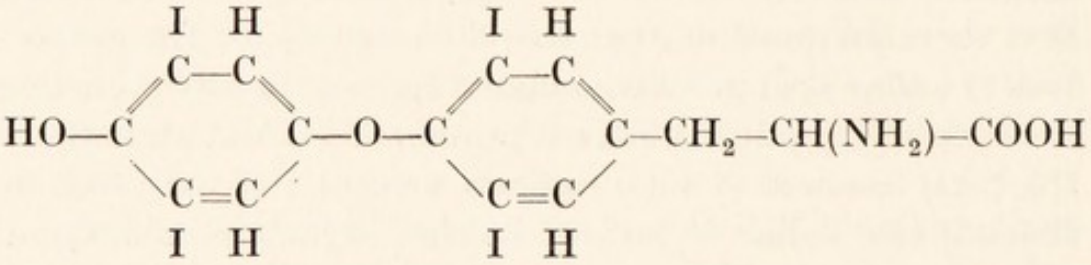
The iodine of sea-weeds seems to be partly in organic combination as diiodotyrosine radicals (149).

Various early attempts to isolate diiodotyrosine from the products of hydrolysed thyroid gland material failed. Harington recently succeeded in doing so (142), while Foster has obtained it from hydrolysed thyroglobulin (107), and Harington has obtained it in the optically active dextro-form by enzymic hydrolysis of thyroglobulin (142).

Thyroxine was isolated in crystalline form by Kendall on December 25th, 1914 (179). 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 the thyroid. 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 oxidized tryptophane derivative containing an indole nucleus, and accordingly termed it " thyroxin " from " thyroid-oxy-indole."

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 (138). The final proof of synthesis was furnished by Harington and Barger (141). 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 [1] :—



Thyroxine, prepared from thyroid tissue, or synthetized, is optically inactive. Harington resolved it into its active dextro- and laevo-components (138) ; 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 (143). Successive treatments by pepsin and trypsin followed by adequate chemical treatment gave a brown powder contain-

[1] Harington's chemical procedures have been fully described in various monographs (140, 27, 144, 280). In 1926 Dakin also proved that 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 (141).

ing 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 $[\alpha]_{5461} = -3{\cdot}8°$.

In the course of his researches Harington prepared *thyronine* ($C_{15}H_{15}O_4N$, thyroxine without iodine), diiodothyronine (with only half the iodine of thyroxine), and dibromo- and tetrabromothyronine (the corresponding bromine derivatives). The physiological properties of these compounds have an important bearing upon certain theories concerning Graves' disease.

The Amounts of Iodothyroglobulin, Thyroxine, and Diiodotyrosine in Thyroid Tissue, compared with its Total Iodine Content. Kendall (179) 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). All, or practically all, the iodine of dogs' and hogs' thyroids is present combined in thyroglobulin, and evidence is steadily accumulating that thyroid tissue contains no appreciable quantity of any other iodine compound (20, 215), even after iodide has been administered (176).

A definite separation of the other two 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 in the hydrolysate.

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 the ratio of thyroxine-iodine to total-iodine varies from 28 to 60 per cent. (28). Leland and Foster (199), 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 29 per cent. of the total iodine (extreme values 14 and 41 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 (179) but have been reported for Australian sheep (83). Kendall and Simonsen showed that a corresponding variation exists seasonally in the thyroxine content of hogs in the United States (179).

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.) There is some recent evidence that proteases can—non-specifically—bring about such changes (cf. p. 28).

Comparison of Thyroglobulin from Normal and Goitrous Thyroids. Cavett, Rice and McClendon (63, 62) have shown that thyroglobulin has a constant amino-acid composition except for thyroxine, diiodotyrosine and tyrosine radicals, whether the thyroid be normal, colloid, adenomatous, or of Graves' disease. Goitre is associated with abnormal thyroglobulin, and the abnormality consists in deficiency of thyroxine and diiodotyrosine radicals with a proportionately greater content of tyrosine radicals. There is no relationship between thyroxine content and basal metabolic rate in colloid and adenomatous goitres, but those from cases of Graves' disease tend to have a thyroxine content inversely proportional to the basal metabolic rate. It would appear that in the goitrous thyroid conversion of tyrosine radicals to those of diiodotyrosine and especially of thyroxine is interfered with.

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. Such comparisons have been extended to include the results from injection of soluble derivatives such as thyroxine.

Various effects have been selected for basis of comparison. Such selection has been critically reviewed by Kendall (179). In order of importance the tests available seem to be: (i) the effect on myxoedematous patients, patients suffering from a definite and preferably a marked deficiency of the thyroid principle; (ii) the effect on oxygen consumption of small animals such as the rat (290); (iii) increase in the resistance of mice to acetonitrile (162); (iv) decrease in growth rate and production of organ hypertrophy in young rats (53). Amphibian metamorphosis is frequently used for such comparisons, but is not so specific. Accurate comparative studies with myxoedematous patients have only recently been made, and will be discussed last.

Results with the Oxygen-consumption Test. Naturally occurring *l*-thyroxine is about three times more potent than *d*-thyroxine; it should, therefore, be about 50 per cent. more active, physiologically, than the ordinary racemic thyroxine obtained from thyroid by hydrolysis with alkali. Pure *l*-thyroxine is less active than the mixture of thyroxine and thyroxine-peptide which Harington obtained by enzymic digestion of thyroglobulin. Thyronine (cf. p. 23) and diiodotyrosine are inactive. Diiodothyronine and tetrabromothyronine (bromothyroxine) only show slight physiological activity (113, 143, 142). Thyroxamine has no activity; the ketonic acid corresponding to thyroxine has about three-elevenths of its activity (57).[1]

[1] Work by Neufeld in my laboratory (263A) has shown that of all the tissues in the body thyroid contains most bromine; this, however, does not appear to be especially associated with the thyroglobulin molecule, and its significance, if any, is not known.

Results with the Acetonitrile Test. Reid Hunt found that while with most species of animals the feeding of thyroid increased susceptibility to acetonitrile (methyl cyanide), this treatment increases the *resistance* of mice to the poison. The cause of the difference between species has not been ascertained, but the effect on mice has been used extensively in studies of thyroid activity.

Using this test, Hunt showed that the activity of different thyroid preparations is closely proportional to their iodine content, and demonstrated that comparisons based on equal iodine dosage were legitimate. Results with the test on this basis have shown that iodothyroglobulin administered orally has about the same activity as desiccated thyroid, but when its solution is given intravenously it is without action. Diiodotyrosine shows only negligible activity (366, 346). Optically inactive (racemic) thyroxine, whether given orally or intravenously, is only about two-thirds as active as thyroid (162, 255). Since thyroxine represents at most only half of the iodine of the gland, and the remainder, as diiodotyrosine, is inactive, we should expect that if thyroxine represented all the activity of thyroid in tests based on equal iodine dosage it would show *greater* activity. Nor does the difference in activity between racemic and *l*-thyroxine completely account for the discrepancy.

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 (53).

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 (270). Diiodotyrosine has no effect in cases of myxoedema and cretinism (323). Thyroxine has the same effect on such cases, qualitatively, as has thyroid itself (179), and Harington's thyroxine-peptide has at least as great a quantitative effect (143).

Recent Tests with Myxoedematous Patients. The results of Means, Lerman and Salter (249, 201) and of Thompson and his co-workers (335) derived from accurate calorigenic studies based upon equal iodine dosage lead to the following conclusions : Synthetic thyroxine in alkaline solution given orally produces 70 to 80 per cent. of the effect of the same dose given intravenously, and a much greater effect than when given orally in neutral suspension. The effects of thyroid, given orally, and thyroxine, given intravenously or subcutaneously, are equal (335, cf. 121). Thyroxine-peptide (49 per cent. iodine) is much more soluble than thyroxine, and produces the same effect when given orally or intravenously. Its effect is equal to that of thyroxine, injected intravenously. *The calorigenic activity of whole thyroid depends on its total organic iodine content*, and not on its thyroxine content. This suggests the paradoxical conclusion that diiodotyrosine, inactive in itself, acquires calorigenic properties when linked to other amino-acids in thyroglobulin.

Furthermore, Salter and Lerman (303) have shown that thyroglobulin from non-toxic, and from iodinized toxic goitres, and the thyroxine-peptide from either, all have essentially equivalent activity in terms of iodine (cf. Palmer and Leland (271)).

Salter and Pearson (304) have recently carried out an experiment which undoubtedly has a bearing upon the synthesis of the active principle in the thyroid. They digested human thyroglobulin by pepsin, removed the thyroxine fraction, and proved that the residual diiodo-

tyrosine-peptone was calorigenically inert. This was concentrated and subjected to peptic synthesis, following Wasteney's procedure. An artificial protein was obtained of large molecular size, containing iodine and with properties somewhat like thyroglobulin. They found that this contained an iodine fraction resembling thyroxine, and that it relieved myxoedema as effectively as thyroglobulin. Their results afford the first experimental evidence that the inactive diiodotyrosine-fraction is a potential source of the active principle and can have activity conferred on it by protease action.

Summary of Results from Tests of Comparison. There is agreement to the following extent: Thyroglobulin and thyroxine-polypeptide have the full activity of thyroid. Pure diiodotyrosine is inactive.

There is a distinct discrepancy between conclusions from tests on animals and on myxoedematous patients regarding the relative activity of thyroxine. The former suggest that it is less active than thyroid (and therefore thyroglobulin), the latter that its activity is equal.

The Active Principle of the Thyroid. Is this principle, by which we must infer the compound secreted from the gland into the blood, thyroxine, thyroxine-peptide, or thyroglobulin itself? Evidence can be advanced in favour of each of the three. Qualitatively thyroxine exhibits all the activities of thyroid. Quantitatively its action is at least of the same order, and may be equal. Thyroxine-peptide is so resistant to enzyme action that when thyroid or thyroglobulin is ingested much of the resulting activity must be due to absorption of this peptide; evidence as to the mode of excretion suggests that no free thyroxine is absorbed from the gut following thyroid administration (18).

On the other hand, the active iodine-compound in blood behaves as if it is a protein (cf. p. 92). Anaphylactic results obtained with guinea-pigs sensitized to thyroglobulin show that at least some thyroglobulin can be absorbed unaltered

from the intestine (146, 19); there is similar evidence that it can pass from the thyroid to the circulation (58), and the claim has been definitely put forward that it is the actual principle which is secreted (20). Yet, while we know that insulin and other proteins with molecular weights of about 35,000 normally pass across animal membranes, it is difficult to believe that a globulin with a molecular weight of nearly 700,000 (225) can normally do so to an appreciable extent.

Harington (139) has advanced a theory which would explain the activity of diiodotyrosine radicals in thyroglobulin. He suggests that the actual principle is a polypeptide molecule with the structure :

(*Thyroxine radical*)—(*Amino-acid radicals*)—(*Diiodotyrosine radical*).

Further work will be necessary to test this theory. In the meantime it seems important to stress again the finding that *thyroid activity is proportional to total iodine content, and not to thyroxine content.*

The Path of Discharge of the Essential Principle. Earlier studies of the secretory process have been reviewed by Marine (239). The recent investigations of Ludford and Cramer (208), Grant (128), Krogh, Lindberg and Okkels (191), and Severinghaus (309), have led to conclusions which, while in general agreement with earlier theories, permit a clearer visualization of the actual mechanism.

Ludford and Cramer employed extreme cold as stimulating factor to the thyroids of epilated rats. The others have taken advantage of the now recognized thyrotropic action of pituitary grafts or injections of specific pituitary extracts to contrast normal and very active thyroids in the amphibia *Amblystoma Jeffersonium* and *opacum*, the duck, guinea-pig, sheep, and Rhesus monkey. Severinghaus' paper presents a critical summary and permits the following conclusions :

The secretory cells of the thyroid pass through phases of activity and rest. They may secrete apically into the adjacent acinus, or basally into the blood stream, and they

also normally transport stored secretion from the acini to the vascular channels at their basal borders.

As a result of undue and prolonged stimulus, these " chief " cells become exhausted, and may degenerate to " Langendorff colloid " cells. In the thyroid of the duck (and probably of other birds) atrophy of these Langendorff cells produces at their sites direct channels of communication between follicular lumen and peripheral circulation and (emergency) release of follicular material secondary to the normal intracellular transmission. The latter is practically the sole procedure in other animals.

Formation of secretion is evidenced by cellular enlargement and formation of intra-cellular colloid vesicles ; the Golgi apparatus enlarges to a prominent network, tending to move apically from its resting position applied to the nucleus. Numerous fuchsinophilic granules are seen in this apical region, and perhaps may be actual antecedents of the colloidal secretion. The latter appears first as droplets in juxtaposition to the Golgi apparatus. In normal resting glands these may be passed apically to the lumen of the adjoining acinus. In hyperactive glands whose colloid content has been discharged, they tend to move basewards, to be excreted directly into the blood.

The Golgi apparatus retains its apical position whether the cell be secreting or transporting colloid, but does not enlarge during transport of material. Thus its size gives some clue to the action in progress at any given time, permitting conclusions as to whether droplets are newly formed or merely in transport.

During transport from the acini the apical region of the cell expands to become dome-shaped. The mitochondria increase in number and size. Numerous intra-cellular colloid vacuoles are present, and may be conspicuous in the basal region.

Grant considers that non-staining and staining materials are probably different stages of the secretory product. Freshly formed secretion and secretion in process of passage through the cell membranes are probably non-staining,

while within the cytoplasm, or within the lumen of the acinus, the secretion acquires chromophilic properties. Severinghaus suggests that the degree of staining may be due to the degree of colloid " concentration " or " dilution," " dilution " being necessary for transport through either apical or basal cell membranes. If these terms be allowed to connote chemical as well as physical changes, both passage through membranes and difference in staining properties are more easily explicable.

The observations of Okkels (269) and Wahlberg (347) are in general agreement. The latter considers that in the normal thyroid the greater part of the parenchyma at any one time is functionally inactive and in reserve.

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 thyroglobulin. 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, for passage to all the cells of the organism. If the demand is excessive, newly formed " principle " may be passed directly to the capillaries without storage.

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 body cells ; this seems the immediate logical conclusion from the depression of heat production caused by thyroidectomy, and the opposite effect which follows thyroid feeding. Two problems immediately suggest themselves :

What is, precisely, the nature of thyroid effect upon cell oxidations? Are all the manifold effects associated with decreased and increased thyroid action traceable directly or indirectly to level of oxidation in the tissues? At least some of these manifold effects must be described, to understand the significance of these questions.

Experimental thyroidectomy produces in animals at all ages a marked diminution of basal and general metabolism. In the young animal general growth, ossification, and development of the sex organs are all retarded. Thymus involution is delayed. The anterior lobe of the pituitary and the cortex of the adrenal gland are somewhat enlarged. The skin becomes thickened; its hairy covering develops imperfectly. There is usually a marked lack of intelligence, indicating involvement of the central nervous system, especially the brain. Body temperature is subnormal. The blazing fire of young life is damped down.

In the adult animal corresponding effects are produced where possible. Muscle loses tone and becomes weaker, and muscular activity is diminished. Sexual function is depressed. The nervous system is affected; dullness and apathy are marked. The skin is dry and hair tends to fall out. Anaemia is usual. Regeneration of tissue is retarded. Body temperature is subnormal, heat production is lowered, and consumption of oxygen and production of carbon dioxide are lessened. The power of heat regulation is decreased. Carbohydrate tolerance is raised (312).

Administration of thyroid or thyroxine produces most diverse effects, increased catabolism in mammals, especially associated with nitrogenous metabolism, increased protection against acetonitrile poisoning in mice (but not in rats), accelerated metamorphosis of amphibian larvae, changed feathering in birds.

When thyroid is fed to a thyroidectomized animal dosage can be adjusted to produce a normal animal—normal, provided that dosage be maintained. When it is fed to a

normal animal, the animal loses weight, with increased excretion of urea and creatine, increased oxygen-use, carbon dioxide production, heat production, and oxidation of carbohydrate (179). The glycogen reserve of the liver is depleted (179); muscle glycogen is also affected. Hyperglycaemia may result (it is not uncommon in clinical hyperthyroidism).

In young rats and rabbits thyroid or thyroxine feeding produces a lessened rate of growth, along with disappearance of body-fat, lessened muscle-bulk, and hypertrophy of heart, liver, kidneys, adrenals, pancreas, spleen, and lymphatic tissue. The animal's thyroid is distended with colloid, bloodless, and thus relatively small—a resting gland (53). Mice behave somewhat similarly (298).

Markedly toxic effects are produced in most animals by continued high dosage, with fatal termination if the treatment is prolonged. It has been shown that in rabbits the toxicity is increased by an increased external temperature (33° as compared with 25°), and death is preceded by a general acidosis which may be due to accelerated and incomplete tissue oxidation (91).

The mechanism of the protective action against acetonitrile in mice (cf. p. 27) is not yet known. Hunt thinks it is due to acceleration of oxidation of the poison to formic acid and thiocyanate. There is evidence that thyroid catalyzes the demethylation of acetonitrile, but that its action ends there (21).

The premature metamorphosis of tadpoles is specifically an effect of iodine rather than of thyroid. Thyroidectomized tadpoles will not metamorphose at all unless iodine in some form is given. But of all iodine preparations thyroid and thyroxine are most effective; the latter is 100 times as effective as diiodotyrosine (179). Yet, in the thyroidectomized, hypophysectomized Colorado axolotl, which, untreated, will not metamorphose, intraperitoneal implantation of powdered crystalline iodine rapidly induces metamorphosis

(166, 342). It seems that the thyroid principle acts, as regards metamorphosis, merely as a purveyor of iodine in very effective but not specific form of combination.

Thyroxine appears to act as a depressant of cell division, since it has been shown to retard the cleavage rate and differentiation of the eggs of the sea-urchin and the ascidian, and of *Paramoecium* (339, 365), an effect not produced by other iodine compounds. Whether this effect can be ascribed to enhanced oxidation is not certain, but there is some evidence that thyroxine increases the level of oxidation of sea-urchin spermatozoa (60).

Most races of fowls exhibit certain secondary sex differences in the feathers of the neck, wing-bow and saddle. When male birds are fed small doses of thyroid, and a patch of feathers plucked in these regions, the new feathers do not show the characteristic male plumage. Larger thyroid dosage produces rapid moulting, and the new feathers show depigmentation (164, 68, 367, 189). There is some evidence associating the gonads with these changes, though no adequate simple explanation is yet available (79, 158). The depigmentation may well be associated with such an increased level of oxidation as would tend to inhibit melan information, and is definitely traceable to the thyroid principle.

These varied phenomena following heightened thyroid action in the organism cannot all be traced to a raised level of oxidation, though in none can proof be yet adduced against this explanation, and in many it seems rational. The *in vitro* experiments to be described immediately lend support to this view. The response to onset of cold weather in areas such as the Central United States also is suggestive of oxidation and heat production by the thyroid. There is usually a physiological enlargement of that gland in farm animals, and its iodine content decreases—due to increased output of the iodine-containing principle (179). A similar functional enlargement has been observed in pigeons.

In vitro Experiments. The surviving hearts of thyroidec-

tomized cats use less glucose than those of normal cats. Those from cats dosed with thyroxine use more, and those of thyroidectomized cats given appropriate doses of thyroxine use approximately the normal amount (10A).

Numerous experiments have demonstrated that surviving tissues of thyroidectomized animals use less oxygen and those of thyroid-fed animals use more oxygen than do normal controls. Mansfeld, in a recent important paper (236) has dealt thoroughly with the earlier European literature. Some typical results may be stated briefly.

Surviving strips of diaphragm muscle from thyroidless rats show a 25 to 30 per cent. oxygen-use below normal (107A) Tissue cells from cretin pups and lambs show a similar decrease, whilst muscle-strips from others given thyroxine for five weeks previously show a marked increase (93A). Blood from alligators treated with thyroxine shows greater oxygen consumption than blood from controls, while when thyroxine is added directly to alligator's blood there is sometimes increased consumption. Thyroxine produces increased consumption of glucose and formation of lactic acid, and a lowered respiratory quotient in these animals (308).

Numerous investigators have employed Warburg's method of measuring oxygen-uptake with minced tissue or tissue slices. Mansfeld has summarized such work admirably, and to his account may be added that of Andrus and McEachern (13, 231). The results indicate beyond doubt that thyroxine (or thyroid) produces acceleration of anaerobic oxidation in tissue cells.

Such results, and those with surviving organs separated from nerve control, suggest a peripheral site of thyroid action. Yet there is considerable evidence supporting central action. Thus, if continued and sufficiently large doses of thyroid are fed to normal dogs, death ensues, but such doses are not fatal to dogs whose sympathetic nerves to the heart have been sectioned (Bohnenkamp and Enderlen, 1931). Patients with brain disease are refractory to thyroxine

(Falta and Högler, Sehle, Leschke). Vererbély (1932) claimed, indeed, that thyroxine produced increased oxygen-use in brain cells, but not in other tissues.

Mansfeld himself has obtained the following important results : Vererbély's results were not obtained when strict asepsis and stricter comparison with controls were employed. Measurements under anaerobic conditions in presence of a hydrogen-acceptor such as methylene blue completely confirmed earlier work, showing that pre-thyroxine treatment increased oxygen consumption of all organs of both warm and cold-blooded animals, while Ahlgren's figure for optimum concentration, 10^{-13}, was confirmed. Increasing the thyroxine concentration above this figure *diminishes* the effect. The conditions in these experiments suggested that the acceleration produced might be specifically that of dehydrogenation rather than oxidation ; this proved not to be the case. Accident led to the discovery that thyroxine-action is not directly associated with the reduction of methylene blue to its colourless base, but is linked with the fore-period of such experiments, in which hydrogen-transfer does not take place. *During the subsequent oxidation-phase thyroxine is inactive.*

Warburg had shown that if ethyl carbylamine, C_2H_5NC, is added to an enzyme system, the aerobic phase becomes an anaerobic one. Mansfeld deduced that in such a condition no fore-period of incubation with thyroxine should be necessary to demonstrate its action, and showed that this indeed is the case, and that in presence of ethyl carbylamine all tissues tested showed increased oxygen-use on addition of thyroxine, with the sole exception of muscle (in which probably lactic acid decomposes the ethyl carbylamine).

Mansfeld then showed that in physiological concentration (and it is important to stress this, as contrasted with effects due to vastly greater, non-physiological dosage) thyroxine does not increase glycolysis, but rather depresses it (while, correspondingly, addition of glucose lessens the increased

oxygen-use due to thyroxine). He confirmed earlier results that where thyroxine does increase oxidation there is a parallel increase of production of ammonia. Autolysis, largely decomposition of protein, is increased by the presence of thyroxine. He concludes that *thyroxine acts on the cells themselves, primarily in facilitating changes in protein metabolism*, and that probably an increase in some of the resulting products leads to the increase in oxygen-use. Such a conclusion agrees with that of Haffner (1927), who, finding that potassium cyanide does not inhibit the action of thyroxine in accelerating metamorphosis in tadpoles, concluded that it acts on the anaerobic phase of cell life.

This part of Mansfeld's work relates to study of the effects of thyroxine outside the intact organism. It is to be noted that in this type of experiment the tissue cells or some large proportion of them suffer some degree of damage in the experimental procedure.

He next attempted to find an explanation for the fact that after administration of thyroid or thyroxine there is a latent period of twelve to twenty-four hours before increased oxygen consumption can be demonstrated in the intact organism, a fact completely at variance with its immediate action on the isolated cell. He has found that when the two kidneys of a rabbit are removed by successive nephrectomies, and thyroxine is injected into the animal between the two operations, the second kidney shows a definitely increased oxygen consumption, but that if, initially, both kidneys are denervated, there is no thyroxine effect. Hence it would seem that thyroxine can only reach the cells of an undamaged organ if its innervation is intact. This suggested to Mansfeld that thyroxine is taken up by the nervous system and transferred to tissue cells through the peripheral nerves in a manner comparable to that of tetanus toxin and certain viruses. In support of this view he has found that when the two hind limbs of a frog are carefully removed and laid in a moist chamber, and the living end of one ischiadic nerve is

allowed to dip into a Ringer solution, whilst that of the other dips into a Ringer solution containing thyroxine in concentration 10^{-12}, after the preparation has been kept for twenty to forty hours at 16° to 18° if the gastrocnemii are minced and oxygen consumption measured in Warburg's apparatus the " thyroxinized " muscle shows a 20 to 32 per cent. increase in oxygen consumption above that of the control. The observation of Schittenhelm and Eisler (1927) is quoted in support : they found that after administration of thyroxine to an animal the iodine content of the middle brain and tuber cinereum is increased ten-fold, though it drops to normal values within twenty-four hours.

Therefore Mansfeld concludes that thyroxine (and presumably also the actual thyroid-principle, whatever that be) passes by the nerve fibrils to the interior of tissue cells, reaching the zone of least aerobic oxidation, the zone where its own action can be most effective.

The experiments of Andrus and McEachern (13, 231) are in general agreement with the *in vitro* results that have been quoted. They noted further that the increased respiration of tissues of animals rendered hyperthyroid is not as great as would be expected from the increase of metabolism in the intact animal, and no new nor abnormal mechanisms are involved. They also find that increase in tissue glycolysis is not the fundamental cause of increased oxygen consumption in hyperthyroidism.

Summary. The results following thyroidectomy and the restoration of the thyroidectomized animal to normal state by administration of thyroid or thyroxine in appropriate dosages are strongly suggestive of control of oxidation level by the thyroid principle. Many of the results induced by artificial hyperthyroidism are susceptible of a similar explanation ; other actions seem more remote, or specific. (The clinical pictures associated with human hypothyroid and hyperthyroid states also present features which seem untraceable to mere control of grade of oxidation (248).)

Experiments with surviving tissues show definitely that thyroxine (and presumably the thyroid principle) increases their consumption of oxygen, and thus their heat production. There is strong evidence that its catalytic action is applied to some anaerobic stage of intracellular metabolism not directly associated with oxidation or dehydrogenation, but not improbably concerned with the deamination of protein metabolites. In addition there is good suggestive evidence, which it is to be hoped will be rigidly tested as soon as possible, that in the intact animal the thyroid principle reaches the interior of the tissue cells by the route of the nervous system, and the nerve fibrils penetrating the cells. If this interesting hypothesis proves true, at least a partial explanation will have been afforded of the curious lag in thyroid action following its administration therapeutically or experimentally.

It seems logical to conclude, at the present stage of our knowledge, that the varied effects associated with thyroid activity all involve increase in oxidative processes, but are not themselves necessarily the results of the heightened level of oxidation.

Non-thyroid Catalysts of Metabolism. Recent work by Magne (235), Tainter and Cutting (329), Dodds (88) and others has shown that various nitrophenols and similar compounds markedly increase the oxidative processes in the organism. The compounds chiefly studied have been 2 . 4 . dinitrophenol and dinitro-o-cresol. They increase the oxidative processes of all tissues. With sufficient dosage body temperature is increased, and there is marked sweating. With still higher dosage animals and man may die following extreme hyperpyrexia. There is evidence that fats are oxidized in preference to carbohydrates, while protein metabolism is not so much affected. Moderate persistent dosage produces and maintains a high basal metabolic rate (+ 30 to + 50 per cent.) without undue symptoms other than steady fall of body weight.

The action of these compounds shows a little, but only a little, resemblance to that of thyroid. Liver and muscle glycogen are diminished, but tadpole metamorphosis is not accelerated, and there is no beneficial effect on myxoedema even when the basal metabolism is maintained above normal for a considerable time (93, 329, 88).

These drugs are being extensively used in the treatment of obesity, with good results. But all writers stress the necessity for great caution in their use and continued control of the patient. Several fatalities have been reported. The susceptibility of dogs with experimental diabetes is greatly increased. Evidently marked caution is required in using this method of treatment with obese diabetics.

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.)

Gley, writing in 1926, could find no convincing evidence in favour of this view (119, cf. 174). But favourable evidence is accumulating.

According to Sunder-Plassmann (325B), each thyroid cell is under sympathetic nerve control through a terminal reticulum. If impulses from the central nervous system are abnormally strong, no matter through what cause, a hyper- or dysfunction ensues. Under pathological conditions the terminal reticulum is destroyed and the thyroid is out of control (cf. also p. 95 footnote).

Nonidez (266) adduces anatomical evidence that the blood supply of the thyroid is under close control of the nervous system, which may thus indirectly influence the rate of secretion and of discharge of its principle. Bachromejew and Ter-Ossipowa (16) have observed histological changes in the thyroid suggesting increased activity, following stimulation of the peripheral end of the superior laryngeal nerve.

In earlier work Nonidez (1931) found evidence for the existence of a specific thyroid nerve made up of fibres from the superior cervical ganglion of the sympathetic and para-sympathetic fibres from the superior laryngeal branch of the

vagus. Mr. Donald Ross, working under the direction of my colleague, Professor V. H. K. Moorhouse, has confirmed and extended these findings for the dog (the data are in course of publication). He finds that in most dogs the origins of the thyroid nerve are those stated by Nonidez, but that occasionally the vagus fibres arise from the ganglion nodosum, or the vago-sympathetic trunk, instead of the superior laryngeal branch.

Ross has further shown that stimulation of this thyroid nerve consistently slows the flow of blood through the gland, but has been unable to demonstrate any definite histological changes following continued nerve stimulation in acute experiments. His results strongly support the view that *there exists an important indirect control of the thyroid gland through nervous regulation of its blood supply.*

It has been shown that after bilateral splanchnectomy, splanchnico-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 (296).

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 VIII).

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 (92). Attention may be drawn to one or two phases dealt with in recently published papers.

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 (37), 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 (268), by Bierring (26), and by Talbot (331).

Impedance Angle Measurements. Claims that the so-called " impedance angle " (related to the impedance by the body to an alternating current) is more accurate in diagnosis of abnormal thyroid states than basal metabolic rate determinations (44) do not seem to be justified (297, 159).

Estimation from Pulse-Rate and Pulse-Pressure. Attempts have been made to determine the basal metabolic rate from formulæ based upon pulse-rate and pulse-pressure. While some degree of relationship exists, the potential error is too great to give that certainty of information required from a diagnostic test (284, 72, 282).

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 (9, 327, 133), that of students of South Carolina averages 10 per cent. below (287), and similar results have been obtained for students in Florida (337). (However, similar results were also obtained for those in the more temperate surroundings of Minneapolis (73)). Other findings for places in temperate climates are in almost complete agreement with the standards (129, 54).

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 (94). Results for Japanese are conflicting (341, 330, 234). 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 (341). Eskimos in the Baffin Bay district gave values averaging 33 per cent. higher than the standards (145). 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 (24). Low values have been obtained for Australian aborigines (352).

While the precise causes of these racial differences cannot be stated, differences in diet are undoubtedly an important factor. Thus the low figures obtained for medical students in Madras— — 12 per cent. for males ; — 16 per cent. for females—have been attributed to low protein diet and ready muscular relaxation (188). Nevertheless, Benedict, in a recent review of the literature (23), says that the existence of a racial factor can no longer be doubted, and he regards as especially noteworthy the findings for female Tamils (17 per

cent. below the standards) and for male Mayans (8 per cent. above). (Cf. also (340).)

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 (92).

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 (86).

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 (92).

Basal Metabolism in Disease. The relatively large correction 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, in chronic encephalitis with Parkinsonism and in intestinal obstruction. 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 (92). It is generally assumed that any change from normal in a patient with pituitary disease is due to a pituitary-thyroid interrelationship (cf. Chapter VIII).

Bearing directly upon the association of abnormal basal rate with abnormal thyroid function is the finding that an additional injection of 0·1 mg. of pure human thyroglobulin to the daily dose raises the rate 10 $\pm$ 5 per cent. (97).

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.

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

[1] For an example of a fully differentiated classification, see Joll (174).

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 (69, 104).

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 (174).

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 " (174).

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. Marine, in a recent review (241), still considers that there is inadequate support for such views.

The goitre of mountainous regions has been variously termed parenchymatous goitre, adenoparenchymatous goitre, simple hyperplastic goitre and chronic hypertrophic goitre. According to McCarrison (221), 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 (362). They consider, on probably insufficient evidence, that the ultimate atrophy is the cause of myxoedema in adults (cf. p. 70). de Jong believes that lymphadenoid goitre is not a true goitre, but a chronic thyroiditis (185).

Joll has dealt very fully with the pathological histology of simple goitre (174).

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. 14). 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 (105), Argentina (244), Hungary (33), and China (8). There is complete agreement between these surveys; the inverse relationship definitely exists. Japan is practically non-goitrous. The iodine con-

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
Switzerland					*Switzerland.*				
Effingen	1·0	7	28–108	64	Kaisten .	62	11 (10 G.)	4–29	19
Italy.					Hunzenschwil	56	12 (10 G.)	5–28	17
Ligurian Coast	0	3	94–140	112	*Norway.*				
Norway.					Hostvedt	60	4	26–69	40
Vik i Sogn	0	6	107–240	173	Vittingfoss .	55	8	14–105	48
					Ljöterud .	54	5	19–41	29
					Meheia. .	54	5	10–62	39
Samoa.	0	12	–	302	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

G.: from goitrous individuals; N.G.: from non-goitrous individuals.

sumption of the Japanese is more than double that of any other people (226).

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 (224). The correlation is not quite so marked in Alberta, Canada (350).

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 (150). 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 (224).

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 (105, 209, 149).

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. 222, 218).

The Effects of Administration of Iodine Compounds in Preventing and Benefiting Endemic Goitre. Before dealing specifically with the subject named in the heading, 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.

Prout appears to have used iodine in the treatment of goitre as early as 1816, five years after its discovery (326), Coindet used it in 1820, and Boussingault in 1833 concluded that the iodine in natural salts used in certain districts in South America acted as a preventive of goitre (229). Prévost advocated its employment as a preventive of goitre in 1849. During the following decade the treatment was used to some extent in Switzerland, Austria, and Italy, the 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 (181).

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 (184), Norway (264), Italy (260), Austria (172), New Zealand (175) 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 (181, 277, 221).

McClure's analysis of the results in Michigan following ten years' prophylactic use of iodine shows that now only 1 per cent. of the school children have goitre, as compared with 35 per cent. in 1924 (228).

Keith (178) 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. Pig litters were usually hairless, dying within twenty-four hours. All cows were slightly goitrous. All calves from two year old heifers were goitrous, most of them died, and the remainder became cretins. Subsequent calves were somewhat less affected. Mares became goitrous, and, after they had been in the valley three years, their colts were goitrous. These were frequently carried twelve or even thirteen months, and then did not survive. Poultry brought into the valley appeared to thrive, but their eggs, though containing embryos, failed to hatch.

Babies born in the district had goitre. Almost every woman coming into the valley developed it within a few

months, and bachelors with no access to fresh milk were similarly affected.

Mr. John Ronayne, one of the chief farmers of the district, made a series of careful observations of these effects, and sought advice about treatment. He was referred to Marine, who advised iodine, as tincture or iodide. The results appeared miraculous. All goitres disappeared from the animals and there was no further trouble in rearing stock or fowls. Keith, in 1922, five years later, could not find a goitre in animal or human being. The cost of treatment was negligible. Two dollars' worth of tincture of iodine annually sufficed Ronayne to keep 100 cattle, 12 horses, 30 pigs, and 200 chicken, and their progeny free from goitre.

The thyroids of wild animals in this district appeared to be normal, while, prior to the iodine treatment, it was observed that goitre in all animals tended to recede in summer and autumn, and animals born in the fall were able to live.

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 (99) 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 administra-

tion 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 (358).

The sole important exception to the beneficial use of iodine is New Zealand. While an early survey showed an inverse proportionality between iodine of the diet (or the urine) and incidence of goitre (151), and while iodine treatment at first appeared to give as good results as it has elsewhere (175), a more recent report (313) shows that the inverse relationship no longer exists, and in certain districts there has been a marked increase in goitre in school children. Although the investigators state that there has been no change in environment (including water supply and food supply) in the five years' interval between the two surveys, it would appear quite probable that a closer investigation might reveal some other varying factor, such as changed calcium intake, and that, to counteract this factor, a still larger iodine intake is desirable.

Some Potential 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 (220) 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 (115, 70). Others have suggested factors which might prevent the absorption of iodine from the gastro-intestinal canal, such as its presence in an unassimilable form (178), or chronic digestive infections (275).

(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 (305, 182), who form but a small minority of those who have published results concerning the treatment.

Stott (322) has studied the distribution and cause of endemic goitre in the United Provinces (India). Graves' disease is absent, but hypothyroidism 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. 58.)

Straub considers that iodine deficiency is the chief factor in the development of goitre in the Hungarian plain, and that calcium does not play any part (322A).

Several investigators have claimed to be able to isolate various specific organisms from goitrous thyroid glands (300, 56, 80); it has been further claimed that dogs inoculated with such isolated organisms will develop goitre (80).

McCarrison (216), 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 that there is a racial factor; the highest incidence is amongst children of Central European and Jewish parentage. It is probable, however, that an unbalanced diet may

constitute the actual factor. Cabbage is frequently a predominant constituent of their diet (cf. p. 59). The diets of many Jewish children are poorly balanced and too rich in fat (1). The frequency of goitre in the Carpathians has also been attributed to excessive use of cabbage in the diet (325A).

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. Numerous investigations led to quite contradictory results, but proof that such deficiency actually can produce goitre seems at length to have been furnished by Levine, Remington and von Kolnitz (202, 204), who have shown that a diet containing 15γ of iodine per kilogram, providing rats with only 0·14γ per day, produces goitre in 35 days or less. Such goitrous glands show marked hyperplasia, lack of colloid and low iodine content. The minimum amount of iodine which definitely prevents goitre is 1 to 2γ per day.

Mellanby (251) has obtained goitres in pups when the mothers were kept on a diet low in iodine content during pregnancy, and the young, after weaning, were kept on a similar diet.

Hellwig has revived an old theory that excess of calcium salts in a diet may cause endemic goitre, a theory associated with belief that water from limestone sources is goitrigenic. He has produced hyperplastic goitre in rats fed on barley and given 2 per cent. calcium chloride solution as sole source of drinking water (147). In more recent work (148) he claims that moderate excess of iodine added to such diets results in colloid goitre; marked excess of iodine in prevention of goitre. Juanita Thompson's experiments are nearer to normal conditions (333). She finds that in rats fed diets deficient in iodine there is gross enlargement of the thyroid

associated with hypertrophy and hyperplasia ; these changes sometimes revert to atrophy. Addition of calcium carbonate to the diet results in greater and more rapid increase in size of the thyroid and a more marked hyperplasia, while all such changes can be prevented by increasing the dietary iodine.

Krauss and Monroe's experiments (187) also support the view that a high calcium content of the diet acts as an auxiliary factor in production of goitre.

McCarrison (217) 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 production 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 (356).

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.

Later work by various investigators may be summed up as follows: Cabbage grown at different places and at the same place at different times exhibits different grades of goitrigenic activity. In the Eastern United States goitrigenic properties appear in the growing cabbage early in November. The iodine content is not a factor (356). Cabbage grown in India (219) and in Great Britain (318) also shows goitrigenic properties, varying with season. New Zealand cabbage is goitrigenic, but to a less degree (149).

Brussels sprouts and cauliflower are goitrigenic, but various other members of the Brassica group of vegetables are not (356). Soy bean and ground nut are goitrigenic to rats (209). Rats seem to be more resistant to such agencies than are rabbits (168).

Cabbage dried *in vacuo* or in air, or extracted with ether or acetone, loses its goitrigenic power. The specific agent is not readily soluble in water even at 100° C. Mild acid hydrolysis does not destroy it; alkaline hydrolysis destroys it to a slight extent (356).

The active material is believed to be glucosidic in character, but attempts to extract such a glucoside have been unsuccessful. Since, however, it is known that cyanides are components of the glucosides in such vegetables, Marine and his associates injected various organic cyanides into young rabbits. Goitres were produced, and even exophthalmos (242). Marine believes that such goitres are produced through the intermediation of the pituitary (cf. Chapter VIII).

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 p. 84).

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 (with possible association with a deficiency of vitamin A), some goitrigenic factor (probably 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 (174). 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 possible that sporadic goitre may also be accounted for by some one or other of the causes just listed. Nevertheless, there seems to be the possibility of a genotypical factor, which renders certain families more susceptible to these causes (253).

Marine's considered opinion concerning the etiology of goitre, written in 1935 (241), should be quoted : " Goitre is a deficiency disease due to an insufficient supply of iodine. This iodine deficiency may be relative or absolute and may result from (i) factors that increase the needs of the body for thyroxine, such as puberty, pregnancy, the menopause, certain infections and intoxications, exposure to cold, excess of certain substances in the dietary, including fats, proteins, and calcium (the ratios of the latter with phosphorus and magnesium are also important), and deficient oxidation (for instance, thyroid reaction in anaemias or in the presence of oxygen deficiency such as occurs at high altitudes), (ii) factors that interfere with the absorption or utilization of a normal intake of iodine, and (iii) factors that bring about an abnormally low intake of iodine . . ."

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 " (150). " 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 " (172).

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 or 2 parts 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 (105). This, spread over 10 grams of salt (an average daily intake), gives a ratio of 1 : 550,000. A similar comparison in New Zealand (151) 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 (105).

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. 51) ; 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 are of the right order.

Eggenberger's conclusions, presented to the Second International Goitre Congress in 1933 (225) are that the necessary quantity for daily use is 1 or 2 micro-grams per kg. body-weight, and that if the average daily intake is less than 1 microgram the danger of goitre exists. If about 2 micrograms per kg. be ingested there is then no danger of goitre, even if susceptibility is increased by infectious disease or high fat or high cabbage diet. Iodized salt in proportion of 1 to 100,000 will give this safe ratio.

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 : 5,000 ratio suggest beneficial effects to normal animals rather than the reverse. Rats and pigs grow faster(137, 100). When a little iodide is fed continuously to sows during pregnancy the litters are improved and grow more rapidly (357). Cattle and goats give an increased yield of milk (306). Previously sterile cows produce normal calves (274). The increase in rate of growth is attributed to a slight depressant effect of small dosage on metabolic activity (153, 207).

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 (186), 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 (237) and von Jauregg (172).

" 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 : 100,000) which experience has shown is quite efficient in prophylaxis (51).

Too great a stress on the potential danger has led to restrictions in the use of iodized salt in Austria and elsewhere, restrictions deplored by authorities on goitre (173, 106, 14). Fischler (106) reports that in one district in Bavaria in 1928, when iodized salt was in full use, only 6 per cent. of the children were goitrous, while in 1933, after the mischievous propaganda against it had lessened its use, 52 per cent. were goitrous.

Perhaps the best argument in favour of iodized salt, in spite of possible risk to patients with non-toxic adenoma, is the ten years' report on treatment in Michigan (228). While for the first two years the number of cases of toxic adenoma operated on increased, subsequently the number of cases of both toxic diffuse and toxic nodular goitre operated on have rapidly and steadily decreased, whence it seems reasonable to assume that both types of toxic goitre are less apt to occur when there has been no previous enlargement of the thyroid. With this may be compared McClendon's finding (223) that in Japan, although the country as a whole is almost non-goitrous where the iodine intake is unlimited, yet in certain districts removed from the sea and without easy facility of communication both non-toxic and toxic goitres exist, in similar ratios to those found elsewhere.

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 (279, 183, 103). There would seem to be some slight possibility of thyroid imbalance ultimately resulting from such treatment (53). Joll (174) 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. 56).

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

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.)

report on the first case in 1920 (261). 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 (283); 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 (362) 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 conclusions of McCarrison (cf. p. 59) 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 (197). Anginal pain sometimes occurs following thyroid administration (103). 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 (334).

The importance of bone-age studies in diagnosis of childhood myxoedema has been stressed by Lisser, Shelton, and others (55).

Thyroid Dosage in Myxoedema. This will vary with the grade of the myxoedema, to which the basal metabolic rate is the most accessible clue. In all probability it is wise to start with such a dosage as, continued, will slowly restore a normal basal rate, several weeks being required to bring this about. While individual requirements will undoubtedly vary (cf. Chapter I) the analysis of results by Means and Lerman (248) from study of a large number of cases with complete myxoedema undoubtedly provides a useful guide. The salient features of their data are reproduced in Fig. 3 (slightly altered from their diagram), and it is necessary to insist, with them, that it presents a generalization, portraying approximately what happens in most cases, and not precisely what happens, unless by chance, in any single case.

Although 3 . 5 . diiodothyronine is much less active than thyroxine, its use has been suggested in myxoedema. A dosage of 50 to 75 mg. per day, orally, has been found to restore to normal and control severe cases. This is over fifty times the requisite corresponding dose of thyroxine. The action of diiodothyronine may be due to partial conversion to thyroxine by the thyroid. The dosage quoted does not produce toxic symptoms, and the compound is stable and easily obtained (10).

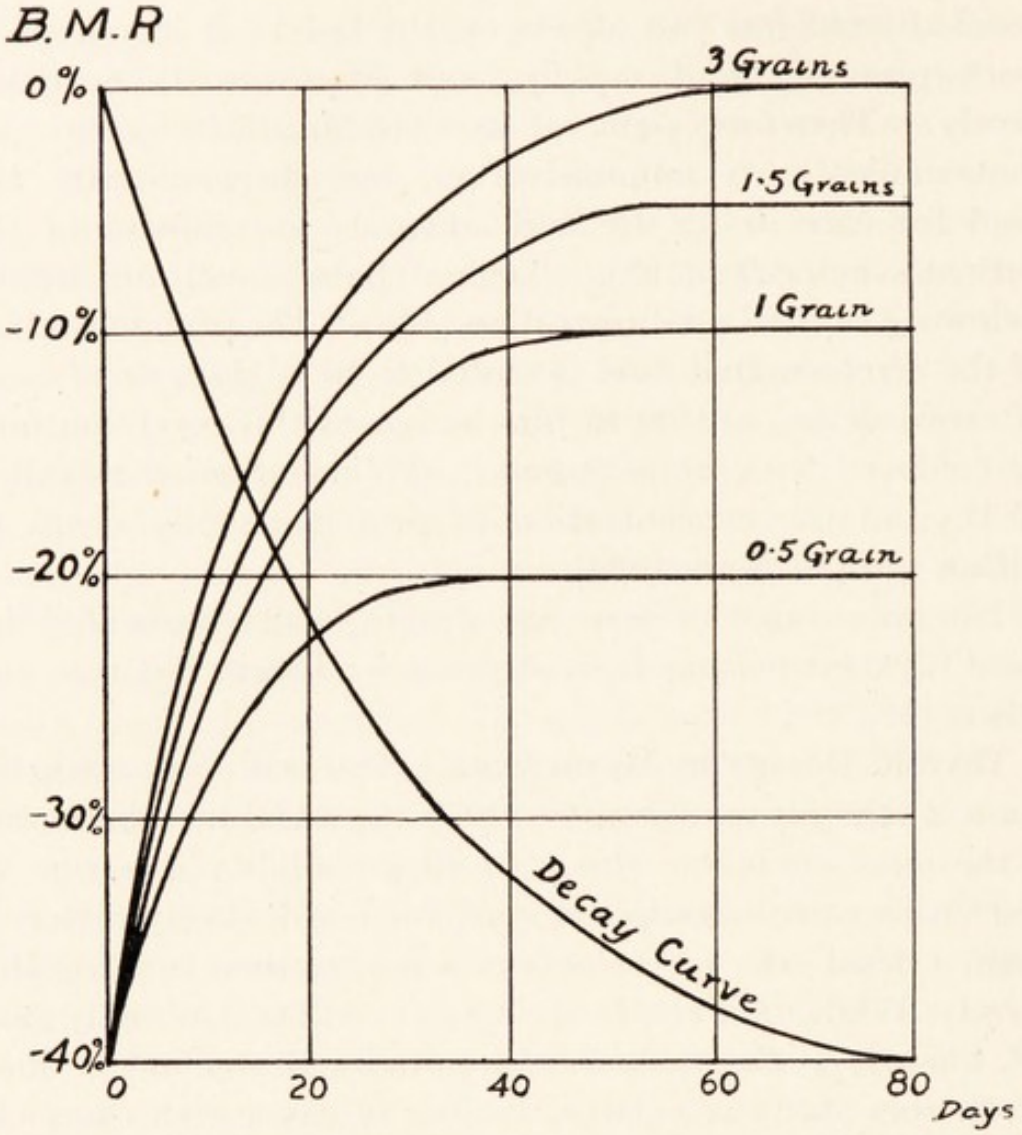


FIG. 3.—Diagram showing the approximate relationships between basal metabolic rate, and daily thyroid dosage (in grains of thyroid, U.S.P., containing 0·2 per cent. iodine), in patients initially with complete myxoedema. The decay curve indicates the B.M.R. response to be expected following discontinuance of thyroid administration to a balanced myxoedematous patient, or complete extirpation of the thyroid of a person with a normal B.M.R. (Modified from Chart 1, Means and Lerman, *Arch. Int. Med.*, 1935, lv, 1.)

Non-myxoedematous Hypothyroidism in Adults. Attention has been drawn to this syndrome by numerous recent writers (353, 232, 233, 319, 49, 194, 354). 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 subnormal 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 had, in the hospital to which I am attached, some years ago a number of pupil nurses who exhibited a definitely low basal rate and various minor symptoms, resulting from voluntary undernutrition due to the then current fashion of " reducing." Compulsory correct dieting caused the disappearance of this type of case (77, 310).

Atypical forms of myxoedema have been described, accompanied by rheumatoid pains, or severe menorrhagia or metrorrhagia, anaemia, or obesity (32). 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

[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 (71, 103).

depressed psychosis. Irritability and excitement may be sufficient to suggest a disordered mentality; thought distortion, with hallucinations and delusions, may suggest dementia praecox (109).[1]

Indian writers report that the abortion frequent in women exhibiting hypothyroidism can often be prevented by administration of thyroid (258).

The Heart in Myxoedema. An enlargement of the heart sometimes accompanying myxoedema was apparently first described by H. Zondek in 1918 (370), and has since been referred to by a number of other clinicians (101, 16, 125, 245, 116). The enlargement can be greater than in any other condition; there is often a resemblance to a pericardial effusion.[2] The heart-change is characterized by generalized dilatation, which may in part be due to oedema of heart muscle. Its action is sometimes very indolent, and there may be cardiac insufficiency. The electrocardiogram shows a low T wave. There is often remarkable shrinkage towards normal size, with restoration of normal action, following thyroid therapy. Digitalis therapy has no effect. Auricular fibrillation has been reported in one case, with a basal rate of —41 per cent. In this case thyroid therapy had no effect on the fibrillation (349).

Cretinism. A good example of both physical and mental benefit following administration of thyroid to a cretin from an early age has recently been reported by Close (65). The excellent results are well shown by the photographs in Fig. 4.

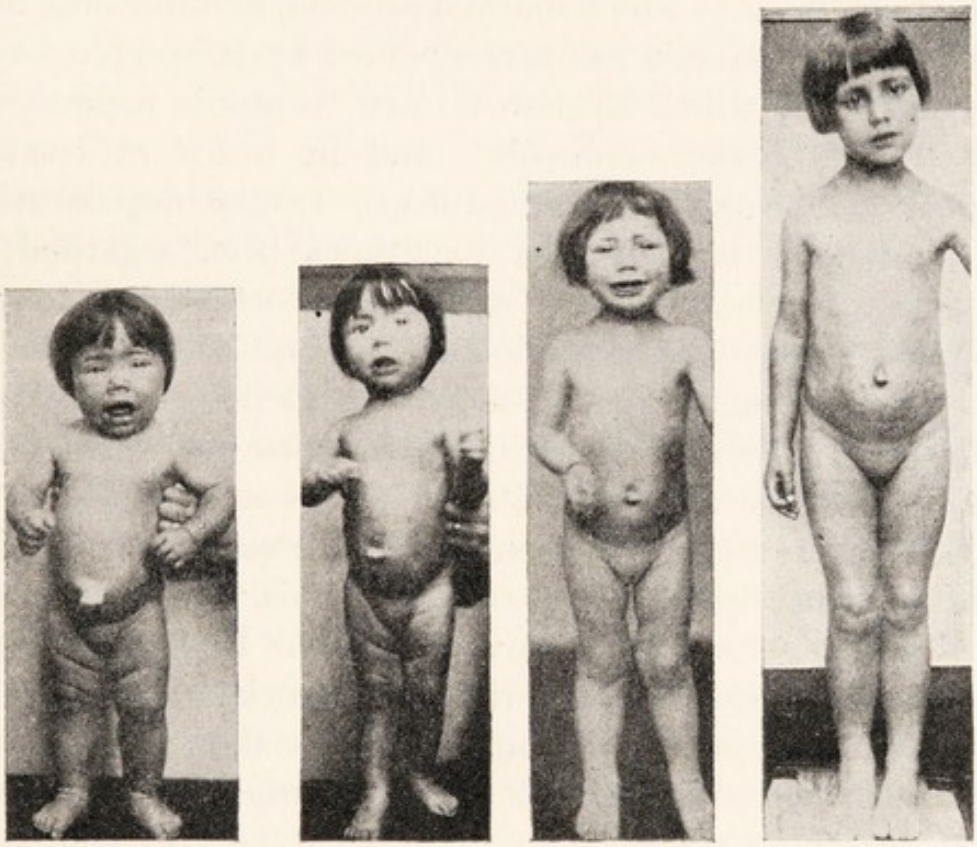
While there are individual variations in the intellectual level reached by children under treatment, good results are obtained with those who have normal birth and developmental histories, and have developed thyroid deficiency later

[1] 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 (161).

[2] In at least some cases the apparent enlargement is due to chronic pericardial effusion. These also respond to thyroid therapy (110).

in life following infectious disease, provided treatment is persistent (48).

Thyroid therapy in underdeveloped children is stated to produce a definite increase in height and in dental and bone



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

FIG. 4.—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.)

development, even though the basal metabolic rate is not increased (338).

The Thyroid and Rickets. Thyroidectomy in very young animals is said to produce a condition strongly simulating rickets ; this condition is not benefited by feeding cod-liver oil (193). There is some evidence of lowered thyroid function in human rickets, as judged by a very low blood iodine value, 2 to 5γ, instead of over 7γ per cent. Administration of vitamin D

preparations not only improves the rickets, but restores the blood iodine to normal limits. Injection of thyroxine into rachitic rats heals the rickets (265).

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 (98). 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 (17).

The changed etiology does not necessarily exclude thyroid treatment, which has produced excellent palliative results for several years in such cases (cf. 82) ; Boothby, however, considers that it is not indicated unless there is an associated myxoedema (38).

The Hyperthyroid State

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 → hyper-

trophy → 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.

The opposing views of Marine (238, 239) and of Plummer (278, 36) concerning the unity or otherwise of hyperthyroid states have still to be reconciled. Marine considers that hyperthyroidism is but one entity, a disease of the nervous system in which the visceral nervous system is most prominently involved, and which is characterized by a profound disturbance of the regulatory control and functional interactions of all organ activities, its most prominent manifestations being increased metabolism of thyroid origin, general asthenia, tachycardia and moderate thyroid enlargement. He insists that it is necessary to look beyond the thyroid for the primary disturbance. He admits that the disease may well be divided into *acute* and *chronic* forms, but prefers to reject such terms as *toxic adenoma*, *adenomatous goitre with hyperthyroidism* and *thyrotoxicosis*. He considers that the histological changes are constant, but not specific, and that the presence of hyperplasia of lymphoblastic tissue (cf. Plummer's views) should not be stressed, since it occurs also in Addison's disease and in " status lymphaticus," and may be merely an antagonistic or compensatory reaction. He believes (adversely to Warthin and others), that the disease can be acquired, especially by women, in middle life, though cases in early life may be associated with an inherited or constitutional condition.

