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**RECENT ADVANCES IN  
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# RECENT ADVANCES IN ENDOCRINOLOGY

By

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FOURTH EDITION

With 67 Figures, including Three Plates



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MY FRIENDS  
THE MEDICAL PROFESSION  
OF WINNIPEG



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## PREFACE TO THE FOURTH EDITION

IN the three and a half years which have elapsed since the publication of the third edition the volume of papers dealing with endocrine work has increased rather than slackened. A second important journal in English devoted solely to endocrinology has recently commenced publication. Numerous valuable monographs have appeared, dealing with particular phases of the subject; of these I have endeavoured to take full advantage. The valuable sections on hormones in the yearly volumes of the *Annual Review of Biochemistry*, and the new *Annual Review of Physiology* have been of great service in directing attention to the chief papers in an overwhelming mass of literature.

In this edition the chapter dealing with the gonads has been very largely rewritten, and an account of the endocrine control of reproduction can now be presented in completer form than can any other section of the subject. The chapter dealing with the pituitary has also been considerably revised, and, for the first time, it is possible to report considered arguments in favour of a lesser, rather than of a greater number of hormones of that important gland.

Each of the other chapters has been revised to greater or lesser extent. New advances of importance now reported are the preparation of synthetic iodoproteins with thyroid activity, the proved beneficial use of dihydrotachysterol in treatment of hypoparathyroidism, a clarification of clinical hyperparathyroidism, the broadening of the conception of diabetes mellitus, the isolation of the hormones of the adrenal cortex, with the determination of their chemical nature, the demonstration that glutathione produces the peculiar growth effects attributed to thymus preparations, and the therapeutic use of implants of crystalline tablets of insoluble hormones.

Several new figures have been added, for which due acknowledgment has been made in the legends attached to them. I am much indebted to Dr. John Parks of Washington, D.C., for further information concerning the case shown in Fig. 41. I am also greatly indebted to Professors D. L. Thomson and J. B. Collip for their kindness in allowing me to read their review of "Hormones" for the 1940 *Annual Review of Physiology*, prior to its publication.

I wish once more to acknowledge the continued courtesy and co-operation of Messrs. J. & A. Churchill Ltd.



## 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.

A. T. CAMERON.

WINNIPEG, 1933.

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# RECENT ADVANCES IN ENDOCRINOLOGY

## CHAPTER I INTRODUCTION

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

The history of endocrinology as an exact branch of science scarcely antedates the present century; the name itself is still younger. Until chemical studies progressed sufficiently to result in isolation of several of the "internal secretions," and to 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 term *hormone* (from Gk. *hormōn*, rousing or setting in motion), originally proposed by Bayliss and Starling, has been universally adopted for endocrine compounds, though in its



original sense it scarcely applies to all of them. Other suggested terms as *chalone* and *autacoid* are no longer used. *Endocrinology* (Gk. *endon*, within ; *krinein*, to separate) is generally accepted as the name of that branch of science which concerns itself with the hormones, while the glands concerned are *endocrine glands*, and their "internal secretions" are *endocrine secretions*.

The terminology applied to the hormones themselves is gradually becoming simplified and more definite, as these compounds are gradually being obtained in pure crystalline form. There is still too great a tendency to coin new names to avoid those employed by the larger pharmaceutical companies. Many of these firms are now employing the scientific names, as well as those they use for patent purposes. The physician is, therefore, more easily able to apply his own knowledge to the selection of his therapy.

Hormone production is associated with the thyroid, parathyroid, pituitary, and adrenal glands, the islet tissue of the pancreas, the mucous membrane of the upper part of the intestine, and the gonads. The thymus and pineal probably produce hormones. Various claims have been made for others, to which short reference will be made.

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.

Replacement treatment by grafts has proved to be only of transient benefit. Claims have been made that suitable cultures of endocrine glands give better results when engrafted (4), but such procedures are obviously tedious.

A useful addition to methods of treatment is the implantation of crystal pellets (suitably sterilized) of potent hormones. A single implant can produce a desired effect for weeks. The method has proved of service with the hormones of the gonads and the adrenal cortex. The essence of the method consists in selecting material of very slight solubility, so that tablets of insulin, fairly soluble, are useless for the purpose (2), though the injection of insoluble protamine zinc insulin achieves the same purpose in much lesser degree.

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, 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 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.

Precision of dosage of preparations from the anterior pituitary is and will remain difficult, till complete separation of the hormones of that gland has been achieved; at present all such preparations are mixtures of several hormones in uncertain amounts.

Pratt, some years ago, published a thoughtful paper on this subject of dosage in endocrine disorders (3). He pointed 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 compounds. He laid 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."

Complete knowledge of the chemical constitution of certain hormones has led to attempts to improve upon nature. Oestradiol was produced in the laboratory and shown to be more potent than the natural oestrone, but, later, oestradiol was proved to be the true hormone of the ovary. Desoxycorticosterone was a laboratory product before it was isolated from the adrenal cortex. In those two cases nature was ahead of man's efforts. More recently Dodds and his collaborators have found synthetic oestrogens unrelated chemically to those produced in the ovaries (cf. Chapter VII) and this type of research holds promise.

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 hormones 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 hormones it is necessary to know something of the other glands which they control, and the hormones which these secrete. It is at present easier to deal with these other glands and their hormones 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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## CHAPTER II

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#### 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 chapter VIII.



### 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 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



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

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."

Rienhoff (294), from an accurate study, including injection experiments, and a reconstruction of the thyroid gland (cf. Fig. 1), concludes that the lymphatic system of the gland is a closed system, playing no rôle in the transmission of its hormone.

Jackson (170) has studied 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, has drawn 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 stressed 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 made 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 physiologically hyperplastic active glands are the rule. 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."<sup>1</sup>

Baillif (372) has presented evidence, from the study of thyroids of rats subjected to the stimulus of cold, that the interfollicular epithelial cells (characterized by a cytoplasm filled with lipide granules) are capable of producing new follicles whenever stimuli activate the gland.

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 been mentioned (cf. p. 8). The physiological changes in size of the gland, brought about by seasonal changes in temperature evoking increased or decreased heat production (cf. p. 30), are accompanied by histological changes. Somewhat similar alterations occur in the thyroids of women during the menstrual cycle.

<sup>1</sup> Hoar, in a recent study of the thyroid gland of the Atlantic salmon, has shown that in the smolt stage it presents a hyperplastic appearance, and that later it changes to a state resembling that of colloid goitre (390A).



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

TABLE I  
*Distribution of Iodine in Nature*

Material.	Iodine Content.	Authority.
Rocks (Europe)—	$\gamma$	
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 <i>et al.</i>
“ “ (N.Z.) . . . . .	0.0–0.2	Hercus, Benson, Carter.
Mineral waters (Switzerland) . . . . .	0.12–63.0	v. Fellenberg.
Atmosphere ( <i>per cubic metre</i> ) . . . . .	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 <i>et al.</i> (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 <i>et al.</i> (214).



connection with the storage of iodine. The limits in the amount found in (desiccated) thyroid are 0.01 and 1.16 per cent. . . . Other tissues in mammals contain less than 0.001 per cent." (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). Numerous modifications have been suggested in recent years, but cannot be dealt with here.

The table on p. 11, 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 materials unless otherwise stated.

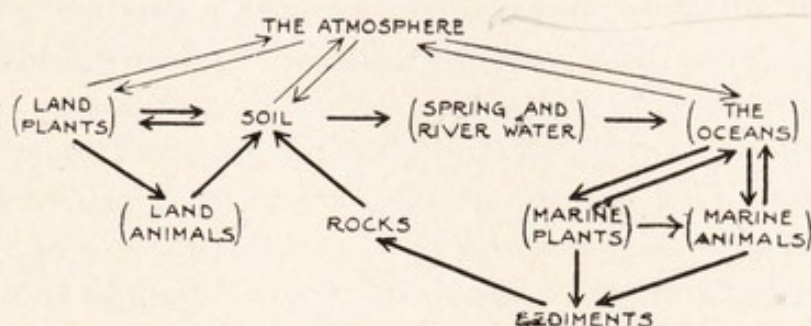
According to v. Fellenberg the iodine content of soils is much higher than that of rocks which, by weathering, have produced these soils (224). He concludes that the soil receives iodine from water percolating through it, such (rain) water obtaining its iodine from the atmosphere. He has shown that the 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).

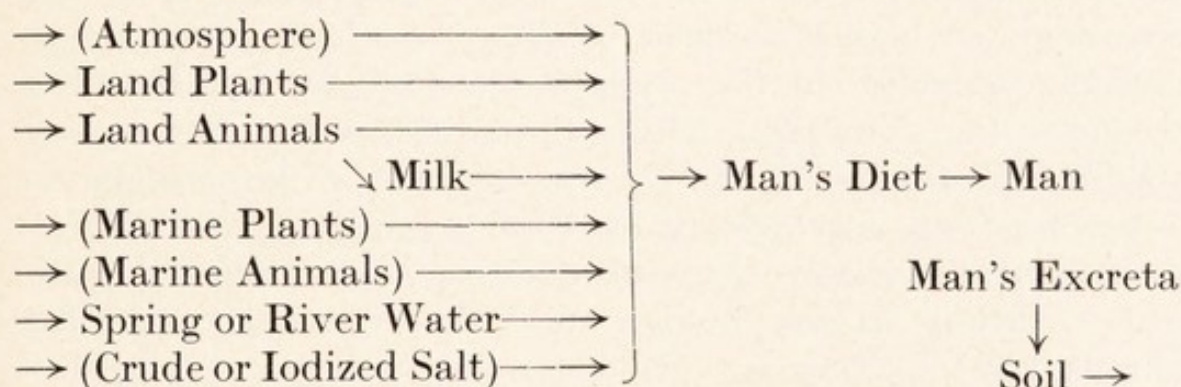


In it the main channels of iodine movement are represented by thicker lines.



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 altered 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:—



(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.<sup>1</sup>

**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 <sup>2</sup>**

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 unlikely.

<sup>1</sup> The human thyroid gland in Iceland is unusually small, and, correspondingly, is unusually rich in iodine, averaging 0.083 per cent. in fresh tissue. The high iodine-intake from a diet rich in iodine is presumably the cause (411).

<sup>2</sup> Blanchard, Péneau and Simonnet (27), and Harington (140), have published monographs which give a full account of the chemical and pharmaco-dynamical properties of these compounds.



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

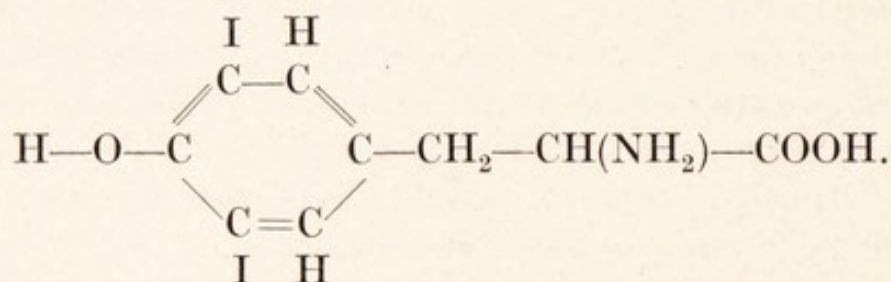
Pure thyroglobulin is a white amorphous powder. Its composition appears to be constant, except for an iodine content varying from 0 to 1.7 per cent. Successive extractions of the same thyroid material yield preparations with diminishing iodine content, suggesting that the thyroglobulin present is a mixture 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 dogs' 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.

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

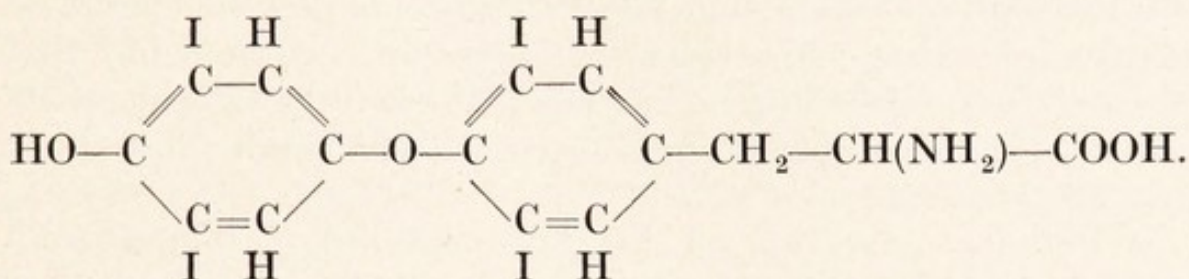
Various early attempts to isolate diiodotyrosine from the products of hydrolysed thyroid gland material failed. Harington recently succeeded in doing so (142), while Foster has obtained it from hydrolysed thyroglobulin (107), and Harington has obtained it in the optically active dextro-form by enzymic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

**Thyroxine** was isolated in crystalline form by Kendall on December 25th, 1914 (179). 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<sup>1</sup> :—



Prepared from thyroid tissue, or synthetized, it is optically inactive. Harington resolved it into its active dextro- and laevo-components (138); laevo-thyroxine possesses 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 containing somewhat over 30 per cent. of iodine. This was not affected by erepsin; it proved to be a mixture of some free thyroxine and a larger amount in combination as a tri- or tetra-peptide. The latter is strongly resistant to enzymic hydrolysis.

Crystalline laevo-thyroxine melts at 235° C. with decomposition. Its specific rotation (5 per cent. concentration in 2 : 1 alcohol-*N* NaOH) is  $[\alpha]_{5461} = -3.8^\circ$ . (Cf. also 384.)

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.

<sup>1</sup> 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).



**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); in adult human thyroids it averages 29 per cent. (199) and in those of new-born infants 20 per cent. (401).

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). 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.



Cavett (63, 62) has studied the (Van Slyke) nitrogen distribution, and the tyrosine and tryptophane radical contents in normal and goitrous human thyroglobulin. His results indicate that with the exception of tyrosine, thyroxine, and diiodotyrosine the amino-acid radical distribution in the thyroglobulin molecule is the same, whether it is obtained from a normal, colloid, or adenomatous gland, or a gland from a patient with Graves' disease. His data seem to indicate that tyrosine radicals, present at certain points in the protein molecule, are capable of conversion to diiodotyrosine and thyroxine. This process is interfered with in goitrous glands, especially as regards formation of thyroxine radicals.

Thyroglobulin from colloid glands of untreated patients is very deficient in radicals of the iodine-containing amino-acids, while its content of tyrosine radicals is proportionately greater. After treatment of cases of toxic adenoma and of Graves' disease with Lugol's solution the diiodotyrosine radical content is increased even to amounts above normal, that of thyroxine radicals is less affected, and the content of tyrosine radicals is proportionately decreased.

### Synthetic Iodo-Proteins and Thyroid Activity

When iodine is allowed to react with solutions of such proteins as egg-white and casein, it combines with the protein molecule. Oswald, by hydrolysing such iodised proteins, obtained amounts of diiodotyrosine of the same order as from gorgonin and spongin (cf. p. 16), but Ingvaldsen's experiments on determining recovered diiodotyrosine from addition of known amounts to protein material suggest that all such figures are too low (167).

Since the discovery of thyroxine a number of attempts have been made to obtain, from hydrolysates of iodized proteins, fractions exhibiting the biological activity of thyroid. Abelin's work (5) from 1933 onwards has seemed to suggest that certain fractions do indeed exhibit such activity.

The experimental indication that thyroid activity parallels total-iodine rather than thyroxine-iodine (p. 24), and Salter and Pearson's work stress the importance of such experiments. Salter and Pearson (304, cf. 409) digested human thyroglobulin with pepsin, removed the thyroxine fraction, and could find



no thyroid-activity in the residual diiodotyrosine-peptone. Solution of this was concentrated and subjected to peptic synthesis, following Wasteney's procedure. An artificial protein was produced, containing iodine, and possessing properties akin to those of thyroglobulin. It yielded an iodine-containing fraction resembling thyroxine, and it relieved myxoedema as readily as thyroglobulin does.

Foster, Palmer and Leland (384) consider that synthesis of protein was not truly effected in these experiments. However, some confirmation is afforded by the important recent work of Ludwig and von Mutzenbecher (395). They have also been able to effect a peptic synthesis on peptic hydrolysates of thyroglobulin and of iodized casein, the resulting protein exhibiting thyroid activity. Moreover, as will be seen, they can prepare active material from diiodotyrosine itself.

Ludwig and von Mutzenbecher have shown that when proteins such as casein are allowed to take up iodine at low temperatures to the saturation point (100 gm. of casein will unite with some 50 gm. of iodine) the iodized protein shows little or no thyroid activity. But when such experiments are carried out under conditions simulating those present in mammalian tissues ( $37^{\circ}$  C., and a *pH* between 7 and 9 afforded by bicarbonate solution), and the iodization is not allowed to proceed to completion, compounds are produced exhibiting marked thyroid activity (as shown by effect on basal metabolism, and by loss of body weight when administered to guinea-pigs). Moreover, from hydrolysates of these compounds both thyroxine and diiodotyrosine can be isolated with ease, although enzymic hydrolysis has, in their hands, so far not permitted the isolation of an optically active thyroxine.

They have obtained such results not only with casein, but also with plasma proteins, silk-fibroin, and edestin, and indeed it would appear that any protein containing an appreciable number of tyrosine radicals should thus be able to form thyroxine radicals.

(In addition to thyroxine and diiodotyrosine they have isolated from the digests mono-iodo-tyrosine, a compound not previously known, and have shown that it is easily convertible to tyrosine and to diiodotyrosine. Its preparation from thyroglobulin has not yet been demonstrated.)



The authors state that their results indicate that at low temperatures iodization leads only to the formation of mono- and diiodotyrosine radicals, but at higher temperatures oxidation processes are involved, with ether-formation, and so thyroxine radicals are produced.

They find, further, that when diiodotyrosine is dissolved in one equivalent of 0.1*N* sodium hydroxide, and kept at 37° for one or two weeks, then diluted with 9 volumes of water, and sulphuric acid added to weak acidity, fine brown flecks separate. These are dissolved in 2*N* sodium hydroxide, and the solution is extracted with butyl alcohol. From the extract, by appropriate treatment, crystalline thyroxine has been obtained.

Harington and Rivers have already confirmed the important aspects of this research (389). They have been able to obtain 100 mg. of crystalline thyroxine from 100 gm. of iodized casein. The mechanism is not enzymic; boiled casein solutions also yield thyroxine.

These illuminating experiments still leave a number of problems unsettled. How do cold-blooded animals produce thyroxine radicals? Why, if conditions simulating those in mammalian tissues are so favourable to thyroxine formation, is this formation limited to thyroid tissue in vertebrates? (Of course, in these experiments elementary iodine is used, while the thyroid handles iodide.) It is also to be noted that the yields of thyroxine from hydrolysates of artificial iodoproteins are much less than those from thyroglobulin.

### **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 of 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 twice as potent as racemic thyroxine obtained from thyroid by hydrolysis with alkali. Pure *l*-thyroxine is, according to Gaddum (113), less active than the mixture of thyroxine and thyroxine-peptide which Harington obtained by enzymic digestion of thyroglobulin, though Foster states (384) that the calorogenic activity of thyroid is quantitatively accounted for by the thyroxine radicals it contains. Thyronine (cf. p. 17) 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).<sup>1</sup>

<sup>1</sup> 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.<sup>1</sup>

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

<sup>1</sup> Escamillo (383) has recently reviewed the literature; he concludes from his own experiments with hyperplastic and other human thyroids, that the reaction cannot be used as an aid to diagnosis of thyroid function.



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 iodinated toxic goitres, and the thyroxine-peptide from either, all have essentially equivalent activity in terms of iodine (cf. Palmer and Leland (271) ).

*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. 80). 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. (Cf. also p. 27.)

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 is necessary to test this theory, and it must still be emphasized that the exact nature of the thyroid hormone has yet to be determined.

**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), Severinghaus (309), Uhlenhuth (416), and others, 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 thyrotrophic 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 intracellular 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 intracellular 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.

Baillif (372) has also given a clear account of secretion in the rat's thyroid.

McClendon (227), from direct observation of thyroid tissue in the ultracentrifuge and the centrifuge microscope, and from histological preparations of the tissue fixed immediately after removal from the centrifuge, considers that thyroglobulin can pass between the cells of the follicle under stimulus of the thyrotrophic hormone of the pituitary (cf. p. 383), or under some mechanical force. But even if thyroglobulin can so reach the circulation no explanation is available as to how a hormone with a molecular weight of 700,000 could pass across an animal membrane into the cell in which it produces its effect.

Not an  
insuper-  
differe

Thyroid, cultivated *in vitro* in the Carrel-Lindbergh apparatus secretes iodine, chiefly in non-protein combination, into the perfusion fluid, under stimulation of the thyrotrophic hormone (377, 372A).

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 hormone 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 hormone 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

simply decreased tissue differentiation



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 ( $33^{\circ}$  as compared with  $25^{\circ}$ ), 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. 23) 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 melanin formation, and is definitely traceable to the thyroid hormone.

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. Mansfield (236) has summarized 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.<sup>1</sup> 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. Apparently 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 appears to be 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

<sup>1</sup> Haarman (387) is in general agreement, but finds that the optimal concentration lies between  $10^{-14}$  and  $10^{-16}$ .



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.

Mansfeld has 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 states 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. This suggests that thyroxine can only reach the cells of an undamaged organ if its innervation is intact and suggested to Mansfeld that thyroxine is taken up by the nervous system and transferred to tissue cells through the peripheral nerves. In support of this view Mansfeld states that 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  $16^{\circ}$  to  $18^{\circ}$  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. 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.

These later experiments of Mansfeld, and the conclusions he bases on them, cannot be accepted until strong confirmatory evidence becomes available. Actually, other recent work is adverse to his views. Oberdisse and Roda (400), in careful experiments, cannot find that kidney denervation affects the thyroxine action on kidney tissue. Without new supporting work Mansfeld's intriguing theory of the carriage of the thyroid hormone by nerve paths is unacceptable.

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 hormone) increases their consumption of oxygen, and thus their heat production. There is 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.

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 have been 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. 83 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 parasympathetic fibres from the superior laryngeal branch of the vagus. Ross and Moorhouse (407) have confirmed and extended these findings for the dog. They find that in most dogs the origins of the thyroid nerve are those stated by Nonidez, but that frequently the vagus fibres arise from the ganglion nodosum, or the vago-sympathetic trunk, instead of the superior laryngeal branch. The thyroid nerve terminates in branches which enter the gland with branches of the superior thyroid artery.

Stimulation of the thyroid nerve consistently slows the flow of blood through the gland. Histological changes following continued nerve stimulation in acute experiments could not be demonstrated. Their results support the view that *there exists an important indirect control of the thyroid gland through nervous regulation of its blood supply* and thus of the output of hormone into the general circulation.

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 hormone in the tissue cells (296).

*There is definite evidence that the thyroid secretion is under the control of one of the hormones 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  $\pm 15$  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.<sup>1</sup>

**Standards.** The normal standards of Aub and DuBois for heat production per square metre of body surface have been 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 formulae based upon pulse-rate and pulse-pressure. While some degree of relationship exists, the potential error is too great to give that

<sup>1</sup> Macias (396) has shown, and his results have been confirmed by Perrill and Jones (403) that, provided the oxygen content of the inspired air is not allowed to fall below 10 per cent., atmospheric air can be employed in basal metabolism machines and accurate results obtained; special adjustments may be necessary to increase the volume capacity of the instrument under such conditions, and, where oxygen is easily obtainable, its use is obviously preferable.



certainly 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). Altitudes up to 8,720 feet appear to have no influence on basal metabolism (390 B).

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), but there seems good evidence that, as far as basal metabolism is concerned, they retain their racial characteristics even when born and brought up under different climatic conditions (373). 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). According to Heinbecker Eskimos in the Baffin Bay district gave values averaging 33 per cent. higher than the standards (145), but Levine (393) found that 18 out of 23 Eskimos examined under ideal conditions gave normal rates bearing no relationship to race or climate. 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).)

Campos (376) has recently published (in English) a valuable and critical account of determinations of the basal metabolism of different races in tropical and subtropical countries ; his paper includes a full bibliography.

Under-nutrition can markedly affect the basal rate. Moderate under-nutrition does not produce an appreciable effect, but 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).

According to Rubenstein (408) during the menstrual cycle there is a regular fluctuation within  $\pm 5$  per cent. of the mean, the lowest values occurring just before the mid-period, and the highest a few days before menstruation.

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). Hanna (388) states that to the end of the fifth lunar month values remain within high normal levels ; at the ninth lunar month figures of from +14 to +16 per cent. are obtained, and on the ninth day post-partum basal metabolism is again normal.

**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, cardiac disease, 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 when man is injected with pure human thyroglobulin an addition to the daily dose of 0·1 mg. 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 <sup>1</sup>:

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

<sup>1</sup> For an example of a fully differentiated classification, see Joll (174).



(which is generally considered a hyperthyroid state) appear to exhibit no hyperthyroidism. Again, some malignant tumours of the thyroid are associated with hyperthyroidism.

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

### Inflammatory Conditions of the Thyroid

In the rare instances when the thyroid is influenced by the toxins of acute infections the colloid may diminish or disappear, the cells lining the follicles may degenerate or desquamate, and there may develop increased vascularity and hyperplastic changes in the epithelium. The latter may become sufficiently conspicuous to resemble those seen in certain stages of Graves' disease. The basal metabolic rate may be increased. Administration of iodine lessens the effect (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 has been suggested 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 (241) 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. 59). 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. 11). 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

TABLE II

*Iodine Content of Human Urine in Non-Goitrous and Goitrous Districts*

Non-Goitrous Districts.					Goitrous Districts.				
District.	Goitrous Children.	No. of Urines N.G.	Urine-Iodine.		District.	Goitrous Children.	No. of Urines.	Urine-Iodine.	
			Ex-tremes.	Mean.				Ex-tremes.	Mean.
	Per cent.		γ	γ		Per cent.		γ	γ
<i>Switzerland</i> Effingen	1.0	7	28-108	64	<i>Switzerland.</i> Kaisten	62	11 (10 G.)	4-29	19
<i>Italy.</i> Ligurian Coast	0	3	94-140	112	Hunzenschwil	56	12 (10 G.)	5-28	17
<i>Norway.</i> Vik i Sogn	0	6	107-240	173	<i>Norway.</i> Hostvedt	60	4	26-69	40
					Vittingfoss	55	8	14-105	48
					Ljöterud	54	5	19-41	29
					Meheia	54	5	10-62	39
<i>Samoa.</i>	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.

of goitre have been made for goitrous and non-goitrous districts in Switzerland (105), Argentina (244), Hungary (33), and China (8). There is excellent agreement between these surveys ; the inverse relationship definitely exists. Japan is practically non-goitrous. The iodine consumption 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. In 1925 there was 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. 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, iodide 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 in 1934 only 1 per cent. of the school children had goitre, as compared with 35 per cent. in 1924 (228).

Keith (178) reported in 1924 the development of goitre in a whole community and its farm stock in Pemberton Valley, British Columbia, and the rapid abolition of the goitre in animals and man by administration of iodine. Evvard (99) has reported similar good effects with farm animals (cf. also 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. 49.)

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. Crotti, the outstanding proponent of a fungus as causative agent of goitre, has given a detailed account of his work in his monograph (80).

McCarrison studied the incidence of goitre in a large rat colony in India (216) and traced its occurrence to an ill-balanced diet, and especially to deficiency of vitamin A. Most of the goitres were lymph-adenoid in type, and were not benefited by administration of iodine.

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 (cf. p. 50). 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 15 $\gamma$  of iodine per kilogram, providing rats with only 0.14 $\gamma$  per day, produced goitre in 35 days or less. Such goitrous glands showed marked hyperplasia, lack of colloid and low iodine content. The minimum amount of iodine which definitely prevented goitre in these rats was 1 to 2 $\gamma$  per day. (Cf. also 406.)

Sharpless (410) has produced hyperplastic goitre in rats on a diet containing 75 per cent. raw soy bean flour and 60 to 80 $\gamma$  of iodine per kilogram, a reasonably large amount. Additional iodide prevented the goitre.

Excellent controlled experiments have been carried out by Palton (402), who found that a synthetic ration containing 0.145 mg. of iodine per kilogram produced marked (hyperplastic) goitre in chicks within twelve weeks, while when the iodine content was raised to 5 mg. per kilogram no goitre occurred.

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 Goitrogenic 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. The work has been chiefly carried out from 1928 onwards by Webster and Chesney in Baltimore and Marine in New York, and their respective co-workers (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 goitrogenic 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 goitrogenic activity. In the Eastern United States goitrogenic 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 goitrogenic properties, varying with season. New Zealand cabbage is goitrogenic, but to a less degree (149).

Brussels sprouts and cauliflower are goitrogenic, but various other members of the Brassica group of vegetables are not (356). Rats seem to be more resistant than rabbits to such agencies (168), but Blum has succeeded in producing goitre in both rats and guinea-pigs on a cabbage diet (375). He has also observed that the young from female rabbits fed cabbage during pregnancy are goitrous. His paper contains some excellent illustrations. Soy bean and ground nut are goitrogenic to rats (209, cf. 410).

Cabbage dried *in vacuo* or in air, or extracted with ether or acetone, loses its goitrogenic 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 iodide fed. These experiments with such hyperplastic glands are believed to throw light upon the causation of so-called "iodine-Basedow" (see p. 73).

**The Causes of Endemic Goitre.** At present it is not possible to state the etiology of endemic goitre accurately and precisely, but it seems justifiable to conclude that it can arise from more than one cause. The possible causes include lack of iodine, too great a calcium content in the diet, an unbalanced diet (with possible association with a deficiency of vitamin A), some goitrogenic 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 goitrogenic 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).

Ucko (415) has marshalled the evidence against the theory of iodine insufficiency. The British Committee on iodine deficiency and thyroid disease (379), from studies of the relationship of the iodine contents of water, milk, and pasture to the occurrence of endemic goitre in two English districts, confirm the relationship of environmental deficiency of iodine to incidence of goitre, but consider that there is no conclusive proof that this is the sole cause. Wahlberg (417) in a recently published study of goitre in Finland, considers that both relative deficiency of iodine and excess of calcium play rôles, which are, however, probably more aggravating than causal. He thinks that other



important factors are low standard of living and familial disposition, while other factors may still be unknown.

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.** The amount of iodide added to salt has varied in different countries from 1 part of sodium or potassium iodide to from 5,000 to 200,000 parts of salt. Some idea of the optimal amount is given by studies of the iodine content of foods in goitrous and non-goitrous districts (105, 151), and from comparisons of urine-iodine content in such districts.

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 micrograms 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 hyperthyroidism. 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.<sup>1</sup>

**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. 45).

### The Hypothyroid State

Little advance has been made in recent years in the study of hyperthyroidism, 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

<sup>1</sup> Read has studied the incidence of Graves' disease throughout the United States. His findings indicate its presence in regions where endemic goitre is unknown, suggesting that there is no common etiology to the two conditions (405).



thyroid restores myxoedematous individuals to comparatively normal physical and mental health, so long as that administration is maintained, and improves and may completely restore the normal physical condition of cretins, although its effect on their mentality depends upon commencement of treatment at a very early age.

**Myxoedema.** Murray, who was the first to administer thyroid to myxoedematous patients (in 1891), made a final report on the first case in 1920 (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.

If the conclusions of McCarrison (cf. p. 50) and of Williamson and Pearse (p. 42) 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 necessary before their views can be accepted.

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



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.)



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 widely different pictures presented by myxoedematous patients are well indicated by the series of 5 cases reported by Ravin (404).

The importance of bone-age studies in diagnosis of childhood myxoedema has been stressed by Lissner, 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). Implantation of thyroxine pellets does not control myxoedema, since absorption is insufficiently rapid (385A).

**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

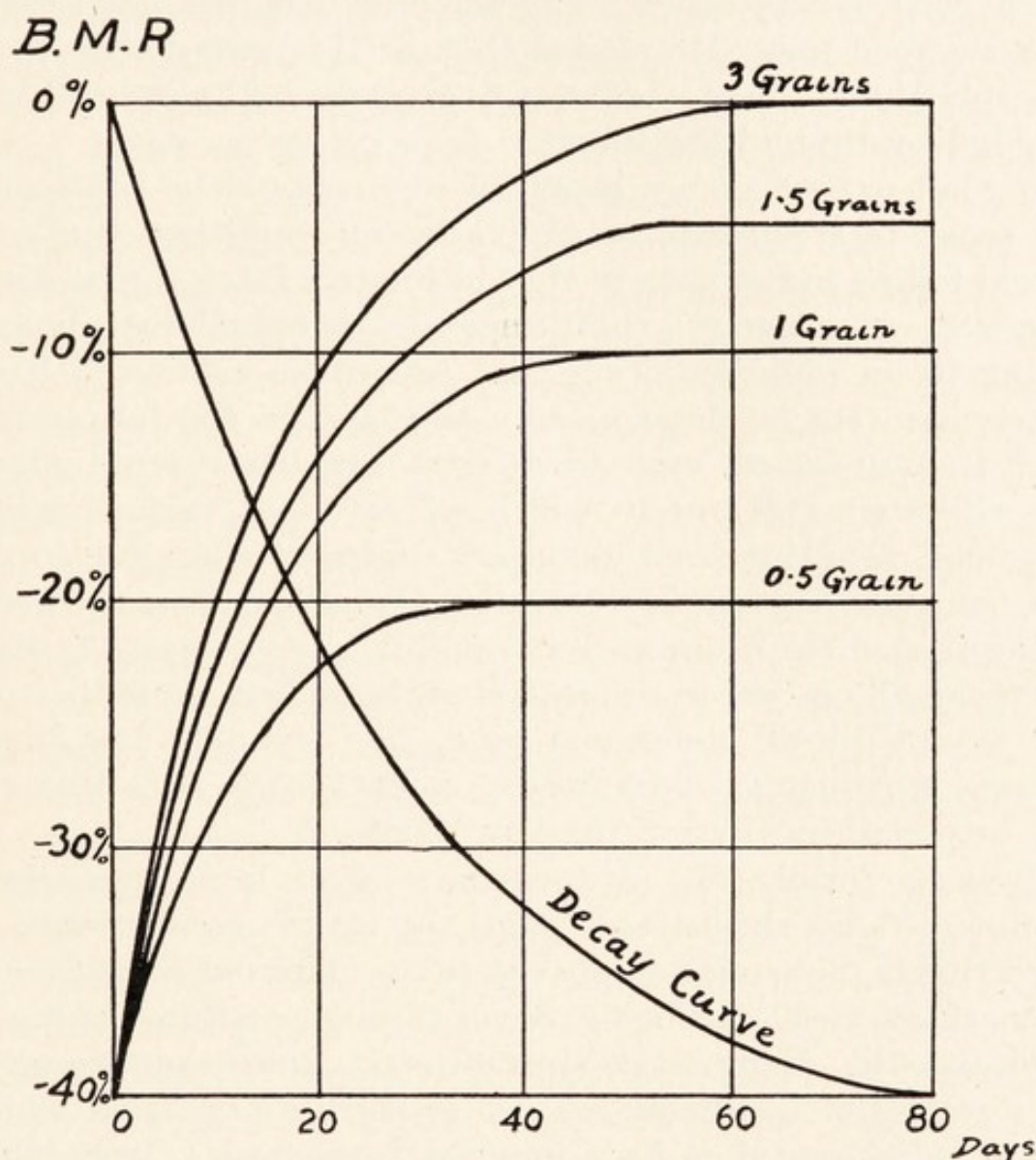


FIG. 3. Diagram showing the approximate relationships between basal metabolic rate, and daily thyroid dosage (in grains of thyroid, U.S.P., containing 0.2 per cent. iodine), in patients initially with complete myxoedema. The decay curve indicates the B.M.R. response to be expected following discontinuance 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.)

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.<sup>1</sup>

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).

Occasionally a case of masked hypothyroidism presents itself with complaint of abdominal pain, and exhibits low blood pressure, leukopenia, and low basal metabolism; it is dramatically benefited by thyroid therapy (373A).

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 depressed psychosis. Irritability and excitement may be sufficient to suggest a disordered mentality; thought distortion, with hallucinations and delusions, may suggest dementia praecox (109).<sup>2</sup>

<sup>1</sup> 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).

<sup>2</sup> 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).



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

Two cases of keratoderma of hands and feet, associated with a hypothyroid state, and showing marked improvement following thyroid treatment, have recently been reported (378).

**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, 15, 125, 245, 116). The enlargement can be greater than in any other condition; there is often a resemblance to a pericardial effusion.<sup>1</sup> 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 been reported by Close (65), whose excellent results are well shown by the photographs in Fig. 4. Kerley (392) has recorded an excellent series of cases under observation from infancy to early adult life; Gesell (385) has also published a similar interesting clinical study.

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 in life following infectious disease, provided treatment is persistent (48). (Cf. also 394.)

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

<sup>1</sup> In at least some cases the apparent enlargement is due to chronic pericardial effusion. These also respond to thyroid therapy (110).



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

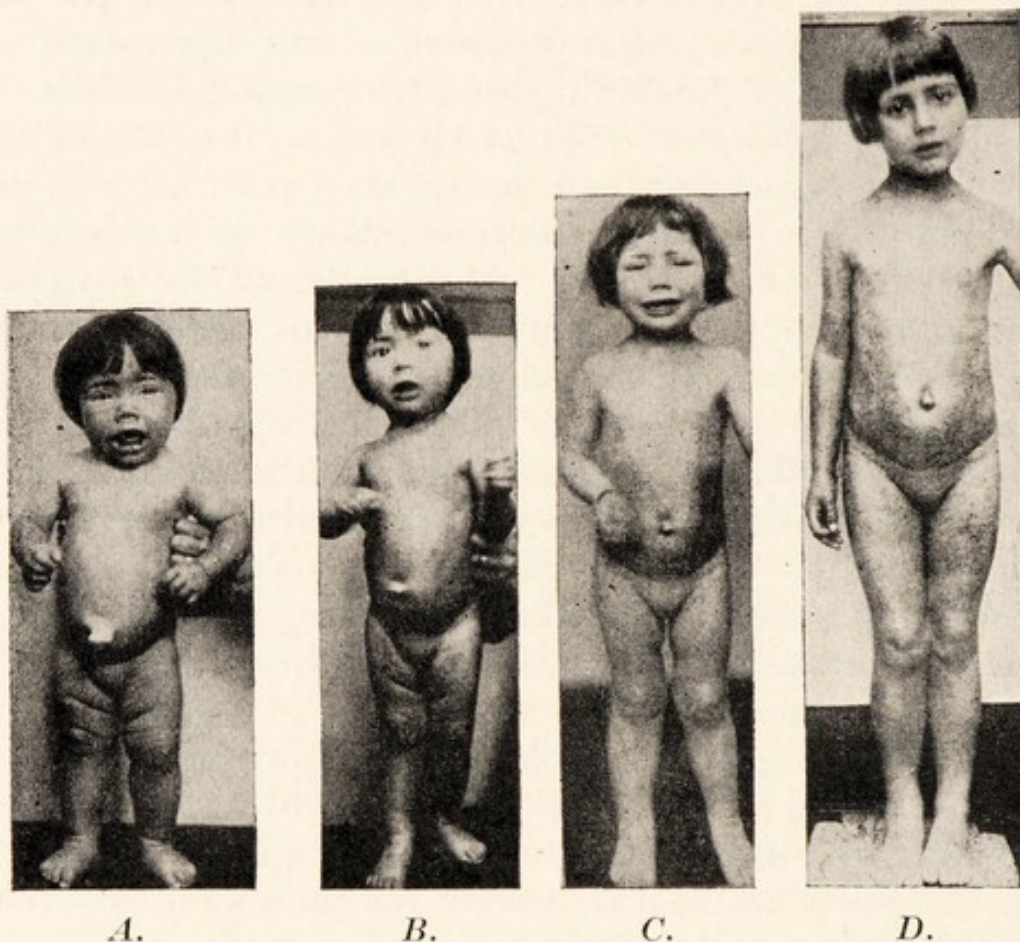


FIG. 4. A case of cretinism successfully treated with thyroid. A. Eighteen months old. Large umbilical hernia. Just before treatment commenced. B. Twenty-three months old. The hernia has disappeared. C. Thirty-eight months old. D. Sixty-six months old. Weight, 46 lb. Height, 46 inches. Practically normal, both physically and mentally. (From Close, *Guy's Hospital Reports*, 1932, lxxxii, 155.)

judged by a very low blood iodine value, 2 to 5 $\gamma$ , instead of over 7 $\gamma$  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  $\rightarrow$  hypertrophy  $\rightarrow$  hyperplasia  $\rightarrow$  exhaustion atrophy *or* involution to colloid state  $\rightarrow$  hypertrophy  $\rightarrow$  hyperplasia  $\rightarrow$  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 view) 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 believed 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 pointed 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.<sup>1</sup>

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."



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*

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.).

*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*."

With this, however, may be contrasted Means' opinion (398). "Although, within the group, at least two fairly clean-cut different types emerge, Graves' and Plummer's, we have given up any attempt at more than tentative sub-division because of the great number of intermediate forms connecting the two extreme types by an unbroken series and because of the absence of any evidence that more than one etiologic factor produces the clinical picture."



Since many writers on hyperthyroid diseases do not accept the differentiation, some part of the literature is difficult of analysis. Some assistance in differentiation and in considering etiology may perhaps be obtained by comparing the results of thyroid administration to animals and man (pure hyperthyroidism) with the symptoms and signs in exophthalmic goitre (Graves' disease) and in toxic adenoma (adenomatous goitre with hyperthyroidism, secondary toxic goitre), as shown in Table III, based largely on Boothby (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. 35).



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 nasopharynx, 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).

Malnutrition (induced, for example, by an excessive reducing régime for obesity) may cause or precipitate a thyrotoxic condition (399).

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.) In "masked hyperthyroidism" the hyperthyroid condition is obscured, either through absence or but slight indication of expected features, as just indicated, or by their overshadowing through some secondary condition, such as heart failure. In some of such cases the basal metabolism fluctuates round a high normal value, and striking manifestations of hyperthyroidism are absent, though the chronicity of the condition may ultimately affect the heart deleteriously, producing, for example, auricular fibrillation. Other cases may show complete absence of nervous symptoms (cf. 397).

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 apparently markedly so in the young of some species of fish (386), 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 thyrotrophic hormone 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 consider-



able 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.

Smelser (412) finds that injection of thyroxine produces beneficial effects in cases of exophthalmos artificially produced in thyroidectomized guinea-pigs by injections of anterior pituitary extracts. Daniels (380) attributes the production of exophthalmos to stimulation of centres in the diencephalon by the thyrotrophic hormone of the pituitary.