In his earlier papers, in considering the etiology of the disease, he stressed potential interrelationships with the adrenal cortex and the gonads ; in a recent paper he has outlined a possible intermediate channel through the anterior pituitary (cf. Chapter VIII).

Plummer believes that there are two distinct entities in hyperthyroidism, exophthalmic goitre (Graves' disease)

associated with thyroid hyperplasia (diffuse hypertrophy), and a hyperthyroid state developing from non-hyperplastic goitre—localized hypertrophy, an adenoma. The latter may therefore be termed " hyperfunctioning adenomatous goitre." Mixed types of gland are possible, and approximately 20 per cent. of cases of Graves' disease are superimposed on old adenomatous goitres. The onset of Graves' disease is relatively acute, and the cause fairly definite. It is characterized by the nervous phenomena and the eye symptoms. In " hyperfunctioning adenomatous goitre " the toxic symptoms—nervousness, tremor, loss of strength and weight —develop slowly and insidiously over a period of years.

The hyperfunctioning of exophthalmic goitre is considered to be caused by hyperfunction of the whole gland, while that of adenomatous goitre is caused by a localized reaction in the gland.

Plummer points out that 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 1912–21. There were only three second resections. Of 4,992 cases of exophthalmic goitre, 326 came to subsequent second operation.

Plummer considered that in Graves' disease the thyroxine produced by the thyroid was not completely iodized, and the incomplete product was more toxic and accelerated metabolism more rapidly. Hence he was led to administer Lugol's solution post-operatively, with the idea of stimulating complete iodization, and thereby he almost completely abolished post-operative deaths. Subsequently he used the treatment pre-operatively with still further benefit. We now know that his conception of an incomplete thyroxine was unsound, and based upon the then faulty conception of its constitution. It has been shown that the activity of incompletely iodized thyroxine is of the same type, but much

less in degree than that of thyroxine itself, while no change has been produced in thyroxine which causes development of any toxic properties (139). The benefit resulting from the use of Lugol's solution remains a proved fact, although, as will be seen later, the way in which this benefit is produced remains unexplained.

Whether or not Plummer's theory of two types of hyperthyroidism is correct (and even Marine is forced to admit some degree of differentiation) it 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 (174): "It is convenient . . . to make a distinction 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

[1] Rienhoff (294) 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."

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

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.).

goitre), as shown in Table III, based largely on Boothby (34), Joll (174), and Sharpey-Schafer (312).

Graves' Disease was first described by Parry in 1786, then by Flajani (1802), Graves (1835) and Basedow (1840), all independently (312). 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 VIII).

Observations which support a bacterial origin, such as the reported isolation of specific organisms (157, 196), or the production of hyperthyroid conditions following experimentally produced infections (364, 252), 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 (198).

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 (363, 369, 316, cf. also 165, 42). The nature of the nervous control of the thyroid is still not clear (cf. p. 41).

That a constitutional factor exists, as Warthin originally suggested (although perhaps merely as an inherited thyroid weakness as Cockayne thinks (67) 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.* 114, 95) 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 made a careful clinical study of the disease in children (307), 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.

In adult cases the constitutional and neural aspects of the

disease and its association with psychic traumatism are sometimes emphasized (41, 42), but possibly the peculiar nervous and psychic manifestations can be as satisfactorily explained by exaggeration of customary reactions of emotionally unstable patients, due to the disease (334), or to relationship with sex epochs (154). The possibility that endogenous organic cyanides play a *rôle* cannot be excluded (cf. Chapter VIII).

Studies of the variable electrical excitability of the median nerve following operations for hyperthyroid conditions (136), and of the respective blood pictures in Graves' disease and in induced hyperthyroidism (161), 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 (42), 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. (Cf. also 124.)

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 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 (24A).

Exophthalmos cannot be considered as a condition peculiarly associated with hyperthyroidism. It is true that it can be produced in some, though not in all, laboratory animals by artificial hyperthyroidism (192) and that it

occasionally results in human beings from continued thyroid overdosage (40). Yet poisoning with methyl cyanide produces exophthalmos in thyroidectomized rabbits, while injection of the thyrotropic principle of the pituitary will produce it in both normal and thyroidectomized guinea-pigs (cf. Chapter VIII). There are a number of authenticated cases of Graves' disease in which, after removal of the thyroid, exophthalmos subsequently developed, at periods varying from three to twelve months, with no later improvement. In several of these the basal metabolic rate was below normal, while one exhibited definite myxoedema (368).

Justin-Besançon (177) has recently reviewed the literature of exophthalmos critically. He considers that the condition exemplifies a neuro-vegetative disturbance. When cats are successively injected with ephedrine and pilocarpine a considerable degree of exophthalmos is induced without marked mydriasis, *i.e.*, the typical eye condition of Graves' disease. Injection of thyroxine alone will not produce this effect, but when it is injected along with ephedrine and pilocarpine the thyroxine enhances their effects. Thus the results of Justin-Besançon indicate that the hyperthyroid individual is unusually susceptible to such disturbances of the sympathetic nervous system as may result in exophthalmos.

He has shown further that administration of corynanthine (an isomer of the alkaloid yohimbine) causes retrogression of the exophthalmos in such experimental animals, and has obtained good results by oral administration of this drug in some proportion of post-operative cases of Graves' disease.

Naffziger (262) has described surgical treatment of cases of progressive exophthalmos following thyroidectomy.

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 (34) : " 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 (56) but seems unlikely. There is some evidence that in women adenomata increase with an increasing number of childbirths (307A).

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 (35), 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. 60) will probably throw further light on this condition. Children exhibiting the condition recover without operation following cessation of the iodine treatment (103).

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 (163, 205, 301, 12, 11, 75, 328).

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. 52, 65). 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

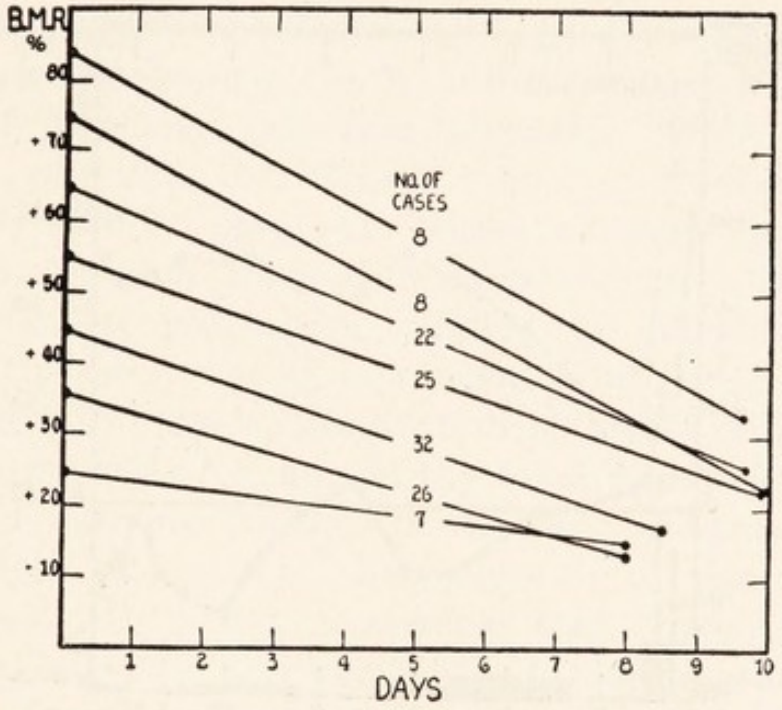


FIG. 5.—Response to iodine in Graves' disease. The average basal metabolism before is compared with that after the usual course of iodine in the form of Lugol's solution. The abscissae represent the average time required for the characteristic response. A total of 128 cases was divided into groups according 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.)

several times daily (263, 207, 78). 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

[1] According to Joll (174), Waller (351), 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.

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

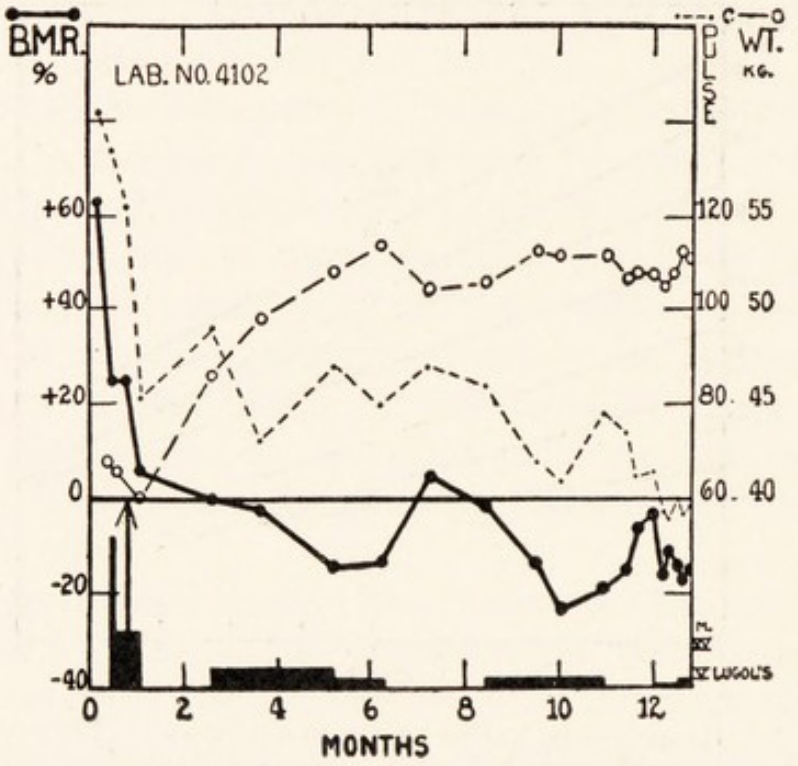


FIG. 6.—Iodine responses in a case of residual thyrotoxicosis following subtotal thyroidectomy (arrow) for exophthalmic goitre. The case was characterized by residual nervousness, which disappeared under the iodine treatment. (From Means, Thompson, and Thompson, *Trans. Assoc. Am. Physicians*, 1928, xliii, 146.)

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" (179). The beneficial results have been extensively and completely confirmed.

Means, Thompson, and Thompson (250) write that the

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 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. 5. 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. 6. 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 recurrence might be prevented by judicious occasional use of iodine over a long period following the thyroidectomy (102), while its careful use in *recurrent* Graves' disease has been proved beneficial (134).

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 (334). 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 administration 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 169).

A certain proportion of patients are considered to be refractory to iodine treatment *ab initio*. Means and Lerman (248) consider that such refractoriness is not real and that these patients are already fully "iodinized."

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 (334, 344, 336, 348). 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 (106A), iodized fat or sodium iodide given with a concentrated mixture of vitamins A and D (281, 6, 108), sajodin (calcium iodobehenate) (112), ethyl iodide (inhaled) and potassium iodide (200).

A number of European investigators have reported favourably on the use of diiodotyrosine in treatment of Graves'

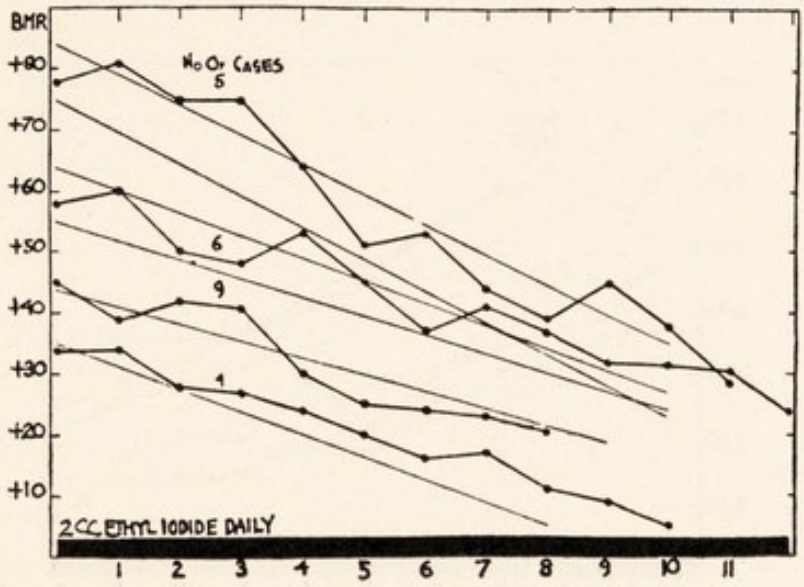


FIG. 7.—Comparison of the metabolic rate changes produced by Lugol's solution (cf. Fig. 5) and ethyl iodide in Graves' disease. The cases are grouped in accordance with the resting levels, each ten-point interval constituting a group. (From Lerman and Means, *Am. J. Med. Sci.*,. 1931, clxxxi, 745.)

disease. Like Lugol's solution, it is extremely doubtful if it has any proper *rôle* in treatment other than pre-operative (cf. 132, 85). However, as Laroche (195) points out, it is better tolerated than Lugol's solution, while, on account of its relationship to the thyroid principle (cf. p. 30), smaller doses are needed (139).

Lerman and Means (200) studied the effects of inhalation 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. 7

and 8. 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

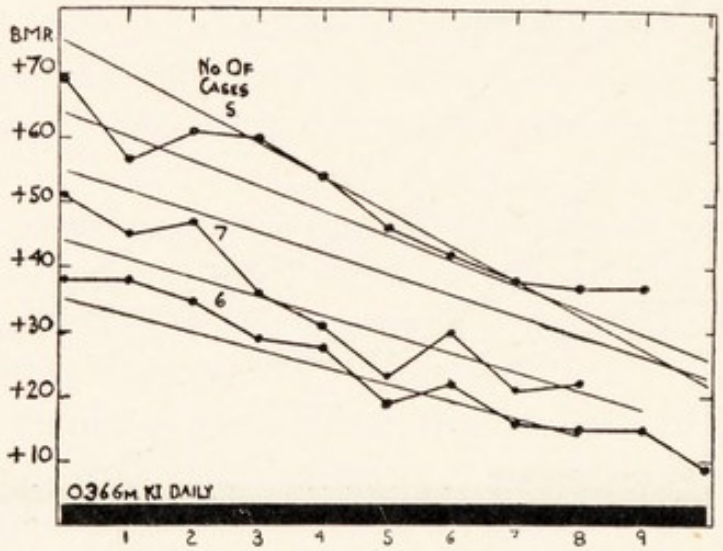


FIG. 8.—Comparison of the metabolic rate changes produced by Lugol's solution (cf. Fig. 5) and potassium iodide in Graves' disease. The cases treated by the latter are arranged in three groups, with initial rate less than + 45, between 45 and 59, and + 60 per cent. or over. (From Lerman and Means, *Am. J. Med. Sci.*, 1931, clxxxi, 745.)

school have expressed the opinion that no benefit is conferred (179). Since the condition is closely related to pure hyperthyroidism, artificially produced (cf. p. 80), it is pertinent to note that administration of Lugol's solution confers no protection against thyroxine dosage in animals or in man (324, 289, 192, 59). Yet there seems to be definite evidence that it depresses metabolism in some cases in certain non-thyroid conditions, including pernicious anaemia (243) and lymphatic leukaemia (111), even though it is stated to have no appreciable effect on normal man (243, 285, 315,

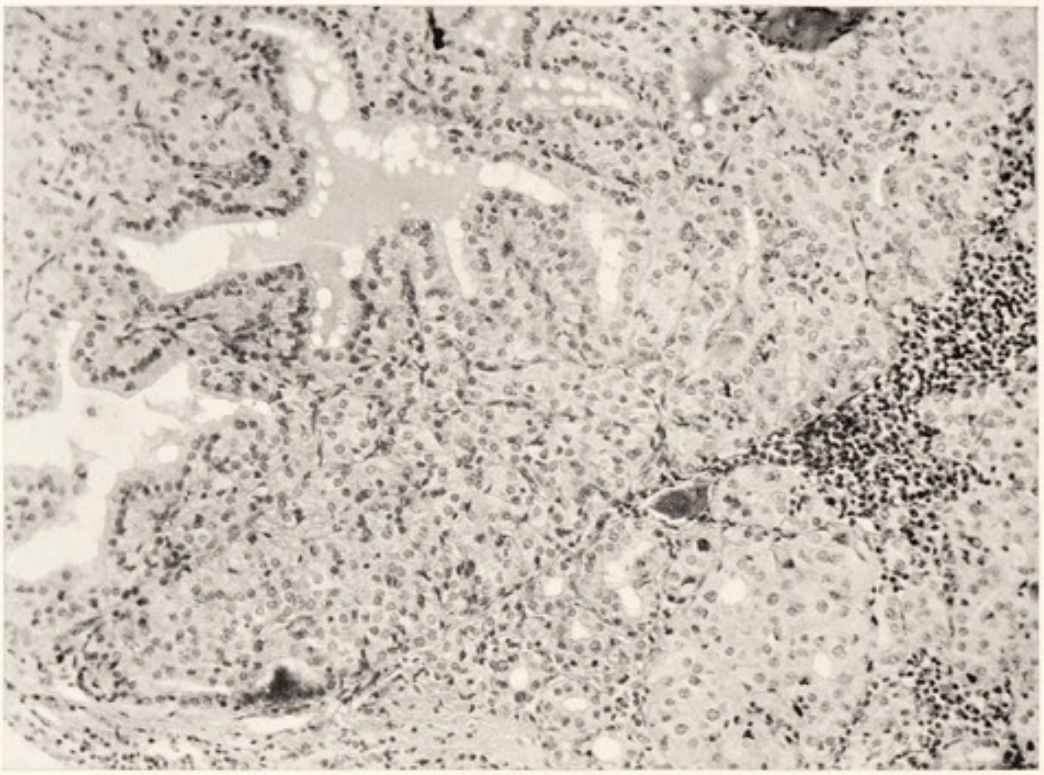


FIG. 9.—Graves' disease untreated with Lugol's solution. The acini are for the greater part filled with hyperplastic epithelium. Absorption of colloid, especially along the line of contact with the epithelium. To the right there is a small collection of lymphoid tissue. × 130. (Photo-micrograph and description by Professor William Boyd.)

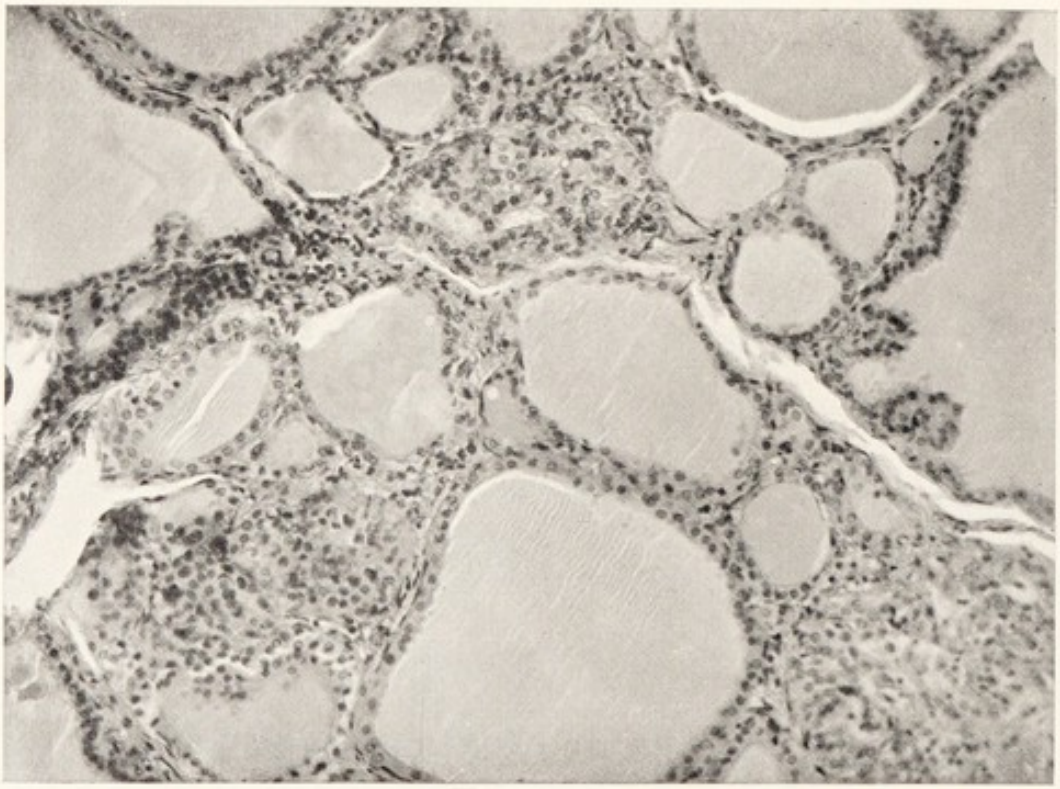


FIG. 10.—Graves' disease treated for a short time with Lugol's solution. A few epithelial buds are seen at the right, but most of the hyperplasia has disappeared, and the acini are filled again with colloid. × 130. (Photo-micrograph and description by Professor William Boyd.)

[*To face p.* 91.

206). Certain writers state definitely that it is just as effective in toxic adenoma as in Graves' disease (276, 50). Jackson (169A) believes that while its effect is not constant nor specific, it is beneficial in the majority of cases.

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. 78). Marine (240) suggested that the beneficial action depends upon the rapid formation of colloid, which mechanically blocks the secretion of thyroxine into the general circulation. (Cf. also (272).)

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 (293, 295, 61, 359). (Certain observers are not in complete agreement with this view (147, 257).) 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. 9 and 10).

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 (156).

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 (213), whose own studies are amongst the most important. The average iodine content of normal 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.[1]

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. (Cf. also Dodds (87).) A typical result is shown in Table IV. (D.H., female, aged twenty, with definite Graves' disease) (213).

[1] Measurements of the iodine content of the cerebrospinal fluid suggest that most of the blood iodine is in non-dialysable, organic combination (230).

Perkin, Lahey and Cattell (272) have obtained similar results, but on a somewhat lower level of iodine content. They find that the value in normal people seldom exceeds 10γ. A moderate proportion of hyperthyroid cases show normal blood iodine, and these cases are more severe and do not respond so well to pre-operative iodine treatment. At operation the thyroids of such patients often show primary hyperplasia with irregular involution. Usually, following subtotal thyroidectomy, there is a striking decrease in blood iodine within three months, but when this does not happen

TABLE IV

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

Date.	Lugol's Solution. Daily Dosage.	B.M.R.	Blood-Iodine. In-organic.	Blood-Iodine. Organic.	Remarks.
72.1928	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

there is generally clinical evidence of persistent hyperthyroidism.

They have suggested an iodine tolerance test, in which 37·5 mg. iodine in Lugol's solution is administered orally, and blood-iodine is estimated at 0, 0·5, 1, 1·5 and 2·5 hours thereafter. Hyperthyroid cases show a curve depressed below that for normals. Watson has modified the test, and injects 0·25 mg. of iodine (in Lugol's solution) per kg. body-weight, intravenously (354A).

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 (25).[1] 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 (25).

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 (131).

These chemical studies indicate that the output of the thyroid 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.

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

[1] It is interesting in this connection to note that major (non-thyroid) surgical manipulations lead to marked hyperactivity of the thyroid gland, evidenced by considerable increase in blood iodine, which may persist for several days (230).

the system, not produced through the thyroid. It has no permanent effect on the cause of Graves' disease (250).[1]

Recent cytological studies, especially those of Okkels (269) and Wahlberg (347) can now be contrasted with those made on physiologically stimulated glands in animals (cf. p. 30). The results are in general agreement with those based on histological and chemical investigations. There is no apparent qualitative difference in the mechanism of secretion in the normal thyroid and in the thyroid in various pathological states. In absence of clinical symptoms of a thyrotoxicosis, the Golgi apparatus of the follicular cells is not enlarged, whether the condition be ordinary parenchymatous goitre or nodular goitre. Cases of toxic goitre, almost without exception (and all cases of Graves' disease), show enlarged and often markedly enlarged Golgi apparatus, indicating marked hypersecretion. This enlargement persists during pre-operative iodine treatment, indicating that the hypersecretory activity also persists, although accumulation of colloid material indicates that follicular storage has for a time replaced discharge of the active principle into the circulation. Okkels is of the opinion that the cytological appearance of the cells in thyroid disease is more consistent with actual clinical conditions than are the ordinary pathological-anatomical classifications.

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 (41) and disputed (247, 47). Benefit has been stated by various clinicians to follow the use of gynergen—ergotamine tartrate (7, 317), physostigmine salicylate (41), potassium permanganate (267), and sodium or ammonium fluoride (122).

[1] Friedgood (111) has compared the effect of Lugol's solution on the basal metabolic rate, and the symptomatic response to it, in Graves' disease, on the one hand, and in chronic lymphatic leucaemia, polycythaemia vera, acromegaly, and pernicious anaemia, on the other. Such effects and responses are similar in kind, but less constant and less in extent in the latter diseases than in Graves' disease. The general similarity suggests (i) an underlying hyperactive state of the sympathetic nervous system in all these conditions, (ii) the beneficial effects in chronic lymphatic leucaemia, etc., and also in Graves' disease are probably not produced through the thyroid, and (iii) Graves' disease is not primarily a disease of the thyroid gland, but the sympathetic nervous system appears to play a major *rôle* in it and in chronic lymphatic leucaemia.

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 (299, 311, 43) and of the concentrated principle (see Chapter V). Good results have also been claimed following administration of insulin (336, 120). Claims have been made for A-P-L and theelin therapy (55).

The formation of anti-thyroid compounds in the organism, compounds which antagonize the action of its principle and which are apparently normally present in blood, will be dealt with in Chapter X. A therapeutic treatment has evolved in connection with these presumed compounds, and will be referred to there.

Curious claims have recently been made that copper, arsenic and other mineral salts are beneficial in hyperthyroid states (152, 259); the beneficial action of copper salts in experimental human hyperthyroidism is denied (254).

Roentgen-ray and radium treatment are advocated with varying degrees of enthusiasm by different writers. Diathermy is stated to be of no great value (171). The most generally expressed opinion concerning X-ray treatment is that it is more suitable when the toxaemia is moderate than for severe cases (292, 117, 291, 273). (According to Joll (174) it may be used in early cases associated with great 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 (256): 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 (130, 155).

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. Various figures have been

published (66, 174), those from specially trained teams naturally being lower. De Courcy (84) considers the average mortality to be about 1 per cent.; Hyman and Kessel consider it to be much higher for the whole operated population (165).

As regards late results of operative treatment, exophthalmos usually lessens but does not always disappear. Nervousness is invariably improved but not always banished. Most, but not all patients gain weight. Some are not improved (90, 256, 109). Graham and Wallace (126), surveying the late results in 125 cases, report that 90 per cent. were rendered fit for work. The four patients in this series who died were all chronic cases who had been unrelieved by medical treatment.

Fenger (105A), 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.

Thyroidectomy in Non-thyroid Conditions. Since in patients with congestive heart failure and a normal basal metabolic rate the basal velocity of the blood flow is greatly lowered, while in myxoedema it may be similarly slowed in absence of symptoms or signs of congestive heart failure (the diminished circulation being adequate to the diminished body needs), complete thyroidectomy has been practised on a number of patients with congestive heart failure or angina pectoris, in whom there was no evidence of an abnormal thyroid condition. Good results are claimed. It is stated that thirst ceases and oedema disappears; patients can make some degree of exertion without palpitation or dyspnoea, and can sleep without sedatives. Though

the circulation is not improved, it suffices for the needs of the lowered metabolism (30, 203 ; cf. 46, 81, 59). Symptoms of myxoedema tend to develop within two or three months and the blood cholesterol steadily rises. Control of distressing symptoms can be established by administration of thyroid in amounts varying from one-tenth to one-half grain daily (31).

There is evidence that a more critical attitude is developing towards this treatment, especially in cases of congestive heart failure. Thus Clark, Means and Sprague (64) consider that after results indicated that the operation was only satisfactory in one-fourth of their 19 cases.

One case of aleukaemic lymphatic leukaemia has been similarly treated with apparently good results, although it is admitted that the improvement may have been due to a remission in the severity of the disease and not to the unusual treatment (30).

Complete thyroidectomy has been performed in cases of severe, uncomplicated diabetes mellitus (361, 302), and, although a marked increase in the carbohydrate tolerance resulted, it is doubtful if the results support the use of such procedure.

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 (355). (Cf. also 96 and 314.)

Joll states that in areas where endemic goitre is prevalent malignant disease of the thyroid is relatively common (174).

Administration of Thyroid in Various Conditions

Good results have been reported in hebephrenic dementia praecox (161). A considerable number of gynaecological cases which exhibit as symptoms menorrhagia, and sterility or abortion (separately or in combination) are apparently relieved by administration of thyroid and by no other treatment (74). Thyroid administration has proved of some service in the treatment of cataract (180), in some proportion of cases of arthritis (hypertrophic type) (135), and, in slight

dosage, in treatment of certain types of alopecia (123); it has no beneficial effect when given to senile rats (160). Local application of thyroxine appears to be of some service in the treatment of otosclerosis and similar types of deafness (127).

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

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 (286), 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 very little of the mechanisms by which thyroid tissue forms the thyroxine radical, and can only shrewdly 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 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

THE PARATHYROID GLANDS [1]

Introduction

THE parathyroid glands in man, usually adjacent to the dorsal surface of the thyroid, show variations both in number and location. In somewhat more than half the cases examined by Heinbach (55) four glands were found, and somewhat more than half the glands found were opposite the middle third of the thyroid. Heinbach considers the usual description of a "superior" and an "inferior" pair (27) is misleading. The glands vary in size from 3 to 15 mm. long and 2 to 3 mm. broad and thick, and are yellowish-red to brown-red in colour. Small accessory glands are by no means uncommon, especially near or embedded in the thymus (27).

The glands are relatively very vascular. They are each

[1] Much of the recent work has been reviewed by Thomson and Collip (122), 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. Shelling (114) has published a still more recent review.

supplied by a special arteriole from a thyroid artery ; from it sinus-like capillaries come into close relationship with the cells themselves (113).

Hermann (55A), from an extensive study both of human cadavers and of fresh autopsy material, finds that in man on each side of the neck a branch of the inferior thyroid artery joins with a branch from the recurrent laryngeal nerve (given off at the point at which this nerve crosses the main branch of the artery), to form a " stalk " to an inferior parathyroid gland, and similarly a branch of the superior thyroid artery is joined by a fine filament from the external branch of the superior laryngeal nerve to form a " stalk " to a superior parathyroid gland. He states that these four stalks are constant in number, though each may lead to more than one gland. Some of the nerve fibres terminate in the vessel walls ; many penetrate between epithelial cells, forming nodular endings (103). Transplantation experiments suggest that the glands can function adequately in absence of all nervous connections (103, 77).

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 (113, cf. 88A). 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 (113, 26). Small colloid vesicles are sometimes found ; the number of these also increases with age (cf. also 74, 85, 89).

Cytological studies of rats' parathyroids indicate that the condition of the mitochondria and Golgi apparatus varies with and is related to the secretory activity of the cells (104).

The definite association of those acute manifestations

which we call tetany with experimental removal of the parathyroids is due to Gley (43). Vassale and Generali (128) 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 122).

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 (81). 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 (80).

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*

[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 (6), Vincent (130), Sharpey-Schafer (113), and Shelling (114).

parathyroidectomy in all mammals, and in birds (122). Variations in susceptibility to parathyroidectomy in different species are probably traceable to dietary differences (114). When tetany 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. Small doses of curare temporarily abolish parathyroid tetany in dogs, indicating the association of the nerves with the hyperexcitability (54).

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 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 (11). 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*, 48, 32).

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 psycho-neurotic patient convalescent from encephalitis lethargica (7), cases associated with continued pain from cholelithiasis and cholecystitis (47, 87), or from retention of urine (94), or from the prolonged discomfort of a pelvic condition (87). Even too violent exercise taken when in poor physical condition, or crying spells associated with a neurosis, have produced symptoms of tetany (47). 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 (82). Prolonged immersion in hot baths can set up a hyperpnoea which may induce tetany (75).

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 (87).

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 (31). However, the blood phosphates are also depressed, whereas, following parathyroid extirpation, they are slightly increased (38). Many of the symptoms suggest dehydration and anhydraemia (52). 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 (88). A similar condition in cows and ewes following a period of close confinement has been described (30, 118). Dehydration may be a factor in all such cases (52).

Magnesium deprivation can lead to tetany, and the so-called "grass tetany" of cattle is probably of this type (114).

Tetany is produced in a proportion of young white rats fed desiccated thyroid; it often is apparent after a few days' treatment (16). 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 both clinical and experimental evidence that hyperthyroidism leads to increased excretion of calcium, and it seems probable that this can occasionally produce such a lowering of blood calcium as to induce tetany (122). Administration of thyroid or thyroxine to rats increases the excretion of calcium, chiefly through the intestine, and sets up a negative calcium balance which is restored to normal by sufficient calciferol (98). Patients with Graves' disease excrete calcium and phosphate to a greater extent than normal in both urine and faeces, although the increased excretion is mainly through the latter channel; this altered metabolism is not due to a deficiency in vitamin D (4, 5, 45).

An interesting case—a woman of forty-eight—illustrates such "hyperthyroid tetany." She had a thyroidectomy, apparently for Graves' disease, more than twenty years ago. The hyperthyroidism recurred after sixteen years, with a second operation three years later. Subsequent to the second operation symptoms of tetany gradually developed, but were controlled for a while with viosterol and a high calcium diet. She was admitted to the Winnipeg General Hospital in January, 1934 (two years after the second operation), with symptoms of both tetany and hyperthyroidism. Her blood calcium at two determinations was 6·6 and 6·9 mg. per 100 c.c. of serum, and her plasma inorganic phosphorus 4·1 and 4·3 mg. Her basal metabolic rate was + 39 per cent. At operation a hyperplastic thyroid mass was removed. Following operation, the blood calcium slowly rose without special treatment, until three weeks later the figure was 8·4 mg. All symptoms of tetany had disappeared, and were still absent some months later.

There appears to have been a degree of parathyroid removal or destruction in the first two operations, which in itself was insufficient to cause tetany, but, when accentuated by the recurring hyperthyroidism and the increased excretion of calcium caused thereby, resulted in a sufficiently low blood calcium for tetanic manifestations. When the additional factor was removed, tetany ceased. I am indebted to Dr. Gordon Fahrni for permission to mention this case.

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, and, in fact, McLean (84) has recently advanced evidence against it. If it 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 (122).)

Blood Calcium [1]

The calcium of human blood occurs wholly, or almost wholly, in the plasma. Results indicating its presence in the

[1] Thomson and Collip have dealt very thoroughly with the partition of calcium in the blood (122).

red cells in any but negligible amount are due to inaccuracy of technique (122). 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 at least 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. Since calcium plays a definite *rôle* in clotting it seems quite possible that the equilibria between 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 absolutely 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 (42), so that they obviously should be standardized in all ultra-filtration experiments (72). Since this has only recently been realized it is not surprising that dialysis experiments have not led to very concordant results. Cantarow has discussed their validity critically (*90*).

The more recent determinations, based upon a combination of compensation dialysis and ultra-filtration under pressure, have indicated that 68 per cent. of the calcium is dialysable in blood serum of rabbits (*370*, *517*), and 45 to 55 per cent. (*517*) or 42 to 58 per cent. (78, *317*) in that of man, dogs, and cattle.

It is still a matter of doubt whether the cerebrospinal fluid

can be regarded as produced solely by filtration through the membranes of the choroid plexus (40, 119, 88, 39). However, accepting the view that the calcium of the fluid can be regarded as corresponding to the diffusible calcium of the plasma, 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 (8), 46 to 51 per cent. in dispensary patients (*369*), and 45 to 55 per cent. in normal men (*87*). (Cf. also (117).)

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 (122), although recent evidence suggests that the albumin fraction is involved (8). 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 (95) and by Thomson and Collip (122). *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 (102), 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. A suggestion that it is present in some citrate-like combination is no longer supported (122). 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.

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. or more per 100 c.c. have been made, based upon different experimental procedures (122, 95); McLean and Hastings consider that the value lies between 4 and 5 mg., almost entirely accounting for the diffusible fraction (83).

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. 112) 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.[1]

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. 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 (122). 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,

[1] Tuzioka (127) claims that thyroparathyroidectomy in the dog markedly diminishes the output of bile and of bile salts, while Hoshizima (58) finds that the tetany of such animals can be partly or completely prevented by daily administration of bile acids, though the blood calcium is not restored to normal.

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 (22). MacCallum, considering this earlier work, wrote in 1924 concerning the therapeutical results (79) : " 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 (51) 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.[1] Collip achieved this in 1924.

His method consisted essentially in boiling fresh minced glands with dilute hydrochloric acid for from thirty to sixty minutes, cooling, and removing fat, then adjusting the *p*H to 8·0 or 9·0 until suspended material had dissolved, and again to 5·5, when a precipitate formed. This was filtered off and the active principle salted out of the filtrate, redissolved in dilute alkali, and purified by similar procedures.

Various modifications have been suggested, without material improvement (122, *9*). (For details of the methods see Harrow and Sherman (53).) So far the active principle has not been obtained in pure crystalline condition.

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

[1] " Aside from suggestive work by Berman, Hanson, and some others nothing thoroughly conclusive as evidence of a parathyroid hormone was presented until Collip arrived on the scene " (53).

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 associated with the presence of a primary amino-group (126).

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 (122). 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 (22): During the first twenty-four hours, while the blood calcium is rising steadily to a peak of about 20 mg. per 100 c.c. serum, the animal has occasional attacks—commencing some hours from the start of the injections—of vomiting and diarrhoea, and may manifest uneasiness of manner, but otherwise appears normal. The

peak height of calcium may be maintained for several hours ; it then starts to fall. Occasional attacks of vomiting and diarrhoea continue. The animal becomes more and more depressed ; respiratory distress may be noted.

As the blood calcium falls inorganic phosphorus rises and more serious symptoms appear. Vomiting continues. The animal commences to pass blood by the bowel. Blood urea and non-protein nitrogen increase greatly. Blood volume diminishes and the blood thickens. Its coagulation time diminishes. (Blood samples are obtained from peripheral

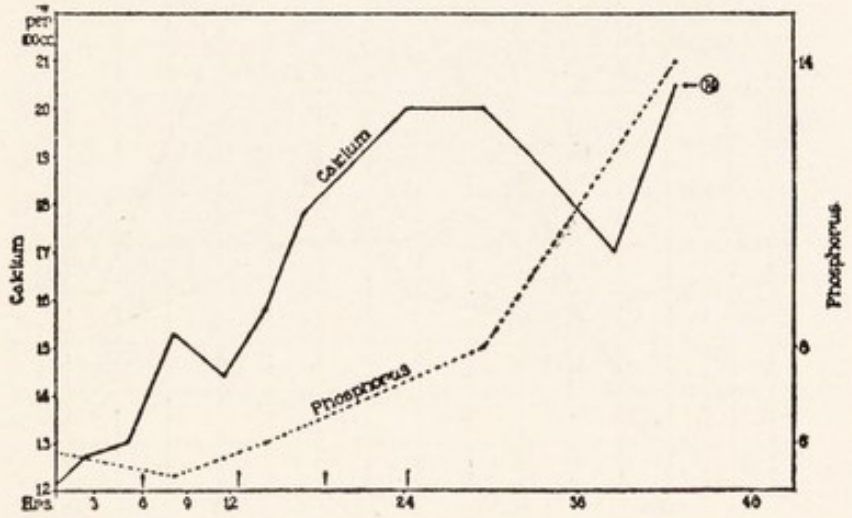

FIG. 11.—The blood serum calcium and whole blood inorganic phosphorus curves in continued parathyroid overdosage in the normal dog. (From Collip, *Medicine*, 1926, v, 22.)

veins only with difficulty.) The kidney practically ceases to function.

A number of these changes are illustrated in Figs. 11 and 12.

Studies of carbon dioxide content, combining power and *p*H of the blood plasma indicate that there is a condition of compensated alkalosis on the first day, which passes into a condition of compensated acidosis, and this changes to uncompensated acidosis just prior to death.

Post-mortem examination discloses marked congestion of the alimentary canal, and presence of blood in the stomach

and intestine. Calcification has been observed—especially in the space of Bowman's capsule and lumina of the tubules of the kidneys, and also in the walls of the lesser arteries and the Küppfer cells of the liver (122).

This pathological picture of acute effects following overdosage can be almost exactly paralleled by combined injection of calcium chloride and acid sodium phosphate

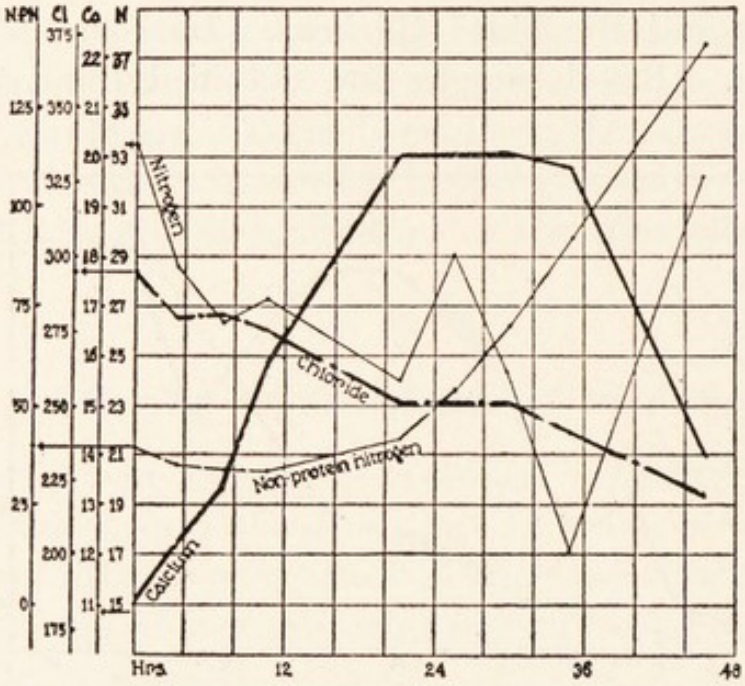

FIG. 12.—The blood serum calcium, whole blood chloride, nitrogen and non-protein nitrogen in the normal dog as affected by repeated injections of potent parathyroid extracts and frequent bleedings. (From Collip, *Medicine*, 1926, v, 23.)

(NaH_2PO_4), whence the actual symptoms may be ascribed to coincident hypercalcaemia and hyperphosphataemia (122).

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*, 122). The response of man is similar to that of the dog, although he seems more resistant

to overdosage (122, *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 (122).

Shelling considers that many of the effects of acute hyperparathyroidism are due to continued diuresis and the resulting anhydraemia, so that rational treatment of such condition is to replace water and electrolytes and to combat

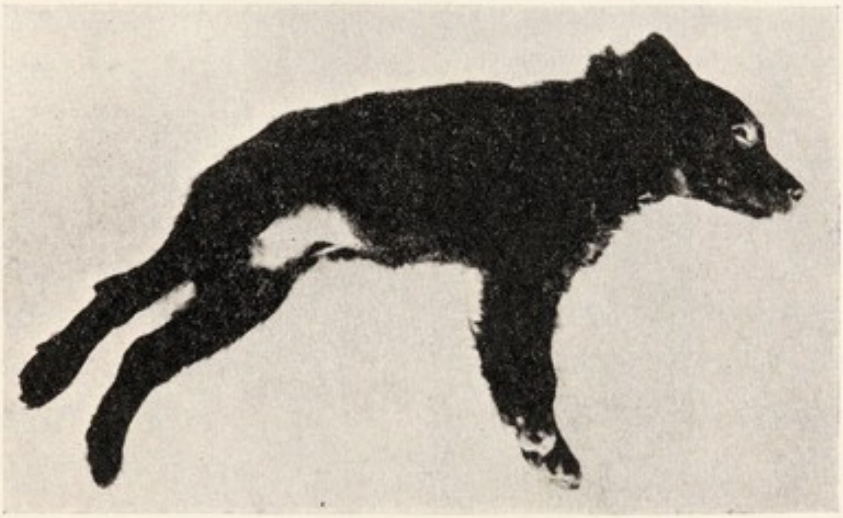

FIG. 13.—Dog in tetany, fifty-nine days after thyroparathyroidectomy. (From Collip, *J. Biol. Chem.*, 1925, lxiii, 400.)

acidosis (in absence of vomiting) or alkalosis (following vomiting) (114).

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 (97). The explanation appears to lie in the precise mechanism of action of the principle (cf. p. 134).

The Parathyroidectomized Animal. Injection of the active

extract into such 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

FIG. 14.—The same dog as in Fig. 13. Complete recovery three hours after subcutaneous injection of a potent parathyroid extract. (From Collip, *J. Biol. Chem.*, *loc. cit.*)

level. The slight increase in blood phosphate produced by extirpation of the glands disappears (22). 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. 13 and 14. His results have been completely confirmed by numerous investigators.

According to Reiss (102), 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 (22). Man suffering from parathyroid deficiency following operative procedures responds to treatment as satisfactorily as does the dog (cf., however, p. 137).

The curious finding has recently been published that " parathormone " is ineffective in controlling the tetany of parathyroidectomized rachitic rats (70).

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 may cause a slight rise in the cerebrospinal fluid calcium, tending to be somewhat more delayed than that in plasma (*83*). 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 indepen-

dently, and that the level of diffusible calcium is not entirely dependent on the total serum-calcium level.

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. 118). Following the administration of a potent parathyroid extract (parathormone) there is a slight increase in blood magnesium, antecedent to the rise in blood calcium (108, 46).

Thomson and Collip (122) have covered all the literature 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 (22). 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 (122). 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 (33, 97).

The measurement of calcium in the parotid saliva of the dog has been suggested for standardization (3). Another suggested method is based upon the protection against narcosis due to magnesium sulphate, conferred on mice by parathyroid extract (34).

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 (22, 122, *143*), and final disproof is afforded by the experiments of Saunders (105).

Vitamin *D*, *calciferol*, $C_{28}H_{43}OH$, 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. Other investigators have shown that when large doses are administered parathyroidectomized dogs can be maintained in good health at normal calcium level, and that the treatment is beneficial when tetany follows human parathyroidectomy (122).

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*). Shelling (114) criticizes Taylor's experimental evidence in support of direct relationship as vitiated by inadequate control of dietary factors, and supports the view that the parathyroids and the vitamin are antagonistic.

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 (17) or depresses the excretion of calcium into the intestine (121). There is no conclusive evidence that the parathyroid principle affects the absorption of calcium (122). 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 (17).

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. 111).

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.

Much argument has taken and is taking place as to whether parathyroid action is primarily on calcium or on phosphate metabolism. The argument in favour of the latter view has been well set out by Shelling (114), but since there is a complete interrelationship the argument is rather academic than practical.

There is good 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 Robinson, 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 (86). In many other less drastic events bone is denuded of some proportion of its store (122).

It is most probable that the primary action of the parathyroid principle on bone results in liberation of calcium and phosphate by some direct stimulating action (122). Such theory of direct action is not completely accepted. Various other theories have been advanced, generally complicated ones (cf. 114). Yet the histological studies quoted below seem to afford definite proof of direct action. From this it would seem to follow that 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) (122).

Histological evidence supports the theory of direct action. 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. 139).

The characteristic effect of the parathyroid principle on the bones of rats occurs after bilateral nephrectomy, and therefore cannot be ascribed to an action on the renal threshold for phosphate (23).

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. 116). 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. 109; also 122, *25*).[1]

[1] Shelling's results are not in complete agreement. He considers that the nature of the response depends on the degree of dosage of the parathyroid principle and the calcium and phosphorus contents of the diet (115).

The experiments of Pugsley (97) 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 (111, 24, 107). But when it is given in small amounts over long periods increased calcium deposition in bone results, the cortical tissue becoming denser and thicker (106).

Selye has shown (109) 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 (123) that the parathyroid principle does not increase the solvent power of blood plasma for the calcium compounds of bone.[1,2]

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 concentration of the blood circulating in bony tissues. When

[1] Ortenberg (92) 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.

[2] Wilder (136) has suggested that the amount of principle secreted by the parathyroid glands determines the sensitivity of the organism to the action of vitamin *D*.

the glands are extirpated denudation does not occur and the blood calcium falls.[1]

The very high blood plasma calcium in laying hens seems to depend on the integrity of the parathyroids (124, cf. 63).

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 (100). 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. 131). 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 (12). He administers frequent doses of calcium lactate, with cod liver oil, and finds that parathyroid extract is seldom needed.

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

[1] It has been claimed that when sufficient vitamin *D* is administered to parathyroidectomized pups (71), and rats on optimal calcium diet (114) 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 ; nevertheless the life of these animals can scarcely be considered as normal.

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. 127), and Selye's work indicates that it is due to a reversal of parathyroid effect (cf. p. 134). According to Reed and Seed (101) large doses of irradiated ergosterol are equally effective and patients do not become refractory to this treatment. Shelling (114) thinks that special attention should be paid to the calcium/phosphorus ratio in the diet in such cases, and that milk and cheese are contraindicated as large sources of calcium, since their high phosphorus content can render the calcium useless. He thinks that too great an amount of phosphorus in the diet can account for refractoriness to continued parathyroid therapy. Margaret Hoskins' experiments on parathyroidectomized rats kept on diets of different calcium/phosphorus ratios support his views (59).

In the treatment of tetany, whether of parathyroprivic or of idiopathic origin, Snapper claims that a preparation termed " A. T. 10 " is of great value (116). This material is a by-product from the irradiation of ergosterol, and has been fractionated and freed from vitamin *D* (56). Its effect is less rapid, but more lasting, than that of " parathormone," and is cumulative, and since it produces hypercalcaemia it should only be used in cases in which the blood calcium can be checked. Parathyroidectomized dogs have been maintained in normal condition for over a year by this agency, without appearance of toxic effects.

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 (69, 6, 125). The administration of parathyroid extract has been found beneficial. Shannon (112) 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 the rare condition, 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 above normal. 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 literature has been thoroughly reviewed by Hunter (60, 61), Cuthbertson and Mackay (28), Jessop (66) and Shelling (114).

Generalized Osteitis Fibrosa is progressive, with pain (referable to the bones and joints of the lower extremities, and to the spine), 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 are common. Metastatic calcification is not infrequent. Thirst and polyuria are fairly common.

The disease was differentiated from osteomalacia by von Recklinghausen in 1891 and associated with a parathyroid tumour by Askenazy in 1904. Mandl, in 1926, was the first to operate and remove a tumour. There is a steady increase in diagnosis and operative removal of these tumours. Up to June, 1934, eighty-two such cases had been reported ; in almost all the results have been good. In the majority there is only one tumour ; rarely, tumours of two glands are found. Sometimes none can be found at first operation though later

operation may discover one. The tumour is seldom palpable. The largest so far recorded measured 7·5 × 5·0 × 1·8 cm., and weighed 26·2 grams ; it was situated behind the trachea. Tumours have been reported in the jaw (18A) and completely embedded in the thyroid (129). The size bears no relation to the severity of the bone lesions ; in a severely crippled patient the tumour only weighed 1·3 grams.

Radiographs of patients frequently show greatly diminished density of bone shadow, and pictures comparable with those seen in osteomalacia and generalized carcinomatosis. Histological examination of the bone from autopsy material shows lacunar resorption, apposition, fibrosis of

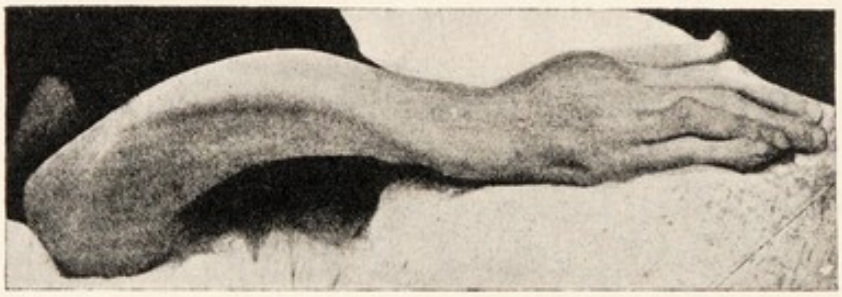

FIG. 15.—Antero-external curvature of forearm and large bony swelling on dorsum of right hand from a case of generalized osteitis fibrosa. (From Hunter, *Proc. Roy. Soc. Med.*, 1931, xxiv, 489 ; Clin. Sect.)

marrow, and formation of osteoclastomata and cysts. There is a generalized osteoporosis.

Chemical study of such patients shows usually a high blood calcium, low blood inorganic phosphate, and markedly increased excretion of calcium and phosphorus. Figures recorded for serum calcium vary from 10·6 (one patient only ; actually on successive days the figures were 11·5, 11·0, 10·6 and 11·0, indicating a tendency to slight increase) (135) up to 29·4 mg. per 100 c.c. (114). Plasma phosphorus varies from 1·0 to 2·5, except in the rare cases associated with calcium salt deposits in the kidney parenchyma (see below) when higher figures may be found. Plasma phosphatase is often high.

Onset of the disease is insidious. It may last many years. One of Albright's cases had a history of thirty-nine years (1). It is commonest in middle adult life, but can occur even in young children (114).

Operation abolishes pain in almost all cases. Restoration of calcium metabolism to normal occurs with varying rapidity. A hypocalcaemia frequently develops, and latent

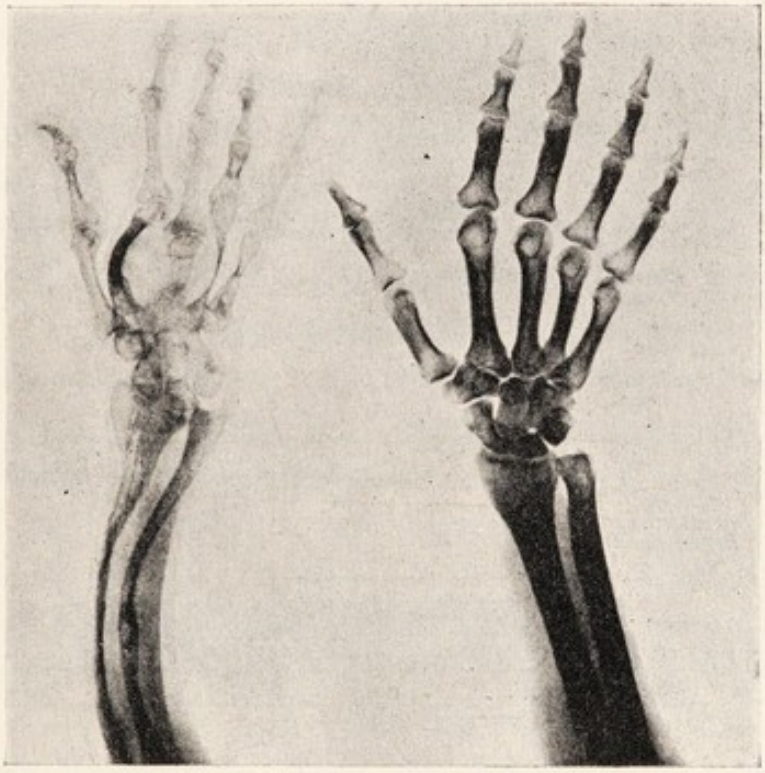

FIG. 16.—Controlled radiograph of right hand and forearm (cf. Fig. 15). (From Hunter, *Proc. Roy. Soc. Med.*, *loc. cit.*)

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. 15 and 16 depict the typical bony curvature and diminished density of bone shadow seen in one of Hunter's cases. Fig. 17 shows the changes in blood calcium and phosphorus in the same case. Excellent illustrations of the extreme deformity which can occur, and the degree of recovery possible in such an extreme case following removal

of a tumour, are given in a paper by Quick and his colleagues (99). The recalcification of bones is sometimes marked (14).

Experiments with animals confirm hyperparathyroidism as the cause of the disease. The typical picture has been produced in dogs, guinea-pigs and rats (*260*, 65, 68, 134, 67, 91). In a normal human subject sufficient symptoms have

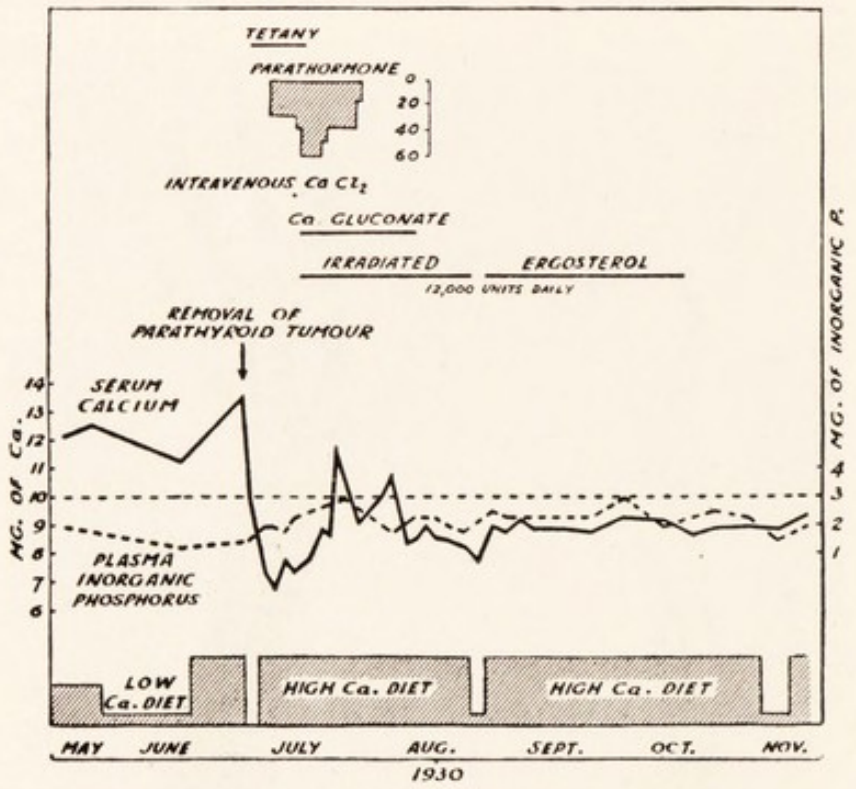

FIG. 17.—Chemistry of the blood of Hunter's case of generalized osteitis fibrosa. The blood was examined for two months before and for five months after operation. The low calcium diet was that given during the investigation of the calcium balance. The high calcium diet was not weighed. (From Hunter, *Proc. Roy. Soc. Med.*, *loc. cit.*)

been produced by somewhat less drastic overdosage to confirm the relationship (61, 68). Selye's studies (p. 134) afford final confirmation.

When the diagnosis is certain, surgery is the most obvious and reliable method of treatment. Presumably because it has been shown that intravenous injection of sodium phosphate lowers blood calcium (93), acid sodium phosphate has

been administered in generalized osteitis fibrosa, and good results claimed (1), but it seems likely that such treatment may actually aggravate the condition (64). X-ray treatment of the parathyroids has been reported of value in one case (29), but is probably inadvisable (9, 64).

Two trends have recently been evident in papers dealing with hyperparathyroidism. One has been a tendency to associate it with diseases other than generalized osteitis fibrosa, and to operate, removing one or more normal parathyroids. The other has been to subdivide true hyperparathyroidism into different types, especially stressing types with renal involvement.

Definitely high blood calcium is occasionally found in acute gout, in arthritis deformans and in polycythaemia vera (15). Possibly because of such findings, cases of arthritis have been operated on with removal of one or more parathyroids (cf. 41), while on more general grounds similar operation has been recommended in various diseases involving bone (cf. 132). Bauer (9) has appraised such views critically and definitely. He emphasizes the fact that hyperparathyroidism is a *generalized* skeletal condition, and a distinct disease due to a parathyroid adenoma, while arthritis and Paget's disease are not generalized and are not due to hyperparathyroidism. He does not believe that any patient should be subjected to parathyroidectomy unless sufficient evidence has been gathered from the history, physical examination, X-ray examination and metabolic studies to leave no doubt as to the correctness of the diagnosis. (Cf. also 25, 90, 49.)

Stress has been laid on the view that hyperparathyroidism is constantly associated with a demonstrable adenoma, and therefore that normal parathyroids should never be removed, it being very doubtful if any benefit accrues from such operations (19, 64). However, hyperplasia of the parathyroids, in absence of tumour, undoubtedly does occur. Its occurrence in osteomalacia, senile osteoporosis, rickets,

and renal rickets can be regarded as compensatory to a primary disturbance in the metabolism of lime salts (114), but in certain cases which were clinically hyperparathyroid Albright (2) found at operation no tumour, but multiple parathyroid enlargement, with uniformly greatly enlarged cells. He considered that seventeen of the 105 cases at that time reported in the literature came within this category, that surgical removal is only of doubtful value in these, and that possibly the condition is associated with the parathyrotropic factor of the pituitary (cf. Chapter VIII).

Castleman and Mallory (18) have made a detailed histological study of the parathyroids in twenty-five cases of hyperparathyroidism, contrasted with those from 150 normals. They definitely distinguish between hyperplasia and neoplasia. The first is characterized by diffuse uniform enlargement of all the glandular tissue. The localized tumours are usually of one gland, or part of one gland, rarely of two glands. (Their study confirms the monophyletic theory of the origin of the various cell-types of the parathyroid, and supports the view that the oxyphil cell is an inactive involution product (cf. p. 111).) They have reviewed the operative or autopsy findings of 137 other cases in the literature, and find no reason to alter their conclusions. They point out that these cases exemplify the fact that the parathyroids can hypertrophy to a greater extent than any other type of tissue—in hyperplasia to 100 times the normal size, and in tumours to many hundred times.

They find a very rough proportionality between the height of blood serum calcium and degree of parathyroid enlargement. Thus, in five cases the average total parathyroid volume was 255 cubic mm., and the serum calcium was less than 12 mg. per 100 c.c. ; in nine cases the average volume was 3,830 cubic mm. and the serum calcium 12 to 14 mg. ; while in eight cases the average volume was 16,000 cubic mm. and the serum calcium always over 14 mg. As the size of the tumour increases the proportional effect of unit volume

on the blood calcium becomes rapidly less and less, and the relation is, in fact, almost logarithmic.

Their analysis (in 1935) of the literature shows twenty-two cases of multiple hyperplasia and 140 cases of neoplasia, of which 128 were of single glands.

From the studies of Albright (1) and others, it seems possible to differentiate several different types of clinical hyperparathyroidism. These include (i) the classical type (von Recklinghausen's disease of bone), in which skeletal symptoms predominate, decalcification, cysts, and tumours are found in the bones, and eventually fractures occur; (ii) the osteoporotic type, with generalized decalcification, but no cysts or bone tumours; and (iii) hyperparathyroidism especially associated with kidney lesions. The third type is by no means the rarest, and it has indeed been suggested that in every case of urinary lithiasis the possibility of hyperparathyroidism should be carefully considered (20), especially since, in such cases, bone changes may not be detectible (21).

When the kidneys are involved, the earliest symptoms may be associated with renal stones of the calcium phosphate type, and, secondarily, with pyelonephritis. Less often calcium phosphate can be slowly deposited from the kidney tubules in the parenchyma, the concretions eventually leading to inflammatory changes, sclerosis, and contracted kidneys. In the latter condition the blood phosphate may not be below normal, and may even be slightly increased. In a case reported by Elsom (36) the earlier symptoms were for a long time regarded as rising from an atypical form of glomerulonephritis.

In these cases involving the kidneys, although recurrency of stone formation is probably prevented by operative removal of a tumour, and the general condition of the patient is markedly improved, the kidney damage—as measured by the urea clearance test—is probably not markedly repaired (36).

The experiments of Goadby and Stacey (44) suggest that

the parathyroid principle acts directly on the kidney to produce a phosphate diuresis, and those of Morgan and Samisch (89) also suggest a specific effect on renal tissue. While the possibility of direct action of an excess of parathyroid principle on the kidneys cannot be excluded, it seems more probable that kidney involvement, when it occurs, is definitely due to a chronically heightened calcium and phosphate excretion. In a large proportion of individuals the kidneys can handle an increased excretion without undue difficulty, so that the patient is first seen because of symptoms associated with bone changes. In others, for some still unascertained reason (Shelling (114) considers that insufficient diuresis is the cause) the kidneys fail to carry the extra load, and calcium phosphate is precipitated as stones or in the parenchyma fairly early in the life of the tumour, and perhaps even before the degree of bone destruction is sufficient to permit detection in the X-ray picture. In agreement with this it is frequently found that the blood phosphatase of this type of patient is within normal limits, while such a view is not inconsistent with that of Albright, that the degree of bone involvement is an index to the duration of the disease and not to its severity.

Lahey has recently written (73): "We must all, particularly the orthopaedist and the urologist, be on the lookout for hyperparathyroidism in patients complaining of pain in the back or extremities, in patients with diffuse neuritis or arthritic pains made worse on motion, in patients with progressive loss in stature, and in patients with kidney stone." Nevertheless it is important to remember that a raised blood calcium is in itself only suggestive of hyperparathyroidism; it can occur in arthritic and other conditions (cf. p. 143), in multiple myeloma, and in metastatic carcinomatosis of the skeleton (114).

Castleman and Mallory (18) in analysing 162 cases in the literature (including their own 25 cases) found that 75 showed osteitis alone, 14 renal stones alone, and 55 both osteitis and

renal stones. (Information was incomplete concerning 18 cases.)

Malignant Tumours of the Parathyroid. Eighteen cases have been reported (50) but in only one of these was there definite evidence of hyperparathyroidism accompanied by decalcification of the skeleton (133, 131).

Marble-bone disease, a condition of extreme brittleness of the bones, seems to be associated with chronic hyperparathyroidism. A typical case has been described (96) 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. 134). Some evidence against this view has been reviewed by Shelling (114). (Cf. also Ellis (35).)

Selye (110) 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. (Cf. also 115, 76.)

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 (37). 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 (62). Similar treatment has been employed in radium poisoning, but is too slow to be useful (114).

Since the parathyroid principle induces diuresis (*4*, *5*, *251*, *495*) it has been employed in nephrosis and similar conditions associated with oedema, and clinical improvement has been reported (114), oedema tending to disappear. Favourable effects have been reported following subcutaneous injection in subcutaneous fibrositis and cellulitis, although there is no obvious biochemical basis for the treatment (57).

Two cases of severe essential purpura haemorrhagica (thrombocytopenia purpura) have been apparently cured by the production of marked hypercalcaemia, by repeated injections of parathyroid extract (78A). In both cases toxic symptoms of overdosage were induced—vomiting, followed by weakness, apathy, and lethargy.

(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 (122).)

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CHAPTER IV

THE ISLETS OF LANGERHANS AND INSULIN

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. 123, 173).

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 as a source of choline (16) is also capable of living for a number of years. Hédon's dog, a classic example, was kept alive 57 months (cf. p. 179).

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 dog (128). 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 (123), 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 Kollman (17), 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. (Cf. also Boldyreff (207).) Some evidence is accumulating in favour of the existence of a functional relationship between the islet and acinar tissue of the pancreas (204).

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 (145).

The islets appear to be under control of the vagus (123, 89, 203). La Barre (108) 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 [1]

Very powerful insulin preparations have been obtained by various procedures. Certain of these are probably 80 or 90 per cent. pure.[2, 3]

Crystallization is recognized as a necessary step in the preparation of any compound in pure condition. Insulin was first crystallized by Abel in 1926. 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. 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. Harington and Scott 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 $C_{45}H_{69}O_{14}N_{11}S$. It gives the biuret, Pauly, Millon, and ninhydrin reactions, Sakaguchi's test for arginine, and positive tests for cystine, but tryptophane radicals, the sulphydryl group, and carbohydrate 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 21 per cent.,

[1] The chemistry of insulin has been recently reviewed by Jensen and Evans (98), who give a complete bibliography.

[2] For methods of preparation of insulin, see Harrow and Sherwin (82).

[3] 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. 30).

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, hydroxyproline, methionine and glycine appear to be absent, nor has any constituent foreign to the ordinary protein molecule been detected. Proline and phenylalanine are present; the presence of valine is doubtful. All the sulphur, 3·2 per cent., is present in disulphide linkage. So far 88 per cent. of the constituents of the insulin molecule has been accounted for.

Insulin crystals are well defined; they seldom exceed 0·01 mm. in diameter. The compound appears to be dimorphous, one form belonging to the rhombohedral class and showing double refraction, and the other showing a more equant habit and no double refraction.

Crystalline insulin is optically active and laevo-rotatory. The actual rotation varies markedly with the *p*H of the medium. 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.[1] Insulin dissolves easily in dilute acid and alkali and in 90 per cent. phenol. It is slightly soluble in 80 per cent. alcohol. Its isoelectric point is at *p*H 5·3–5·35.

Crystalline insulin preparations contain a trace of zinc or some similar element. When the purest available amorphous insulin is crystallized from ammonium acetate buffered solution by means of addition of zinc, cobalt, or cadmium salts, the metal contents of the ash from such crystals are proportional to their atomic weights, and indicate, for example, an insulin containing 0·52 per cent. of zinc, corresponding to about three atoms of zinc in the insulin

[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 (146). The amount excreted does not exceed 1·5 clinical units per 100 c.c. of urine (27).

molecule (171). Beef pancreas contains about 20 mg. of zinc per kg. fresh material, but the amount is not proportional to the insulin present, and there is insufficient evidence as yet to associate zinc with the function of insulin (212). Zinc salts actually seem to delay insulin action (cf. p. 186).

Abel 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 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 phenol solution by dilution with water. This work has not been confirmed. Such views of active prosthetic groups, suggested by Willstätter's theory of enzymic activity, 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. 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. 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. A definite, small amount of iodine inactivates insulin immediately ; this is probably due to oxidation of its disulphide linkages. Numerous other studies of reversible and irreversible inactivation suggest that if there is a specially active group in the molecule it probably is a cystine-glutamine derivative (98, 98A). Digestion experiments with different enzymes (trypsin, pepsin) indicate that the slightest degree of destruction of the protein molecule results in loss of activity.

When insulin is treated with iodine, the latter is taken up by tyrosine radicals in the usual fashion ; this change largely abolishes the physiological activity, which is restored proportionately to removal of iodine by catalytic reduction (220A).

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. 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. 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. 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.

Such results suggest that there is but one insulin, and have,

therefore, some bearing on the sensitivity reactions of certain diabetics to insulin.

Crystalline insulin has the same therapeutic effect as commercial preparations when injected into human diabetics in equivalent dosage.

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 (4). 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 (35) 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 (114); headache, dizziness, lack of muscular control (147), 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 (123). 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 (58, 62, 70). This normal difference tends to disappear in the diabetic, and the severer the diabetes, the more closely the curves approximate (52, 70, 156), illustrating loss of power to utilize glucose by the tissues. This power is restored by the action of insulin (123, 109, 41). Mann and Magath (129) 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 (123).

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 (123).

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. Lawrence (111, 233A) and Joslin (100) support the view that its action is limited to glycogen formation. Macleod (125, 126) believed that its action is much less limited, and is concerned with the formation of some intermediate substance from glucose, which can be either oxidized or polymerized to glycogen.

Experimental data still give no decisive answer to these questions (56, 20, 162, 117, 40). Interpretation of results is rendered difficult by the normal cycle of exchanges between liver and muscle, the long recognized shift of glycogen from liver to muscle *viâ* blood glucose, on the one hand, and the more recently recognized shift in the reverse direction through the intermediation of lactic acid and through the action of fatigue or adrenine (39), a cycle which operates in the diabetic as well as in the normal animal (88).

It has been shown that the effective concentration of insulin is of importance in determining whether liver glycogen be stored or not. Small doses result in storage ; larger, non-physiological and convulsive doses lead to depletion of the liver glycogen (59).

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 yet be stated. If the latter be not the case, it obviously follows that glucose, to be oxidized, must be first transformed to glycogen.

Recent studies suggest that the precise action of insulin is facilitation of the reaction in the tissues between pyruvic and glycerophosphoric acids (233).

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 (28, 95). This anomalous effect is not produced by crystalline insulin, and must therefore be attributed to impurities in the commercial insulin preparations (65, 199). Extracts of pancreas have been shown to produce hyperglycaemia when injected intravenously (124, 66), 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 (65) 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."

Certain results which follow the injection of insulin, such as the increased excretion of allantoine in normal dogs, and of uric acid in the Dalmatian coach hound (232) are due to the increased output of adrenine resulting from insulin stimulation (209).

Control of Insulin Secretion. Claims have been made, but as yet on inadequate grounds, that the islet-tissue is under the control of a pituitary "pancreatropic" principle (cf. Chapter VIII). Increase in blood glucose in itself evokes increase in insulin output, independent of extrinsic innervation of the pancreas (213).

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 (80) 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 (149) and by Newburgh and Marsh (141). 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 diets have the further advantage of being cheaper, and more easily obtainable and prepared. The 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.

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.

It was shown many years ago by Hamman and Hirschman that if two consecutive doses of glucose are given to a healthy subject the degree of hyperglycaemia following the second is less than that from the first (78). The explanation which is usually accepted of this—the so-called "Traugott-Staube effect"—is that offered by Macleod (123), that the first dose of sugar sensitizes the islet-mechanism, so that the second dose calls forth insulin more readily.

Sweeny, in 1927 (183), determined the sugar tolerance curves of normal individuals during starvation, and on high-fat, high-protein and high-carbohydrate diets respectively. He found that fat diets and starvation lowered sugar tolerance, while high carbohydrate diets increased it, and considered that the former lessened the sensitivity of the islet-mechanism, while the latter improved it.

In 1929 Porges and Adlersberg published a monograph dealing exhaustively with their experimental and clinical work (151). Studies on non-diabetic patients gave results similar to those of Sweeney. Tolerance curves on patients kept for some time on a low carbohydrate diet, or a diet rich in fat, showed higher peaks and delayed returns to normal (while sometimes there was even an induced glycosuria) when contrasted with those for patients kept on a mixed diet. 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.

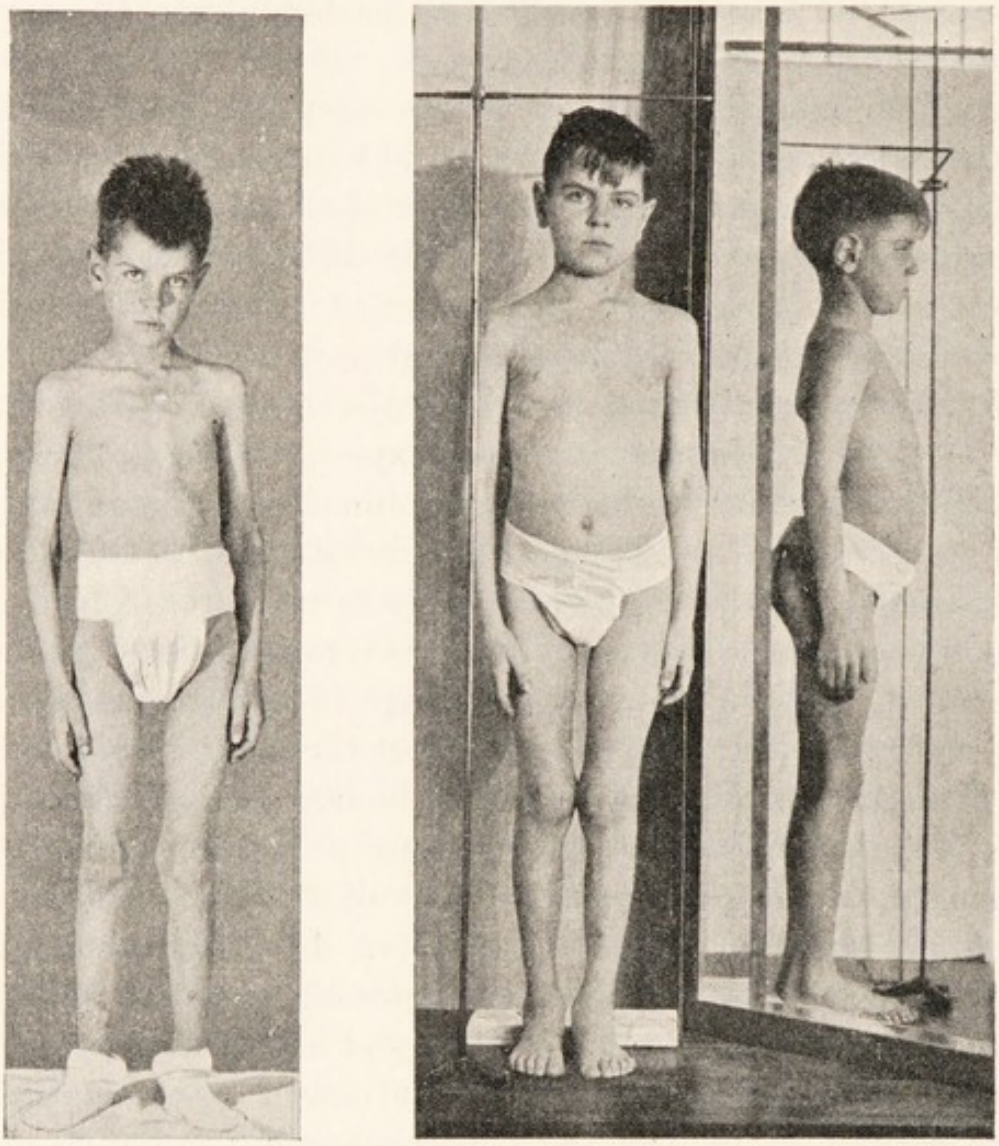
Little fat is fed (even as little as 50 grams), in a diet yielding a total calorie value of 3,000–4,000 calories. The

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 (158) 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 stresses a low-calorie, rather than a low-fat diet. 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 or abolish the need for it. He has recently reported on fifty cases kept on this treatment for five years or longer.

Sansum (170) 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. In a recent paper (74) he reports on seventy patients who have maintained such a diet for seven years. All showed increased well-being and physical fitness; forty-two showed an increased sugar tolerance. Geyelin (215) has obtained equally good results over a ten-year period.



A. B.

FIG. 18.—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. 32, 131). A very good example of the beneficial effect of such a diet in diabetic children is shown in Fig. 18.

Good results on diets with " higher " or normal carbohydrate content have been reported by a number of clinicians (165, 67, 97, 152, 246). (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.) It is the experience of most advocates of such diets that when patients are changed from a high fat, low carbohydrate to a low fat, high carbohydrate diet, insulin requirement is not only not increased, but is frequently decreased. Various suggestions have been put forward to account for this apparent paradox. Greater stimulation of the islets by the greater amount of carbohydrate, or by an increased ratio of liver glycogen to liver fat (169, 151), has been suggested. Rabinowitch (158) seems to consider that diabetes may be due not to hypoinsulinism, but to inability to utilize insulin, but this view affords no explanation of the paradox. Eason and Lyon (47) deny that the paradox exists, and claim that the increased tolerance is really due to a lower caloric intake, and thereby to partial inanition. They have tested diets with different carbohydrate-fat ratios, but the same caloric value, and find that with such diets increased carbohydrate needs increased insulin. Their findings seem in the nature of a minority report and certainly do not agree with those of Ellis and of Himsworth, now to be recorded.

Ellis (50) treated a number of severe cases of diabetes with glucose and insulin, given hourly, and with no other food, for a number of days. He found that 600 grams of glucose daily could be tolerated with no greater insulin than on a restricted diet, while in some cases there was a marked reduction in the amount of insulin necessary. In one—an extreme case—before this special treatment was instituted,

192 units of insulin per day were necessary, while on the twenty-first day of the treatment only 9 units were required. In no case was there any exacerbation of the diabetes.

Himsworth (87) has carried out careful experiments on normal healthy subjects which completely confirm the results of Hammon and Hirschman, Sweeney, and Porges and Adlers-

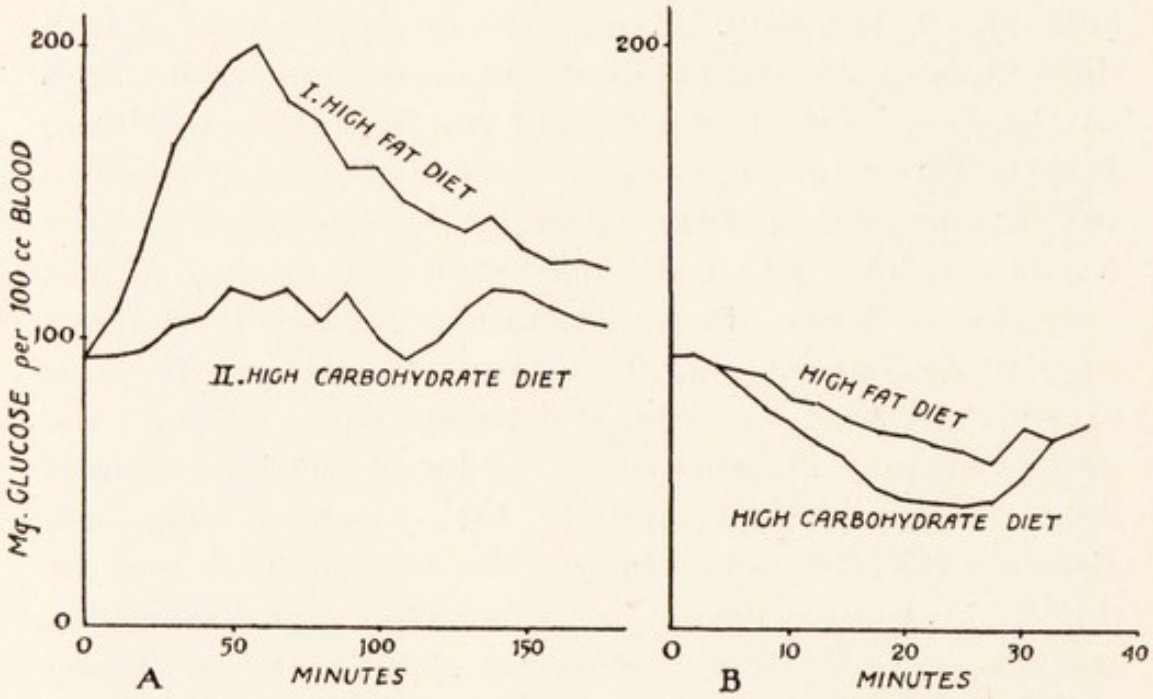


FIG. 19.—A. Two sugar tolerance curves after 50 grams of glucose, determined on the same healthy subject: (i) when accustomed to a high fat diet; and (ii) when accustomed to an equicaloric high carbohydrate diet. B. Two blood sugar curves following intravenous injection of 5 units of crystalline insulin. Both were obtained on the same healthy subject, the one during a period of high fat diet and the other when on an equicaloric high carbohydrate diet. (After Himsworth, *Brit. Med. J.*, 1934, ii, 57.)

berg. Glucose tolerance curves were determined on normal individuals habituated to a high fat, low carbohydrate diet, and contrasted with curves on the same individuals subsequently habituated to a low fat, high carbohydrate diet of equal caloric value. Typical results are contrasted in Fig. 19, A. They show definitely that the high carbohydrate diet increases the tolerance, and the high fat diet decreases

it. (The values are for capillary blood.) Himsworth also contrasted, again on the same subjects, the relative effects of injecting 5 units of crystalline insulin during each of the two states (habituation to high fat and to high carbohydrate respectively). Typical results are shown in Fig. 19, B. It is clear that *the effect of the same dose of insulin is greater on high carbohydrate than on high fat régime.* The increased tolerance is due not to change in calorie value nor to change in ketogenic-antiketogenic ratio, but solely to the increased amount of carbohydrate in the diet (223).

In discussing these results, Himsworth refers to the important finding of Allan in 1923 (5) with depancreatized dogs. There is no direct linear relation between the carbohydrate ingested and the amount of insulin needed to care for it and prevent glycosuria. The relationship is approximately logarithmic. The greater the relative amount of carbohydrate, the greater is the amount metabolized by each unit of insulin.

In the intact animal increased blood sugar leads to an insulin secretion which automatically holds it within normal limits (203, 64). In the diabetic with partial function the same response exists, but is not so active nor so successful. With high carbohydrate *régime*, such secreted insulin (or injected insulin), can care for relatively greater quantities of carbohydrate. This in itself indicates that but little more insulin should be necessary for increased carbohydrate, but does not explain why less is needed, nor have we any explanation for the logarithmic relationship itself. Why should insulin, at different levels of carbohydrate metabolism, be able to care for different amounts of carbohydrate ? Himsworth postulates an unknown, intermediate factor which governs the susceptibility of the organism to insulin, and in some way activates it. Insulin-resistant cases suffer from a deficiency of this factor, as well as of insulin. He divides diabetics, in consequence, into two classes, (i) insulin-sensitive, suffering from hypo-insulinism (a type

which easily develops hypoglycaemic symptoms), and (ii) insulin-insensitive, the diabetes being due entirely or partly to deficiency of the unknown factor (224). One wonders whether this interesting hypothesis can be related to the existence of the insulinotropic principle of the duodenum postulated by Laughton and Macallum and by LaBarre (cf. Chapter IX).

In the state of diabetic coma, in which the organism can well be considered as over-saturated with fat and the products of fat catabolism, the frequently increased resistance to insulin may well be attributed to that fat saturation itself, and administration of large doses of glucose, seems, as suggested by Himsworth, to be the logical treatment to increase sensitivity to insulin and gives good results (cf. also 113).

The view-point of the average diabetic clinic, as expressed by various recent reports, indicates a slower but a steady change towards increased carbohydrate. In a recent review of this dietary problem (101) Joslin states that Adlersberg and Porges have somewhat modified their original treatment, that Falta is adverse to low fat diet, and that he himself, in agreement with von Noorden, believes that the diet for an average adult should approximate to 140 grams of carbohydrate, 70 grams of protein and 90 grams of fat, although he somewhat negatives his own belief by adding that if diabetics can take care of more than 140 grams of carbohydrate with reasonable dosage of insulin, they should be given more.

Lawrence has published an account of a simple form of treatment which he claims gives excellent results (112). He gives from 100 to 150 grams of carbohydrate, carefully controlled in amount and balanced with insulin, and allows the patient to suit himself as to fat and protein intake.

Liver Rhythm. The conclusion of Forsgren and others (57, 92, 2) that there is a rhythmicity in liver function, with alternate and not coincident activity as regards glycogen formation and storage, and bile secretion (the former occurring chiefly at night), if it be correct, suggests an explanation

for the varying degrees of hyperglycaemia and glycosuria of diabetics at different times of the day, not entirely explicable by the incidence of meals, and Nöllerström (143) suggests that insulin dosage should be adjusted to this rhythm rather than to meal hours.

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 " (100).) Increased blood cholesterol has been suggested as one of the causes of arteriosclerosis (9) and as predisposing to diabetic gangrene and therefore of bad prognostic value (157, 196). Rabinowitch (237) stresses this for the young diabetic and believes there is evidence that high carbohydrate—low fat diet delays development of cardiovascular diseases in such cases. Joslin (99) 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.

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 (130) 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 (185) 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 slight, but only slight, improvement in two diabetic children following such operation.

His results are suggestive, but little definite conclusion can yet be drawn from them. Mass ligation of the pancreas near the head is without effect (107). It is obvious that such surgical treatment does not lead to sufficiently rapid and

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 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.

definite improvement to suggest that it should be widely employed.

Treatment of Diabetes with Staphylococcic Toxoid. Diabetics with focal infection in the upper respiratory tract exhibit a staphylococcic factor. Surgical removal of the focus induces slow improvement. Hence the effect of staphylococcic toxoid has been tested, and it is reported that weekly injections of toxoid lower the amount of insulin required (68).

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 (12, 85, 8). 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 (75). 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 (116), 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 (71).

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 (194). 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 (153). 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 (52).

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 (138).

Cases of hyperthyroidism frequently exhibit a glycosuria, but the simultaneous occurrence of hyperthyroidism and diabetes mellitus is rare (102).

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 (134) 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 (190). 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. In adults obesity is certainly a predisposing factor, as many writers, and especially Joslin (100) have stressed. But the cause of the diabetes is probably to be traced, not to the obesity itself, but to some one or more of the factors which have led to that obesity. (Hess has shown that high fat, or high fat and carbohydrate in the diet of rainbow trout causes a fatty degeneration of the pancreas with reduction of islet tissue (222).) Himsworth speaks of a dietary disposition. He has attempted to determine what were the diets of diabetics prior to their diabetes (223), and considers that he has obtained evidence that they had a relatively high fat content and diminished carbohydrate content. Since he has shown (p. 170) that such diets impair sugar tolerance and insulin sensitization, their chronic effects obviously possess potentialities as causative factors of diabetes. He has further attempted a correlation between the incidence of diabetes and the diet of different races, nations, and social classes, and obtains supporting evidence for his thesis, although it is doubtful if he has satisfactorily explained the absence of diabetes in Eskimos (cf. 245, 238).

Such an explanation based on diet does not apply to the child diabetic who is, as Joslin points out, seldom obese. General bacterial infections are probably seldom the direct cause, since they might be expected to precipitate the severest grade of diabetes suddenly, 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 (105) ; 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 " (159, cf. also 137).

It has been suggested, on experimental grounds, that the susceptibility to infection on the part of the diabetic is due to a disordered cell-nutrition closely associated with diminution of cell-glycogen reserve (240).

de Candia (208A) speaks of a hypoplastic-thymic-lymphatic constitution as a causative factor in severe (constitutional) diabetes of childhood.

Acute pancreatitis leads to disturbances of islet function in a large proportion of cases ; only a few of these develop a true diabetes (188, 166, 14, 184).

Murray-Lyon finds a hereditary incidence in about 16 per cent. of diabetics (138) ; Cammidge finds it in 40 per cent. (208). The existence of a hereditary predisposition is indicated by the more frequent occurrence of diabetes (of the same grade of severity) in similar than in dissimilar twins, and it has been suggested that the potentiality for developing the disease is transmitted as a simple Mendelian recessive (187, 198A, 234).

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 (100)) 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. 69), 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 (83) (providing choline). 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. 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. 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.

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, 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 (198) 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 (25): "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 (86) 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 (142). Avery (10), 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 (154). Similar effects were not produced by injection of insulin into fatty tissue in normal rats (164).

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" (100). 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 (189, cf. 121). Foetal mortality is not lessened by such insulin treatment (176) and exact control of the pregnant diabetic is necessary to prevent undue demands on the islet tissue of the foetus (46). In fact Priscilla White has recently written that prevention of the death and decay of the overripe foetus of the diabetic mother is a challenge to the obstetrician and the research worker in diabetes. She considers that premature delivery by Caesarian section is the solution (247). Izquierdo (96) 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 " (53), or the lack of a " co-enzyme " (111). Himsworth's theory concerning resistance to insulin has been quoted (cf. p. 171). It has been suggested that obesity lessens the response of the diabetic to insulin (13).

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 (110): "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 (136) 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 (135). The claim has not been substantiated, and there is obviously potential danger of toxic action on the kidneys from the phosphotungstate (110). 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 (181). The claim is not supported (15). Administration with

liver extract is said to favour absorption from the stomach (15).

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 (191, 93).

Insulin Administration by Inunction. Claims have been made that this procedure yields good results, both in the experimental animal (221) and with diabetic patients (236). A considerably greater dosage is required than by subcutaneous injection.

Protamine Insulinate. The greatest forward step in insulin therapy during the past few years has been the preparation of protamine compounds of insulin by Hagedorn and his collaborators at Copenhagen (220), and their demonstration that these permit greater and easier control of the diabetic patient, especially that patient who responds to insulin treatment by pronounced oscillation of blood sugar. As they point out, injections of insulin are at best only a poor imitation of nature's continuous mechanism, and serious disturbances do not usually follow such sudden and discontinuous dosage only because the organism possesses additional means of regulating the blood sugar. They therefore attempted to discover a procedure whereby absorption of insulin from the tissues, following injection, could be slowed down, and thereby its action rendered more continuous. They tried to find a derivative which, unlike the hydrochloride, should be only sparingly soluble in tissue fluids. Insulin, like other proteins, is amphoteric, and capable of combining with both acids and bases. Since its salt with nucleic acid had an iso-electric zone—the zone of least solubility—more acid than that of insulin itself, this suggested that a compound with a base might be more effective. Compounds with kyrin, histones, and globin proved unsuitable; those with protamines seemed promising. The compounds with clupeine, scombrine, and salmine (the protamines from herring, mackerel, and salmon sperm,

respectively) proved to be still too soluble in tissue fluids, but a new protamine prepared from the sperm of the rainbow trout *Salmo irideus* had only a very slight solubility at *p*H 7·3, and was therefore tested extensively on clinical patients, during the last two years. To quote from the investigators themselves, the results " have shown that the sharp peak effect, usually seen three or four hours after the injection of ordinary insulin, is largely avoided by the use of protamine insulinate. Furthermore, the effect . . . is more prolonged—roughly about twice as long as that of ordinary insulin. Without diminishing the number of injections, we can by this means diminish the blood sugar fluctuation, reduce or suppress the glycosuria, and reduce the ammonia excretion, and at the same time reduce the risk of the occurrence of hypoglycaemia."

The injection is painless. Protein reactions do not occur, and there is no local reaction or other ill effect. Sometimes the needed dosage is lessened. The protamine compound is of no special value for patients whom insulin controls satisfactorily, and it is unsuitable for acute conditions such as coma, on account of its slower action.

Root, White, Marble and Stotz (241) have tested the treatment on fifteen patients and confirm the Danish clinicians. The latter used a diet containing 100 grams of carbohydrate. The Americans obtained equally good results with higher carbohydrate diets (130 to 140 grams); they note that Hagedorn's plan of spacing the carbohydrate 40, 40, and 20 per cent. over the three meals is advantageous. Where only two injections are needed per day it is found best to administer insulin in the morning and protamine insulinate at night. Further reports of good effects have been made by the Toronto school (229) and by Lawrence and Archer (233A).

The effect of the insulinate in lengthening and dampening down the insulin action is shown in Fig. 20.

Administration of the new compound has some very

definite disadvantages. It is not indefinitely stable. Therefore every few days it is necessary to add protamine and sodium phosphate (for buffering effect) to insulin under sterile conditions. Furthermore, it is doubtful if the supply of protamine from the particular species of fish can be increased sufficiently to permit a large supply. Neverthe-

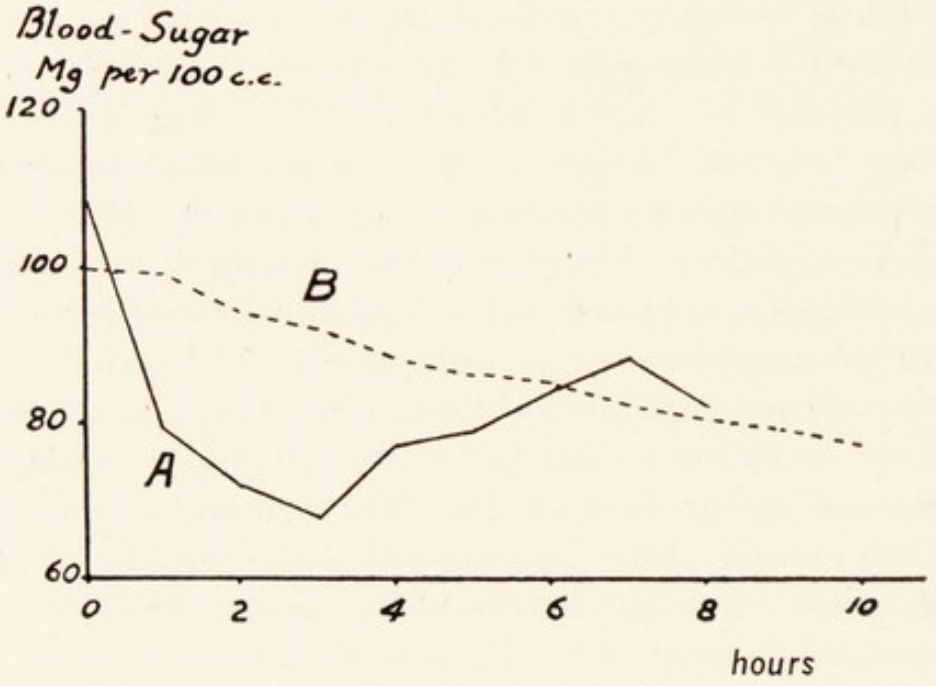


FIG. 20.—Contrast of the effects of insulin and protamine insulinate on the blood sugar. Miss M. G., normal, aet. 25, nurse-dietitian. Both tests after an over-night fast. No food taken during the tests. A. October 23rd. Effect of 8 units of insulin (Lilly) given subcutaneously. B. October 25th. Effect of 10 units of protamine insulinate (equivalent to 7·3 units of insulin (Lilly)). (After Root, White, Marble and Stotz, *J. Am. Med. Assoc.*, 1936, cvi, 180, Fig. 1.)

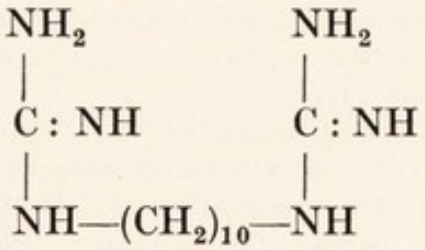
less, now that this method of improving insulin action has been demonstrated, it should be possible to produce other compounds of insulin with like properties.

In this connection the observations of Scott and Fisher (243) are also of importance. Testing the effect of zinc salts on the action of insulin (cf. p. 157) they have found that in concentration of 0·1 per cent. zinc greatly delays and prolongs the effect on rabbits, while it appears

to exaggerate the delaying effect of protamine insulinate. Spermine from beef pancreas has effects somewhat similar to protamines.

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 (192) 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 (60) :



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 (72). More recent results lead to no more favourable conclusions, either regarding synthalin or Frank's later preparation, dodecamethylene

diguanidine (synthalin B) (60), 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 (20), and liver glycogen is caused to disappear (168). Not only is the respiratory quotient not increased (84) but oxidation seems actually to be inhibited and the formation of lactic acid increased (180).

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 (20, 106, 19). Comparison of various synthalin homologues shows that the toxicity and hypoglycaemic effects run parallel (18).

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 (118), 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.

Labbé (231) has reviewed the use of vegetable " insulinoids " in diabetes, and believes that a concentrate from the radicles of germinated barley has been shown both by experiments on animals and by its employment in clinical diabetes to produce definite hypoglycaemic effects, with diminution of the signs and symptoms of acidosis. It is considered to be effective when given either subcutaneously or orally.

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.

Dietary Substitutes in Diabetes. Some years ago *intarvin* was introduced into diabetic dietaries. This is the glyceride of margaric acid, a C_{17} fatty acid, and it was supposed to be of benefit, since it could not give rise to acetoacetic acid in the organism. It has fallen into disuse.

" Sionon," d-sorbitol, the alcohol corresponding to glucose, has been recommended as a sweetening agent, but does not replace carbohydrate and is costly (148, 163).

Such substitutes are, of course, unnecessary if the diabetic is correctly treated.

Pseudo-hypoinsulinism due to Antagonistic Secretions

It has been claimed that after injection of insulin has depressed the blood sugar of an animal, injection of an extract of the posterior pituitary gland will elevate it (29, 42). Simultaneous injections of pituitrin and insulin can be so adjusted that the blood sugar remains unaltered (42). The effect seems to be the result of a direct antagonism; apparently the so-called " oxytocic principle " of the pituitary is concerned (127). There is, however, some evidence against this supposed antagonism (197).

Some proportion of acromegalics exhibit glycosuria; a smaller proportion exhibit a true diabetes mellitus (43). Since acromegaly is associated with hypersecretion of the *anterior* pituitary, the relationship indicated by such observations is distinct from the antagonism just referred to. Certain cases which exhibit concurrent acromegaly and diabetes sometimes recover from the latter spontaneously (39), or after removal of a pituitary tumour (49). de Wesse-

low (246A) has furnished evidence that in some proportion of elderly obese " diabetics " the glycosuria is due to excess of a pituitary factor in the circulation, rather than to deficiency of insulin.

Cammidge (34) 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 or indirect relationship between some one or more of the internal secretions of the pituitary and insulin is now well established. Consideration must be deferred to Chapter VIII.

Hyperinsulinism and Hypoglycaemia

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 (80).

Since Harris' early observations numerous cases of hyperinsulinism have been described in the literature. 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.

Very varied symptoms are associated with clinical hypoglycaemia, although marked cases exhibit a fairly definite syndrome (172, 63) comparable with the sequelae of removal of the liver in dogs, reported in Mann and Magath's classic experiments (128) and with the symptoms observed in diabetic patients following overdosage with insulin (81). Most of the symptoms are probably traceable to disturbances originating in the central nervous system, due to the fact that it is peculiarly susceptible to glucose starvation from low blood sugar, since it has no store of carbohydrate (91).

Experimental evidence is becoming available that such brain starvation does actually occur (211, 205). It has been shown that chronic insulin intoxication in rabbits and dogs produces marked, though not specific, histological changes in brain tissue (244, 218).

Wauchope has published an excellent review of the subject (193). He lists the symptoms according to relative time of onset : (i) fatigue and lassitude, restlessness, malaise ; (ii) (due to compensatory secretion of adrenine) pallor or occasional flushing, cold clammy perspiration, palpitation, tremor, often hunger or thirst, fear ; (iii) senses clouded, with frequently behaviouristic resemblance to alcoholic intoxication, bravado, negativism, hallucination ; (iv) convulsions and paralysis, with loss of memory ; (v) coma ; and in the extreme cases (vi) death.

Transitions from one stage to the next may be gradual or rapid. The attack can be completely aborted at any stage by administration of carbohydrate. It may be arrested at any stage even without treatment, and there seem to be no permanent ill-effects. But the hypoglycaemia tends to exhaust the glycogen stores and so predisposes to another attack, and recurring attacks tend to be more severe.

In the severe attacks " 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 " (63).

Symptoms develop at variable levels of blood sugar. Extremes of 28 and 70 mg. per 100 c.c. have been reported. The precise values are of course complicated by the fact that many ordinary methods of estimation give too high values for glucose by from 20 to 30 mg. It has even been reported that the " true " sugar value in rabbit's blood at the incidence of insulin convulsions is zero (45). Wauchope, using what I have elsewhere termed " enhanced-glucose " values (32), considers as approximately correct generalizations : normal fasting value 100 mg. per 100 c.c. blood ; initial symptoms 80 mg. ; severe symptoms 50 to 45 mg. ; death at about 25 mg. (193). Some of these values are almost certainly too high, even for " enhanced-glucose."

There is evidence, not yet quite convincing, that symptoms can occur in some cases associated with a marked sudden fall of blood sugar level, rather than a fall to a specifically low level (193, 182). Such cases, if they do occur, are rare.

In many cases of hypoglycaemia the causative factor cannot yet be definitely stated. It seems therefore better at present to undertake no rigid classification, and the cases will only roughly be subdivided into (i) those of true hyperinsulinism, associated with tumour of the islets, either benign or malignant, (ii) cases not associated with hyperinsulinism, and (iii) cases in which the cause of the hypoglycaemia is uncertain.

Cases Associated with Tumour of the Islets. The first definite case was reported by Wilder, Allan, Power, and Robinson in 1927 (200). 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 examina-

tion 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. A similar case has very recently been reported from the same clinic (235), in which also an extract of the liver metastases produced an insulin-like effect. Similar cases have been reported elsewhere (*e.g.*, 115, 186) though without biochemical examination of the metastases. 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 (94) 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.

McClenahan and Norris (119) 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 (*e.g.*, 202, 43, 36, 61, 104). The tumours are more frequently benign than malignant, though Judd and Rynearson (227) consider them to be either malignant or pre-malignant.

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 (139) reported a case exhibiting marked hypoglycaemia and convulsions; at subsequent autopsy the liver was found to be riddled with carcinoma. Crawford (41A) 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.

Judd (228) has reported two cases of marked spontaneous hypoglycaemia associated with decrease of hepatic function and a cirrhotic condition of the liver.

In milder degree liver deficiency seems responsible for hypoglycaemia associated with "recurrent vomiting" in children, through some degree of fatty degeneration (103), or with phosphorus poisoning (122), or with acute yellow atrophy of the liver (54), or in chloroform poisoning in dogs (22). Lowered blood sugar also occurs along with parenchymatous changes in the liver caused by arsphenamine, by hydrazine, or by the fungus *Agaricus bulbosus* (150A). The occasional cases of hypoglycaemia seen in pernicious vomiting of pregnancy (33) are also probably traceable to undue depletion of liver glycogen during the pregnant state.

Many cases seem due to unusual depletion of the carbohydrate depôts ; in at least some of these cases there may be *deficiency in the liver capacity for storage of glycogen* (cf. 77), perhaps the opposite condition of von Gierke's disease.

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. 32.)

Hypoglycaemia of Uncertain Cause. (*a*) *Hypoglycaemia Possibly Associated with Hyperplasia of Islet Tissue.* Cases have been reported in which exploration did not reveal a tumour, but partial resection of the pancreas was performed, and benefit claimed (55, 3, 161). Yet overactivity of islet tissue is difficult to demonstrate in absence of tumour ; the

beneficial results from such resections are never as definite as those following removal of a tumour. Berry (206) has, however, reported a carefully controlled case, in which more than half the pancreas was removed, and sugar tolerance was undoubtedly improved. The case of a premature child born of a diabetic mother exemplifies simple hypertrophy and hyperplasia. It exhibited a somewhat low blood sugar and died on the third day. Autopsy showed apparent hypertrophy and hyperplasia of the islets (73). In a similar case (239) a living child was removed by Caesarian section, and kept alive by sugar administration. At birth the mother's blood sugar was 0·28 per cent., that of blood from the umbilical cord was 0·19 per cent., and that of the baby's blood was 0·04 per cent. The baby exhibited some hypoglycaemic symptoms. The hypoglycaemia reported in a child of eight months may have had a similar origin (230).

(*b*) *Hypoglycaemia Associated with Various Conditions.* Many cases have been treated by dietary measures, with varying success. In absence of operation, the cause of the hypoglycaemia is, of course, uncertain. Successful treatment may suggest a hyperinsulinism due to hyperplasia of the islets (63, 174, 120), but this cannot be regarded as established.

Hypoglycaemia, sometimes sufficiently recurrent to produce persistent symptoms, has been associated with severe burns (76), premature labour (150), adrenal insufficiency, including Addison's disease (7, 160), pituitary tumours (201), menstruation (195), chronic infections, recurrent bilious attacks, neurasthenia, etc. (33, 51).

An association between anginal pain and hypoglycaemia seems possible (179); cases have been reported of cardiac pain due to insulin overdosage (133). Sippe has recently published a study of five cases which he regards as hypoglycaemic angina of the cardiac type. In such cases effort produces precordial pain and a feeling of exhaustion, both relieved by

rest. The pain and exhaustion may occur some time after the exertion (whereas in angina pectoris the distress occurs immediately). The pain may be of true anginal type or in the nature of a constant ache referred to the precordial area (178).

In myxoedema hypoglycaemia may occur in absence of symptoms (193).

Goldzieher (216) has analysed the findings in 112 cases which he considers as chronically hypoglycaemic, and stresses (probably too much) hypopituitarism and hypothyroidism as potential causes.

The drop below fasting value frequently seen towards the end of a sugar tolerance test on a normal individual is undoubtedly due to a slight degree of physiological hyperinsulinism, the end result of the stimulus of the glucose meal. In cases of renal glycosuria the extra loss of sugar resulting from the lowered kidney threshold can apparently sometimes lead to hypoglycaemic symptoms (193).

Association of Hypoglycaemia with Abnormal Mental States. Shih-Hao and Hisao-Chien (175), 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 (33), 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 had 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 (167) has reported three cases exhibiting severe hysterical attacks ; one progressed to an epileptiform state, which proved to be due to hypoglycaemia. McGovern's case (120) 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 carbo-

hydrate 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 (55): "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.

A case with visual hallucinations and mild but definite catatonia has been reported by Greenwood (219). Gray and Burtness (217) stress headache, often of the migrainous type, as a condition associated with a blood sugar level between 60 and 90 mg. per 100 c.c. Such headache is completely or partly relieved by frequent carbohydrate meals and has been reproduced by induced hypoglycaemia.

Differentiation and Treatment of the Causes of Hypoglycaemia. Harris (80) 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 (80, 63, 120, 33).

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. Every patient should be dieted to suit his own particular needs, and should be taught food values just as is the diabetic. Harris recommends that an adult of average height and weight should be given about 2,250 calories, made up of 90 to 120 grams of carbohydrate, 60 to 75 grams of protein and the rest fats (cream and butter), vitamins and salts being properly cared for (80, 174). John (225) recommends insulin and a high fat diet in the functional type of case.

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. Judd and Rynearson (227) stress the danger that such tumours may become malignant, and that delayed operation may lead to the finding of an inoperable condition.

It would seem justifiable to conclude that in all cases where very low blood sugar values (25 or 30 mg. per 100 c.c.) are found recurring even occasionally in a series of tests, where there is no good response to dietetic treatment, and especially where the history shows rapid onset, or increasing severity of the condition, laparotomy is called for.

Gray and Burtness (217) have suggested an "insulin tolerance test" for hypoglycaemia, in which after a twelve to sixteen hours' fast intravenous injection of 0·01 unit of insulin per kg. body weight is given. This idea is based on Collip's concept that the parenteral administration of any endocrine principle is inversely proportional to its concentration of the body (cf. p. 6), and thus theoretically applies only to cases of hyperinsulinism, and not to all cases of hypoglycaemia. The authors claim that diabetic patients

show a greater fall of blood sugar below the fasting value than do normal persons, and hypoglycaemic patients a lesser fall than normal. They state that patients experience no discomfort during this test. From a few tests carried out under my direction I am unable to corroborate the last statement.

The Use of Insulin in Non-diabetic Conditions

Glucose-insulin therapy is of proved benefit in numerous non-diabetic conditions, through the stimulation of appetite induced by insulin. Some of the claims presented in the literature undoubtedly require confirmation.

Excellent results have been obtained with non-diabetic tuberculous patients, the majority of whom, even in severe cases, show increase of appetite and gain of weight and strength (38, 144, 44, 6, 11). Many cases of malnutrition have benefited (177, 132, 24, 37), but Freyberg thinks the benefit is due to suggestion (214). It is of benefit in congestive heart failure and especially with patients with intractable angina pectoris. The opinion has been advanced that anginal pain is related to faulty carbohydrate metabolism in heart muscle, which is corrected by the insulin (179).

Insulin promotes the fattening of chronically thin people to optimal weight. A dose of, at maximum, 10 units, given thrice daily twenty to thirty minutes before meals, leads to rapid gain of weight, increased well-being and less nervousness, and thus acts as an admirable tonic. The gain in weight is demonstrably due to actual increase in fat deposits, the fat cells becoming enlarged (21).