Brain (39) has described a condition which he terms "exophthalmic ophthalmoplegia," and considers can arise spontaneously, associated with slight symptoms of thyrotoxicosis, or can occur after subtotal thyroidectomy. He has further reported a case in which the patient (suffering from paralysis of the ocular sympathetic as a result of syringomyelia) developed Graves' disease with exophthalmos, and concludes that the sympathetic nervous system therefore plays no part in the production of the exophthalmos. (The patient is stated to have recovered under medical treatment.)

Naffziger (262) has described surgical treatment of cases of progressive exophthalmos following thyroidectomy. (Cf. also 414.) Brunton (375A) has recently discussed the anatomical mechanism involved in exophthalmos.

**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 child-births (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. 51) 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, 382).

### 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. 45, 55). Trousseau, in 1863, accidentally employed tincture of iodine in a case of Graves' disease and got good results. Between 1920 and 1925 several papers were published recording definite clinical improvement and lowered basal metabolic rate in patients with Graves' disease, following small doses of potassium iodide administered several times daily (263, 207, 78).



The introduction of Lugol's solution by Plummer led to the abandonment of treatment with potassium iodide.<sup>1</sup>

Plummer originally introduced use of Lugol's solution as part of the post-operative treatment in Graves' disease; the beneficial results were so striking that its use was extended to the pre-operative preparation of the patient; at once thyroidectomy by a skilful surgeon became an almost negligible operative risk. "When Lugol's solution is given in exophthalmic goitre, there may be a marked drop in the basal

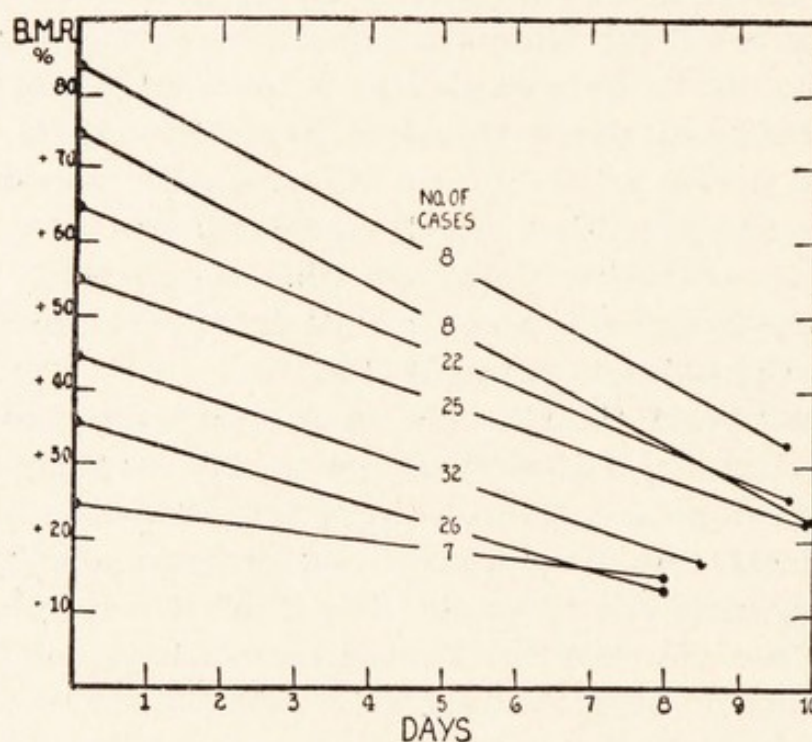


FIG. 5. Response to iodine in Graves' disease. The average basal metabolism before is compared with that after the usual course of iodine in the form of Lugol's solution. The abscissae represent the average time required for the characteristic response. A total of 128 cases was divided into groups according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

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

<sup>1</sup> 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.



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

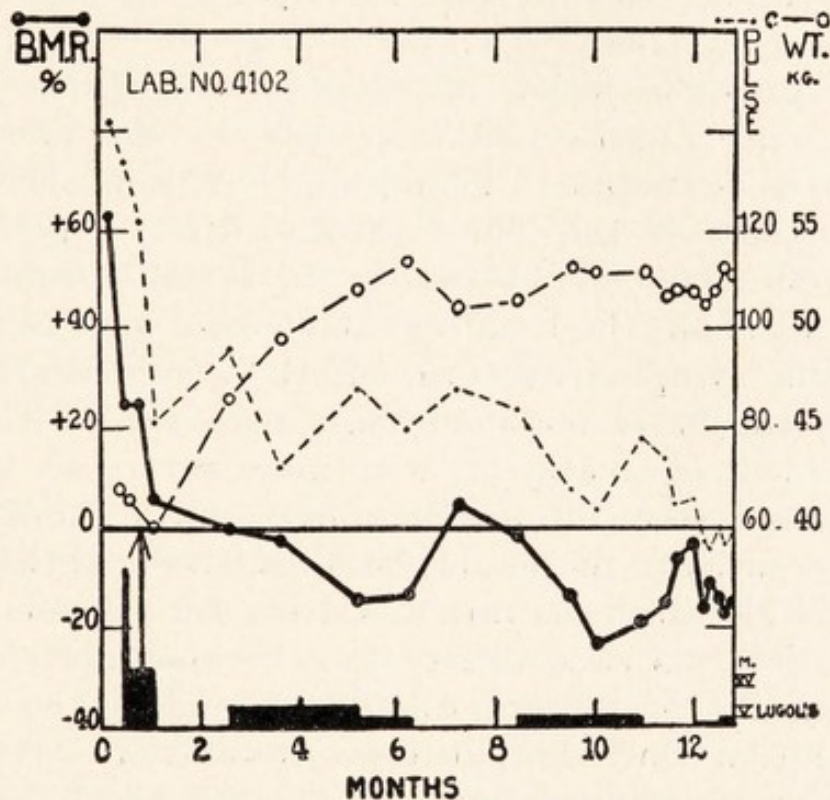


FIG. 6. Iodine responses in a case of residual thyrotoxicosis following subtotal thyroidectomy (arrow) for exophthalmic goitre. The case was characterized by residual nervousness, which disappeared under the iodine treatment. (From Means, Thompson, and Thompson, *Trans. Assoc. Am. Physicians*, 1928,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

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).<sup>1</sup>

In the great majority of cases *prolonged (pre-operative) treatment with Lugol's solution leads to development of a refractoriness to iodine*. Thompson has 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

<sup>1</sup> Davison and Aries (381) believe that, if sufficient thyroid gland has been removed at operation, and if the patient has previously been adequately prepared, immediate post-operative use of iodine has no rational basis, but think such use is still definitely indicated in incomplete operations such as polar ligations.



refractory to iodine treatment *ab initio*. Means and Lerman (248) believe 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' 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 hormone (cf. p. 17), 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 school have expressed the opinion that no benefit is conferred (179). Since the condition seems closely related to pure hyperthyroidism, artificially produced (cf. p. 69), it is pertinent to note that administration of Lugol's solution confers no

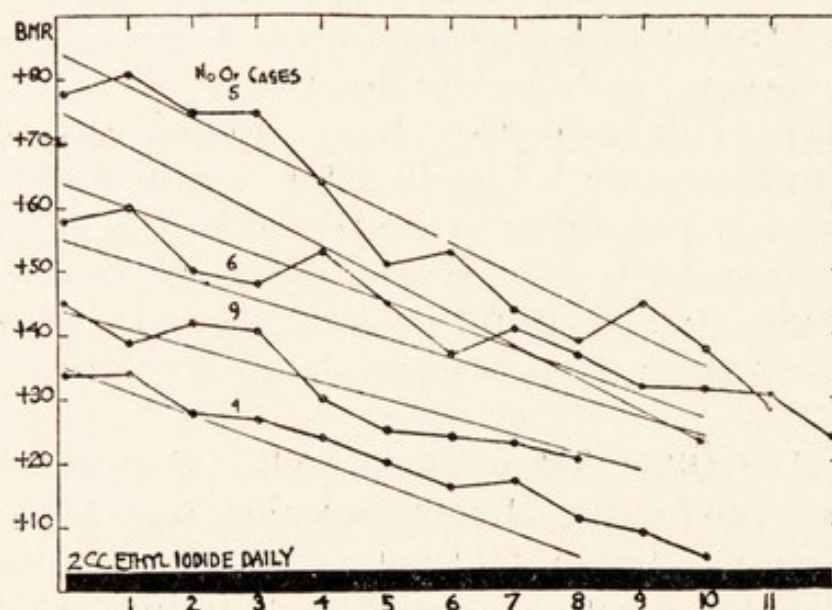


FIG. 7. Comparison of the metabolic rate changes produced by Lugol's solution (cf. Fig. 5) and ethyl iodide in Graves' disease. The cases are grouped in accordance with the resting levels, each ten-point interval constituting a group. (From Lerman and Means, *Am. J. Med. Sci.*, 1931, clxxxi, 745.)

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, 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 extrathyroid. 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

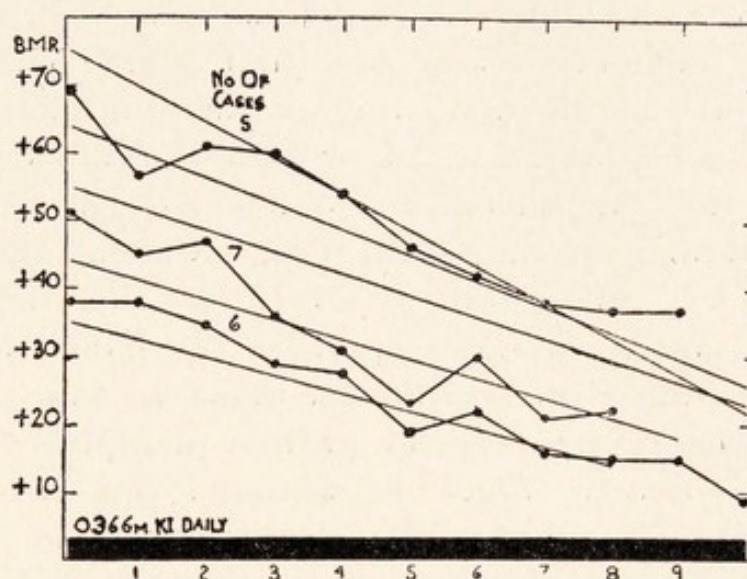


FIG. 8. Comparison of the metabolic rate changes produced by Lugol's solution (cf. Fig. 5) and potassium iodide in Graves' disease. The cases treated by the latter are arranged in three groups, with initial rate less than +45, between 45 and 59, and +60 per cent. or over. (From Lerman and Means, *Am.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

correction of a condition in which an abnormal thyroxine was being produced in the gland; this view cannot be upheld (cf. p. 67). 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 the 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 Professor William Boyd, a typical picture of a thyroid section from an untreated case of Graves' disease (old material) is contrasted in Figs. 9 and 10 with an average picture obtained after correct treatment with Lugol's solution.

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).

According to Lunde (213) the average iodine content of normal man varies from 9 to 13 $\gamma$  per 100 c.c. blood; there are certain seasonal fluctuations. (Wider extremes have been reported, seldom exceeding 8 to 17 $\gamma$ .) Cretins show a lower figure, 6 $\gamma$  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). The amount of lipoid-iodine present is negligible. Normally, the inorganic iodine varies from 7 to 12 $\gamma$ , the organic iodine from 1 to 4 $\gamma$ .<sup>1</sup>

In untreated patients with Graves' disease, while the inorganic fraction is not much affected, the organic fraction is markedly increased. After treatment with Lugol's solution the inorganic fraction is of course vastly increased, but the organic fraction is *decreased* to normal or nearly normal limits, concurrently with the fall in basal metabolic rate and symptomatic improvement. A typical result is shown in Table IV. (D.H., female, aged twenty, with definite Graves' disease) (213). (Cf. also 87.)

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 10 $\gamma$ . Baumann (374) finds still lower normal figures. A moderate proportion of hyperthyroid cases show normal blood iodine, and these cases

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



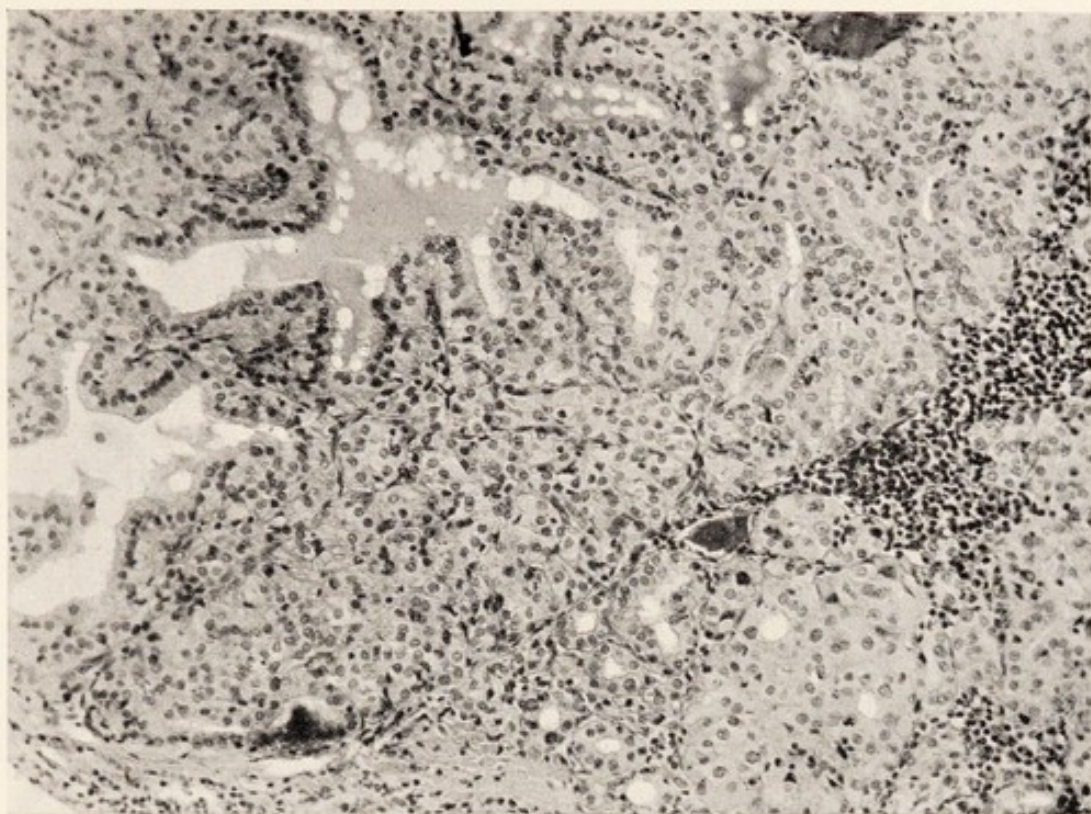


FIG. 9. Graves' disease untreated with Lugol's solution. The acini are for the greater part filled with hyperplastic epithelium. Absorption of colloid, especially along the line of contact with the epithelium. To the right there is a small collection of lymphoid tissue.  $\times 130$ . (Photo-micrograph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

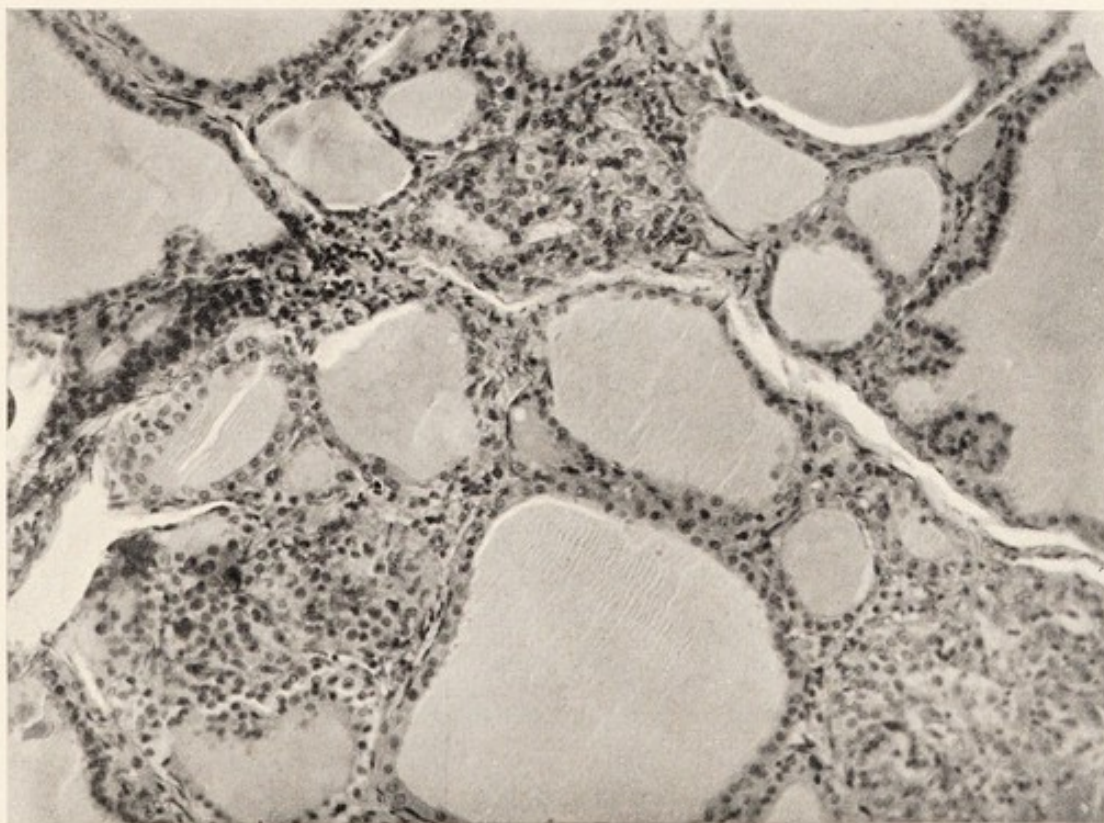


FIG. 10. Graves' disease treated for a short time with Lugol's solution. A few epithelial buds are seen at the right, but most of the hyperplasia has disappeared, and the acini are filled again with colloid.  $\times 130$ . (Photo-micrograph and description by Professor William Boyd.)







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 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

TABLE IV

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

Date.	Lugol's Solution.	B.M.R.	Blood Iodine.		Remarks.
	Daily Dosage.		In-organic.	Organic.	
7.2.1928	10 drops, 4 times	+ 80%	15 $\gamma$	36 $\gamma$	—
8.2.1928	"	—	94 $\gamma$	23 $\gamma$	—
13.2.1928	"	+ 40%	377 $\gamma$	1 $\gamma$	—
20.2.1928	"	+ 7%	252 $\gamma$	3.4 $\gamma$	—
21.2.1928	"	—	—	—	Subtotal
23.2.1928	"	+ 72%	243 $\gamma$	6.7 $\gamma$	thyroidectomy
7.3.1928	Iodine stopped	—	—	—	— Symptom-free

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). His results are in general agreement. Injected iodine disappears from the circulation less rapidly in hypothyroid patients. There is, however, no definite correlation between iodine tolerance and basal metabolism values. (His tolerance curves show, for normal, hypothyroid, and hyperthyroid patients, considerable overlapping, so that evidently the clinical application of the test has distinct limitations.) McCullagh and McCullagh (230) have also discussed the diagnostic value of blood iodine determinations.



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).<sup>1</sup> 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 hormone 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 hormone 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 the system, not

<sup>1</sup> 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).



produced through the thyroid. It has no permanent effect on the cause of Graves' disease (250).<sup>1</sup> (Saturation of the thyroid with iodide may perhaps have some degree of parallelism with saturation of proteins by iodine. Cf. p. 20.)

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. 25). 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). The rationale for most of such treatment is difficult to understand.

<sup>1</sup> 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.



In support of Marine's view 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 presumed to antagonize the action of its hormone, will be dealt with in Chapter X. A therapeutic treatment has evolved in connection with these presumed compounds, and will be referred to there.

Kasakow (391) has published some extraordinary results of administration of what he terms "lysats," mixed degradation products of various endocrine and other glands.

Other curious claims have 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). A case has been reported in which the patient is said to have recovered completely after rest and a high fat diet (413).

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. (Cf. also 390.)

At the present time there is no medical treatment which will re-establish thyroid balance in adults 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 hormone.

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 <sup>1</sup>

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#### 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 (69) 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 (34) 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 (34).

The glands are relatively very vascular. They are each supplied by a special arteriole from a thyroid artery; from it sinus-like capillaries come into close relationship with the cells themselves (135).

Hermann (71), from an extensive study both of human

<sup>1</sup> Thomson and Collip (145) thoroughly reviewed the literature to 1932, and most of the earlier references will be found in their bibliography. Shelling also reviewed the subject in 1935 (136).



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 (125). Transplantation experiments suggest that the glands can function adequately in absence of all nervous connections (125, 93).

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 (135, cf. 108). 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 (135, 32). Small colloid vesicles are sometimes found; the number of these also increases with age (cf. also 90, 101, 109).

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 (127).

The definite association of those acute manifestations which we call tetany with experimental removal of the parathyroids is due to Gley (51). Vassale and Generali (150) 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 have dealt critically and satisfactorily with apparent exceptions (145).

MacCallum and Voegtlin in 1909 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. 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 (97).

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). 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 (145).

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 such tests as Trousseau's and Chvostek's.<sup>1</sup>

Tetany is almost invariably produced following *complete* parathyroidectomy in all mammals, and in birds (145). Variations in susceptibility to parathyroidectomy in different species are probably traceable to dietary differences (136). 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 (68).

Some proportion of cases of spontaneous human tetany are associated with hypoparathyroidism (cf. 23); the majority probably are not. Tetany 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

<sup>1</sup> 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 (10), Vincent (152), Sharpey-Schafer (135), and Shelling (136).



acidosis in the organism) or of calcium salts. More permanent relief is conferred by continued administration of an active concentrate of vitamin D (cf. p. 121).

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 study of idiopathic steatorrhoea it was found 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 (14). Severe vomiting or continued gastric lavage, in adults, may lead to tetany.

Low plasma calcium is not necessarily accompanied by tetany. Young rats in winter frequently exhibit low blood calcium levels in absence of tetany (22). Greenberg states that when a diet very low in calcium is fed rats, a peculiar syndrome is induced, with serum calcium varying from 4.4 to 6.6 mg. per 100 c.c., but tetany does not occur (57).

In 1920 Collip and Backus and Grant and Goldman 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 (145).

A number of clinical cases have been reported in which such hyper-ventilation was the immediate cause, and generally the only conditioning factor. Such include tetany occurring during a paroxysm of hyperpnoea in a psychoneurotic patient convalescent from encephalitis lethargica (11), cases associated with continued pain from cholelithiasis and cholecystitis (58, 106), or from retention of urine (114), or from the prolonged discomfort of a pelvic condition (106). Even too violent exercise taken when in poor physical condition, or crying spells associated with a neurosis, have produced symptoms of tetany (58). 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 (98). Prolonged immersion in hot baths can set up a hyperpnoea which may induce tetany (91).



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 (106).

Tetany can be experimentally produced in animals by intravenous injections of sodium or potassium phosphate (145). 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 (38). However, the blood phosphates are also depressed, whereas, following parathyroid extirpation, they are slightly increased (45). 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 (107). A similar condition in cows and ewes following a period of close confinement has been described (36, 140). Dehydration may be a factor in all such cases (66).

Magnesium deprivation can lead to tetany, and the so-called "grass tetany" of cattle is probably of this type (136, cf. 56).

Tetany is produced in a proportion of young white rats fed desiccated thyroid; it often is apparent after a few days' treatment (19). 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 (145). 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 (120). 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 (8, 9, 54).

An interesting case—a woman of forty-eight—illustrates such "hyperthyroid tetany." She had a thyroidectomy, apparently for Graves' disease, at the age of twenty-seven. 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.

On the other hand, Cope and Donaldson (33) have reported a case of simultaneous hypoparathyroidism and hyperthyroidism, in which, during periods of marked thyroid activity, the blood serum calcium rose towards normal, and the signs of tetany diminished, although the negative calcium and phosphorus balance increased.

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 (100) 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, yet there is only slight increase in blood phosphate. Changes in hydrogen-ion concentration will undoubtedly change the equilibrium between the different phosphate ions ( $\text{H}_2\text{PO}_4'$ ,  $\text{HPO}_4''$ , and  $\text{PO}_4'''$ ) 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 (145).)

### Blood Calcium

The calcium of human blood occurs wholly, or almost wholly, in the plasma. Results indicating its presence in the red cells in any but negligible amount are due to inaccuracy of technique (145). It seems unlikely that in normal blood the envelope of the red cells is appreciably 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 so 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 (49), so that they obviously should be standardized in all ultra-filtration experiments (88). Since this has only recently been realized it is not surprising that the earlier dialysis experiments have not led to very concordant results.

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, and 45 to 55 per cent. or 42 to 58 per cent. in that of man, dogs, and cattle (145).



In the normal animal cerebrospinal fluid calcium corresponds to the diffusible fraction of plasma calcium. When, however, through experimental conditions (thyroparathyroidectomy, injection of parathyroid hormone, etc.), or pathological conditions, the level of plasma calcium is increased or decreased, the cerebrospinal fluid calcium does not reflect the change (110, 21).

The distribution of calcium in plasma appears to be governed by its protein and phosphate content and its *pH* (cf. 21 for references). About 50 per cent. is held in protein combination (the non-diffusible fraction, as determined by dialysis experiments), whilst almost all the remainder, according to McLean and Hastings (99), is present in ionized form. All the combined calcium is easily set free from its combination, as the use of oxalate for the prevention of clotting indicates.

In hypercalcaemic conditions (as in hyperparathyroidism) and in hypocalcaemic conditions (as following parathyroidectomy) blood plasma calcium is distributed between diffusible and non-diffusible forms very similarly to its normal distribution (138, 70).

### The Effects of Parathyroidectomy

It is important to remember that not only is the blood plasma calcium depressed, with resulting tetany, but, concomitantly, the plasma phosphate is elevated, while urinary phosphate excretion is diminished.

Chronic effects cannot easily be studied in most species of mammals, since, on the one hand, complete parathyroidectomy rapidly causes death, while, on the other hand, partial parathyroidectomy is followed rapidly by sufficient regeneration to restore normal conditions. In the rat, however, although accessory parathyroids are usually absent, extirpation of the glands is seldom fatal, and chronic effects are thus ascertainable. Bones become somewhat decalcified; analyses show them to be low in ash, calcium, and phosphorus, though relatively high in magnesium (145); such results are not easily explained, since in hyperparathyroidism the bones are also denuded of calcium. Erdheim first showed that the teeth are affected, becoming opaque, brittle, and distorted (145). Stour, Chandler, and



Tweedy (142) find that there is, in parathyroidectomized young rats, an initial increased calcification of the dentine, and subsequently defective calcification and formation of both dentine and enamel, in the nature of a fluctuation in the degree of calcification, causing the brittleness.

Parathyroidectomized rats show an increased appetite for calcium salts (126).

Evans, Szurek, and Kern (43) report that when thyroparathyroidectomized dogs are kept free from tetany by administration of calcium lactate or gluconate, after a varying period this treatment can be discontinued without tetany developing, although blood plasma calcium and phosphorus remain at tetany levels. Such dogs have been kept for nine months in good condition. There is no significant change in blood sodium, potassium, magnesium, or chloride during this period. The authors believe that some neuro-muscular adjustment permits this survival.

### **The Preparation of an Active Parathyroid Extract**

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

The earlier attempts to obtain active extracts of the gland have been reviewed by Collip (28). MacCallum, considering this earlier work, wrote in 1924 concerning the therapeutical results (96): "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 (65) 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.<sup>1</sup> Collip achieved this in 1924.

His method consisted essentially in boiling fresh minced

<sup>1</sup> "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" (67).



glands with dilute hydrochloric acid for from thirty to sixty minutes, cooling, and removing fat, then adjusting the pH 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 (145). (For details of the methods see Harrow and Sherman (67).) 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 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 dialyse through a collodion membrane. There is evidence that its activity is associated with the presence of a primary amino- or acid-amide-group (148).

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. Intravenous injections produce their maximum effect earlier—in four to eight hours—and this is relatively slight (145).



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 (28): 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;

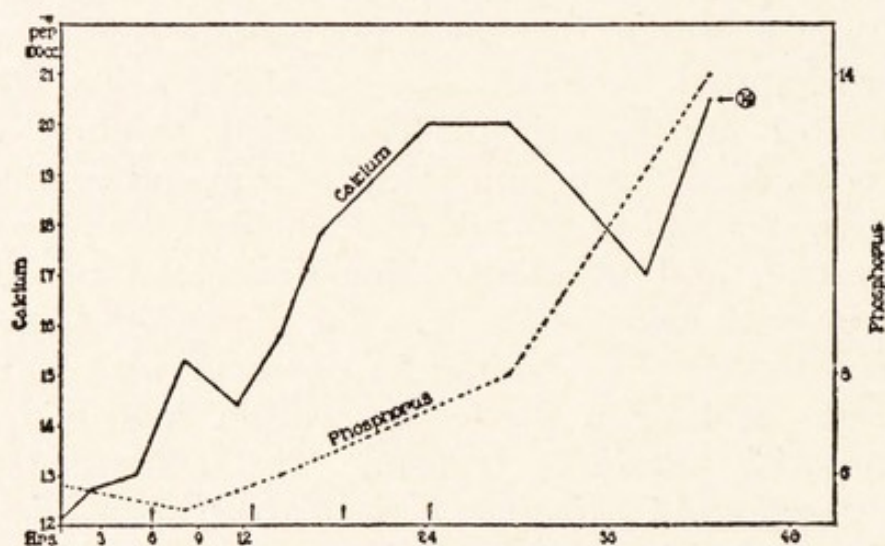


FIG. 11. The blood serum calcium and whole blood inorganic phosphorus curves in continued parathyroid overdosage in the normal dog. (From Collip, *Medicine*, 1926, v, 22.)

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 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 pH 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 (145).

This pathological picture of acute effects following overdosage

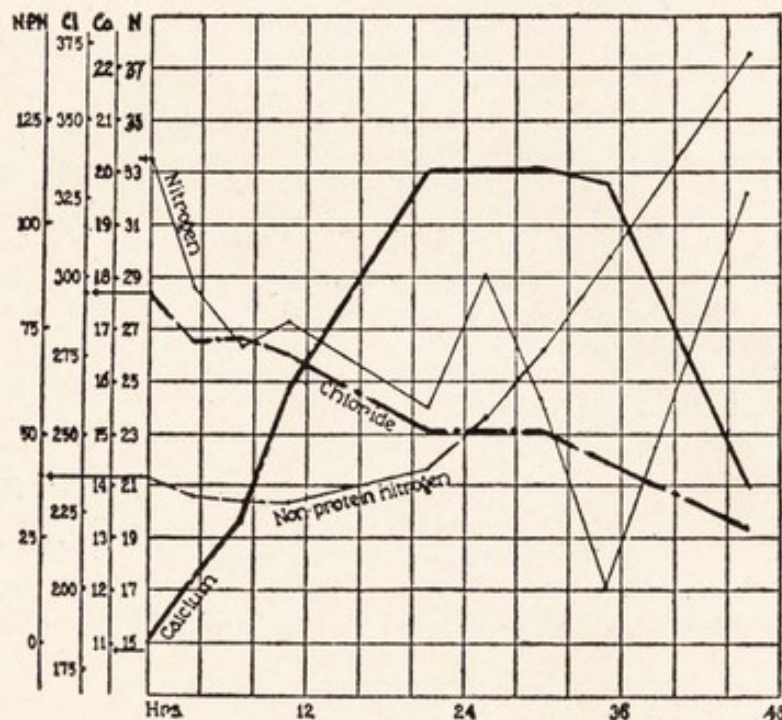


FIG. 12. The blood serum calcium, whole blood chloride, nitrogen and non-protein nitrogen in the normal dog as affected by repeated injections of potent parathyroid extracts and frequent bleedings. (From Collip, *Medicine*, 1926, v, 23.)

can be almost exactly paralleled by combined injection of calcium chloride and acid sodium phosphate ( $\text{NaH}_2\text{PO}_4$ ), whence the actual symptoms may be ascribed to coincident hypercalcaemia and hyperphosphataemia (145).

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 (145). The response of man is similar to that of the dog, although he seems more resistant to overdosage (145). 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 (145).

Shelling considers that many of the effects of acute hyperparathyroidism are due to continued diuresis and the resulting anhydraemia leading ultimately to anuria, azotaemia, and



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

death, so that rational treatment of such condition is to replace water and electrolytes (136).

It has been suggested that the effect of the parathyroid principle is primarily upon the blood phosphate (146); the potential interrelationship between plasma calcium and phosphate has already been mentioned (cf. pp. 105 and 107). Following the administration of a potent parathyroid extract (parathormone) there is a slight increase in blood magnesium, antecedent to the rise in blood calcium (130, 55). The effect on calcium and phosphate is almost immediate. Within the first hour urinary phosphate excretion increases, blood plasma inorganic phosphate falls, and calcium increases (93A).

Chronic treatment with parathyroid extracts gives results in animals simulating human osteitis fibrosa (cf. p. 123).



**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 (145). Rats also appear to develop an immunity



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.*)

(119). The explanation appears to lie in the precise mechanism of action of the principle (cf. p. 118).

Taylor (144) has succeeded, by giving gradually increasing doses of parathyroid extract to dogs, starting with 2 units per day, and increasing the dose at weekly intervals, in producing in two or three months a state of tolerance to the hormone, in which the dogs remained apparently normal and with normal serum calcium. At this stage of tolerance to doses which usually



prove fatal the dogs were also immune to massive doses of irradiated ergosterol. Serum from these dogs, injected into others along with parathyroid hormone, failed altogether to alter the normal response to the hormone (thus giving no evidence of anti-hormone production; cf. p. 419).

**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 level. The slight increase in blood phosphate produced by extirpation of the glands disappears (28). Collip 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. (Cf. also 124.)

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 (28). Man suffering from parathyroid deficiency following operative procedures responds to treatment as satisfactorily as does the dog (cf., however, p. 121).

According to Kozelka parathyroid hormone is ineffective in controlling the tetany of parathyroidectomized rachitic rats (86).

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

Collip 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 (28). 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 (145). The method can only be considered as roughly accurate.

Burn 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 found no appreciable rise, so that the method seems unsound (145).

Hanson 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 (145).

A somewhat tedious but possibly accurate procedure has been recently suggested based upon increase in urinary calcium when rats are injected with parathyroid extracts (39, 119). The response to various doses of the same preparation is said to be proportional to the logarithm of the dose. Using such a method, it is suggested that a rat unit be defined as one-tenth of the amount of hormone needed to produce an increase of 1 mg. in the total urinary excretion of calcium (147).

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

An old theory, thoroughly discredited (28, 145, 128), that the parathyroids are concerned with the destruction of guanidine compounds, and that parathyroid tetany is a guanidine tetany, is still further disproved by the observation of Anders and Myers (7) that the blood content of guanidine compounds was normal in 8 cases of parathyroid tetany.

Vitamin D, whether the natural product  $C_{27}H_{43}OH$ , from cholesterol, or calciferol,  $C_{28}H_{43}OH$ , prepared artificially by irradiating ergosterol, is an important agent in the control of calcium metabolism, and the type of action it produces has led to a theory of interrelationship between the vitamin and the parathyroid hormone. 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 (artificial) vitamin, if marked, leads to hypercalcaemia, and to deposition of calcium salts in various sites.

Hess and his co-workers considered that the vitamin stimulated the parathyroids to secrete the hormone, and thus achieved control of calcium metabolism. They could not produce hypercalcaemia by administration of the vitamin to parathyroidectomized monkeys and dogs. Later investigators,



however, showed that sufficiently large doses of the vitamin will maintain such animals in good health with normal blood calcium, while the treatment has been proved beneficial when tetany follows human thyroidectomy (145).

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 investigated this point carefully ; his animals in which all tissue liable to contain accessory parathyroids had been removed usually developed tetany which could not be relieved by the vitamin, however excessive the dosage. Other investigators did not obtain such definite results (145). Shelling (136) criticized Taylor's experimental evidence in support of direct relationship as vitiated by inadequate control of dietary factors.

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 (20) or depresses the excretion of calcium into the intestine (143). There is no conclusive evidence that the parathyroid principle affects the absorption of calcium (145). 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 (20).

**The Function of the Parathyroid Hormone.** 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. 99).

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 (136), but since there is a complete interrelationship the problem is difficult to settle.

There is good evidence that the hormone 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 been summarized by Kay (cf. 145).

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 (103). In many other less drastic events bone is denuded of some proportion of its store (145).

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 (145). Such theory of direct action is not completely accepted. Various other theories have been advanced, generally complicated ones (cf. 136). 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) (145).

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. 123).

When the injections are continued over a long period<sup>1</sup> the rats pass into a stage of apparent immunity to the parathyroid principle, which is, however, actually a state of increased tolerance (cf. p. 113). 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. 131, 145).

The experiments of Pugsley (119) are in chemical agreement with these findings. In such rats prolonged injections lead first to increased calcium excretion, but finally to decreased excretion.

Shelling's results (137) are not in complete agreement; he considered that dosage and the calcium and phosphorus content of the diet condition the response. Burrows (18) seems in general to confirm Selye's conclusions, although his work, except from the anatomical standpoint, seems somewhat uncritical.

Selye showed further that if only very small doses of the hormone 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 (133, 30).

<sup>1</sup> Chronic experimental hyperparathyroidism frequently produces a chronic nephritis in rats, due apparently to deposition of calcium phosphate within and without the kidney tubules with obstruction, leading to atrophy of some nephrons and dilatation of others (25A).



But when it is given in small amounts over long periods increased calcium deposition in bone results, the cortical tissue becoming denser and thicker (129).

Selye has shown (131) 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 hormone 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 (145) that the parathyroid principle does not increase the solvent power of blood plasma for the calcium compounds of bone.

The theory that the primary action of the hormone is on phosphate metabolism has been mentioned (p. 117). There has been, within recent years, an attempt to associate this action with excretion of phosphate through the kidneys. Although Collip (29) showed that the characteristic action on bone occurs after bilateral nephrectomy in rats, yet Tweedy (49) can find no evidence that parathyroid extract (or, indeed, massive doses of calciferol) produces any action on blood and tissue calcium in such experimental animals, and considers that mobilization of the calcium stores of the body by the hormone depends on kidney function.



Goadby and Stacey (53, 52), and Morgan and Samisch (109) have brought forward experimental evidence in favour of the view that the function of the hormone is to produce phosphate diuresis through a specific effect on renal tissue, and much of the clinical evidence from studies of cases of hyperparathyroidism can be similarly interpreted. But, as pointed out on p. 117, the close interrelationship between calcium and phosphorus metabolism renders the subject almost polemical.

Our present knowledge therefore only permits the statement that the parathyroid hormone controls bone deposition and denudation either directly, or indirectly, through control of phosphate excretion by kidney tissue.<sup>1</sup> The rough constancy of blood calcium and inorganic phosphate probably depends on a series of equilibria between rates of absorption and excretion of calcium and phosphate, and degrees of bone deposition and denudation, such equilibria being controlled by the parathyroid hormone and vitamin *D*, probably acting independently. Excess of the hormone produces (by direct or indirect action) undue denudation of bone and increased blood plasma calcium, and decreased plasma phosphate (due perhaps to increased phosphate diuresis), while deficiency of the hormone stops denudation of bone, and blood calcium falls.<sup>2</sup>

The very high blood plasma calcium in laying hens seems to depend on the integrity of the parathyroids (79).

### Treatment of Hypoparathyroidism

Human hypoparathyroidism is seen most commonly as a condition following thyroidectomy. Not infrequently a

<sup>1</sup> Brull and Carbonesco have very recently shown that if a dog is parathyroidectomized, and, forty-eight hours later, a kidney from it is transplanted, it excretes much less phosphate than does one transplanted from a normal dog, and perfused with the same blood at the same time. Thomson and Collip incline to the view that the controls of bone and kidney by the hormone are distinct and independent functions (144A).

<sup>2</sup> It has been claimed that when sufficient vitamin *D* is administered to parathyroidectomized pups (87), and rats on optimal calcium diet (136) 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.



transient post-operative latent tetany is observed, accompanied by a slight fall of blood calcium (122). When open manifestations occur, they can be controlled by oral administration of calcium lactate or gluconate, or, if severe, by administration of vitamin *D* or injection of parathyroid extract. Boothby (15) recommends frequent doses of calcium lactate with cod-liver oil, and finds that parathyroid extract is seldom necessary. It but seldom happens that so much parathyroid tissue is removed or irretrievably damaged that persistent tetany results. Even after a long interval hypertrophy of a trace of remaining parathyroid seems to be possible (145).

While the estimation of that degree of manifestation of tetany calling for record probably varies in different surgical clinics, with good surgery such manifestation is rare. In 1934 the Crile clinic reported an incidence of 1.3 per cent. in 11,500 cases of thyroidectomy, in 1931 the Mayo clinic 1.5 per cent. in 13,300 cases, and in 1937 the Lahey clinic 0.2 per cent. in 13,000 cases (142).

Lisser and Shepardson (145) have shown that continual administration of parathyroid extract in a case of persistent tetany sets up a gradual tolerance, and that increasing the dosage to control the tetany finally becomes ineffectual, so that death may result. Such acquired tolerance accords with experimental results (cf. p. 113), and may be due to a reversal of the effect of the hormone (cf. p. 118), though Shelling attributes it to high dietary phosphorus (136), a theory to which Margaret Hoskins' experimental findings on rats kept on diets with different calcium/phosphorus ratios lend some support (75).

Cantarow has reported (23) a case of severe chronic idiopathic tetany due to parathyroid insufficiency, in a young girl, which did not respond to vitamin *D* therapy, but did respond satisfactorily to high dosage of parathyroid hormone combined with high calcium and relatively low phosphorus intake, during five and half years. Ultimately, however, this *régime* failed. Anderson and Lyall (7A) claim that normal blood plasma calcium and phosphate levels can be maintained in hypoparathyroid patients by diets low in phosphorus and high in calcium (supplemented by calcium lactate) without vitamin *D* or other therapy.