Insulin-glucose is of value in combating acidosis, recurrent vomiting and acute intestinal intoxication in children (69), and the acidosis developing in prolonged narcosis produced for therapeutic purposes in certain types of mental disease (155). It is said to be a useful adjunct in the treatment of

drug addiction (26, 210) and peptic ulcer (226, 31). Good results in coeliac disease have been claimed by several writers (cf. 247).

Several writers claim that it is useful in certain dermatoses, especially those associated with a disturbance in carbohydrate metabolism (24, 1, 140). Benefit has been claimed in cases of hyperthyroidism, arteritis (24), typhus, chronic uraemia, cholelithiasis, and melanosarcoma with metastases (48). Claims that this therapy is useful in pernicious vomiting of pregnancy are denied (79).

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CHAPTER V

THE ADRENAL GLANDS

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, three series of facts have been generally recognized for a considerable time.

These glands are composed of two separate types of tissue, which in mammals constitute their *cortex* and *medulla*; in elasmobranch fishes these tissues exhibit no form of union. Transition stages are seen in the amphibia. 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 (124) 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 (124), Sharpey-Schafer (104), Goldzieher (44), 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 (125).

[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 (124, 44).

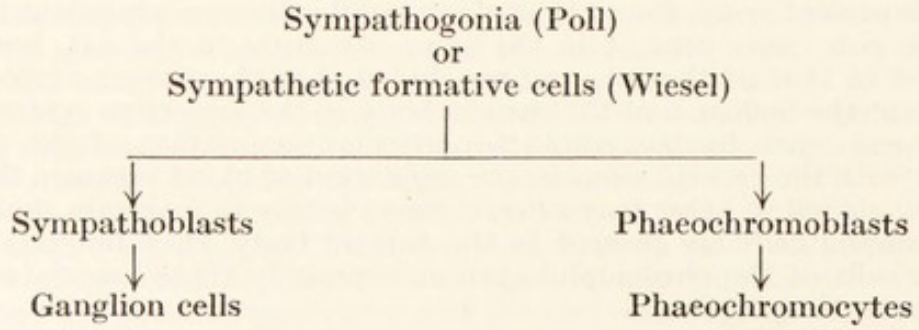
The medulla consists of "a solid cell-mass permeated by sinus-like blood vessels. . . . There can be little doubt that

[1] Christianna Smith (110), 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.

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" (104).

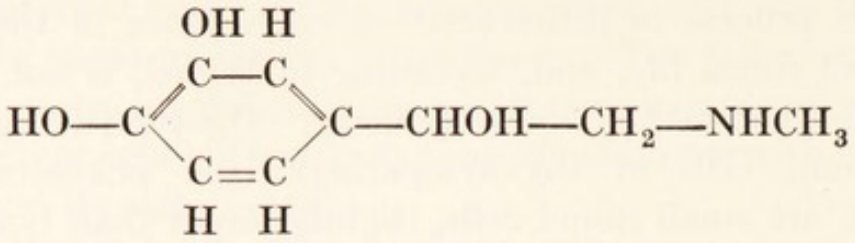
Rabin (93) has summarized the embryonic development succinctly. "The suprarenal cortex develops from the splanchnic mesoderm. Developmentally, and in most respects functionally, also, it may be considered a separate organ. . . . The immediate anlage of the suprarenal medulla, and the anlages of the remainder 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 (124). 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. (For methods of preparation see Harrow and Sherman (49).)

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 (29) 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.

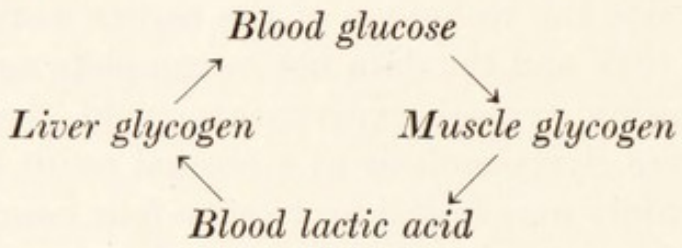
In earlier work, perfusion experiments with the livers of such cold-blooded animals as the frog and turtle demonstrated that adrenine increases glycogenolysis with resulting formation of glucose. The effect is not so marked or so regular when mammalian livers are used. It has been assumed that the same 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 (31), 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 (101). 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 (31).

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 (31).

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 (31), 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 (29) :



(The results of certain other investigations are not in complete agreement with these conclusions (32, 100, 39).)

Cori (29) 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.

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 (29). 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 (29).

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 (29). The action of adrenine on glycogen is probably only a lytic one (134).

Hrubetz (150) showed that when rabbits are injected subcutaneously with varying doses of adrenine, the blood sugar reaches its maximum value one and a half hours after injection, independent of the dose, and does not return to normal level till more than four hours after the injection. Rise of blood sugar level is proportional to dosage with dosages varying from 0·05 to 0·2 mg. per kg., after which the effect becomes relatively less.

The Calorigenic Action of Adrenine. The term was introduced by Boothby and Sandiford (11) 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 (30). 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 (19). It is produced rapidly and ceases rapidly, following cessation of injection.

The Formation of Adrenine in the Adrenal Medulla. Various earlier claims made for different suggested precursors (87, 119, 71) have been disproved (86, 38, 102). A claim has

been made, on inadequate grounds, that there is a circulation of adrenine in the organism (112).

Their chemical relationship suggests strongly that adrenine is formed from tyrosine. Schuler (171), testing various potential precursors with surviving adrenal medulla tissue in Ringer's solution, and using Folin's reaction as a test for adrenine, found that phenylethylamine increased the reaction slightly, and tyramine markedly. Tyrosine and phenylalanine produced no effect. The adrenine-like nature of the product was proved by blood-pressure experiments. Schuler considers that tyramine is probably formed in the kidneys by decarboxylation from tyrosine, and is then transformed by the adrenals (172).

One of the functions of ascorbic acid (vitamin C) in the adrenals may be to stabilize adrenine, since it tends to prevent its oxidation (147).

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 (114, 20)).

Stewart (114) 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 (20) 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."

Recent work tends to harmonize the views of Stewart and Cannon. 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.

Vincent and Thompson (126) showed that Cow (33) 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 (123, 35, 36). 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 (13).

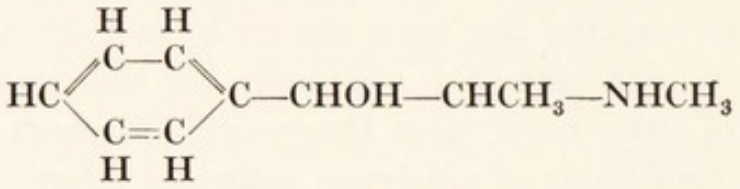
Cannon (19) has recently summarized the evidence in favour of discontinuity of adrenal secretion, but admits that " there is no logical antagonism between the ' tonus ' theory and the ' emergency ' theory." Since even such minor exercise as walking has been shown to call forth a definite secretion of adrenine (21), 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 (77). 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 (78). 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 (34) 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 (26).

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.

Various related compounds with comparable effects have been synthetized.

Chemical Transmission of Nerve Impulses. While the evidence is convincing that adrenine is a true endocrine compound, secreted by the adrenal medulla into the adrenal vein, and there-

after producing its sympathomimetic action in the tissues to which it is carried, strong evidence is also now available that *impulses transmitted by the sympathetic and parasympathetic nerves are then chemically transmitted by substances liberated at the nerve endings, which thereupon act upon the effector structures.* Adrenine itself, or a compound closely related to it, is believed to be responsible for transmission from the great majority of the sympathetic fibres, and acetylcholine, $CH_3.CO.O.CH_2.CH_2.N(CH_3)_3.OH$, for transmission from parasympathetic fibres.

These compounds, so produced, can scarcely be regarded as endocrine, yet their behaviour is so closely related to that of the product of the adrenal medulla that a short account of their actions seems desirable. This is largely taken from recent papers by Dale (37) and McSwiney (80).

Injection of acetylcholine produces very exactly the various effects which result from stimulation of different parasympathetic nerves. It has been isolated from the spleen, and possibly from other organs, so that it may be considered a normal product of the organism. It has not yet been isolated from blood. It is not only very unstable, but the blood contains a specific esterase which hydrolyses it rapidly to choline and acetic acid. Progress in proving its presence in relation to nerve transmission has only been possible since the discovery that eserine (physostigmine) inhibits the action of the specific enzyme. Hence, whenever eserine intensifies or prolongs a nervous effect, there is ground for believing that acetylcholine is concerned in the transmission of that effect ; cumulative evidence is convincing.

The effects attributable to the intermediation of acetylcholine include vagus inhibition on the heart (Loewi) and probably most of the actions produced through the vagus (Dale), the stimulation of salivary secretion (Babkin and others), the transmission of stimuli through the splanchnic nerves to the adrenals (Feldberg and Minz), and the transmission of intestinal peristalsis (Le Heux). It seems probable that it is the agent by which impulses are passed across the synapse of the ganglion cell, it may be concerned with the transmission of nervous excitation to voluntary muscle, and even with transmission of impulses in the central gray matter of the brain (Dale).

When in a cat, adrenalectomized and deprived of all other chromophil tissue, the lower end of the sympathetic chain is stimulated, something passes into the blood which produces adrenine-like actions (Cannon), including a rise in blood sugar (133). Cannon believes that he has sufficient evidence to distinguish this from adrenine itself, and has termed it " sympathin." Complete removal of the chain of sympathetic ganglia abolishes the phenomenon.

When the cervical sympathetic nerve is stimulated, this substance appears in the aqueous humour of the eye. It has been

shown to be a catechol derivative with an aminated side chain (Bacq). It is therefore so closely related to adrenine that convincing evidence will be necessary to disprove its identity with the latter. Cannon, however, refuses to admit identity, and even asserts the existence of two "sympathins," one excitatory and one inhibitory (139).

Since there is not complete parallelism between the two chemical mechanisms and the two divisions of the autonomic nervous system, Dale suggests that nerves might be classified as "cholinergic," or as "adrenergic," according to the chemical substance which is liberated to transmit their impulses.

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. 250), 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 (44).

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 (44) 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 (93), 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 (93).)

Rabin (93), Labbé (69), and Goldzieher (43) have summarized the literature of such cases. About forty have been reported. Some are much better authenticated than others. Typical authenticated cases are :

Labbé's cases (69). 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.

Rabin's case (93). 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 supra-

renal 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.

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 (84), 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 (105), reported the case of a woman,

[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."

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 (92) 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.

In a case reported this year from the Mayo Clinic (151), in which the blood pressure varied from 90/70 between attacks to 280/160 or similar figures in attacks, palpation

of the right kidney suggested tenderness, and at operation a large tumour 10·5 cm. in diameter was removed ; there was a fringe of cortex at the lower border. The tumour was filled with necrotic material and apparently many haemorrhages had taken place into it. Its total weight was 240 grams ; one half of it yielded 120 mg. of crystalline adrenine. The patient made a complete recovery.

Volhard (127) 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 (122) 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.

Cases of paroxysmal hypertension have been reported in which X-ray examination suggested which adrenal was involved, and the indication was proved correct at subsequent successful operation (60).

Hicks (53) has described a case in which a tumour—a phaeochromocytoma—was discovered at post-mortem examination attached to an adrenal by a short fibrous pedicle through which it received its blood supply. Extract of the tumour, injected intravenously into a dog, produced a marked pressor effect. The patient had not exhibited hypertension. In a similar case reported by Rogers (94) hypertension was present.

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 (93) has reviewed the literature of such tumours, and considers that Mayo's case should perhaps be included among them (cf. also 69, 166).

A case of malignant phaeochromocytoma of the adrenals has been reported by King (64). Although the tumour contained typical chromophil cells, which were also present in some of the many metastases, the patient had exhibited normal blood pressure.

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 (44).

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 (103). Oppel (89) 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

Results following Extirpation. 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 " (124, cf. also 4).

Biedl showed, in 1910, that removal of the " interrenal body " (cf. p. 208) in elasmobranch fishes produced a very similar series of symptoms. His results have recently been fully confirmed by Kisch (65). 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.

The rat seems more resistant to double adrenalectomy than do most mammals ; this is probably associated with the more frequent presence of accessory cortical bodies in this animal. The mortality rate thus varies greatly in different laboratories (131), age at operation being also an important determining factor (176) ; comparison of results is rendered difficult. Chronic effects can be more easily determined in the rat. Levy Simpson and Korenschevsky stress the invariable presence of decreased appetite ; certain other effects are undoubtedly secondary to this. Growth is impaired and there is poor fat deposition, delayed involution of the thymus, and increase in weight of the secondary sex organs (173) ; degeneration of the second convoluted tubules of the kidney has been observed (174).

Metabolic Changes. During the penultimate stages the blood urea and non-protein nitrogen rise, while blood glucose falls. The inorganic phosphate of the plasma gradually increases, while the carbon dioxide capacity decreases (96, 129). One of the most definite effects is a change in the relative proportions of the mineral constituents of the blood. There is a marked fall in sodium content, a lesser fall of

chloride (and correspondingly increased excretion of these constituents in the urine), while potassium and magnesium contents are increased (6, 75).

In the frog, after muscular asthenia is present, there is, according to Moschini, a marked drop in the creatine-phosphate content of muscle (161).

The significance of these and other changes will be discussed in the section dealing with the function of the cortical principle.

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 especially 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.)

Hartman (50, 51) also uses cats and employs a two-stage

operation. Swingle and Pfiffner employ both cats and dogs (116).

Stewart and Rogoff extracted adrenals with excess of 0·9 per cent. saline containing a little glycerol, with subsequent addition of alcohol, and fractionation of the extract with benzene. They showed that injections of such extracts into both dogs and cats definitely lengthened the survival period, indicating that these extracts contained the active principle of the cortex. This they termed *interrenalin* (96, 115, 113).

Hartman extracts adrenal cortex material with ether (which removes very little adrenine), evaporates off the ether, extracts the residue with warm 80 per cent. alcohol and chills the extract. Inert material separates and is removed. The alcohol is distilled off *in vacuo*, and the residue is dissolved in water and sterilized. It is almost free from adrenine.

Continued injection of this extract into adrenalectomized cats keeps them alive indefinitely. Adrenalectomized young female rats have been kept alive to maturity and have reared normal litters. When such adrenalectomized rats are exposed to cold the body temperature falls and they may die. Injection of the extract restores their normal reactivity to cold. Hartman terms the active principle *cortin ;* this name is gradually being generally accepted.

Swingle and Pfiffner obtain their active preparation by extraction of the adrenals with ethyl alcohol, and subsequent treatment of the extract with benzene and acetone, discarding residues, then distribution between 70 per cent. alcohol and petroleum ether, transference of the alcohol fraction to 95 per cent. alcohol, filtration through permutit (which removes adrenine), and transference to water. The extract so obtained appears to be somewhat more potent than that of Hartman (79). It also keeps adrenalectomized dogs and cats alive for indefinitely long periods.

Various methods, based on the same general principles, have been employed by Zwemer, Kutz, Grollman and Firor, and others, and potent extracts obtained.[1]

Hoskins (57, 41) 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 has increased 20 to 30 mm. mercury, and the diastolic pressure showed corresponding increases. The patients also showed some increase in body weight and red cell count.

An active extract has been prepared from the interrenal bodies of skates (143).

Crystalline products have been obtained from the adrenal cortex by various workers (*e.g.*, 45, 62), but it is still doubtful if pure cortin has been obtained crystalline. Kendall (153) claims to have separated nine closely related compounds, including degradation products and derivatives, and thinks that three, to which he ascribes the formulae $C_{26}H_{36}O_5$, $C_{21}H_{34}O_5$, and $C_{21}H_{30}O_5$, are of functional significance. Pfiffner and Wintersteiner (164) have also obtained a number of crystalline compounds with somewhat similar formulae, but find them all to be physiologically inactive. Their most potent preparation so far (165) is a pale yellow syrup, a homogeneous non-crystalline fraction assaying 400 dog-units per mg. (cf. p. 237), soluble in ethyl and methyl alcohols, acetone, chloroform, and other lipoid solvents, and glacial acetic acid, and sparingly soluble in ether, water, and benzene. It is probably nitrogen-free.

Physiological Properties of the Cortical Principle. These have been studied by Hartman (50, 51), Swingle and Pfiffner (116), Britton (17), Wilson (128A), Houssay (58), and others.

[1] For full details of the various methods of preparation of the cortical principle see Harrow and Sherwin (49).

Many of their findings are in agreement, although there is still marked disagreement as to the function of the principle, as revealed by such findings. In most of the extracts used in such work 1 c.c. is equivalent to 40 grams of whole gland.

Adrenalectomized cats, injected daily with a small dose (0·5 to 1·0 c.c.) of active extract, have been kept in perfect

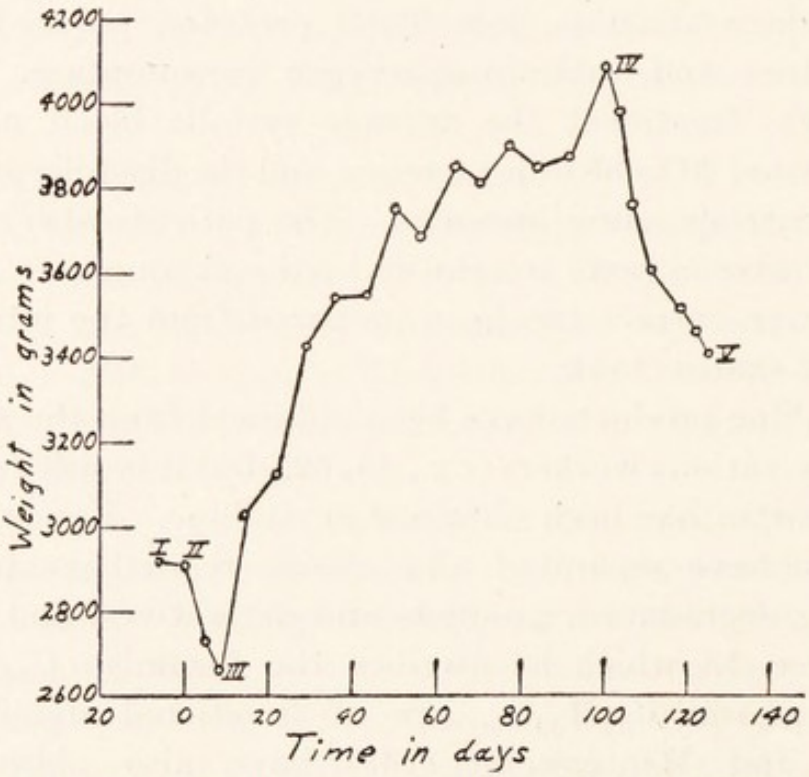


FIG. 21.—The weight chart of a bilaterally adrenalectomized cat treated with an active extract of the cortico-adrenal principle, following the exhibition of severe symptoms of adrenal insufficiency. I. Right adrenal removed. II. Left adrenal removed. III. Animal prostrate; treatment begun. IV. Treatment discontinued. V. Death from adrenal insufficiency. (From Pfiffner and Swingle, *Endocrinology*, 1931, xv, 338.)

health for 100 days, when they were sacrificed to demonstrate 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

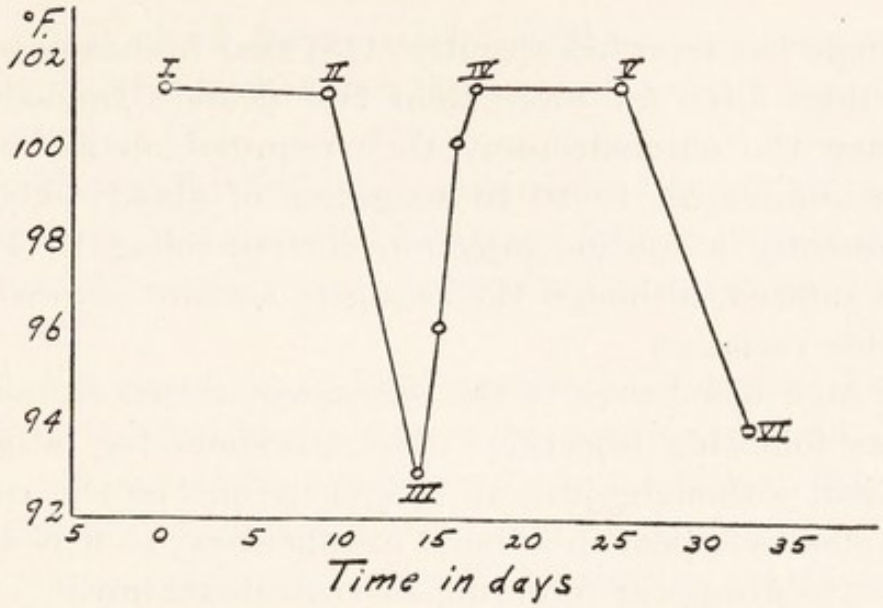


FIG. 22.—Rectal temperature chart of a bilaterally adrenalectomized cat treated intraperitoneally with an active extract of the cortico-adrenal principle, following the exhibition of severe symptoms of adrenal insufficiency. I. Second adrenal removed. II. No symptoms. III. Prostrate; treatment begun. IV. No symptoms. V. Treatment discontinued. VI. Animal prostrate (died of adrenal insufficiency). (From Pfiffner and Swingle, *Endocrinology*, *loc. cit.*)

FIG. 23.—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.)

injections in larger and more frequent dosage brings complete restoration. Similar results have been obtained with dogs.

Swingle has reported recently (118) that he has now kept three dogs alive for more than two years. Immediately following the adrenalectomy they required an amount of extract equivalent to 20 to 40 grams of gland daily, but subsequently a routine injection corresponding to 4 to 8 grams sufficed, although the requisite amount showed considerable variation.

The high blood urea of the adrenalectomized animal disappears following injection. The threshold for fatigue is increased, although, just as fatigue is one of the earliest symptoms to appear in adrenal insufficiency, so it is among the last to disappear following successful treatment.

The effects of deficiency and administration of the principle are well shown in Fig. 21 (showing changes in body weight) and Fig. 22 (showing changes in body temperature). The perfect condition of adrenalectomized animals following prolonged replacement therapy is demonstrated in Fig. 23.

It is clear that cortin is effective by mouth, whether in the form of adrenal cortex tissue itself or in concentrated extract. But oral administration is not nearly as effective as parenteral injection. Swingle (118) states that his extract is only one-twelfth as effective by oral route. Grollman and Firor claim that theirs is one-fifth as effective (47). They state that this is due to rapid inactivation of the principle in alkaline medium. For similar reasons fresh or desiccated adrenal gland is not very effective when given orally, while it is toxic unless the medulla is completely removed. They find that, as tested on adrenalectomized dogs, cortex tissue heated rapidly to 100° C. can be used successfully.

Intraperitoneal injections act rapidly. Cats in extreme pre-terminal prostration definitely improve in from fifteen to thirty minutes. Convulsions are abolished and the animals attempt to sit up. Within an hour they may walk about and appear almost normal. Within two hours they may take food. For complete restoration of such cats, a twenty-four-hour dose equivalent to 0·125 to 0·25 gram of

fresh whole gland per kg. body weight is necessary, representing at least 2,000 times the amount present in a normal cat's adrenals (17).

It is noteworthy that the maintenance dose is always much greater than that present at any one time in normal glands. The recovery dose from pre-terminal prostration is much greater still.

Large excess of the cortical extract is non-toxic. Definite effects on normal animals have been reported (and denied (156).) It is said that capability for exertion is increased. Hypertrophy of the anterior pituitary is produced in young rats, but no changes have been observed in the thyroid or adrenals. Statements concerning the gonads are conflicting, and will be referred to in connection with adrenal tumours.

The principle is excreted in urine in minute quantities. It has been estimated that 1 litre of urine contains the amount in 0·5 gram of gland (46, cf. 91).

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 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) " (116).

Kendall (62) criticizes the method on account of the great variability shown in the response of adrenalectomized dogs.

Kutz has suggested a method of assay using rats (68), and Everse and de Fremery (40) another based on the decreased rate of production of fatigue of muscle produced by injections of the principle into anaesthetized adrenalectomized rats.

Function of the Cortical Principle. There are at present two antagonistic schools of thought concerning this function.

One, of which R. F. Loeb (156) is a protagonist, stresses the control of sodium ions, and attempts to relate the various changes associated with cortical deficiency to the loss of effective control of sodium metabolism. The other school, of which Britton is the leading protagonist, considers the chief, or even the sole function of cortin is the control of carbohydrate metabolism, all other effects associated with the adrenal cortex being secondary (14, 16). Swingle and Pfiffner's view that the chief function is regulation and maintenance of a normal volume of liquid within the vascular system (116) is not necessarily antagonistic to the theory of sodium control. A satisfactory decision as to which of these theories is most accurate cannot yet be made.

Our knowledge concerning the effects following complete adrenalectomy has been supplemented by studies on animals controlled by cortin therapy following extirpation of both adrenals, and then allowed to develop insufficiency by withdrawal of this therapy. Loeb has recently summarized the results (156 ; cf. 130).

At a varying period in the initial stages of adrenal deficiency, through increased excretion of sodium, associated especially with chloride and with water, dehydration results, and, later, anhydraemia. The excretion of sodium is so great that it must come from interstitial fluid as well as from blood. The blood plasma volume decreases ; its non-protein nitrogen and urea, and to a lesser extent the plasma proteins, increase. The concentration of sodium in the plasma falls, that of chloride somewhat less, and that of potassium rises ; the sodium and urea changes are interrelated (157).

There is evidence of disturbed renal function, indicated both by blood changes and kidney functional tests. Whether or not the effect on the kidneys is prior to or subsequent to the undue loss of sodium is not clear.

Later events, as Loeb points out, may well be the sequels of the loss of sodium and resulting disturbances in mineral

metabolism, and the dehydration. These include loss of weight, refusal of food, and manifestation of shock. If Moschini's observation concerning the drop in creatine-phosphate content of muscle (cf. p. 231) is correct, and applies generally, it may afford an adequate explanation for the asthenia, though it has still to be linked causatively with the phenomena which precede it.

Swingle and Pfiffner would attribute the anhydraemia and circulatory collapse to transudation into the tissues (116), and claim that adrenalectomized dogs can mobilize fluid from their tissues under the action of cortin, even when deprived of food and water during the period of its action. But Harrop (145) claims that he has proved the anhydraemia is at least partly due to renal loss of fluid.

Swingle and Pfiffner stress the resemblance of the penultimate stage in these animals to traumatic and secondary shock in man, and have therefore suggested that cortin may have therapeutic value in treatment of shock (117). Wolfram and Zwemer obtained some evidence that cortin tends to prevent anaphylactic shock (182), and there is further evidence associating cortin with the defence mechanisms of the body (137).

Parkins, Taylor and Swingle (163) find that when normal dogs are subjected to severe trauma to both hind limbs there is only a slight reduction in plasma sodium and chloride even in extreme secondary shock, whereas the healthy adrenalectomized dog balanced with cortin responds to even mild muscle trauma by marked decrease of blood sodium and chloride and circulatory collapse. Such a dog is similarly affected even by intraperitoneal injection of isotonic glucose (which is distinctly adverse to Britton's views) and will succumb unless large doses of cortin are given it. When such traumatic shock is produced in the normal animal, depletion of lipoid and haemorrhagic changes are found in its adrenal cortical tissue (141).

Britton stresses the hypoglycaemia associated with adrenal

deficiency, and maintains that convulsions preceding death from this deficiency are typical of hypoglycaemia and not of anhydraemia, though he admits the chemical changes affecting sodium and other constituents. But he argues that intraperitoneal injection of glucose solutions depresses blood sodium and chloride to much lower levels than occur following adrenalectomy, and without deleterious results. He states (135) that the adrenalectomized opossum and marmot die in hypoglycaemia, with decreased liver and muscle glycogen, and that death cannot be traced to sodium and chloride disturbances, since in these animals plasma sodium and chloride are increased, and their excretion lessened. He admits (175) that cortin may be linked with water balance and indirectly associated with salt balance ; his arguments seem to be somewhat insecurely based on experimental evidence.

MacLean (159) has made a curious observation linking increased salt intake with increased sensitivity to insulin, and thus hypoglycaemia ; this is the converse of the relationship suggested by Britton's theory.

Loeb (156) suggests that Britton's experimental results can be attributed to removal of adrenal medullary tissue. Yet Zwemer and Sullivan (184) have shown that removal of cortical tissue has greater effect on the blood sugar of cats than removal of the medulla, and Evan's studies on increased liver glycogen formation in animals under diminished atmospheric pressure also suggest that certain aspects of carbohydrate metabolism are more closely associated with cortin than with adrenine, since adrenalectomy abolishes such increases while removal of all the medullary tissue does not (141A). Leloir's review of the relationship of carbohydrate metabolism to the adrenals (154) also shows that cortin, directly or indirectly, is concerned with the height of blood sugar, and the synthesis of muscle glycogen.

It is obviously not yet possible to draw final conclusions concerning the function of cortin. But the evidence asso-

ciating it with sodium metabolism seems more clear cut than that limiting its action primarily to control of carbohydrate metabolism, especially when the remarkable effect of salt therapy in the adrenalectomized animal and in Addison's disease is borne in mind (see below and p. 248). There is of course the possibility of more than one unrelated function, and of more than one active principle of the cortex.

Svirbely's observation (178) that cortin does not prolong the survival period of guinea-pigs on a scorbutic diet may prove of importance since it suggests that vitamin C (ascorbic acid) is necessary in adequate amount for the proper function of cortin.

Cortilactin. Adrenalectomized rats will bear young if treated with cortin, but make no attempt to suckle them (51). Swingle and Pfiffner's preparation supports lactation in the dog (116). Hartman attributes the difference to different methods of preparation. In the preparation of cortin fatty substances are removed by chilling to $-12°$ C. If the temperature is only lowered to $-3°$, the resulting extract supports lactation. Hartman concludes that some substance is removed between $-3°$ and $-12°$ which is necessary for milk production. An extract of this fraction was added to cortin and injected into adrenalectomized pregnant female rats. They raised a larger proportion of young. Hartman concludes that there is a second principle of the adrenal cortex concerned with lactation, and terms it *cortilactin* (18) (cf., however, 141B).

Cortipressin. Looney and Darnell (158) claim to have separated from the adrenal cortex by extraction with a solution of equal volumes of alcohol, glycerol and water at *p*H 11 material which after extended oral administration produces a prolonged increase in blood pressure. They have fractionated the active constituent to some extent and have shown that its solubility properties do not resemble those of cortin and that it is not adrenine. They suggest the name *cortipressin* (cf. also 149).

The Adrenalectomized Animal and Salt Therapy. The view that adrenal cortical function is concerned with mineral metabolism and especially that of sodium is greatly strengthened by the results following the administration of sodium salts to animals in a condition of cortical deficiency. It is well recognized that the lives of such adrenalectomized

animals can be prolonged by daily injections of sodium chloride solution (cf. 130), and recently Kendall has claimed (152, 153) that adrenalectomized dogs may be maintained in normal condition by continued administration of sodium chloride, plus sodium citrate or carbonate, without cortin, and if the potassium intake be kept low, such animals can even be carried through a reproductive cycle without cortin. (They are peculiarly sensitive to potassium salts, which precipitate a crisis.) They cannot be maintained indefinitely on sodium chloride alone.

Hypo-Cortico-Adrenalism and 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 (99). It is significant that the latter not only reproduce Addison's original paper in their monograph, but, in agreement with 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. 231). 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 (81) thinks that there may be a racial factor, and that the disease is relatively commoner in Spain.

A Diagnostic Test. By withdrawing salt from the diet of patients suspected of suffering from Addison's disease (and in absence of pigmentation, or with dark-complexioned patients, diagnosis is sometimes difficult) it is possible to produce symptoms of crisis. This provocative test obviously should only be employed in hospitals, and when a supply of a known active preparation of cortin is available (177). A positive result may not be shown for three or four days (162).

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.

The lowered sodium and chloride content of the blood which follows adrenalectomy is also found in severe Addison's disease (74). Glycogen formation is interfered with, both from glucose and lactic acid ; adrenine only slightly mobilizes liver glycogen. Creatinuria is present (179).

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 hope that cortical replacement therapy may become as successful as insulin therapy has in diabetes mellitus. Since, however, adrenal material is much more difficult to obtain and its content of its principle at any time is so much less than its normal output, 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.

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. 236) 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 " (99). Pigmentation lessens. Adrenine and the related ephedrine are not effective when given alone in Addison's disease.

Effects of Cortin and Salt Therapy. Good results have been obtained following administration of any of the *active*

preparations of cortin. Those of Rogoff and Stewart for "interrenalin" (97, 95), of Hartman (51, 52) and others (55, 2, 9) with his preparation of cortin, and of Rowntree and Green (116, 99, 98) and others (120, 107, 3, 22) with that of Swingle and Pfiffner may be cited.

Provided treatment with active preparations of cortin be instituted before a moribund condition is reached, and sufficient extract is available for massive doses when necessary, favourable results are to be expected. Most patients show a striking response within twenty-four to seventy-two hours. Nausea and vomiting stop. Appetite reappears. There is gain in weight and strength. The pigmentation appears to decrease. The patient regains a sense of both physical and mental vigour and well-being. The blood pressure may increase slightly, but this is for the most part a response to increased activity and not a specific effect of cortin.

Since a course of treatment frequently consists of the administration of 40 to 60 c.c. (spread over four to ten days) of an extract of which 1 c.c. represents 30 grams of adrenal cortex (the entire supply from two steers), it is obvious that such dosages suggest that a relatively enormous amount of cortin is requisite. 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."

Intravenous injection is recommended. Intramuscular injection is well borne by some proportion of patients, but subcutaneous injection is too irritating. Various simple preparations have been described, suitable for oral administration (57, 41, 142, 144, 148).

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.

A case of the disease has been successfully carried through pregnancy by combined oral and injection administration of the extract (90).

Knowledge that adrenalectomy affects sodium metabolism not only explains the good results following intravenous administration of saline in Addison's disease, but has also led to more definite therapy. Good results are obtained, at least for a time, by treating cases with high salt diet and specific addition of sodium chloride (up to 10 grams daily) (76, 48). Some proportion of patients respond well to administration of sodium chloride (or of sodium chloride combined with other sodium salts such as the citrate (48, 177), with little or no cortin. The larger proportion require cortin as well, especially in crises. (Weller (180) claims that *glucose* will relieve symptoms in all but the terminal stages.)

Harrop (48) has critically reviewed the treatment of the disease. He considers that the adrenal cortex extract is not so successful as is usually believed, at least when a rigid diagnosis of the disease is adhered to. He thinks that its clinical value as a routine treatment during the remissions of the disease has not been demonstrated, although it is not harmful.

Patients with Addison's disease, in spite of availability of the extract, still die within a fairly short time, although it is doubtful if the effect of continued massive dosage has been sufficiently tested. Snell (111), in summing up the results obtained with the extract at the Mayo Clinic, where 48 cases have been treated between May, 1930, and January, 1934, states that of these 32 have died, and of the 32 at least 12 died in spite of the treatment. He thinks there is a slight but definite gain in length of life.

In view of such criticisms and conclusions, statements as

to dosage cannot yet be regarded as final. Simpson (108) considered that 10 to 20 c.c. of the extract daily should be a satisfactory maintenance dosage. If Harrop's view is correct, such amounts may not be of special value except in crises, when still larger amounts are needed.

The lack of complete success with cortin therapy may quite possibly be due to the lack of marked activity of at least some if not most of the preparations commercially available. Thus Loeb (155) remarks that if adrenal insufficiency in man is not relieved by salt administration, it will not be relieved by commercial cortical extracts given in the usual dosage. (Cf. also Rogoff (168) and Grollman and Firor (142).) There is all the more reason to hope that the synthesis of cortin will be achieved in the near future, so that large amounts of the *pure* compound may be available for clinical use.

Unusual Treatment in Addison's Disease. Slight, if transient, benefit has been reported following grafts of adrenal tissue from dead persons or from sheep (1).

Various investigators have described disturbances in sulphur metabolism following adrenalectomy, and such observations apparently suggested to Rivoire (1932) the desirability of testing the effect of intravenous injection of cysteine. He claimed improvement in one case. Léobardy and Labesse (73) recently reported the results in three cases. Cysteine hydrochloride, 0·01 gram, in a few cubic centimetres of water, sterilized and neutralized with sterile sodium carbonate before injection, is injected daily with occasional cessation for several days. They claim to have obtained excellent results as regards body weight, activity and blood pressure. Pigmentation diminished, but did not disappear. The treatment needs to be continuous. Simultaneous administration of cortical extract produces no added benefit. Rivoire (167) thinks that the treatment has not received the attention it merits, but admits that, since it is now his practice to administer sodium chloride also, it is difficult to say what benefit should be attributed to the cysteine itself.

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. 229, 242). The pigmentation is not invariably present and is not paralleled in adrenalectomized animals.

Nevertheless, there seem to be increasing adherents to the view that Addison's disease is not merely a result of cortical deficiency. Various writers (48, 8) claim that treatment with cortical extract produces no definite effect on hypotension or pigmentation. Nor can results obtained with adrenalectomized animals be paralleled with those on cases of the disease.

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 (72): "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

The extract has been tested on normal persons, psychical effects being ruled out by occasional control doses of saline or brain tissue extract. It seems to produce a capacity for increased effort, a certain composure of the nervous system and a sense of increased well-being. Menstruation is sometimes brought on at a slightly earlier period (52).

Some proportion of cases of nervous asthenia are benefited (52, 98). Statements regarding hyperthyroid conditions are not in complete agreement, although apparently in some cases benefit follows injection of the extract (52, 98, 62, 128). (Cortin counteracts the action of thyroxine on guinea-pigs (161A).)

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 (10). Still more empirical is the use of desiccated adrenal cortex orally in 6 cases of vomiting of pregnancy ; good results were claimed (61).

Hyper-Cortico-Adrenalism

Some clue to the nature of the disease-syndrome which will result from hyperfunction of the adrenal cortex should be obtainable by careful studies of the effects following administration of heavy and continuous doses of active cortical preparations to normal animals. However, 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.

Relationship Between the Adrenal Cortex and the Gonads. There seems definite evidence that adrenalectomy produces in male rats loss of libido and potency and degeneration of

the seminiferous tubules (23), and in 90 per cent. or more of female rats suppression of the oestrous cycle and atrophy of the ovaries (83, 23, 28). In the latter, the cycle can be restored by homotransplants of adrenal cortex (83) or by injection of cortical extract (28, 116, 15).

There is a significant increase in the size of the adrenals, due to cortical hypertrophy, during the breeding season of the ground squirrel; a similar change follows artificial stimulation of the gonads at a time of sexual inactivity. There is an expansion of the reticular zone of the cortex; correspondingly, following castration, degenerative changes occur in this zone (183).

In agreement with these findings, it is recognized that in Addison's disease amenorrhoea, absence of libido, impotence and atrophy of the testes may occur, while treatment with the cortical extract tends to produce return to normal sex function (99, 108).

There is thus excellent evidence that deficiency of the cortical principle leads to depression of gonad function. But there is no convincing evidence that excess of the principle over-stimulates that function.

Britton and his co-workers found that injection of cortical extract produced sexual precocity in rats through precocious maturation of the sex glands. Presence of corpora lutea in the ovaries and hypertrophy of the uterus were observed in female rats at twenty-eight days following two weeks' injection (17). Müller (88) and Klein (66) found that development of the female gonads is depressed, but that of the male gonads is promoted. Cassida and Hellbaum claim that the ovaries are stimulated (24). A number of other investigators have either got negative or uncertain results (cf. 109).

This lack of definite results seems the more surprising when the findings in cases of cortical tumours are considered.

Adrenal Cortical Tumours. Adrenal hyperplasia in foetal life has been suggested as the cause of pseudo-hermaphro-

ditism, but, although it is admitted that such hyperplasia is frequently found at autopsy, the *rôle* of the adrenal in this condition is still uncertain (44). The frequent finding of benign or malignant tumours of the adrenal cortex in cases of virilism and of pubertas praecox drew attention to the possibility that the tumours might be causative factors, and the hypothesis was strengthened by the beneficial results following removal of the tumour in a number of cases. Articles by Hoskins (39) and Goldzieher (44), and more recently by Cecil (25) and Simpson, Kohn-Speyer and Korenschevsky (109) permit some degree of classification of the possible effects of such tumours. The subject has attained added importance in connection with the recent pituitary syndrome described by Cushing (cf. Chapter VIII).

The effects produced by most cortical tumours are positive in kind, suggesting an over-production rather than an under-production of the cortical principle. The nature of these effects varies with age and sex.

In young and adolescent boys what may be termed—after Cecil—the "herculean" type results. There is precocious growth, early muscular development, early ossification, and early dentition. Hair appears early on pubis, face and body. The skin is rough, and acne common. The external genitalia enlarge to adult size. If the tumour develops after puberty this precocious "maleness" is impossible. In most of these cases, with a benign or slow-growing malignant tumour, the result is premature senility and early death.

Rarely, in adult males, the development is towards the female type. The breasts in these rare cases enlarge, and even secrete milk, the testes atrophy, there is loss of libido and development of the female type of obesity. Operation and removal of the tumour in such a case can result in return to normal.

In the female the predominating change is towards maleness. If the tumour occurs before puberty, whether it be an adenoma or a slow-growing malignant tumour, marked

changes towards the adult type occur. The girl becomes fat. She seldom shows unusual muscular development. Hair appears early on the pubis and sometimes on the face. The skin is red, coarse and dry, and acne is common. The vocal cords are enlarged; the voice becomes coarse and ugly. Ossification and dentition may be hastened. The child's mentality is usually normal.

The clitoris enlarges to the size of the penis. The labia majora are enlarged and covered with hair. But the internal genital organs may be smaller than usual. Menstruation does not usually begin at the age of puberty.

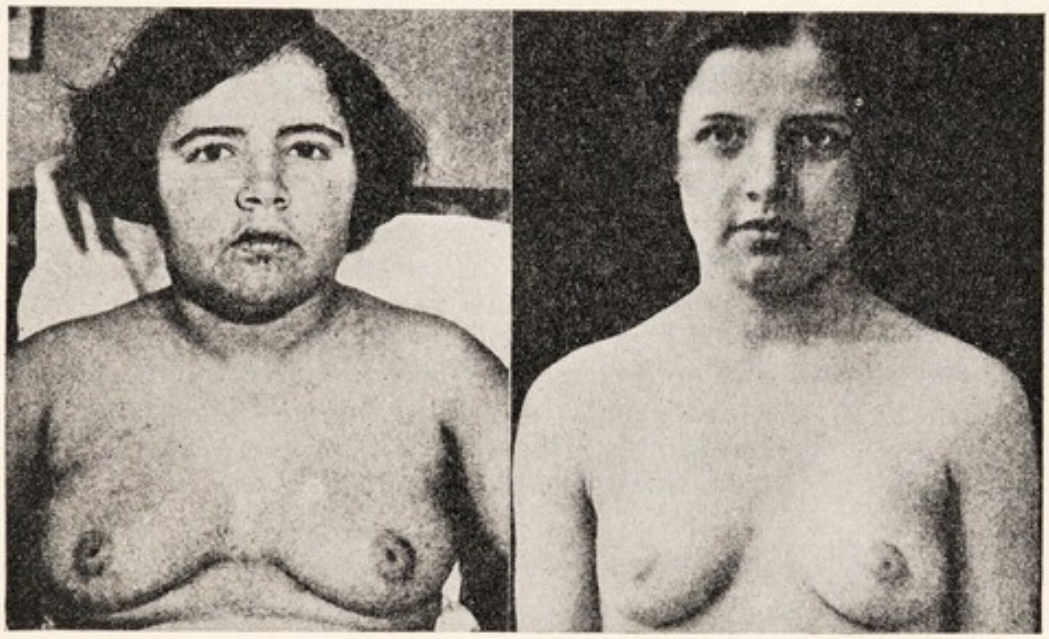
When tumour growth commences after puberty the changes are of a corresponding nature. Menstruation ceases —this is usually the first symptom noticed. There is loss of sexual desire and often of the normal female modesty. Occasionally the patient becomes attracted towards those of her own sex. Hirsutism appears. The pubic hair takes on male distribution. Hair appears on the face and later on legs, arms, abdomen, chest and back. The hair of the head becomes coarse and dry. The general distribution, Cecil points out, is much more profuse than that occurring in most men. The skin is red or brown, and dry. Acne is frequent. Pigmentation (unlike that in Addison's disease) can occur. Striae atrophicae appear on abdomen, hips and thighs. The voice becomes masculine. Clitoris and labia enlarge. The uterus and ovaries may atrophy. The breasts diminish in size. The obesity is striking, with distribution of fat on abdomen, chest, buttocks and hips, but not on arms and legs, and full and unsightly face (with fat in the cheeks, under the chin and in the neck).

Hypertension may be present; if it is, it is not of the paroxysmal type.

A third type exists, in which amenorrhoea is present, with obesity, hypertrichosis, diabetes and hypertension (25, 70, 121). Cecil believes that this syndrome is pluriglandular, and that the pituitary is involved. However, cases un-

doubtedly occur exhibiting all these conditions, in which the pituitary is normal, as far as serial sections indicate.[1] Yet, when control of the adrenal cortex by the pituitary is remembered (cf. Chapter VIII), association of the pituitary cannot be excluded in any of these cases.

Surgical removal of such tumours has now been performed



A B

FIG. 24.—Tumour of the adrenal cortex in a girl of nine years of age. A. Photograph taken fourteen days after removal of the tumour, before any external changes had occurred. B. Taken four months later. For details, see text. (From Kepler, Kennedy, Davis, Walters and Wilder, *Proc. Staff Meetings Mayo Clinic*, 1934, ix, 169; and *Annals of Surgery*, 1934.)

in many cases, especially in girls and women. The results are marked.

The hirsutism disappears (the loss of hair being largely at the menstrual periods). The clitoris, labia and breasts return to normal size; uterus and ovaries resume normal function within a few months. The obesity disappears. The masculine voice persists longest.

[1] Hunter's case (59) comes within this category (12) (cf. also 63), and that of Calder and Porio (138) is an excellent example.

Fig. 24 represents the striking change produced by removal of such a tumour from a patient in the Mayo Clinic (63). This patient commenced to show unusual development of breasts, generalized growth of hair, and deepening and coarsening of the voice at the age of four, with the usual subsequent developments. Mentally she was normal. She was operated on at nine years of age. At that time her height was 4 feet 5 inches, within normal limits, but her weight was 103 lb.—36 lb. over weight. The sella turcica was normal. At operation the right adrenal was half the usual size, but the left was replaced by a large encapsulated cortical adenoma, 6 × 4 × 2 cm.

The first picture (Fig. 24, A) was taken fourteen days after the operation, before any external changes had occurred. Within a week of that time weight commenced to fall and hair commenced to disappear, and later the skin became smooth, the voice higher pitched and the external genitalia smaller. While previous to operation there had been menstrual spotting for a year at two-month intervals, subsequently there was none.

The second picture (Fig. 24, B) was taken four months later. The weight was now only 72 lb. The body proportions were normal except for the breasts, still somewhat of adult type, and the external genitalia, still large. The appearance is more youthful, though still much beyond her years.

As already stated, in numerous recent autopsy reports in the literature an adrenal cortical tumour was found in absence of a pituitary basophile tumour. The evidence excluding pituitary influence in cases such as that just quoted is naturally not so final. Nevertheless, the astonishing change to normal or nearly normal following surgical removal of these tumours strongly suggests that the whole of the signs and symptoms in these cases can properly be referred to the adrenal tumour.

The importance of this point lies in the fact that the syndrome described by Cushing, and associated by him with basophile tumours of the anterior pituitary, appears to be identical with that resulting from cortical tumour. The condition associated with the rare arrhenoblastomata of the ovary is also similar, but obesity is absent and differentiation is possible.

Cecil stresses the necessity of considering whether the opposite adrenal is sufficiently normal to maintain life before

removing the whole of the tumour. The Mayo Clinic overcome possible transient deficiency by treatment with cortical extract for a while after operation.

A possible explanation of the marked sex changes resulting from such tumours is that perhaps they arise, not from differentiated cortical tissue, but from small islands of undifferentiated mesenchymal tissue related in origin to the sex glands and present in the normal adrenal in the region of the capsule (42). Whether this theory could be extended to explain a typical case in which the tumour originated in an accessory cortical body in the neighbourhood of the solar plexus (67) is perhaps doubtful. Nor does it explain the differences in the syndromes associated respectively with adrenal tumours and arrhenoblastomata of the ovary.

Broster and Vines (136) stress the frequent occurrence of virilism without adrenal tumours and even without cortical hyperplasia, and claim good results by removal of the (larger) adrenal. Their data are insufficiently convincing.

Adrenal Denervation

The potential danger following surgical interference with the integrity of the adrenals (except in the presence of an adrenal neoplasm) is exemplified by the history of a case reported by Rogoff (169). The patient, a diabetic, was controlled fairly well by diet and insulin. Becoming aware through the lay Press that certain surgeons were advocating denervation of the adrenals as relief or cure for diabetes, and being assured by his own surgeon that no harm could come of it, he requested the treatment, and following denervation of both adrenals developed Addison's disease rather rapidly; treatment for it was complicated by his diabetes, so that the disease rapidly had a fatal termination.

Rogoff points out that surgical procedure of this nature cannot be expected to be of permanent benefit, since denervation will usually be followed by regeneration of the nerves, while excision of one gland is usually followed by hypertrophy of the other (cf., however, Hartman (146)), so that "surgical intervention with the adrenals for various con-

ditions—Raynaud's disease, spontaneous gangrene, hypertension, epilepsy, gastric ulcer, thyroid disease, diabetes and the like—is to be deprecated." DeCourcy has presented the opposite view for cases of hypertension (140).

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CHAPTER VI

THE THYMUS AND PINEAL GLANDS

Introduction

UNTIL the last two or three years it was possible to make very few definitely accurate statements about either of these glands. While there was evidence associating both with endocrine function, much of it was so confused and contradictory that it was not even possible to state definitely that either the pineal or the thymus was an endocrine gland.

The recent work by Rowntree and his collaborators, working with extracts prepared by Hanson, has led to such striking results that, although no marked degree of concentration of the active principles of these glands can have been attained, and although time has not yet permitted confirmation from other laboratories, it seems desirable to set out these results in some detail. A brief statement of definite knowledge prior to Rowntree's work will first be given.

Earlier Work on the Thymus. The thymus of most mammals is situated in the thorax, but in some species it is found in the neck, and in some in both positions. It is made up of several lobules, each divisible into cortex and medulla. While there is strong resemblance to the structure of lymphatic glands, the medulla is characterized by the presence of the peculiar concentric corpuscles of Hassall, whose origin and function are not known.

The thymus seems to be relatively and absolutely largest during the period of the body's greatest growth ; at puberty involution commences, and the gland gradually atrophies.

Though the histological resemblance of the gland to lymphoid tissue suggested that it merely functions as a large mass of such tissue (18), yet an opinion has long been held that its function is associated with the growth of the organism (5). The earlier literature has recently been reviewed by Rowntree, who gives an extensive bibliography (12).

The first definite advance towards proving the " growth " hypothesis is due to Asher and his colleagues (2, 3), who succeeded in obtaining a concentrated extract, freed from protein and lipoid material, which appeared to accelerate growth in rats when given in daily dosage of 1 mg. Asher considered that the extract contained the active principle of the gland, and termed this *thymocrescin*. The extract was prepared by treatment of calves' thymuses with acetone and ether, then extraction of the residue with water, and fractional precipitation and extraction with alcohol, water, and ammonium sulphate. It was considered to be a sulphur-containing polypeptide. It will be seen that Hanson's preparation is obtained by a very different method. General growth, growth of the skeleton, and growth of the gonads all appeared to be accelerated by thymocrescin. Important is the observation that extracts of lymph glands, prepared in precisely similar fashion, were found to be inactive.

An entirely different extract of thymus was prepared by Temesvary in 1926, and termed *thymophysin*. It was supposed to produce a slight but definite increase in the strength of contractions of the isolated uterus, and has consequently been advocated for clinical use. Many clinicians have claimed good results with it (*e.g.*, 19, 4, 6) while others (*e.g.*, 9) find it of no value.

Earlier Work on the Pineal. The pineal gland is a small, pinkish, cone-shaped structure, situated in the mid-brain, underneath the posterior region of the corpus callosum, and

resting upon the anterior elevation of the corpora quadrigemina (18). There seem to be two types of cells, neuroglia, and " secretory " or ependymal cells. Of the mass of statements in the earliér literature concerning the gland, only two seem certainly accurate, and one of these has no discernible clinical significance. Numerous extirpation and feeding experiments led to results too confusing and contradictory for analysis (15).

A rare syndrome is found in young children, usually boys. They exhibit abnormal growth, associated with some degree of premature genital development, and 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 that organ (18). Perhaps Saphir's experimental results (17) that pineal tissue contains a gonadotropic substance have some bearing upon this syndrome.

If ox-pineal is fed to tadpoles, along with plant food, from the beginning of larval life, about half an hour after each feeding they become sufficiently translucent to permit the beating heart to be visible. This translucency lasts about three hours. The phenomenon persists till metamorphosis (8, 7, 1). Its significance is not known.

Rowntree's Experiments with Hanson's Thymus Extract

The studies of Rowntree and his collaborators (10–14, 4A) introduce the novel procedure of continuous production of endocrine hyperfunction through successive generations. They have been carried out entirely on rats.