Very rarely, parathyroid hormone is quite ineffective in



inducing increased blood calcium. Hunter and Aub (78) noted that one out of seven cases treated for lead poisoning completely failed to respond with an increased blood calcium, although the patient's calcium excretion was increased in usual fashion. Merritt and Bauer (104) obtained no significant elevation of blood calcium in two of seven patients (with normal parathyroids) injected with the hormone. Mathewson and Cameron (102) obtained no response when the hormone was administered in a case of purpura of undetermined origin. Such results have no definite bearing on the treatment of hypoparathyroidism, since in these cases the blood calcium was normal before treatment. The failure to respond is possibly associated with an unusual facility to excrete calcium through the kidneys.

An advance of considerable importance has been the application of *dihydrotachysterol*, one of the products of ultraviolet radiation of ergosterol, to cases of post-operative and idiopathic tetany. This compound was first isolated and used clinically by Holtz (73), and is generally known as "A. T. 10" (Antitetaniemittel Nr. 10). It has since been favourably reported on by a number of clinicians (cf. 132, 95, 116). Jelke (82) has published a good bibliography concerning its use in over 300 cases, mostly of post-operative tetany.<sup>1</sup>

The compound, dissolved in oil, is administered orally. It acts more slowly than the parathyroid hormone, but no immunity to its action is established by prolonged use. It abolishes tetany in two to three days, and somewhat more slowly raises the blood calcium to normal values. It prevents depletion of tissue calcium and causes exogenous calcium to be properly utilized. It has been used successfully in cases which have developed a resistance to parathyroid hormone. Initially, and until tetany disappears, fairly large dosage can be employed; the dose is then rapidly lowered until a small maintenance dose given once in several days suffices. If treatment is stopped the tetany reappears. The treatment has some disadvantages. A. T. 10 is not a harmless compound. Overdosage leads to hypercalcaemia and toxic symptoms. Treatment must be

<sup>1</sup> According to v. Werder (154A) dihydrotachysterol can be regarded as calciferol (vitamin  $D_2$ ) to which two atoms of hydrogen have been added, converting the attached methylene group ( $:CH_2$ ) to a methyl group with elimination of a double bond.



individual, so that, especially in the initial stages, adequate control by repeated determinations of blood serum calcium is essential. The material is expensive.

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 (85, 10, 146). The administration of parathyroid extract has been found beneficial. Shannon (134) 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.

A curious case of temporary idiopathic parathyroid tetany (not associated with rickets) in a seven weeks' old boy is reported by Pincus and Gittleman (118). The infant responded favourably to treatment with parathyroid extract. Cessation of treatment led to renewal of the tetanic convulsions, but after a few weeks, when treatment was discontinued, no tetany ensued, and the patient remained well.

Parathyroid hormone and vitamin *D* therapies are contraindicated in cases of osteogenesis imperfecta, since they produce negative calcium and phosphorus balances in these cases and thus tend to accentuate a functional disturbance already present (64).

### Hyperparathyroidism

Acute and chronic experimental hyperparathyroidism have been described (pp. 110, 112). The corresponding clinical conditions exist and are of great interest. Although clinical hyperparathyroidism is rare, now that it has been thoroughly studied, diagnosis is easier, and cases are being diagnosed more frequently.

The pathology of hyperparathyroidism primarily involves the bones or the kidneys, or both. The relative ease with which the kidneys can dispose of an increased calcium and phosphate load probably determines whether bone lesions or kidney stones first bring the patient to his physician. The cause of the hyperparathyroidism is usually an adenoma of one or sometimes two glands, less often generalized parathyroid hyperplasia.

**Generalized osteitis fibrosa** (von Recklinghausen's disease of



bone) was differentiated from osteomalacia by von Recklinghausen in 1891, and was associated with a parathyroid tumour by Askenazy in 1904. In 1926 the first operation for the condition was performed by Mandl, and a tumour was found and removed (145, 35). The disease 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 (in absence of surgical treatment). All bones may show pathological decalcification with osteoclastomata. Multiple foci of osteitis fibrosa occur, with or without benign giant-celled tumours and cysts. The condition is more frequent in women. Renal

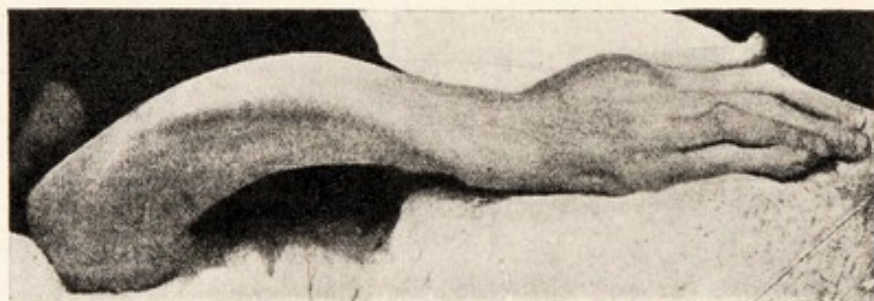


FIG. 15. Antero-external curvature of forearm and large bony swelling on dorsum of right hand from a case of generalized osteitis fibrosa. (From Hunter, *Proc. Roy. Soc. Med.*, 1931, xxiv, 489 ; Clin. Sect.)

calculi are common. Metastatic calcification is not infrequent. Thirst and polyuria are often present.

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 marrow, and formation of osteoclastomata and cysts. There is a generalized osteoporosis. Hodges (72) and Compere (31) have recently dealt in detail with the skeletal changes.

Chemical study of such patients shows usually a high blood calcium, low blood inorganic phosphate, and markedly increased excretion of calcium and phosphorus. Recorded figures for serum calcium vary from high normal to 29.4 mg. per 100 c.c. (136). Plasma phosphorus usually varies from 2.5 down to 1.0 mg. Plasma phosphatase is often high.



Onset of the disease is insidious. It may last many years. A history of thirty-nine years has been recorded (1). It is commonest in middle adult life, but can occur even in young children (136).

Amongst others, Churchill and Cope have recently described a suitable surgical technique (26).

At operation, in the majority of cases only one tumour is found; rarely, tumours of two glands are present. Sometimes repeated operation is needed to find a tumour. They are

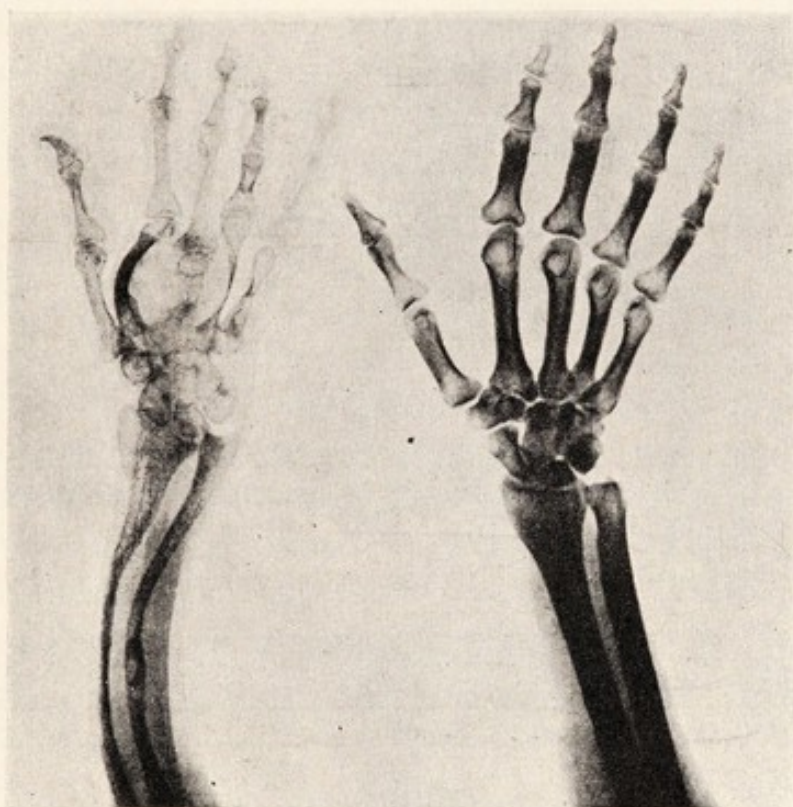


FIG. 16. Controlled radiograph of right hand and forearm (cf. Fig. 15). (From Hunter, *Proc. Roy. Soc. Med.*, *loc. cit.*)

seldom palpable. The largest so far recorded measured  $7.5 \times 5.0 \times 1.8$  cm., and weighed 26.2 gm.; it was situated behind the trachea. Tumours have been reported in the jaw (27) and completely embedded in the thyroid (151). The size bears no relation to the severity of bone lesions; in a severely crippled patient the tumour only weighed 1.3 gm.

Operation abolishes pain in almost all cases. Restoration of calcium metabolism to normal occurs with varying rapidity. A hypocalcaemia frequently develops, and latent and even open tetany may occur. General symptomatic improvement



takes place, and crippled patients may recover sufficiently to be able to walk without artificial aid.

Figs. 15 and 16 depict the typical bony curvature and diminished density of bone shadow, as seen in one of Hunter's cases (76, 77). 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,

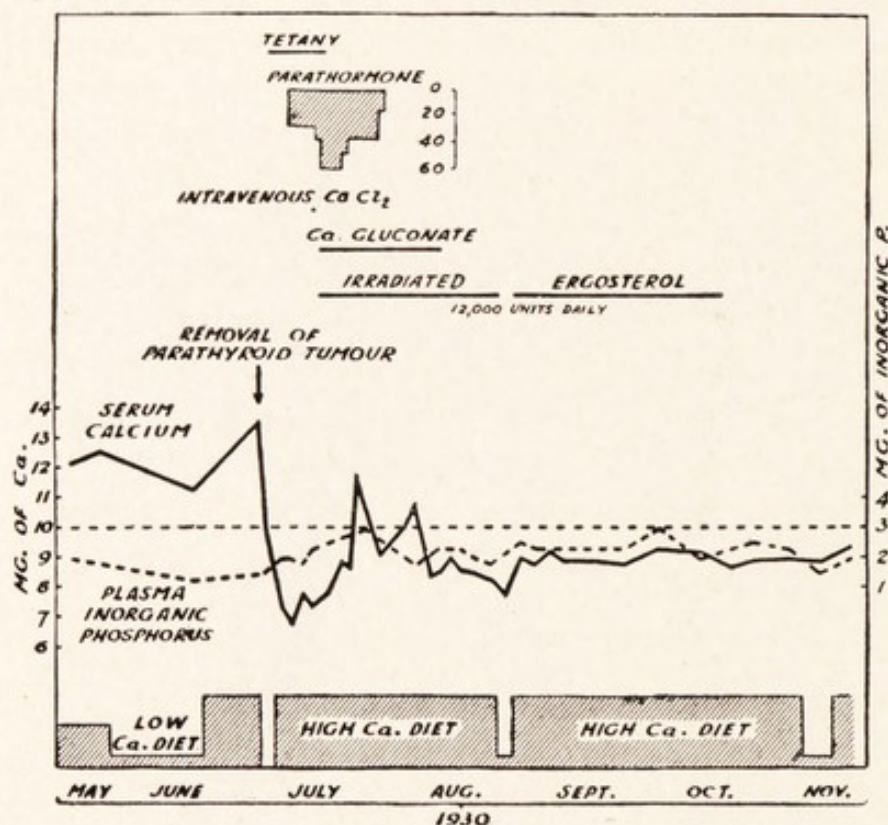


FIG. 17. Chemistry of the blood of Hunter's case of generalized osteitis fibrosa. The blood was examined for two months before and for five months after operation. The low calcium diet was that given during the investigation of the calcium balance. The high calcium diet was not weighed. (From Hunter,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

are to be found in a paper by Quick (121). The recalcification of bones is sometimes marked (16).

A case has been reported in which, after removal of a parathyroid adenoma and improvement, the condition recurred four years later, and at post-mortem another adenoma was found (46).

Experimental chronic hyperparathyroidism has produced in dogs, guinea-pigs, and rats a picture markedly resembling that



of clinical osteitis fibrosa (145, 81, 84, 156, 83, 112), while in a normal human subject sufficient symptoms have been produced by less drastic dosage to confirm the hyperparathyroid nature of the clinical disease (77, 84). Selye's studies suggest the mechanism.

**The Hyperparathyroid-Kidney Stone Syndrome.** Albright first stressed the occurrence of hyperparathyroidism without appreciable bone involvement, but with kidney lesions and stone (1).

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 this type of case blood phosphate may be normal or even slightly increased (1). (In a case reported by Elsom (42) the earlier symptoms were for some time considered as rising from an atypical form of glomerulonephritis.)

It is now generally recognized that in treating cases of kidney-stone potential hyperparathyroidism must be borne in mind (89).

Albright (3) considers that while cases of the classical type with bone disease (and high blood phosphatase) usually develop post-operative hypocalcaemia, the kidney-stone type of case usually does not.

While recurrency of stone formation is probably prevented by operative removal of a tumour, and the general condition of the patient is strikingly improved, the kidney damage—as measured by the urea clearance test—does not seem to be greatly repaired (42).

It is still difficult to estimate the proportion of hyperparathyroid cases exhibiting kidney stones. Castleman and Mallory analysing twenty-five of their own cases and another 119 from the literature in 1935 (24) found that seventy-five showed osteitis alone, fourteen renal stones alone, and fifty-five both osteitis and renal stones. The proportion exhibiting no detectable bone changes will probably prove to be considerably greater when a much larger number of cases can be reviewed. Barney and Mintz (12) report that of their 340 cases of kidney stone hyperparathyroidism was responsible for between 4 and



5 per cent. The symptoms and signs of urinary lithiasis in hyperparathyroid cases do not differ from those of lithiasis from other causes. (Albright has recently discussed the possibility of dissolving kidney stones by introducing "from below" through a catheter solutions of sodium citrate-citric acid and of sodium hexametaphosphate (6A).)

**Hyperplastic Parathyroids.** In considering cases with hyperplastic glands Albright differentiates between primary and secondary involvement of the parathyroids. He has reported several cases of primary idiopathic hypertrophy or hyperplasia (6) in which all the parathyroids are enlarged, apparently through hypertrophy rather than hyperplasia. The cause of the hypertrophy is not known, and there is no evidence involving the pituitary. The degree of glandular hypertrophy is proportional to the hyperparathyroidism, in contrast with findings in cases of adenoma (cf. 24). Surgical treatment is of more doubtful benefit in such cases (2).

Contrasted with this condition is what Albright terms "renal osteitis fibrosa cystica" (4), a rare condition in which, through long-standing severe renal insufficiency, there is marked phosphate retention, while the serum calcium is normal or low, there is severe acidosis, medial arteriosclerosis, calcium deposits round joints, and bone changes like those in osteitis fibrosa but more generalized, while the parathyroid glands are all markedly enlarged. This enlargement is considered to be compensatory, due to the phosphate retention. Albright considers that this syndrome is, for adults, a counterpart of renal rickets (in which he claims that the "rickets" is really an osteitis fibrosa). (Chown has published clinical and experimental evidence which suggests that the nephritic lesions in renal rickets are traceable to hypercalcaemia of parathyroid origin (25). The two views are not necessarily in conflict.)

Drake, Albright and Castleman (37) have shown that repeated parenteral injections of phosphate into rabbits leads to definite hyperplastic enlargement of the parathyroids, and believe that these experimental results support Albright's view.

**Osteitis Fibrosa of Non-parathyroid Origin.** Albright (5) on basis of study of five cases (and thirteen others cited from the literature) has described a syndrome characterized by osteitis



fibrosa disseminata, non-elevated areas of brown pigmentation, and endocrine dysfunction, with precocious puberty in females. Bone changes are localized. Calcium and phosphorus metabolism are normal. The condition is not one of hyperparathyroidism, but is probably due to some embryological or trophic neurological disturbance, rather than to a primary endocrine cause.

**Diagnosis of Hyperparathyroid Conditions.** In considering diagnosis, the classical picture is clear cut. Difficulties arise with border-line cases.

In thirty-five cases in one series (3) blood calcium varied by roughly equal increments all the way from 10·7 to 17·3 mg. per 100 c.c. serum. Nine of these cases had values below 12 mg. The majority of cases have phosphorus value below 2·5 mg. (cf. also 60). Albright (3) gives the following criteria for assistance in diagnosis of border-line cases.

If serum calcium is not definitely high, consider whether the serum phosphorus is persistently low or urinary calcium excretion is increased.

If the presence of kidney stones constitutes the only symptom, and if these are largely composed of calcium phosphate, while there is no obvious cause such as infection or obstruction for their occurrence, then hyperparathyroidism should be seriously considered.

If serum protein is low, while the figure for serum calcium is normal, then the ionized calcium fraction is probably above normal (cf. 99).

If hyperparathyroidism is diagnosed on such grounds as these, then at operation a tumour may or may not be found. Albright reports three unsuccessful cases, although he comments on the possibility of minute, undetectable tumours.

Some other aspects of differential diagnosis may be mentioned. Blood calcium and phosphorus are normal in uncomplicated Paget's disease, calcium is elevated in many cases of multiple myeloma (with normal or slightly high phosphorus), and is sometimes increased in carcinoma with bone metastases (while phosphorus is usually normal) (60, 136).

Hamilton and Highman (62) suggested, as a test for hyperparathyroidism, intramuscular injection into rabbits of 30 c.c. of the patient's blood, which is supposed to cause a rise in the



blood calcium of the rabbit. Gilligan (50) could obtain no results supporting the test.

Definitely high blood calcium is occasionally found in acute gout, in arthritis deformans, and in polycythaemia vera (17). Possibly because of such findings, cases of arthritis have been operated on with removal of one or more parathyroids (cf. 48), while on more general grounds similar operation has been recommended in various diseases involving bone (cf. 154). Bauer (13) has appraised such views critically and definitely. He emphasizes the fact that the bone changes in hyperparathyroidism are generalized, and are due usually to an adenoma, while the changes in 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 31, 111, 59).

**Acute Clinical Hyperparathyroidism.** Hanes (63) has described such a case. A woman of forty-nine gave a history which, in light of present knowledge, indicated that a marked hyperparathyroid condition had existed for at least five years, with definite kidney calcification. During examination, a blood serum calcium of 20 mg. per 100 c.c., with 4.7 mg. phosphorus, changed in three days to 22 mg. (and 4.8). There was a marked nodule of about 2 cm. in diameter at the left lower pole of the thyroid. This was undoubtedly subjected to repeated palpation, which may have been the cause of the final parathyroid intoxication from which the patient succumbed four days later. Death was preceded by marked asthenia, nervousness (in contrast to previous cheerfulness), and slight fever, with generalized aching pain. The cause of death was apparently circulatory failure from the parathyroid intoxication. (The high phosphorus corresponds to the pre-mortal increasing phosphorus in acute experimental parathyroid poisoning; cf. p. 110.) The post-mortem picture strikingly resembled that of dogs and other animals dead from continued parathyroid injections.

At the other end of the scale is an interesting case reported by Fridericksen (47) of tetany in a suckling, with latent osteitis fibrosa in the mother.



### Other Parathyroid Diseases

*Malignant Tumours of the Parathyroid.* Eighteen cases have been reported (61) but in only one of these was there definite evidence of hyperparathyroidism accompanied by decalcification of the skeleton (155, 153).

*Marble-bone disease*, a condition of extreme brittleness of the bones, seems to be associated with chronic hyperparathyroidism. A typical case has been described (115) 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. 118). Some evidence against this view has been reviewed by Shelling (136). (Cf. also Ellis (41).)

Selye (132) 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 137, 92).

### 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 (44). 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 (78). Similar treatment has been employed in radium poisoning, but is too slow to be useful (136).



Since the parathyroid principle induces diuresis (145) it has been employed in nephrosis and similar conditions associated with oedema, and clinical improvement has been reported (136), 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 (74).

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 (94). In both cases toxic symptoms of overdosage were induced—vomiting, followed by weakness, apathy, and lethargy. (Cf. also 40.)

(Parathyroid extracts have been prepared which do not affect the level of blood calcium and which powerfully retard growth. Similar extracts may be obtained from other tissues, and the effect is not specific to the parathyroid glands, nor presumably concerned with their function (145).)

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## CHAPTER IV

### THE ISLETS OF LANGERHANS AND INSULIN

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#### Introduction

THE discovery of insulin by Banting and Best in 1921, working in Macleod's laboratory, and its preparation for clinical use, in which work Collip largely participated, led to rapid strides, not only in the treatment of diabetes mellitus, but also in the elucidation of many of the problems of carbohydrate metabolism. It seems desirable to set forth the main points, concerning which there is reasonable agreement, before considering in detail some of the more recent work (cf. 171, 241).

**Insulin** is a hormone 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 hyperglycaemia results, and if the condition of *hypoinsulinism* persists, all the symptoms and findings associated with classical *diabetes mellitus* ensue. If, on the other hand, through generalized hyperplasia 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 added further support to this view.

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 complete oxidation of the fatty acids to carbon dioxide and water through the stages of butyric acid and acetoacetic acid is gradually replaced by a slower transformation to acetone, a change so slow that acetoacetic acid and beta-hydroxy-butyric acid 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) is also capable of living for a number of years when maintained with insulin.<sup>1</sup>

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 (177). 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 (171), that the islets are

<sup>1</sup> *Choline and Lipocaic.* Depancreatized dogs tend to develop fatty livers. Best and his collaborators showed that the reason inclusion of raw pancreas in the diet of these dogs prolongs their lives is due to its choline content; it was later shown that certain proteins also produce a similar effect (Best and Huntsman, Channon and Wilkinson).

Dragstedt in 1938 prepared an alcoholic extract of pancreas which also maintained such dogs, preventing undue deposition of liver fat; this was considered to contain a specific compound, termed "lipocaic." Best and Ridout (18) have shown that the effect of the extract is only such as could be predicted from its choline and protein content (cf. also 10A).



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 was in part reopened by Bierry and Kollman (19), who, while not denying that the islet tissue has a special function, believed it to be formed from acinar tissue, although they consider that it cannot revert to acinar tissue. They claimed that even in fishes it is impossible to separate islet tissue completely from acinar tissue. (Cf. also Boldyreff (24).) We do not yet know of any direct relationship between the functions of the islet and acinar tissue. While Babkin (11) has demonstrated that the blood-sugar level conditions the enzyme production of the pancreas, hyperglycaemia increasing and hypoglycaemia decreasing the output of enzymes (probably through intermediary nervous action), Moreno (183) concludes from study of the effect of injections of insulin on the flow of pancreatic juice that there is no relationship between insulin and the external secretions. Hebb (104) has found some evidence of an indirect relationship.

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 (196).

The islets appear to be under the control of the vagus (171, 115, 277). La Barre (149) states 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. Etcheverry (61) finds that, although they are under vagal control, the islets, deprived of intrinsic innervation, can still regulate glucose perfectly, and Houssay (119) has grafted a dog's pancreas into the neck of a second, depancreatized dog, and obtained perfect function for many hours with this denervated preparation.



### The Chemical Nature of Insulin<sup>1</sup>

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

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 *pH* 4.2 to 5.3 pyridine precipitates various impurities. By addition of sufficient 0.65 per cent. ammonia the *pH* 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.** Insulin crystals are well defined; they seldom exceed 0.01 mm. in diameter. The compound appears to be dimorphous, though Crowfoot (48) considers that the crystals merely exhibit marked changes of habit.

Crystalline insulin gives the biuret, Pauly, Millon, and ninhydrin reactions, Sakaguchi's test for arginine, and positive tests for cystine. Tryptophane radicals, the sulphydryl group, and carbohydrate radicals appear to be absent. Hydrolysis yields the following percentages of amino-acids: lysine, 2.26; arginine, 3.22; histidine, 8; tyrosine, 12.20; cystine, 12.50; glutamic acid, 30.0; and leucine, 20.0. In addition phenylalanine and proline radicals are present in small but still undetermined

<sup>1</sup> The chemistry of insulin has been reviewed by Jensen and Evans (128), who give a complete bibliography, and, later, by du Vigneaud (256).

<sup>2</sup> For methods of preparation of insulin, see Harrow and Sherwin (102).

<sup>3</sup> 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. 31).



quantities. Eighty-six per cent. of the whole molecule has been accounted for, and there has been found no evidence of the presence of other organic radicals. All the sulphur, 3.2 per cent., is present in cystine radicals.

The molecular weight, as determined by the ultracentrifuge, is 35,100, the molecules being spherical, and, within the limit of error, of the same size as those of Bence Jones' protein, so that insulin can be and is normally excreted through the kidney glomeruli in small amounts (197, 28). On the basis of Bergmann's theory that such a molecule contains 288 amino-acid radicals the figures quoted account for 234, probably 6 each of lysine and arginine, 18 of histidine and cystine, 24 of tyrosine, and 72 each of glutamic acid and leucine.

Crystalline insulin contains a trace of zinc or some similar element (according to the salts present in the crystallizing medium), and such elements as zinc, cobalt, cadmium, and nickel are taken up in amounts proportional to their atomic weights. Zinc insulin crystals contain 0.52 per cent. of zinc, corresponding to three atoms in the molecule (237). Pancreas tissue contains 20 to 40 mg. of zinc per kilogram of fresh material, and it seems likely that its presence has functional significance, though Krebs and Eggleton (147) from *in vitro* studies of oxygen consumption in muscle tissue, consider that the zinc compound is not the physiological form of the hormone.

Crystalline insulin is optically active and laevo-rotatory; the rotation varies markedly with the *pH* of the solution. It dissolves easily in dilute acid and alkali and in 90 per cent. alcohol, and slightly in 80 per cent. alcohol. Its iso-electric point lies between *pH* 5.3 and 5.5.

Numerous attempts have been made to associate the physiological activity of insulin with some particular portion of its molecule; all the experimental results have led to the conclusion that the activity is associated with the molecular architecture of the whole molecule (cf. 256). In so far as further conclusion is possible, there is some evidence that the activity is essentially linked with the disulphide linkage of the cystine radicals.

**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  $23.3 \pm 0.6$  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 summary of the literature dealing with allergic manifestations following injections of insulin was published in 1932 by Allan and Scherer (4). They pointed 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 observations made at the Mayo Clinic, Allan and Scherer stated 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 (36) 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. Corcoran (43) has described a method of rapid desensitization, starting with very frequent but very minute doses.

Other still more unusual toxic manifestations have been recorded, as, for example, a transient haematuria (157); headache, dizziness, lack of muscular control (199), 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 (171). 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 (70, 76, 84). This normal difference tends to disappear in the diabetic, and the severer the diabetes, the more closely the curves approximate (62, 84, 213), illustrating loss of power to utilize glucose by the tissues. This power is restored by the action of insulin (171,



152, 46). Mann and Magath (178) 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 considered that the chief sites of insulin action are the cardiac and skeletal muscles (171).

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 (171), and Yovanovitz (276) has recently stressed the benefit to diabetic patients from the use of fruits rich in fructose, such as plums, pears, and figs.

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 (156) and Joslin (133) support the view that its action is limited to glycogen formation. Macleod (173, 174) 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 (68, 21, 221, 162, 45). 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 (44), a cycle which operates in the diabetic as well as in the normal animal (113).

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 (71).

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 insulin facilitates the reaction in the tissues between pyruvic and glycerophosphoric acids (151) and acts as catalyst in the citric acid oxidation cycle of Krebs (147).

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 (29, 123). This anomalous effect is not produced by crystalline insulin, and must therefore be attributed to impurities in the commercial insulin preparations (78, 270). Extracts of pancreas have been shown to produce hyperglycaemia when injected intravenously (172, 80), 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 (78) 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."



Vitamin B<sub>1</sub> (thiamin) increases the hypoglycaemic response of rats to insulin (30).

Certain results which follow the injection of insulin, such as the increased excretion of allantoin in normal dogs, and of uric acid in the Dalmatian coach hound are due to the increased output of adrenaline resulting from insulin stimulation (38A).

*Control of Insulin Secretion.* There is evidence of control by vagal stimulation, an extra, rather than an essential mechanism (cf. p. 139). Increase of blood glucose above fasting level acts as a stimulus to secretion of insulin, independent of extrinsic innervation of the pancreas (67). One or more of the pituitary hormones exert direct or indirect control (cf. Chapter VIII).

### Terminology of Diseases Associated with the Islets of Langerhans

"Diabetes mellitus," strictly speaking, only names a symptom, and one which is not specific to the disease. Harris (100) has suggested the term *hypoinsulinism* as more appropriate. It is becoming more and more recognized, however, that diabetes mellitus is a disease associated with a disordered carbohydrate metabolism, but which may or may not be associated with hypoinsulinism, though in the majority of cases exhibiting the classical syndrome a hypoinsulinism undoubtedly exists.

Houssay defines diabetes mellitus as a disturbance of the carbohydrate metabolism in which the normal balance of the (endocrine) regulatory factors is altered. He considers that while, in diabetes mellitus, there is always a relative insufficiency of insulin for the needs of the organism, the actual amount secreted may be normal or even above normal (119). Long has enunciated similar views (163). One or more hormones of the anterior pituitary are involved with insulin, and a hormone of the adrenal cortex, and possibly still other hormones, in an endocrine balance, any disturbance of which may result in a diabetes mellitus.

It is well recognized, for example, that some proportion of acromegals exhibits glycosuria, and a still smaller proportion a true diabetes mellitus, and that this diabetes sometimes disappears, either spontaneously as the acromegaly "burns



itself out" (44) or after removal of a pituitary adenoma (cf. 58).

Some discussion of the complicated endocrine control of carbohydrate metabolism is given in Chapter VIII.

Harris (100) recognized in certain patients symptoms which were identical with those resulting from overdosage of insulin, and coined the term *hyperinsulinism* for their condition. In using this term it must be remembered that while hyperinsulinism connotes hypoglycaemia, hypoglycaemia does not necessarily also mean hyperinsulinism.

### 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 (13, 106, 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 summarized the literature critically (93). 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 glycuria*, *diabetes innocens*, and *benign* or *innocent glycosuria*. Of all such terms *negligible glycosuria*, suggested by Leyton (160), 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 (86).

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 (263). 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 (208). 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 (62).

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 (189).

Cases of hyperthyroidism frequently exhibit a glycosuria, but the simultaneous occurrence of hyperthyroidism and diabetes mellitus is rare (136).

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 (185) has reviewed the literature.

### **Diet in the Treatment of Diabetes Mellitus**

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 (201) and by Newburgh and Marsh (192). 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 hormone 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 the reduction of glycosuria to negligible amounts, and also production of a normal level of blood sugar for at least some part of each day, while any dangerous degree of hypoglycaemia must be avoided. The necessary balance is more delicate; its maintenance requires more care.

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 (98). The explanation which is usually accepted of this—the so-called “Traugott-Staube effect”—is that offered by Macleod (171), that the first dose of sugar sensitizes the islet-mechanism, so that the second dose calls forth insulin more readily.

Sweeney, in 1927 (253), 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 (205). 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. Hence the clinical treatment of severe diabetes which Porges and Adlersberg advocated: little fat (even as little as 50 grams), in a diet yielding a total calorie value of 3,000–4,000 calories, of which the caloric value is mainly provided by carbohydrate, the usual amount of protein being given and the necessary insulin.

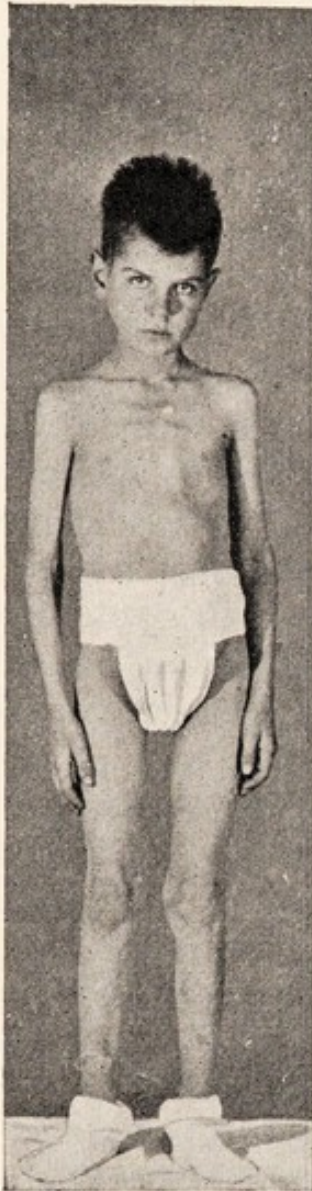
With this *régime* tolerance for carbohydrate gradually increased and less insulin was required. Their diabetics put on weight and liked their diet better.

Rabinowitch (215) 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 many of his cases it was noteworthy that transference from relatively low to relatively high carbohydrate diet—with corresponding diminution in fat—not only did not require increased insulin dosage, but even lessened or abolished the need for it. He has recently reported on fifty cases kept on this treatment for five years or longer.

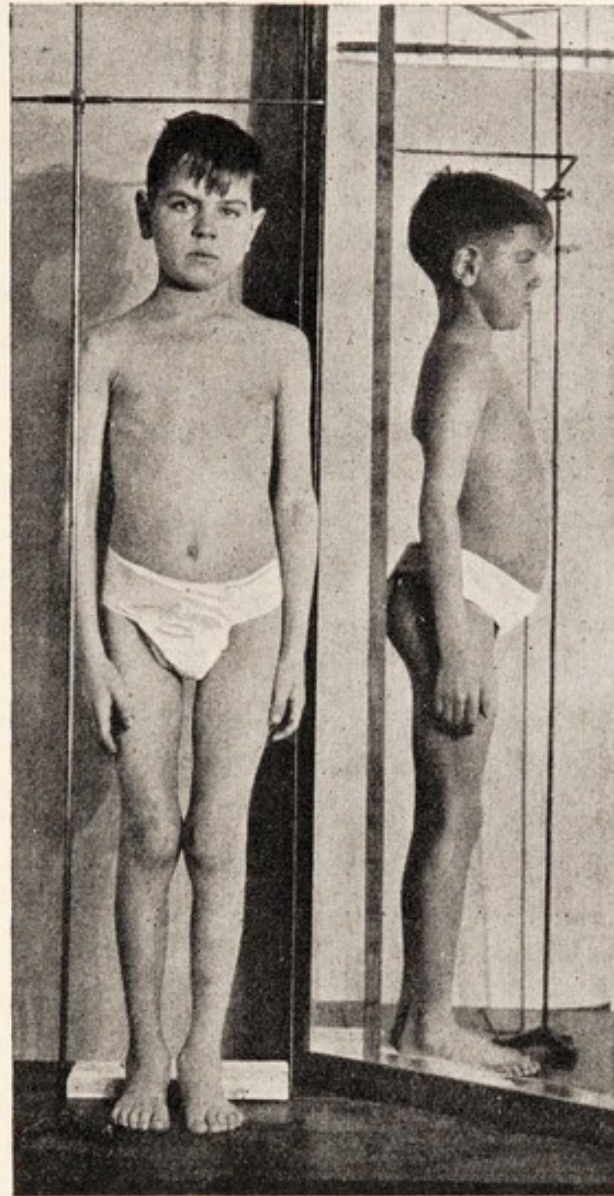
Sansum (236) recommends a carbohydrate to fat ratio of 2 to 1, or even (especially with children) of 3 or 4 to 1, with adequate caloric requirement to maintain normal weight. He obtained excellent results with such diets. In 1933 (236) he reported on seventy patients who had maintained such a diet for seven years. All showed increased well-being and physical fitness; forty-two showed an increased sugar tolerance. Geyelin (79) has obtained equally good results over a ten-year period.

We have obtained, in Winnipeg, excellent results with normal carbohydrate and normal fat diets, fully bearing out





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. (Reproduced by the kindness of Dr. H. Medovy.)



the general principles just described (cf., *e.g.*, 180). 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 many clinicians (cf., *e.g.*, 226, 81, 126, 207, 261). It is the general experience that when patients are changed from a high fat, low carbohydrate to a low fat, high carbohydrate diet, insulin requirement 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 (235, 205), has been suggested.

Ellis (59) 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 (110) has carried out careful experiments on normal healthy subjects which completely confirm the results of Hammon and Hirschman, Sweeney, and Porges and Adlersberg. 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.) (Cf. also 167.) 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 caloric value nor to change



in ketogenic-antiketogenic ratio, but solely to the increased amount of carbohydrate in the diet (111).<sup>1</sup>

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.

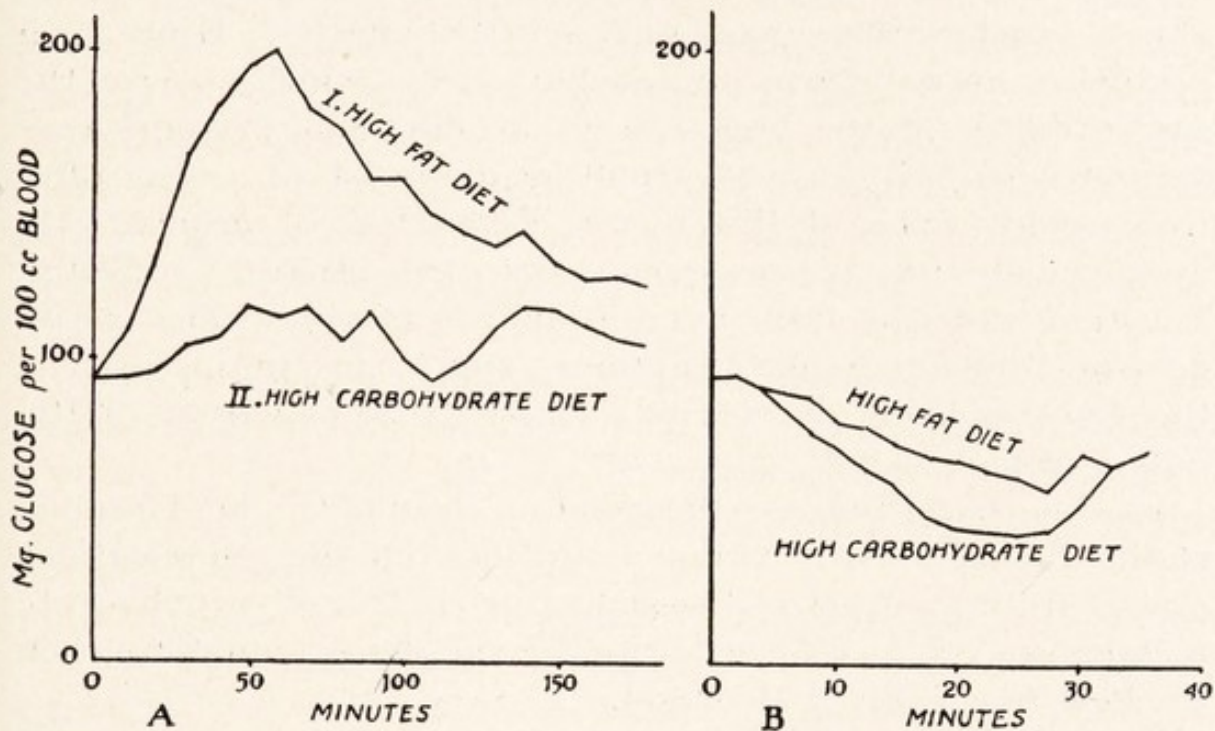


FIG. 19. A. Two sugar tolerance curves after 50 grams of glucose, determined on the same healthy subject : (i) when accustomed to a high fat diet ; and (ii) when accustomed to an equicaloric high carbohydrate diet. B. Two blood sugar curves following intravenous injection of 5 units of crystalline insulin. 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.)

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 (277, 110). In the diabetic with partial function the

<sup>1</sup> Vesa's experiments on diabetics are apparently in contradiction, but these were merely studies of the effects of single meals of fat, or meat, or bread on the effect of an insulin dose, and are in no way comparable (255A). His bibliography is useful.



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. He suggests that 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 (112).<sup>1</sup>

In this connection the theory of an insulintrophic hormone of the duodenum may have application, but the complications arising from an upset of the endocrine control of carbohydrate metabolism (cf. p. 146) may afford a simpler explanation when they are more fully understood.

The view-point of the average diabetic clinic, as expressed by various recent reports, indicates a slow but a steady change towards increased carbohydrate. In a review of this dietary problem in 1933 (134) Joslin stated 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 (154). He gives from 100 to 150 grams of carbohydrate, carefully

<sup>1</sup> In a recent paper (112A) Himsworth has modified his views, and regards insensitivity as due to a retardation of insulin action (and not to a neutralization of that action).



controlled in amount and balanced with insulin, and allows the patient to suit himself as to fat and protein intake.

*Insulin Resistance.* Diabetic patients exhibit very varying response to insulin, which has led to the idea of insulin resistivity.

For example, Wiener (271) has reported the case of an elderly diabetic whose diabetes rapidly became severe, so that he was admitted to hospital in a pre-coma state and needed 3,250 units of insulin in twenty-four hours to prevent coma, subsequently requiring 440 units a day to establish his balance.

Glen and Eaton (85) report a case in which high insulin resistance was exhibited, and claim that injection of the patient's serum into rabbits set up in them active resistance to insulin injections. Dohan (52) was unable to obtain such results.

de Wesselow and Griffiths (265) consider that the degree of resistance to insulin is determined by the general metabolic condition. Increased dietary carbohydrate lessens the resistance, and the more nearly normal the diet the less insulin resistance is likely (cf. also 110, 112).

*Liver Rhythm.* The conclusion of Forsgren and others (69, 117, 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 (194) suggests that insulin dosage should be adjusted to this rhythm rather than to meal hours.

### **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 (153): "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 (187) 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 (186). The claim has not been substantiated, and there is obviously potential danger of toxic action on the kidneys from the phosphotungstate (153). 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 (250). The claim is not supported (17). Administration with liver extract is said to favour absorption from the stomach (17).

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 (259, 118).

**Insulin Administration by Inunction.** Claims have been made that this procedure yields good results, both in the experimental animal (108) and with diabetic patients (211). A considerably greater dosage is required than by subcutaneous injection.

**Insulins with Delayed Action.** The insulin secreted into the circulation from the islets of Langerhans under the action of physiological stimuli is provided in minute amount continuously or semi-continuously. The insulin injected subcutaneously as replacement therapy into the diabetic patient two or three times a day is absorbed into the circulation fairly rapidly, so that the available concentration in the blood at different times shows fluctuations much greater than normal. As a result, the blood sugar of the diabetic patient, even when this *soluble*



insulin is administered under the best conditions, also shows greater fluctuations than normal.

Within the last few years insulin compounds have been prepared of such slight solubility in tissue fluids that their use permits imitation of the normal function of the islets of Langerhans to much greater degree. By contrast with these relatively insoluble compounds, ordinary preparations of crystalline or amorphous insulin are now usually referred to as "soluble insulin."

The iso-electric point of insulin, its zone of least solubility, lies at  $pH$  5.3. Tissue fluid is less acid; its  $pH$  varies from 6.5 to 7.5, from slightly acid to slightly alkaline. In consequence, tissue fluids hold insulin in solution fairly easily.