Hanson's extract is made by treating the neck thymus glands of calves with 0·5 per cent. hydrochloric acid, with application of heat. Most of the experiments have been carried out with an extract prepared in 1930, which, three years later, showed the following characteristics : It is a golden yellow liquid, with a taste and smell resembling that

of bouillon. It has a *p*H of about 5, and is non-toxic in relatively large doses, and non-irritating locally, when

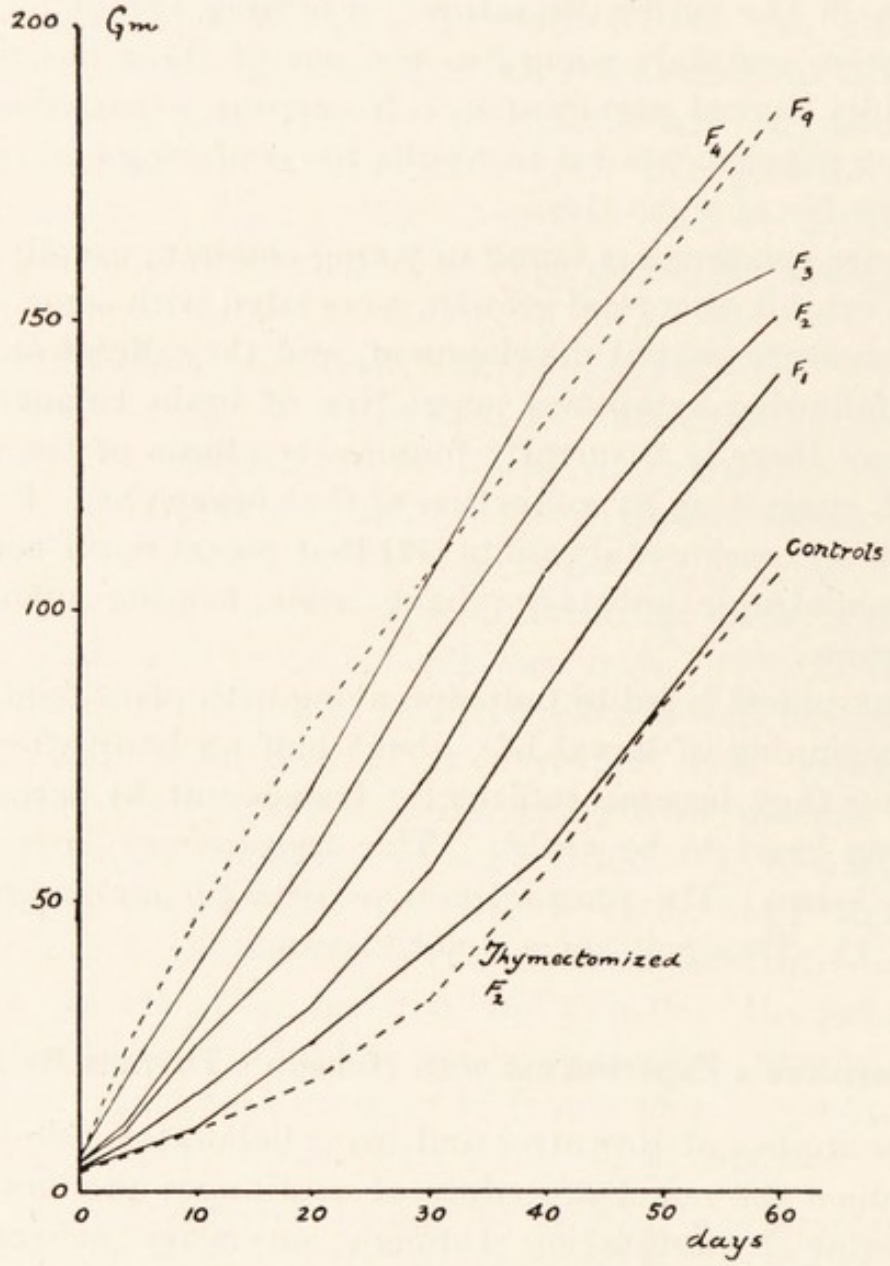

FIG. 25.—Weight curves of thymus-treated and thymectomized rats, contrasted with normals. Constructed from the curves in Rowntree *et al.*, *Arch. Int. Med.*, 1935, lvi, 1, Fig. 5, and *Ann. Int. Med.*, 1935, ix, 359, Fig. 2.

injected. It has remained completely stable over a long period (all preparations do not show this stability); 100 c.c. of the extract, corresponding to 60 grams of raw calf thymus,

contain 5·75 mg. of total nitrogen, 4·0 mg. calcium, 12·8 mg. inorganic phosphorus, 19·0 mg. of lipoid phosphorus, 365 mg. of sodium chloride, 10·0 mg. of cholesterol, 14·0 mg. of uric acid, and 15·8 mg. of reduced and oxidized sulphur compounds calculated as glutathione. Obviously these figures merely indicate the extreme impurity of the extract, regarded as an extract of the active thymus principle, and in addition

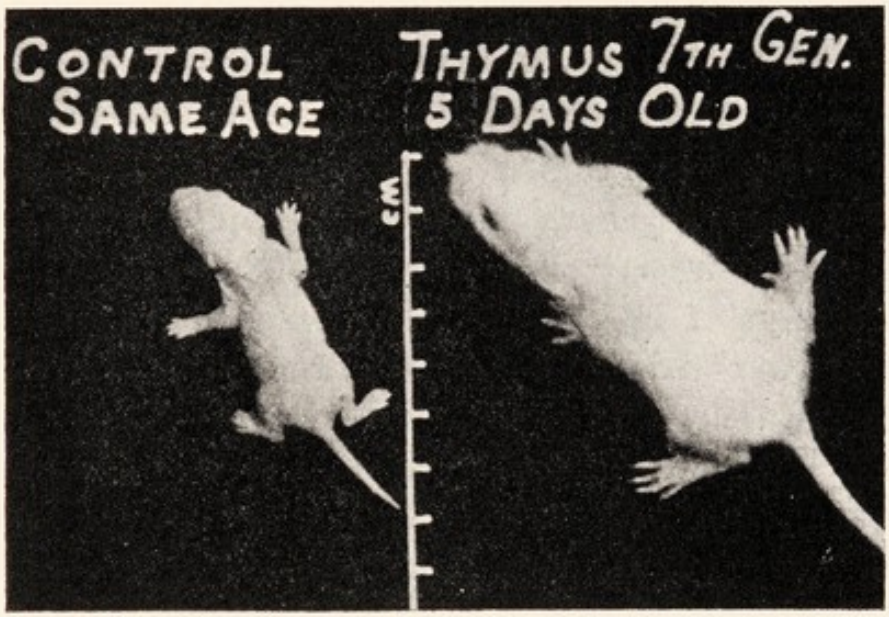

FIG. 26.—Comparison of a five-day old thymus-treated rat of the seventh generation with a control of the same age. (From Rowntree *et al.*, *Ann. Int. Med.*, 1935, ix, 359.)

the indication that probably a relatively large amount of glutathione is present.

Experimental Procedure. A colony of twelve white rats was divided into test and control groups. Litter mates born to the rats in each group were mated in pairs when possible. The rats in the test group have each received by intraperitoneal injection 1 c.c. of the thymus extract daily, even during periods of pregnancy and lactation. Treatment of the young has usually been begun from the sixteenth to the twentieth day after birth. The first generation, the original group (F_0) has received continuous treatment since June 16th, 1933, the second generation (F_1) since September 25th,

1933, the third generation since January 19th, 1934, and so on. At the time of the last report the tenth generation was being studied, and there were several hundred rats under treatment.

Results. In the first generation the test animals became heavier than the controls, bred more frequently, and had larger litters, in which the young averaged a heavier weight. The first six litters from this group were practically normal, but the later litters showed definite precocity, which was greater the later the litter. With each succeeding generation the precocity became more marked. This is well shown in Table V and Figs. 25 to 27.

Psychic precocity is as striking as the physical. Rats of the fifth to tenth generation run about the cage at from two to three days of age, and are as alert as normal rats of sixteen to twenty days of age. Weaning is possible at forty-eight hours, the young rats finding their own food and drink supplies. At this age they can nest for themselves and need no further parental care. They can swim at the third day. Beyond precocity, they show no abnormal behaviour.

TABLE V

Comparison of Thymus-treated Rats with Controls

Generation.	Average Birth Weight.	Ears open.	Teeth erupted.	Hair appeared.	Eyes opened.	Testes descended.	Vagina opened.	Pregnant.	First Litter cast.
	gm.	days	days	days	days	days	days	days	days
Controls	4·6	2·5–3·5	8–10	12–16	14–17	35–40	55–62	80	102
F_1	5·1	2–3	8–9	10–12	12–14	15–29	30–45	70	92
F_2	5·3	2	4–6	4–6	4–6	15–21	23–32	56	78
F_3	5·3	1–2	4–6	4–6	4–6	10–12	21–27	42	64
F_4	5·6	1–2	2–3	2–3	2–3	6–10	18–20	25	47
F_5	5·5	1–2	2	2	2–3	4–6	18–20	40	61
F_6	5·6	½–2	1–2	1–2	2–3	3–10	16–20	46	68
F_7	5·5	Birth	Birth	1–2	1¾–2	3–4	16–18	37	59
F_8	6·5	Birth	Birth	1	1¾–2	3–4	16–18	22	43
F_9	6·0	Birth	Birth	1	1½–1¾	2–3	6	—	—

Studies of the blood of these animals showed a definite increase in calcium and inorganic phosphorus content;

there was no change in haemoglobin and white cell counts. X-ray studies showed, for comparable ages, increase of the skeletons in all dimensions, but particularly in the length of the diaphyses of the long bones, earlier visibility of the epiphyses of the long bones, and earlier calcification and union of the centres of ossification of the long bones and the vertebrae.

The *precocity* of the development is to be stressed. The young of the third and succeeding generations grew and developed physically, sexually, and psychically in an extraordinarily precocious manner. But they did not become giant rats. The growth rate slackens from the end of the second month. The fertility of these rats is increased.

Effects of Thymectomy in Successive Generations. Reports have been made on the results of thymectomy in five successive generations. Growth is retarded in the second and later generations. There is only mild retardation in development ; each stage seems slowed to the longest limit of normal. There is a definite decreased growth rate for the first four or five weeks of life (cf. Fig. 25). Thus, for example, a control rat at eighteen days of age weighed 23 grams, a thymectomized rat of the second generation at the same age weighed 12·5 grams, while, by contrast, a thymus-treated rat of the ninth generation at four days of age weighed 27 grams.

Replacement thymus therapy through four generations completely overcomes the retarding effect of thymectomy, and if pushed vigorously leads to accceleration of growth and precocious development of young. Frequent thymus implants will produce the same effect.

Diseases of the Thymus

Numerous diseases affect the thymus, but scarcely any can be directly associated with it. It appears to be enlarged in Graves' disease and in Addison's disease, and is persistent

in eunuchs and after early castration, while it diminishes in size in wasting diseases, and in starvation and inanition.

Enlargement is specially associated with " thymic stridor " and " thymic asthma " occurring at or shortly after birth, and the so-called " status thymico-lymphaticus," though the existence of these as real entities is by no means certain, let alone their association with the thymus (12).

It is far too soon to know what clinical benefits will accrue from Rowntree's experimental investigations.

Rowntree's Experiments with Hanson's Pineal Extract

These results are a partial antithesis of those with the thymus extract (13–16).

Hanson's Pineal Extract. This is made from beef pineal glands. Several extracts have been prepared, and the potency of these is apparently not yet under control. The most potent extract, which has been used for most of the experiments, is an aqueous acid derivative, probably in the form of a picrate, containing 0·21 per cent. free picric acid. It is described as a slightly turbid, somewhat greenish-looking solution, relatively non-toxic, but somewhat irritating locally. " One cannot . . . escape the impression that it is somewhat of a deterrent to the general good health of the rats . . . " This must obviously be borne in mind in evaluating the results. Glands are extracted with 0·1 per cent. hydrochloric acid, and the extract subsequently treated successively with picric acid and then re-extraction with hydrochloric acid. Experiments have shown that injections of picric acid solutions in corresponding dosage are without effect.

Experimental Procedure. This has followed very closely that employed in the thymus experiments. So far the published data refer to observations up to the sixth generation ; the results are based on a colony of several hundred rats.

Results. In the first generation no effect was noted other than moderate loss of weight, and phenomena suggestive of

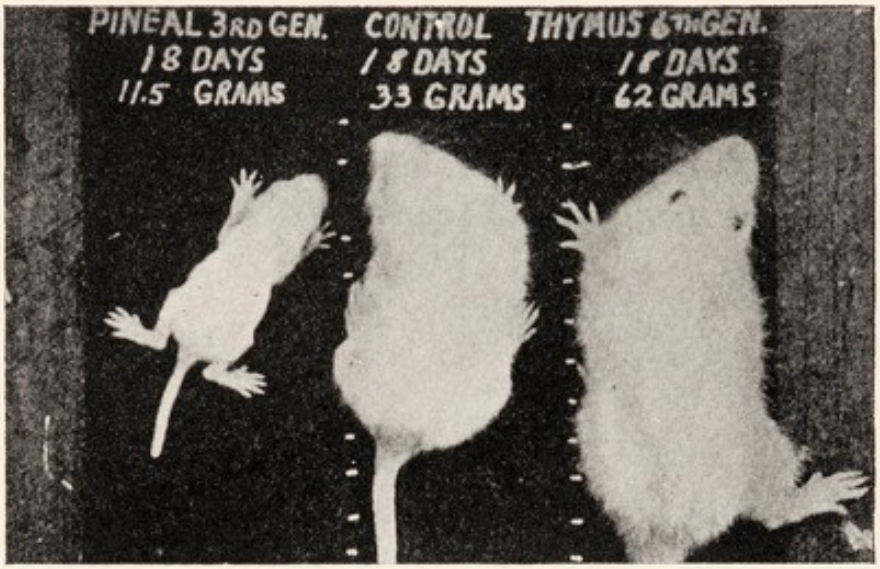

FIG. 27.—Comparison of a thymus-treated rat of the sixth generation, a pineal-treated rat of the third generation, and a control, all eighteen days old. (From Rowntree *et al.*, *Ann. Int. Med.*, 1935, ix, 359.)

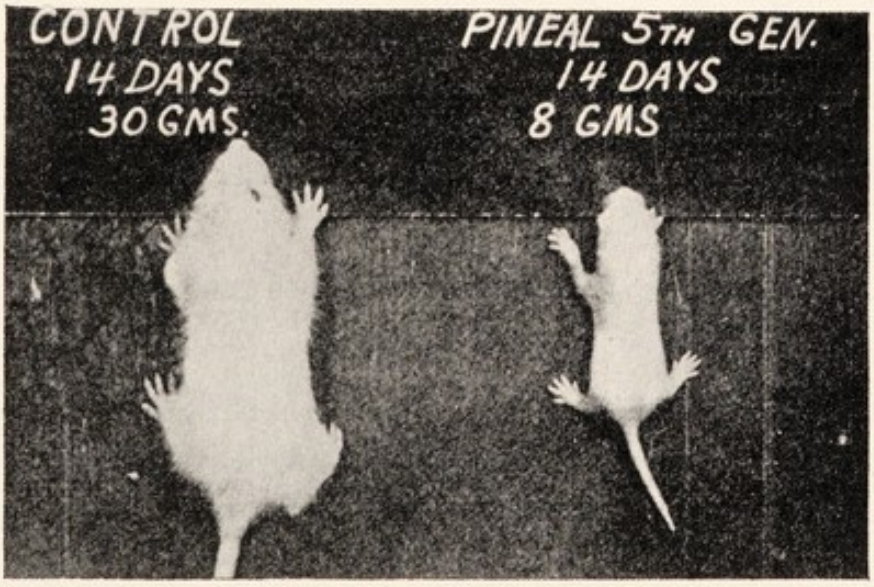

FIG. 28.—Comparison of a pineal-treated rat of the fifth generation with a control of the same age. (From Rowntree *et al.*, *Ann. Int. Med.*, 1935, ix, 359.)

sex excitation and early breeding. In the second generation there was definite retardation in growth with mild precocity

in gonadal development. In subsequent generations these features were accentuated, producing a picture of precocious "dwarfism" with relative macrogenitalism. In addition eye anomalies (ocular diseases and blindness) are common in these animals, which are physically weak and more nervous and irritable than normal.

The dwarfism is permanent, though it becomes less striking as the animals age. The precocity of development is shown

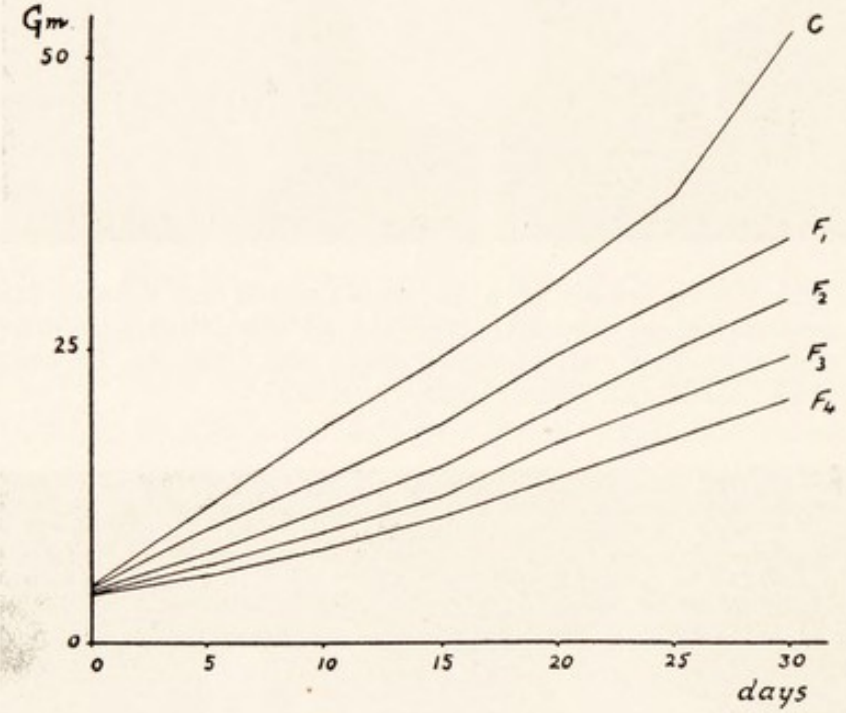

FIG. 29.—Weight curves of pineal-treated rats and controls. After Rowntree *et al.*, *Science*, 1936, lxxxiii, 164, Fig. 1.

in Table VI, in which only the average figures are given, since there is considerable lack of uniformity in individuals. Growth curves are shown in Fig. 29; note also Figs. 27 and 28.

Results from Pinealectomy. Rowntree reports (16) that in a small series of pinealectomized rats (four successive generations) there is no definite evidence of enhanced growth and retarded development, such as might be expected from the results of pineal treatment. The results were inconstant.

TABLE VI

Progressive Development under Pineal Treatment

Generation.	Ears opened.	Teeth erupted.	Fur appeared.	Eyes opened.	Testes descended.	Vagina opened.
	days	days	days	days	days	days
Controls	3	9·0	16	15·5	38	65
F_1	3·3	9·0	13	14·9	22	45
F_2	2·8	9·0	12	13·8	15	37
F_3	2·3	6·9	9	9·8	10	32
F_4	2·0	4·0	5	6·0	5	24

Function of the Thymus and Pineal Glands

Rowntree's most recent comments on his results are that the function of the thymus gland of the parent rat is concerned with the rate of growth and development of the offspring, but that studies of the pineal gland have not yet progressed to the stage which permits any statement as to its function.

One may perhaps wonder if the function of the thymus during foetal life should not also be considered, and in connection with the individual, rather than its offspring.

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CHAPTER VII

THE ENDOCRINE SECRETIONS OF THE ORGANS CONCERNED WITH REPRODUCTION

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. 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 (60). 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 (127) [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.

[Ovulation in the rabbit only follows copulation. " Pseudo-pregnancy " can be induced by sterile mating with

[1] For further details, see Frank (60), Parkes (127), Sharpey-Schafer (150), or Robson (135).

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.]

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 (161).

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 (97).

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 under-developed 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 (97).

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 (161).

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 (97).

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 (154).

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

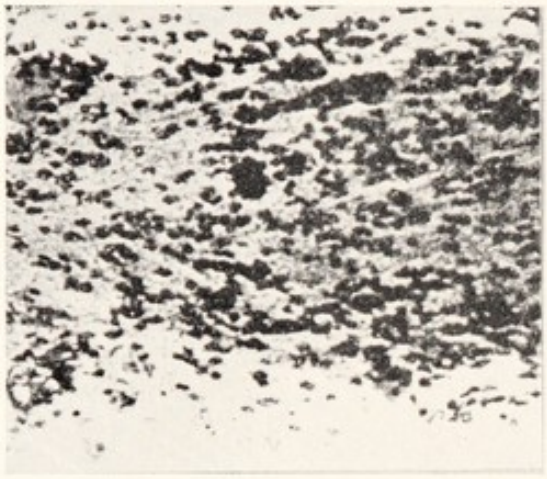


FIG. 30.

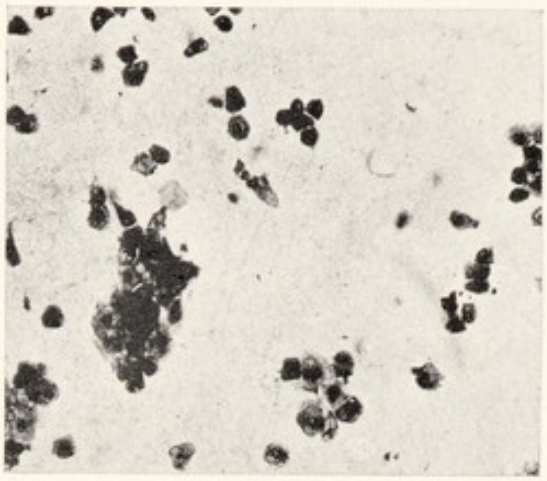


FIG. 31.

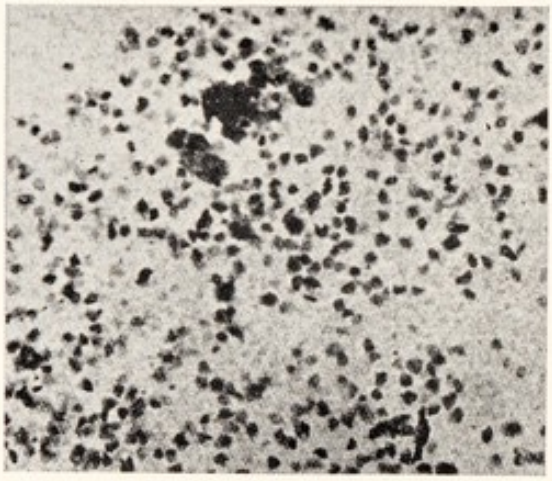


FIG. 32.

FIG. 33.

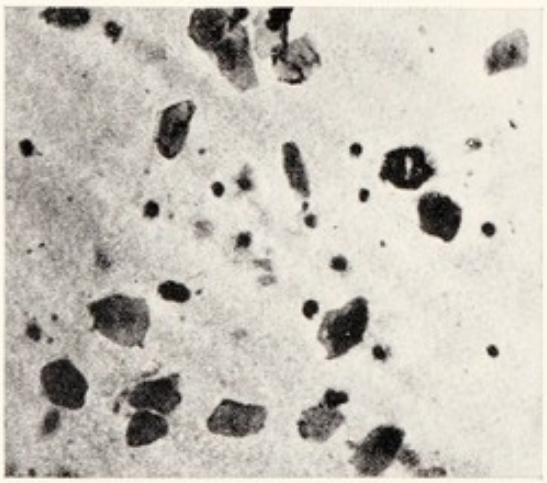


FIG. 34.

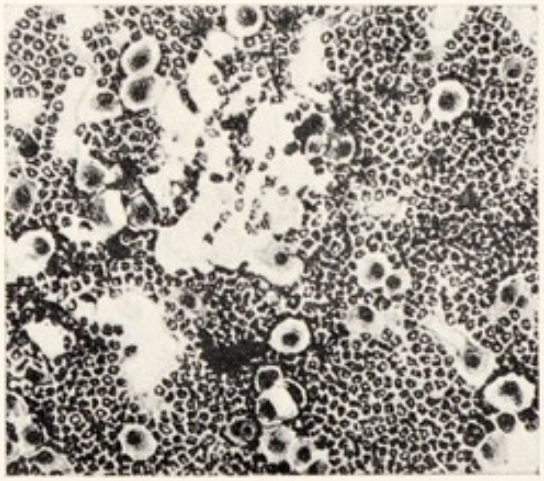


FIG. 35.

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 p.* 283.

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

FIG. 30.—Dioestrous smear: leucocytes in stringy mass. × 40.

FIG. 31.—Pro-oestrous smear: chiefly nucleated epithelial cells with an occasional leucocyte. × 40. Present thirty-five to forty hours after first injection.

FIG. 32.—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. 33.—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. 34.—Early stage of leucocytic infiltration (metoestrum). × 40. Few nucleated epithelial cells have appeared as yet.

FIG. 35.—Late stage of the metoestrum. × 40. Enormous numbers of leucocytes, some cornified scales (in the centre of the field), and many nucleated epithelial cells.

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 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 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. (In women slight changes take place during the menstrual cycle (41, 122, 142A).)

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. 30–35.

Earlier Endocrine Research on the Gonads

Endocrine research on the gonads is now uniquely fortunate in being in the possession of a series of pure endocrine compounds, whose constitution is known, and a number of which have been synthetized. It is possible, therefore, to check the presumed function of these glands accurately, by studies with pure principles uncontaminated with impurities, which, in the case of other glands not infrequently have, and still perhaps are confusing the interpretation of results. Before discussing these pure compounds it will be convenient to give a very brief account of the work which led up to their isolation. For fuller details reference must be made to such symposia as the volume entitled " Sex and Internal Secretions," edited by Edgar Allen (3). For details of the preparation of the less recently isolated compounds Harrow and Sherwin (69) should be consulted.

The Ovaries. Allen and Doisy (1923, 1924), having shown that the vaginal smear test could be used with the rat, by aid of this test succeeded in obtaining a concentrate of the ovarian principle. They aspirated fresh follicular liquor from hogs' ovaries, removed its proteins by excess of alcohol, and subjected the filtrate to successive treatments with lipoid solvents and water, obtaining finally a fraction which was soluble in lipoids, and which induced oestrus in spayed rats and rabbits. They showed that such an active fraction could be obtained from whole ovaries and from placenta. Later on Zondek and Aschheim found that the urine of pregnant women or of pregnant mares is a very rich source of this activity. Numerous names were suggested for the active compound. The earlier terms, such as *oestrin*, *folliculin* and *progynon*, have been largely replaced, since the preparation of a crystalline compound, by *oestrone* (in Great Britain and Canada) and *theelin* (in the United States).

Crystalline oestrone (theelin) was obtained independently and almost simultaneously by Doisy (August, 1929),

Butenandt (October, 1929) and Dingemanse (1930), from the urine of pregnant women.

A second crystalline compound was isolated from the urine of pregnant women by Marrian (1930) and shortly afterwards by Doisy (49, cf. 21). This is now termed *oestriol* or *theelol*. It has been shown to differ from oestrone by the elements of a molecule of water, and is frequently referred to by German investigators as folliculin-hydrate.

Corpus luteum. The earlier theories concerning the function of the corpus luteum have been set out by Hisaw (72), and critically reviewed by Pratt (132). Association of the corpus luteum with endocrine function was suggested by Prenant and von Born; Fraenkel adduced some experimental evidence in support of this view, demonstrating that removal of the corpus luteum of the rabbit leads, in early pregnancy, either to absorption of the foetuses or premature expulsion. Numerous investigators have contributed to the present general acceptance of the endocrine theory.

Results of experimental removal of the corpus luteum suggested that it normally inhibits ovulation during pregnancy. If it is removed early in pregnancy, abortion or resorption of the foetuses occurs in mice, rats, opossum and guinea-pigs; if removed late in pregnancy normal birth of living young may occur in guinea-pigs and rabbits. There seems to be a marked species difference, since in women *early* removal of the corpus luteum may not at all interfere with the pregnancy (132).

Evidence obtained by Loeb and others, working with the guinea-pig, rabbit and bitch, demonstrated that the corpus luteum secretes a substance which sensitizes the uterus (so that it will then respond to mechanical stimuli by formation of decidual tissue).

In addition to the three functions thus suggested by this experimental evidence (inhibition of ovulation, sensitization of the uterus for implantation of the ovum, and maintenance of pregnancy) further evidence was obtained that the corpus

luteum took some part in the development of the mammary glands (cf. 127).

The early accurate work on extracts of the corpus luteum indicated that there was one compound definitely, and a second possibly associated with its function. The first of these, variously termed progestin (Allen), corporin (Hisaw), the beta-factor (Wiesner), lutin (Clauberg), and luteosterone (Slotta), was extracted by lipoid solvents, and produces changes typical of early pregnancy and pseudo-pregnancy in the uterus of castrated rabbits, and also continuance of life and normal development of the embryos of rabbits castrated during pregnancy. In 1934 this substance was obtained in crystalline form independently and almost simultaneously by four groups of investigators, Butenandt and his co-workers, Slotta, Ruschig and Fels, Allen and Wintersteiner, and Hartmann and Wettstein. The question of priority, of secondary importance, has been dealt with fairly by Hohlweg and Schmidt (74). The name " progesterone " has been agreed upon.

In addition Hisaw claims (72) to have obtained an active extract of a substance he terms *relaxin*, water-soluble, and probably peptide in nature. He attributes to it the property of causing the relaxed condition of the pelvic bones, particularly observable in the pregnant guinea-pig. Evidence for the existence of this second compound cannot as yet be regarded as definite, and indeed Burrows (15A) has recently found that the relaxation may be due to such compounds as oestrone and equilin.

Placenta. The presence of oestrogenic material in placenta was demonstrated early. Japanese workers (Hirose, Murata and Adachi) obtained evidence that it also contained a substance which produced numerous corpora lutea in rabbits, in a manner simulating the action of anterior pituitary implants (cf. p. 395). Wiesner (170) obtained placental extracts with similar properties, and Collip developed these studies still further (36).

Collip has shown that when an acetone extract of human placentae is acidified, addition of excess of alcohol fractionates it into two parts containing different principles. The precipitate, purified by repeatedly re-dissolving it in water and re-precipitating with alcohol, gives finally a preparation which produces the pituitary-like effects already described, and which, when injected into immature rats

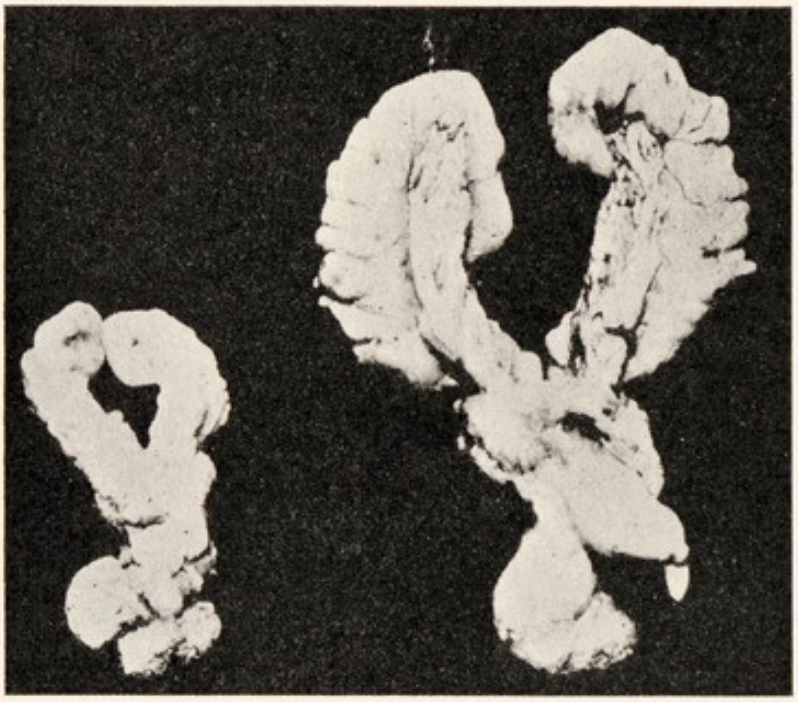


FIG. 36.—Seminal vesicles and prostate of control (left) and experimental adult rat (right) after injection of the anterior - pituitary - like principle (the equivalent of 15 grams of placenta) administered daily except Sundays for forty-two days. (From Collip *et al.*, *Can. Med. Assoc. J.*, 1931, xxiv, 201.)

nineteen to twenty-one days old, produces oestrus in three to five days. Collip termed the active compound in this material the *anterior-pituitary-like*, or A-P-L principle. A-P-L behaves as a protein, has not been crystallized, and has not yet been obtained in definitely pure condition. It must be administered by injection to be effective.

The alcoholic filtrate from A-P-L is concentrated by removal of alcohol, then acidified and extracted with ether. *Emmenin* remains in the aqueous phase, while some oestriol

is present in the ether extract, and has been obtained in crystal form (31, 15), and its identity definitely established. Collip showed that when the emmenin fraction was autoclaved in acid solution it became ether-soluble, suggesting that it is an ester of oestriol (34, 35).

Emmenin is effective orally, as well as by injection. It rapidly produces oestrus in immature rats, but produces no definite changes in the ovaries. It has no effect on the cycles of normal adult rats, or on the normal course of pregnancy or lactation. It has practically no effect on adult castrates. It thus differs from oestrone in this respect and in its greater effectiveness by oral route.

The A-P-L principle also affects the male animal. Marked enlargements of the accessory genital structures are produced, especially of the seminal vesicles and prostate gland (cf. Fig. 36). The weight of the testes is not much affected. Function, rather than hypertrophy, is stimulated (Collip).

As already stated, oestrone is present in the urine of pregnant women in relatively large amount. Collip succeeded in separating concentrates from urine which appeared to correspond with emmenin and the A-P-L principle, and from the former he obtained crystals of oestriol (31).

It is practically certain that A-P-L of the placenta is identical with Zondek's *prolan from urine*. Zondek and Aschheim's earlier work with pituitary extracts showed that these possessed definite gonad-stimulating effects. This work will be discussed in the next chapter. In 1927, having found that preparations from urine were much more potent in producing the gonad effects they were studying, they somewhat rashly assumed that the active urine constituent was identical with that in the pituitary. This assumption cannot be upheld, but the experimental evidence stressing the differences must be deferred until the pituitary compounds have been dealt with. In the meantime the identity of A-P-L and prolan will be assumed, and though Zondek's work on prolan is earlier than Collip's on A-P-L,

the latter term will be used, since a good deal of confusion exists in the literature between urinary and pituitary " prolan " (cf. 32).

A-P-L is concentrated from urine in various ways; essentially the treatment consists of precipitation from acid urine with alcohol and purification of the precipitate by extraction with ether, re-solution in water, and re-precipitation with alcohol. A water-soluble preparation is obtained. Claims have been made (95, 181), but not yet substantiated, that it can be obtained in crystalline form.

The Testes. The biological tests usually 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. Koch (87) has summarized the earlier work. McGee, working under his direction, in 1927, first conclusively demonstrated that a benzene extract can be prepared from testes which causes comb-growth when injected into capons. Later work has shown that the same, or some similar principle is present in the urine of men, and in traces in bull's blood. Extraction with lipoid solvents is an essential feature in the preparation of concentrates from testes or urine. Frattino and Maino (62) have claimed that they have obtained a very active crystalline preparation. Butenandt (16) definitely was the first to obtain pure crystals from urine and to determine the constitution of the compound, which he termed *androsterone*. He estimated that about two million litres of urine contain 1 gram.

Chemistry of the Endocrine Compounds concerned with Reproduction [1]

Two groups of compounds control reproduction in mammals. The first are protein, or protein-like in character,

[1] Fengel has announced (Endocrinology, July, 1936) the isolation of a crystalline nitrogenous compound from the ovaries, producing delayed but prolonged oestrus, and much more powerful than oestrone.

the second are all derivatives of cholesterol, and are very probably formed from it in the mammalian organism.

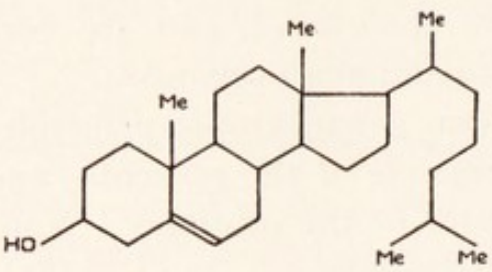
The protein group consists of two gonadotropic principles of the pituitary, the A-P-L principle of the placenta, and *prolactin*, the galactogenic principle of the pituitary. None have as yet been obtained definitely pure.

The Cholesterol Derivatives. Progress in study of this group of substances has been so rapid, and has been contributed to by so many investigators, that no full account is possible here. The most important chemical aspects of the group have recently been clearly summarized by Ruzicka (140), to whose account I am indebted. (Cf. also Tscherning (157).) The constitutional formulae of the members of the group mentioned here are given on pp. 292, 293. All the sex-principles have a characteristic four-ring skeleton, of which the conventional numbering is shown on p. 292. They can be considered either as derived from a C_{19}, hydrocarbon, androstane, with two methyl groups, or a C_{18} hydrocarbon, oestrane, with one methyl group.

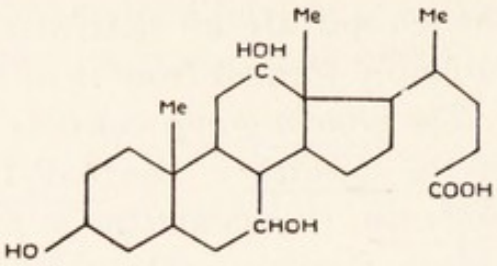
Cholesterol, $C_{27}H_{41}OH$, has a long side-chain, which may be considered as gradually etched away to form the others. Its general distribution in the body, its varied functions associated with fat transport, formation of sebum, etc., and its excretion through the bile, whose bile salts hold it in solution, are moderately well understood. The cholic acids, precursors of these bile salts, are probably formed from it. Cholesterol may be obtained by the mammalian organism partly in the diet, but can also, at least in part, be synthetized by the organism. Ergosterol, and its isomer calciferol (vitamin D), are closely related to it.

Pregnandiol, $C_{21}H_{36}O_2$, has been isolated from pregnancy urine independently by Marrian (110) and Butenandt (17). It has no physiological activity, but is important as an intermediate stage between cholesterol (or the bile acids) and the active gonad compounds.

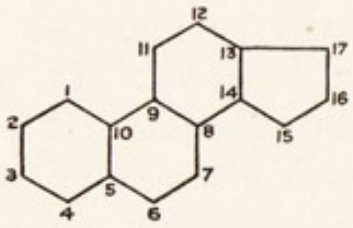
Progesterone, $C_{21}H_{30}O_2$, crystallizes in two isomeric



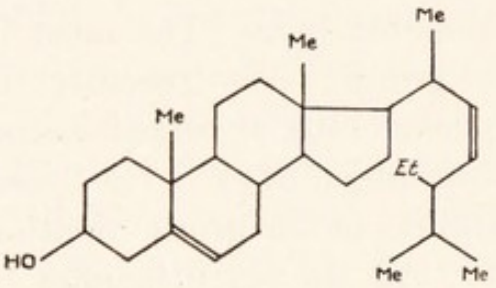
Cholesterol



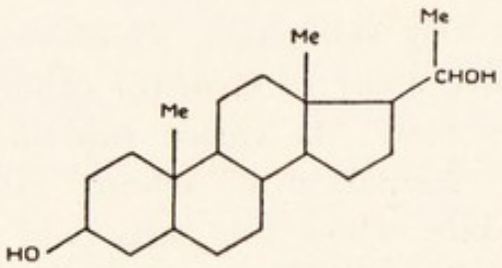
A Cholic Acid



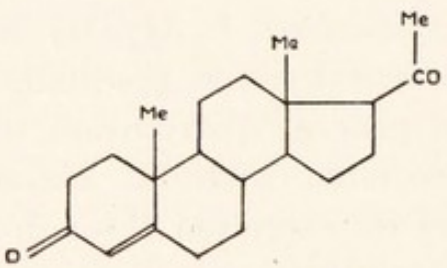
Skeleton Ring Structure



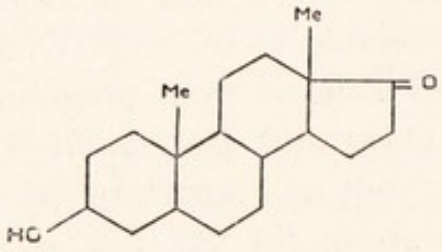
Stigmasterol
(from Soy Bean Oil)



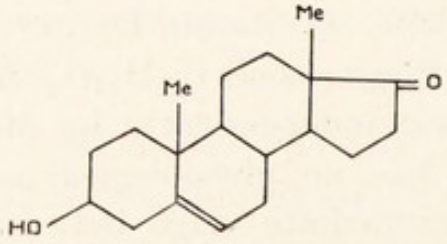
Pregnandiol
(from urine)



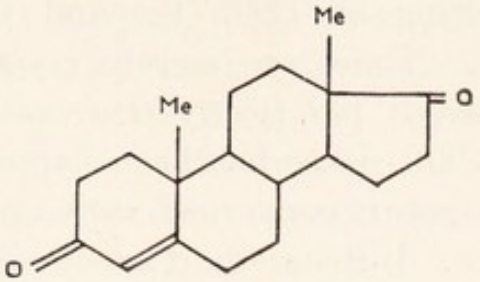
Progesterone
(from corpus luteum)



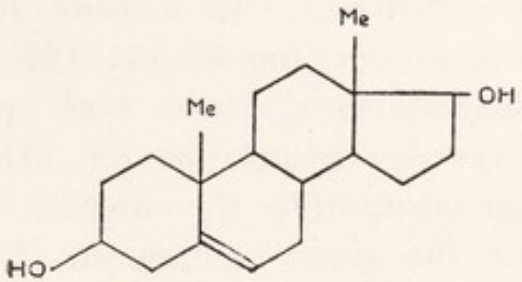
Androsterone
(from urine)



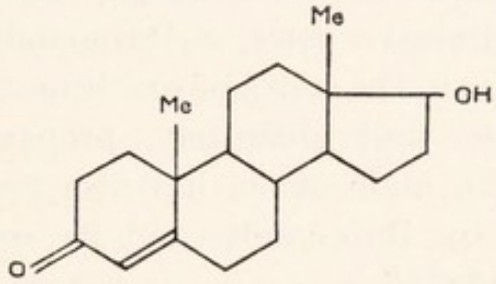
Androstene-3-ol-17-one
(from urine)



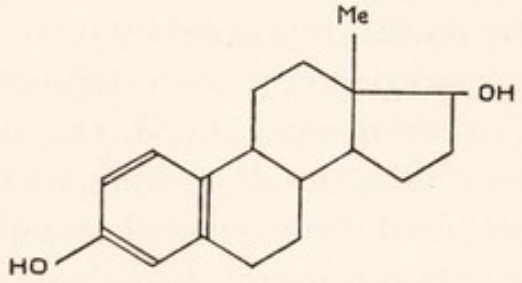
Androstene-3, 17-*dione*
(artificial)



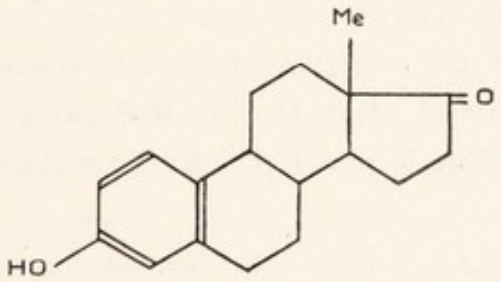
Androstene-3, 17-*diol*
(artificial)



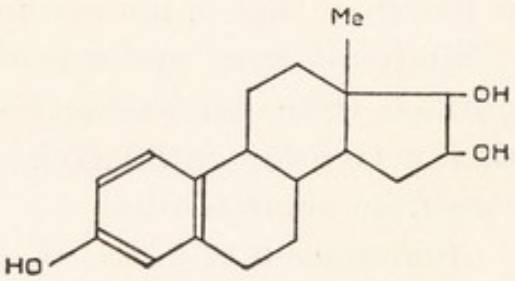
Testosterone
(from testes)



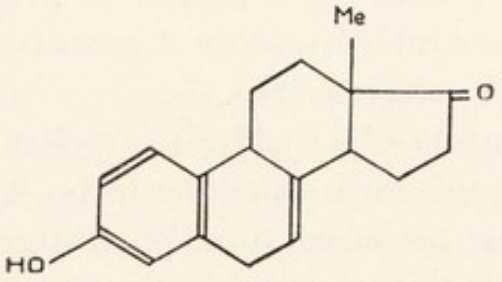
Oestra-3, 17-*diol*
(from ovaries)



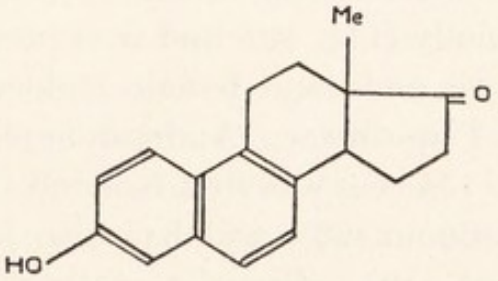
Oestrone (*Theelin*)
(from pregnancy urine, etc.)



Oestriol (*Theelol*)
(from pregnancy urine, etc.)



Equilin
(from pregnant mare's urine)



Equilenin
(from pregnant mare's urine)

modifications, the α-form, melting at 128·5° C., and the β-form, melting at 121–122° C. These are merely crystal modifications, since both possess practically the same physiological activity (cf. 74). The name has been happily agreed upon by the chief investigators concerned with work on the pure compound, Allen, Butenandt, Corner and Slotta (6), and is modified from the earlier terms *progestin* and *luteosterone*. It is probably formed in the organism from pregnandiol, to which it is very closely related, and from which Butenandt has prepared it. More important, for its future availability for clinical service, is Butenandt's preparation of it from stigmasterol, the sterol of soy bean oil.

Androsterone, $C_{19}H_{30}O_2$, the first definitely prepared crystalline product from human male urine, has also been prepared from epicholestanol by Butenandt, and its constitution proved. It melts at 178° C.

Androstene-3-ol-17-one, $C_{19}H_{28}O_2$, was also isolated from urine by Butenandt, and has also been prepared from cholesterol. It melts at 148° C. Its physiological activity is less than that of androsterone (157).

Study of pure androsterone in comparison with highly purified testicular extracts showed that it did not possess their activity quantitatively. This led to Ruzicka's preparation from cholesterol of

Androstene-3,17-dione, $C_{19}H_{26}O_2$, but its physiological properties did not completely correspond with those of the testicular extract (whose principle can actually be formed from it by oxidation). The corresponding di-ol compound.

Androstene-3,17-diol, $C_{19}H_{28}O_2$ has been prepared artificially (141, 19), and is of peculiar interest, since it possesses both male and female endocrine activity (18).

Testosterone, (Androstene-3-one-17-ol), $C_{19}H_{28}O_2$, (melting at 154° C.) was first isolated in pure form from the testes by Laqueur (93), and has also been prepared from cholesterol (141, 19). Ruzicka states that testosterone is twenty to twenty-five times more powerful, physiologically, than

androsterone. Deanesly and Parkes (45) find that it is six times more active on capons, two to five times as active on the prostate, and ten times as active, measured on the seminal vesicles of castrated rats. Laqueur believes that testicular extract contains in addition a still unknown substance, itself inactive, which markedly enhances the activity of testosterone; Deanesly and Parkes agree that testosterone does not account for the complete endocrine activity of the testes.

The similar "female" compounds possess one methyl group only, and show a greater degree of unsaturation.

Oestradiol (dihydro-oestrone, dihydro-folliculin), $C_{18}H_{24}O_2$, the most powerful oestrogenic compound known, was first prepared by Schwenk and Hildebrandt (79) by partial dehydrogenation of oestrone, but has since been obtained by Doisy from pig's ovaries (103), and undoubtedly bears the same relationship to ovarian tissue as testosterone does to the testes. It is stated to be about six times as physiologically active as oestrone. It seems also to be present in the urine of pregnant mares (172).

Oestrone (Theelin), $C_{18}H_{22}O_2$, melts at 250–251° C. It is easily soluble in alcohol, acetone, chloroform and benzene, less soluble in ether, and only very slightly soluble in water. It is fairly easily oxidized. A photomicrograph of Butenandt's crystals is shown in Fig. 37.

Oestriol (Theelol), $C_{18}H_{24}O_3$, has still less activity than oestrone. It is easily soluble in pyridine, less so in methyl and ethyl alcohols, and only slightly in ether. It is soluble in dilute potassium hydroxide, but insoluble in sodium carbonate. It can be converted into oestrone by heating with potassium hydrogen sulphate at 180° C. and 0·02 mm. mercury pressure, when oestrone distils over (16), or by fusing with the same reagent, suspending the product in 0·01 *N* sodium hydroxide, and extracting the oestrone with ether (111).

Equilin, $C_{18}H_{20}O_2$, and *Equilenin*, $C_{18}H_{18}O_2$, have been

derived from urine of the pregnant mare. Both show slight " female " activity (63A).

Thus there exist three groups of compounds in mammalian tissues and urine : (i) progesterone, so far the only compound isolated possessing the activity associated with extracts of the corpus luteum ; (ii) the " male " group, testosterone, androstene-3-ol-17-one, and androsterone, of which testosterone is by far the most active ; and (iii) the

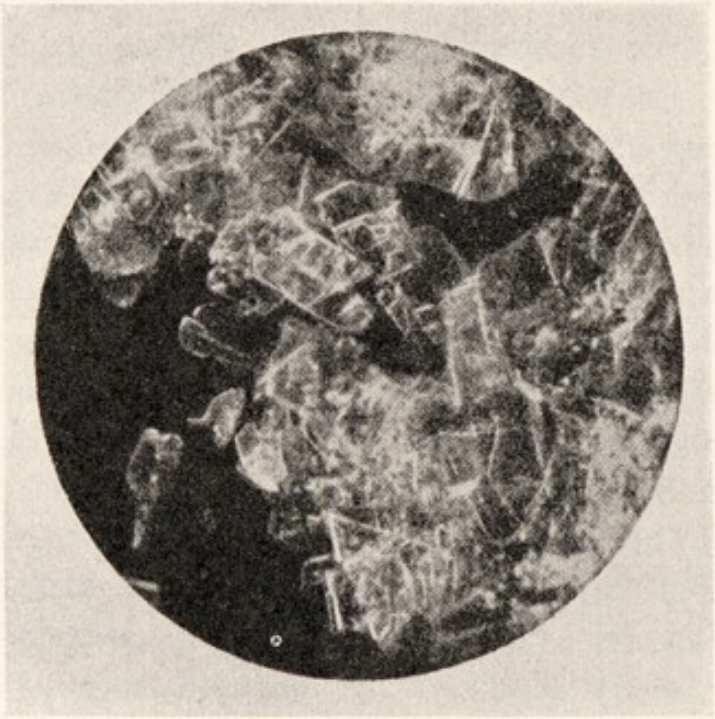


FIG. 37.—Crystallized oestrone from acetic ether. (From Butenandt, *Zeitschr. physiol. Chem.*, 1930, cxci, 127.)

" female " group, oestradiol, oestrone, and oestriol, and, in pregnant mare's urine, equilin and equilenin.

It seems most probable that in the second and third groups testosterone and oestradiol are to be regarded as the essential " male " and " female " compounds respectively, and the others as degradation products, perhaps formed as a result of their interactions. That there is a definite possibility of inter-conversion of " male " into " female " principles is exemplified by Zondek's demonstration that the materials

richest in "female" activity are stallions' testes and urine (180). Stallions' urine definitely contains oestrone (26). Zondek has suggested that the male principle is produced in both sexes, and its degree of conversion is determined by the sex, and to some extent by the species. Ruzicka (140) suggests that androstene-3,17-diol may be Nature's intermediate product, even though it has not been shown to occur naturally; it is easily convertible into both androsterone and oestrone, and can, by dehydrogenation, give testosterone, which could easily be changed to oestradiol.

Emmenin. As already mentioned (p. 289), it has been shown that an emmenin fraction can be obtained from pregnancy urine, and there is evidence that emmenin itself is in some ester-like combination which can be hydrolysed to oestriol.

The recent work of Marrian and his colleagues (30) has shown that emmenin is oestriol glycuronate, and that it is almost certain that during the greater part of pregnancy over 99 per cent. of the oestrogenic material excreted in urine is in this or some similar form of combination, requiring autoclaving in acid solution to liberate oestrone and oestriol. Glycuronates are usually regarded as detoxication products. The physiological activity of emmenin is much less than that of oestrone, and it seems natural to conclude that the formation of oestriol, and then of its glycuronate, are protective measures during pregnancy against over-activity of oestradiol and oestrone.

Present Knowledge of Endocrine Function of the Gonads

Ovarian Function (cf. 4). Three, if not four compounds are concerned in ovarian and placental function, oestradiol, oestrone, oestriol, and emmenin (oestriol glycuronate). Oestradiol would appear to be the actual compound formed in the ovaries and responsible for their endocrine function, and it cannot yet be stated to what extent the others are to

be regarded as functionally, rather than accidentally contributing to the endocrine activities of those glands. On the other hand oestrone, rather than oestradiol, seems to be associated with the placenta. Apparently the physiological actions of oestradiol and oestrone are like in kind, and the results of experiments with the latter can be regarded as applicable to the true ovarian principle.

Any quantitative comparison of the two compounds needs an accurate definition of the biological test used for measurement. Unfortunately minute differences of technique, and perhaps even minute differences in the response of different strains of the same species of test animal, let alone the larger differences resulting from use of different species, have rendered comparisons difficult, so that, as Butenandt has pointed out, for precision it is practically essential that all comparative tests be carried out in the same laboratory by the same people, and on the same strain of the same species of animal.

Allen and Doisy's original *rat unit* of oestrogenic activity 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. Other workers have used slight modifications; different time-spacing of the injections definitely affects the results. A corresponding *mouse unit* has been used, especially by continental workers, and again with various slight modifications.

Two international standards have been set up (in 1935), based upon pure crystalline material. The unit of one is defined as the specific oestrus-producing effect in 0·0001 mg. of oestrone, and of the other, the corresponding effect in 0·0001 mg. of oestradiol benzoate. It has been recommended that incompletely purified preparations or extracts should be labelled in terms of the equivalent weight of pure compounds, and in international units (133A). According to

these definitions 0·1 gram of oestrone contains one million international units. Such units are confusing and misleading, for the therapeutic use of a compound in which apparently very large doses of hundreds of thousands or millions may be needed to attain definite results, and the actual dosage of the order of one or two tenths of a gram is in reality quite reasonable.

Zondek and Aschheim (176) attempted to determine the site of formation of the ovarian principle by the effects produced by implants of different ovarian tissues. Their conclusions apply equally whether the compound concerned is oestradiol or oestrone. They concluded that it is present in the theca interna cells and especially in atretic follicles, but is absent from the follicular granulosa, the ovarian stroma, and the germinal epithelium. It seems to occur in residual ovarian tissue, after removal of large follicles and corpora lutea, in larger amounts than the remaining follicles can account for. It has been found in ovarian cysts.

The presence of oestrogenic compounds in the corpus luteum is still a matter of dispute. Since, especially in the cow, many corpora lutea contain a fluid centre derived from the liquid of the original follicle, such presence may well be accidental (127). But Pratt in particular (132) believes that in the corpus luteum " the elaboration of a hormone identical with the follicular hormone has been shown quantitatively and qualitatively in numerous species, including the human."

The placenta contains large amounts of oestrone, and emmenin, and some oestriol. Oestrone has been found in the foetal membranes, the amniotic fluid, and the umbilical cord, but is absent from extracts of the foetus. Oestrone (and oestradiol) are absent from tissue unrelated to the sex organs. One (or both) are present in the blood of the non-pregnant woman and the oestrous sow—in greatest amount in human blood about the first day of menstruation, after which there is a rapid decrease (60), in larger amounts in menstrual blood (60), and in still larger amounts in human

blood during pregnancy (176). The amount of oestrone in the urine of non-pregnant women is small; it varies with the stage of the menstrual cycle. The relatively large amounts during pregnancy decrease rapidly after parturition.

Zondek's figures for oestrone, in mouse units, give some idea of the *total* oestrogenic activity in various material, whether this is due to oestradiol, oestrone, or oestriol, or to two of these.

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.

Administration of oestrone is effective whether it is given subcutaneously, intravenously, or orally. Of these the subcutaneous approach is most effective (3), and the oral by far the least, although contrasts of effectiveness seem to give very variable results, depending upon the actual biological test used (49).

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. Several injections of oestrone lead to considerable restoration within twenty-four hours. The uterus in the ovariectomized rat, in a similar interval, becomes small and anaemic, and its lumen slit-shaped in cross section; the uterine tubes atrophy. Oestrone restores uterus and tubes rapidly to normal, while uterine contractions increase in amplitude. Ovariectomy induces atresia of the mammary glands, involving ducts, alveoli, and the epithelium of the nipples. All these degenerations can be repaired by injection of oestrone (3, 134). Oestrone produces corresponding changes in the immature young animal, leading to premature oestrus, which is accompanied in the rat and mouse by premature opening of the vagina (3).

All these effects are produced by oestradiol, but in much greater degree for the same dosage. It would thus appear that the specific action of the ovarian principle is to induce growth in the tissues of the accessory genital organs.