The insoluble insulin now chiefly used for diabetics, *protamine zinc insulin*, is the result of studies of Hagedorn and his collaborators in Copenhagen (97), and of observations of investigators of the Toronto school.

Insulin is a protein, and therefore amphoteric, capable of combining with acid or with base. Its compound with nucleic acid has an iso-electric point still more acid than that of insulin itself. Hence Hagedorn prepared compounds with the alkaline proteins, the histones and protamines. Compounds with the protamines clupein (from herring sperm), scombrine (from mackerel sperm), and salmine (from salmon sperm) still proved too soluble in tissue fluids, but the compound formed by insulin with the protamine prepared from the sperm of the rainbow trout, *Salmo irideus*, proved to be only very slightly soluble at  $pH$  7.3, and its extensive trials on diabetic patients gave most favourable results, the insulin effect being prolonged, and there being no rapid sudden drop in blood sugar level (97).

Scott and Fisher (238) found that a concentration of 0.1 per cent. zinc greatly delayed and prolonged the effect of insulin in rabbits, and exaggerated the delaying effect of protamine. As a result, "protamine zinc insulin" has become generally available for the treatment of diabetics.

Both the original protamine insulinate, and its modification with zinc, have been received with a practically unanimous chorus of praise, and the papers recording success with them are far too numerous to list. Root, White, Marble and



Stotz (229) first confirmed the statements of the Danish investigators concerning protamine insulinate, and these statements were further quickly endorsed by the Toronto school (145), Lawrence and Archer (156), Sprague (248), and many others.

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

Some of the important papers dealing with protamine zinc insulin are those of Wilder (272), Rabinowitch (218), McCullagh

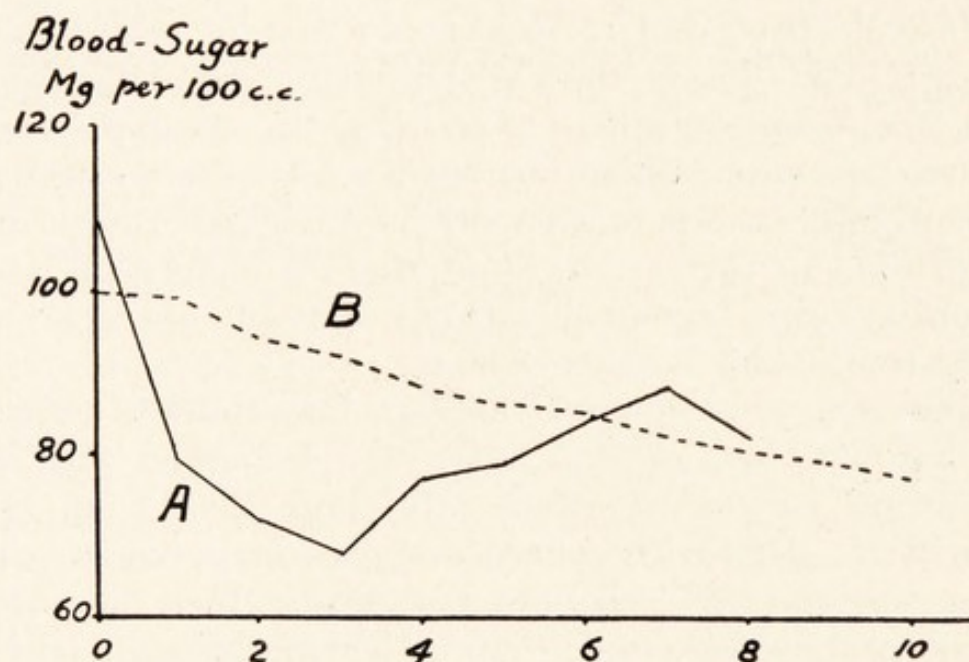


FIG. 20. Contrast of the effects of insulin and protamine insulinate on the blood sugar. Miss M. G., normal, aet. 25, nurse-dietitian. Both tests after an over-night fast. No food taken during the tests. A. October 23rd. Effect of 8 units of insulin (Lilly) given subcutaneously. B. October 25th. Effect 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.)

(166), Dunlop (56) and Lawrence (155). In these the clinical aspects of treatment are fully dealt with.

Protamine insulinate was originally prepared by adding together solutions of protamine, phosphate (as buffer), and insulin, under sterile conditions. The precipitated insulinate was injected in suspension. The mixture was not very stable, and thus needed frequent fresh preparation.

Scott and Fisher (239) have succeeded in preparing satisfactory protamine zinc insulins with various protamines. The



advantage of addition of a trace of zinc salt is twofold. The "insulinate" is stabilized, so that a single solution can now be furnished commercially. In addition, as has been stated, the zinc exaggerates the protamine effect, possibly by still further decreasing solubility (cf. 63).

Addition of a trace of calcium salt also increases stability,

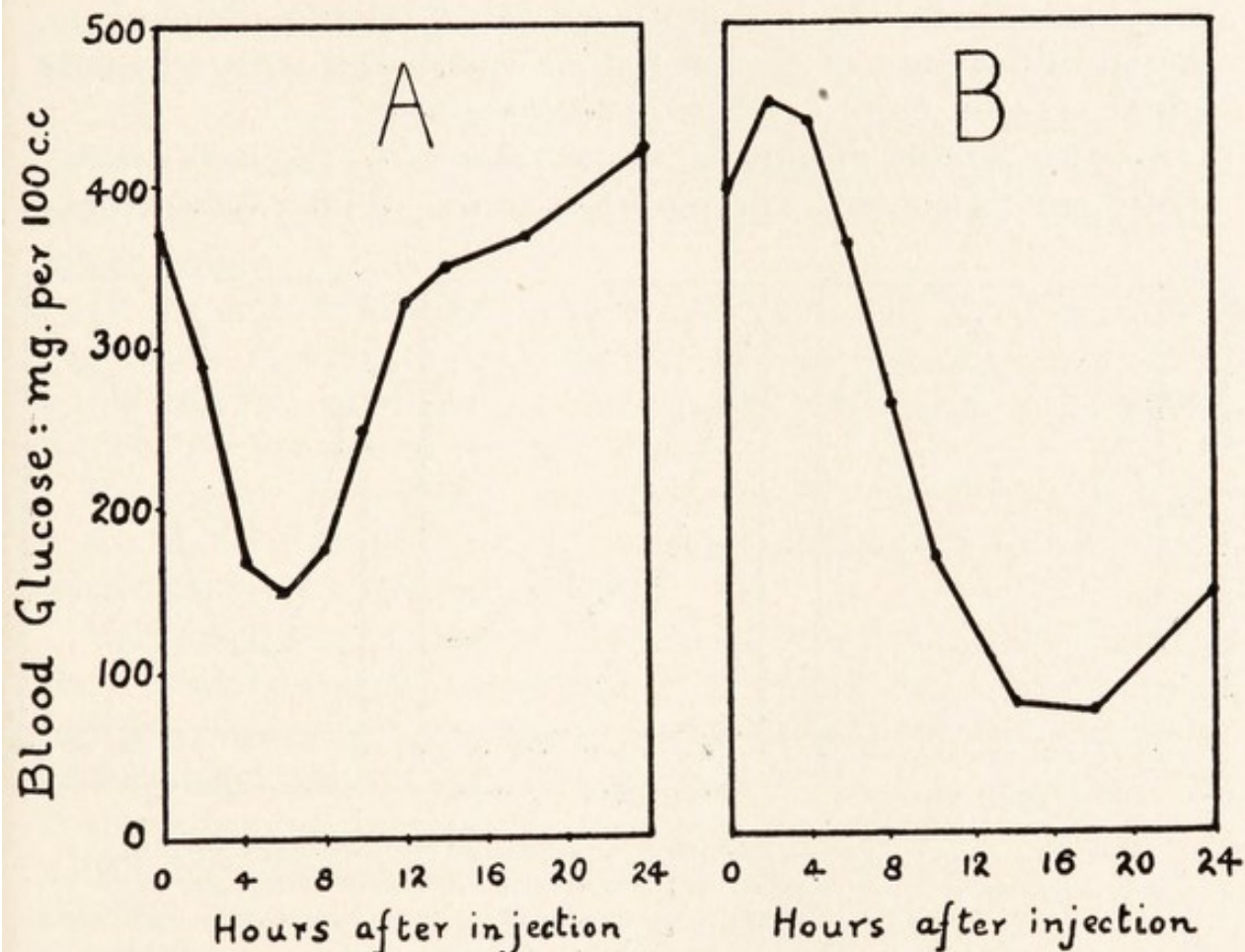


FIG. 21. Comparative duration of action of (A) ordinary insulin, and (B) protamine insulin, as contrasted on the same severely diabetic patient under like conditions. Injection (35 units) at 8 a.m. Breakfast twenty minutes later (30 grams carbohydrate, 18 grams protein, 44 grams fat); no noon or evening meal. At the end of twenty-four hours in test (A) there was marked acidosis; in test (B) there was no evidence of acidosis, and no glycosuria. (After Wilder, *Ann. Int. Med.*, 1937-38, xi, 14.)

but lessens the delaying effect of protamine (272). Such effects are illustrated in Figs. 21 and 22.

Protamine zinc insulin possesses the great advantage over ordinary soluble insulin that, with many cases of mild diabetes,



a single morning injection suffices for the twenty-four hours. In more severe cases it is possible to combine one morning injection of protamine zinc insulin with one of soluble insulin, though mixture before injection is to be avoided, since excess protamine may precipitate the soluble insulin (272).

While the delayed action renders protamine zinc insulin inadequate for treatment of diabetic coma, yet claims have been made that, both in acidotic conditions and in actual coma, the additional use of the protamine compound with adequate soluble insulin improves treatment (218, 272).

As with soluble insulin, opinions differ as to the best dietary treatment to use with the insoluble form. Wilder (272) thinks



FIG. 22. Comparative duration of action of (A) ordinary insulin, (B) protamine insulin in presence of a trace of zinc salt, and (C) protamine insulin in presence of a trace of calcium salt, as tested on the same diabetic patient under comparable conditions. In each case 50 units were injected; food was withheld during each forty-eight-hour period. (After Wilder, *Ann. Int. Med.*, 1937-38, xi, 16.)

that carbohydrate should not exceed 150 grams per day, though he admits that some patients react well to larger amounts. Rabinowitch (218) gets good results with high carbohydrate diet, and considers that protamine zinc insulin improves carbohydrate tolerance.

According to McCullagh (166) a single dose of protamine zinc insulin produces an effect during a period of fifty to sixty-five hours, and maintains its maximum effect twelve to eighteen hours. Obviously a single daily dose can produce a cumulative



effect, which may ultimately lead to hypoglycaemia, but a hypoglycaemia far more delayed than that produced by ordinary insulin. As Wilder (272) has pointed out, this hypoglycaemia sets in so insidiously that the secondary symptoms attributable to adrenaline (cf. p. 170), and acting as danger signals, the tremor, sweating, tachycardia, and increased pulse rate, may not occur. The chief effects noticeable in this delayed hypoglycaemia may thus be only those of cerebral origin—lassitude, fatigue, and headache or nausea—and loss of consciousness is not likely to occur. Yet Bollman's animal experiments (quoted by Wilder) indicate that prolonged hypoglycaemia may give rise to dangerous brain lesions.

Hence it seems desirable that patients using the new insulin should be trained to take greater precautions than before to avoid the effects of fasting, and should take sugar at frequent intervals whenever meals are missed.

Protamine zinc insulin has given equally satisfactory results with diabetic children (269). Comparable effects are given with depancreatized dogs (146).

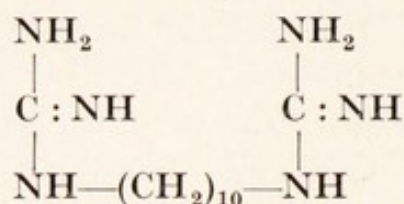
Various other mixtures have been tested, many of which show evidence of the same type of improvement over ordinary insulin. Indeed, it is quite probable that further search may find forms of combined-insulin which will be even more beneficial than protamine zinc insulin. Good results have been obtained with insulin tannate (91) and insulin zinc tannate (127) (though this has a tendency to produce irritant skin reactions in some patients), thymus-histone insulin (91), globin zinc insulin (225, 15A), and spermine zinc insulin (239). Warburton has prepared a compound of hexamethylene tetramine and insulin, which, it is claimed, gives both immediate and sustained effect, and is particularly useful with the young diabetic (64A).

**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 (260) 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. His search led to the synthesis of diguanidinodecamethylene, or synthalin (72) :



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 (87). More recent results led to no more favourable conclusions, either regarding synthalin or Frank's later preparation, dodecamethylene diguanidine (synthalin B) (72), but threw 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 (21), and liver glycogen is caused to disappear (232). Not only is the respiratory quotient not increased (105) but oxidation seems actually to be inhibited and the formation of lactic acid increased (249).

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 (21, 144, 20). Comparison of various synthalin homologues shows that the toxicity and hypoglycaemic effects run parallel (20).

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

Labbé (148) 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.

Large and Brocklesby (150) have shown that an extract from the roots of the Devil's Club (*Fatsia horrida*) has definite hypoglycaemia-producing properties.

**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 (200, 222).

Proferin, a flour largely consisting of a plant protein, has been advocated recently, but is of no particular benefit (198).

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

### 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 (258). 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 (133), 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 (109).) Himsworth speaks of a dietary disposition. He has attempted to determine what were the diets of diabetics prior to their diabetes (111), and considers that he has obtained evidence that they had a relatively high fat content and diminished carbohydrate content. Since he has shown (p. 152) 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.

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 (143); 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" (216, cf. also 188).

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 (227).

Murray-Lyon finds a hereditary incidence in about 16 per cent. of diabetics (189); Cammidge finds it in 40 per cent. (35); Joslin, in 25 per cent. (137). 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 (255, 204).

Mosenthal (184) has attempted to correlate pancreatitis and diabetes. He believes that the chronic pancreatitis in elderly persons, resulting from arteriosclerosis and senescent processes, leads to the slowly progressive diabetes of middle and old age. In chronic pancreatitis from other causes there is usually too little destruction of islet tissue to lead to diabetes. Repeated attacks of mild acute pancreatitis may lead to interference with carbohydrate and fat metabolism and ultimately to the turbulent diabetes of the young. Modern views, however, based on experimental evidence, are tending to regard diabetes mellitus as essentially due to a disturbance in the balance of the hormonal forces controlling carbohydrate metabolism (cf. p. 146), a theory which emphasizes the probability of multiple causes.

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 cases, quoted by Joslin (133)) and in certain hyperpituitary cases, in which the diabetes is not due to hypoinsulinism, complete recovery has so far not been recorded.

The diabetic child affords the most interesting material for prolonged study of the effect of insulin. Priscilla White (266) has 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 favourable prognosis.

Marble, White and collaborators (179) call attention to the not infrequent occurrence of gross enlargement of the liver in children with severe, poorly controlled diabetes (the protuberant abdomen suggests at first sight the possibility of von Gierke's



disease). They present detailed findings in sixty cases, in thirty-one of which the spleen was also enlarged. There are frequent complications, as diabetic coma and acidosis, hypoglycaemic attacks, dwarfism, arteriosclerosis, neuritis, etc., and the dwarfism, protuberant abdomen, and bouts of abdominal pain are particularly striking features. The liver enlargement is primarily due to gross fatty infiltration. It is not due to lack of choline (cf. p. 138), since the feeding of raw pancreas is without effect. The majority of cases treated with protamine zinc insulin show some diminution in the size of the liver.

Priscilla White (268) has recently reviewed a series of 1,250 patients with juvenile diabetes from the angle of Houssay's theory of the nature of the disease (p. 146). She stresses the lack of evidence of a pathological pancreas in these children. She considers that 176 of her cases showed evidence of prolonged pituitary involvement, in almost all cases a hypoactivity. These included ninety-four dwarfs, twenty-two with signs of Fröhlich's syndrome (cf. p. 350), and fifty-one with signs of infantilism. The dwarfism followed and did not precede the diabetes, and was usually not recognized till the fifth year of the disease. It was not the result of treatment and was of pituitary type. It seems possible that in such cases both diabetes and dwarfism are due to pituitary deficiency, and that the hypoinsulinism is only relative.

Such observations further stress the multiple causative factors of diabetes.

As regards the general results of insulin therapy the remarks of Bowen have pertinence (26): "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.

Joslin (138) recently stressed the continuous decline in the death rate of diabetics, although pointing out that it is still much in excess of that of the general population (cf. also 135).

**Arteriosclerosis and Hypertension in Diabetes.** Joslin,



writing in 1928 (133), said, "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." Increased blood cholesterol has been suggested as one of the causes of arteriosclerosis (9) and as predisposing to diabetic gangrene (214, 264), and such hypercholesterolaemia has been viewed as due to persistent use of a high fat diet (cf. 217), a view which has not met with complete acceptance (132).

Hypertension is a frequent accompaniment of diabetic arteriosclerosis, but all the characteristic vascular lesions of diabetes (retinal haemorrhages, coronary occlusion, gangrene) are found in diabetics with normal blood pressure as well as those with hypertension, though presence of hypertension increases the incidence of these lesions. Root and Sharkey (230), in reviewing a number of cases, conclude that premature and excessive development of vascular disease in the diabetic occurs predominantly in muscular arteries under the greatest physical strain, especially in obese patients, and is due to the metabolic changes of the diabetes itself, and probably to a disordered lipide metabolism. Hypertension is an important contributory factor since it imposes additional strain. They conclude further that insulin with modern dietary treatment is lessening the frequency of arteriosclerosis in the legs of children.

Arteriosclerosis is apparently responsible for neuritic symptoms (pain) in diabetes. Sandstead and Beams (234) state that oral administration of sodium chloride relieves such pain. In this connection the observation of McQuarrie (175) is interesting. He finds that excess sodium (as sodium chloride) exerts a favourable influence on the carbohydrate metabolism of diabetic children kept on diets low in potassium, but elevates their blood pressures significantly. Potassium salts have the opposite effect. (MacLean has observed that increased salt intake increases sensitivity to insulin (170A).)

Perhaps the neuritic symptoms are associated with the lowered phospholipide, cholesterol and cerebroside content of the nerves from diabetic patients, which Jordan and Randall (131) believe is associated with arteriosclerosis in the diabetic. Jordan describes fully these neuritic manifestations (131).

**Other Diabetic Complications.** Hepburn and Graham (107) 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 (193). 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 (210). Similar effects were not produced by injection of insulin into fatty tissue in normal rats (224).

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" (133). 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 (257, cf. 169). Foetal mortality is not lessened by such insulin treatment (244) and exact control of the pregnant diabetic is necessary to prevent undue demands on the islet tissue of the foetus (55). In fact Priscilla White has recently written that prevention of the death and decay of the over-ripe 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 (267). Potter and Adair (206) quote statistics which indicate that while the use of insulin has markedly reduced maternal mortality, foetal mortality is still 30 to 40 per cent. Izquierdo (124) 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.

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.

### 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 (100).

Since Harris's 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 (240, 77) comparable with the sequelae of removal of the liver in dogs, reported in Mann and Magath's classic experiments (177) and with the symptoms observed in diabetic patients following overdosage with insulin (101). 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 (116).

Experimental evidence is becoming available that such brain starvation does actually occur (49, 14). It has been shown that chronic insulin intoxication in rabbits and dogs produces marked, though not specific, histological changes in brain tissue (251, 92). Some investigators would trace this to direct action of insulin on



brain tissue (cf. 176). Studies of schizophrenics in induced hyperinsulinism (cf. p. 179) scarcely support this view (cf. 114).

Wauchope has published an excellent review of the subject (262). 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" (77).

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 (54). Wauchope, using what I have elsewhere termed "enhanced-glucose" values (33), 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. (262). 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 (262, 252). Such cases, if they do occur, are rare.



The glucose tolerance curve of hypoglycaemic patients is frequently apparently normal for the first three hours (or may even show an undue rise at the end of one hour); at the fifth or sixth hour such curves usually drop to low levels.

Dorst (53) considers that a certain type of patient, often under weight, exhibiting a clinical picture described as a neurocirculatory and effort syndrome, and giving a low, flat glucose tolerance curve, is not to be classed as hypoglycaemic, and indeed is frequently clinically improved by small doses of insulin, which lead to increased appetite, gain in weight, and a more normal tolerance curve.

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.

Hypoglycaemia of such types has been termed "spontaneous," as contrasted with that induced by overdosage of injected insulin. Wilder (272A) suggests that "paroxysmal hypoglycaemia" is a better term, since the syndrome is characterized by periodically occurring attacks of hypoglycaemia, which are frequently accompanied by actual paroxysms (his paper contains a useful bibliography).

**Cases Associated with Tumour of the Islets.** The first definite case was reported by Wilder, Allan, Power, and Robinson in 1927 (273). The patient exhibited marked hypoglycaemia. His condition was inoperable and became progressively worse until half-hourly doses of glucose were necessary to prevent convulsions. Blood sugar analyses included figures below 0.03 per cent. Post-mortem examination revealed a carcinoma of the islets with metastases in the liver. An extract of these carcinomatous metastatic nodules was made; injected into an animal, it produced insulin action. A similar case has very recently been reported from the same clinic (209), in which also an extract of the liver metastases produced an insulin-like effect. Similar cases have been reported elsewhere (*e.g.*, 158, 254) 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 (120) 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 (165) 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.*, 275, 50, 37, 74, 141). The tumours are more frequently benign than malignant, though Judd and Rynearson (140) consider them to be either malignant or pre-malignant.

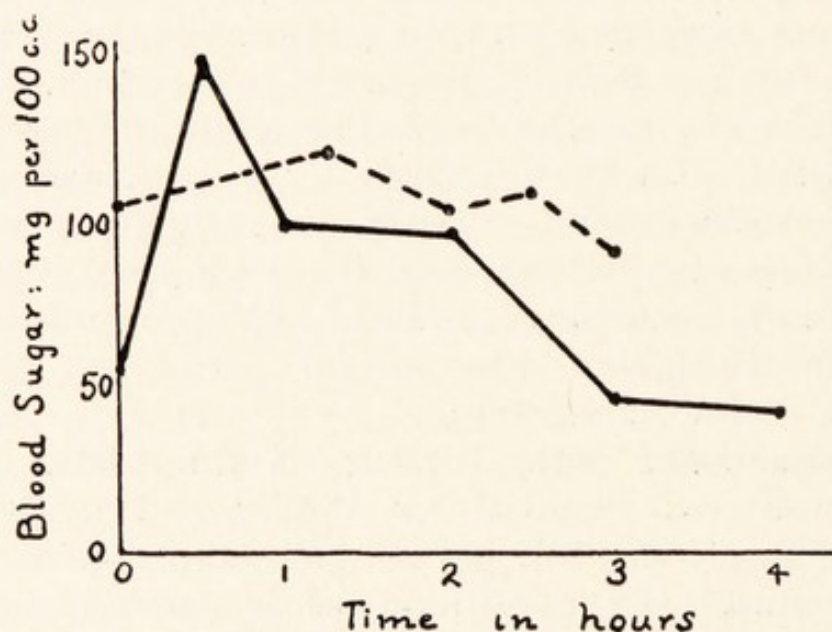


FIG. 23. Hyperinsulinism. The continuous line represents blood sugar values after administration of 50 grams of glucose. Prior to the test the patient had been fed a high carbohydrate diet for a week, while food was withheld for twelve hours immediately prior to the sugar meal. At subsequent operation a small adenoma of an islet of Langerhans was removed. The dotted line represents a similar sugar tolerance curve taken six months later. (After Fraser, Maclay and Mann, *Quart. J. Med.*, 1938, vii, 115.)

Fig. 23 shows sugar tolerance curves before and after removal of an islet adenoma.

Whipple and Frantz (270A), in a useful review of the surgical



treatment of the condition, reported that in two of their six cases multiple islet adenomas were present.

**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 (190) reported a case exhibiting marked hypoglycaemia and convulsions; at subsequent autopsy the liver was found to be riddled with carcinoma. Crawford (47) 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 (142) 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 (139), or with phosphorus poisoning (170), or with acute yellow atrophy of the liver (64), or in chloroform poisoning in dogs (23). Lowered blood sugar also occurs along with parenchymatous changes in the liver caused by arsphenamine, by hydrazine, or by the fungus *Agaricus bulbosus* (203). The occasional cases of hypoglycaemia seen in pernicious vomiting of pregnancy (34) 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. 96), 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. 33.)

**Hypoglycaemia of Uncertain Cause.** (a) *Hypoglycaemia Possibly Associated with Hyperplasia of Islet Tissue.* In a number of cases, where exploration did not reveal a tumour, resection of a portion of the pancreas has been performed, in belief that hyperplasia of the islets was responsible for the hypoglycaemia. Overactivity of the islets due to hyperplasia is difficult to demonstrate, and the earlier surgical efforts were not definitely beneficial. More recently, in cases in which the greater part of the pancreas has been removed, sugar tolerance was undoubtedly improved (cf. 88, 16).

(b) *Hypoglycaemia in Young Children.* Gray and Feemster (90) reported in 1926 the case of a premature child born of a diabetic mother, which exhibited somewhat low blood sugar and died on the third day. Autopsy showed apparent hypertrophy and hyperplasia of the islets. In a similar case Randell and Rynearson (220) removed a living child by Caesarian section, and kept it 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 0.04 per cent.

Hartman and Jaudon (103) have reviewed 286 cases of hypoglycaemia in the St. Louis Children's Hospital over a fifteen-year period, and draw the following conclusions: Hypoglycaemia not infrequently occurs in normal new-born infants during the first four or five days of life, and in them seems to be due to an imperfectly developed regulatory mechanism which creates a state of relative hyperinsulinism. At the same period the children of diabetic mothers may show a more severe condition, with, sometimes, development of convulsions and collapse. This is probably due to increased physiological hyperactivity of the islets (from the hyperglycaemic maternal stimulus) rather than to hypertrophy or hyperplasia. It can be controlled in emergency by adrenaline or intravenous glucose, with the prophylactic use of supplemental milk and carbohydrate feeding immediately after birth. The natural tendency to hypoglycaemia in the newborn is intensified by adrenal or intercranial haemorrhage.

On the other hand, a case of pregnancy complicated by



hyperinsulinism in the mother has been reported (159), in which the child was apparently normal (while the pregnancy reduced the sugar tolerance of the mother).

Rector and Jennings (223), from a study of eleven cases, consider that the rare occurrence in children of hypoglycaemia with recurrent convulsive manifestations is usually found between the ages of one and three years, and is due to a functional hepatic disorder of intermittent character, associated with a temporary depletion of the glycogen reserve, and in most cases is amenable to conservative treatment. Intravenous glucose abolishes the convulsions and a diet high in carbohydrate prevents their recurrence. (Graham and Hartman's case (88), a girl only twelve months old, exhibiting only 18 mg. per cent. capillary blood sugar, and benefiting from resection of most of the pancreas, would seem to indicate that not all hypoglycaemias in this age group derive their hypoglycaemia from the same cause.)

(c) *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 (77, 242, 168), but this cannot be regarded as established.

Hypoglycaemia, sometimes sufficiently recurrent to produce persistent symptoms, has been associated with severe burns (94), premature labour (202), adrenal insufficiency, including Addison's disease (7, 219), pituitary tumours (274), menstruation (100), chronic infections, recurrent bilious attacks, neurasthenia, etc. (34, 60).

It should be remembered that the hypoglycaemia in Addison's disease is only slight, while the marked hypoglycaemia sometimes met with in hypopituitary conditions (272), and generally present in von Gierke's disease, is not accompanied by symptoms. In myxoedema also hypoglycaemia may occur in absence of symptoms (262).

An association between anginal pain and hypoglycaemia seems possible (247); cases have been reported of cardiac pain due to insulin overdosage (182). 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 (246).

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 (262).

**Association of Hypoglycaemia with Abnormal Mental States.** Shih-Hao and Hisao-Chien (243), 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 (34), 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 (231) has reported three cases exhibiting severe hysterical attacks; one progressed to an epileptiform state, which proved to be due to hypoglycaemia. McGovern's case (168) showed frequent attacks of amnesia and coma, often accompanied by convulsions of epileptoid type. During the convulsions the blood sugar fell to 0.03 per cent. Treatment with carbohydrate every hour warded off attacks for a period of eighteen months.

Of particular significance is the psychiatrist's report in a case reported by Finney (65): "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 (95). Gray and Burtness (89) 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.

Gilmour and Walton (83) have reported a case of islet adenoma, verified at operation, in which the symptoms were first thought due to an intracranial lesion, a view supported by the findings of a ventriculogram and raised globulin content of the cerebrospinal fluid.

Aitken (3) draws attention to marked residual mental deterioration occasionally noted after operation, and possibly due to prolonged malnutrition of brain tissue. Prolonged experimental hyperinsulinism in animals indicates such a possibility (cf. p. 169).

That the neurological manifestations of hypoglycaemia are due to complex factors of which the hypoglycaemia is but one is suggested by the observation of Hrubetz (121) that administration of phenobarbital along with insulin to rabbits prevents development of convulsions, although the hypoglycaemia is produced as usual.

#### **Differentiation and Treatment of the Causes of Hypoglycaemia.**

Harris (100) 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 (100, 77, 168, 34).



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 (100, 242). John (129) recommends insulin and a high fat diet in the functional type of case.

As Wilder (273) points out, the exercise of the hypoglycaemic needs control; increase in exercise needs appropriate increase in food intake.

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 (140) 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 (89) 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 effect of parenteral administration of any endocrine principle is inversely proportional to its concentration in the body (cf. p. 4), 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. Fraser (73) supports the test. Baudouin states that injection of insulin into myxoedematous patients gives curves of the hypoglycaemic type, which are changed to normal type by thyroid administration.

### **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 (40, 195, 51, 6, 12). Many cases of malnutrition have benefited (245, 181, 25, 38), but Freyberg thinks the benefit is due to suggestion (75). 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 (247).

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 (22).

Insulin-glucose is of value in combating acidosis, recurrent vomiting and acute intestinal intoxication in children (82), and the acidosis developing in prolonged narcosis produced for therapeutic purposes in certain types of mental disease (212). It is said to be a useful adjunct in the treatment of drug addiction (27, 39) and peptic ulcer (130, 32). Good results in coeliac disease have been claimed by several writers (cf. 267).

In 1935 Sakel (233) published his method of treating cases of schizophrenia by repeatedly inducing hypoglycaemia to the stage of convulsions by injections of insulin. Very many papers



have appeared during the past four years, dealing with Sakel's method. The general conclusions seem to be that the treatment is distinctly beneficial, although the degree of persistence of the cure and the extent to which it exceeds spontaneous remissions still need careful evaluation. It definitely decreases the period of hospitalization (cf. 125, 42). Protamine zinc insulin is relatively inefficient for such treatment (85A).

Robinson (228) has reported good results in six out of nine cases of acute alcoholic psychosis, achieved by similar treatment.

The use of insulin as a dressing for chronic indolent ulcers has been suggested (161), but apparently without justification (122).

Several writers claim that it is useful in certain dermatoses, especially those associated with a disturbance in carbohydrate metabolism (25, 1, 191). Benefit has been claimed in cases of hyperthyroidism, arteritis (25), typhus, chronic uraemia, cholelithiasis, and melanosarcoma with metastases (57). Claims that this therapy is useful in pernicious vomiting of pregnancy are denied (99).

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## CHAPTER V

### THE ADRENAL GLANDS

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#### Introduction

CONCERNING the normal function of the paired glands whose close anatomical juxtaposition to the kidneys has led to the name *suprarenal* or *adrenal* glands, 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.<sup>1</sup>

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 (147) 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.<sup>2</sup>

"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.

"Chromophile bodies"<sup>3</sup> are found in close relationship to the ganglia of the sympathetic nervous system in elasmobranch fishes. In mammals the relative total amount of chromophile tissue seems to increase. Some part of the carotid body,<sup>4</sup> and

<sup>1</sup> For an account of the earlier work on the adrenal glands, see Vincent (147), Sharpey-Schafer (127), Goldzieher (51), or the articles in Barker's "Endocrinology and Metabolism" (5). Cf. also Grollman (56).

<sup>2</sup> 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 (148).

<sup>3</sup> 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 "chromophile" 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.

<sup>4</sup> Christianna Smith (134), in a study of the origin and development of the carotid body, found that chromophile 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 chromophile cells are present in the carotid body, they function as do other cells of the chromophile system, especially those associated with sympathetic ganglia.



the whole of the abdominal chromophile body, are made up of chromophile tissue. The largest mass of all is the adrenal medulla, but the proportion of chromophile cells in the two adrenal glands to total chromophile 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 chromophile 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 (147, 51).

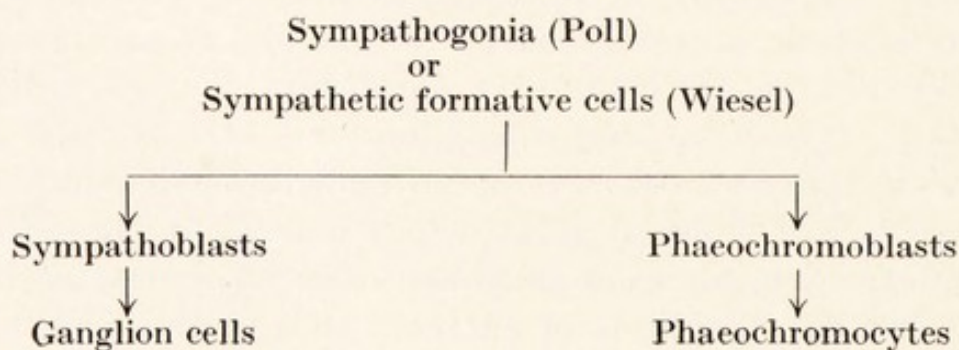
Zwemer (159), in agreement with previous investigators, finds that the cortex grows from without inwards, new cells being formed at the periphery, and cells being destroyed in the reticular zone. The glomerular cells arise from indifferent cells resembling those of connective tissue, and transform to the specific, lipide-rich, spongiocytes. As the cells secrete, the ratio of nucleus to cytoplasm is greatly decreased. Lipide material is probably extruded from the cells in droplet form. Acute demand causes discharge of material from mature cells. Prolonged demand stimulates formation of new cells. (Cf. also 7A.) It will be shown in Chapter VIII that the adrenal cortex is under control of an adrenotrophic hormone of the pituitary. The Golgi apparatus, particularly in the cells of the fasciculate zone, becomes shrunken after hypophysectomy, and is restored by injections of extracts rich in the adrenotrophic



hormone, while injection of these extracts into normal animals causes hypertrophy of the Golgi apparatus (111A).

The medulla consists of "a solid cell-mass permeated by sinus-like blood vessels. . . . There can be little doubt that the materials (secreted by the cells) find their way directly into the blood within the blood spaces. The cell-protoplasm contains chromophile granules which vary in size and amount in different cells. These cells are stained brown with chromic acid and its salts" (127).

Rabin (111) 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 state 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.”

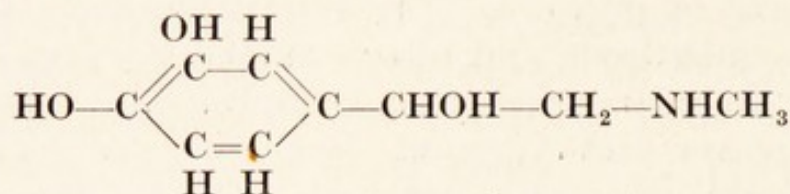
Staining reactions have shown that the adrenal gland is particularly rich in vitamin C, ascorbic acid (it was one of the sources from which Szent-Györgyi originally isolated the vitamin). According to Glick and Biskind in the calf's adrenal the fascicular region is richest in the vitamin, the medulla and the glomerular zones contain less (50).

### 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 (147). 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 (62).)

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 most frequently 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 (30) 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

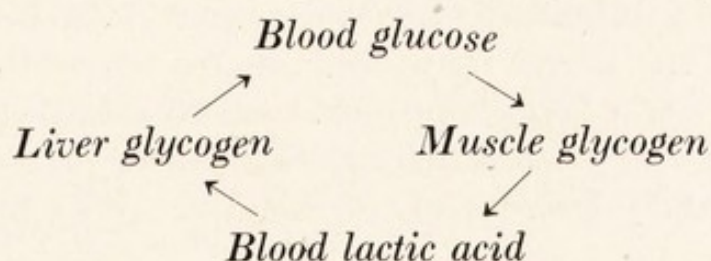


this has only recently become available, since the technique of the earlier work was open to criticism (32), and the data not in complete agreement.

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 (122). 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 (32).

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 (32).

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 (32), 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 (30):





(The results of certain other investigations are not in complete agreement with these conclusions (33, 121, 44).)

Cori (30) 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 (30). 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 (30).

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 (30). The action of adrenine on glycogen is probably only a lytic one (12).

Hrubetz (72) 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.



An interesting observation of Da Silva on cats, confirmed by Marenzi and Gerschman on dogs (100), is that intravenous injection of small doses of adrenine leads to an immediate increase of blood plasma potassium; in two or three minutes the potassium content has returned to normal. This effect is not produced in hepatectomized animals, suggesting that the liver is the source of the extra potassium.

**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 (31). 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 (23). It is produced rapidly and ceases rapidly, following cessation of injection.

**Formation and Destruction of Adrenine.** Their chemical relationship suggests strongly that adrenine is formed from tyrosine. Schuler, testing various potential precursors with surviving guinea-pig adrenal medulla tissue in Ringer's solution, and using Folin's reaction as a test for adrenine, found that phenylethylamine increased the reaction negligibly, 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 (171). Holtz (69) has confirmed the enzymic production of tyramine in the kidneys, and has succeeded in isolating it from kidney tissue in the form of a benzoyl derivative. Devine has shown that in the bullock adrenal phenylethylamine is the probable precursor (40A).

One of the functions of ascorbic acid (vitamin C) in the adrenals may be to stabilize adrenine, since it tends to prevent its oxidation (67). Scorbutic guinea-pigs do not react to



injections of adrenine, but treatment with ascorbic acid restores the normal action (157).

Vertebrate tissue, such as liver, intestine, and kidney, contains an oxidase which brings about oxidation of adrenine, tyramine, and aliphatic amines. Adrenine is converted to methylamine and an aldehyde (113, 9).

**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 twenty 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 (137, 24) ).

Stewart (137) 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 (24) 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."

More recent work tends to harmonize the views of Stewart and Cannon. One of the 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 (149) showed that Cow (34) 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." Hence the older experiments in which only the adrenal veins were clamped or ligatured led to fallacious conclusions, since adrenine could still leak out through the collateral circulation. They showed 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 (148, 36, 37). 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 (23) 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 (25), obviously the difference of view-point 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

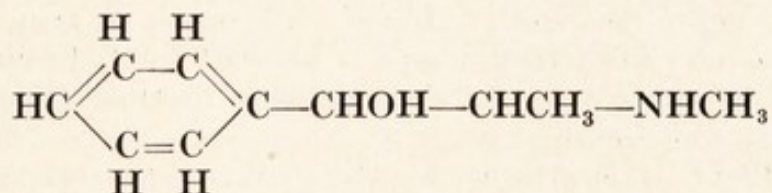


leads to glycosuria and increased secretion of adrenine, and the slight hyperglycaemia which follows the smoking of tobacco is attributable to the same intermediation (95). In certain states of emotional tension in mental patients sugar tolerance curves show a delayed return to normal fasting values, and this effect is also traceable to adrenine action (96). 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 (35) 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 (29).

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 synthesized.

**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 thereafter 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,  $\text{CH}_3\cdot\text{CO}\cdot\text{O}\cdot\text{CH}_2\cdot\text{CH}_2\cdot\text{N}(\text{CH}_3)_3\cdot\text{OH}$ , 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 (39) and McSwiney (98).

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 from the white cells of blood, and possibly from other organs, so that it may be considered a normal product of the organism. 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 (97A), 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 chromophile 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 (10). 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 (26).

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

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 chromophile tissue. Goldzieher has reviewed the favourable evidence (51).

Hyperfunction of the adrenal medulla, with chronically increased functional activity, if the condition existed, might be expected to produce a persistently heightened blood pressure, with some degree of hyperglycaemia. Such a condition, associated with hyperplastic medullary tissue, has not yet been proved to exist (cf. 51).

On the other hand, hyperfunctioning adenomatous tumours of chromophile tissue produce *paroxysmal hypertension*, in which, from time to time, the (diastolic) blood pressure rather suddenly rises from normal to 200 or more mm. of mercury, with various acute and distressing symptoms, and, after a variable but relatively short time, sinks back to normal. Somewhat more than forty cases of this syndrome have been reported, most of them well authenticated by the finding of a tumour at autopsy or operation. The subject has been well reviewed by, amongst others, Rabin (111) and Donzalet (42).

The condition was first recognized at post-mortem by Robert in 1899, was first diagnosed during life by Vaquez and Donzalet in 1926, and was first successfully treated by surgical interven-



tion by C. H. Mayo in 1927, since when surgery has been successfully utilized in a number of cases.

The onset of the syndrome is usually but not always insidious, the attacks of hypertension gradually increasing in severity and in frequency, accompanied by shivering and pallor, and followed by palpitation, rapid pulse, perspiration and cyanosis of the extremities. A hemiplegia may ultimately develop, some degree of albuminuria is not uncommon in the later stages, and the patient ultimately dies in an acute attack or in coma. At autopsy a varied pathology is found, with a tumour of one or the other adrenal, or, more seldom, of one of the accessory chromophile bodies.

These tumours consist of abnormal proliferations of mature phaeochromocytes, in Rabin's terminology (111), 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 (111).)

The phaeochromocytoma or paraganglioma is usually benign, perfectly encapsulated, and does not give rise to metastases. It may be as large as an orange. The cellular structure is like that of the normal adrenal medulla, and, histologically, suggests active secretion.<sup>1</sup>

Shipley's case (128) is fairly typical. A woman aged twenty-six suffered from paroxysmal attacks of hypertension of increasing frequency, while severe occipital headaches became

<sup>1</sup> 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."



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  $9 \times 7 \times 3.5$  cm., and was completely encapsulated. Macroscopically, it was a tumour of the medulla, microscopically a paraganglioma.

In a case reported from the Mayo Clinic (76), 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.

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 (75).

Hicks (67A) 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 (114) hypertension was present.

A case of malignant phaeochromocytoma of the adrenals has been reported by King (82). Although the tumour contained typical chromophile 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 (51).

### 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" (147, cf. also 4).

Biedl showed, in 1910, that removal of the "interrenal body" (cf. p. 188) in elasmobranch fishes produced a very similar series of symptoms. His results have recently been fully confirmed by Kisch (83). 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.



Maes (99) has shown that corresponding changes also occur in the adrenalectomized frog.

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 (cf. 52). The mortality rate thus varies greatly in different laboratories (2), age at operation being also an important determining factor (133); 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; degeneration of the second convoluted tubules of the kidney has been observed (131).

*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 (118, 156). 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, 90).

In the frog, after muscular asthenia is present, there is, according to Moschini, a marked drop in the creatine-phosphate content of muscle (106).

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 in attempts to prepare active extracts of the hormone it is essential to use as biological test the prolongation of the life of an adrenalectomized animal.



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 (64, 65) also used cats and employed a two-stage operation. Swingle and Pfiffner employed both cats and dogs (140).

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* (118, 138, 136).

Hartman extracted adrenal cortex material with ether (which removes very little adrenaline), evaporated off the ether, extracted the residue with warm 80 per cent. alcohol and chilled the extract. Inert material separated and was removed. The alcohol was distilled off *in vacuo*, and the residue dissolved in water and sterilized. Such preparations are almost free from adrenaline.

Continued injection of such an 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 termed the active principle *cortin*.

Swingle and Pfiffner obtained 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 appeared to be somewhat more potent than that of Hartman (97). Extracts of this type also keep 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.<sup>1</sup>

Hoskins (70, 46) 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 (60).

**Physiological Properties of Cortical Extracts.** These have been studied by Hartman (64), Swingle and Pfiffner (140), Britton (15), Wilson (154), Houssay (71), and others. 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 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 injections in larger

<sup>1</sup> For details of various methods of preparation of the cortical principle see Harrow and Sherwin (62).



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

Swingle reported in 1934 (141) that he had then 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,

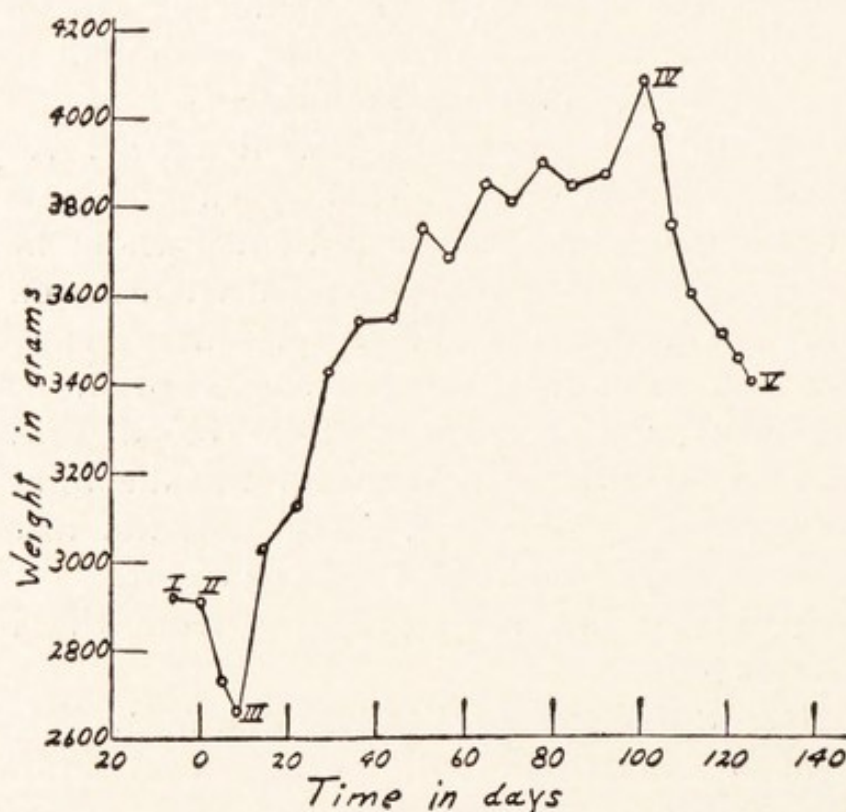


FIG. 24. The weight chart of a bilaterally adrenalectomized cat treated with an active extract of the cortico-adrenal principle, following the exhibition of severe symptoms of adrenal insufficiency. I. Right adrenal removed. II. Left adrenal removed. III. Animal prostrate; treatment begun. IV. Treatment discontinued. V. Death from adrenal insufficiency. (From Pfiffner and Swingle, *Endocrinology*, 1931, xv, 338.)

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. 24 (showing changes in body weight) and Fig. 25 (showing changes in body temperature). The



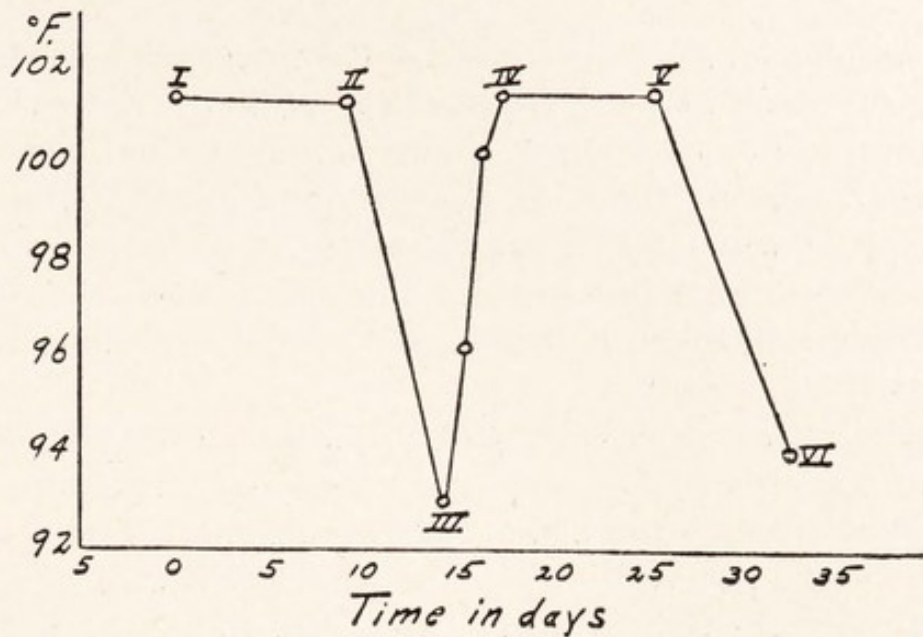


FIG. 25. Rectal temperature chart of a bilaterally adrenalectomized cat treated intraperitoneally with an active extract of the cortico-adrenal principle, following the exhibition of severe symptoms of adrenal insufficiency. I. Second adrenal removed. II. No symptoms. III. Prostrate; treatment begun. IV. No symptoms. V. Treatment discontinued. VI. Animal prostrate (died of adrenal insufficiency). (From Pfiffner and Swingle, *Endocrinology*, loc. cit.)



FIG. 26. 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.)

perfect condition of adrenalectomized animals following prolonged replacement therapy is demonstrated in Fig. 26.

It is clear that cortical extracts are effective by mouth. But oral administration is not nearly as effective as parenteral injection. Swingle (141) states that his extract is only one-



twelfth as effective by oral route. Grollman and Firor claim that theirs is one-fifth as effective (58). 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 (15).

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 (93)). It is said that capability for exertion is increased. Both in normal dogs and in man there is an initial fall in excretion of sodium and chloride following injection of the extract, with a slightly increased excretion of potassium. After repeated injection this effect is no longer produced (65). Hypertrophy of the anterior pituitary is produced in young rats, but no changes have been observed in the thyroid or adrenals. Action on the gonads will be dealt with later.

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 (41, cf. 110).

**Assay of Extracts.** 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)" (140).

Kendall (78) criticized the method on account of the great variability shown in the response of adrenalectomized dogs.

Kutz suggested a method of assay using rats (85), and Everse and de Fremery (45) another based on the decreased rate of production of fatigue of muscle produced by injections of the principle into anaesthetized adrenalectomized rats. Various modifications of this method have been used. All seem open to criticism (cf. 66).

Schachter and Beebe (123) suggest the guinea-pig as test animal, describe a method of bilateral adrenalectomy, and claim that results are very constant for different guinea-pigs.

**Chemistry of the Compounds of the Adrenal Cortex.**<sup>1</sup> Chemical studies of the adrenal cortex have been made especially by Kendall, Reichstein, Wintersteiner, and their respective colleagues (cf. 101, 155, 66). A number of crystalline compounds have been obtained, some of which are physiologically active, possessing in varying degree what can at present best be termed "cortin-like activity." Some have properties allying them with the sex-hormones, while others are physiologically inert. It is not yet proved whether any one of these can be regarded as the true "life-preserving" hormone of the cortex, nor to what extent if any others can be regarded as intermediate stages in the preparation of the true hormone, nor indeed is it at all clear how many hormones are elaborated by the adrenal cortex.

Different groups of workers gave their crystalline compounds different terms. The confusion arising from this is gradually

<sup>1</sup> During the past few years excellent reviews of the chemistry and physiology of adrenal cortical hormones have been prepared by Marrian and Butler (101), Wintersteiner and Smith (155), Miescher (104b), and Evans (43), while in the Cold Spring Harbor Symposium on hormones in 1937 (66) there is adequate and detailed presentation of the different viewpoints by the protagonists themselves, with illuminating discussions. To avoid an exhausting list of references, advantage will be taken of the bibliographies of the reviews, and references to these will include references to their bibliographies.



being clarified. All these compounds are sterol derivatives, and can be regarded as derived from cholesterol.

The following is a partial list of those possessing cortin-like activity.

Pregnene-11,17,21-triol-3,20-dione ( $C_{21}H_{30}O_5$ ), Reichstein's compound M, and probably identical with Kendall's compound F, has about half the activity of corticosterone.

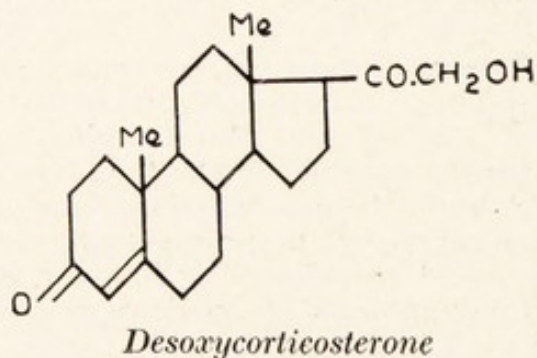
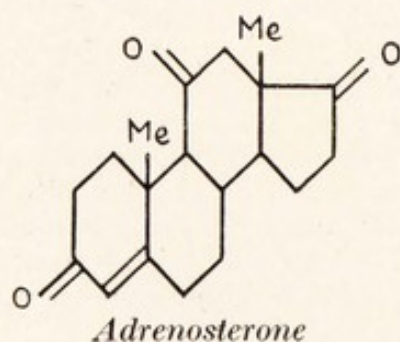
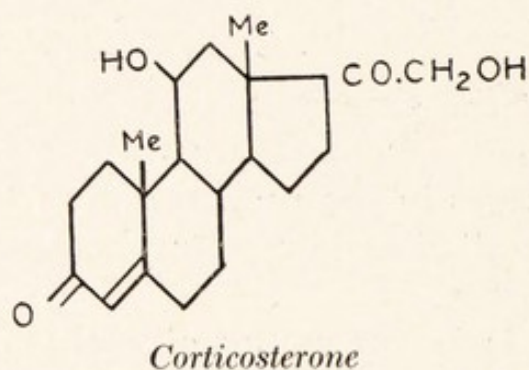
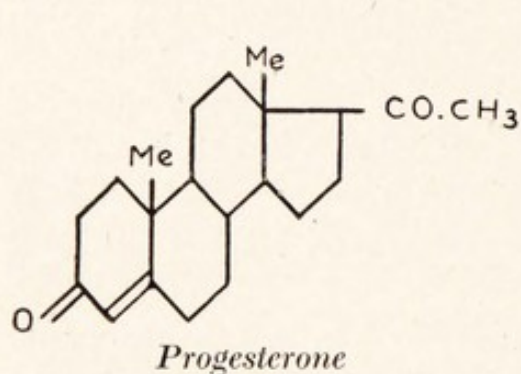
Pregnene-11,21-diol-3,20-dione ( $C_{21}H_{30}O_4$ ), *corticosterone* (originally termed by Reichstein compound H) is Kendall's compound B.

*Desoxycorticosterone*, ( $C_{21}H_{30}O_3$ ) was first prepared synthetically by Reichstein, but has now been obtained by him from the adrenal cortex. It is said to be much more potent than corticosterone.

Dehydrocorticosterone ( $C_{21}H_{28}O_4$ ), pregnene-21-ol-3,11,20-trione, according to Kendall, has some physiological activity.

In addition, Reichstein (112) has isolated androstene-3,11,17-trione, and has shown that it has androgenic activity, causing comb-growth in capons (cf. p. 263); he has named it *adrenosterone*.

The formulae of some of these compounds are shown below, with that of progesterone to stress their relationship with the sex-hormones. They should also be compared with the formulae of the latter shown on pp. 265, 266.





The relative activity of such compounds is not yet clearly determined, since different laboratories use different biological tests, some of which are perhaps open to criticism (cf. p. 211). Nor is it yet certain that any of the crystalline compounds yet isolated is responsible for the full activity of the hormone or hormones of the adrenal cortex.

Grollman claims to have prepared a crystalline compound, not yet completely characterized, which is much more active than corticosterone (66).<sup>1</sup>

It has been shown that esters of desoxycorticosterone with the lower fatty acids increase its potency but not the duration of its effect; its palmitic acid ester prolongs the effect of a single injection for more than three weeks (104A).

**Functions of the Adrenal Cortex.** There are at present numerous theories concerning the function or functions of the adrenal cortex. These are not independent of one another, but differ chiefly in their conceptions of what should be regarded as the primary function and what are secondary effects dependent on that function. The principal protagonists of each theory do not appear to have modified their views during the past several years, but each is continually presenting fresh evidence in favour of his own view, so that the subject is becoming increasingly a matter of argument. Until the actual hormone or hormones have been definitely established, and it or their *primary* physiological function determined, it is unlikely that any final decision can be made.

A clue to the function of the hormones of the cortical tissue perhaps lies in the sequence of events following production of an experimental deficiency. The effects of such deficiency have been fairly thoroughly studied, not only in the deficiency

<sup>1</sup> Hartman has succeeded, by prolonged fractionations of alcoholic extracts of adrenal cortex material between water and ether, in separating "cortin" (which passes into the ether) and what he terms the "sodium-factor." "Cortin," free from the sodium-factor, maintains the health of adrenalectomized animals, while the sodium-factor is responsible for sodium retention, and has a potency of the same order of magnitude as desoxycorticosterone. The sodium-factor is present in both medulla and cortex (65A).

Repeated injections of adrenal cortical extracts into normal animals, whether intravenously or intraperitoneally, gradually produce less and less effect. The refractoriness so developed is due to the sodium-factor, and is apparently an immune reaction (65A).



immediately following double adrenalectomy, but also in the controlled deficiency which ensues when cortical extracts are withheld from previously balanced adrenalectomized animals (cf. 93, 158).

In the dog, for example, within a few days after adrenalectomy, or after cessation of maintenance treatment, the following events occur: increased excretion of sodium and of chloride, accompanied by decreased blood plasma sodium, and chloride or bicarbonate or both, increased plasma potassium, increased blood urea and non-protein nitrogen, and a slight increase of plasma protein; some degree of hypoglycaemia; decreased plasma volume (to an anhydraemia) accompanied by dehydration, and decreased rate of blood flow; loss of appetite, refusal of food, and loss of body weight; fall of blood pressure, drop of body temperature, muscular weakness, death. Many of these changes are obviously dependent on others preceding them. The interdependence of several, for example, those concerning sodium and potassium, is so close as to render the determination of the *initial* change most difficult, and it is therefore possible to argue, very logically, that any one of several different initial changes can set up the whole syndrome.

Swingle stresses the anhydraemia, and therefore believes that the primary function of the cortical hormone is to control blood volume. Loeb, Harrop and others stress the excretion of sodium, and consider that the hormone essentially controls sodium metabolism. Zwemer stresses the increase of blood potassium, and believes this precedes and causes sodium loss; he believes the primary function is control of tissue potassium. Hartman views maintenance of electrolyte balance as the important function. Britton stresses the hypoglycaemia, and considers that all the other changes are secondary to interference with carbohydrate metabolism, control of which is the primary function of the cortical hormone. Verzár suggests that the hormone is essential for phosphorylation of carbohydrate compounds, and particularly of vitamin B<sub>2</sub>, so that in its absence this flavine cannot form the "yellow-ferment" essential for so many important oxidation-reduction processes. Jimenez-Diaz believes that the essential function is control of the kidney, and that loss of function leads to loss of the kidney's power to form ammonia, whence the loss of power to retain



sodium, this setting in train the other changes. (Cf. 101, 155, 43.)

It is possible that ultimately it will be found that the function of the "cortin-like" hormone of the adrenal cortex is the control of some essential *reaction*, rather than, specifically, the metabolism of sodium, or potassium, or carbohydrate, or something else. From this point of view Verzár's hypothesis is most interesting, and it is important to note that Pijoan and Oberg have recently confirmed his finding that adrenalectomized animals can be maintained in health by administration of flavine phosphate, but that "cortin" cannot maintain the life of the adrenalectomized rat fed on a diet freed from vitamin B<sub>2</sub> (43).

It is now certain that the adrenal cortex, in addition to producing one or more hormones with cortin-like action, also produces others whose properties resemble those of the sex-hormones (one being identical with the hormone of the corpus luteum). In addition, it has been proved that this sex-hormone can function in place of "cortin."

Injection of pituitary extracts and of A-P-L (cf. p. 261) has prolonged the lives of adrenalectomized cats (Swingle), while rat pituitary implants somewhat prolong the lives of adrenalectomized female rats (Emery and Schwabe; Cavanaugh and Gaunt). Since ovariectomy prevents this effect on rats it must be produced through stimulation of the gonads and thus through the sex-hormones (101, 155).

Thorn and Harrop and Thorn and Engle showed that the sex-hormones oestradiol, progesterone, and testosterone, when injected into normal dogs, diminish excretion of sodium and chloride through the kidneys and increase potassium excretion; since sodium therapy ameliorates adrenal deficiency (see below), it might seem that beneficial effects of the sex-hormones on adrenalectomized animals were only indirect, produced through conservation of sodium.

Nevertheless, it has been shown conclusively by a number of different investigators that crystalline progesterone will prolong the lives of adrenalectomized rats, ferrets, and dogs (48, 143, 55); 1 mg. per day usually suffices for female rats (125A). It should be remembered that desoxycorticosterone is 21-hydroxyprogesterone, so that it is scarcely surprising that progesterone



itself has recently been isolated from extracts of ox cortical tissue (7); thus this progestational hormone of the corpus luteum is also a hormone of the adrenal cortex.

On the other hand, there are numerous evidences of the control of gonadal functions by the adrenal cortex. Adrenalectomy produces in male rats loss of libido and potency, and degeneration of the seminiferous tubules (19), and in the majority of female rats suppression of the oestrous cycle and atrophy of the ovaries (103, 27), which can be restored by homotransplants of adrenal cortex (103) or injection of cortical extracts (168, 14).

In Addison's disease, a disease due to cortical deficiency (see p. 219), amenorrhoea, absence of libido, impotence, and atrophy of the testes may occur, while treatment with potent cortical extracts tends to restore normal sex function (120, 129).

Conclusions drawn from early experiments in which animals were given large doses of cortical extracts were conflicting, as far as the sex glands were concerned. Recent work has given more definite results. Extracts have been prepared from horse, ox and kangaroo adrenals, which exhibit progestational and oestrogenic activity (Callow and Parkes, Allen and Bourne, and others, cf. 101, 155). This is no longer surprising, now that progesterone has been isolated from adrenal cortex, but, in addition, crystalline desoxycorticosterone has been shown to produce progestational proliferation in the endometrium of the uteri of immature rabbits, and has, weight for weight, between 10 and 15 per cent. of the activity of progesterone (145).

Reichstein's compound *adrenosterone* has been referred to. This, it will be remembered, has activities which link it with the male sex-hormone, and not with progesterone. Its structure is very similar to that of testosterone (cf. p. 266).

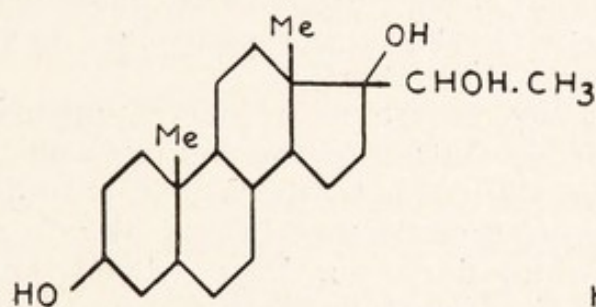
Hoffmann (cf. 155) claims to have prepared from the adrenals a *gonadotrophic* hormone, which, unlike the pituitary gonadotrophic hormones, is non-protein, while it is not soluble in lipide solvents. Injections are said to produce increase of weight of the rat ovary, follicular stimulation, luteinization, etc.

Further information concerning potential hormones from the adrenal with gonadal activity is being obtained by studies of the urine of women exhibiting the virilism syndrome usually

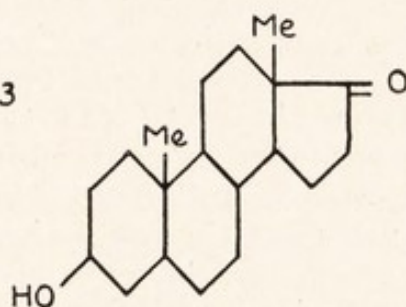


associated with adrenal cortical tumours (cf. p. 228). Cahill *et al.* (21) showed that in several of such cases large amounts of an oestrogenic hormone were excreted. Callow (22) isolated a relatively large amount of dehydro-isoandrosterone (an androgenic compound) from the urine of a young girl with virilism and an adrenal tumour. Burrows, Cook and Warren (19) isolated the weakly androgenic androstadiene-17-one from the urine of a male patient with an adrenal tumour, and exhibiting feminism.

Butler and Marrian (20) isolated pregnane-3,17,20-triol from the urine of two women exhibiting virilism. It disappeared from the urine after removal of an enlarged adrenal; it is not a normal urinary constituent. The same investigators, working with concentrated extracts of women with virilism and adrenal tumours, have obtained several crystalline compounds, including the pregnane-triol just mentioned (which apparently is inert), and the androgenic compound isoandrosterone (3-hydroxyetioallocholane-17-one).



*Pregnane-3, 17, 20-triol*



*Isoandrosterone*

While there can be little doubt that the formation of these compounds is associated with the abnormal adrenal condition (usually a cortical tumour), yet it would be wrong, until they have been isolated from cortical tissue, to claim that they are actually produced in that tissue. They might well be excretory products, bearing a similar relationship to an adrenal precursor to that which pregnanediol does to progesterone (cf. p. 271).

While in at least one case of virilism the tumour tissue has been shown to be entirely or partially deficient in cortin-like activity, yet Anderson and Haymaker have prepared extracts from the blood and urine of patients with Cushing's syndrome (cf. pp. 229, 362) which prolonged the lives of adrenalectomized rats, and which therefore were apparently rich in "cortin" (43).



It is obvious that at the present time no simple statement concerning the hormones of the adrenal cortex and their functions is possible. Two hormones, or two series of hormones, are undoubtedly produced, one with cortin-like action, the other with gonadal properties. Each seems to possess some of the physiological activity typical of the other, due undoubtedly to their close chemical relationship. Each is quite probably convertible to the other in cortical tissue.

Certain experimental data led to theories that the adrenal cortex elaborated hormones other than "cortin," which were primarily concerned with lactation (cortilactin), and with blood pressure (cortipressin) (18, 94). The majority of investigators appear to disagree with these views (cf. 47, 66).

*Selye's Theory of an Alarm Reaction.* During the last three years Selye has published a large number of papers concerning what he has termed the "alarm reaction" (126). He has observed in experiments, chiefly on the rat, that if the organism is subjected to an unusual and severe but non-specific stimulus (such as continued exposure to temperatures just above freezing-point, handling of the intestine, or poisoning with sub-lethal doses of such compounds as atropine or morphine), a "general adaptation syndrome" develops, which can be divided into two or three stages.

Between the sixth and twenty-fourth hour after application of such a stimulus there is rapid involution of the thymus and lymph glands, spleen and liver, loss of cortical lipides and chromophile substance from the adrenals, accumulation of transudates in pleura and peritoneum, decrease of muscular tone, drop of body temperature, and ulcer formation in the digestive tract. Urine output is decreased, and hypoglycaemia and hypochloraemia appear.

Subsequently, the adrenal cortex enlarges, regaining lipide granules, and the medullary chromophile cells become distended with vacuoles. Blood sugar and chloride return to normal values. Later on the gonads become atrophic, degranulated basophile cells appear in the anterior pituitary, the thyroid tends to become hyperplastic, and secretion of milk ceases in the lactating animal.

Under continuous stimulation, if the stimulus be not too severe, the experimental animal builds up a resistance, so that ultimately the appearance and functions of the organs return practically to normal. But with still further treatment, after from one to three months the animal loses this resistance, hypoglycaemia and hypochloraemia return, and the animal finally succumbs in a stage of exhaustion.

Selye considers that much of the response to the stimulus is due to liberation from the different tissues of relatively large quantities of histamine or of something with histamine-like action (Kendall and Ingle disagree with this view, cf. 155).



Adrenalectomy sensitizes the rat to such stimuli (and to the action of histamine) but prevents thymus and lymph gland involution (unless the animals are dosed with sodium chloride). Apparently the effect on the thymus is not due to adrenine, nor to "cortin", though removal of the adrenal medullas (leaving functioning cortex) prevents thymus involution.

Gradually increasing dosage of one stimulus to a point where large dosage will no longer cause thymus involution does not prevent a second different stimulus from causing a severe thymus effect.

At present Selye's experiments, however interesting, seem insufficient to render his conclusions convincing. His experimental procedures are too far removed from normal degrees of dangerous stimuli to permit deductions as to normal mechanisms. There is, however, some evidence that in various clinical states (even influenza) there is an increased (and detectable) output of the cortical hormone in the urine, which does lend some support to the view that increased activity of the adrenal cortex forms part of the protective mechanism of the organism (149A).

**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. 158), and recently Kendall has claimed (79, 80) 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 monograph of Rowntree and Snell published in 1931 (120). 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 gastrointestinal manifestations. Stomach and intestinal ulceration is often found, just as in adrenalectomized animals (cf. p. 206). 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." In at least some cases the patient dies in hypoglycaemic coma (3, 151).

**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. A case of amyloidosis of the adrenals, associated with tuberculosis of other tissues, has been reported (104). Carcinoma does not seem to be a cause. Marañon (102) thinks that there may be a racial factor, and that the disease is relatively commoner in Spain.

**Diagnostic Tests.** 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 (135). A positive result may not be shown for three or four days (108).

Another test for which claims have been made is the administration of a special diet for three days, containing 0.95 gram of chloride-ion, 0.5 gram of sodium, and 4.1 gram of potassium, with, in addition, the administration of 0.033 gram of potassium (as citrate) per kilogram on the afternoon of the first and again on the morning of the second day. If the concentration of chloride-chlorine in the morning specimen of urine on the third day exceeds 225 mg. per cent., adrenal insufficiency is probable; if it is less than 125 mg. per cent., adrenal insufficiency is unlikely (38). (The only trial of this test which I have seen gave figures midway between these limits.)

**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 ( $97^{\circ}$  to  $98^{\circ}$  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 (89). Glycogen formation is interfered with, both from glucose and lactic acid; adrenine only slightly mobilizes liver glycogen. Creatinuria is present (142). A deficiency of vitamin C may be present (153).

**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. We are yet far from such achievement.

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 has been 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. We now know the adrenal cortical hormone produces its effect when taken orally.<sup>1</sup>

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

<sup>1</sup> Rowntree has recently reported that of three mild cases dating back to this period, one died after eighteen years of the disease, another who has exhibited it for fifteen years, is still alive, and in the third, who developed it seventeen years ago, the disease seems to have cleared up completely, although only earlier types of therapy were employed (118A).



tions of cortin. Those of Rogoff and Stewart for "interrenalin" (115), of Hartman (64), and others with his preparation of cortin, and of Rowntree and Green (120) and others 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 commercial preparations are now available.

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 cheaper (synthetized) hormone are available, that ultimately 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 (109).

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) (91, 61). Some proportion of patients respond well to administration of sodium chloride (or of sodium chloride combined with other sodium salts such as the citrate (61, 135), with little or no cortin. The larger proportion require cortin as well, especially in crises.

Within recent years, and especially since the promulgation of theories that the adrenal hormone controls potassium, rather than sodium metabolism, stress has been laid on diets poor in potassium. Wilder (152) has pointed out that in considering such dietary treatment the relationship between the potassium and sodium intake needs to be estimated. The average diet contains about 4 grams of potassium per day, and the patient with Addison's disease needs, to offset this amount, about 18 grams of sodium chloride and 5 grams of sodium citrate. If the potassium intake is diminished to 1.6 grams daily, the sodium requirement is much lessened, but diets so deficient in potassium will also be deficient in calcium, phosphorus, and the vitamin B complex, which will have to be added. Wilkinson and Himsworth, while agreeing that high potassium intake is dangerous, do not believe it necessary to strive after diets containing such small amounts of potassium, and so deficient in other requirements (132).

Some idea of present attainment in the treatment of Addison's disease is given by Greene's critical appraisal of the results with 34 cases treated between 1930 and 1937, with active "cortin" extracts and salt therapy (54). He considered that only in 11 of the 34 cases had there been a definite marked prolongation of life due to the treatment, while only 4 were still alive when he reported. Wilkinson has reported slightly better results (132). The variation in results in different clinics is undoubtedly in part due to the extract used, and to the frequently doubtful value of commercial extracts (92, 116, 59).

But, as an example of what can be done in the treatment of Addison's disease, a quotation from Himsworth is illuminating (132). A man of thirty-four, with a history of acute



disease for two years, and presenting an exceptionally severe condition, needs 25 c.c. of cortin (as "organon") and 20 grams of sodium chloride daily to maintain him. "But on such a dose for the last eighteen months he has been remarkably well. He can drive 200 miles a day by car, in Switzerland he was able to ski and climb 3,000 feet up an easy mountain, and he has recently held a responsible if not arduous post of daily routine work. His clinical condition is not, however, uniformly stable at this relatively high level, and is always liable to remission as a result of mild infections. On the whole he is capable of living a quiet life and his appetite is sufficiently good to permit him indulging an interest in the table. This patient has found that his own subjective feelings of health are better guides to treatment than a knowledge of the behaviour of his blood electrolytes. If his appetite is not good he increases the dose of cortin; when he feels well again he gradually reduces the dose to his usual level of 25 c.c."

*Desoxycorticone Acetate Therapy.* Since the synthesis of desoxycorticone it has been used successfully in treatment of Addison's disease, a result which is understandable now that it has been isolated from the gland, and therefore is, after all, a natural hormone. (As judged by rat tests it seems one of the most powerful hormones of the adrenal cortex.)

A recent Symposium of the Royal Society of Medicine (132) gives an interesting account of its successful employment, as contrasted and associated with cortin extracts and salt therapy. It is injected in solution in arachis or sesame oil, intramuscularly, in dosage of 5 mg. per c.c.

It seems to be agreed that it is definitely beneficial, though in crises intravenous cortin may also be necessary, to supply other factors from the gland. It can at least replace cortin in considerable degree, and 5 mg. is probably equal to 15 to 30 c.c. of commercial preparations (such as "organon"). It obviously has the great advantage of lessened cost to the patient.

An interesting development is the insertion of tablets of the compound in subcutaneous tissue. A tablet of 50 mg. was so inserted into one of Simpson's patients, and produced a maximum effect for a period of two months (132).

**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 seems 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. 204, 214). The pigmentation is not invariably present and is not paralleled in adrenalectomized animals.

It is not yet certain that Addison's disease can be regarded as purely and simply hypo-cortico-adrenalism. Results obtained with adrenalectomized animals are not entirely paralleled by 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 (87): "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 (64).

Some proportion of cases of asthenia are benefited (64, 119), perhaps indicating in these a mild adrenal cortical deficiency. Such cases show a deficient storage of vitamin C. Their blood pressure changes parallel their improvement and the intensity of the therapy (53).

Statements regarding hyperthyroid conditions are not in complete agreement, although apparently in some cases benefit follows injection of the extract (64, 119, 78, 150). (Cortin counteracts the action of thyroxine on guinea-pigs (107).)

On the ground that Paget's disease is an entity representing disturbance of bone metabolism through imbalance between the parathyroid glands and adrenal cortex (there being excessive function of the former), Berman administered a crude extract of the cortex along with high calcium diet to 18 patients, and claims benefit in 16 of them (8). Still more empirical is the use of desiccated adrenal cortex orally in 6 cases of vomiting of pregnancy; good results were claimed (77).

### 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 (140), but the possibility of insufficient period of treatment cannot be excluded.

*Adrenal Cortical Tumours.* Adrenal hyperplasia in foetal life has been suggested as the cause of pseudo-hermaphroditism, but, although it is admitted that such hyperplasia is frequently found at autopsy, the rôle of the adrenal in this condition is still uncertain (51). There is a possibility that the syndrome now to be dealt with can also develop even during foetal life (cf. 74). 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 (44) and Goldzieher (51), and more recently by Cecil (28), by Simpson, Kohn-Speyer and Korenschevsky (130) and by Cahill (21) 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 some hormone. 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 (88). Operation and removal of the tumour in such a case can result in return to normal (68).

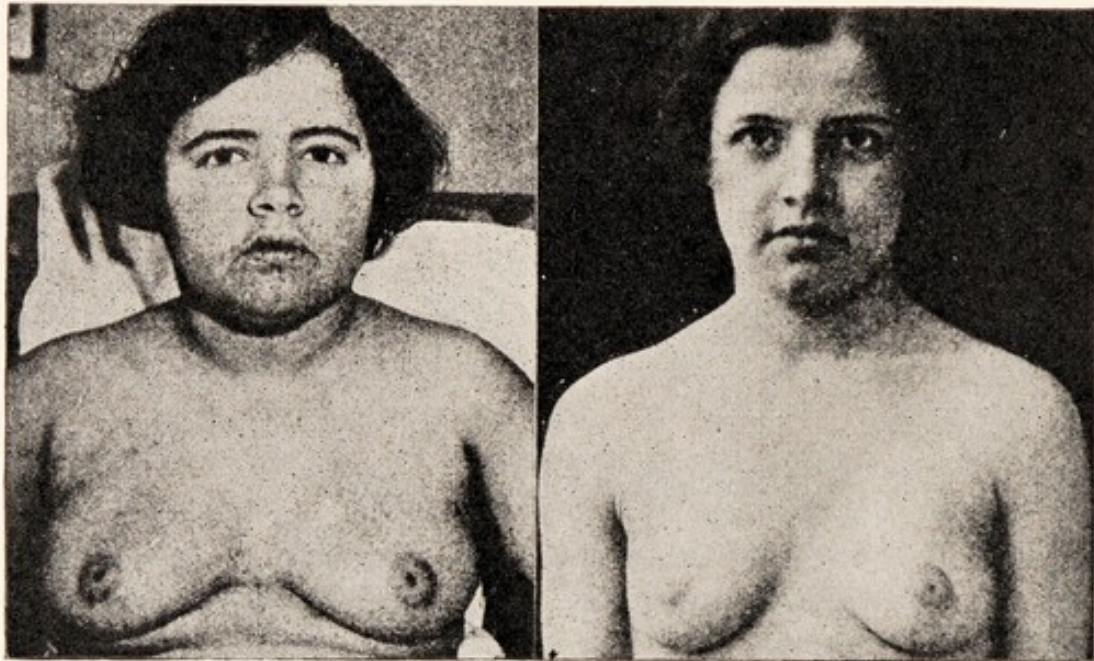
In the female the predominating change is towards maleness. If the tumour occurs before puberty, whether it be an adenoma or a slow-growing malignant tumour, marked changes towards the adult type occur. The girl becomes fat. She seldom shows unusual muscular development. Hair appears early on the pubis 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. (But Kepler (81A) has reported a case of menstruation in a nineteen-month-old



girl, in whom, at operation, a tumour of the left adrenal cortex was found.)

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.



A

B

FIG. 27. 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.)

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 (28, 86, 144). Cecil believes that this syndrome is pluriglandular, and that the pituitary is involved. However, cases undoubtedly occur exhibiting all these conditions, in which the pituitary is normal, as far as serial sections indicate.<sup>1</sup> 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 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.

Fig. 27 represents the striking change produced by removal of such a tumour from a patient in the Mayo Clinic (81). 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,  $6 \times 4 \times 2$  cm.

The first picture (Fig. 27, 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. 27, B) was taken four months later. The weight was now only 72 lb. The body proportions were normal

<sup>1</sup> Hunter's case (73) comes within this category (cf. also 81), and that of Calder and Porio (21A) is an excellent example.



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, in many respects closely resembles that resulting from cortical tumour (cf. Chapter VIII, p. 362). The condition associated with the rare arrhenoblastomata of the ovary has similar features, but obesity is absent and differentiation is possible (cf. p. 300).

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.

Whether the tumour is associated with the right or with the left adrenal can sometimes be ascertained by injection of air and X-ray examination (21; cf. 29A). When, as with many of the adenocarcinomas found in these cases, metastases develop (usually to the lungs), these also function in the same way, and in several cases recurrence has occurred after successful operation (21).

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 (49, 56). 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 (84) 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 (16) stress the frequent occurrence of virilism



without adrenal tumours and even without cortical hyperplasia, and claim good results by removal of the (larger) adrenal. Broster has dealt with the surgical aspect (17). It seems, however, as with other endocrine glands, surgical decrease of hyperplastic tissue is seldom as successful as removal of a tumour.

Vines has developed a differential stain—the Ponceau fuchsin stain—which he claims stains adrenal cortical cells in cases of virilism a vivid red, as contrasted with normal cells which take up the counter stain, aniline blue (cf. 17). Cahill does not entirely confirm this statement (21).

### 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 (117). 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 (63)), so that "surgical intervention with the adrenals for various conditions—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 (40).

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## CHAPTER VI

### THE THYMUS AND PINEAL GLANDS

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#### Introduction

EVIDENCE from earlier work on the thymus and pineal is confused and contradictory. Within the past few years Rowntree and his collaborators, using extracts at first prepared by Hanson, have obtained a number of very striking results, which merit some detailed description, although their interpretation is still incomplete, and more confirmation from other laboratories is still needed.

*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 (27), yet an opinion has long been held that its function is associated with the growth of the organism (11). The earlier literature has recently been reviewed by Rowntree, who gives an extensive bibliography (20).

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 lipid 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, but that its activity is probably due to the sulphur-containing glutathione. General growth, growth of the skeleton, and growth of the gonads all appeared to be accelerated by thymocresin. Important is the observation that extracts of lymph glands, prepared in precisely similar fashion, were found to be inactive. (A useful *résumé* of Asher's work has been given by Gudernatsch (12).)

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.*, 28, 6, 13) while others (*e.g.*, 18) 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 (27). There seem to be two types of cells, neuroglia, and "secretory" or ependymal cells. Of the mass of statements in the earlier 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 (21).

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 (27). Perhaps Saphir's experimental results (24) indicating that pineal tissue contains a gonadotropic substance have some bearing upon this syndrome, though Tarkhan's work (26) does not support this view.

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 (17, 14, 1). Its significance is not known.

### Experiments with Hanson's Thymus Extract

The studies of Rowntree and his collaborators (19, 8) 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 dilute hydrochloric acid. Most of the earlier experiments were carried out with definitely crude extracts with varying degrees of activity and stability (22). Steinberg (25) has lately published details of the present method of preparation. Fresh thymus glands of two- to six-week-old calves are immediately minced into 1 per cent. hydrochloric acid, and more acid is added to give a ratio of 1.5 grams of tissue to 1 c.c. of acid. The mixture is then heated rapidly to 68° C. Protein coagulates and sediments, leaving a clear, pale yellow, supernatant fluid. This is syphoned off, 0.2 per cent. chlorobutanol is added as preservative, and the extract is stored at 40° C. It is protein-free and contains just over 2 per cent. of dissolved solids.

It loses its activity fairly rapidly, and completely within five months. When it is stored at room temperature the activity is lost more quickly.

Such extracts are rich in iodine-reducing compounds. Fresh extracts contain over 20 mg. of ascorbic acid, 40 to 70 mg. of glutathione, and a few milligrams of cysteine per 100 c.c. (23). These constituents have recently been shown to have an important relation to the action of the extracts.