Following castration in women injection of oestrone produces definite changes in the vagina—increase in secretion, and gradual replacement of epithelial by squamous cells (126).

Pituitary implants produce the same effects as oestrogenic compounds on the secondary sex organs of the immature animal (cf. p. 395), but are inactive on the spayed animal, indicating that their effect is indirect, through increased output of oestradiol from the ovaries.

The heightened voluntary activity of female rats just before and during oestrus is usually attributed to an increased secretion of the oestrogenic principle, since no such cyclical activity occurs in ovariectomized animals, while it is restored to them by ovarian transplants (3). Injections of oestrone are only partially effective in producing this restoration.

Oestrogenic compounds produce no effect on the heart, blood-pressure, and general metabolism, and no stimulating effect on the ovaries, though there is some evidence that follicular development may be inhibited. In the normal adult female growth of the accessory sex organs tends to be accentuated ; large enough dosage may completely eliminate the degenerative stage of the vaginal cycle, while 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. Oestrus is induced during lactation—when normally the cyclic changes in ovaries and genital tract are suspended for a while—and, transiently, in old animals after sexual function has ceased.

Injection into pregnant animals in sufficient dosage may terminate pregnancy by abortion or resorption of the embryos in utero. The dosage necessary to produce this effect increases as gestation proceeds (3). Long continued

injections depress the reproductive cycle and interfere with lactation (137, 163). They cause atrophy of the ovaries (92). The gonadotropic function of the pituitary is inhibited (cf. 121), so that continuous oestrus results in such animals as the rat (127).

Oestrone injections produce testicular degeneration in adult male animals, and inhibit normal gonadal growth in immature males (2, 163); the effect is probably produced through the anterior pituitary.

The physiological actions of *oestriol* are qualitatively like those of oestrone, but are quantitatively less, following subcutaneous injection; the oral effect of oestriol is one-half to one-third of that following subcutaneous injection (49). Very varied figures for its activity appear in the literature. These are partly due to the test used for comparison, and perhaps partly to slight admixture of oestrone in the material tested (22). Probably the former explanation applies to the discrepancy in activity of two very pure preparations, one of which was found by Butenandt to have an activity of 75,000 mouse units per gram (22), and the other by Marrian, an activity of 7·6 million mouse units per gram (111). It has been suggested by Butenandt, and it seems quite probable that the physiological activity of oestriol is really due to its partial reconversion in the body to a more active compound. Such a hypothesis, if true, might well account for marked variations under slightly differing conditions. This is further borne out by the observation of Dorfman, Gallagher and Koch that oestrone is vastly more effective in producing uterine hypertrophy in the rat, while oestriol is the more efficient of the two in causing opening of the vagina in the immature rat (50).

The precise mechanism of the action of these oestrogenic compounds is not known. They may, as Allen suggests (3), act either by direct stimulation of the accessory genital organs to induce cell division and heightened function, or directly stimulate the vascular control mechanism, pro-

ducing hyperaemia. The first hypothesis seems more probable.

Corpus luteum. The action of progesterone in stimulating the uterus, etc., has been referred to (p. 296). The important observations of Knaus (85) on the pseudo-pregnant rabbit showed that at the same time interval after copulation, about thirty-two hours, the response of the uterus to pitocin of the posterior pituitary commenced to decrease, and the lutein cells of the commencing corpus luteum became visible in microscopic preparations. Obviously progesterone produced from these cells counteracts in some way the action of pitocin. The view is supported by the further observation that experimental removal of the corpus luteum in these rabbits was followed by normal contraction response to pitocin in nine or ten hours. Robson (136) has studied this action further and finds that the inhibition of spontaneous uterine contraction of the rabbit uterus by progesterone is only exhibited *in vivo*, not *in vitro*.

It has been shown that the dual properties attributed to the principle of the corpus luteum are actually produced by crystalline progesterone (5).

Corpus luteum extracts are assayed for progesterone by the production of progestational proliferation of the uterus of immature or pseudo-pregnant rabbits, under defined conditions. The unit based on the former is approximately one-half of that based on the latter (175). The international unit is defined as the specific progestational activity of 1 mg. of crystalline progesterone (133A).

A further complication in the interrelationships of the genital tract may exist, if, as some work suggests, there is a specific endocrine compound elaborated by the *uterus*.

Hysterectomy in rabbits inhibits the development of oestrus and brings about changes in the structure of the ovary tending to degeneration. Auto-transplants of endometrium limit these changes (148). On the other hand, continued artificial distension of the uterus in rats following Caesarian section also inhibits oestrus, producing enlarged pituitaries and enlarged ovaries;

the latter contain large corpora lutea but no mature follicles, suggesting that the procedure inhibits involution of corpora lutea and maturation of follicles (144). Such observations suggest the possibility that the uterine endometrium elaborates an endocrine principle of its own.

The placenta elaborates the A-P-L principle in the chorionic villi (cf. 28). Since these are associated with the foetus, and since positive pregnancy tests depending on the presence of this principle in the urine of the pregnant woman (cf. p. 329) can be obtained at seven to ten days after implantation of the ovum in the uterine wall, the A-P-L principle must be regarded as associated with the ovum, and in that respect the most fundamental of all the endocrine principles.

It is not so easy to decide concerning the formation of oestrone in the placenta. Evidence such as that adduced by v. Probstner (133) suggests actual formation. He reports a case in which, early in the pregnancy of a thirty-year-old woman, the ovaries—or dermoid cysts replacing them—were removed, with a subsequent normal pregnancy, continued presence of oestrone in the urine during the pregnancy, and a normal amount of it in the placenta. However, in the similar case reported by Allan and Dodds (1B) the double ovariectomy was followed by no change in the A-P-L content of the urine, but a definite decrease in oestrone content.

So far there is no evidence to suggest that emmenin can be formed elsewhere than in the placenta. It is only associated with the placenta and the urine of pregnancy. While it seems likely that both oestrone and oestriol are produced from oestradiol to diminish its physiological potency, there is as yet no evidence directly associating oestradiol with the placenta.

Selye, Collip and Thomson (145) believe that in the rat the placenta must produce progesterone, since the uterus shows distinct progestational changes and the mammary glands are maintained in well developed condition for as long

as six days after the simultaneous removal of the ovaries and all the embryos.

Studies of the effect of pre-operative administration of A-P-L on human ovaries obtained at operation indicate that A-P-L does not affect primordial or early follicles, and that it acts primarily on maturing and mature follicles, increasing degenerative changes and probably producing cystic degeneration (67). Prolonged injections of pregnancy urine into female swine produce similar effects (159).

Menstruation. The relationship of the oestrogenic compounds and progesterone to menstrual bleeding is indicated by the experiments of Hisaw (73), Engle and Smith (56) and Corner (39). In the mature monkey *cessation* of prolonged oestrone injections is followed by bleeding. Injection of progesterone stops this, but it commences three to five days after progesterone is stopped, while injection of oestrone at this stage has no effect. Good grounds are adduced for believing that the same effects hold true for women.

Testes. Crystalline testosterone has all the qualities of the testicular principle, when tested on castrate rats. Androsterone seems deficient in effect on the seminal vesicles and penis. The difference in comparative effect of the two, as exhibited by different tests, has been mentioned (p. 106), and is stressed further by the work of Callow and Deanesly (23) and of Korenschevsky (88). It is of course unlikely, if androsterone is to be regarded as a degradation product of testosterone, that its activity should parallel precisely that of its precursor. (The international unit of activity is defined as that of 0·1 mg. of crystalline *androsterone* (133A).)

An instance of the closely related functional effects of the male and female principles is the demonstration by Collip (33) that testosterone can stimulate the development of mammary glands in the rat, and the production of dense milk in them.

According to McCullagh (106) androsterone fails to maintain a castrated animal in a completely normal state. It produces prostatic hypertrophy in normal rats. He has

presented evidence that the ether-insoluble fraction of testes contains a substance, which he terms *inhibin*, which produces atrophy of the secondary sex glands in the normal male rat, and a cessation of oestrus in females. (Cf. also Teem (155).)

Carcinogenic and Growth Stimulating Actions of Oestrogenic Compounds

Various compounds with a similar chemical constitution to the sex principles, such as calciferol (vitamin D) possess some oestrogenic action. Still more interesting is the fact that more distantly related compounds prepared from tar, such as dibenzanthracene and benzpyrene, which are active carcinogenic agents, also display some oestrogenic activity (48, 37).

The converse carcinogenic activity displayed by the naturally occurring oestrogenic compounds has recently been reviewed by Leo Loeb (99), who points out that while carcinogenic hydrocarbons may affect a great variety of tissues, the endocrine compounds are limited in carcinogenic action to the tissues which they normally control. He considers that both groups of compounds bring about cancerous transformations of tissues indirectly, but by differing mechanisms.

It has been shown by the work of Loeb himself and of others that endocrine compounds of the ovary, in association with or controlled by certain hereditary factors, are responsible for the origin of mammary carcinoma in mice. If in mice belonging to strains with a known high incidence of mammary cancer the ovaries are extirpated at the age of three to four months, the cancer incidence falls to zero, or almost zero, depending on the particular strain. Ovariectomy at two months invariably prevents cancer development, but at eight to ten months it has no effect.

Experiments attempting to increase cancer-incidence in non-cancerous strains, by injection of oestrogenic compounds,

have met with some success. Lacassagne (91), using, apparently, the powerful benzoate of oestradiol, has succeeded in producing mammary carcinoma in male mice, in whom normally it does not occur.[1]

Important recent contributions bearing on the general problem of induced growth have been published by Collip and by Zondek. The induced changes reported are chiefly production of uterine metaplasia and adenomata in endocrine glands.

Selye, Thomson and Collip (146) noted in 1935 that chronic oestrone injections into female castrate rats produced fairly rapidly a more or less complete metaplasia of the cylindrical epithelium with cornification in the uterus. In the paper just published by McEuen, Selye and Collip (107) they chronicle the results of chronic injection of oestrone into five male and six female rats for periods of well over 300 days. The age of these animals at commencement of the injections was three to four months. (They note that in the female animals oestrus was maintained practically throughout the whole period.)

At post-mortem the female rats showed extreme hypertrophic fibrosis of the horns of the uteri, with squamous metaphasia of the epithelium (five animals), all had enlarged pituitaries, three of which had large cavernous adenomata of the anterior lobe, one a small adenoma of the intermediate lobe, and several vacuolization of the posterior lobe. All had multiple mammary milk cysts; one mamma showed an

[1] Incidental observations have been made of the production of sarcoma in mice, following injection of oestrogenic material (Cori, *J. Med. Res.*, 1927, xlv, 983) or implantation of ovarian grafts into castrates (de Jongh and Korteweg, *Acta brev. néerl.*, 1935, v, 126). Gardner, Smith, Strong and Allen (*Arch. Pathol.*, 1936, xxi, 504), have now reported development of spindle-cell sarcomata in all of five male mice, following a prolonged course of oestrone injections and a further prolonged course of injections of " keto-oestrin benzoate " (apparently oestrone benzoate). The injections were made under the skin of the back, and the tumours developed at the site of injection. They were only obtained in mice of a strain of known high incidence of mammary cancer in females. These tumours were transferable to animals of the same or related strains.

adenofibroma whose scirrhous tissue had invaded the spaces between fibres of the pectoral muscles. Five animals had enlarged adrenals, and in one a cystic adenoma of the adrenal cortex was found.

In the males the secondary sex organs were atrophied. The pituitaries were all enlarged, and two had cavernous adenomata in the anterior lobe.

Collip believes that the hereditary factor can be ruled out, since incidence of spontaneous tumour is extremely rare in his rat colony, and the animals for this experiment were chosen by random selection.

Zondek (179) found that administration of oestradiol to rats over periods of fourteen to nineteen weeks inhibited pituitary function to such an extent that dwarfed animals with hypoplastic genitals resulted. The pituitaries of male animals were enlarged up to four times the normal size. One female animal, which had been given 280,000 mouse units, had a tumour of the anterior pituitary twenty times the size of the normal gland, and large enough to produce signs of pressure on the brain and optic nerve. Pituitary enlargement was not produced in rabbits similarly treated.

Endocrine Control of Human Reproduction

The facts so far presented, and still to be presented in the therapeutic sections of this chapter, and in the next, now permit the limning in of a picture presenting the salient features of the endocrine control of reproduction.

The facts to be presented in the next chapter will show that in the young animal the developing pituitary puts out many compounds, as a result of whose endocrine action general growth proceeds, and various other endocrine glands, including the gonads are stimulated steadily and continuously.

In the male mammal development of the testes and their descent into the scrotum are due to this pituitary stimulation.

Both spermatogenetic and interstitial elements are controlled. In man there is steady development to puberty. At, or just prior to puberty, the cells producing testosterone (probably the interstitial cells) either commence their function or increase it markedly, so that development of the prostate and seminal vesicles commences, and of those changes in secondary sex characters which indicate maleness —the breaking voice, the typical hair distribution, etc.— while concurrent stimulation of the sperm-producing cells by the appropriate pituitary hormone has brought these to the stage at which man is ready to take his share in the reproductive cycle. He remains continuously fertile for a much longer period than his counterpart, nor is his fertility in any way cyclical.

In the young female mammal, including the young girl, as a result of continuous pituitary stimulation, the ovaries steadily increase in size, and a proportion of their Graafian follicles grow rapidly. Towards puberty, either through summation or increase of pituitary stimulus, the growth of certain of these follicles becomes still more rapid; they extend inwards, and bulge the surface of the ovary outwards. The output of oestradiol is consequently also increased; it circulates in the blood, and as a result of its stimulating effect the female secondary sex organs, uterus, vagina, clitoris, mammae, enlarge, and the feminine sex characters become more pronounced.

Ultimately, some one of the follicles ruptures; its leading competitors atrophy but still continue to produce oestradiol. The rupturing follicle discharges its ovum, and this first ovum in almost all cases will remain unfertilized, passing slowly down the adjacent Fallopian tube to the uterus and dying in its passage.

As a result of the increased output of oestradiol the basal membrane of the uterus proliferates rapidly. (In conditions where it is subjected to excessive amounts of oestradiol the marked proliferation can even result in cystic degeneration

and one form of intermenstrual bleeding (79).) This proliferation constitutes the first stage of uterine change in the menstrual cycle.

Blood passes into the ruptured follicle, now a haemorrhagic follicle. It changes its character. The cavity is invaded by connective tissue, while special lutein cells develop, and these we may well suppose are the agents by which the progesterone of this changed body, the corpus luteum, is produced. Progesterone acts upon the hypertrophied mucous membrane of the uterus, so that it becomes glandular in character. It inhibits the response of uterine muscle to pitocin. This effect, first demonstrated by Knaus on the rabbit (p. 303), he was also able to demonstrate for woman by an ingenious experimental method (85, cf. also 59, 130). Furthermore, since it was possible to demonstrate that in normal woman the inhibiting effect of progesterone first becomes demonstrable fifteen days before the next menstrual haemorrhage, it follows that ovulation, the immediate cause of the formation of a corpus luteum and production of progesterone, is also some fifteen days before the next menstrual period. The clinical studies of both Knaus and Ogino (85) are in agreement with this conclusion that human ovulation precedes menstruation by fifteen days. (Cf. also Latz and Reiner (94), who quote other confirmatory evidence, and Knaus (86), who replies to adverse criticism.) This conclusion has an obviously important bearing on the control of conception.

The uterus regains its response to " pituitrin " fourteen days later, just before the menstrual period.

During these fifteen days the corpus luteum waxes and wanes, and the output of progesterone increases to a maximum and then diminishes. Rapid development of new Graafian follicles is halted, through depression of pituitary activity traceable to the action of oestradiol, perhaps aided by progesterone. Oestradiol output is continued, not only from the atrophying follicles, but, in woman, perhaps also

from the corpus luteum itself. The breasts swell, an effect also probably due to the combined actions of oestradiol and progesterone (77).

The whole purpose of the uterine changes is the preparation of a nest for the fertilized ovum. If a menstrual cycle is completed, Nature has failed in this attempt to reproduce, and the wasted preparations are removed. The diminished output of progesterone from the regressing corpus luteum gradually ceases to control the uterus ; not improbably there is diminution of oestradiol production also. Thus stimulation to functional activity of the uterus is withdrawn, and the hypertrophied tissue is swept away by the haemorrhage of menstruation, leaving the original basal membrane to be stimulated again in the next cycle.

Decreased output of oestradiol and progesterone removes the inhibition on the anterior pituitary, which again exerts its gonadotropic action ; the ovaries are again stimulated, Graafian follicles again enlarge rapidly, and in due course one is elected to initiate a new cycle. The first few cycles are irregular and long, but gradually a regular rhythm sets in, which, however, is never as regular as the average woman believes, and which is subject to many influences, endocrine, climatic, emotional, an influence of varying exercise, and so on.

If living and sufficiently active spermatozoa are in, or come into the neighbourhood of an ovum within a few hours of its discharge from its follicle (and their life and activity are conditioned by the period of incubation within the female abdomen, whose temperature kills them within two or three days (85), and if fertilization of the ovum follows (through some physico-chemical process inadequately explained by the term " chemotaxis "), the developing ovum so changes its envelope that, after a passage of six or eight days through the Fallopian tube, and perhaps one or two days within the uterus itself, implantation of the changed ovum within the wall of the uterus, once more prepared for

this "nidation," is possible and usually occurs. Almost immediately thereafter some part of the outer surface of the ovum—the future chorionic membrane of the placenta—commences to produce the A-P-L principle. This passes into the maternal circulation and so influences the ovary that the corpus luteum is stimulated to further growth and continued existence. The actions of progesterone are thus maintained. The uterus remains flaccid and is easily distended by the growing uterus.

Adequate evidence is not yet available as to how long the effect of progesterone is necessary to maintain pregnancy and prevent abortion or resorption. It would appear that its influence is less necessary in women than in the lower mammalian species (cf. p. 286). As long as the corpus luteum persists during pregnancy we may assume, until there is good evidence to the contrary, that it functions in the usual way.

The vastly increased output of oestrogenic material in the urine of pregnant women suggests either an increased production of oestradiol and a changed method of disposal, or a primary production of oestrone in the placenta. The formation of emmenin, and the fact that esters of the oestrogenic compounds are excreted (cf. p. 297) suggest that some adjustment of oestrogenic activity is achieved by what virtually amounts to a detoxication.

Whatever is the active oestrogenic agency, depression of gonadotropic activity of the pituitary persists, ensuring absence of ovulation, while stimulation of breast development continues.

(The experiments of Selye, Collip and Thomson on the rat (145) suggest that the placenta determines the length of pregnancy either by its effect on the corpus luteum, or by its own progesterone production, or by both.)

Towards the end of pregnancy the uterus regains its tone and its reaction to pitocin of the pituitary. It seems a natural supposition to conclude that at childbirth pitocin induces

labour through its normal action in stimulating uterine contraction. The evidence in favour of this view is inadequate ; it has even been shown that in the experimental animal parturition can occur in absence of the pituitary. Yet it has also been shown that the pitocin activity of blood is depressed during pregnancy, but is increased above normal at term (27), and further that *free* oestrone appears to sensitize the uterus to the action of pitocin, and free oestrone, instead of its esters, becomes available just before parturition (137A, 111A).

The endocrine control of reproduction does not cease at childbirth ; the food-supply of the child has been prepared. The mammary glands have been stimulated to the stage of storage of secretory products (and possibly oestrone has inhibited actual secretion of milk (137)). Prolactin, from the anterior pituitary, stimulates an actual flow of milk, while its stimulus is further enhanced by the nervous reflexes set up through the act of suckling (147). Moreover, maternal behaviour seems itself to be under the control of prolactin (cf. Chapter VIII).

Ovarian function recommences, Graafian follicles develop once more, and ovulation follows, but the menstrual cycle may be disarranged for awhile and incomplete, perhaps also through the agency of prolactin (47). Sooner or later, if no second pregnancy commences, the regular rhythm of menstruation is restored, and the reproductive sequence of short or long cycles continues until, at a relatively early age, the menopause brings it to a close, perhaps, as Zondek suggests (178) through exhaustion of suitable ovarian follicular material ; the primary cause is associated with the ovary itself (2).

A small percentage of women exhibit a peculiar though regular cycle in which ovulation does not occur, but a form of menstruation does take place. The cycle is usually a little long. Its peculiarity appears to lie in a specific inability of the follicles to rupture. They persist, and the ova die, and then the follicles degenerate. There is in consequence a

sudden marked reduction in output of the oestrogenic principle, a reduction which in itself can lead to that haemorrhagic sloughing off of the uterine mucosa which constitutes menstruation. The abnormality in these sterile women can only be detected by examination of the uterine mucosa shortly before an expected period. It will show the proliferation of the first half of the cycle, due to oestradiol, but not the second stage of change due to progesterone (125). In these cases there is no loss of uterine sensitivity to pitocin (86).

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 (117) 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 oestrone on the male reproductive system, and the proved diminution of the gonad-stimulating principle in the pituitaries of female

animals following injections of oestrone. 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 principle of the corpus luteum controls certain uterine changes leading to implantation of the ovum, prevents further ovulation, and maintains pregnancy, and has, 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.

Since, during pregnancy, the potent oestradiol of the ovary no longer circulates in the organism, and even the less potent oestrone is converted to some still less active ester, it would appear quite possible that hyperactivity of ovarian endocrine function could produce pathological effects, if this were not prevented in such a manner. Evidence of such a hyperactivity, as a cause of disease, remains to be established, although the experiments of Collip and of Zondek (cf. p. 307) suggest that chronic hyperactivity may well lead to a marked pathology. Hyposecretion 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 amenorrhoea, 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 under-

secretion to abortion, especially at the earlier stages of gestation. From oversecretion of the testicular principle we might expect a " hypermasculinization," and from under-secretion 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 " (131).

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 (131). The changes in the vaginal epithelium during the cycle, so useful for experimental control and so definitely related to ovarian function in the lower mammals, are only slowly being defined and utilized clinically in woman (41, 151, 142A). Objective criteria which can be employed are the condition of the secondary sex organs and sex characteristics.

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 (131) and Rowe (139) 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.

Castration. Prepuberal castrates are rare among women. The available evidence is in agreement with that concerning experimental castration in young animals—there is 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 flushes, 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 (131).

Werner (165) has made a careful comparative study of fifty-three castrates, ninety-six women in the menopause, and forty-eight having involutional melancholia. He classifies the subjective symptoms as nervous (nervousness, excitability, irritability, headache, etc.), circulatory (hot

flushes, tachycardia, vertigo, etc.), and general (lassitude, constipation, menstrual disorders, etc.), and concludes that they are accompaniments of ovarian hypofunction, or non-function, and points out that there is a striking parallelism in the symptoms of the menopause and of involutional melancholia.

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. 280). A recent study by McCartney (102) 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 (139) 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.

Toxaemias of Pregnancy. It has been stated (152) that the placentae of toxaemic and eclamptic patients contain excessive amounts of the A-P-L principle and tend to be low in oestrogenic material. The functional significance of this is not yet clear.

Tumours of the Gonads. Our knowledge of ovarian tumours has recently been summarized by Novak (123A),

whose conclusions are largely based on studies of his own cases.

Granulosa cell tumours, possibly adenomas, but usually tumours of low-grade malignancy, when occurring in young children lead to precocious puberty and menstruation, with the corresponding secondary sex manifestations. The accelerated feminization is due to increased production of oestrone by the constituent cells of the tumour. Removal of the tumour tends to a return to the normal condition of the child.

In adult women, even when beyond the menopause, such tumours can produce through excess of oestrone a hyperplasia of the endometrium associated with periodic bleeding (pseudo-menstruation).

The much rarer *arrhenoblastomata* lead to defeminization and masculinization. These tumours are believed to originate in certain undifferentiated cells occurring in the region of the rete ovarii (the female homologue of the testis). The clinical manifestations vary. In the extremer cases amenorrhoea results, the breasts flatten and atrophy, a heavy growth of hair appears on face, chest, abdomen and lower extremities, the contours of the body take on a male appearance, the voice deepens, and the clitoris hypertrophies to penis-like proportions. Absence of obesity and striae atrophicae permit differentiation from the syndromes associated with adrenal cortical tumours and basophile tumours of the pituitary. Milder cases may show only amenorrhoea or amenorrhoea with hypertrichosis. The tumours are of low malignant type and may show a resemblance to testicular structure or be atypical. Removal of the tumour leads to slow regression of symptoms.

Seminomata may have some endocrine aspect, since they are the characteristic type found in cryptorchids and pseudohermaphrodites, but the Brenner tumour, arising from early undifferentiated cells which are neither male nor female, has no endocrine significance.

The most interesting type of tumour—from an endocrine view-point—associated with the testis is teratoma of the chorionepithelioma type. Heaney, writing in 1933 (70), found 131 cases in the literature. Of these 123 were primarily associated with a testicle and only 8 were extra-testicular, although even these probably had their origin in the urogenital anlage. When the urine of such patients is tested by Zondek and Aschheim or Friedman's test, markedly positive results are obtained, comparable with those given by the urine of women with chorionepithelioma, especially if metastases are present (cf. p. 331). Usually during life these tumours produce no marked symptoms of endocrine character.[1] Entwisle and Hepp (57) have, however, reported a case in which a very small tumour of the testicle was accompanied by enormous metastases throughout the body ; marked gynaecomastia was produced. At post-mortem examination the pituitary showed histological changes identical with those found in pregnant women.

Treatment of Gonadal Disorders

Ovarian Therapy.[2] Marrian and Parkes (112) 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 (60).

[1] Evans (58) has reported an unusual result obtained with the urine from such a case. Tested on male rats, it produced a very marked development of the seminal vesicles. It stimulated development of the pigeon's testis, which is peculiarly insensible to endocrine stimulation.

[2] Cf. Novak (124) and Corner (38).

An account was given in the previous section of the present theory of control of the menstrual cycle, and the difference between " interval bleeding " and true menstrual bleeding was pointed out. Neglect of this difference has undoubtedly led to unjustifiable claims of benefit from ovarian therapy. In considering such therapy, confusion can arise in describing dosage. The international unit, based on experiments with small animals, becomes cumbrous and misleading when used clinically, as Kaufmann has stressed (79). It is desirable to remember that 25 mg. of dihydro-oestrone (oestradiol) produce the same effect as 100 mg. of oestrone (and 200 or more mg. of oestriol), all these being the equivalent of 200,000 mouse units (between 50,000 and 200,000 rat units), and 1,000,000 international units.

Numerous claims have been made that relatively small dosage of oestrone is effective in inducing menstruation in cases of primary and secondary amenorrhoea. Pratt criticizes such claims, and believes that favourable results are due to psychotherapy (131). Amongst the most carefully controlled cases are those reported by Werner and Collier (166), who have treated eight castrates with intact uterus, and claim that a total of 2,800 rat units given in slowly increasing doses produce an endometrial growth approximating to that in normal women, bleeding (which seems to be rather of the " interval " type than true menstrual bleeding), enlargement of genital tract and breasts, relief from subjective symptoms and increased libido. One patient bled cyclically in a normal manner on four occasions. (Cf. also (114).)

Nevertheless, such claims seem to fall short of certainty when compared with the systematic results described by Kaufmann in a brilliant lecture to the Royal Society of Medicine in February, 1934 (79), results fully confirmed by Dodds and Gardiner Hill in the discussion following this lecture, and in agreement with those reported by Clauberg (29), Rock (138), and Loeser (100). (Cf. also (123, 90, 51A).)

Kaufmann has strikingly confirmed the calculation of

Marrian and Parkes already quoted. Using dihydro-oestrone in a solution such that 4 c.c. contained 25 mg. (1,000,000 international units), he first established true menstruation in a castrated woman by intramuscular injection of, in all, 125 mg., spread over several months, followed by injection of thirty-five rabbit units of progesterone. In a second case similar results were obtained with 300,000 mouse units of the benzoate ester of oestradiol, and five injections each of 6 mg. of pure progesterone (80). It is interesting to note that Loeser (199) estimates that the human ovary secretes monthly (oestradiol corresponding to) 0·125 to 0·15 gram of oestrone, and 40 to 50 rabbit units of progesterone.

Kaufmann has obtained definitely successful results in the following different types of case.

Primary Amenorrhoea of Long Standing. In these cases there is arrested development of the uterus, which is frequently extremely small, and treatment must be aimed not at producing menstruation, but at stimulation of the uterus to grow. Hence, large doses of oestrone or dihydro-oestrone are necessary. Kaufmann achieved success in three of five cases. Doses totalling between 200 and 300 mg. of the dihydro-compound were necessary. One case failed to respond even with total dosage of 375 mg. (Cf. also 98.)

Secondary Amenorrhoea. Proof of success requires establishment of true menstruation. Kaufmann treated a group of forty patients by simulation of the normal ovarian stimulus as closely as possible. One cubic centimetre of the solution (6·25 mg. of dihydro-oestrone) was injected on each of the 1st, 4th, 8th, 11th and 15th days of a month, and then on each of the 19th, 20th, 21st, 22nd and 23rd days seven rabbit units of progesterone. In almost all these cases true menstrual bleeding commenced on the second day after the last injection of progestin. After artificial menstruation had been so induced for three consecutive months, treatment was stopped. Complete regular spontaneous menstruation then often occurred during several months, although Kauf-

mann considers that in cases of severe disturbance of ovarian function recurrence of amenorrhoea is to be expected after a few months.

In a second series he gave dihydro-oestrone only. Haemorrhages, partly "interval" and party true-menstrual, were produced.

Exceptionally Severe Secondary Symptoms Following Castration. Smaller dosage is frequently sufficient in such cases, and should always be first employed. If benefit is not obtained, large doses should be given for two weeks, of the order 12·5 mg. dihydro-oestrone per week, then 2·5 mg. weekly. Remarkable results are produced. The flushings, profuse sweating, almost complete insomnia and nervous disturbances disappear very rapidly.

Uterine Haemorrhage of Ovarian Origin. The anatomical basis is almost always cystic hyperplasia of the uterus, and Kaufmann has shown, from treatment of a castrate with excessive doses of dihydro-oestrone, that this cystic hyperplasia is the result of excessive ovarian stimulation. Hence, administration of oestrone is a mistake. He finds that correct and highly efficient treatment consists in the administration of progesterone. He has had excellent results in fifty cases. Most of them required 5 to 10 rabbit units spread over five days. Occasionally much larger dosage, 60 to 80 units, was needed.

Kaufmann's results seem to place ovarian therapy on a sound basis. The only difficult problem is the cost to the patient (138).

Dr. Gilbert Adamson (1A) has drawn attention to the resemblance in disturbances in certain patients following childbirth, and in certain cases of dysmenorrhoea to those at the menopause, and has obtained good results by the use of oestrone in such cases. (Cf. also Schneider (142B).)

Gonorrhoeal Vulvovaginitis in Childhood. Oestrone has been advocated, usually by oral route, and in rather varied dosage by different clinicians. The basis of the treatment is

the epithelization of vaginal mucous membrane by oestrone. The majority who have used the treatment report good results in most cases (96, 129, 120) but relapses occur, and at least one series of unsuccessful results has been reported (173), while it is too early to know the after-results of such therapy.

Hall and Lewis (68) have shown that in immature monkeys injections of oestrone change a definitely alkaline vaginal fluid to a highly acid one, and they point out that a similar but less abrupt change takes place in girls at normal puberty; they suggest that this change may explain the favourable results of the oestrone treatment in infantile gonorrhoeal vaginitis. (Cf. 96A.)

Good results have been reported following the oestrone treatment of senile vaginitis (76).

Haemophilia. It has been suggested that, on account of its method of transmission, haemophilia might respond to treatment by oestrone. This has been tested by Birch (11), who has claimed that the treatment is successful. Other apparently successful results have been reported, but the most recent reports are unfavourable (13, 14, 159). Chew (28A) studied two cases for ten and thirteen months respectively, and found that no benefit was obtained from oestrone, progesterone, or A-P-L preparations. (Eley, Green and McKhann (52) claim to have obtained good results by use of a globulin solution made by extracting human placentae with isotonic saline, and some subsequent degree of purification.)

The same type of reasoning has led to similar treatment in cases of retinitis pigmentosa, a disease commoner in males than in females, and similar claims of benefit have appeared (169). Less clear is the reason for application of this therapy in a case of complete loss of head and body hair in a male patient. Renewed growth of hair followed the treatment (83).

Good results have been claimed by the use of progesterone in cases of *habitual or threatened abortion* (12), and of primary

dysmenorrhoea (174). It is stated that oestrogenic treatment of cystic diseases of the breast is beneficial (42A).

Emmenin Therapy. Campbell and Collip (25, 24) have described beneficial effects from administration of the placental principles.

Emmenin produces no benefit in primary amenorrhoea. In secondary amenorrhoea the results depend on its type. When there is a steady progressive decline of flow, with lengthening of cycles, or even complete amenorrhoea of not too long duration, emmenin is effective in the majority of cases, and, when effective, restores menstruation after an average of twenty-three days' treatment. If the amenorrhoea is over eighteen months' duration, the treatment is ineffective. Cases in which amenorrhoea suddenly interrupts apparently regular normal menstrual cycles are refractory to the treatment.

The majority of cases of dysmenorrhoea and polymenorrhoea are benefited, but intermenstrual pain and menorrhalgia are not. There is no effect on menopausal symptoms, nor on menorrhagia or metrorrhagia.

The dosage in secondary amenorrhoea 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 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.

Similar results have been obtained by Watson (164) and Goldberg and Lisser (64). MacFarlane (108) and Stewart (153A) claim improvement in some proportion of menopausal cases.

Campbell has reported that fifteen childless patients became pregnant when disturbed ovarian function was

corrected with emmenin (24). (It has been claimed that emmenin is beneficial in migraine (13).)

A-P-L Therapy. 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. 38. In cases of metrorrhagia the continuous uterine bleeding has been in some measure controlled, but a tendency to establishment of

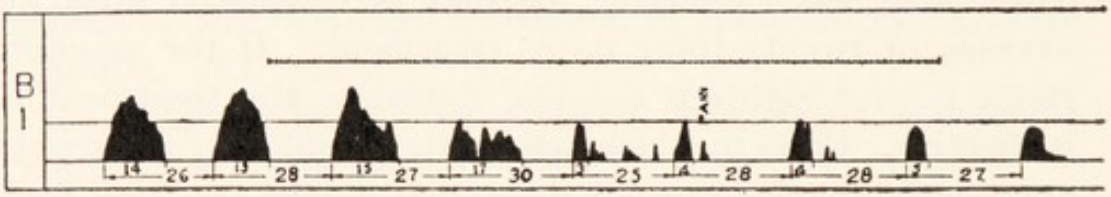


FIG. 38.—Administration of anterior-pituitary-like principle in a case of menorrhagia. A woman aged 35, married six years, no children. Menstruation began at 14; regular every twenty-eight days. In March, 1930, the periods began to be prolonged to thirteen to fifteen days. She complained of feeling giddy 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.)

normal cycles has not been observed. Intervals of amenorrhoea may appear. Successful treatment is illustrated in Fig. 39. Henderson (71) has recently reported a large measure of success with this therapy.

Claims have been made that severe functional bleeding can be controlled by administration of Zondek's " prolan " (122, 115). The identity of this with the A-P-L principle brings these claims into agreement with those for the latter. Frank, however, believes that prolan is ineffective (61).

A number of clinicians (*e.g.*, 142, 66, 1, 153, 14A) have reported favourable results following the treatment of cases

of *undescended testicles* with A-P-L preparations from urine ; this treatment was suggested by the experiments of Engle with immature monkeys (54). A-P-L is said to be beneficial in the treatment of acne vulgaris (94A).

Ovarian Transplants. The literature has been reviewed by Thorek (156), who considers that such transplants improve waning physical and psychic conditions and retard onset of symptoms of senility. No claim for rejuvenation is made. The transplants are said to function for several years.

Testicular Therapy. The logical use of the testicular principle would seem to be for replacement therapy in senility.

FIG. 39.—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.)

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 (116) : " 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 (139) : " 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 (162), yet Pratts' 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." 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 " (131).

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 the crystalline testicular principle will permit 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.

One or two reports have appeared in the literature, concerning the results following injections of potent extracts. Benjamin (7) has claimed good results, based mainly on subjective improvement. McCullagh (105) 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. 327), 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. (Cf. 104.)

Tests 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.

Their method has been subjected by them to various modifications. As at present employed by them, the technique is as follows (134, 6A) : 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 oestrone (theelin) 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.

The test is not given by the urine of pregnant rats, mice, rabbits, bitches, cats, cows, swine and elephants (55), nor with monkeys, though there is good evidence that it is given by pregnancy urine of the higher apes (182).

Summarizing the results obtained with his procedure to 1931 (176) Zondek claims an error of only 1 to 2 per cent., in 5,515 tests by thirty different groups of observers.

Various modifications have been suggested, including that by Friedman and Lapham (63) in which rabbits are used as test animals. Comparison of various procedures, supposed to be diagnostic of pregnancy, indicate that Friedman's procedure is most satisfactory for general use (167, 128, 9).

In Friedman's test advantage is taken of the fact that rabbits only ovulate after copulation, or pseudo-copulation. Adult female rabbits of any age, preferably over 5 lb. in weight, and which have been segregated from both males and other females for three, or better, four weeks, are suitable test animals. (Rabbits which have just littered can be used, and, in emergency, rabbits shown by preliminary laparotomy to have ovaries devoid of haemorrhagic follicles.)

Slight modifications of the test are employed in different laboratories, but usually 5 to 8 c.c. of unconcentrated morning urine are injected into an ear vein twice, with a twenty-four-hours' interval between, and the rabbit killed and autopsied after a further twenty-four hours. One or more haemorrhagic follicles indicate a positive result. Only macroscopic examination is necessary. The best urine preservative seems to be boric acid.

Using operative technique, rabbits can be used twice,

though it has been reported that further use tends to increase the degree of error. No aseptic precautions are necessary.

The degree of error is about the same as that for the original Aschheim-Zondek test with mice, but the much easier technique and the greater rapidity with which the result is obtained have led to wide adoption of the rabbit test.

These tests depend upon the production of the A-P-L principle in the chorionic villi of the placenta. Sufficient is secreted to give a positive result seven to ten days after embedding of the impregnated ovum. Positive results are given in cases of ectopic pregnancy (although the degree of error is somewhat greater) (65), with hydatidiform mole, and in extreme degree with chorionepithelioma (one three-hundredth of a c.c. may give a positive result). For the same reason, those types of teratoma of the testis akin to chorionepithelioma also give markedly positive results, especially when metastases have developed. In cases of dead foetus positive results may be given until complete separation of the placenta. After discharge of a mole, positive results may continue for over a month without subsequent development of a chorionepithelioma. An ensuing negative test should be checked at intervals (113).

The test is probably the most accurate biological test known. It is gradually being made quantitative, with a view towards increasing its efficacy in testing for chorion-epithelioma and teratoma of the testis. The literature dealing with the test is already large ; only a few recent references can be given (11, 118, 82, 40, 44, 109, 40A).

A test has been devised by Bercovitz (8) in which a drop of the patient's blood serum or citrated blood is dropped into one eye, and that compared with the other eye, dilatation or contraction signifying a positive result. King (81) concludes that the test is not sufficiently reliable for doubtful cases, and the effect should be merely regarded as an interesting phenomenon.

A test has been described depending on extrusion of ova in the cloaca of the toad (149).

A test has been based on increased presence of histidine (78), but it appears to be neither so accurate, nor applicable as early

in pregnancy as the Aschheim-Zondek and Friedman tests (143), while it is given by the urine of patients with grave hepatic lesions (160).

Changes in the length of the oviduct of the female Bitterling, through action of urine of pregnancy added to the water containing the fish, have been suggested as suitable for a biological test for pregnancy; not only would adequate supplies of this fish be difficult to ensure, but it appears also that the test is not specific and not very accurate (84, 125A). Male urine gives it (84).

A test has been suggested based upon the premature opening of the vaginal orifice of immature rats. It does not appear to be quite as accurate as the Friedman test (75).

The uterine response to intravenous injection of pitocin has also been suggested as an early test for pregnancy (168). The softened pregnant uterus shows an increase in consistency.

Prediction of Sex. It has been claimed that injection of the urine of pregnant women into immature male rabbits enables prediction of sex of the child, since if the foetus is female the testes of the rabbit show enlargement and ingestion, and microscopic evidence of increased spermatogenesis, while if the foetus is male no testicular changes occur (51). Such results have not received confirmation (42, 119).

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CHAPTER VIII

THE PITUITARY GLAND

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 (158, 187, 41). The following brief statements are taken chiefly from Bailey's description (12).

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" (44).

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 (133) states that the pars intermedia is practically absent in the adult human pituitary, but Brander (24) finds that it is extremely variable in extent and arrangement.

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]

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 " (12).

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

[1] Engelbach (58) 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 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)."

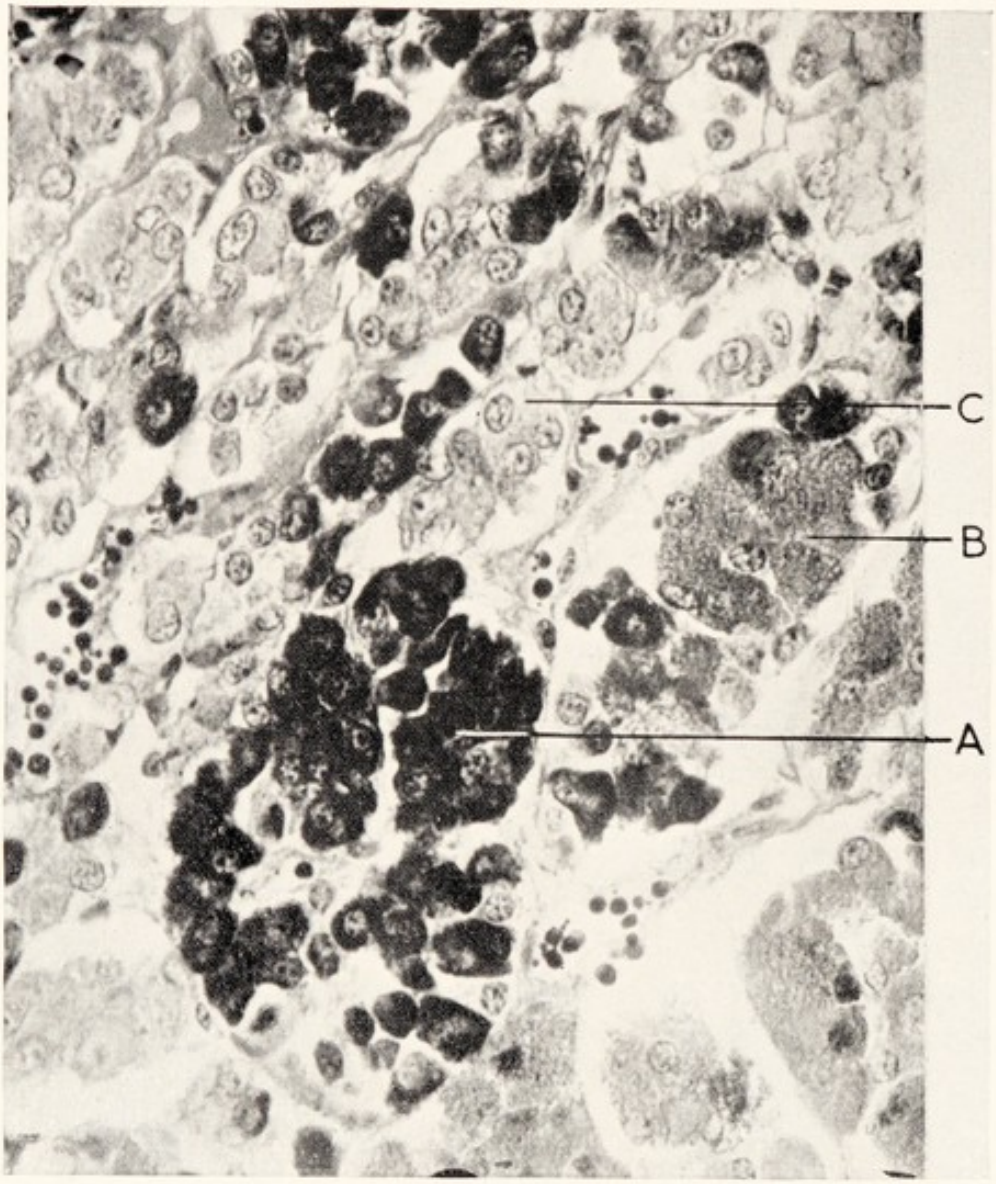

FIG. 40.—Section of anterior lobe of human pituitary, stained with aniline blue and acid-fuchsin. *A*, acidophile cells, dark-staining and clustered; *B*, basophile cells (intermediate shade); *C*, chromophobe cells (faint shade). × 400. Photo-micrograph by Professor William Boyd.

[*To face p.* 341.

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.

There are very few photomicrographs in the literature which really reveal the different histological appearances of the three types of cells. Excellent representations are given by Boyd (23) and by Roussy (145). Through the kindness of Professor William Boyd I am able to present a most excellent photomicrograph (Fig. 40) which well illustrates the relative distribution of the three types of cells.

According to Rasmussen (132), the distribution of these three types of cell in the anterior pituitary of man 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

He finds a very similar distribution in the pituitaries of non-pregnant women (averaging 43 per cent. alpha, 7 beta and 50 chromophobe), while pregnancy causes no significant change.

Wolfe has presented evidence of cyclic variations in the three types of cells, in the rat, dog, and sow, corresponding to the different phases of oestrus.

Certain so-called *cells of pregnancy* and *cells of castration* have been described as occurring in these two conditions (cf. 58, 203, 209). While there is some evidence for the existence of the latter, Rasmussen has been unable to identify specific " pregnancy cells." There is an enlargement of the pituitary during pregnancy, but it is not due to hyperplasia of any one of the three types of cells.

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 (44) 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."

Biedl has suggested that the chromophobe cells are " mother cells " from which both basophile and acidophile cells are derived. This theory has been beautifully proved by Severinghaus (155). He has shown that the chromophobe cells of the rat's pituitary can be separated into two distinct types by reason of differences in their Golgi apparatus. That of the first type corresponds to the Golgi apparatus of the acidophile cells (a filamentous net), while that of the second corresponds to the Golgi apparatus of the basophile cells (a ring). He can find no evidence of change from the alpha to the beta type, but he shows further, from studies of the pituitaries of castrated rats, that chromophile cells can revert to chromophobe cells. The castration cell has the typical basophilic type of Golgi apparatus (155).

Susman (228), from study of autopsy material, believes that the three types of cells are only phases of a single type, and seems to conclude that the pituitary has a normal function associated with cell growth, and an emergency function leading to pituitary cell exhaustion. His technique can scarcely be compared with that of Sevringhaus.

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 (26). 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 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.

It has been suggested by Houssay (84), 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] Evidence is accumulating that the posterior and intermediate parts secrete their own specific compounds. This view is particularly supported by Geiling's studies, and will be referred to later.

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 (44).

The first important observation bearing upon the function of the posterior pituitary was that of Oliver and Schafer in

[1] Atwell has studied the interrelationship between the pars intermedia and nervosa, and possible specific action of the pars tuberalis (11).

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 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 (33) 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 expansion 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 showed that these actions were due neither to an adrenine-like compound nor to histamine. They were first attributed to a single compound (1), but this view was gradually proved to be wrong (54, 150, 53), and in 1928 Kamm and his associates provided indisputable evidence of the separation of the pressor and oxytocic principles (94, 27).

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*.

The pressor principle is responsible for the diuretic-antidiuretic action of pituitary extracts (94, 74). In normal animals, not under anaesthesia, the predominant effect is suppression of flow of urine (28). The beneficial effects produced on patients with diabetes insipidus are due to this principle, oxytocin being without effect (74, 172), and it has been suggested from comparative studies that the effect is due to stimulation of water re-absorption by the thin segments of the loop of Henle of the kidney tubules (30). Adolph (196) concludes from studies on the frog that the diuretic action of small doses of posterior pituitary extracts is due to improvement of the general circulation, while the anti-diuretic effect of larger doses is due to direct action on the afferent arterioles

of the glomeruli. There is also evidence that the stimulating effects on the smooth muscle of the intestine (74) are due to pitressin.

Gulland has studied the partial destruction of pitocin by nitrous acid, and its inactivation by various enzymes ; his results indicated a possible peptide character (77) and that it plays a rôle in an oxidation-reduction system (78). Pitressin is said to be richer in cystine radicals than pitocin ; it appears to contain a disulphide linkage (151) ; both are rich in phenolic (presumably tyrosine) radicals (186). Stehle (171) has suggested a modified method of separation of the two principles.

Pitocin appears to produce the full effect of pituitary extract in obstetrical practice (27).

The effect on blood coagulation is probably not due to any specific principle, since purification lessens or abolishes it (99).

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 (146).

The Intermediate Part

The existence of a constituent in " pituitrin " causing contraction of the melanophores of the frog's skin by no means proves that this constituent is present in the posterior lobe. Separation of the intermediate part from the posterior lobe is not easily effected. Zondek (193) prepared an aqueous extract of what he terms " intermedin " ; this he believes to be secreted by the pars intermedia, and this acts

on frog's melanophores, and, further, has a specific effect on the pigment-forming cells of certain fishes. When it is injected into the minnow *Phoxinus laevis* an intensely red colour appears between the fins. In cattle pituitaries it is present in the pars intermedia to a much greater extent than in the posterior lobe, while only a trace of it is extractable from the anterior lobe.

Zondek considers that " intermedin " leaves the pituitary by the path of the pituitary stalk. It seems to be present in traces in blood and to have some effect on water metabolism so that certain cases of diabetes insipidus have responded well to treatment with preparations containing it (192). However its complete separation from vasopressin has not been established (173).

Geiling's work definitely associates the melanophore principle with the pars intermedia, and stresses the closer association of that tissue with the anterior lobe of the pituitary, than with the posterior lobe. He prepared tissue cultures from the three parts of the pituitary of the mouse and rat (214). Extracts of material from cultures of the pars intermedia had no effect on blood pressure (vasopressin absent), but had a marked melanophore-expanding effect when injected into frogs. Cultures of the posterior lobe (admixed with a ragged edge of the pars intermedia) had both vasopressor and melanophore effects.

In marine mammals the pars intermedia seems to be absent. Wislocki could not find it in the porpoise (232), and Valsö (184) reported it absent in the blue whale, and stated that " intermedin " is formed in the anterior lobe of this animal. Geiling has studied the pituitary of the finback and sperm whales very carefully (213), in association with MacCallum, Wallen-Lawrence, and Riddle, and has shown that there are only present a large anterior and a smaller posterior lobe, separated by a septum. No pars intermedia could be found. The posterior lobe contains no melanophore principle, but there is a plentiful supply of it in the anterior

lobe. The posterior lobe contains the usual amount of vasopressin, but much less oxytocin, and both of these appear to be elaborated in the posterior lobe itself. The anterior lobe contains the gonadotropic, thyrotropic, and adrenotropic principles (cf. p. 358) but a very low prolactin content.

The Posterior Pituitary as an Endocrine Gland

Pitressin and pitocin unquestionably produce specific pharmacological effects. Further evidence is necessary before it can be assumed that they are endocrine principles, secreted from the gland under physiological conditions.

While the presence of adrenine in blood has been reasonably demonstrated, there is as yet no definite proof that the pressor and oxytocic principles normally reach it (cf., however, *e.g.*, Brull (25)). Various writers have suggested that the colloid and hyaline material in the pars tuberalis can be considered as a secretory product which passes up the pituitary stalk into the third ventricle, and Cushing (44, 46) especially has stressed Herring's view that epithelial cells stream from the pars intermedia to the posterior lobe and are there transformed to hyaline material—the Herring bodies—which he believes contains the endocrine principles of the posterior lobe, that normally are either carried to act directly on the nervous centres in the tube, or pass into the infundibular cavity and there act on nerve cells. He has expressed the view that the streaming cells are basophilic in character, and that in certain diseased conditions—hypertension, pituitary basophilism, eclampsia—there is marked increase of such cells and of hyaline material in the posterior lobe.

Marcano (112), while believing that this wandering of basophile cells does occur, can find no relationship between the height of blood pressure in cases of hypertension, and the degree of apparent cell-wandering (cf. also Beato (200)).

Biggart (203) cannot correlate basophile invasion of the posterior lobe with essential hypertension or with eclampsia.

Final disproof of Cushing's views is afforded by Geiling, who has found that in the whale (213) and chicken (206), both without a pars intermedia, Herring bodies are still present in the posterior lobe, which obviously cannot have originated in the pars intermedia. Further, Gersh (215) has found that the Herring bodies are merely artefacts. They are protein material changed to hyaline by fixative, and are separable from the pressor principle. They cannot be considered as the secretion of the posterior lobe.

It is therefore almost certain that the pressor and oxytocic substances associated with the posterior lobe are produced in that lobe, and it seems most probable that the pituicytes, specific to that tissue, are associated with the production. It does not follow that the path of discharge of these compounds and even of one or more of those associated with the anterior lobe may not be through the pituitary stalk. Geiling considers, however, that the path of discharge is probably into the general circulation through veins originating from sinusoids in the buccal part of the pituitary, and from capillaries in the neural portion; these 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 (122, 121).

Pathological states sometimes virtually ablate the posterior pituitary (cf. Fig. 41, p. 358). Such natural human experiments have led to interesting observations. Craniopharyngiomas usually compress the pituitary stalk. Large chromophobe adenomas, confined within the sella, compress the posterior lobe, but leave the hypothalamus unaffected. Patients with these tumours almost always exhibit a lowered blood pressure, especially those with craniopharyngiomas (44).

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.

There is, however, a marked species difference. Application of the silver clip technique to the dog by Mahoney and Sheehan (224) resulted in a state of diabetes insipidus; in pups this was accompanied by retardation of growth, sexual infantilism, and adiposity, suggesting interference with discharge of the secretions of both lobes. But in the monkey there resulted no polyuria, no polydipsia, and no change in basal metabolic rate or blood sugar level, or urinary excretion of nitrogen and bases. Mahoney and Sheehan point out that there are important anatomical differences in the pituitary stalk and the downward extension of the third ventricle in these two species, and they conclude that in the monkey obstruction of the stalk does not interfere with nourishment of the pituitary or the discharge of its endocrine compounds.

Raab (130), in his studies on fat metabolism, showed that extract of the posterior pituitary lobe is particularly effective when injected into the ventricle, and Cushing (44) confirmed this by tests on patients in pronounced hypopituitary states, who had all been previously operated on for pituitary adenoma. Light and Bysshe (103) obtained similar results with monkeys. It seems therefore probable that the endocrine principles of the pituitary participate (with variations in different species) in the vasomotor and other activities of the inter-brain.

Observations reported in the previous chapter suggest that the oxytocic principle is physiologically associated with uterine contractions, and with the termination of pregnancy.

The evidence is by no means definite, but seems to indicate that pitocin may at least be one of the factors (perhaps not an essential factor) normally associated with the mechanism of parturition.

Thus the available evidence in favour of the view that the posterior lobe of the pituitary is endocrine in function is suggestive, but not final. It seems extremely unlikely, however, that the presence of two specific compounds with powerful pharmacological actions is not associated with a definite physiological purpose.

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 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 demonstrated that ablation of the dog's pituitary produced marked but only transient polyuria, provided the base of the brain was uninjured during the operation, while damage to the base of the brain bordering on the pituitary resulted in marked and persistent polyuria, even though the pituitary was not damaged; this polyuria was not controllable by injections of pituitary extracts (33) (cf. also Bailey and Bremer (13)). Delayed adiposity and genital atrophy can occur in these cases.