*Earlier Experiments.* 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

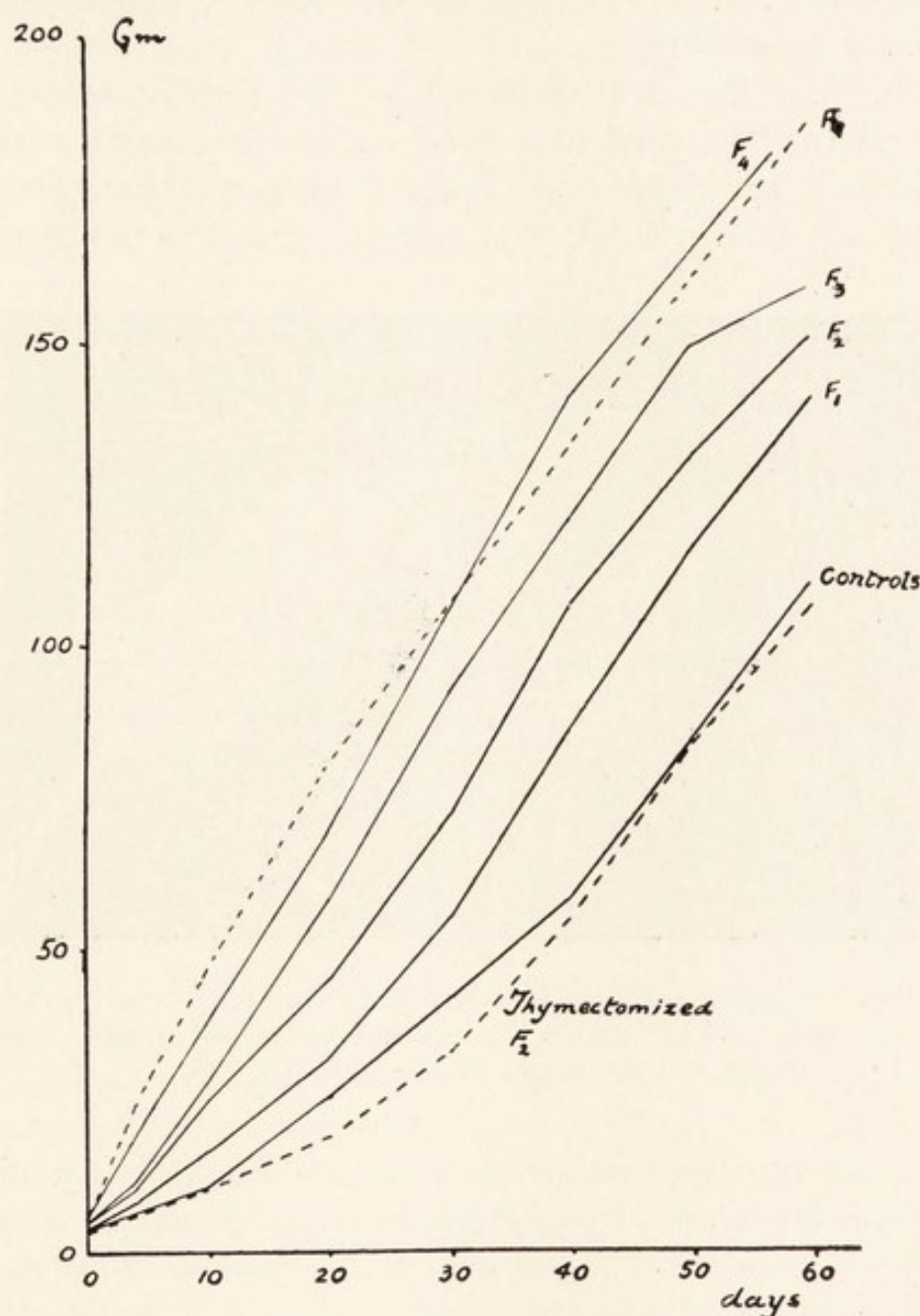


FIG. 28. Weight curves of thymus-treated and thymectomized rats, contrasted with normals. Constructed from the curves in Rowntree *et al.*, *Arch. Int. Med.*, 1935, lvi, 1, Fig. 5, and *Ann. Int. Med.*, 1935, ix, 359, Fig. 2.

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 ( $F_0$ ) 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. Many hundred rats have been 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,

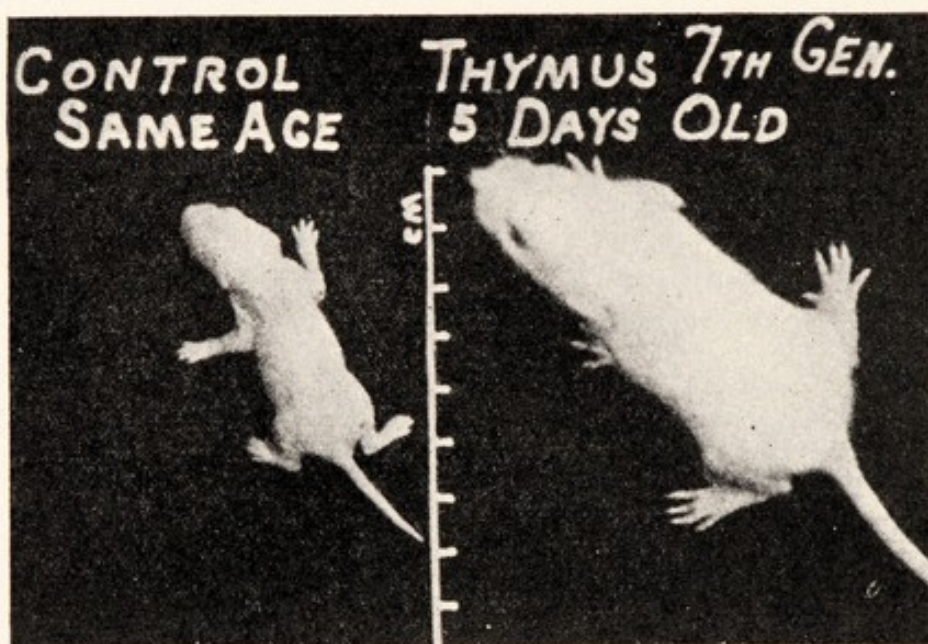


FIG. 29. Comparison of a five-day-old thymus-treated rat of the seventh generation with a control of the same age. (From Rowntree *et al.*, *Ann. Int. Med.*, 1935, ix, 359.)

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. 28 to 30.

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 <sub>1</sub>	5.1	2-3	8-9	10-12	12-14	15-29	30-45	70	92
F <sub>2</sub>	5.3	2	4-6	4-6	4-6	15-21	23-32	56	78
F <sub>3</sub>	5.3	1-2	4-6	4-6	4-6	10-12	21-27	42	64
F <sub>4</sub>	5.6	1-2	2-3	2-3	2-3	6-10	18-20	25	47
F <sub>5</sub>	5.5	1-2	2	2	2-3	4-6	18-20	40	61
F <sub>6</sub>	5.6	$\frac{1}{2}$ -2	1-2	1-2	2-3	3-10	16-20	46	68
F <sub>7</sub>	5.5	Birth	Birth	1-2	1 $\frac{1}{2}$ -2	3-4	16-18	37	59
F <sub>8</sub>	6.5	Birth	Birth	1	1 $\frac{1}{2}$ -2	3-4	16-18	22	43
F <sub>9</sub>	6.0	Birth	Birth	1	1 $\frac{1}{2}$ -1 $\frac{3}{4}$	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.

*Later Experiments.* Rowntree wrote in 1936 (22) that while growth is accelerated to the seventh generation, and differential development to the tenth, "Our data would seem to indicate that the limit in the influence on both growth and development has been attained perhaps, and that little increase beyond the present limits can be expected in the future."

He has obtained some suggestion of precocious development following use of acid extracts of other lymphoid glands (22). He has shown that frequent homologous thymus implants in parent rats produce an effect similar in kind but less in degree than that obtained by injections of the extracts (9).



In the earlier work both parent rats were injected. Subsequently it has been found that only the female parent needs the injections (23).

It has now been shown that precocious growth and development, similar in kind to the effects of the thymus extracts, is produced by daily intraperitoneal injections of solutions of glutathione into the parent rats, while somewhat similar results have been obtained with solutions of ascorbic acid and cysteine (23). Rowntree considers that there is some difference between the effects of these pure compounds and those of the extracts.

Similar results with glutathione have been obtained by Lee and Ayres (15). Shaffer and Ziegler (24A) have isolated glutathione from calf thymus glands in yield of 0.28 gram per kg.

*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. 28). 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 acceleration of growth and precocious development of young. Frequent thymus implants will produce the same effect (7).

*Chiodi's Results.* Chiodi, working in Houssay's laboratory, has repeated Rowntree's work with injections of a thymus extract of the Hanson type, and with thymectomy in successive generations (4).

While his published curves for the injection experiments suggest some degree of precocious growth in the second generation, later generations show no definite effect, and he concludes that no effect is produced. He finds that thymectomy produces no change in growth rate in successive generations, and, indeed,



his published curves indicate that any change is in the direction of increased, rather than of decreased rate of growth.

While the difference between Chiodi's and Rowntree's injection experiments can well be attributed to differences in the extracts used, especially since Rowntree has indicated that many of his own extracts have proved to be non-potent (22), the difference in their results following thymectomy cannot be so easily explained.

Chiodi has also attempted to determine the interrelationship between the thymus and other endocrine glands (5). He can find no connection between gonad function and physiological involution of the thymus. Thyroidectomy seems to lead to atrophy of the rat thymus, but adrenalectomy produces no effect within thirty days.

(Low (16) obtained little or no effect on the thymus of rats to which various endocrine preparations were administered, although he states that a combination of thyroid and oestrogenic extract renders the animals liable to infection, which leads to involution of the thymus.)

### 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 (20).

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

### Experiments with Pineal Extract and Pinealectomy

These results are a partial antithesis of those with the thymus extract (21).

*Hanson's pineal extract* was a hydrochloric acid-picric acid extract of beef pineal glands, crude in nature. Rowntree's



earlier work was carried out with such extracts, whose potency varied.



FIG. 30. 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. 31. 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.)

Steinberg has recently published details of the preparation of the more refined extracts now used (25). Beef pineal glands are collected in acetone, and the dehydrated, defatted material



is dried and powdered and extracted with 0.4 per cent. hydrochloric acid, the extract heated to 75° C., sodium carbonate added till flocculation is complete, and a clear golden yellow supernatant fluid is then syphoned off, preserved by addition of 0.2 per cent. chlorobutanol, and stored at 40° C. It is relatively stable, protein-free, and contains no iodine-reducing substances.

*Experimental Procedure.* This has followed very closely that

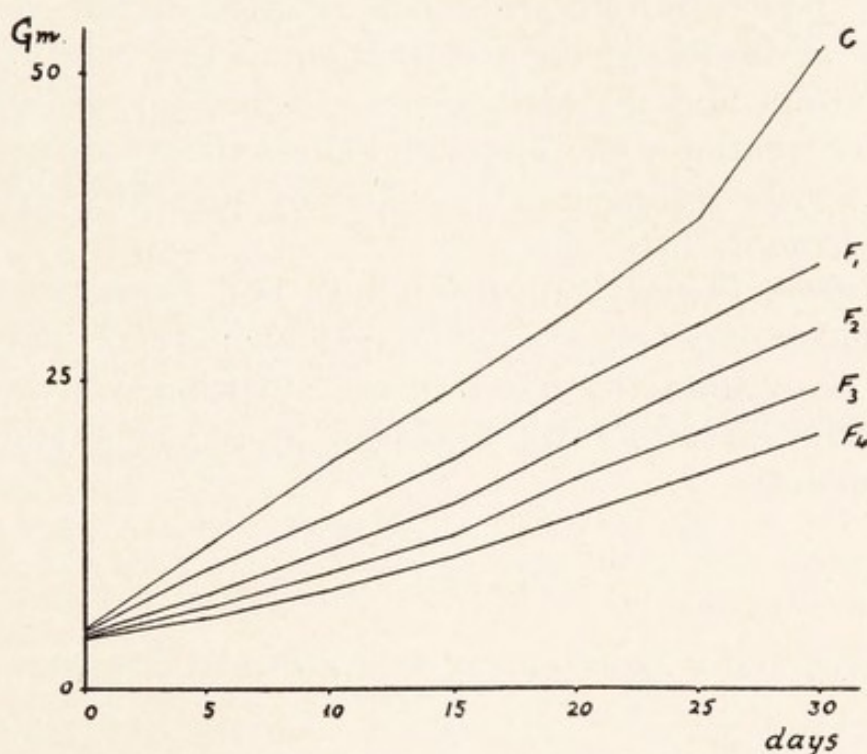


FIG. 32. Weight curves of pineal-treated rats and controls.  
(After Rowntree *et al.*, *Science*, 1936, lxxxiii, 164, Fig. 1.)

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 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 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. 32; note also Figs. 30 and 31.

Einhorn and Rowntree (10) have recently summarized the results of their experiments with pineal extract: "The daily intraperitoneal administration of 1 c.c. of pineal extract to successive generations of rats has resulted in an increase of frequency of breeding, an acceleration in the rate of somatic differentiation, and a retardation in the rate of growth in the offspring of treated rats. These biological effects are progressive in character, becoming more marked in successive generations under treatment."

*Results from Pinealectomy.* Einhorn and Rowntree (10) find that pinealectomy in successive generations of parent rats produces no noteworthy effect in the offspring, which, further, are not affected either by injection of pineal extract or by pineal implants.

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	3.3	9.0	16	15.5	38	65
F <sub>1</sub>	3.3	9.0	13	14.9	22	45
F <sub>2</sub>	2.8	9.0	12	13.8	15	37
F <sub>3</sub>	2.3	6.9	9	9.8	10	32
F <sub>4</sub>	2.0	4.0	5	6.0	5	24

**Function of the Thymus and Pineal Glands**

In spite of the striking results of Rowntree's experiments with thymus extracts, the parallel effects produced by glutathione and the discordance between his results with thymectomy experiments and those of Chiodi demand suspension of judg-



ment as to conclusions bearing upon the *in vivo* function of thymus tissue.

Nor can any definite statement yet be made concerning the pineal gland.

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## CHAPTER VII

### HORMONES OF THE ORGANS CONCERNED WITH REPRODUCTION

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#### Introduction

*Gonadal Development and Cyclical Changes.* The hormones 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 hormones 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 (62). 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 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 (and current opinion does not associate such interstitial tissue with ovarian hormones). 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 unstriated 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 lipide 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 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 turgescient 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 (163)<sup>1</sup> :

<sup>1</sup> For further details, see Frank (62), Parkes (163), Sharpey-Schafer (191), or Robson (171). Marshall, in a recent Croonian Lecture to the Royal Society of London (138), has reviewed critically present knowledge of the



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 and ferret only follows copulation. "Pseudo-pregnancy" can be induced by sterile mating with a vasectomized male. Ovulation is then followed by development of normal corpora lutea, modification of the uterine mucosa, and hypertrophy of the mammary glands (conditions typical of the early stages of actual pregnancy). Pseudo-pregnancy is probably due to a nervous reflex set up through copulation, and acting through the anterior pituitary to produce ovarian development and formation of corpora lutea. When pseudo-pregnancy is produced by similar procedure in the rat and mouse, the lives of the corpora lutea are prolonged and the next oestrus delayed.]

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 inter-tubular connective tissue, and forming conspicuous isolated groups of cells in man.

*Castration and Implantation.* Much early information was gained concerning the functions of the gonadal hormones by study of the effects of extirpation and of grafting. Such

causes determining sexual periodicity in mammals and birds, and the relationship of the internal sex rhythm to seasonal changes and external environmental phenomena in general. These often—especially exposure to sunlight (and probably to the ultra-violet rays of that light)—act enteroceptively through the nervous system and probably through the hypothalamus upon the anterior pituitary, and through it upon the gonads. Marshall concludes "in all the higher animals sexual periodicity, while conditioned by the environment, is regulated in its successive phases by the combined integrative action of the nervous and endocrine systems."



experiments afforded information of great value concerning the hormonal control of the secondary sex organs and characters.

The experiments of Nussbaum on the frog in 1912 produced reasonable evidence that the sex characters of the male are controlled by a specific hormone 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, then mating changes ensue normally. The absence of nervous connections from such a graft indicated that the effect was due to a testicular hormone (211).

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 (117).

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 was gained by studies of the Skopecs, a Russian religious sect who practise castration in the first decade of life. Following such early castration the adult castrate has small and underdeveloped penis, prostate, and seminal vesicles. Masculine distribution of hair does not develop. The beard is absent. The limitation of hair in the pubic region is feminine. Obesity may or may not be present. The larynx is an enlarged infantile larynx, and the voice of the prepuberal boy persists throughout life. The skeleton shows some characteristic changes. Growth of the long bones persists beyond the usual time; the castrate



tends to be tall through disproportionate length of leg. The general intelligence is not specially influenced, but apathy is a characteristic feature. Post-puberal castration produces less marked effects (117).

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 are not affected by castration (211).

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.

Generally speaking, gonadal implants tend to restore the secondary sex organs of castrated animals 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 (117).

*Vaginal Smear Tests.* 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 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 (199).

In the guinea-pig the period of oestrus lasts about twenty-four hours and occurs very regularly every fifteen to seventeen days. Throughout the twenty-four-hour period fluid is abundant in the vagina. For the first six to twelve hours (during which period the female will accept the male), the fluid is a fairly clear, frothy mucus. It gradually increases in quantity until it fills the lumen of the vagina. During the second stage, two to four hours, the fluid presents a cheesy appearance, and during the third stage, five to ten hours, it slowly becomes more liquid and serous. A fourth stage is also differentiated, in which there may be slight bleeding. Following this period of sexual activity the "vaginal closure membrane" grows over the vaginal opening (a change specific to the guinea-pig). If this membrane be broken during the dioestral period the vagina is found to contain only a scanty amount of slimy fluid, poor in cells.

Smears prepared from the vaginal fluid at the different stages show such characteristic differences in appearance as to be diagnostic of the exact sexual state of the animal.







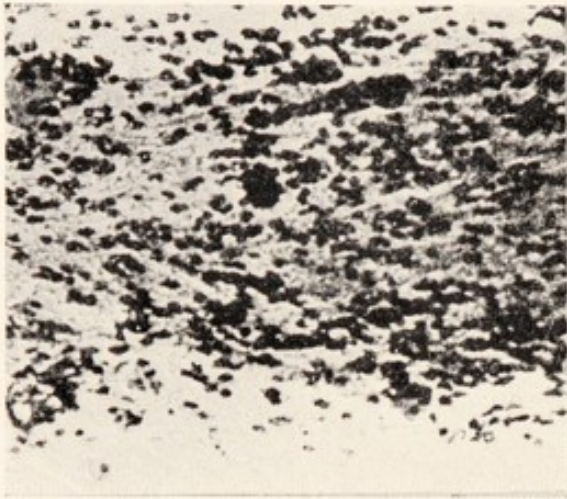


FIG. 33.

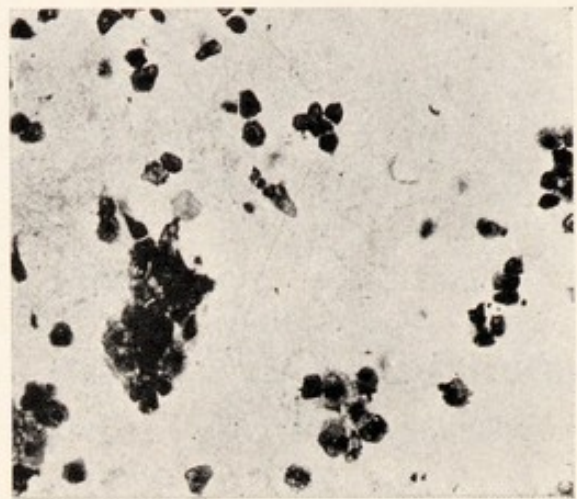


FIG. 34.

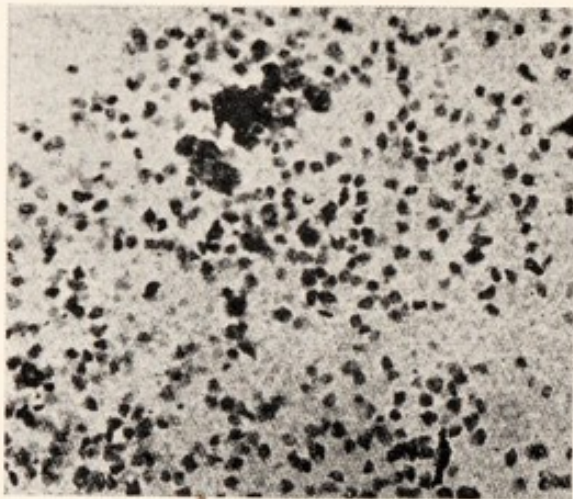


FIG. 35.



FIG. 36.

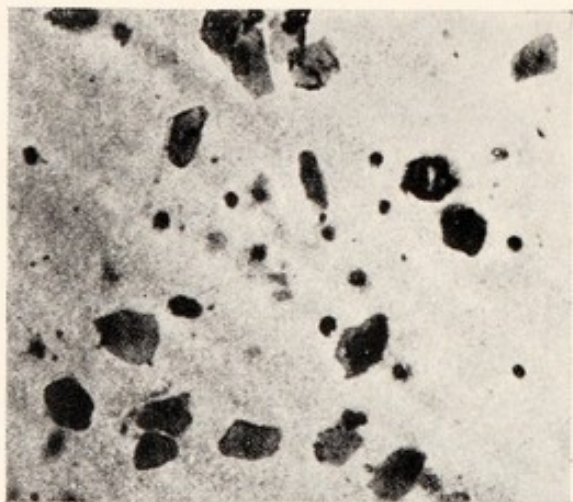


FIG. 37.

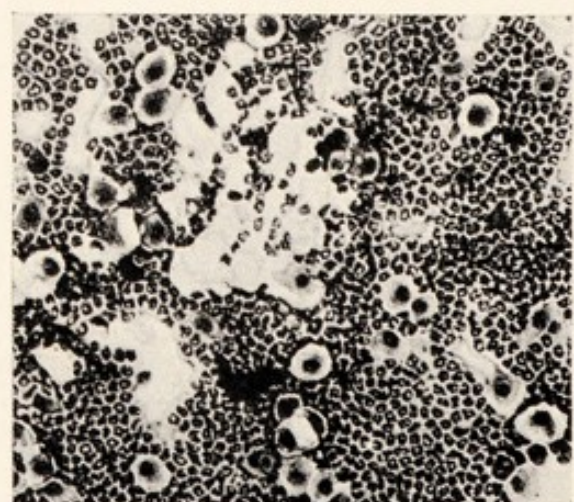


FIG. 38.

PLATE II: 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. 257.)



In the first stage the mucous fluid contains an abundant mass of cells, of a squamous type and showing considerable plasmolysis with bent and wrinkled cell-membranes. Their nuclei are very small and pycnotic; the protoplasm has degenerated and does not stain well; it exhibits a reticular structure. These cells, derived from the wall of the vagina, predominate over all others at this stage.

Towards the end of the first stage and at the beginning of the second there are also present some elongate, cornified cells, without nuclei, which are desquamated from the more

FIG. 33. Dioestrous smear: leucocytes in stringy mass.  $\times 40$ .

FIG. 34. Pro-oestrous smear: chiefly nucleated epithelial cells with an occasional leucocyte.  $\times 40$ . Present thirty-five to forty hours after first injection.

FIG. 35. Oestrous smear: non-nucleated cornified epithelial scales.  $\times 40$ . 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. 36. Flat, cornified elements of the oestrous smear stage.  $\times 250$ . Eosin stains these cells a brilliant red. Although the site of the former nucleus is apparent, all basophilic staining reaction has been lost.

FIG. 37. Early stage of leucocytic infiltration (metoestrus).  $\times 40$ . Few nucleated epithelial cells have appeared as yet.

FIG. 38. Late stage of the metoestrus.  $\times 40$ . Enormous numbers of leucocytes, some cornified scales (in the centre of the field), and many nucleated epithelial cells.

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 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.

Papanicolaou first definitely showed the existence of some degree of rhythmicity of the human vaginal smear (160) ; a cyclical change occurs shortly before menstruation and lasts six or seven days, while during the rest of the cycle the epithelium is quiescent (205).

The importance of the vaginal smear test lies in the 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. 33–38.

*Hormones of the Ovaries.*<sup>1</sup> 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 lipide

<sup>1</sup> Before discussing the chemistry of the pure hormones of the gonads it is desirable to give a brief account of the work which led to their isolation. For fuller details the reader should consult such symposia as that edited by Edgar Allen (3), and for details of the preparation of the less recently isolated compounds Harrow and Sherwin (86).



solvents and water, obtaining finally a fraction which was soluble in lipides, 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 material exhibiting this activity.

At first it was believed that only a single compound was responsible for the oestrogenic activity, and numerous names, such as oestrin, folliculin, and progynon, were given to this hypothetical compound by different investigators. Since the preparation in crystalline form of more than one active compound, such names have been almost completely discarded.

Crystalline *oestrone* was obtained independently and almost simultaneously by Doisy (August, 1929), Butenandt (October, 1929), and Dingemans (1930) from the urine of pregnant women. Doisy termed it *theelin*. In 1930 Marrian isolated a second active compound from the same source; Doisy obtained it a little later (49, cf. 23). This compound, *oestriol*, was termed *theolol* by Doisy, and differs from *oestrone* by the elements of a molecule of water. The terms *oestrone* and *oestriol* are based on a formal nomenclature now applying to a whole series of such compounds, and are now generally used. In 1935 Doisy showed that the still more active compound *oestra-di-ol*, already synthetized, was naturally present in ovarian tissue.

*Hormone of the Corpus Luteum.* The earlier theories concerning the function of the corpus luteum have been set out by Hisaw (93), and critically reviewed by Pratt (167). 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, and that if it is removed during pregnancy, abortion will occur in some, though not in all species.



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) some evidence was obtained that the corpus luteum took part in the development of the mammary glands (cf. 163).

Early accurate work on the corpus luteum indicated that its hormone is extractable by lipide solvents. Such extracts produced 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. The hormone was variously termed progestin (Allen), corporin (Hisaw), the beta-factor (Wiesner), lutin (Clauberg), and luteosterone (Slotta). 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 (94). The name "progesterone" has been agreed upon.

*Hormones of the Ovum and 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. 378). Wiesner (218) obtained placental extracts with similar properties, and Collip developed these studies still further (37).

Collip showed when an acetone extract of human placentae is acidified, addition of excess of alcohol fractionates it into two parts containing different hormones. 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 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*

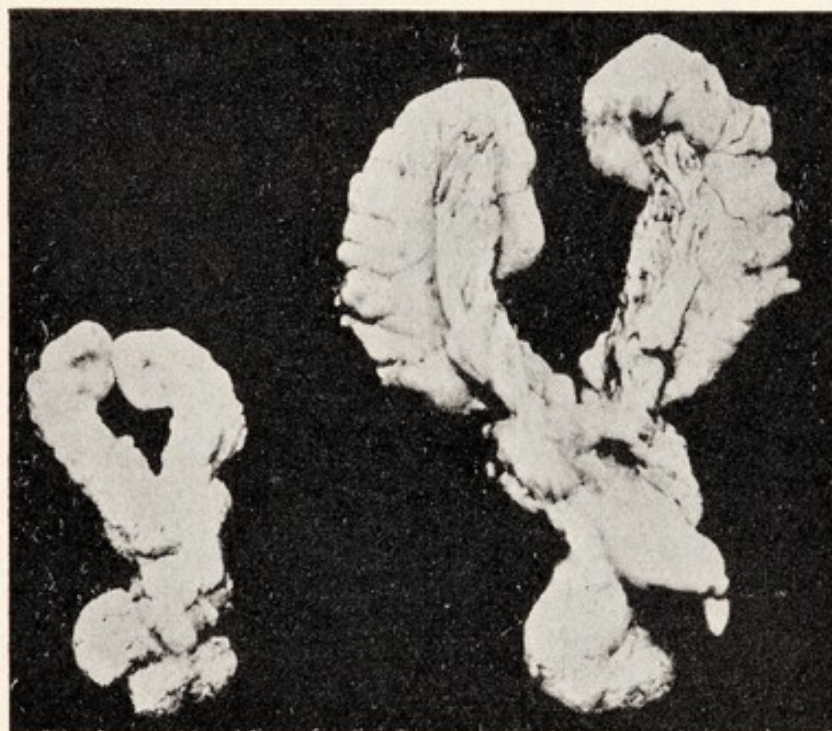


FIG. 39. Seminal vesicles and prostate of control (left) and experimental adult rat (right) after injection of the anterior-pituitary-like principle (the equivalent of 15 grams of placenta) administered daily except Sundays for forty-two days. (From Collip *et al.*, *Can. Med. Assoc. J.*, 1931, xxiv, 201.)

remains in the aqueous phase, while some oestriol is present in the ether extract, and has been obtained in crystal form (34, 13), 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 (35, 36). Later work suggests that it may be a glucuronide.

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 apparently 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. 39). 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 (34).

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 will be dealt with later. In the meantime the identity of A-P-L and urinary prolan will be assumed, and, since a good deal of confusion exists in the literature between "urinary" and "pituitary" prolan, the term prolan will not be employed. Since A-P-L is produced by chorionic tissue derived from the ovum, it can truly be regarded as the primordial hormone, and Fluhmann (60) has recently adopted Hamburger's very rational suggestion in terming it the *chorionic gonadotrophic hormone*.

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. It has not yet been obtained in crystalline form.

*Hormones of 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 (106) summarized the earlier work. McGee, working under his direction, conclusively demonstrated in 1927 that a benzene extract can be prepared from testes which causes comb-growth when injected into capons. Later work showed that the same, or some similar principle is present in the urine of men, and in traces in bull's blood. Extraction with lipid solvents is an essential feature in the preparation of concentrates from testes or urine. Frattino and Maino (65) claimed that they had obtained a very active crystalline preparation, but have never published details. 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. Subsequently *testosterone*, much more active, was isolated from testes.

### Chemistry of the Hormones concerned with Reproduction

Two groups of compounds control reproduction in mammals. The first are protein, or protein-like in character, being the two gonadotrophic hormones of the anterior pituitary, the lactogenic hormone "prolactin" of that gland, and the chorionic hormone A-P-L of the placenta, or, more strictly speaking, of the ovum. The second group are all derivatives of cholesterol, which is probably their parent substance in the mammalian organism<sup>1</sup>; they are lipid in character.

These cholesterol derivatives and related compounds are conveniently divided into three sub-groups, (i) *androgens* (*i.e.*, compounds with androgenic properties, conferring maleness) and their derivatives, (ii) *oestrogens* (*i.e.*, compounds with oestrogenic properties, conferring femaleness) and their derivatives, and (iii) *progesterone* and its derivatives.

The protein group will be dealt with in some detail in the next chapter, though some account of A-P-L will be included in this.

**The Cholesterol Derivatives.** Since the crystallization and

<sup>1</sup> Schramm and Hanisch (185) have observed that addition of a colloidal cholesterol solution to minced guinea-pig ovarian tissue increases its oxygen consumption in a specific manner, suggesting that the cholesterol has been oxidized.



identification of oestrone in 1929, preparation of pure natural and artificial androgenic and oestrogenic compounds, and others related to them but physiologically inert, has been extraordinarily rapid. In addition, a number of synthetic oestrogenic compounds has been prepared, which are not derivatives of cholesterol. The earlier work was largely carried out by Butenandt and Ruzicka, who both, and also Tscherning, published useful summaries of this work in 1936 (20, 178, 206). More recently the chemistry of this series of compounds has been reviewed by Marrian (201, 134), by Koch (104), and by many others.

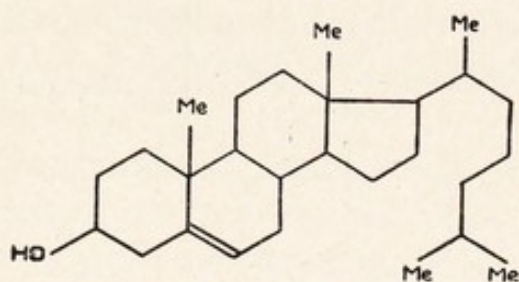
The constitutional formulae of some important members of the series are given on pp. 265 and 266. All the sex-hormones derived from cholesterol have the characteristic four-ring skeleton of that compound, of which the conventional numbering is shown on p. 265. The androgens and progesterone can be regarded as derived from a  $C_{19}$  hydrocarbon, androstane, with two methyl groups, and the oestrogens from 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 synthesized by the organism. Through dehydro-cholesterol the natural vitamin  $D_3$  is derived from it, while ergosterol and its derivative calciferol, the synthetic vitamin  $D_2$ , are closely related to it.

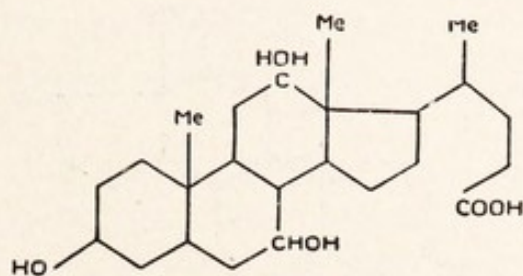
### Naturally Occurring Androgens

*Testosterone* (androstene-3-one-17-ol),  $C_{19}H_{28}O_2$  (melting-point  $154^\circ C.$ ), was first isolated in pure form from the testes by Laqueur (112) and has been prepared from cholesterol (179, 19). It is the most powerful natural androgenic substance known, being six or more times as active as androsterone by the capon comb test, two to five times as judged by the prostate test,

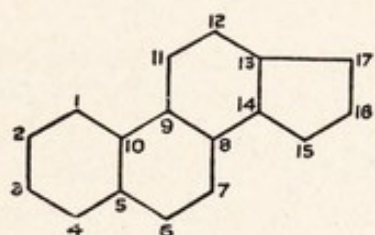




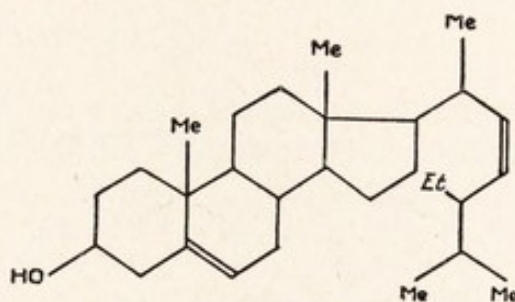
*Cholesterol*



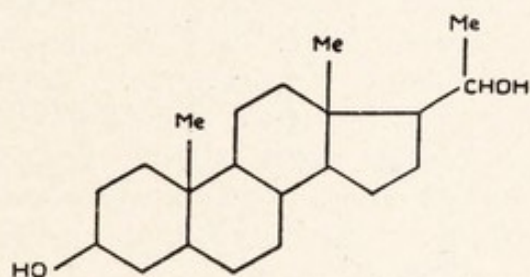
*A Cholic Acid*



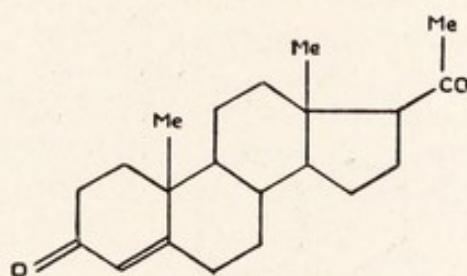
*Skeleton Ring Structure*



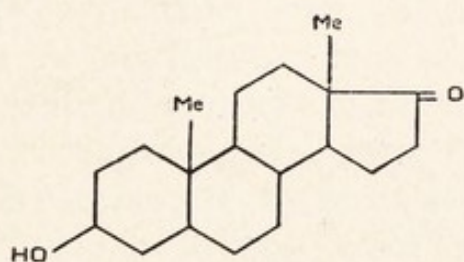
*Stigmasterol*  
(from Soy Bean Oil)



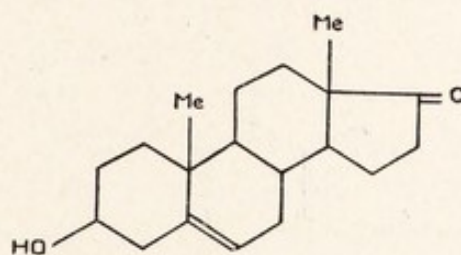
*Pregnanediol*  
(from urine)



*Progesterone*  
(from corpus luteum)

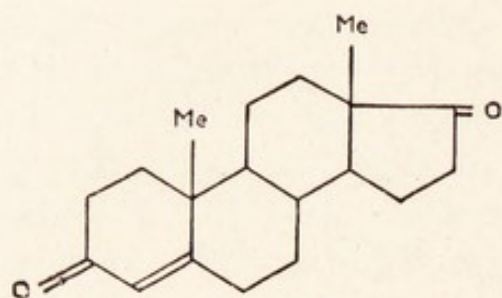


*Androsterone*  
(from urine)

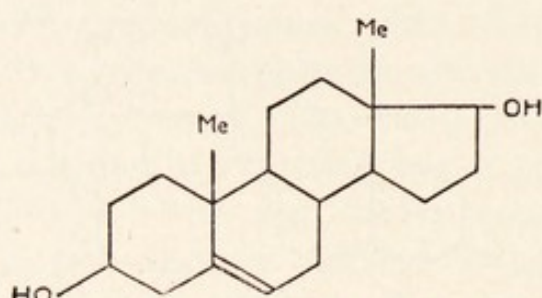


*Androstene-3-ol-17-one*  
(from urine)

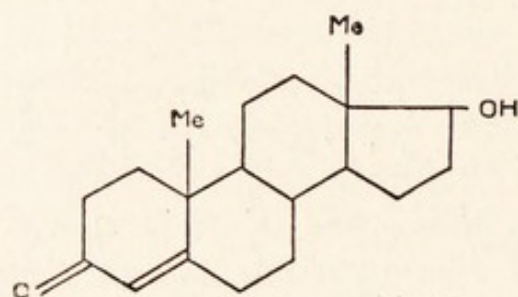




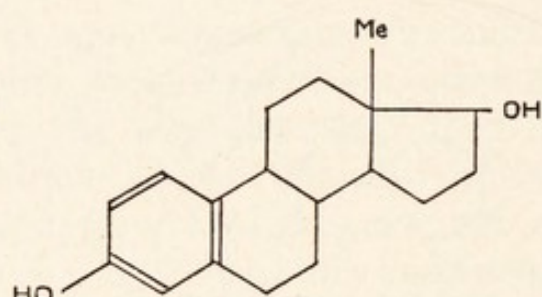
*Androstene-3, 17-dione*  
(artificial)



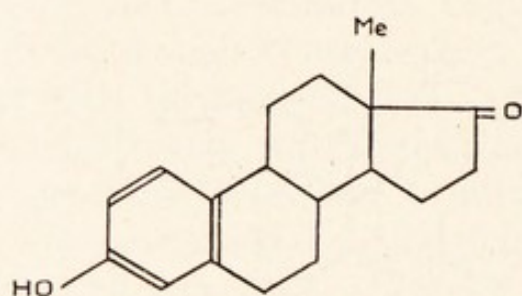
*Androstene-3, 17-diol*  
(artificial)



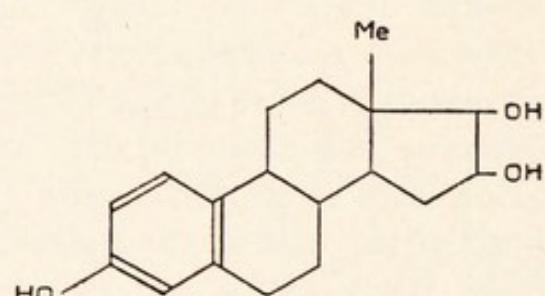
*Testosterone*  
(from testes)



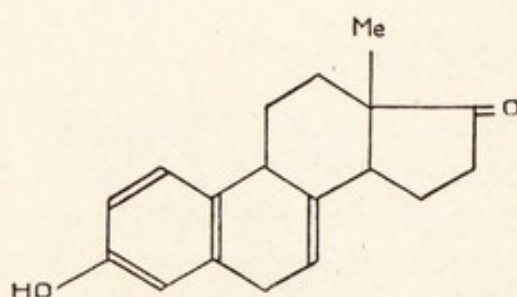
*Oestra-3, 17-diol*  
(from ovaries)



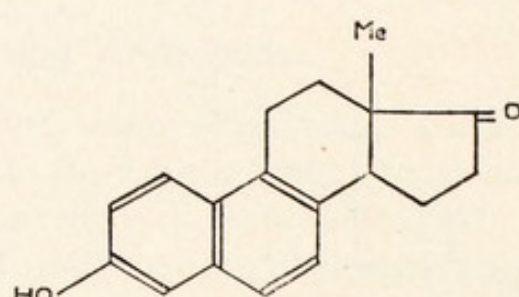
*Oestrone (Theelin)*  
(from pregnancy urine, etc.)



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



*Equilin*  
(from pregnant mare's urine)



*Equilenin*  
(from pregnant mare's urine)



and ten times as judged by the action on the seminal vesicles of castrated rats (44). Certain of its esters show greater prolonged potency.

*Androsterone* (androstane-3-ol-17-one),  $C_{19}H_{30}O_2$ , the first androgen to be obtained in crystalline form, was prepared from urine, and later from epicholesterol by Butenandt, who proved its constitution. It melts at  $178^{\circ}C$ .

*Dehydroisoandrosterone* (androstene-3-ol-17-one),  $C_{19}H_{28}O_2$ , is also present in human male urine, from which it was isolated by Butenandt. It melts at  $148^{\circ}C$ . Its physiological activity is about one-third that of androsterone (104).

In addition to these, Laqueur believes (and Deanesly and Parkes, and others have supported his view) that testicular tissue contains a still unknown compound, Laqueur's *X-substance*, which, while neither androgenic nor oestrogenic itself, materially enhances the activity of testosterone, though not needed to activate it. It is stated to be also present in liver, adrenals, ovaries, wheat seedlings, and urine, and is lipide-soluble. It has been purified to the extent that a 0.5 mg. daily dose is definitely effective in accentuating testosterone action on rats. The X-substance behaves like a highly unsaturated fatty acid (104, 66). But it may prove to be no specific substance, but merely a naturally occurring metabolite with the power to enhance the activities of testosterone and other compounds.

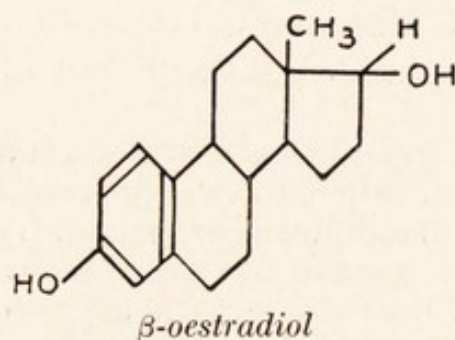
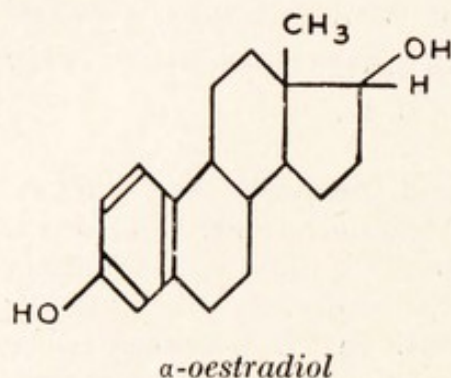
Koch, in 1937 (104), listed some thirty compounds (including certain esters) which exhibit androgenic activity. Many of these have only been prepared in the laboratory. A number of them have potency of the same order as androsterone. Such a synthetic compound as androstene-3,17-diol is of interest, since it is both androgenic and oestrogenic (179, 19, 18).

### Naturally Occurring Oestrogens

*Oestra-di-ol* (oestradiol, dihydro-oestrone, dihydrofolliculin),  $C_{18}H_{24}O_2$ , is known in two forms. The alpha form is chiefly responsible for ovarian activity, and was first prepared by Schwenk and Hildebrandt in 1933 (99) by partial dehydrogenation of oestrone. It was later isolated by Doisy and his colleagues from sow ovaries (124) and, very recently, from the urine of pregnant women (50B). Wintersteiner has obtained both compounds by reduction of oestrone, the alpha-form being produced in greater yield (215), while he has also isolated the beta-form from the urine of pregnant mares. The alpha-form is stated to be about six times as physiologically active as



oestrone; the beta-form has much the less activity of the two (215, 21). The two isomers are easily separated, since the alpha-form is precipitated by digitonin, and the beta-form is not. The first crystallizes in prismatic needles and melts at  $176^{\circ}\text{C}$ ., the second crystallizes in thick prisms and melts at  $218^{\circ}$ . Their solutions in alcohol have specific rotations (for  $18^{\circ}$  and D) of  $78^{\circ}$  and  $56.7^{\circ}$  respectively. Their formulae are shown.



*Oestrone* (Theelin),  $\text{C}_{18}\text{H}_{22}\text{O}_2$ , melts at  $250\text{--}251^{\circ}\text{C}$ . 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. 40.

*Oestriol* (Theelol),  $\text{C}_{18}\text{H}_{24}\text{O}_3$ , 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  $180^{\circ}\text{C}$ . 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 (136).

*Equilin*,  $\text{C}_{18}\text{H}_{20}\text{O}_2$ , and *Equilenin*,  $\text{C}_{18}\text{H}_{18}\text{O}_2$ , have been derived from urine of the pregnant mare. Both show slight "female" activity (71).

*Emmenin*. As already mentioned (p. 261), 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 hydrolyzed to oestriol.

The work of Marrian and his colleagues (32) indicates that emmenin is oestriol glycuronide, 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 other ester form of combination, requiring autoclaving in acid solution to liberate oestrone and oestriol. Glycuronides 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 glycuronide are protective measures during pregnancy against over-activity of oestradiol and oestrone.

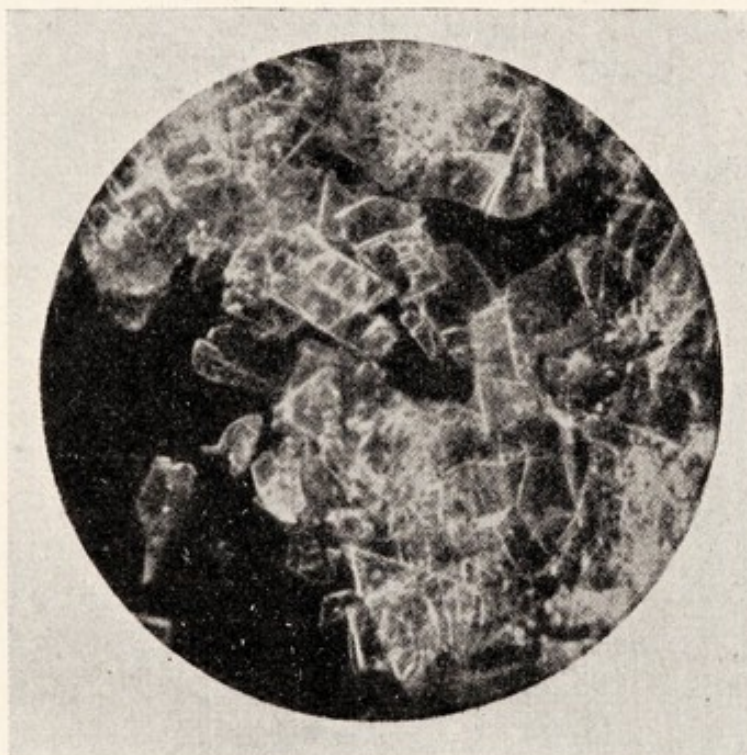


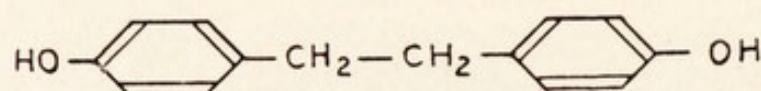
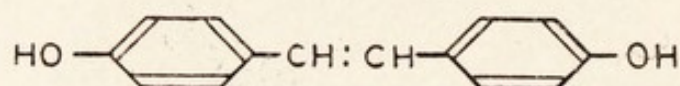
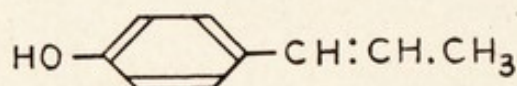
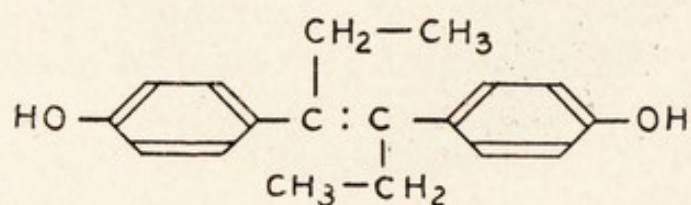
FIG. 40. Crystallized oestrone from acetic ether. (From Butenandt, *Zeitschr. physiol. Chem.*, 1930, exci, 127.)

**Synthetic oestrogens** have been especially studied by Dodds and his collaborators (39, 49). They have found active oestrogens among the derivatives of diphenylethane and diphenylethylene. Anol (p-hydroxy-propylenebenzene) and 4 : 4'-dihydroxy-diphenyl also have oestrogenic activity, so that evidently the phenanthrene nucleus is not essential for such activity. The most potent compounds they have so far obtained are *stilboestrol* and *hexoestrol*.

Stilboestrol (diethyl-stilboestrol, and, in full, 4 : 4'-dihydroxy- $\alpha\beta$ -diethylstilbene) has an activity three times as great as that of oestrone (and almost comparable with that of oestradiol) and



the additional therapeutic advantage that it is relatively more effective than either of these when administered orally. Its action is similar to that of oestrone on the uterus of the ovariectomized rat, on the mating reaction, on the vagina and uterus of immature rats, and on nipple growth in the guinea-pig (but it shows less action on the mammae than does oestrone). Its esters behave similarly to those of the natural oestrogens, the effect being slightly lessened, but prolonged. The dipropionate is especially effective.

*Diphenylethane**Diphenylethylene**Anol**Stilboestrol*

The compound 4 : 4'-dihydroxy- $\gamma\delta$ -diphenyl- $\beta\delta$ -hexadiene has activity about equal to that of stilboestrol, while hexoestrol (4 : 4'-dihydroxy- $\gamma\delta$ -n-hexane) seems to be even more active, producing full oestrus response in rats in a dose of less than 0.2 $\gamma$ .

### The Progestational Hormone and its Derivatives

*Progesterone*,  $\text{C}_{21}\text{H}_{30}\text{O}_2$ , crystallizes in two isomeric modifications, the  $\alpha$ -form, melting at 128.5° C., and the  $\beta$ -form, melting at 121–122° C. These crystal modifications both possess practically the same physiological activity (cf. 94). It has been prepared by Butenandt from stigmasterol, the sterol of soy bean oil.



*Anhydro-oxy-progesterone*, a synthetic product, the pregneneinolone or ethinyl-testosterone of Inhoffen and Hohlweg, and Ruzicka, is, according to Emmens and Parkes (56), equally active in progestational properties whether given orally or by injection. Its activity is about one-tenth of that of progesterone. In addition it shows some oestrogenic activity (causing growth of the immature uterus) and weak androgenic activity, as judged by the comb test.

*Pregnanediol*,  $C_{21}H_{36}O_2$ , was isolated from the urine of pregnant women independently by Marrian (133) and Butenandt (17). It has no physiological activity, but is one of the chief excretory products of progesterone, and its estimation in urine has proved to be of important physiological and diagnostic value. It is excreted as sodium pregnanediol glycuronide (see below) in human urine, and has also been isolated by Marker, Kamm and McGrew (222) from urine of the pregnant mare.

### Excretion of Sex Hormones and their Degradation Products

While beta-oestradiol has been isolated from the urine of pregnant mares (92) and oestradiol from that of pregnant women (50B), definite proof of the excretion of unchanged testosterone does not yet appear to be available.

Androsterone is the principal androgenic compound excreted in the urine of men and women. It has been isolated in crystalline form from women's urine to the extent of 1.3 mg. per litre, while dehydroisoandrosterone has been obtained in yield of 0.2 mg. per litre. These figures are comparable with the yields from men's urine (25). Excretion of androgens in male children is slightly higher than in female children of the same age (152A).

According to Koch, man excretes daily 63 to 68 international units (capon units) of androgens and 9 to 12 $\gamma$  of oestrogens (calculated as oestrone), woman 42 to 56 I.U. of androgens and 18 to 36 $\gamma$  of oestrogens; Hamblen (83) gives lower figures for androgens, for normal women. Male castrates excrete very little androgen, eunuchoids' excretion is below normal, in cases of gynaecomastia there is no excess excretion of oestrogens, but in cases of virilism in women there is a tendency to low excretion



of oestrogens and somewhat increased excretion of androgens (105), which is probably in whole or in part accounted for by androgenic compounds from the adrenal cortex (cf. Chapter V, pp. 211, 217). Indeed, all the androgens of normal women's urine may be of extra-ovarian origin (cf. 83, 24).

All androgenic material in fresh male human urine is present in conjugated form (125). Androsterone and dehydroisoandrosterone are excreted as glucuronides (Koch and others). Oestrone is excreted as a conjugated sulphate in the urine of pregnant women and pregnant mares (201, 181, 22): one may reasonably presume that it is present in this form in the urine of non-pregnant women also. Oestriol is excreted as a glucuronide; it has been isolated as sodium oestriol glucuronide from pregnancy urine (33).

However, at the mid-interval of the menstrual cycle there is a sudden appearance of free oestrogen in the urine, possibly due to its liberation in the follicular fluid from the ruptured follicle at ovulation, and peritoneal absorption and excretion too rapid to permit complete conjugation. Free oestrogen is also present in slight amount just before the onset of menstruation (158).

Zondek has shown that stallion's urine (and testes) are relatively very rich in oestrogenic material (229); the urine definitely contains oestrone (29). The urines of the stallion, ram and bull contain relatively little androgenic material (Koch).

Venning and Browne (209) showed that progesterone is excreted in urine as sodium pregnanediol glucuronide, and have devised a method of estimating the latter, by which they have shown that its excretion commences within forty-eight hours of ovulation in women, and ceases twenty-four to forty-eight hours before menstruation commences. They have also shown that the compound is excreted during pregnancy. Their work has been confirmed by numerous observers (*e.g.*, 78, 221, 200). Wilson, Randall and Osterberg (221) state that the pregnanediol compound may be excreted in the late proliferative stage of the endometrium, indicating that there is an interval of time before progesterone produces the differentiation (progestational) stage. During pregnancy there is an average daily excretion of 5 to 10 mg. during the first four months, which then rises rather sharply to an average of 25 mg. in the fifth month, and then more slowly to an average of 54 mg. in the ninth month (though there



are large variations in the actual figures for different women). The excretion falls to less than 5 mg. by the fourth day post partum. (Their results indicate that during the first four months of pregnancy the corpus luteum is responsible for the progesterone, and, subsequently, the placenta.)

In addition to pregnanediol it has been shown by Marker, Kamm and McGrew that during pregnancy women also excrete the isomeric allopregnanediol, and traces of epi-allo-pregnane-3-ol-20-one (a compound which possesses androgenic activity of the same order as androsterone) and epi-pregnane-3-ol-20-one, all of which must be considered as probable degradation products of progesterone (222).

**The Chorionic Gonadotrophic Hormone, A-P-L.** Evidence strongly indicating the non-identity of A-P-L with either of the gonadotrophic hormones of the anterior pituitary will be submitted later. In the meantime it will be assumed that this hormone is produced primarily by some part of the fertilized ovum, immediately after it becomes embedded in the uterine wall (or that of one of the tubes), and, subsequently, from the corresponding foetal part of the placenta, and continues to be produced from this source until detachment of the placenta, either at normal parturition or at abortion.

As has been stated, two sources of the hormone are available, the placenta itself, as Collip showed, and the urine of pregnant women, as Zondek first demonstrated.

Collip purified A-P-L from placenta to the stage at which it could be used therapeutically; the purified material behaved as a protein. Bischoff and Long (7), studying a preparation from urine of pregnancy, found that it was stable to moderate excess of nitrous acid, formaldehyde, acetaldehyde, and iodoacetate. They could obtain no evidence of the existence of more than one gonadotrophic hormone in urine of pregnancy. Gurin, Bachman and Wilson (77), by preliminary absorption on benzoic acid, and extraction with aqueous acetone or 50 per cent. alcohol, obtained highly purified preparations (containing from 1,000 to 3,000 minimum effective doses per milligram as tested by the Friedman test on rabbits) and showed that these preparations contained a carbohydrate polypeptide with the properties of a mucoid. Hexosamine and acetyl radicals were present; pentose, ketohexose, and uronic acid groups absent.



The purest preparations contained two hexose radicals per hexosan. The non-hexosamine radicals appeared to be galactose.

A gonadotrophic hormone is present in the serum of the pregnant mare, which probably is formed in placental tissue, but which appears to differ from A-P-L, possessing activity more closely resembling the combined actions of the two gonadotrophic hormones of the anterior pituitary (cf., *e.g.*, 154B). Cartland and Mason (28) have made highly concentrated preparations of this hormone. Rinderknecht (169) also effected marked concentration, by precipitation with sulphosalicylic acid and fractional precipitation with acetone, obtaining finally a white powder, soluble in water.

The hormone in the blood serum of pregnant women resembles in activity, and is probably identical with A-P-L (10).

### Functional Activities of the Hormones Concerned with Reproduction <sup>1</sup>

*Introduction.* The hormones concerned are, it will be remembered, the follicle-stimulating and luteinizing hormones of the anterior pituitary, similar hormones from chorionic tissue, prolactin, the androgenic hormones of testicular origin in the male, the androgenic hormones of adrenal cortical or other origin in the female, the oestrogenic hormones in the male and in the female, and progesterone of the corpus luteum (while perhaps the formation of some progesterone in the adrenal cortex—cf. Chapter V, p. 215—should not be forgotten).

<sup>1</sup> The rapid publication of small, fragmentary papers, necessitated in part, perhaps, by forced competition, is nowhere better exemplified than in physiological studies of the gonads. The confusion which results from this mass of material, often contradictory, has been fortunately partly dispelled by fairly frequent, admirable reviews, such as those of Marrian and Butler (135), Wintersteiner and Smith (222), and Freud, Laqueur and Mühlbock (66) in successive volumes of the *Annual Review of Biochemistry*, and of Evans (57) in the corresponding *Annual Review of Physiology*, and of Koch, Corner, Newton, and Snyder, in *Physiological Reviews*. In these, in the annual articles by Sevringhaus in the *Year Book of Neurology, Psychiatry and Endocrinology* (189), in numerous articles in Vol. V of the *Quantitative Symposia of Quantitative Biology* (201), and the recent monographs of Reynolds (168) and of Fluhmann (60) there are copious references, and these reviews will therefore be largely quoted to avoid an unwieldy list of references in this volume. It is quite impossible to deal with the whole literature here.



Many of these can act alone; others, for example progesterone, need for their action tissues prepared by other hormones. In still other cases, as, for example, when both oestrogens and androgens are present, normal results may depend perhaps upon the maintenance of some degree of correct balance between them, while differences in relative amounts may be the determinative factor for sex (cf. 57).

### Reproductive Activity in the Female Animal

*Events Preceding Ovulation.* Under the influence of the follicle-stimulating hormone of the anterior pituitary some proportion of the follicles of the ovary enlarge, with potential production of oestrogen. Ultimately the stimulation effects rapid growth of a number of Graafian follicles, with resultant rapid increase in the output of oestrogen, and then one or more of these follicles rupture—the actual number varying in different species—with discharge of one or more ova. The infundibulum of the apposed Fallopian tube is alternately exposed to each side of the neighbouring ovary, and ciliate movements assist the transfer of ova to the tube (168).

The actual rupture of a follicle seems due to increased osmotic pressure of its own fluid contents (194). Its ovum is slowly extruded in an oozing mass of fluid (168). The process apparently needs no nervous control, for it can occur in apparently normal fashion after all nerve connections to the ovary have been severed, and has occurred in transplanted ovaries of many species. Yet potential nervous influences producing, for example, delay in ovulation, cannot be entirely disregarded (Hinsey, 201).

*Site of Production of the Ovarian Hormone, and its Nature.* Zondek and Aschheim (226) concluded from study of the comparative effects produced by implants of different ovarian tissues that the hormone is present in the theca interna cells, and especially in atretic follicles, but is absent from the follicular granulosa, ovarian stroma, and germinal epithelium. Corner, in a recent critical review of the available evidence (42), concludes that the site of production is most probably the theca interna of follicles of all sizes.

It is usually considered that the actual hormone of the ovary



is oestradiol, and that oestrone is produced from it (and is perhaps to be regarded, since it is relatively so much less active, as the first degradation product of oestradiol). If this be the case, then in some species some oestradiol must be converted to oestrone within the ovary itself, since, for example, Doisy has shown that 1 kg. of sow ovary contains 0.014 mg. of oestradiol and 0.010 mg. of oestrone, the oestrone being responsible for 15 per cent. of the total oestrogenic activity. On the other hand, cow ovaries do not appear to contain oestrone (214). Smith and Smith have obtained evidence that the human ovary can not only convert oestradiol to oestrone, but also oestrone to oestradiol (193).

*Sequels to Ovulation.* The formation of corpora lutea in the ruptured follicle has already been described (cf. p. 251). Progesterone is probably formed in the lutein cells, the cells specific to the corpus luteum.

The unruptured Graafian follicles continue to produce oestrogenic hormone. Corpora lutea of certain species produce oestrogen also. This is not surprising, since these corpora lutea contain both epitheloid layers of the follicular wall. The corpora lutea of the sow yield 60 to 80 rat units of oestrogen per kilogram of fresh tissue, those of women 2,000 to 3,000 rat units or even much more per kilogram (42).

The above data suggest the need for oestrogenic activity concurrently with that of progesterone.

*Physiological Activities of Oestrogens and Progesterone, in Absence of Pregnancy.* Oestrogens stimulate the early growth of the uterus and maintain it; the atrophy of the uterus which follows ovariectomy, and which sets in after the menopause, can be prevented by administration of oestrone or oestradiol (3, 168).

Oestrogens prepare the uterus and tubes for the stimulation of progesterone which follows the first, and each succeeding ovulation; the oestrogenic hormone is responsible for the pre-ovulatory growth of the uterus in each oestrus cycle (168). The principal effects produced are a generalized hyperaemia and, following this increased vascularity, increased growth of the endometrium, with mitoses in glands and epithelium of this tissue. In the atrophying gland, after ovariectomy, these changes begin a few hours after injection of oestrogen, and are



accompanied by increased oxygen consumption. The tubes are similarly affected. During this pre-ovulation period (in woman and the female monkey) the uterus contains little or no glycogen (168, and Allen, 201).

Progesterone is responsible for the post-ovulatory changes in the uterus; oestrogenic stimulation must precede to enable a response to it. For normal post-ovulatory development of the uterus concurrent action of both progesterone and oestrogen seems necessary, though excess of oestrogen prevents proper endometrial response to progesterone (168).

Progesterone produces secretory changes in the endometrium, still further augments its blood supply, and, by some mechanism still unknown, causes deposition of glycogen (for nutritive purposes), especially in the most superficial glands of the corpus uteri (168).

The actions of oestrogen and of progesterone are directly on the cells of the uterus. Very minute doses of crystalline progesterone introduced directly within the lumen of the uterus of the rabbit, previously sensitized with oestrone, produce the characteristic progestation proliferation of the endometrium (131).

Changes in the vaginal epithelium indicated by those in the vaginal smear, and resulting from the effect of oestrogen, have already been dealt with (p. 256). Mucification of the vaginal epithelium may be due to synergistic action of balanced amounts of oestrogen and progesterone (222). Experimental administration of oestrogen causes a lowering of the *pH* of the vaginal fluid of monkeys, while subsequent treatment with progesterone leads to a rapid rise of *pH*; such a rise generally precedes menstruation (52). Changes in glycogen content under such treatment indicate that lactic acid production may determine the *pH* (222).

The patterned co-ordinated muscular activity in tubes and uterus which Reynolds stresses (168) is initiated by oestrogen. Contraction waves commence in the myometrium of the tubes and spread to the uterus. When these changes are produced in the experimental animal, such motility follows the initial hyperaemia by a latent period of definite length, which is not affected by increase of dosage, and is induced by oestradiol, oestrone, or oestriol. Progesterone inhibits this rhythmic



motility. Quiescence of uterus and tubes sets in at variable periods after ovulation in different species. It is abolished by experimental removal of the corpora lutea and therefore of progesterone. Knaus has shown that in pseudo-pregnancy in the rabbit—which is in the post-ovulatory state—the uterus will not contract following injection of pituitrin. At this stage also, injection of oestrogen does not lead to motility. But after removal of the corpora lutea (or after hypophysectomy which results in their atrophy) normal response to oestrogen or to pituitrin quickly follows (168). (However, presence or absence of corpora lutea does not affect uterine response to posterior pituitary extracts in the rat, mouse, guinea-pig and cat (57).)

Recent important work throws a little light upon the chemical changes accompanying the physiological phenomena just described. Pincus and Zahl (165) found that if oestrone is administered to a pseudo-pregnant rabbit, or to an ovariectomized rabbit that is being treated with progesterone, oestriol appears in the urine. This does not occur in hysterectomized animals, so that the uterus is essential for the change from oestrone to oestriol. Smith and his confreres (195) have plotted curves of the excretion of total oestrogen, oestrone, and oestriol during the menstrual cycle, and during pregnancy in women, and their results are in harmony with the assumption that oestradiol of the ovaries is converted to oestrone, and that the degree of conversion of oestrone to oestriol depends on the amount of progesterone present. Browne and Venning (14, 210) have shown that when progesterone is injected intramuscularly into a patient with intact uterus 40 to 46 per cent. is recoverable in the urine as sodium pregnanediol glycuronide. In hysterectomized patients so treated no pregnanediol is recoverable, though if pregnanediol itself is injected the same proportion is recovered. Hence it is evident that uterine tissue is needed to bring about this change, and it is probable that almost all progesterone is normally converted to pregnanediol in the uterus.<sup>1</sup>

The progesterone-pregnanediol and oestrone-oestriol changes

<sup>1</sup> At least in males some other tissue can transform progesterone to pregnanediol. Buxton and Westphal (23A) have observed that when 30 mg. of progesterone were given daily to two men with Addison's disease and to one normal man, sodium pregnanediol glycuronide could be isolated from their urine. Adult monkeys given progesterone do not excrete pregnanediol. Female rabbits and cats do not normally excrete it.



are obviously interlocked. Marrian (134) believes that progesterone acts as a hydrogen-acceptor in the formation of oestriol from oestrone.

Oestrogens stimulate the development of the mammary glands, though the mechanism of action, and whether it be direct or exercised through the pituitary, is still not definitely known (cf. 153). However, the observation of MacBryde (122) that application of oestrogen in ointment to breasts stimulates their growth, and that if the treatment be confined to one breast the growth is limited to that breast strongly suggests that the action is direct.

The female pattern of hair distribution is due to the action of oestrogens. This specific pattern develops precociously in young girls with granulosa-cell tumours which produce oestrogen (cf. p. 298), while no abnormal distribution occurs in women with anovulatory cycles (cf. p. 289), so that it does not appear to be in any way under the control of progesterone. It may be assumed that other female sex characters are controlled by oestrogens.

Some curious observations have been made by Hamilton (84), which indicate that tanning of the skin by ultraviolet rays of sunlight is due to the production of some colourless precursor which needs the presence of gonadal hormones for a photograph-like "development," both androgens and oestrogens being effective.

The deep reddening and swelling of the "sexual skin" (covering the external genitalia) of monkeys is the result of oestrogenic stimulation, and has been produced in ovariectomized monkeys and baboons by injections of oestrogenic extracts (3); it is maximal at time of ovulation (60). The nasal mucosa of the intact monkey responds to oestrogen in a specific manner, especially in the middle and inferior conchae which exhibit reddening and swelling. In normal animals this nasal sex activity occurs cyclically and pre-menstrually. A similar phenomenon occurs in women (148).

Oestrogens injected into rats of both sexes initially cause pituitary enlargement, and can thus indirectly through pituitary stimulus produce formation of corpora lutea in female animals, enlargement of the adrenal cortex, and increased thyrotrophic activity and basal metabolism. Selye and Collip



found that when oestrone is administered to hypophysectomized rats whose ovaries were maintained in normal condition by administration of anterior pituitary extract, enlargement of the ovaries was produced, suggesting direct action of oestrogen on the ovary. Chronic treatment of normal animals with oestrone causes atrophy of the gonads, presumably through inhibition of the gonadotrophic activity of the pituitary (135). (Cf. also 44A.)

*Fate of Ova.* Fertilization of ova must occur within a relatively short period after their liberation, or does not occur at all. Two factors limit the possibility of fertilization within this time-interval, the death within two or three days of spermatozoa at the temperature of the body (103), and early changes at the outer surface of the ovum, which becomes enveloped by a layer of protein nature, rendering it impermeable to spermatozoa (168).

Whether fertilized or unfertilized, ova pass down the tubes, at first rapidly under the influence of the rhythmic contractions, then more slowly, when progesterone from the newly formed corpora lutea has inhibited the motility. A definite time is needed for each species, to permit adequate preparation of the uterus for nidation. Thus if motility of the tubes, in rabbits and mice, is unduly increased by injection of oestradiol, passage of fertilized ova can be accelerated, but they thereupon disintegrate in the fluid of the unprepared uterus (57).

Passage through the tube lasts three days in the monkey, after which the fertilized ovum remains six days in the uterus "bathed in the nutritive juices furnished by the maternal glands" (88). The time periods in woman are probably about the same.

Under normal conditions the unfertilized ovum dies during passage, and passes out of the uterus. The fertilized ovum, after a period varying with the species, penetrates the surface of a uterine endometrium properly prepared for its reception by progesterone. If fertilization does not occur, the lower mammal returns to a condition of anoestrus; primates menstruate.

*The Cycle of Pregnancy.* The fertilized ovum reaches the lumen of a uterus prepared for nidation by the combined action of oestrogen and progesterone, and well stored with glycogen; as just stated, after a period varying with the species the ovum



embeds itself in the endometrium. In man and the chimpanzee implantation is interstitial, in the monkey superficial (87). With this embedding the long cycle of pregnancy commences.

In the higher species of mammals the ovarian cycle is in abeyance during pregnancy, presumably through chronic oestrogenic stimulation of the anterior pituitary and resulting depression of the gonadotrophic function of that gland. Ovarian follicles grow and mature, but do not ovulate as a rule. (In the rhesus monkey there may be one or two abortive cycles which seldom reach the stage of bleeding. Two cases of women with bicornate uteri have been reported, in whom periodic bleeding during pregnancy was observed.) The lower species of mammals vary in this respect. The oestrous cycle continues in the rat, and some stages of it occur in the guinea-pig. Ovulation can be induced experimentally in the mouse without abortion following. It occurs spontaneously in the horse, while in the cow follicles tend to enlarge, with occasional oestrus (197).

During pregnancy in the rabbit both tubes and uterus remain quiescent for a long period, and activity of the tubes no longer spreads to the uterus, which does not exhibit spontaneous motility until late in pregnancy. Growth of the uterus is due to a combination and alternation of hormonal stimuli, and of the distension stimulus of growing foetuses and their placentae (168) of combined uterine and foetal origin.

The placenta contains glycogen, whose storage in this tissue, as in the uterus, is probably to be traced to action of progesterone. The placenta also contains oestrogens, and progesterone.

According to Doisy, 25 per cent. of the oestrogenic activity of the human placenta is due to oestrone, the remainder to non-ketonic compounds (presumably chiefly oestriol and its esters). Oestriol, and its ester emmenin, have been prepared from placenta. Both oestrone and oestradiol are present and have been isolated (214, 50A). It does not necessarily follow, of course, that these compounds are formed in placental tissue. But when ovariectomy is performed on the pregnant mare at the 200th day of pregnancy, her urine continues to contain oestrogenic compounds, which must therefore have an extra-ovarian source. And there is evidence, though it needs confirmation, that oestrogenic compounds are also present in



the urine of pregnant women after double ovariectomy. Newton is of the opinion that the balance of evidence indicates that placental tissue can produce oestrogens, but is insufficient to show that it produces the large amounts excreted during human pregnancy (154). Corner believes that to the extent that production of oestrogens is extra-ovarian, it is almost certainly in the placenta (42). But Reynolds considers that there is no evidence for the placental production of oestrogens in the mouse, rat, or rabbit (168). It may be remarked that emmenin is only associated with the placenta and the urine of pregnancy, and that there is no evidence to suggest that this ester of oestriol is formed elsewhere than in the placenta.

It has been mentioned that, following embedding of a fertilized ovum the corpus luteum persists and enlarges (p. 252), though the need for its persistence seems to vary in different species. Snyder (197) has recently reviewed the evidence bearing on this particular point. In the opossum, rabbit, and mouse the corpus luteum is essential throughout pregnancy. In the guinea-pig its removal does not necessarily lead to abortion after the ova are implanted. In the cat, ovariectomy at the forty-sixth day of pregnancy leads to abortion, but at the forty-ninth day does not. In women ovariectomy at the thirty-fourth day, and in the female rhesus monkey at two months, does not necessarily lead to abortion. In the mare and female donkey there is no corpus luteum present in the latter part of pregnancy.

The corpus luteum, when present, continues to produce progesterone. In sows biological tests demonstrate the presence of that compound in corpora lutea until the last ten days of pregnancy (57).

The question at once arises: Is a supply of progesterone needed throughout pregnancy in species which do not need a persistent corpus luteum, and if so, from what other source is it available?

It will be remembered (cf. p. 272) that in women pregnanediol is excreted throughout pregnancy, and in amount which rises sharply during the fifth month, and then continues to rise, its excretion ceasing abruptly just after parturition, which certainly suggests a placental origin. As Newton points out (154), after the sixtieth day the amount excreted is greater



than at any time during the menstrual cycle. Further, the ratio of oestriol to oestrone in urine continually increases during pregnancy (66), indicating that progesterone is functioning normally. Newton states that the presence of small amounts of progesterone has been definitely demonstrated in placental tissue (154). It seems very probable that, at least in some species for which continuing corpora lutea are not essential, progesterone is needed throughout pregnancy, and must be available from other tissues. It is a natural supposition that the placenta takes on this function vicariously, but final evidence is still needed (cf. 168).

The duration of pregnancy is to some extent under experimental control. Delay can be caused in mice by suckling young, or by injection of progesterone, and in rats by injection of extracts of the anterior pituitary. Such delay results in a parallel delay in development of the foetuses and in parturition (197). If rabbits, normally at term on the thirty-second day, are injected with A-P-L on the twenty-fifth day, ovulation is induced, with production of fresh corpora lutea, and parturition is delayed to the fortieth day. (That the delay is due to the new supply of progesterone is shown by the fact that injection of crystalline progesterone itself will cause it (91).) The foetuses continue to grow, and remain alive for three days past normal term, then die, but the placenta still functions after their death, so that onset of labour cannot be due to changes in the foetus, or the placenta, or to mechanical distension of the placenta (197, 107).

The pharmacological action of oxytocin of the posterior pituitary in causing uterine contraction *in vitro* (cf. p. 334) suggests that oxytocin may well be a normal factor in induction of parturition. The evidence in favour of this view is inadequate, and it has even been shown that in the experimental animal parturition can occur after hypophysectomy. Yet it has also been shown that the oxytocic activity of the blood is depressed during pregnancy, but is increased above normal at term (30), and further that free oestrone appears to sensitize the uterus to the action of oxytocin, and that free oestrone, instead of its esters, becomes available just before parturition (6, 173, 136A). Furthermore, just before parturition the excretion of oestriol in urine decreases, and that of oestrone increases (66). Smith

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suggests that this increase of oestrone is the determining factor leading to onset of labour (195). (A case is on record which showed a marked rise in excretion of free oestrogen before abortion at the fourteenth week (159).) While therefore there is very much in favour of the view that oxytocin is involved in normal parturition (cf. also 197), yet Reynolds considers that the actual mode of contraction of the uterus in labour does not resemble that induced by oxytocin (168).

The endocrine control of reproduction does not cease at birth of young; their food supply has been prepared. Oestrone has stimulated directly or through the pituitary the mammary glands to the stage of storage of secretory products. Prolactin, from the anterior pituitary, stimulates an actual flow of milk, and its stimulus is enhanced by nervous reflexes set up through the act of suckling (172, 188). Maternal behaviour itself seems to be under control of prolactin (cf. Chapter VIII).

**Hormonal Control of Reproduction in Women and Female Monkeys.** A simple straightforward account of the endocrine factors concerned with reproduction in female animals is very difficult because of the marked differences between the oestrus cycles of the lower mammals and the menstrual cycles of primates, and further, because of lack of sufficient knowledge of many factors needed for adequate comparison of hormonal relationships during pregnancy of different species. In the foregoing paragraphs matters have been dealt with which seem to be common to a number of different species. In this section certain aspects of the subject will be discussed, particularly relating to the primates and especially to woman.

The initial stimulation of gonadal development under pituitary stimulation is the same in primates as in lower mammals. The young girl is virtually asexual for seven or eight years, and then in order, with increasing production of oestrogen, commence the development of the female pelvis, the breasts, and pubic hair, and ultimately the first menstruation occurs (60), to be followed by others at irregular but decreasing intervals until cycle follows cycle with a moderate degree of regularity. The early cycles may include ovulation, but not infrequently ovulation does not occur.

The general sequence of events in the genital tract from ovaries to vagina is very similar throughout mammals.



Dickenson's results from rectal palpation of women show that in them the same relationships hold. Motility of the uterus is greatest during maturation of the follicle (when oestrogen output is maximal). It subsides to quiescence as the corpus luteum forms and produces progesterone, and shows a secondary increase in activity just before menstruation (when progesterone output is decreasing) (168). (The high degree of sensitivity of the human uterus to progesterone is exemplified by the specific relief afforded by a single minute dose in the great majority of cases of "after-pain" (168).)

The uterus undergoes the same types of change in primates as in the lower mammals. The basal layer of its endometrium responds to oestrogen, generating, and in each new cycle regenerating a functional layer, which is subsequently converted by progesterone action to a receptacle suitable for a fertilized ovum. If no fertilized ovum nests within it the functional layer is cast off and the cycle is finished by menstruation.

*Menstruation.* Certain types of bleeding must be clearly differentiated from menstruation. So-called "mid-interval bleeding" of women and female monkeys, the pro-oestrous bleeding of the bitch and the oestrous bleeding of the cow occur at about the time the Graafian follicle reaches full development and ruptures. In addition, both bitch and cow, and also the guinea-pig, exhibit some bleeding which in point of time corresponds to the menstruation of primates (60).

The bleeding at the end of anovulatory cycles in women and monkeys can be described as pseudo-menstruation for the sake of distinction, though as far as is at present known, only histological examination of the uterine endometrium (and absence of excretion of pregnanediol) enable it to be differentiated from true menstruation.

Prior to menstruation glandular cells in the endometrium degenerate and secretion ceases. Markee transplanted a uterine graft of a macaque monkey to its eye. In the first part of the menstrual period, under oestrogenic stimulation, this graft showed a rhythm of alternate vasodilatation and vasoconstriction of certain areas; at the time of ovulation vasodilatation was marked. Prior to menstruation vasoconstriction occurred for a day or so, blanching the graft, then small arteries



dilated and ruptured, the haematomata produced increased in size, and finally free blood escaped (60).

The second (progestational) phase of the uterine endometrium needs progesterone for development and also for maintenance. If nidation does not occur, the corpus luteum regresses, and output of progesterone lessens. Menstruation follows cessation of its stimulus. Much evidence supports this view.

Venning and Browne's demonstration that excretion of pregnanediol and therefore production of progesterone ceases just before menstruation illustrates the close time relationship. When progesterone is administered in the second half of the cycle, in both women and female monkeys, the next expected period is definitely delayed (222). During surgical operations in women excision of a recently formed corpus luteum results in menstrual bleeding within forty-eight hours, although mere manipulation of the ovaries which does not affect such a corpus luteum does not hasten menstruation. But if, following excision of such a corpus luteum, progesterone is injected, menstruation only occurs in from three to six days (147). Massive doses of oestrogen do not have this effect (217).

Since there is some increase in oestrogen output just prior to menstruation (66), and since free oestrone (not esters) then appears, Smith believes that the bleeding of menstruation is due to this accumulation of free oestrone, itself due to its non-conversion to oestriol from lack of progesterone (193).

Menstruation is undoubtedly under nervous influences to some extent. Emotional states, such as fear of pregnancy and anxiety, may inhibit the menstrual flow in women (202), but the channel of influence, whether through the anterior pituitary, the ovary, or the uterus itself, cannot yet be stated (Hinsey, 201).

Any who have had reasonably large experience with the Friedman pregnancy test must have encountered a number of cases where the report of a negative result to an unmarried woman previously exposed to risk of pregnancy was rapidly followed by menstruation, and this time-sequence occurring two or more weeks after a missed period seems too definite to be attributable to chance. In such cases there seems no reason to suppose that ovulation was delayed, and one must conclude that the second stage of the cycle was unduly prolonged through some nervous control associated with anxiety.

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women (see Pryde BMS (1941)) who have no



In the anovulatory cycle menstruation or pseudo-menstruation occurs after the usual time-interval, but cannot be caused by cessation of progesterone production. Diminishing output of oestrogen due to regression of follicles seems to be the probable cause. (Genital haemorrhage of the new-born, with accompanying enlargement of uterus and breasts, can be similarly accounted for, by the stimulus of oestrogenic hormone from the maternal organism, and the sudden arrest of that stimulus at birth (60).)

*Time of Ovulation.* Ogino (103) and Knaus (103), from accurate studies of menstrual histories of many women, and correlation of occurrence of pregnancy with known times of coition, drew the conclusion that ovulation must occur about fourteen or fifteen days before the next expected period, and that, in order that pregnancy can result, coition must occur a little prior to, or just following ovulation. These conclusions were apparently supported by the results of ingenious experiments by Knaus on women (in which uterine contractions were recorded by means of inserted balloons kept under low pressure). He believed that he was able to show that the same refractoriness of uterine muscle to pituitrin stimulation existed in women during the second half of the cycle, as in the rabbit.

Such conclusions, supporting the theory of the existence of a so-called "safe-period" in women, during which coition cannot lead to pregnancy, have naturally an important bearing both on avoidance of pregnancy and on the induction of pregnancy in some proportion of apparently sterile women. Knaus' experiments on women have been repeated by a number of investigators, some of whom have claimed to confirm them, others to disprove his results. Reynolds (168) comments on all such work that investigators who obtained results in disagreement with those of Knaus did not sufficiently follow his directions, the main type of error of experiment being use of too large balloons under too great an internal pressure, and thus capable themselves through this pressure of setting up uterine contractions. Reynolds considers that Knaus' results are, in general, accurate, although the actual time interval set by Knaus is too rigid. Such a view is supported by many more recent observations of quite varying character.

C. G. Hartman (88, 87) has carried out a series of important



studies on female monkeys, and has demonstrated that the menstrual cycle so closely resembles that of women that conclusions about one species may reasonably be transferred to the other. Thus, for example, in the young adolescent monkey the length of cycle is very variable, as it is in young girls. In older monkeys and in women there is less time-spread, the average length of cycle being four weeks in both, though individual monkeys show less regularity than individual women.

The time of ovulation in monkeys can be accurately detected by rectal palpation, and occurs on the average on the thirteenth day of a twenty-eight-day cycle, that is, fifteen days before the next expected period. In almost all the animals studied ovulation occurred between the eighth and sixteenth day of the cycle. Hartman concludes that in the monkey there is an absolutely safe period during which conception cannot occur; the fertile days are from the eighth to the twenty-first of the cycle.

Various autopsy studies on women, whose menstrual histories were known and in which ova were recovered and the follicles which had produced them were studied, have set ovulation at between the eleventh and thirteenth day from commencement of previous menses (142).

The electrical potential of the body, measured from finger-tip to finger-tip of each hand, changes at the mid-interval (15). Rock (175) showed that when laparotomy was performed on a patient just after this change had occurred, a fresh ovulation was found—on the fourteenth day of the cycle; Burr has recorded a similar case (15).

Temperature studies by Zuck lend further support to ovulation at such a period (230).

Browne and Venning's findings concerning the initial time of excretion of pregnanediol (cf. p. 272), and data pertaining to the occurrence of mid-interval pain, and mid-interval haemorrhage are all in good agreement with limitation of the time of ovulation to within a relatively short period during the cycle.

Reynolds' recent statement undoubtedly represents the present view of the majority of investigators. "The occurrence of ovulation . . . for cycles of average length (28 to 30 days) . . . is generally from the 12th to the 18th day. Of course it is



inevitable that variations occur, due to diversity of the length of cycles in most individuals" (168).

While the whole theory of hormonal control of the cycle indicates such an ordered sequence of events that menstruation may be expected to follow ovulation in an ordered manner and after a definite lapse of time, yet, as has been already pointed out (p. 286), emotional influences seem undoubtedly able to extend this interval, by a mechanism still unknown.

*Anovulatory Cycles.* In the monkey anovulatory cycles are the rule during the summer months—the non-breeding season—and are frequent after pregnancy and during lactation (87).

Not only is a condition of amenorrhoea more common in adolescent girls during the summer months, but there is evidence that anovulatory cycles frequently occur. In cases where early coition is frequent, yet pregnancy is not usual until several years after the menarche, fertility gradually increasing (60).

The endometrium remains in the pre-ovulatory stage, and curettage towards the end of the cycle detects the condition. There are no clinical manifestations, and, as already mentioned, the lengths of cycles and of bleeding periods are normal. The cause of this failure to ovulate is not certainly known, but may lie in lack of appropriate pituitary stimulation (cf. 60).

Such cycles are by no means uncommon during the post-partum period. In a recent study of 194 cycles of forty-seven women, all initially nursing but with regularly occurring menses, 106 were found to be anovulatory, and eighty-two ovulatory as judged by endometrial biopsies. In certain cases the studies were carried beyond the stage of weaning, without affecting the type of result. It was found that the time of appearance of the first ovulatory cycle in these women was irregular, and further that occurrence of an ovulatory cycle does not predispose to another ovulation in the succeeding cycle (113).

*A-P-L as a Hormone of Placental Tissue.* As has already been stated, preparations of A-P-L from placental tissue or from urine of pregnant women produce effects on the ovary practically identical with those of the luteinizing hormone of the anterior pituitary. But in absence of the pituitary of the animal under test, such preparations produce no maturation of follicles, and no corpora lutea. Prestimulation of the ovary is needed by the



follicle-stimulating hormone of the pituitary before A-P-L can produce its effect. It produces in males an increase in size and number of the interstitial cells of the testes.