In the rat, as in man, there is above the pituitary a dural diaphragm, perforated for passage of the stalk, so that nervous tissues and the pars tuberalis above, or the body of the pituitary below, can be subjected separately to experimental damage. Smith (164), 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 (137) 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.

Such results appear to suggest that two distinct syndromes can be produced, one associated with the hypothalamus, and the other strictly with the pituitary, but Cushing (44)

stresses the abundant nerve supply and circulatory connections of the two regions and the consequent effect of tuberal injuries on the pituitary.

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 (44) 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.

Fisher and co-workers (69), studying the effects of experimental lesions in the cat, and considering a theory put forward by von Hann (cf. 138) that normal water metabolism is maintained through an antagonistic effect of principles of the posterior and anterior lobes of the pituitary,

conclude that the supraoptico-hypophyseal system regulates the secretion of the anti-diuretic principle. An injury to this system at any one of three points—supraoptic nucleus, tractus supraoptico-hypophyseus, and pars posterior—leads to a deficiency of the anti-diuretic substance, and since this compound acts on the kidney, preventing it from secreting an excessive amount of urine, any deficiency leads to a primary polyuria, which is followed by a secondary and compensatory polydipsia (cf. also 139).

Biggart (20), from post-mortem studies of three cases of diabetes insipidus, in two of which there were lesions both of the pituitary and the hypothalamus, while in the third—the only one of the three which did not respond to pituitrin, —only the pars tuberalis and the hypothalamus were affected, considers that pitressin acts directly on the nucleus tuberis and supra-opticus, since these were undamaged in the first two cases.

The results of Mahoney and Sheehan already quoted (p. 351) suggest that Fisher's theory does not apply to the monkey.

It is obvious, in any case, that the current views concerning the anti-diuretic function of the posterior pituitary are based upon acceptance of the theory that it is an endocrine gland constantly secreting vasopressin.

Good results have been reported in treatment of diabetes insipidus following administration of posterior lobe pituitary powder intranasally (225, 204, 115).

Daily administration of amidopyrone (pyramidon), alternated every fourth day with pitressin, is said to produce good results in the treatment of diabetes insipidus (93).

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 (58). According to Skipp, subcutaneous injections of extract of the posterior pituitary may be beneficial (160A).

It has recently been suggested that undue secretion of the 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 (49). (Cf. 218.)

Dodds (51A) states that extracts of the posterior pituitary contain a compound (not pitocin, but possibly pitressin) which, injected subcutaneously or given orally to laboratory animals, produces severe lesions in the acid-bearing area of the stomach. The animals usually recover in one or two weeks. The effect seems due to a temporary inhibition of secretion of hydrochloric acid, since, in these animals, histamine produces no free acid in the gastric juice. A profound anaemia is also produced apparently due to increased blood destruction, and is accompanied by a marked leucocytosis. The effect is specific to the posterior pituitary.

Various writers have suggested that the toxaemias of pregnancy, especially eclampsia, might be due to hypersecretion of the posterior pituitary principles. Cushing has reviewed the literature (45). There is no convincing evidence.

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 hypofunction. At the present time our knowledge of the anterior pituitary is based largely upon clinical studies of diseases associated with its abnormal states, and on implantation and injection experiments in animals, although biochemical knowledge of the endocrine principles is making rapid progress. 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, and to deal with the biochemical results afterwards.

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.

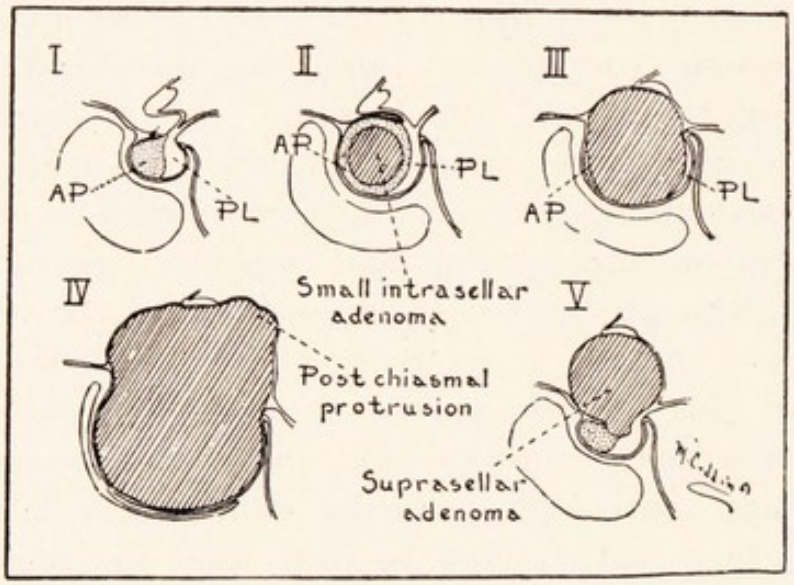

FIG. 41.—A series of drawings to illustrate the mechanical effects of an expanding pituitary adenoma. I. The normal pituitary gland and optic chiasm. II. A small intrasellar adenoma with only a slight expansion of the sellar. III. A larger adenoma beginning to stretch the chiasm—a little anterior lobe 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.)

It is perhaps of some service at this point to anticipate certain results of experimental and clinical studies. There is now a considerable amount of evidence that the anterior pituitary lobe elaborates a number of distinct endocrine compounds, which (i.) control growth, (ii.) control the gonads, (iii.) control the thyroid, (iv.) control the parathyroids, (v.) control the islets of Langerhans, (vi.) control the adrenal cortex, (vii.) control the secretion of milk, and

(viii.) control fat metabolism. It is obvious that, since there are only three types of cells, one or more of these must have a multiplicity of functions. Moreover, no other function can be associated at present with the chromophobe cells than being precursors of the others. There is some evidence that the acidophile cells furnish the growth principle, and less certain evidence that the basophile cells elaborate the principle (or principles) controlling the gonads. Beyond this we have no pertinent evidence.

Tumours of the alpha cells affect growth. Those of the beta cells are apparently associated with gonadal disturbances. Tumours of the chromophobe cells depress pituitary activity through neighbourhood-pressure effects. Tumours of any type can exert such pressure effects leading to depressed function of cells not present in the tumour. 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. 41.

Anterior Pituitary Insufficiency; Simmonds' Disease; Splanchnomicria. Excellent reviews of the literature concerned with this disease have been recently published by Calder (31) and by Silver (161). 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),

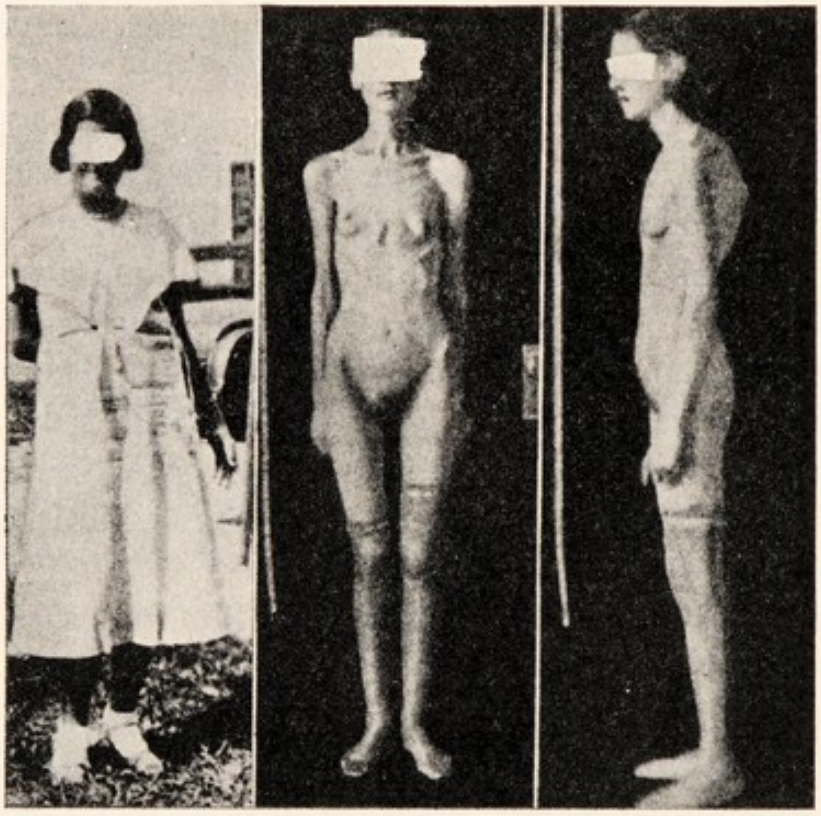

FIG. 42.—Case of Simmonds' disease. *Left:* patient in July, 1933, aged twelve, weight 142 lb. About seven weeks before onset of illness. *Centre and right:* patient in June, 1934, aged thirteen, weight 85 lb. About nine months after onset of illness. (From Dunn, *J. Nerv. Mental Dis.*, 1936, lxxxiii, 166.)

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.

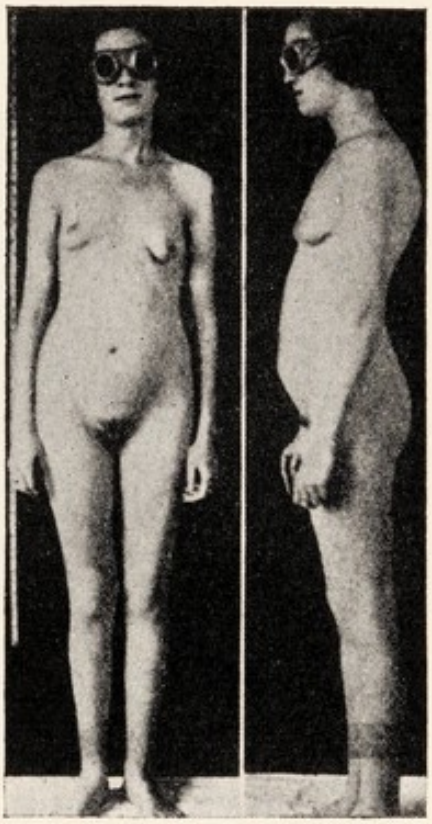

FIG. 43.—The same patient as in Fig. 42. In October, 1934, after twenty-two weeks of combined anterior pituitary and oestrogenic therapy. (From Dunn, *loc. cit.*)

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 (cf. 1A). 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.

It seems possible that more than one syndrome is included under this description; the published reports of benefits

from a very varied therapy strengthen such a view. In a number of cases beneficial results have followed continued injection of extracts of anterior pituitary (58, 136, 32, 111). An excellent example of such a case is reported by Dunn (208), is shown in Figs. 42 and 43. An apparently very similar case has been reported by Moehlig in 1936 (118). This girl was unresponsive to a varied endocrine therapy, including A-P-L, but active pituitary extracts were apparently not given by injection. Bulger and Barr (29A) have reported unsuccessful endocrine therapy in several cases.

FIG. 44.—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.)

Cases have been reported which responded well to A-P-L (81, 216, 217) and even to an adrenal cortex preparation (220).

v. Bergmann has described a number of cases characterized by leanness and a tendency to emaciation, which responded to pituitary treatment (201), and Wahlberg (189) suggests for this type the term *asthenia gravis hypophyseogenea*.

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 (57) 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.

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 (57). It possibly should merely be considered as the childhood form of the Lorain-Levi syndrome.

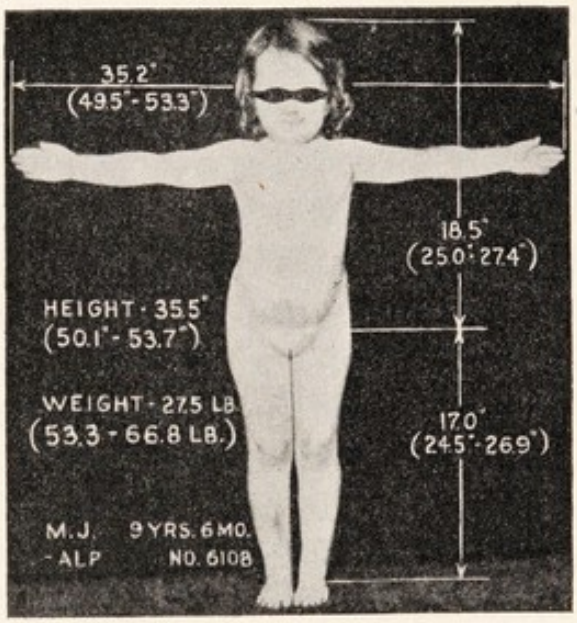

FIG. 45.—Comparison of the actual body measurements of the pituitary dwarf of Fig. 44 with the normal maximum and minimum measurements for her age. (From Engelbach, *Endocrinology*, *loc. cit.*)

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 $35\frac{1}{2}$ inches, her weight $27\frac{1}{2}$ 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. 44 and 45). 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. 394). 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.) A later report on this child states that as a result of two years' treatment with a somewhat crude extract of bovine pituitary, injected intragluteally, she has grown 5·4 inches. Her average for the four years preceding treatment gave a predicted growth of only 2·3 inches, while the average growth for a child of her age is 4·6 inches. Her facial expression has altered towards the normal appearance of her years (159). Equally good results have been reported in a number of other cases exhibiting retarded growth due to pituitary deficiency (59, 159, 148, 207).

It is possible that in replacement therapy of this type crude extracts may be more beneficial than the purified principles, since, as Collip has pointed out, it is unlikely that in any case only one principle needs replacement.

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 either 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 (17) 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 (57). 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 (57).

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 (35). 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 (135).

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.

Rachmann (131) recommends that the treatment of Fröhlich's syndrome should consist of the systematic combined administration of prolan, oestrone and thyroid, controlled by determinations of the basal metabolic rate. He believes that X-ray treatment of the pituitary should be restricted to cases exhibiting compression symptoms.

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 (18). 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

FIG. 46.—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.)

recorded by Behrens and Barr (16), 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 now two sisters and two brothers 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. . . .

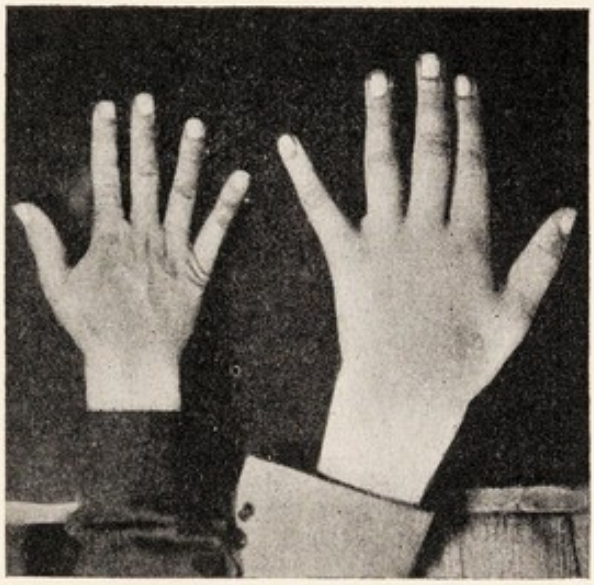

FIG. 47.—Hand of patient (Fig. 46) compared with that of a man 6 feet in height. Noteworthy are the long, lightly tapering fingers and the delicate, fine skin. (From Behrens and Barr, *ibid.*, p. 124.)

" 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. 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.

" 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

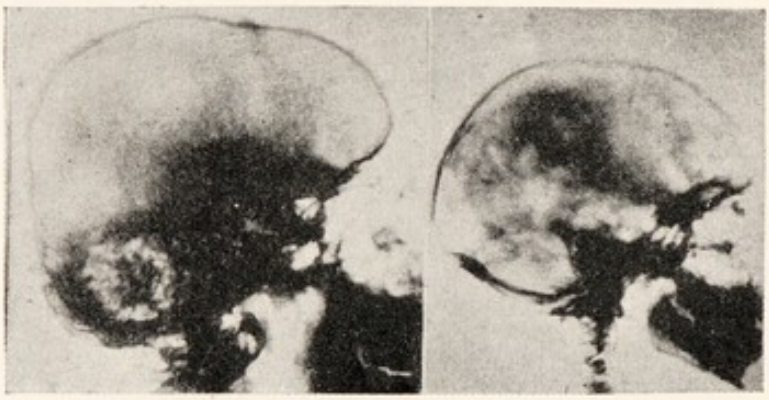

FIG. 48.—X-ray photograph of skull of patient (Fig. 46) at age of eleven, compared with that of a normal boy of the same age. There is an extraordinary development of the mastoid air cells. The sella turcica measured 2·5 cm. anterior-posteriorly ; it has been outlined with dots to indicate its extent. (From Behrens and Barr, *ibid.*, p. 122.)

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 shaped in spite of their size. Many of the essential points of the description are illustrated in Figs. 46 to 49.

" 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, measur-

ing 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. 49 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."

He is now (1936) 8 feet 4 inches high at the age of eighteen,

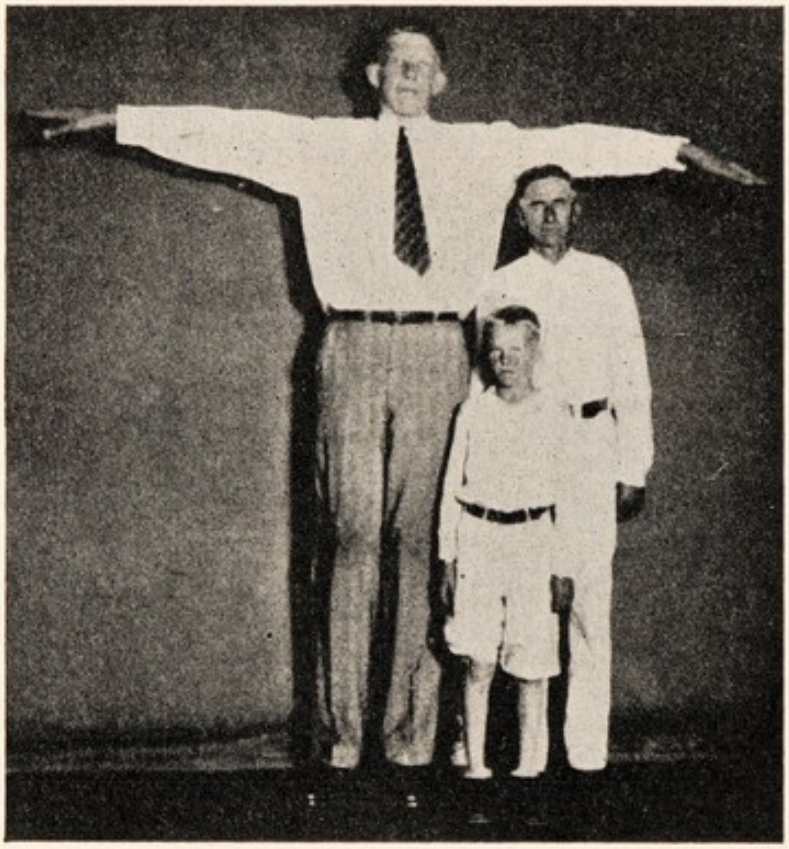

FIG. 49.—The patient at the age of 13½, shown standing with his nine year old brother, and his father, whose height is 5 feet 11 inches. (From Behrens and Barr, *ibid.*, p. 125.)

with a weight of 390 lb., and has not escaped the fame of appearing in the World News on the silver screen.

Engelbach (57) 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 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.

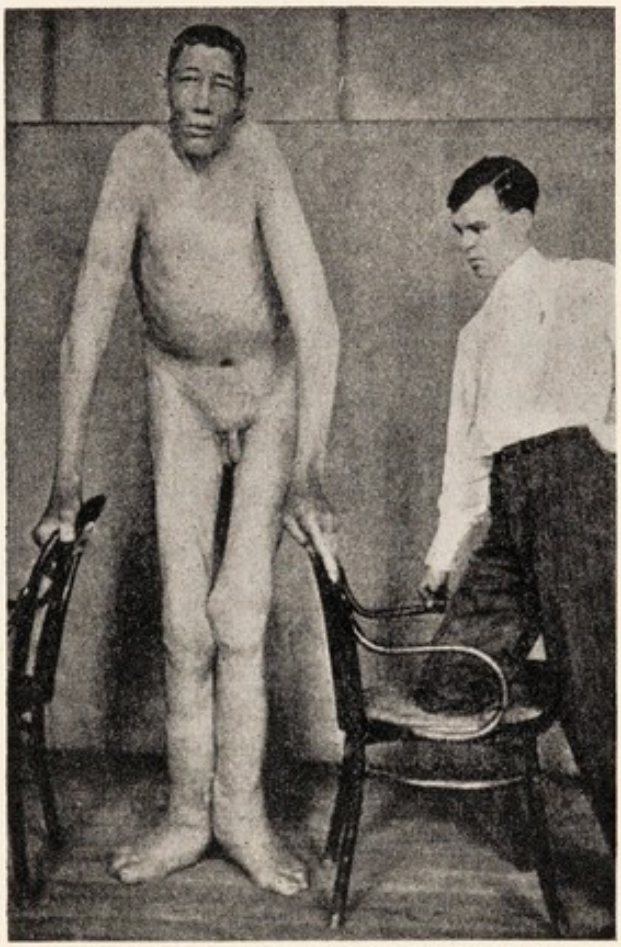

FIG. 50.—A case of gigantism. Final stage. Note the narrow chest, large joints, hypotrichosis, and the large size of the hands compared with that of the normal person of height 68 inches. (From Cushing, "The Pituitary Body and its Disorders," Lippincott, 1912, case XXXII.)

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 (48), a man aged thirty-six, "an extraordinary prototype of the folk-lore giant—overcome by his own size." His appearance is shown in Figs. 50 and 51. 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 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. 50). 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. 51). 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

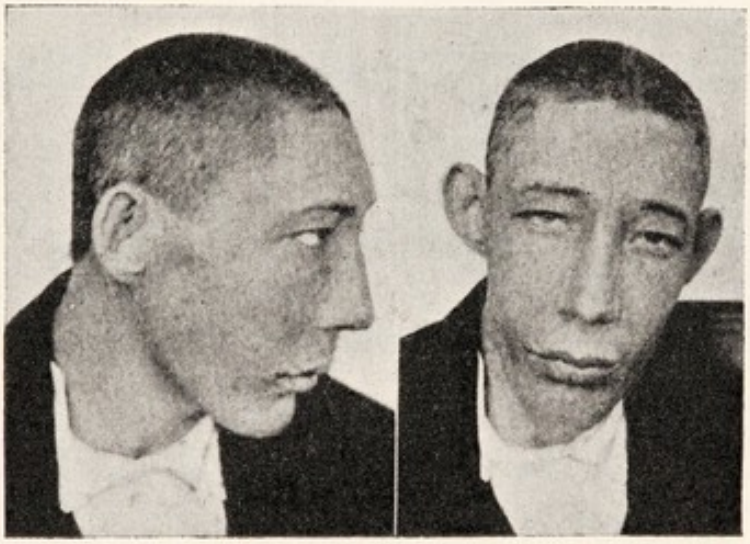

FIG. 51.—The same patient as in Fig. 50. Exhibiting a maxillary, rather than the mandibular prognathism of the acromegalic. (From Cushing, *loc. cit.*)

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 (48).

A possibility of successful treatment is seen in results quoted by Cushing (45). In two boys showing giant-like

rapidity of growth the growth-curves were arrested by X-ray treatment of the pituitary.

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

I. II. III. IV.

FIG. 52.—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.)

hypertrophy. The gradual onset of the facial hypertrophy is beautifully shown in the photograph of Cushing's case XXX. (48), reproduced in Fig. 52. 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.

X-ray examination generally indicates an enlargement of the sella turcica, though in the case presented in Fig. 52 such enlargement was not present.

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 (50).

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 overdevelopment 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 (104, 97).[1]

[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 (14).

It is interesting to note that in the adenoma of acromegaly the Golgi bodies of all the cells, whether chromophobe or acidophile, are acidophilic in type (45), indicating, in accordance with the findings of Severinghaus, that the whole adenoma is composed of acidophile cells and cells which can be changed to acidophile.

Cushing's Pituitary Basophilism. Cushing has recently suggested that the syndrome usually associated with the adrenal cortex is really due to an adenoma of the basophile cells of the anterior pituitary (43, 45). This syndrome is characterized by (i.) a rapidly acquired, peculiarly disposed and usually painful adiposity, confined to face, neck and trunk, (ii.) a tendency to become round-shouldered, even to measurable loss of height, (iii.) a sexual dystrophy, shown by early amenorrhoea in women and ultimate functional impotence in men, (iv.) a tendency to hypertrichosis of face and trunk in females and pre-adolescent males, (v.) a dusky or plethoric appearance of the skin, with purplish liniae atrophiae, and various other symptoms, including hyper-tension, abdominal pains, fatiguability and ultimately extreme weakness. (Cf. 120.)

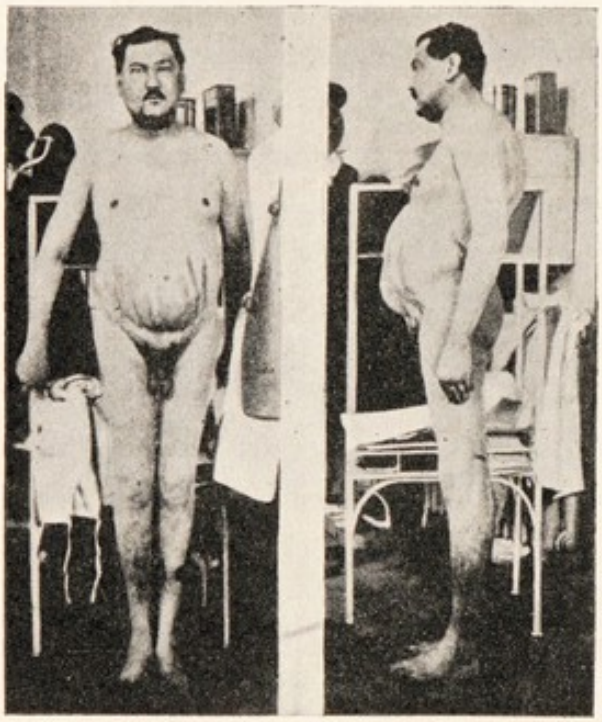

FIG. 53.—Dr. Raab's patient with verified basophilic adenoma of the pituitary. (From Cushing, *Bull. Johns Hopkins Hosp.*, 1932, 1, 137.)

A number of such cases found in the literature, and some under his own observation, including one predicted clinically by Teel (176), have shown at post-mortem examination a pituitary of normal or almost normal size (the sella turcica

is not enlarged), but which contained, as revealed by serial sections, a small adenoma composed of basophile cells. Frequently there is no definite lesion of the other endocrine glands, although the adrenals are generally enlarged and may contain small adenomata, regarded by Cushing as secondary. The thyroid may also be enlarged.

The syndrome appears to be commoner in women than in men, but is as definite in the latter. Raab's case (130) is

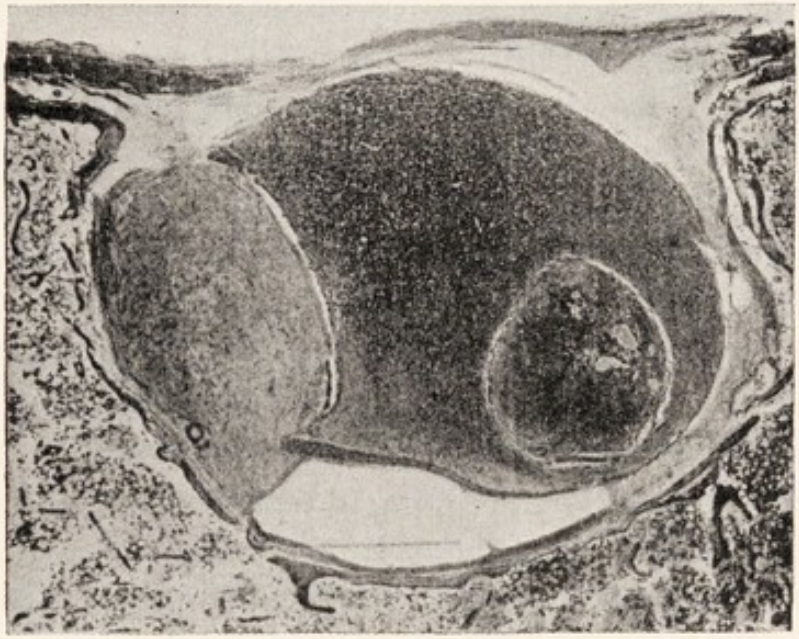

FIG. 54.—Cross section of the pituitary from a case of pituitary basophilism. (From Bishop and Close, *Guy's Hospital Reports*, 1932, lxxxii, 143.)

shown in Fig. 53. It is fully quoted by Cushing (43), and well illustrates the picture in man. Hair distribution and genitalia are normal, the peculiar obesity is obvious from the picture, the broad flame-shaped striae of dark red colour are prominent. This patient was admitted to hospital complaining of headaches and marked gain of weight. A few weeks later he developed severe pain in the lumbar vertebral column, and he died shortly afterwards from acute sepsis, following a streptococcal infection of the hand. Although before death X-ray examination had suggested

some enlargement of the intrasellar space, at autopsy the pituitary was found to be scarcely enlarged, but a basophile adenoma had almost entirely replaced the posterior and had destroyed about two-thirds of the anterior lobe. The vertebral pain was accounted for by an osteoporosis of extreme degree, involving the vertebral column and long bones.

The size of the tumour appears to vary considerably in different cases. A typical picture of such a tumour is shown in Fig. 54, and it is at once obvious from it why in these cases no definite enlargement of the sella turcica occurs.

Cushing considers that a number of the symptoms, including the hypertension, pigmentation and terminal weakness, may be due to secondary adrenal involvement.

A steady stream of reports on cases of Cushing's disease is appearing in the literature (*e.g.*, 21, 118, 42, 174).

It is clearly difficult to differentiate between this syndrome, especially when it occurs in women, and that associated with adrenal cortical tumour. Bauer (15) stresses the likeness between the two syndromes. Frank (72) reports two cases, apparently typical of Cushing's disease, which, at post-mortem, showed adrenal cortical tumours, but no pituitary tumours. (Cf. also Lescher and Robb (101).) Kraus (96) suggests that the pituitary adenoma may be merely secondary. Walters and Wilder (191), reviewing seven cases of the syndrome at the Mayo Clinic, found strong evidence that only two were pituitary basophilism, and the other five were due solely to tumours of the adrenal cortex.

MacCallum and co-workers (222) have described a case typical in most respects of Cushing's disease, in which at post-mortem examination the tumour was found to be closely associated with the pars intermedia. The tumour cells gave no staining reaction with copper haemotoxylin, thus resembling the cells of the pars intermedia, while the basophile cells of the anterior lobe stain black with this reagent.

Crooke (205) states that certain basophile cells exhibit a

hyaline change suggesting altered physiological function. Such cells are scarcely ever seen in normal pituitary glands, but are quite common in the basophilism-adrenocortical syndrome, whether that be due to a basophile adenoma, or an adenoma of the adrenal cortex.

When the close control exerted by the pituitary over the adrenal cortex is remembered (cf. p. 413), the possibility cannot be excluded that the primary stimulus in all these cases arises in the basophile cells of the pituitary, and that hyperplasia or adenoma of these, or hyperplasia or adenoma of the adrenal cortex, are merely variations of what is really a single pituitary-adrenal cortex syndrome.

It seems possible that if a pituitary lesion can be recognized sufficiently early, application of X-ray treatment to the pituitary may be beneficial. Cushing quotes good results in a girl of fifteen, in whom the onset of the disease was acute (45).

It is difficult to believe that the obesity of " pituitary basophilism " is ascribable to neighbourhood pressure from the small tumour on non-pituitary tissue. It must either be due to direct influence on other pituitary tissue 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 (14). 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 optic chiasm, if the tumour was of sufficient size, affected vision. Successful surgical intervention restored both sight and sexual function to normal (44, 82). Careful X-ray therapy gives good results in some proportion of cases (79).

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 (50).

Chromophobe adenoma is rare in childhood. Cushing has reported a case of combined craniopharyngioma and chromophobe adenoma which had been under his observation for eight years. The girl was first operated on at the age of ten, and both tumours removed. Prior to operation the basal metabolic rate was — 36 per cent.; subsequently it rose to — 24 per cent. Six years later, at second operation in 1930, more adenoma was removed. A year later the basal rate was — 19 per cent. In 1932 replacement therapy was attempted, with the growth principle. Appetite was improved and there was gain of weight, but at the end of 110 days there had been no gain in height. Treatment was stopped and the patient retrogressed.

Experimental Investigations of the Function of the Anterior Lobe

The number of different endocrine compounds formed in the anterior lobe is still a matter of opinion. While claims for additional ones still continue to appear, occasional conservative criticism suggests that even claim for the separate existence of the growth principle may be unjustified (cf. Riddle (140)). Conclusions concerning these compounds must be indirect as long as they are not obtained in pure condition. Since, apparently, they are all proteins or protein-like, and probably closely related, such purity may

be long of attainment, and the separate existence of certain of these principles may remain a matter of argument.

It is impossible in a book of this size to do justice to the tremendous amount of research that is being published in this field. Attention can only be drawn to some of the outstanding features which stress the manifold control exercised by the anterior pituitary on the general activities of the body.

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. 359). 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 held the contrary view (158, 58). It seems doubtful if these differing views are more than a difference of opinion as to the cause of death.

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. 353). He further noted that animals which survived for some time showed retardation in general and sexual development, development of adiposity, and an increased 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 (158).

Section of the stalk leads to somewhat parallel changes which are probably traceable to interference with the blood supply of the anterior pituitary (52). Smith (165) 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 (166) 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 (38) 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. 406). In immature rats the thecal cells of the ovary are transformed to "deficiency cells," evidence of a definite action of the pituitary on the ovary long before maturity (154).

Thus from deficiency of pituitary principles, produced by

extirpation, there is evidence that these principles are concerned, directly or indirectly, with growth, gonadal development, carbohydrate and fat metabolism, thyroid function, and secretion of milk.

Artificial Hyperfunction. Oral administration of anterior pituitary has no definite effect. The effects of excess of the

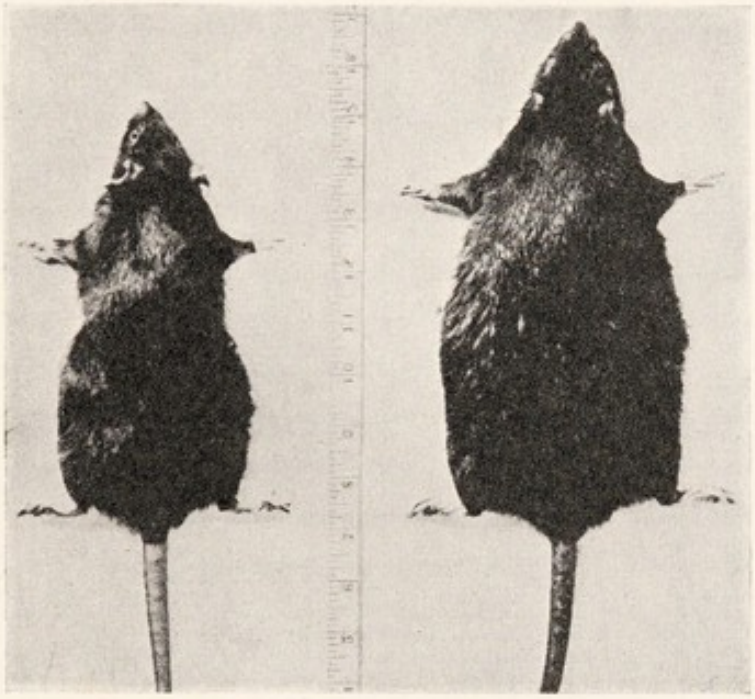

FIG. 55.—Photograph at time of autopsy (at somewhat over 400 days of age) of two female rats whose growth curves are shown in Fig. 56. The rat on the right received daily intraperitoneal injections of anterior lobe extract for over a year. The rat on the left is the untreated litter-mate control. (From Evans, *Harvey Lectures*, 1923–24, p. 212.)

pituitary principles have been studied by observing the cumulative effects of daily transplants (single transplants are without effect) and of daily injections of various extracts. Conclusions have been confirmed by correlating the results with those from replacement therapy in hypophysectomized animals. Evidence is now definite that there is control of growth and the gonads, and of the thyroid and other endocrine glands. It will be less confusing to deal with each of

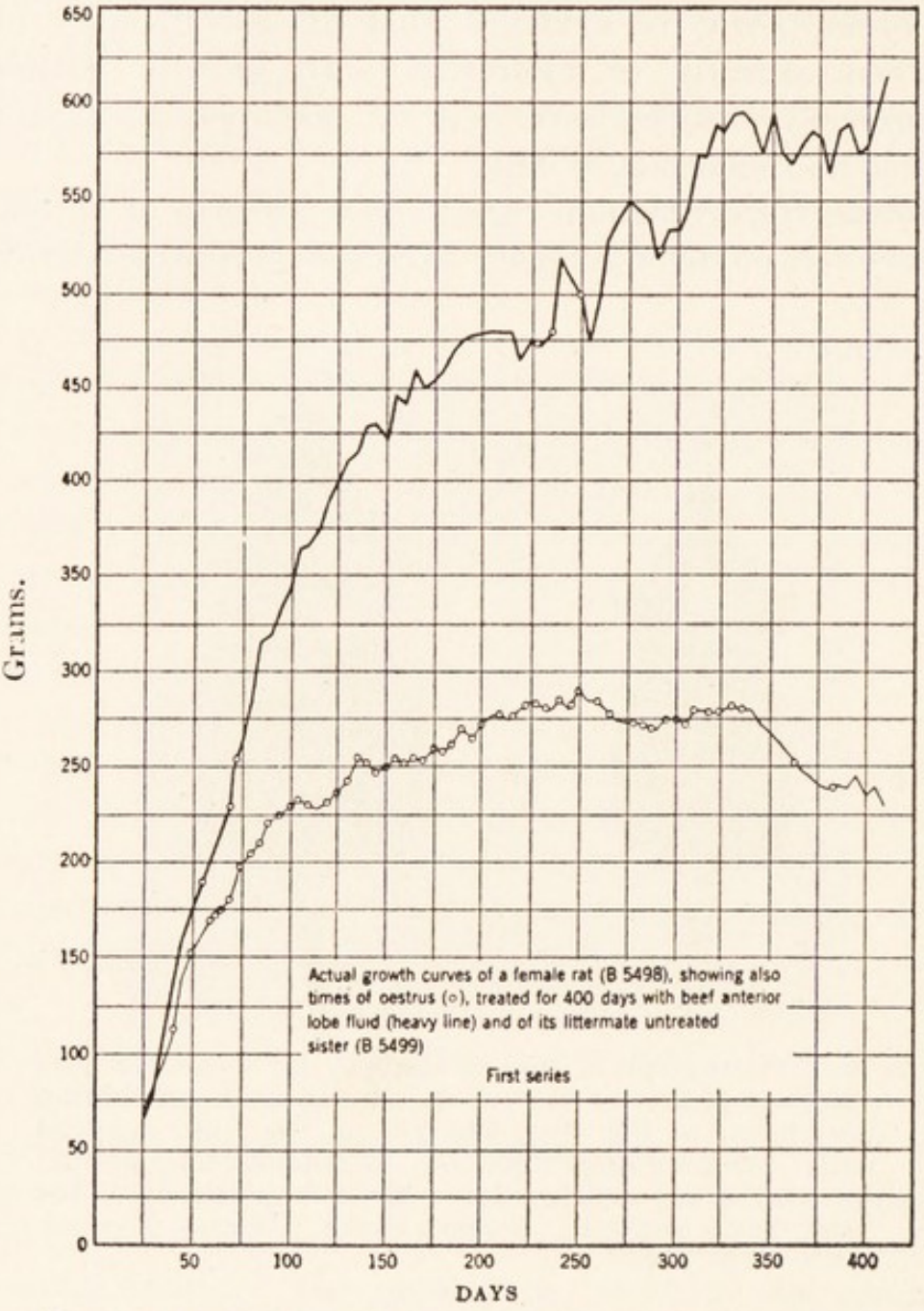

FIG. 56.—Actual growth curves of the two female rats shown in Fig. 55. That of the treated rat is given in heavy line. (From Evans, *loc. cit.*)

these separately, and they will be discussed roughly in the order of discovery.

The Growth Principle. *Evidence for its Existence.* Evans and his co-workers showed, in a series of publications commencing in 1921, that injections of potent pituitary extracts

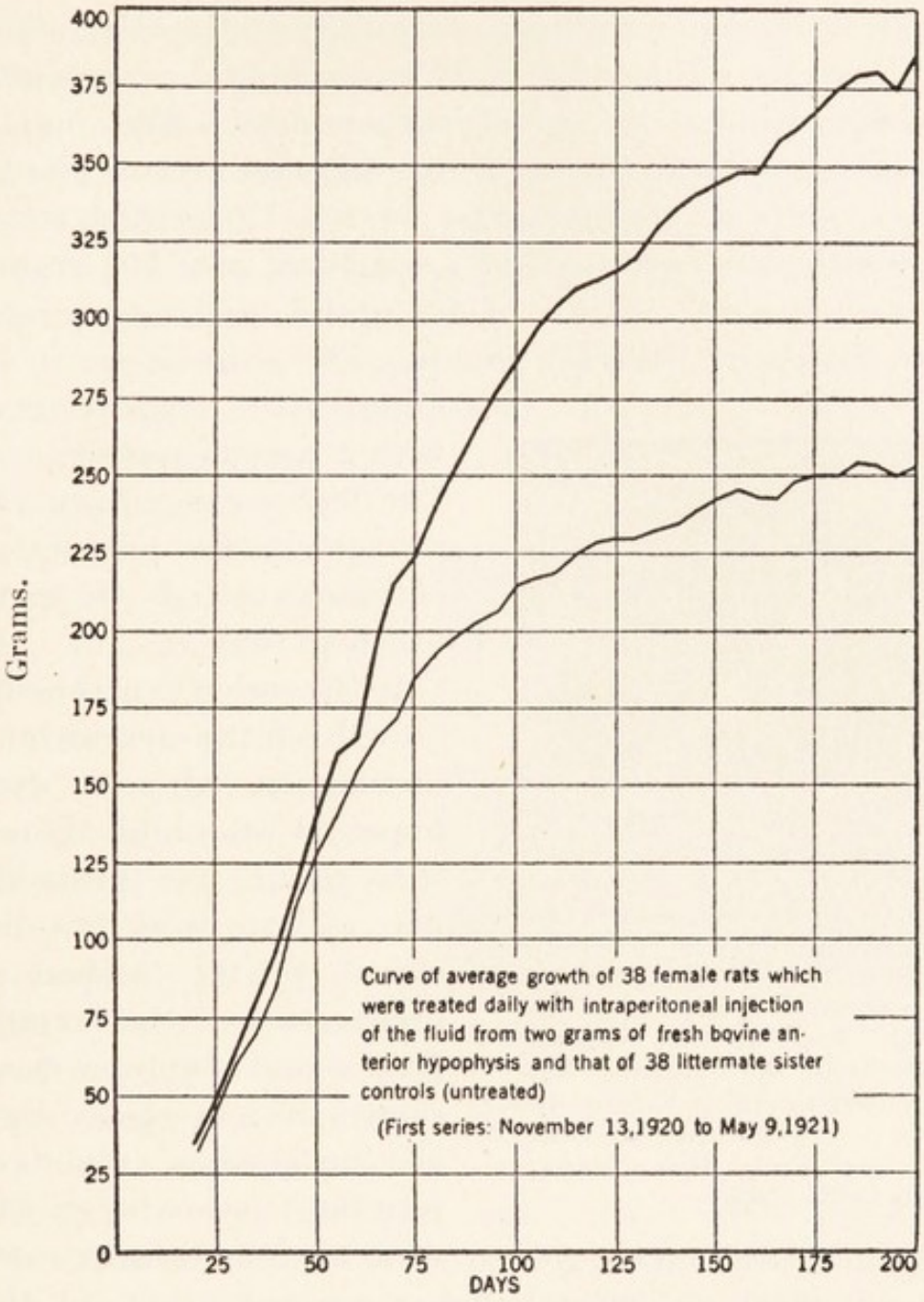

FIG. 57.—Curve (thick line) of average growth of thirty-eight female rats which were treated daily with intraperitoneal injections of the fluid from 2 grams of fresh bovine anterior pituitary, contrasted with the curve (thin line) of thirty-eight untreated litter-mate sister controls. (From Evans, *loc. cit.*)

into rats produced gigantism (62). 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. 55–57. Under such treatment female rats have reached a weight of over 700 grams, as compared with controls of 300 grams, and males, over 900 as compared with 450 grams. The animals are symmetrically proportioned, with a normal metabolism. The degree of gigantism corresponds to the production of human beings 10 to 12 feet high (63).

FIG. 58.—Effect of continued injections of the growth principle of the anterior pituitary. Litter-mate bulldogs, 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.)

In the earlier 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 (65).

Cushing, Teel, and co-workers have published a series of important studies. They used a sterile alkaline extract of beef pituitary. This contained several active principles, as is evident from their results.

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 (127). Nitrogen-

retention, and prolonged diminution of blood non-protein nitrogen, was produced in dogs (179).

An experiment on bull-dogs was carried through to the death of the experimental animal, and the details have been published in full (126, 178). It shows perfectly the gigantism, ultimately an enfeebled gigantism, produced by prolonged and marked hyperpituitarism.

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 dog from this time for fourteen months, with gradually increasing dosage. The changes in appearances are shown in Figs. 58–61.

FIG. 59.—The same two animals eight months later. Treated animal on right. (From Putnam, Benedict and Teel, *ibid.*, p. 1710.)

After three and a half months' treatment the lower jaw and skull in the experimental animal 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.

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-

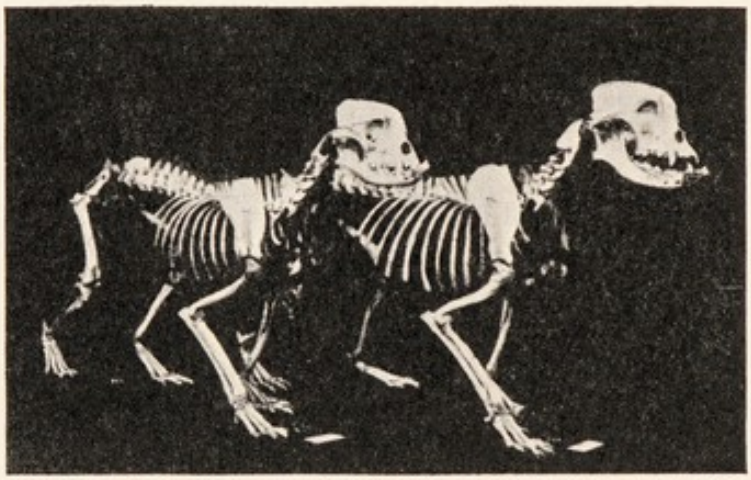

FIG. 60.—Skeletons of the treated and untreated animals at the end of the fourteen months' experiment. Treated animal on right. (From Teel and Cushing, *Endocrinology*, 1930, xiv, 158.)

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 ultimate skeletal changes are well shown in Fig. 60. 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. 61.

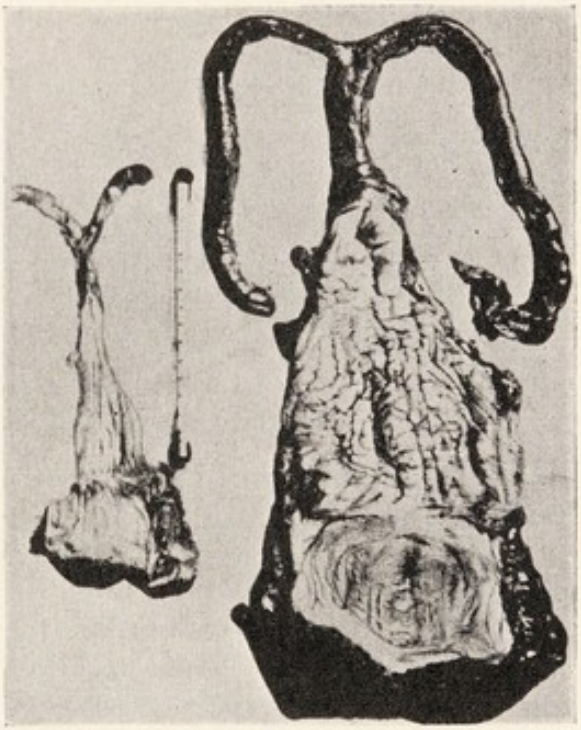

FIG. 61.—Vaginae, uteri and left ovaries of the two animals. The vaginae have been laid open by an incision along the anterior wall. Note the rugosity and thickness of the specimen from the treated animal (right). (From Putnam, Benedict and Teel, *loc. cit.*, p. 1719.)

The pituitary was the same weight as that of the control.

The alkaline extract of Cushing thus produced definite effects on growth, on the gonads, on the thyroid, and possibly on the adrenal cortex.

Evans and his coworkers (68) have pointed out that the apparent production of acromegaly in these experiments (the gigantism is definite) is open to the criticism that the bull-dog itself is normally of acromegalic type. They have themselves produced very definite results with hypophysectomized dogs. A typical experiment is pictured in Fig. 62.

A puppy bitch was hypophysectomized at eight weeks of age. Removal of the pituitary was complete, as evidenced by failure of growth during the next four weeks and by microscopic examination of the base of the skull at autopsy.

Daily intraperitoneal injections of an impure growth extract were then commenced. After 31½ weeks of these injections the hypophysectomized animal was larger than its control. (Oestrus changes occurred just prior to this time, while at autopsy the ovarian follicles showed considerable development, the uterus was somewhat enlarged, and the thyroid showed marked hyperplasia.)

Experiments with mice have shown that the growth

A. B. C.

FIG. 62.—A. Puppy bitch H-68 was hypophysectomized at eight weeks of age; H-70, litter-mate male control. B. Appearance four weeks later. Injection of growth extract commenced. C. Appearance after two months of injections. (From Evans, *et al.*, *Memoirs of the University of California*, 1933, xi.)

principle produces correctly proportional growth of the whole body, as judged by the relative weights of the chief organs, and the percentage dry weight, fat content, ash content and calcium and phosphate content of the ash (189).

Thus the studies of dwarfs, giants and acromegalics, extirpation experiments, and those of Evans and Cushing and their co-workers which have just been outlined, afford convincing evidence that the anterior pituitary elaborates a principal controlling growth.

Restoration of normal growth has been demonstrated

following pituitary implants into hypophysectomized (dwarf) tadpoles (3), and into a strain of dwarf mice (168).

The Achondroplastic Dwarf. Although achondroplasia is usually considered to be associated with the germ-plasm itself, from time to time the theory is advanced that the pituitary may be a causal factor. Evans and his associates (68) have ruled out the possibility of pituitary involvement. Among breeds of dogs the dachshund typifies this distortion of relative lengths of trunk and limbs.

They have injected daily a preparation of the growth principle,

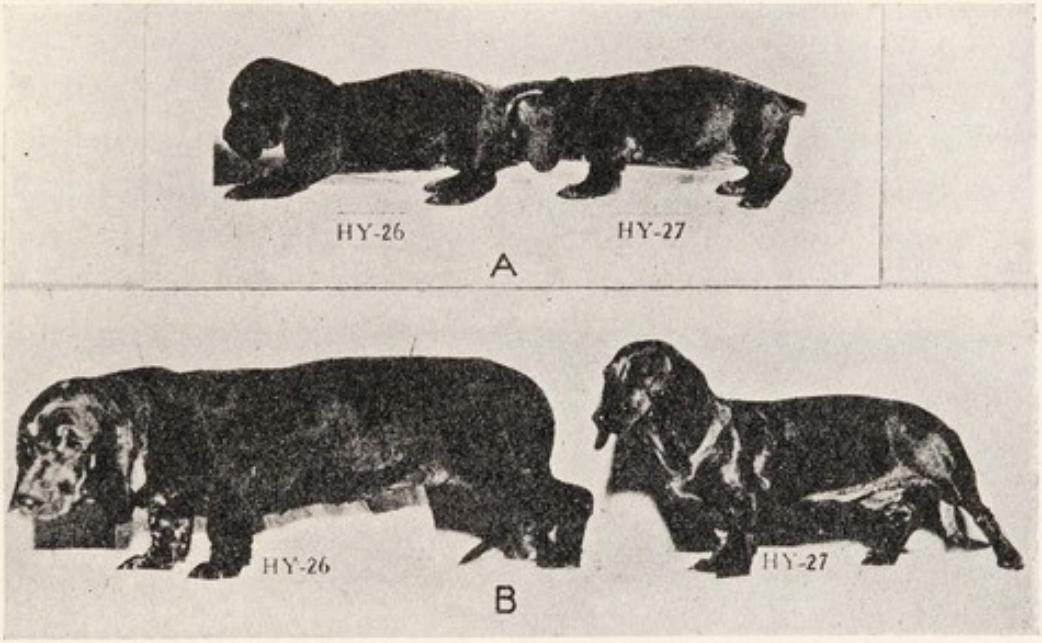

FIG. 63.—A. Litter-mate female dachshunds six weeks old. B. The same animals eleven and a half months old. HY-26 had been injected with the growth principle for thirty-five weeks. Note the elongated body, huge head, and redundant skin. (From Evans *et al.*, *Memoirs of the University of California*, 1933, xi.)

freed from the gonad-stimulating principle, into a number of dachshunds, commencing with very young animals. In each case a litter-mate served as control. The animals increased greatly in size over the controls, gigantism being definite, but still retained the achondroplastic form of the short extremities. A typical pair (control and experimental animal) is shown in Fig. 63.

Preparation and Properties of the Growth Principle. The gradual purification of the growth principle has been due

to successive progress made by Evans, Teel (working with Cushing), Van Dyke (185), Collip (39) and others (cf. 80). Probably much more work will be necessary before the compound is completely freed from other pituitary principles and obtained in pure crystalline form.

Evans' purest preparation (68) is made by extracting pituitary tissue with alkali, precipitating the extract with acetone (the precipitate also contains the gonadotropic principle) and extraction with 95 to 98 per cent. acetic acid, which destroys the gonadotropic principle but does not affect the growth principle. Acetone is added to the extract in presence of quinine sulphate, and the growth principle is thrown down, completely freed of gonadotropic principle.

Collip (39) extracts anterior pituitary with alkali, acidifies and filters. Ammonia is added to the filtrate to 1 per cent. concentration, and then calcium chloride and sodium phosphate to give a suspension of calcium phosphate, which carries down the active principle. It is extracted with very dilute alkali, the *p*H adjusted to 6·5, ammonia added to alkalinity, and the material concentrated *in vacuo* to *p*H 7·5 to 8. A semi-crystalline material separates. This represents between 0·1 and 0·2 per cent. of the original material. One milligramme or less injected twice daily in alkaline solution into completely hypophysectomized rats produces marked growth.

This product has no effect on the thyroid or on basal metabolism. Sometimes an effect on the adrenal is observed, indicating presence of a trace of the adrenotropic principle. It has no effect on the gonads, but apparently a trace of prolactin is present.

The growth principle appears to be a protein. It is easily decomposed. It is resistant to acid, but is unstable to alkali, which in higher concentrations liberates hydrogen sulphide (68). Van Dyke (185) has suggested the name *phyone* (Gk. *Phyo*, I cause to grow). There is some evidence that it is a specific stimulant of protein anabolism (149).

The Gonadotropic Principles. *Evidence for their Existence.* The retardation of sexual development and genital atrophy produced by removal of the pituitary have already been referred to (pp. 383, 384).

Zondek and Aschheim and Smith and Engle 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.

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.

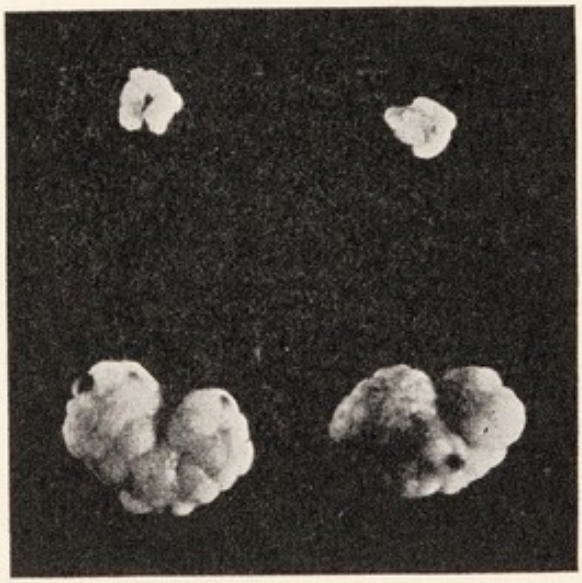

FIG. 64.—Above. Ovaries of littermate control rat. Weight, 21 mg. Below. Ovaries of experimental rat after thirty implantations of fresh rat pituitary gland over eighteen days. Weight, 340 mg. (From Collip, *Proc. California Acad. Med.*, 1930.)

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. Superovulation invariably occurs. Such results are illustrated in Figs. 64 and 65. The uterus corre-

sponds in weight to that of normal animals maturing at normal time, and structurally the uterus and vagina are typical of the adult animal.

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.

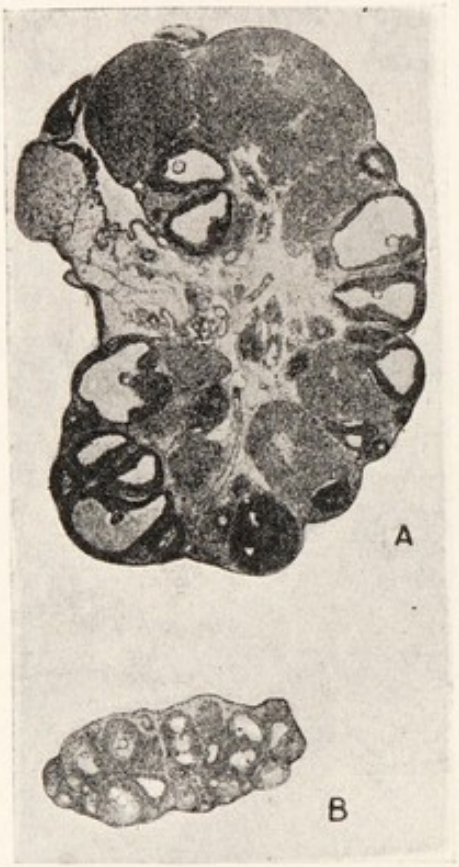

FIG. 65.—A. Cross section of ovary of rat. Sexual maturity induced on the twenty-seventh day of life, following four daily transplantations of anterior pituitary lobe of the rabbit. B. Ovary of untreated, litter-mate control. (From Smith and Engle, *Am. J. Anat.*, 1927–28, xl, 188.)

The 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 oestrus; the first pregnancy is similarly delayed. This is possibly due to reciprocal action between the ovarian and pituitary secretion concerned (60).

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 " (65).

In addition to the work just quoted, the experiments of Cushing and Teel (p. 389 and Fig. 61), and of Evans (p. 387), with impure growth preparations, afford additional proof of gonadotropic action, while Collip's and Evans' later work with purer growth principle preparations proves that the two principles are not identical.

The evidence associating the gonadotropic principles with the basophile cells is strong, but not yet final. The chief clinical evidence available is that from studies of pituitary basophilism (p. 378). Reversely, castration in man leads to marked increase in both the basophilic and chromophobe cells, with cells present in stages of transition, while vacuolization and colloid in the basophile cells suggest storage of the gonadotropic principle (19); similar changes occur in the rat (209). This is in line with the evidence just quoted that pituitaries from castrated rats and rabbits are richer than usual in gonadotropic material (cf. 169).

Zondek, using implantation methods in mice (194), finds that the principles are absent from the posterior pituitary of cattle, and are only present in slight amount in their pars intermedia. They are present in traces in the posterior pituitary of man, but absent from brain tissue surrounding the third ventricle.

The whole of the large and rather confusing recent literature relating to the gonadotropic principles has recently been critically reviewed by Smith (167) and Collip (40). They

are largely in agreement, and their conclusions must be considered as valid, as far as facts are at present available. Their reviews are fully documented, and should be referred to for this voluminous literature.

There is evidence that gonadotropic substances are present (i) in the anterior pituitary gland, (ii) in human urine and blood during pregnancy, (iii) in human urine and blood after ovariectomy and the menopause, (iv) in certain tumours of the reproductive organs, (v) in the blood serum of mares about the middle of pregnancy, and (vi) in the blood of certain lower mammals after castration.

Zondek's postulation of two different types of gonadotropic compound, Prolan A and Prolan B, must be accepted. The first type is gametokinetic or gamogenic. It stimulates male germ cells, ova, and granulosa. It produces follicular stimulation. A compound of this type is the " follicle-stimulating-hormone " (F.S.H.), a term frequently encountered in the literature.

The second type stimulates the theca-cells of the ovaries to become theca-lutein cells, and can stimulate the interstitial cells of the testes. (Collip states definitely that the sperm cells are not affected, but Smith and most students of this particular point are not in agreement.) This second type is a " luteinizing hormone."

The evidence permitting these conclusions has accumulated from several sources. What Collip terms the " maturity hormone complex " of the anterior lobe of the pituitary has been fractionated by several groups of workers (Fevold and Hisaw, Wallen-Lawrence, Evans and his colleagues) and a fraction obtained with pure type (i) action. The second fraction (type (ii) action) has not yet been completely purified from the first.

The urine of women after menopause or ovariectomy contains a type (i) compound only. The pituitary of long castrated horses yields this type alone, or at most with a trace of the other.

Preparations from such sources produce stimulation of follicles in female hypophysectomized rats, without luteinization ; in immature rats, mice and monkeys there is, following stimulation of follicles, a *delayed* luteinization due to their own pituitaries. These preparations restore to normal the seminiferous tubules of the atrophied testes of hypophysectomized male rats.

The gonadotropic fraction from the placenta or urine of pregnancy (the A-P-L principle) produces an effect practically identical with that of the luteinizing fraction of the anterior pituitary, but it produces no follicle maturation and no corpora lutea in absence of the pituitary. Thus it requires for its own action pre-stimulation by a type (i) compound. In males it produces an increase in size and number of the interstitial cells. As noted already, it is still a matter of dispute whether it has any effect on sperm cells.

The follicle-stimulating and luteinizing principles of the pituitary seem to be present in different proportions in different species of animals (104).

Evans (64) has confirmed Riddle's statement that while the testis of the immature pigeon is peculiarly sensitive to the pituitary principle, it is insensitive to A-P-L.

Collip (40) sums up conservatively : " It seems necessary at the present time to postulate two hypophyseal gonadotropic hormones, one follicle stimulating, and one that luteinizes the theca and the mature granulosa while it has no effect on the immature granulosa cells. The so-called prolan A of menopausal urine appears to consist chiefly of the former, or at least to resemble it closely, whereas the placental hormone of pregnancy urine (" prolan A " plus " prolan B " of Zondek's original terminology) is more comparable, in its biological relations, to the luteinizing fraction."

A separate principle has been postulated for the specific stimulation of ovulation. Smith considers the evidence for its existence inadequate. He also believes that it is unnecessary to assume the existence of a " synergic factor " in the pituitary,

separate from the two accepted gonadotropic principles. Such a factor had been postulated to account for an apparent enhancement of the action of the A-P-L principle by addition of low dosage of pituitary extract.

Friedgood and Pincus (211) have adduced evidence that the rate of secretion of the follicle-stimulating principle can be increased experimentally by faradic stimulation of the sympathetic fibres which innervate the anterior pituitary. Their results suggest that the cervical sympathetic nerves may at least be partially responsible, during coitus in the rabbit, for stimulating the anterior pituitary to release its gonadotropic principles in increased amounts.

According to Witschi and Pfeiffer (233) mating instinct is associated with the luteinizing principle.

Preparation of the Gonadotropic Principles. Wallen-Lawrence (190) has effected a moderately complete separation. Sheep pituitaries are dehydrated with acetone and powdered. The powder is extracted with very dilute ammonia, just acidified with acetic acid, and the fluid portion precipitated with alcohol. The precipitate is extracted with very dilute acetic acid, and the extract, after removal of inert material by neutralization, and then further by addition of alcohol to 40 per cent. concentration, is cooled to -6° C. The precipitate which forms is centrifuged off. It consists of the luteinizing factor, with a little of the follicle-stimulating factor. To the filtrate alcohol is added to 55 per cent. concentration ; the precipitate which then separates at -6° contains the follicle-stimulating but not the luteinizing factor.

The Thyrotropic Principle. *Evidence for its Existence.* Marked effect is produced on the thyroid both by induced hypopituitarism and by induced hyperpituitarism. Much of the experimental evidence has been reviewed by Thomson and Collip (180).

When the pituitary is extirpated in frog tadpoles development of the thyroid ceases (p. 384). 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 (182) or adult toads (91) provoke hyperfunction of their thyroids. The

thyroid atrophies following hypophysectomy in young rats (38).

Attention was focussed on the possible existence of a thyrotropic principle by the independent discovery by Loeb (105) and Aron (10) in 1929 that hyperplasia of the thyroid gland resembling the histological picture in Graves' disease could be produced in guinea-pigs by injections of anterior pituitary extract. This has since been confirmed by many investigators for many mammals and birds. Only a portion of this work can be mentioned, but the results are in complete agreement. Collip (40) gives a full bibliography.

Schockaert has carried out very accurate studies on the duck (152, 153). 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 (cf. 34).

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'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 (90).

The decrease in iodine content of the thyroid under thyrotropic stimulation is paralleled by decrease in thyroxine content, indicating a definite discharge of the thyroid principle (71); furthermore, the active iodine fraction of the blood is increased (76, 36, 195). According to Loeser, the thyrotropic principle even controls the taking up of iodine by the thyroid (106).

The autopsy on the giant bull-dog 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. 390).

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 (50).

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 (70) 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. (50). (Cf. also (22).)

In acromegaly, on the other hand, the rates are either normal or high (22). Cushing and Davidoff (50) 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).

Houssay and Artundo have proved that the pituitary exerts its influence on the basal metabolic rate through the thyroid, for they find removal of the pituitary lowers the basal rate, but subsequent removal of thyroid lowers it still further, while after initial thyroidectomy removal of the pituitary does not affect the basal rate (85).

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 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 (114). It has been shown by a number of investigators (170, 182, 105) 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.* (Cf. also Friedgood (210) who states that sodium iodide can temporarily inhibit the effect on the basal metabolic rate.)

Marine (114) 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.

Typical hyperplastic changes have been produced *in vitro* by suspending slices of dog's thyroid in serum saturated with oxygen at body temperature, and adding a thyrotropic concentrate (55). Hence the action is direct. This direct action is also shown by the fact that the thyrotropic principle stimulates thyroid transplants as rapidly as non-transplanted thyroid. The action must take place through the blood stream, and not through a nervous mechanism (113, 86, 95).

Administration of pituitary extracts containing the thyrotropic principle produce an increase in the basal metabolic rate of all patients with functioning thyroid tissue. No effect is produced in patients with marked myxoedema, but in those with mild myxoedema the basal rate can be raised to normal, patients with non-toxic goitre can be rendered toxic, and the condition of patients with Graves' disease is made worse by such treatment. The effect is always temporary, and the basal rate always returns to the pre-treatment value or even lower, while second courses of treatment fail to affect the basal rate (cf. Chapter X); thyroid and thyroxine are still effective in this refractory stage (229).

Preparation and Properties of the Thyrotropic Principle. Most of the experimental work with this principle has been done with fairly crude extracts prepared variously by saline suspensions or acid or alkaline extractions (cf. 80). Probably the purest preparation yet available is that of Anderson and Collip (6). They commence with the filtrate and washings from the calcium phosphate precipitate formed during the preparation of the growth principle (cf. p. 394). These are repeatedly precipitated with ammonium sulphate and the precipitate dissolved in alcohol or acetone, until finally a pure white protein-like substance is obtained. This may contain traces of the adrenotropic principle, but the growth principle is absent.

This product is readily soluble in water and dilute acids and alkali, but is insoluble in lipoid solvents, though soluble in aqueous alcohol, ether and pyridine. It is stable in

powdered form, but decomposes in aqueous solution. Statements concerning its resistance to heat vary. It does not dialyse.

It raises the basal metabolic rate of guinea-pigs and rats, and protects mice against acetonitrile poisoning. When administered to rats, it increases the excretion of calcium, corresponding to that which occurs in hyperthyroidism (cf. p. 117), while it increases the creatine output just as does the administration of thyroxine (124, 125), and depletes the liver of the guinea-pig of glycogen, another thyroid effect (56). It decreases the serum cholesterol content in rats and dogs (123). When the extract is administered to hypophysectomized rats, it prevents the atrophy of the thyroid which usually follows such operation (6).

All known thyroid effects are produced, so far as the material has been tested for them. Rowland and Parkes (147) have described a method of assay.

The Lactogenic Principle, Prolactin. The existence of this principle has been firmly established by Riddle and his collaborators, and by other groups of workers who have suggested other names, such as *galactin* (73, 117), and *mammotropin* (221).

Evidence for the Existence of the Principle. Riddle obtained, by isoelectric precipitation of an acid extract of anterior pituitary tissue, a fraction which stimulated development of the crop-gland in male, female, or castrate pigeons (142). Crude extracts of anterior pituitary produce copious lactation in virgin and in dry goats (61), in virgin heifers, and in normal bitches (110). That lactation depends on pituitary function is shown by the fact that when lactating rats are hypophysectomized lactation stops (38), while the extract is effective in producing lactation after hypophysectomy and after castration. This also holds true for bitches. Male dogs, after pre-treatment with oestrogenic principle, reacted to alkaline extracts of anterior pituitary by a copious flow of milk (219).

Preparation and Properties of Prolactin. Bates and Riddle (199) extract pituitary tissue with 60 to 70 per cent. aqueous ethyl alcohol. The alcohol content is raised in the extract, and the *p*H adjusted to 6·0. The gonadotropic, thyrotropic, and lactogenic principles are all precipitated. Separation of prolactin from the others is effected by taking advantage of its insolubility between *p*H 3 and 4, in presence of sulphates, and, further, through its solubility in aqueous alcohol. Bates and Riddle estimate that about 70 per cent. of the prolactin present in the original tissue can be thus obtained in one fraction, uncontaminated by gonadotropic or thyrotropic principle, and that it can be considered as approximately pure.

As already indicated in the previous chapter, lactation is a complex process. Development of the mammary glands to the stage of storage of their secretion seems brought about by oestrone (or oestradiol) and progesterone ; actual flow of milk is produced by prolactin, and aided by subsequent nervous mechanism set up through the act of suckling. There is some evidence that the oestrogenic compounds depress the formation of prolactin during pregnancy (226).

Prolactin is without influence on the immature mammary gland, and must follow adequate oestrogenic stimulation (212, 5, 219). A fairly large amount of it seems to be secreted daily in the milk of lactating women (221).

The physiological actions of prolactin have been extensively studied by Riddle and his colleagues (143). They have found excellent evidence associating it with four (not un-related) functions. It produces lactation in mammals, after the mammary glands have been previously stimulated by oestrogenic principles. It produces the related crop-gland response in pigeons. It represses the active mature gonads of birds, and is quite possibly the agent which represses ovulation during pregnancy and lactation in mammals. It induces " broody " behaviour—the incubation (nesting) instinct—in fowls, and is therefore probably

associated with maternal behaviour in mammalian species, including woman, and even in some reptiles, amphibians, and fishes. It has, indeed, been shown that the injection of prolactin induces definite maternal behaviour in virgin rats, as shown by the retrieving, cuddling, and protection of young in their vicinity, and by nesting (144).

Similar results have been obtained with male rats, following chronic administration of bovine pituitary implants (although the picture is somewhat confused by the statement that thyroidectomy will also induce them) (223).

These varying effects induced by prolactin are given by none of the other pituitary principles, and obviously all are associated with the care and feeding of the very young.

Prolactin produces a marked calorigenic action; the mechanism in unknown (227).

Riddle has summarized the work on prolactin to date (141) as follows :

" The hormone prolactin elicits a related series of responses though quite different tissues are involved in these responses. Despite differences in the responding tissue a unity or organization is observed in the circumstances that all responses relate to feeding or care of offspring.

" Certain hitherto unanalyzable aspects of cyclic behaviour find their more immediate explanation in the periodic release of prolactin from the animal's pituitary gland.

" It is thought that for the first time in the psychic sphere a normal development or response is found to rest upon a succession or chain of hormonal actions. In this case the series runs estrin—progestin—prolactin.

" In an otherwise fully equipped animal the birth of an instinct as a response to a hormone seems to warrant the conclusion that to this animal the hormone temporarily adds a new element of consciousness."

Claims have been made that the clinical use of prolactin gives beneficial results (98, 230). It is unlikely to have commercial application. It will increase the milk yield of

lactating cows and goats 25 to 50 per cent., but following cessation of its injection the milk yield falls to previous levels, nor will it bring back to lactation cows which have " gone dry " (209A).

The Diabetogenic Principles. Clinical evidence for the association of a deranged pituitary function with deranged carbohydrate metabolism has already been quoted (p. 190). That a specific pituitary principle existed, associated with the latter, was strongly suggested by the work of Houssay and of Geiling and their respective co-workers. Actual proof of the existence of such a principle is largely due to Houssay. The early work of these two schools demonstrated such facts as the following : Removal of the pituitary in dogs enhances the hypoglycaemic action of insulin. When the anterior pituitary is removed in the toad, subsequent pancreatectomy does not produce diabetes, but pituitary implantation can then produce it. In the dog, after removal of the whole pituitary, pancreatectomy only causes a mild diabetes with long survival. Injection of anterior pituitary extract into normal dogs induces glycosuria, hyperglycaemia and ketonuria (88, 75 ; cf. also 129, 183).

The antagonistic action between the pituitary and insulin seems to exist in most vertebrates, as it has not only been demonstrated for the dog and toad (Houssay, Geiling, Barnes (134)), for the cat (107), but also for a fish (119), for various bathrachians and a snake (90). Further work has shown that two separate principles exist, one specifically concerned with carbohydrate metabolism, the other in some way controlling ketone-body formation. Before discussing these separately, it seems desirable to describe that now classical animal, the " Houssay dog."

This dog, without pituitary and without pancreas, though it is not entirely free from the symptoms which follow pancreatectomy, may live for months without specific treatment, but it gradually becomes more and more undernourished, and finally, extremely cachectic and dies. Such

a dog has survived nine months in Collip's laboratory. The Houssay dog shows an exaggerated hyperglycaemic response to a meal, and may exhibit glycosuria, though ketonuria is reduced to a minimum. It is a very sensitive test object for the diabetogenic effects of pituitary extracts, and is also very sensitive to insulin. It can be balanced by careful combined treatment with insulin and anterior pituitary extract; the balanced dog resembles the pancreatectomized animal balanced with insulin (40) but has a higher respiratory quotient (202, 160). It can store liver glycogen (40).

The Blood Sugar Raising Principle. *Evidence for its Existence.* After loss of its pituitary an animal is very susceptible to prolonged fasting, which may even lead to a fatal hypoglycaemia, although the fed animal has a normal blood sugar. A similar dangerous hypoglycaemia has been observed by Wilder in human hypopituitary states (40). The hypoglycaemia of the acromegalic has been referred to (pp. 189, 377). Lucke found low blood sugar in a pituitary dwarf, and this sugar level was raised by injection of a pituitary extract.

Hypophysectomy decreases the liver glycogen of the toad and rabbit, while administration of an alkaline extract of the anterior pituitary inhibits this decrease (40).

Administration of glucose to the Houssay dog leads to an increase in the respiratory quotient, suggesting that the inhibition of carbohydrate oxidation in diabetes is due not only to lack of insulin, but also to lack of the pituitary principle.

The diabetogenic extract of pituitary acts in absence of pancreas, thyroid, ovary, pituitary, splanchnic nerves, lumbar sympathetic chain and adrenal medulla. Repeated injection produces in normal, or hypophysectomized, or thyroidectomized, dogs, after some days, hyperglycaemia, glycosuria, ketonuria, hyperlipaemia, hypercholesterolaemia, marked resistance to insulin and a prolonged heightened sugar tolerance curve. The effect is regarded as specific (90).

The principle is thermolabile, is slowly destroyed at 50° C., and rapidly at 80°. It is slightly soluble in 50 per cent. alcohol, and it will not dialyse (90). Doisy has shown that it is present in urine (116), and diabetic urine is a good source of it. Houssay has prepared it from human urine by adsorption on kaolin, and elution of the adsorbate with dilute alcohol ; its activity was proved on the Houssay dog (40).

When a potent extract is injected along with insulin, it lessens the insulin effect. It increases the resistance of the hypophysectomized dog and the Houssay dog to insulin.

It can be differentiated from the ketogenic principle (see below) by ultra-filtration, and it produces no thyrotropic action (108). Lucke (108) could not separate it from the growth principle, while Evans could not separate it from prolactin (61), so that it is probably identical with neither.

The mechanism of its action is not known, but there is a marked latent period following its intravenous injection. Its effect is immediate following injection into the spinal fluid, which supports Lucke's view that it acts on the nerve centres controlling carbohydrate metabolism (40).

Collip (40) has discussed the possible identity of this principle which raises blood sugar with the ketogenic principle. He is convinced of their non-identity, and points out that when fractionating anterior pituitary preparations the former is always associated with the growth principle and is separated from the latter, which is found associated with the thyrotropic principle ; there is sufficient evidence that neither is identical with the growth or thyrotropic principles themselves.

The Ketogenic Principle. *Evidence for its Existence.* Alkaline pituitary extracts increase ketonuria in rats (Burn and Ling, 1928) and ketone bodies in the blood (Hoffmann and Anselmino, 1931). Such findings led to the suggestion of a special " fat metabolism hormone," " orophysin " or " ketogenic hormone." While the authors of these names do not agree as to the single entity of the compounds they

sponsor, it seems rather unlikely that more than one exists, although one hesitates now to be too conservative about the pituitary. Collip (40) believes that the term " ketogenic hormone " more accurately indicates the action of this compound, than a term loosely suggesting control of fat metabolism. Funk in 1933 showed that it is present in urine.

Preparation and Properties. Anselmino and Hoffmann (197) desiccate fresh pituitary material with acetone, extract the dried powder with water at room temperature, and precipitate the active principle from the extract by addition of 6 to 9 volumes of alcohol. These procedures are repeated. (They obtain it from blood serum by addition of 9 volumes of 96 per cent. alcohol, and from urine by addition of 4 to 5 volumes of 99 per cent. alcohol, subsequently proceeding as with pituitary material.) The purified aqueous solution from the alcohol precipitate is submitted to ultra-filtration at *p*H 8 to 9. The blood-sugar-raising principle will also pass into an ultra-filtrate, but only in neutral or slightly acid solution, so that ultra-filtration under the conditions specified removes all the other pituitary principles, leaving only a solution of the ketogenic compound. The yield from 10 grams of acetone-dried pituitary is of the order 60 mg. The yield from 60 litres of diabetic urine is roughly equivalent.

Injection of an active preparation of the ketogenic factor greatly increases the ketone bodies in blood, especially β-hydroxybutyric acid, thus leading to an increased ketonuria. It produces its effects in thyroidectomized rats and therefore cannot be identical with the thyrotropic factor. It decreases blood lipoids, while the blood-sugar-raising principle increases them (40). It increases liver glycogen, even when thyroxine is simultaneously injected ; it depresses the basal metabolic rate and increases the specific dynamic action of protein.

Other Suggested Diabetogenic Factors. Raab has claimed that a compound " lipoitrin " exists, which increases blood lipoids.

Anselmino and Hoffmann believe in a special "pancreatropic" principle, controlling the islets of Langerhans. The evidence for these is as yet inadequate (40).

The Adrenotropic Principle.[1] *Evidence for its Existence.* It has been seen that in experimental giant animals adrenal lesions may occur, and further that in pituitary basophilism there may be adrenal cortex involvement. The close resemblance between the syndromes of pituitary basophilism and adrenal cortex tumour in itself suggests a close interrelationship.

Smith (1930) and Evans (1932) showed that marked atrophy of the adrenal cortex follows hypophysectomy in the rat, and that this can be repaired by pituitary implants or injections of pituitary extracts.

Evidence for the separate existence of an adrenotropic principle of the anterior pituitary is found in the work of Evans, Houssay, Anselmino and Collip. Evans (68) showed that certain pituitary extracts prevent or restore degenerative changes in the adrenal cortex of hypophysectomized animals, particularly in the zona fasciculata and zona reticularis. Such injections produced an increase in cell cytoplasm and partial recovery of lipoid granules. He was able to show that the gonadotropic principle was not involved, but could not exclude the growth principle.

Houssay (89) has shown that hypophysectomy produces atrophy in these two zones of the adrenal cortex, while the glomerular zone hypertrophies. The medulla is not affected in structure or adrenine content. Actual hypofunction of the adrenal cortex, as shown by symptoms, was not demonstrable. Injection of an anterior pituitary extract produced increase in weight and a total hypertrophy in the adrenals of the dog. Such action can be produced in absence of the

[1] This compound is concerned with the adrenal cortex and not with the medulla. Hence the term "adrenotropic" is too inclusive, and "interrenotropic" has been suggested instead. Since mammals do not possess an interrenal gland, this term is also open to criticism.

pituitary, thyroid and gonads, and after section of the splanchnic nerves.

Anselmino and Hoffmann (7) use as a biological test for the principle increase in size of the adrenal cortex of the castrated infantile female mouse and increase in number and size of the cell elements in the fascicular and reticular zones. By the use of this test they have shown that the principle can be separated by ultra-filtration (through 8 per cent. acetic acid collodion) from the gonadotropic, thyrotropic, parathyrotropic, growth, lactogenic and fat metabolism principles. It is present in the acid ultra-filtrate, which only contains in addition the diabetogenic principle. The substance is water soluble, but insoluble in lipoid solvents. It is precipitated from aqueous solution by excess of alcohol or acetone. Its properties suggest a relatively small molecule.

Collip (40) criticizes results obtained with animals with intact pituitaries, since he points out that adrenal cortical hypertrophy can also result from administration of numerous non-specific toxins.

Collip found that in treating hypophysectomized animals with crude thyrotropic extracts the adrenals were frequently restored to normal along with the thyroid, but as the purity of the thyrotropic preparations increased the effect on the adrenals became less. Hence he tested extracts from the alcoholic mother liquors from which most of the thyrotropic principle had been removed (cf. p. 405), and found that they had excellent adrenotropic activity. From a 75 per cent. acetone soluble fraction he obtained on concentrating in the aqueous phase at *p*H 5 to 6 a fine flocculent precipitate, which was removed, extracted with dilute ammonia, and the ammonia removed from the extract by vacuum distillation. The residue, tested on hypophysectomized rats, had no thyrotropic activity, but restored the atrophied adrenal cortex to normal in daily doses of a quarter of a milligram.

This extract has no effect on growth or on the gonads,

does not possess the A-P-L complementary factor, and Collip considers it as pure a preparation of any pituitary principle as he has been able to obtain (37).

Extracts of anterior lobe of the pituitary have been found beneficial in certain cases of Addison's disease. The effect is presumably due to the adrenotropic factor, and limited to those cases in which destruction of adrenal cortical tissue is not complete (231).

The Parathyrotropic Principle. *Evidence for its Existence.* Houssay and Sammartino (87) showed that atrophy or degenerative lesions are found in the parathyroids of the majority of dogs following extirpation of the pituitary. They also occur in depancreatized dogs, but less frequently, and less intensely. The frequency and intensity are maximum in dogs whose pituitary and pancreas have both been removed.

Anselmino and Hoffmann (9) have shown that injection of anterior pituitary extract into male rats produces enlargement of the parathyroids due to increase of the "chief" cells. Injections of such extracts into dogs and rats cause an increase in blood calcium. This increase does not occur in parathyroidectomized rats. Injection of anterior pituitary extracts increase the serum calcium in the cat and toad (157).

Hertz and Kranes (83) report that the injection of such extracts (or of pregnancy urine) into rabbits produces enlarged and more vascular parathyroids; they consider that the histological changes indicate hyperplasia.

Certain of the symptoms described in some of the cases of pituitary basophilism, such as kyphosis, lordosis, multiple fractures, vertebral pain, etc., suggest a comparison with the hyperparathyroid syndrome, and cases have even been reported as exhibiting typical osteitis fibrosa cystica (118A, 60A), along with hyperplasia of the parathyroids (83). Albright (2) has suggested that 16 per cent. of the reported cases of hyperparathyroidism exhibit parathyroid hyperplasia (rather than tumour) which may be due to undue

stimulation by the parathyrotropic principle of the pituitary (cf. Chapter III).

Clinical use of Anterior Pituitary Principles. This can only be considered as in its commencement.

Clinical trials of the concentrated extracts now available have given some good results (cf. pp. 362, 364, 367, 408, 415).

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 (127, 100, 102, 66).

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CHAPTER IX

SOME ACTUAL AND PRESUMPTIVE ENDOCRINE PRINCIPLES

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

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 (26). 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 (27). (Cf. also Cunningham (7).)

Mellanby has recently (1932) modified his procedure, omitting the use of bile salts. One kg. of fresh mucous 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 (1) 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 appears to contain no cyclic amino-acid radicals, and to behave as a base. In their latest report they claim to have obtained crystalline secretin, a polypeptide with a molecular weight of about 5,000, analysing to 46·08 per cent. C, 6·93 H, 16·39 N, and 0·68 S, with one atom of sulphur in the molecule (16).

Scott and Still (33) have found some evidence for the existence of a pro-secretin.

According to Florey and Harding (11) the secretion of Brunner's glands is under the control of secretin.

Nothing is known of any condition associated with hyper- or hypofunction of secretin.

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 (28) has 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). Brown and Smith (4) have found a histamine-like substance in the gastric juice.

Babkin has shown that injection of histamine produces selective stimulation of the parietal cells of the gastric mucosa and in addition an inhibitive action on the "vagal" phase of gastric secretion (3, 2).

Evidence is accumulating that histamine should be classed as an endocrine principle.

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 (34). (Cf. also 37, 18.)

The Insulinotropic Principle of the Duodenum. 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 (35). Laughton and Macallum prepared an extract from the duodenal mucosa freed from protein and peptone, and still showing the activity described by Heller (24). This extract, when injected into depancreatized dogs, was inactive. This suggested that the effect is produced through increased output of insulin. A more concentrated extract was made by extracting a desiccated preparation of duodenal mucosa with acid alcohol, evaporating the extract, and extracting the residue with hydrochloric acid; calcium phosphate was added to the acid extract and the solution adjusted to *p*H 7·8 to 8. The precipitated calcium phosphate carried down the active principle, and was dried and desiccated for use.

Laughton and Macallum state that this preparation has no hypoglycaemic action on normal animals, but controls experimental hyperglycaemia in them. It has no effect on the hyperglycaemia in totally depancreatized dogs, but lessens that following the administration of glucose to partially depancreatized animals.

Good results have been reported following the clinical use of this extract, given orally, in diabetes mellitus (9).

La Barre and his colleagues (23) have proceeded from the observation of Freud and Saadi-Nazin (1926) that the intraduodenal injection of dilute hydrochloric acid provoked both a

flow of pancreatic juice and a diminution of blood sugar; the latter was attributed by Zunz and La Barre to a hyperinsulinaemia (1928). La Barre has succeeded in separating the agent producing the hypoglycaemia from secretin of the duodenal mucosa by two methods, ether extraction (it passes into solution) and peptic digestion (it is scarcely affected by pepsin). He terms it *incretin*. While in normal animals it appears to act by stimulating insulin output, it is also hypoglycaemic in action when injected intravenously or fed to the completely depancreatized dog, and such animals have been kept alive for some months by this treatment.

While there are distinct differences in the reports by the Canadian and Belgian physiologists, it seems very probable that they are dealing with the same substance.

Villikinin is said to be an endocrine excitant for the intestinal villi (21).

The Haematopoietic Principle. The work of Minot and Murphy, Castle, and others, has proved that pernicious anaemia is a deficiency disease. Castle postulated an intrinsic factor from the gastric mucosa, and an extrinsic factor from the diet, which interacted during gastric digestion to give the active haematopoietic principle. The recent work of Greenspon (14) suggests that the effect attributed to the extrinsic factor is in reality an adsorption of pepsin to protein during digestion, whereby digestion of the protein-like intrinsic factor is prevented. Ungley and Moffett (36) do not support this view, It is still uncertain whether the haematopoietic principle should be considered as endocrine in character, though reaching its site of action by the unusual route of the gastric secretion and absorption and passage to the liver, where, possibly, it undergoes some change before storage and subsequent transfer to the bone-marrow for functional use.

Dakin and West (8) have obtained and examined a very pure and potent liver extract. It is slightly and slowly digested by pepsin, more completely by erepsin. They believe it to be an anhydride type of glucosamine peptide. It contains lysine, arginine, glycine, leucine, hydroxyproline, and aspartic acid radicals, no phosphorus, and no sulphur.

Other Suggested Principles

Haberlandt's "Heart-hormone." Haberlandt has published numerous papers (12), in which he claims that a specific "heart-hormone" exists, which will stimulate the non-beating (frog's) heart to movement. Oppenheimer (29) finds that the active substance in such experiments is not specific.

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* (6, 13). Kraut and Frey's extract is termed by them *kallikrein* (22), while Santenoise has termed his preparation *vagotonine* (32, 12); the term *padutin* is also used for this substance. *Carotidin*, from the carotid gland, may be similar (5).

A Liver Detoxicant. Claims have been made for a specific detoxicant in the liver, *yakriton* (33).

"Plant Hormones." Specific substances exist in plants, with hormone-like action, in that they are translocated from the cells which form them to other parts of the plant to produce their actions. They control plant-growth, and have been termed "auxins." They are probably of simple composition.

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CHAPTER X

ENDOCRINE INTERRELATIONSHIPS

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. Accurate knowledge will depend ultimately on studies of the effects of administration of one or more *pure* endocrine compounds, combined with the effects of surgical removal of one or more endocrine glands, without damage, or with controlled damage to other structures.

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. While Cushing's dictum (6) that "all pituitary syndromes are essentially polyglandular" perhaps conveys too limited an impression of pituitary activity, increasing knowledge suggests that the activities of all the other endocrine glands may be governed by that of the pituitary, and may also react upon it in their turn. The present facts concerning these interrelationships have been given in Chapter VIII. Evidence was presented there that the pituitary controls or helps to control, through secretion of its specific compounds, the thyroid, the ovaries and testes, the adrenal cortex, the islets of Langerhans (by antagonism), and the parathyroids, while still others of its principles exercise some degree of control over general growth, carbohydrate metabolism, fat metabolism, milk secretion and maternal behaviour and the watershifts of the organism. It will be sufficient here to recall the most important features of the endocrine interrelationships.

The Anterior Pituitary and the Thyroid. In Chapter VIII

it has been shown that a specific compound, so far termed the *thyrotropic principle* or *hormone* of the pituitary, stimulates the thyroid to activity, and, pathologically, to over-activity. If, through any cause, this principle is secreted in too great an amount, then hyperthyroidism is produced, at least transiently, while in absence or insufficiency of the principle hypothyroidism is a consequence.

The relationship between pituitary and thyroid is not entirely one-sided. 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 (28). Man and other animals with endemic goitre or cretinism have enlarged pituitaries. Marine has shown that changes in the pituitaries of rabbits in whom parenchymatous goitres have been produced by cabbage diet or by methyl cyanide are practically identical with those following thyroidectomy. There is an increase in size of all glandular cells, and partial or complete disappearance of stainable granules in the acidophile cells. These changes are prevented by administration of thyroid or iodine to the goitrous rabbit, and of thyroid, but not iodine, to the thyroidectomized rabbit, so that the effect on the pituitary must be due to lack of the thyroid principle. Marine considers that the thyroid secretion affects the pituitary as strikingly as the thyrotropic principle affects the thyroid (56).

When thyroid is fed to normal rats the pituitaries become subnormal in weight. There is said to be a slight increase in the percentage of basophile cells, and a definite increase in their size and granular content, accompanied by a change in staining properties. The changes are most marked in animals in which suppression of the oestrous cycle is most evident, suggesting a triangular complexity (33).

It is very doubtful if observations of this nature are sufficient to justify certain differentiations such as Engelbach (7), 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. 416), 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 Anterior Pituitary and the Gonads. The relationship has been discussed fully in Chapter VIII. It will be recalled that the gonadotropic principles stimulate the ovaries to maturation and formation of corpora lutea, and the concomitant production of oestradiol, and, in the male, stimulates the testes to mature growth, and elaboration of testosterone. It has also been pointed out that excess production of the ovarian principle appears to depress the corresponding pituitary function, and that this view is supported by such experiments as the injection of oestrone into immature male animals, when, presumably through depressed pituitary function, the male genitalia remain infantile (cf. p. 314).

The Anterior Pituitary and the Adrenal Cortex. There is definite evidence that the adrenal cortex is directly under the control of the adrenotropic principle of the pituitary (Chapter VIII). The close interrelationship is also exemplified by the almost complete identity of the syndromes associated with adrenal cortical tumours and pituitary basophilism (Chapters V and VIII).

The Anterior Pituitary and the Parathyroids. Some one of the pituitary principles exerts a measure of control on the parathyroids, so that excess of it leads to increased output of the parathyroid principle and depression of blood calcium (cf. Chapter VIII). It has recently been suggested that renal rickets is primarily due to pituitary mal-function, with possible parathyroid involvement (36 ; cf., however, 59).

The Anterior Pituitary, the Islets of Langerhans, and the Adrenal Cortex

In Chapter VIII evidence was presented that the pituitary secretes a compound, the blood-sugar-raising principle, which directly antagonizes the action of insulin on carbohydrate metabolism, and some account was given of the Houssay dog, which, minus both pituitary and pancreas, can live for a fairly long period without treatment, in a state of very mild diabetes. There is as yet no definite evidence that the pituitary directly controls the production of insulin by the islet tissue.

The balanced pituitary-islet control of carbohydrate metabolism, suggested by these facts, only represents one part of the endocrine control of that metabolism. Both adrenine and cortin play a part, the former directly, in regulating glycogen shifts between liver and muscle and *vice versâ*, the latter in a manner which is still undetermined (*vide* Chapter V). Moreover, there is definite evidence that —probably indirectly—increase of insulin in the blood stimulates and is followed by increase of adrenine also, and

there is some further evidence that adrenine also stimulates the secretion of insulin (49, 35, 42), so that in considering insulin effects on metabolism it is necessary to consider in how far adrenine has intervened in these effects.

That extirpation of the adrenals impedes the appearance of hyperglycaemia following pancreatectomy has been recognized by numerous investigators, since Zuelzer's observations in 1907 (cf. Leloir (50) and Long and Lukens (53)). Hartman and Brownell (45) noted that experimental diabetes is less marked in animals suffering from adrenal insufficiency, including cortin insufficiency. Long (52) has found that the adrenalectomized depancreatized cat with barely adequate cortin to maintain the adrenal deficiency will remain alive for periods up to twenty-eight days, definitely longer than the survival period following pancreatectomy in the cat. It presents a picture of mild diabetes very similar to that of the Houssay animal, but hypoglycaemic incidents are common. Injection of anterior pituitary extracts is without effect on this animal. Long suggests that the ketogenic activity of the anterior pituitary may be mediated through the adrenal cortex. He has maintained a similar dog for five weeks with cortin, and with a lowered insulin requirement. Lukens and Long removed adrenals, pituitary and pancreas from two cats, which survived for fifteen and twenty-one days respectively, in a condition similar to that of the Houssay dog (54). In all these cases cortin was administered, so that it seems difficult to associate lack of cortin with the results. Long suggests that either insufficient cortin was given to produce a total diabetic response, or else that his cortical extracts did not contain another unknown compound of the adrenal cortex, specifically concerned with carbohydrate metabolism.

In toads Houssay has shown that the diabetogenic action of the pituitary is independent of the adrenals, since, after removal of pituitary, adrenals, and pancreas, injection of the anterior pituitary of the toad leads to diabetic hyper-

glycaemia. Somewhat similar results have been obtained with dogs that were only partially adrenalectomized (46).

The hypophysectomized animal responds as usual to induced insulin hypoglycaemia by liberation of adrenine into the blood, but this (and also injected adrenine) fails to mobilize liver glycogen and thus to restore blood sugar to normal level. Injection of a suitable anterior pituitary extract not only increases the resistance of the animal to insulin, but also its response to adrenine. These results suggest that the mechanism of pituitary action in this respect is associated with the rendering of liver and muscle glycogen susceptible to action of adrenine. Thyroid action is excluded from these interactions, since thyroidectomy and thyroid administration do not affect them (38, 30). On the other hand there seems to be no interference in the hypophysectomized animal's ability to store glycogen in liver and muscles (34). Such results seem to show some parallelism with the abnormalities in v. Gierke's disease, suggesting that there may be a pituitary involvement in that condition.

It is obvious that much further work will be necessary to unravel these complexities.

Adrenal Interrelationships

The relation between the pituitary and adrenal cortex has been dealt with.

The Adrenal Cortex and the Gonads. The depressed gonadal function in experimental adrenalectomy and in Addison's disease indicates that the adrenal cortex exercises some control over the gonads. The virilism and hirsutism frequently accompanying functioning tumours of the adrenal cortex afford supporting evidence, although, as has been pointed out in Chapter V, conclusive evidence is still lacking that hypergonadism or that precocious puberty can be induced by injections of cortical extracts into normal animals.

The Adrenal Cortex and the Thyroid. As has been pointed out in Chapter II, it has been suggested 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 obtained moderately good results from administration of adrenal cortex to patients with Graves' disease, while, though the evidence is not quite definite, use of potent adrenal cortical extracts has apparently proved to be of benefit in some cases (cf. Chapter V). Cortin lessens the effect of thyroxine on nitrogen metabolism.

The Adrenal Medulla and the Thyroid. Thyroidectomy lessen the response of the cat's denervated heart to adrenine ; administration of thyroxine restores the normal response, whilst if sufficient thyroxine is given to raise the basal metabolic rate above normal, a still greater response is given to adrenine. It seems probable that the interrelationship indicated is not a direct one, but that the effects found are due to an altered degree of sensitization of the structures on which adrenine acts, rather than to a changed output of the adrenal medulla. The results support the use of thyroidectomy in treatment of the embarrassed heart, as in angina pectoris (cf. p. 97), because the diminution of response to various stimuli necessitates the use of less oxygen, and the heart muscle has a greater opportunity to recover (55).

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 (13), 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.

Although, when the combination exists, hyperthyroidism usually precedes diabetes, Bruger (31) has reported a case in which diabetes definitely preceded the hyperthyroidism by three years; the latter accentuated the diabetic condition to such an extent that a fasting blood sugar of 1·5 per cent. was attained, while very large doses of insulin were needed for control.

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 (22). Administration of thyroid is often beneficial in such cases with a low basal metabolic rate but no specific hypothyroid symptoms (44).

There is a little experimental evidence. Injections of oestrone into rats, rabbits and dogs lead to changes in the thyroid in the nature of a colloid goitre or suggesting hypofunction (2, 15).

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. 13). 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.

The Parathyroids and the Pancreas

Pancreatectomy lowers the blood calcium and augments blood phosphate, suggesting a depressed parathyroid function. The result is not affected by subsequent hypophysectomy (41). Definite lesions of the parathyroids are produced, quite different from those following hypophysectomy (47).

Other Interrelationships

The control of general growth, of lactation, and of fat metabolism by different compounds secreted by the anterior pituitary has been referred to already. Such control is probably direct, and not through some other endocrine tissue, so that it scarcely comes within the scope of this chapter.

It seems possible that relationships exist between certain endocrine compounds and some of the vitamins. One such possibility, a suggested control of the parathyroids by calciferol (vitamin D), has been discussed in Chapter II.

It has recently been suggested that there is a relationship between chronic adrenal insufficiency and pellagra; if this is correct, it would follow that a deficiency of vitamin B_2 (G) is involved in the former condition (20).

It may well be of significance that the two chief storehouses of ascorbic acid (vitamin C) in the tissues are the anterior pituitary and the adrenal cortex, although its presence in large amounts in these tissues may merely indicate that it is required for the

formation of their specific compounds by two glands which function at a very high level, since this vitamin is a powerful oxidative catalyst.

There is some evidence that the endocrine system plays an important *rôle* in determining individual susceptibility to allergy phases (61).

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 (29) and by Joslin and Lahey (14).

Wilder found 15 true diabetics 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. 437). 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 (29, 22).

Rowe and Lawrence (23) 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 all of the remaining four exhibited a combined pituitary-thyroid dysfunction, in which in light of present knowledge, inter-

relationship cannot be considered as excluded, their results illustrate the great rarity of true pluriglandular conditions.

Rowe has summed up the matter still more recently (22): "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."

Anti-endocrine Compounds

An Anti-thyroid Compound. Early attempts to treat hyperthyroid conditions by therapeutic measures included the use of serum of thyroidectomized animals (Ballet and Eniquez; Möbius), or their milk (Lanz), or their meat (Sorgo), or the serum of myxoedematous patients (Bunghart and Blumenthal), or the "immune serum" from goats fed on thyroid preparations (Lépine). Treatment of this nature, at first acclaimed, was later viewed more critically, and such measures fell into disrepute (cf. Falta-Meyers (9)), although "anti-thyreoidin Möbius" seems to have been used for a long period.

Such therapy seems based on an underlying idea of the presence in blood or tissues of a substance antagonistic to the thyroid principle, even though the different investigators named seem to have had quite contradictory views as to the mechanism whereby the amount of it might be increased. Recent work suggests that this underlying idea is perhaps a sound one.

It is rather difficult to understand why removal of the thyroid should enhance the serum content of this antagonist. Yet Oberdisse and Thaddia (19) state that anti-thyreoidin Möbius, given orally to normal rats, lowers their gaseous metabolism, and that it produces the same result in rats

artificially rendered hyperthyroid, while administration of the dry powder of normal sheep or horse serum seems to have little or no action. The latter statement differs from the claims now to be set forth.

If it be accepted that thyroid action in itself tends to set up a counterbalancing action through production of some specific antagonist, then we may well expect to find the latter in blood and tissues of normal and still more of hyperthyroid animals. Such a theory seems to be the basis of Blum's work. (It is perhaps to be remembered in this connection that Blum is practically the lone upholder of the thyroid detoxication theory.)

Romeis (21) suggested that a specific protective compound exists, and Blum found evidence that it is decreased in Graves' disease (4), while according to Eufinger (8) it is also decreased in pregnancy and eclampsia. Romeis found that if he added blood along with thyroxine to water containing tadpoles, the well-known acceleration of metamorphosis and retardation of growth produced by thyroxine did not occur. Blum (4) has employed a dried-blood preparation, " Hämokrinin," in treatment of Graves' disease, and has prepared a concentrate " Katechin," sold under the name of " Tyronorman." Herzfeld (11) claims to have obtained good results with this preparation in sixteen of eighteen cases of Graves' disease. The basal metabolic rate falls, the pulse slows, and the body weight increases. The subjective symptoms improve and the exophthalmos lessens, but the size of the goitre is scarcely affected. The improvement generally commences in the second week of treatment, but treatment must be continuous if the benefit is to be maintained. In such treatment marked dietary restrictions are enforced. It is difficult to understand such dietary stringency if the treatment is specific. Cf. also (32). Zondek appears to doubt the usefulness of " Tyronorman " (63).

Blum describes his " catechin " as a water-soluble, heat-resistant compound, which is practically non-dialysable. He

thinks it is formed in the liver and passed into the blood. Anselmino and Hoffmann (1), estimating the amount of material necessary to neutralize the effect of a definite dose of thyroxine on the rat, find that foetal blood is richer than normal human blood, and the latter richer than blood from pregnant women and patients with Graves' disease, and than the blood of cattle, sheep and swine. Beef marrow and ovary and the human foetal liver are especially good sources.

Perhaps bearing on the above is the observation of Morgan and Ivy (18) that a condition simulating cretinism could be produced in young rabbits, following injections with an antiserum from hens injected with a saline *in vivo* extract of rabbits' thyroids.

Anti-pituitary Compounds. It seems to have been observed several years ago that when large doses of pituitary extracts are given for somewhat long periods the very definite effects on the thyroid become quantitatively less—the thyroid regresses to normal size, the basal metabolic rate ceases to rise, and then falls, and the blood iodine content lessens (Korenshevsky (16), Loeb (17), Verzahr (27), etc.). Various explanations of this definite result have been put forward (cf. Friedgood (10), Thurston (26)), all tending to suggest the setting up of an immunity through some protective mechanism.

Collip and Anderson have carried out the most complete studies so far published (5).

When the purified thyrotropic principle is injected into rats (normal or hypophysectomized) or guinea-pigs, there is (cf. Chapter VIII) an increase in the basal metabolic rate, but it returns to normal level within two or three weeks. If the injections are continued, the basal rate continues to fall, and may even reach the low level attained in the hypophysectomized rat. (In eleven normal rats the rate averaged — 29 per cent. at the end of the thirty-seventh day of injection.) Marked increase of dosage does not then cause any rise in the rate, nor does increasing the thyroid volume

through transplants. Since such transplants are ineffective, the phenomenon cannot be attributed to exhaustion of the thyroid by the treatment.

The serum from such rats, injected along with the thyrotropic principle into hypophysectomized rats (which are especially sensitive to the latter), inhibits its action. Saline extracts of liver and spleen from such " immunized " rats also have this inhibitory action, but normal rat or horse serum does not possess it. The active serum is even effective in preventing the action of the thyrotropic principle on normal rats (cf. also 23A).

The " immunized " rats still have a hyperplastic thyroid, and still respond normally to feeding with small doses of desiccated thyroid, or injections of thyroxine, so that the agency causing the cessation of response to the pituitary principle is distinct from Blum's preparation. It is not anti-thyroid, but is anti-thyrotropic.

Four weeks' daily injections of thyrotropic principle into a fifteen-year-old mare produced a serum which definitely inhibited the action of the principle in the hypophysectomized or the normal rat. This serum also itself depresses the basal metabolic rate of normal animals.

Hence there is definite evidence of an anti-thyrotropic compound produced in the organism to prevent the action of excess of the thyrotropic principle, and present in blood.

The solubility properties of this compound resemble those of insulin, and it can be concentrated by making an extract of blood serum with 66 per cent. acetone and raising the acetone concentration to 92 per cent., when the " anti " compound is precipitated. Boiling an acid solution for three minutes decomposes it.

Collip concludes from his results that the compound is not formed in the pituitary gland.

He and his co-workers (24) have also obtained evidence that chronic overdosage with the follicle-stimulating principle of the pituitary and also with the A-P-L compound of urine

produces similar cessation of their effect (although in each case the animal remains sensitive to the other gonadotropic principle, illustrating their essential difference (cf. Chapter VIII). Cf. also (62). Riddle reports that pigeons become refractory to prolactin (57).

In a recent review (37) Collip has summarized the further work in his laboratory : Definite evidence has been obtained of the existence of an anti-ketogenic principle. Long-continued treatment of rats with pituitary extracts rich in the ketogenic factor practically abolished in them the acetonuria of fasting. Also normal animals pre-treated with serum from resistant animals manifested no appreciable ketonuria when fasted or injected with a ketogenic extract. Resistant rats developed practically no ketosis from massive doses of phloridzin, although the usual glycosuria was produced ; control animals had marked ketosis, and those given higher doses died.

Bearing upon the whole problem are Collip's observations that the blood serum of certain patients contains the anti-follicle-stimulating factor, while that of others (and also their urine) contain an anti-ketogenic factor ; there is some evidence that the serum of myxoedematous patients may contain an excess of anti-thyrotropic factor. Scowen and Spence (58) find that normal rabbit and human sera have a slight antithyrotropic action, but that this is not shown by serum from patients with Graves' disease.

Neither in Collip's laboratory nor elsewhere has it so far been possible to demonstrate the existence of compounds antagonistic to oestrone, to the parathyroid principle, and to insulin.

A number of investigators have considered that these "anti" reactions are really of immunological, of antigenantibody character (*e.g.*, 39, 51, 60, 40, 38A, 61A). Collip writes (37) : "We do not believe that this latter possibility affords an adequate explanation for the anti-hormone responses observed, for the following reasons : (i) The studies of Dr.

Carl Bachman on anti-A-P-L serum have shown that the anti-hormone effect does not parallel the anti-body content. (ii) Rats have been made resistant to the maturity hormone of rat pituitary by continued implantation of rat pituitary. (iii) Rats made resistant to the ketogenic principle by a long period of daily injections of an extract made from ox anterior lobes have been shown by Mr. Peter Black to be equally resistant to the ketogenic extract made from sheep or pig anterior lobes. (iv) There is spontaneous occurrence in the serum of certain individuals of a substance capable of inhibiting an anterior lobe principle. Thus positive inhibition of the maturity principle, of the thyrotropic principle, and of the ketogenic principle has been observed."

There is no definite evidence as to the site of formation of these " anti " compounds. However, removal of the pituitary or the thyroid does not prevent formation of the anti-thyrotropic substance, and anti-A-P-L sera have been produced in castrated rats and rabbits. The " target organ," in Collip's phrase, does not appear to be the site. Different investigators have suggested, on rather slight grounds, that organs such as the pineal or the adrenals may be the source of certain of these compounds.

The benefits claimed for the therapeutic use of an anti-thyroid preparation have been mentioned ; they cannot be considered as completely substantiated. It seems not unlikely that more definite results may be achieved with the anti-pituitary preparations, especially if it be true for example that there is an excess of anti-thyrotropic substance in the blood of myxoedematous patients and an absence of it from that of cases of Graves' disease.

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 the degree of dilution of the blood and its concentration of electrolytes, especially sodium and chlorine; (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) carbohydrate metabolism, acting as an antagonist to insulin; (v) the parathyroids and, through them, calcium and phosphorus metabolism; (vi) lactation and maternal behaviour; (vii) general growth of all tissues; (viii) fat metabolism; and (ix) the water exchanges of the body; even this list is incomplete.

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.

Another illustration of unsuspected relationships is available from an interesting study by Billings (3), which indicates that voluntary activity in woman is greatest in the post-menstrual period, when the blood content of oestrone is lowest, and decreases slowly as the latter increases; whether or not such relationship is due to a direct action of oestrone on the smooth musculature, as Billings suggests, it

indicates the way in which the actions of daily life may be affected by varying endocrine activity.

The existence of specific anti-endocrine compounds, produced apparently in order to check undue endocrine activity, indicates both a hitherto unsuspected system of balanced controls, and at the same time unsuspected possibilities of variations due to different degrees of production of these " anti-compounds " in normal individuals.

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 (12) has presented a conservative statement of possibilities in this direction.

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