Newton has recently marshalled the evidence in favour of the production of this hormone by the placenta, and against its production by the anterior pituitary (154).

Pituitary implants from pregnant women appear to possess no gonadotrophic activity, although their urine is rich in gonadotrophic A-P-L. Urine from cases of hydatidiform mole and of chorionepithelioma is still richer in A-P-L, although, especially in the latter condition, there is no known association with increased pituitary activity.

The results of Kido's experiments are very suggestive. He has shown that when human chorionic villi are transplanted to the anterior chamber of a rabbit's eye, a positive A-P-L test (presence of haemorrhagic follicles) is shown by the rabbit's ovaries, and if that rabbit's urine is injected into a second rabbit its ovaries also show a positive test for A-P-L (154).

Very striking also are the results of Gey, Seeger and Hellman (70). They cultured placental tissue obtained at hysterectomy from a three-months' pregnant woman in a medium of 40 per cent. human cord serum, 10 per cent. beef embryo extract, 10 per cent. balanced salt solution, and 40 per cent. chicken plasma. Using the Aschheim-Zondek pregnancy test on twenty-one-day-old-rats, they found that placental cells produce a substance which behaves like A-P-L, and, when cultured for over two months, still retain this power, which possibly resides specifically in the Langhans' cells. They also found that a hydatidiform mole, similarly cultured for a month, produced the same A-P-L effect. Similar experiments with foetal pituitary tissue gave negative results.

As indicating the association of A-P-L with the ovum itself, there is evidence that when fertilized human ova two or three weeks old are implanted into rabbits, haemorrhagic follicles are produced in their ovaries. A-P-L appears in human female urine very shortly after nidation of a fertilized ovum, that is, as soon as material from the ovum can possibly reach the maternal circulation. It is thus detectable by the Friedman pregnancy test within a few days after a missed period. The amount rapidly increases during the first two months of

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pregnancy, then falls to a lower level, but persists until the placenta is completely detached at parturition or at abortion.

The term "chorionic gonadotrophic hormone" used by Hamburger and by Fluhmann obviously describes it. While its function cannot be regarded as definitely settled, the nature of its action strongly suggests that that function is to maintain an active corpus luteum and thus a constant supply of progesterone for the maintenance of the uterus of pregnancy.

A-P-L appears in the urine of the pregnant chimpanzee between the twenty-fifth and thirty-fifth day of gestation, and disappears between the 100th and 130th day. It is present in the urine of the pregnant monkey for one week only, between the eighteenth and twenty-fifth days (87).

It has already been pointed out (p. 274) that the gonadotrophic hormone of the blood of the pregnant mare is not identical with A-P-L, but is more powerful, possessing properties of both gonadotrophic hormones of the pituitary. It suddenly appears in the blood at the time when maternal and foetal tissues first make definite contact, almost certainly indicating its placental origin, and it disappears from the blood some hundred days later (154). This compound is not excreted in the urine. (Claims have been made that it has been successfully used to produce experimental ovulation in women in whom laparotomy was about to be performed and that its use has demonstrated that under such marked stimulation follicles can grow, rupture, release ova, and become corpora lutea in from twenty-four to thirty-six hours (44).)

A gonadotrophic hormone is present in the foetal part of the pig's placenta, and such a hormone is sometimes present in cow's urine between the thirty-eighth and 150th day of pregnancy (154).

*The Menopause.* Permanent cessation of menstrual cycles occurring in women in middle life may well be due, as Zondek suggests (227), to exhaustion of suitable ovarian follicular material. It is obviously a physiological process, though it is not infrequently accompanied by pathological manifestations.

With the cessation of ovarian response to pituitary stimulation, through lack of follicles to respond or to respond normally (212A), there is a fairly rapid cessation of oestrogenic output,



with resulting atrophy of the uterus and corresponding changes in the vagina (cf. 60).

9 **Reproductive Activity in the Male Animal.** In the male mammal development of the testes and their descent into the scrotum are due to stimulation of the gonadotrophic hormones of the anterior pituitary. Both spermatogenetic and interstitial elements are controlled. In man there is a 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, typical hair distribution, etc.—while concurrent stimulation of the sperm-producing cells by the appropriate pituitary hormone has brought these to a 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.

The available evidence suggests that testosterone, in performance of its endocrine function, is degraded to androsterone and dehydroisoandrosterone, which possess much less activity, and are rapidly excreted.<sup>1</sup>

Testosterone is relatively less effective in depressing the gonadotrophic activity of the pituitary than are the oestrogens, while its effect on the pituitary of male animals is much less than on that of females (104).

**Vicarious Activities and Anomalous Phenomena.** It seems desirable to stress these by dealing with them under a separate heading, since at least a number of them present puzzles whose solutions may influence our interpretation of many aspects of gonadal endocrinology.

Vicarious activities are generally to be explained by similarity of chemical constitution, and are probably of little importance

<sup>1</sup> Callow has isolated androsterone from the urine of a eunuchoid under treatment with testosterone, along with aetio-cholane-3-ol-17-one, and has shown that urines of both normal men and women contain amounts of these two compounds of the same order. On the other hand, since Callow and his colleagues have shown that the excretion of dehydro-isoandrosterone is not increased when testosterone is administered to a eunuchoid, but is increased in patients with adrenal cortical tumours, this compound is probably formed from some one of the compounds of the adrenal cortex, and not from testosterone (cf. 203A).



from the point of view of the normal functioning of the organism. Some instances follow.

Testosterone can stimulate the development of mammary glands in the rat, and the production of dense milk in them (41). Various androgens exhibit some progestational activity if given in sufficient dosage (222). Testosterone will maintain pregnancy, with resulting birth of live foetuses, in rats ovariectomized during the latter half of pregnancy (72).


Desoxycorticosterone, though chemically more closely related to progesterone than to oestradiol, is stated to be oestrogenic in the human female, since when it is administered in the post-menopausal state there results the typical oestrogenic type of vaginal smear (180); the oestrogenic activity of the urine of post-menopausal women may thus really be due to compounds from the adrenal cortex. 72

Progesterone is androgenic. It will maintain and stimulate the prostate and to a lesser extent the seminal vesicles of castrated male rats, and increase the size of the clitoris of female rats (75).

The excretion of oestrogens by males and of androgens by females (cf. p. 271) seems anomalous, though it may indicate that each serves as a balancing agent for the correct functioning of its opposite. The most outstanding example of the anomaly is the richness of stallion's urine in oestrone, and its origin in the stallion's testis is indicated by its absence from the urine of the gelding (57). Correspondingly, extracts of sow ovaries have been shown to be androgenic in that they produce comb growth in capons (66).

The similar embryological origin of testes and ovaries suggests that the production of their specific compounds may well be conditioned by factors external to them, such as environment, and this is exemplified by the experiments of Hill (222), who has shown that when mouse ovaries are grafted into the ears of castrated male mice, they effect restoration of atrophied seminal vesicles and prostate, although abdominal transplants will not. The results thus indicate that lower temperature simulating that within the scrotal sac stimulates production of androgen by the ovary.

It has been shown that administration of testosterone to eunuchoids increases their excretion of both androgens and





oestrogens, the increase paralleling the physiological effect, and thus suggesting some relationship between normal testosterone activity and oestrogen formation in the male (95). Similar results have been obtained with immature monkeys (51).

### Abnormal States Associated with the Gonadal Hormones

**Introduction.** Such states can arise as primary results of hypo- or hyperactivity of endocrine functions of the gonads, or dysfunction, if intersexuality can be so regarded, or the gonads may only be secondarily affected and the resulting changes in their endocrine activities thus merely produce secondary effects. The distinction is of importance for correct therapy. It is also of convenience to consider in this section the potentialities of the gonadal hormones in connection with the production of cancer.

**Intersexuality.** Earlier work suggested that there was an antagonistic action between androgens and oestrogens. Moore (146) and others considered that such antagonism was indirect and exerted through depression of output of gonadotrophic hormone from the pituitary.

That the antagonism exists is definite. Injection of oestrogens depresses male sex organ development and causes prostatic hypertrophy; the latter effect is counteracted by androgen (104). Similarly, androgen, injected in sufficient amount, suppresses the oestrous cycle (222), while, when testosterone is injected in adequate dosage into women, atrophic changes are produced in the vaginal mucosa (57).

Recent important work by Greene and Ivy (73) and others seems to indicate potential mechanisms for production of certain intermediate sex forms. When pregnant female rats are injected with oestrone or oestradiol just prior to birth of young, the female offspring develop hypospadias, which indeed can be produced in the new-born young themselves by injection of minute doses into them. Male offspring are not affected. When pregnant female rats are injected with testosterone the female offspring show either arrest of development of the vagina or become free-martins, exhibiting structures resembling prostate and seminal vesicles, the general effect being to inhibit development of structures from the Mullerian duct and



to stimulate development of those from the Wolffian duct. Again, the male offspring are not affected.

**Hypo- and Hyperactivity of Gonadal Hormones.** Since, during pregnancy, oestradiol does not seem to circulate in the organism, and the less potent oestrone is largely converted to oestriol and to their still less potent esters, it would seem that even normal oestrogenic activity must be damped down to allow pregnancy to proceed normally; this, of course, affords no evidence that hyperactivity can induce diseased conditions. Nevertheless, there is evidence that chronic oestrogenic hyperactivity produced experimentally can cause a marked pathology characterized by cystic hyperplasia of the uterus in woman (cf. p. 313) and by uterine metaplasia and occurrence of adenomata in various endocrine glands in rats (cf. p. 304). Chronic oestrogenic hypoactivity, on the other hand, should lead to changes comparable but less in degree than those occurring at the climacteric or following castration—disturbance of the menstrual rhythm even to amenorrhoea, and a tendency to atrophy of uterus and vagina.

Nothing definite is known of conditions associated with an overproduction of progesterone; its underproduction in the early stages of pregnancy might well be one cause of abortion.

Oversecretion of testosterone could produce a hyper-masculinization, undersecretion a persistence of infantile sex characters, perhaps associated with some degree of obesity, as judged by the effects of castration.

In endocrine disorders of women associated with ovarian function clinical data based upon subjective observations are very liable to error, through inexperience, inaccurate observation, and sometimes even intentional suppression of fact. Regularity of menstrual flow exists far more rarely than patients state, yet accurate knowledge of the rhythm and amount is most useful when available (166). Subjective symptoms in men, such as degree of interest in the opposite sex, and of potency, are also particularly open to criticism, especially in considering the possibility of hyperfunction (cf. 166, 176). The effect of the psyche can be a pre-eminent source of error in uncritical examination.

The critical evaluation of data, applied recently by Hamblen to consideration of the results of gonadotrophic therapy (80),



applies in general to study of diseases in women associated with abnormalities of gonadal hormones. He lists the possible objective observations as (i) whenever possible, which is, of course, very seldom, direct inspection of ovaries *in situ* at laparotomy; (ii) microscopic study of uterine endometria obtained at biopsy or curettage; (iii) investigations of cytological characteristics of vaginal fluid and of specimens of vaginal epithelium obtained at autopsy; (iv) determination of hormonal titres of blood and urine; (v) objective observations on alterations in the genital system and the accessory sexual system, and (vi) X-ray studies of the skeletal system. In addition diagnostic checks can be provided by study of ovaries or uterus removed at operation, when such operation is carried out.<sup>1</sup>

<sup>1</sup> It is convenient to add some brief notes here on the available biological and chemical tests. They are often potentially useful, of course, in controlling treatment, as well as for diagnostic purposes.

The existence of some degree of rhythmicity in the human vaginal smear has been demonstrated (cf. p. 258), and Papanicolaou and Shorr (161) have shown that the smear test can be used to indicate the effect of oestrogenic treatment at the menopause.

Biological tests based upon the original Allen-Doisy test with rats are available for both urine and blood. For blood two somewhat differing techniques are available, due respectively to Frank and to Fluhmann. They both need a considerable amount of blood, so that a series of tests on the same patient is undesirable, while the results they yield are not entirely in agreement (60).

Chemical tests simple enough for clinical use, and which would differentiate between, and yield sufficiently quantitative data concerning the different oestrogens, are not yet available for blood or urine; the presence of these compounds in ester combination adds to the difficulty of estimation. Nevertheless, it is to be expected that before long such tests will be available, for both oestrogens and androgens. Recent papers by Callow (24) and by Smith *et al.* (196) give some idea of present progress.

The excretion of pregnanediol is available as a gauge of the degree of function of the corpus luteum (cf. p. 272). But it is important to remember, in employing it for diagnostic purposes, that, as Hamblen (82) has pointed out, correct pregnanediol glycuronide excretion depends on four factors, (i) the presence of one (or more) normal corpora lutea in the ovary, (ii) a normal endometrium to transform progesterone to pregnanediol, (iii) normal hepatic function to conjugate the pregnanediol, and (iv) normal renal function for its excretion. Failure of any one of these factors will lead to abnormally low excretion.

The Zondek and Aschheim test for pregnancy, using mice (170, 4) and the Friedman modification, using rabbits (67), are in reality tests for the chorionic gonadotrophic hormone A-P-L, and therefore yield positive results with urines from cases of pregnancy, incomplete abortion, hydatidiform mole, and chorionepithelioma (as well as with certain cases of teratoma of the testicle in man). They are remarkably accurate, as is also the



Of the above, (iv) and (v) apply also for men.

*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 (166).

Werner (213) has made a careful comparative study of fifty-three castrated women, 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. 254). A recent study by McCartney (123) 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

corresponding test depending on extrusion of ova in the cloaca of the South African clawed toad, *Xenopus laevis* (190), though the use of the latter is limited by the availability of that species of toad. The numerous other tests, chemical and biological, for which claims have been made during the past few years that they are diagnostic of pregnancy, have fortunately been shown to be thoroughly inaccurate with sufficient rapidity to prevent much harm from their use.



usually not purposeful, and were cold, passive, and moody. Some retained sexual function, but without libido. (McCartney has found that a large porportion of schizophrenic patients have abnormal gonadal endocrine function.)

Rowe's studies (176) 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.

There is some evidence that women show an increased excretion of androgen at the menopause (83). Castrated men excrete only traces of androgens and oestrogens, eunuchoids low normal amounts or less, cases of gynaecomastia the usual amounts of oestrogens, but androgen excretion may be low (101). Old men and women excrete only small amounts of androgen (66).

*Tumours of the Gonads.* Novak has summarized present knowledge (155, 156); his 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 oestrogen by the constituent cells of the tumour. Removal of the tumour tends to a return to the normal condition of the child.

Parks (164) has recently published an account of an excellent case. The girl apparently developed normally until just over four and half years, when enlargement of breasts and growth of pubic hair were first observed. Three months later slight menstrual bleeding occurred. After another four months she was admitted to hospital, then weighing 51 lb. and with a height of 46 inches, and thus about the size of an eight-year-old child (cf. Fig. 41). Breasts and nipples were well developed. There was abundant growth of pubic hair but no axillary hair.



The facial expression was more mature than her age. X-ray examination suggested a bone age of at least ten years. Mentality corresponded with age. At operation a right ovarian tumour of granulosa cell type was removed, measuring  $8 \times 5 \times 4$  cm. On the second post-operative day a profuse menstrual flow began, which lasted forty-eight hours. This

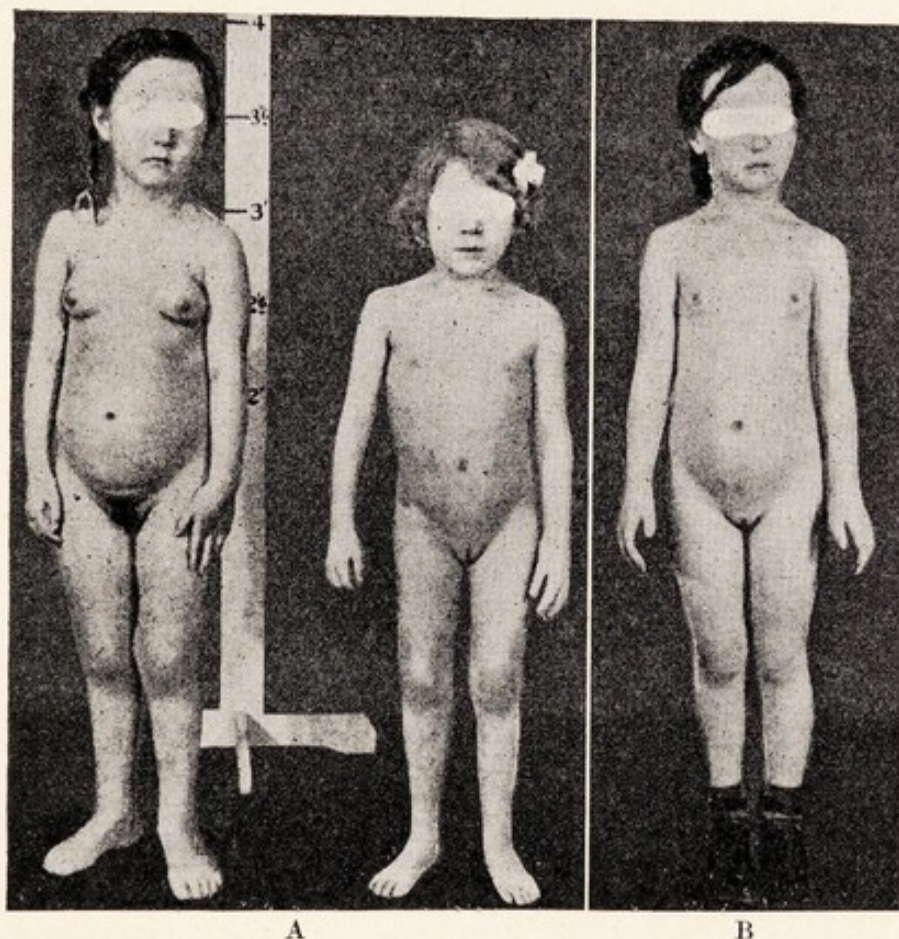


FIG. 41. A. Precocious secondary sex characters in a five-year-old girl with a granulosa cell tumour of the right ovary. Precocious skeletal growth as contrasted with that of a normal five-year-old patient. B. Recession of the secondary sex characters, fifteen months after operation. (From Parks, *Am. J. Obst. Gyn.*, 1938, xxxvi, 674.)

well illustrates the relationship of pseudo-menstruation to the removal of oestrogenic stimulation, and supports the view that the bleeding in anovulatory cycles is also due to this cause (cf. p. 287). There was complete recovery, with recession of secondary sex characters within fifteen months (see Fig. 41 B). Dr. Parks informs me that on November 4th, 1939 (almost four years after operation) this child was nine years old, weighed  $76\frac{1}{2}$  lb., was  $51\frac{1}{2}$  inches high, and was the youngest, tallest and



brightest girl in her grade at school. There had been no return of the secondary sex characters present prior to operation.

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), while it must be borne in mind that chronic oestrogenic stimulation of the post-menopausal endometrium possibly predisposes to adenocarcinoma (156).

Curtis (42) has reported a case in a woman of thirty-six, which is of some interest in that pregnancy occurred six months after removal of the tumour, indicating that prolonged excessive oestrogenic stimulation seems to produce no permanent damage on the reproductive system.

Granulosa cell tumours occasionally produce hyperfeminization effects (156).

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. Usually absence of obesity and striae atrophicae permit differentiation from the syndromes associated with adrenal cortical tumours and basophile tumours of the pituitary, although one case has been reported which rather strongly resembled Cushing's disease, with obesity present, but silvery-greyish-white striae; differential diagnosis was only definitely made at post-mortem examination (27). 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. These tumours usually occur between the ages of twenty and thirty; they are rarely found later. The youngest case yet reported was in a girl aged fifteen (155).

In cases of *hydatidiform mole* and *chorionepithelioma* there is a greatly increased production of A-P-L, and pregnancy tests



afford a means of diagnosis, and, after expulsion of a mole, of ascertaining whether a chorionepithelioma develops at a sufficiently early stage for successful surgery (cf. 43A).

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 (89), 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. Usually during life these tumours produce no marked symptoms of endocrine character. Entwisle and Hepp (58) 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.

A condition suggesting *hyperactivity of the corpus luteum* has recently been reported by Hamblen (81). The patient exhibited during the last phase of her cycles ecchymotic lesions, poly-hypermenorrhoea, dysmenorrhoea, and menstrual headache, and excreted abnormally large amounts of sodium pregnanediol glycuronide. Intensive oestrogenic therapy reduced this excretion, and the symptoms disappeared.

*Primary and Secondary Endocrine Effects.* The varying causes of menstrual disorders illustrate the differentiation stressed at the beginning of this section. Fluhmann discusses this subject very thoroughly (60).

Amenorrhoea is physiological when it occurs prior to the menarche (provided that be not delayed), during pregnancy and lactation, and following the menopause. Otherwise it is pathological. It is a primary effect through loss of endometrium of the uterus at hysterectomy, or through extensive necrosis following severe puerperal infection (being in this case due to a non-endocrine cause).

So-called primary amenorrhoea is a secondary effect through non-development of the uterus, through lack of oestrogenic



stimulation, presumably through lack of gonadotrophic stimulation from the pituitary.

Amenorrhoea is a secondary effect when lack of ovarian stimulation arises from non-endocrine causes such as malnutrition, morphine addiction, alcoholism, wasting diseases such as severe diabetes or tuberculosis, emotional disturbances, change of climate, etc. In some of these conditions the pituitary is also presumably in the line of communication between primary cause and end result, but the original cause is non-endocrine.

It may be a secondary effect in various diseases primarily affecting other endocrine glands than the ovary, for example, Fröhlich's and Simmonds' diseases and acromegaly, initially affecting the pituitary, hyperthyroidism, cortical adrenal tumour, etc.

Oligomenorrhoea and hypomenorrhoea are manifestations of primary or secondary ovarian deficiency, and causally akin to amenorrhoea.

Polymenorrhoea is probably associated with vascular disturbances due to pelvic inflammatory diseases, fibromyomata of the uterus, etc., and is therefore probably of non-endocrine origin. Hypermenorrhoea (menorrhagia) may be of endocrine, but is more usually of non-endocrine origin, being associated with such conditions as pelvic inflammatory disease, pelvic lesions which interfere with uterine contractility, post-partum or post-abortion inflammation, certain blood dyscrasias, etc.

Metropathia haemorrhagia, associated with lack of ovulation and a hyperplastic endometrium, is usually if not always due to over-oestrogenic stimulation, arising from cystic ovaries, granulosa cell tumours, ovarian sarcomata or fibromata, and like causes. It can be induced experimentally in women and in female monkeys by excessive oestrogenic stimulation.

**Carcinogenic and Growth-stimulating Actions of Oestrogenic Hormones.** Various compounds with a similar chemical constitution to the sex principles, such as calciferol (artificial vitamin D), possess some oestrogenic action. Still more interesting is the fact that more distantly related compounds prepared from tar, such as benzpyrene, and the related dibenzanthracene, which are active carcinogenic agents, also display some oestrogenic activity (46, 38).



The converse carcinogenic activity displayed by the naturally occurring oestrogenic compounds has recently been reviewed by Leo Loeb (118, 119), 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 (111), using, apparently, the powerful benzoate of oestradiol, has succeeded in producing mammary carcinoma in male mice, in whom normally it does not occur and has more recently obtained somewhat similar results with stilboestrol (110).

Incidental observations have been made of the production of sarcoma in mice, following injection of oestrogenic material (40) or implantation of ovarian grafts into castrates (98). Gardner (69) has 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.

Androgens seem to counteract the carcinogenic properties of oestrogens. Murray showed in 1928 (150) that male mice bearing ovarian grafts can develop spontaneous mammary



tumours, though normal males do not do so. Lacassagne's results have just been quoted. Nathanson and Andervont (151) point out that such results suggest an antagonism between androgens and oestrogens, as regards carcinogenesis. Murlin (149) finds that androgens extracted from urine, and also chemically pure androsterone propionate in small dosage, will inhibit the growth of the primary Brown-Pearce epithelioma implanted in the testicle of a mouse, and lessen the incidence of metastases. Oestradiol monobenzoate has little effect, nor have pure androsterone and dehydroisoandrosterone (indicating that the urinary extract must contain some other effective steroid). The dosage is important; larger doses of testosterone propionate increase the metastases.

Some important recent contributions have stressed production of uterine metaplasia and adenomata in endocrine glands.

Selye, Thomson and Collip (187) 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. McEuen, Selye and Collip (129) 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 metaplasia 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 adenofibroma whose scirrhus 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.

McEuen (130) treated rats for long periods with oestrone, usually in conjunction with various forms of skin irritation, and found that the occurrence of tumours histologically diagnosed as malignant was more frequent in animals so treated than in controls.

Lipschütz and Vargas have produced uterine and extra-uterine tumours in the guinea-pig by subcutaneous injections of tablets of oestradiol benzoate. These tumours are similar to those produced by a long course of injections of oestrogens, but may appear as early as two and half to three weeks after the implantation of a tablet (117A; cf. 117B). Such results obviously indicate a potential danger from the clinical use of this procedure, unless the size of the tablet is carefully controlled.

Zondek (228) 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.

### Hormonal Treatment of Gonadal Disorders

**Introduction.** During the past few years the evaluation of such hormone therapy has tended to become somewhat more critical. The armamentarium includes preparations containing the gonadotrophic hormones of the pituitary, A-P-L of the human placenta or urine of pregnancy, and the gonadotrophic hormone of the pregnant mare's blood, the oestrogenic compounds (including synthetic substances with hormonal action), progesterone, and the androgenic compounds. These have been utilized with varying success in cases in which hormonal deficiencies exist (substitution therapy), and in certain other types of case where production of known hormonal effects may be beneficial.



As has been pointed out, correct therapy generally depends on accurate knowledge of the nature of the deficiency and of the initial fault which needs rectification.

**Standardization of the Hormones.** It is essential for correct therapy that the preparation used be correctly standardized. This can be most easily done when pure crystalline material is employed, and its dosage measured in terms of milligrams and not of biological units (cf. 48). That is unfortunately not yet possible for the gonadotrophic hormones. Furthermore, it is necessary for proper control of dosage to be able to compare accurately the relative effects of administration of the oestrogenic compounds by injection and orally, while results from implantation of crystalline tablets of oestrogens and androgens need further examination, and comparison with those obtained by repeated injections, to enable accurate control of dosage in this valuable new procedure.

The following standards are in present employment (201) :

*Oestrogens.* The international unit (I.U.) of oestrone is the specific oestrus-inducing activity of 0.0001 mg. of oestrone. The international unit of oestradiol benzoate is the specific oestrus-inducing effect of 0.0001 mg. of that compound.

The Allen-Doisy rat unit is the quantity of oestrogen necessary to produce oestrus—as judged by the vaginal smear test—in an ovariectomized rat weighing 120 to 160 grams, when three injections are given subcutaneously at four-hour intervals, the sum of the three constituting the unit. The Doisy rat unit is the quantity of oestrogen needed to induce oestrus, when given subcutaneously in three divided doses at four-hour intervals, as determined by cornified vaginal smears in 74 per cent. of at least twenty spayed rats.

Corresponding mouse units have been frequently used.

Collip's oral day unit of emmenin is the minimal amount which consistently produces oestrus in from three to five days, when given to nineteen to twenty-one-day-old immature rats for three days. His day unit is the corresponding amount which produces the same effect when injected subcutaneously.

*Progesterone.* The international unit is the specific progestational activity of 1 mg. of crystalline progesterone.

The Corner-Allen rabbit unit is the minimal amount of an extract which, given in five daily doses subcutaneously to a 3 to 4 kg. rabbit spayed eighteen hours after coition, will produce in the uterus a progestational proliferation similar to that of the eighth day of pregnancy.

*Androgens.* The international unit is the androgenic activity of 0.1 mg. of androsterone.



Gallagher and Koch's bird unit is the minimal amount which, administered subcutaneously, produces an average increase of 5 mm. in length and height in at least five of ten brown Leghorn capons. Butenandt's bird unit is the amount causing a 15 to 20 per cent. increase in comb area in three white Leghorn capons. The bird unit of Freud, de Fremery and Laqueur is the minimal daily amount which, given in two injections daily for four days, produces by the fifth day an increase of 15 per cent. in comb area of over 50 per cent. of test capons.

*Gonadotrophic Hormones.* The rat unit for A-P-L is the minimal quantity which, when given subcutaneously twice daily for three days to a twenty-six-day-old female rat, causes formation of corpora lutea at the end of 96 to 100 hours. (Similar units have been defined for the pituitary hormones and for that from mare's serum, and international units have now been based on standard preparations (cf. 34A).)

*Additional Notes on Standards.* Evaluation of comparative biological units varies according to the precise technique used in different laboratories. Mazer and Israel (139) recently published the following comparisons in rat units: 1 mg. of oestrone is equal to 500 to 1,000 rat units, 1 mg. of oestriol to 150, 1 mg. of oestradiol to 6,500, and 1 mg. of oestradiol benzoate to 5,500 rat units.

Very approximately, 25 mg. of oestradiol can be regarded as equivalent to 100 mg. of oestrone or more, 200 mg. of oestriol or more, 200,000 mouse units, between 50,000 and 200,000 rat units, and 1,000,000 international units. International units based on experiments with small animals tend to become cumbrous and misleading when used clinically, and therapeutic doses of oestrogenic compounds, when expressed in milligrams, do not seem unduly large. Thus Marrian and Parkes (137) calculated that if 200 mouse units of an oestrogen are needed to produce complete oestrus in the spayed mouse, 400,000 units should be necessary to produce the corresponding changes in woman. This would indicate that 50 mg. of oestradiol may be a therapeutically effective dose, which is in agreement with accurate clinical observations.

As far as possible, in mentioning dosage in this section, I have reconverted biological units to milligrams.

Very varied figures for the activity of oestriol appear in the literature, and no international unit has been fixed.

The relationship between activity of equal weights of oestrone and oestradiol cannot be regarded as settled. The method of assay makes great difference. A recent determination, in which the assay was based on increase of weight of the immature rat uterus, indicated that as far as this activity is concerned, oestradiol is twenty times as active, weight for weight, as is oestrone (114). By this method, further, the activity of oestriol could not be determined at all.

Emmens (55) has recently discussed many of the variables which affect standardization of oestrogens and androgens.

In a recent paper Green and Ivy (74) tested the effects of emmenin,



oestriol glycuronide, and oestriol on immature and on castrate rats, and obtained essentially similar results, indicating that the ovaries did not affect these results. They concluded from their results that none of these three compounds are converted to more potent oestrogens by the ovaries, and further that oestriol glycuronide and emmenin are more potent by mouth than by subcutaneous injection, while oestriol is somewhat the reverse.

The activity of stilboestrol, as contrasted with oestrone, varies greatly by the method of administration. When both are tested on spayed mice or guinea-pigs, stilboestrol is 1.25 times more powerful, weight for weight, by subcutaneous administration. Administered percutaneously or orally, both are less active, but stilboestrol is only one-third as active percutaneously, and four or five times as active orally (115).

It has been already pointed out that such esters as the benzoate, etc., tend to give a slightly lessened but much more prolonged effect.

As regards progesterone, the Corner-Allen rabbit unit is approximately equal to the international unit, the Clauberg unit is from one-fifth to one-third of this, and itself is equal to three of the so-called German clinical units (60).

Frank and Berman (63) have recently critically reviewed the tests available for gonadotrophic hormones, and concluded that none are more than roughly quantitative, while those based on ovarian and uterine weight increases are useless for A-P-L. Heard and Winton (90) have also reviewed the literature, and have suggested the use of the adult rat in dioestrus produced by a diet deficient in vitamin B factors, the production of vaginal oestrus being considered a positive result. They claim this test gives fairly accurate results both for A-P-L and the hormone in pregnant mare's serum.

It is evident that many further studies of comparative standardization are needed for accurate use of the varied material now available for therapy.

*Implantation Dosage.* Geist (69B) finds that pellets are not as efficient as implants of crystals. A thick avascular capsule slowly forms about implanted pellets of oestrogenic hormones and gradually slows down their therapeutic effect. The local tissue response is much less for implants of crystals.

Warwick and Parkes (212AA) find that progesterone is absorbed from large tablets implanted subcutaneously at an average rate of about 20 per cent. per month, so that 50 mg. tablets will supply about 10 mg. per month. (They also report that free desoxycortosterone is absorbed from implanted tablets more than twice as rapidly as the corresponding acetate. Four 70 mg. tablets of the free compound will give a minimum daily dose of 4 mg. for a month.)

Such observations stress the need for considerably more experimental data before implantation therapy can be effectively controlled.

**Oestrogenic Therapy.** Mazer and Israel (139), using urinary



excretion of oestrogens by castrated women as a test, checked occasionally by direct measurement of oestrogens in their blood, found that a single hypodermic dose of 2 mg. of oestrone or 0.2 mg. of oestradiol benzoate in oil maintained the normal level of blood oestrogen for four days, and was completely eliminated by the end of the fifth day. A daily hypodermic dose of half the above amount produced blood values above normal, while a daily oral dose of half the amount maintained normal values. Obviously, therefore, smaller doses, incapable of maintaining normal blood levels, can scarcely produce much therapeutic effect. Large doses of oestrogens appear to have no deleterious effects on fertility. (Mazer and Israel state that following oral administration oestrone is absorbed more rapidly than oestriol.)

In cases exhibiting *primary amenorrhoea of long standing* there is arrested development of the uterus, which is frequently small and infantile and must be stimulated to grow; large doses of oestrogen are needed. Kaufmann achieved success in three of five cases (99) with doses of oestradiol totalling 200 to 300 mg.; one case received 375 mg. without success. Mazer and Israel report a similar degree of success with similar dosage (139). They conclude from the results of Kaufmann, Clauberg, and their own that the minimum quantity of oestrogen capable of evoking an adequate proliferative phase in the uterine endometrium of a castrated woman is, in terms of oestradiol benzoate, about 10 mg. per month. Smaller amounts may eventually produce some bleeding, not of menstrual type.

In *secondary amenorrhoea* treatment obviously should depend on the cause of the amenorrhoea. Progesterone may or may not be needed. A number of successes have been reported, using oestrogen only (cf. 99, 139).

In the less common condition of *hypomenorrhoea* Mazer and Israel (139) claim good results with two of six women who had taken daily for three months 1.5 to 8 mg. of oestriol (or a corresponding amount of emmenin), and three of eight women who had received injections of 1 to 2 mg. of oestradiol benzoate at four-day intervals for about three months; apparently normal menstruation ensued for at least fifteen months after cessation of treatment. In two of the latter three patients



curettage showed presence of normal premenstrual endometrium.

Emmenin, in similar dosage, has been only occasionally successful in *dysmenorrhoea*. Larger doses of oestradiol benzoate have given a somewhat greater proportion of successes (139), though such relief may only be temporary (60). The rationality of oestrogenic treatment of *oligomenorrhoea* does not seem obvious, but there is some evidence of good effects following prolonged low dosage with, for example, emmenin (60).

Pregnancy has resulted in a number of patients with disturbed ovarian function, after treatment with emmenin had corrected that function (26).

Especially as regards treatment of amenorrhoea, however, different schools of clinicians evidence from their writings considerable difference of opinion. Such results as those just quoted indicate that in some proportion of cases of primary and secondary amenorrhoea oestrogenic therapy is beneficial, correcting the associated uterine atrophy. (In the still smaller number of cases where permanent cure results, this is probably to be attributed to disappearance of whatever primary cause led to diminished output of ovarian hormone.) Nevertheless, Frank, writing in 1937, considered that no useful purpose is achieved by prescribing oestrogen for amenorrhoea (64). Writing more recently, Novak (157), while admitting that the prevailing treatment of amenorrhoea is unsatisfactory, thinks that in some cases oestrogenic therapy can remedy a quantitative deficiency in the patient, and may even sensitize an underdeveloped endometrium to the stage where it will respond to the patient's own small supply of hormone.

*Menopausal Syndrome.* The benefits of oestrogenic therapy are most apparent when it is administered to patients suffering from severe symptoms (as flushing, profuse sweating, insomnia, and nervous disturbances) of the artificial or natural menopause. These symptoms appear to be related to increase in the output of the gonadotrophic hormones of the anterior pituitary. This increase is suppressed by administration of fairly large doses of oestrogen, and the symptoms are ameliorated or disappear (see 139 for references). A dosage of 2 mg. of oestradiol benzoate injected every fourth day has proved effective (139), but treatment must be prolonged beyond the disappearance of the



severer symptoms, with gradually reduced dosage over a period of several months, or the symptoms recur. Some patients are not benefited.<sup>1</sup>

Ault, Hocter and Werner (5) consider that oestrogenic therapy can practically be regarded as specific in involutional melancholia, and record a series of cases showing 92 per cent. recovery with such treatment. They believe this therapy is indicated for any woman who, at the menopause, has disturbing mental aberrations, whether mild or severe, and also that it is beneficial in relieving distressing symptoms of the climacteric in other types of psychoses. Jones, MacGregor and Tod (97) also report good results in a series of seventeen cases of depression at the menopause. Somatic symptoms were relieved in all; six made good recovery within five months. Five mg. of oestradiol benzoate were given twice weekly, 60 to 140 mg. in all in different cases.

Oestrogenic therapy has also been beneficial in treating psychotic disturbances resembling those at the menopause, sometimes seen in certain patients following childbirth, and in others with dysmenorrhoea (2, 184).

*Atrophic rhinitis* is benefited by local treatment with oestrogen in corn oil (148). Intranasal oestrogen insufflation seems to produce some improvement in many cases of *constitutional deafness* (148).

*Gonorrhoeal Vulvovaginitis in Childhood.* Oestrogenic therapy is definitely beneficial. The basis of this treatment is the epithelization of vaginal mucous membrane produced, but conversion of an alkaline vaginal fluid to one definitely acid may be an essential factor (79, 116, 208). Relapses may occur, and at least one series of failures has been reported (224), not improbably due to insufficient dosage, or insufficient length of treatment. Oral therapy has been largely used, but recently vaginal suppositories have been stated to be the most effective method of treatment. Mazer and Israel (139) consider that the optimal dose is, in terms of oestradiol benzoate, 0.2 mg. given

<sup>1</sup> Mazer and Israel (139) record control of the diabetes mellitus which had developed in three such patients, solely by use of at least 0.4 mg. of oestradiol benzoate every fourth day, no insulin being necessary. Levy Simpson (192) has stated that the carbohydrate tolerance is normally somewhat lessened at the climacteric, and can be restored to normal by administration of oestrone.



hypodermically every other day for not less than six weeks. They give a good account of the literature. While there is some evidence that such relatively large doses of oestrogen given to young girls produce transient stimulation of secondary sex characters, etc., such as occasional enlargement of the breasts, rarely some slight growth of pubic hair, and still more rarely some uterine bleeding, these effects disappear completely on cessation of treatment (139).

Good results have been reported following oestrogenic treatment of *senile vaginitis* (95A). Topical application in a lanoline medium is satisfactory (59).

In *mazoplasia* (painful and nodular breasts) and nipple-bleeding oestrogenic therapy is sometimes beneficial (139). It is distinctly useful in cases of *dead foetus*. Reynolds states that successful removal was effected in ten of a series of twelve cases (168 ; cf. 96).

Oestrogenic therapy does not produce abortion between the seventh and fourteenth week of normal pregnancy. It will not induce premature labour. It possibly has some effect in cases of uterine inertia (cf. 96), but so has acetylcholine (168).

Oestrogenic therapy is of no value in haemophilia (9, 11, 207, 31). Claims of benefit in treatment of retinitis pigmentosa (216) and of alopecia (102) seem improbable. Emmenin therapy is claimed to be beneficial in migraine (9).

*Choice of Oestrogenic Agent.* It is doubtful if anything very definite can be said at present concerning differential choice of the oestrogens, if adequate dosage be given for the selected method of administration. Esters only prolong action when injected, not when given by oral route (55A).

Concerning stilboestrol, Winterton and Macgregor (223) obtained results paralleling those with natural oestrogens in fifty cases. Oral administration is as effective as intramuscular. There is occasionally a slight nausea. A number of similar reports have already been made (cf. 8A). It is also effective in senile vaginitis (100).<sup>1</sup> Hexoestrol seems less toxic than stilboestrol (8A).

<sup>1</sup> Selye (186) finds that in *very large* dosage both natural oestrogens and stilboestrol are toxic to mice and rats, inducing marked icterus through liver damage, gastric ulcers, etc. Oestrone and oestradiol are less toxic than stilboestrol. (Cf. also Noble, 154A.) Testosterone is less toxic, and to some extent opposite in its action (186A).



*Ovarian Transplants.* The literature dealing with this method of treatment has been reviewed by Thorek (204), who considers that such transplants improve waning physical and psychic conditions and retard onset of symptoms of senility. They do not rejuvenate. They are said to function for several years.

**Progesterone Therapy.** This is of definite benefit in uterine haemorrhage of ovarian origin, of which the anatomical basis is almost always cystic hyperplasia of the uterus. Kaufmann (99), by treatment of a castrate with very large doses of oestradiol, showed that such cystic hyperplasia is the result of excessive oestrogenic stimulation. He obtained excellent results in treating this type of case with progesterone, and found that 5 to 10 rabbit units (probably about 2.5 to 5 mg.) spread over five days suffice. Occasionally much larger doses are needed. (Cf. also 132.) The use of oestrogen is obviously contraindicated.

Good results have been claimed by use of progesterone in cases of habitual or threatened abortion (8, 168, 68), and of dysmenorrhoea through stopping contractions of uterine muscle (225, 168, 60).

Progesterone in small dosage gives striking relief in fifteen to twenty minutes in abolishing the after-pains of labour (168). Lubin and Clark (121) found that 1 rabbit unit (presumably 1 mg.) produced complete relief in forty-eight out of fifty-five cases.

Robson and Paterson (174) considered that administration of progesterone in twelve cases of pre-eclampsia seemed to produce some clinical benefit.

**Combined Oestrogen and Progesterone Therapy.** Kaufmann (99) succeeded in establishing true menstruation in a castrated woman by intramuscular injection of 125 mg. of oestradiol, spread over several months, followed by injection

Papers are beginning to appear in the literature, cautioning against the indiscriminate administration of stilboestrol until its toxicology shall have been more intensely studied (cf., *e.g.*, 67A, 191A, 164A). Kurzrok considers that its therapeutic use is definitely limited on account of the persistent nausea and occasional vomiting it induces in a large proportion of patients (109A).

Auchincloss and Haagensen (4A) caution against potential danger of inducing carcinoma by excessive administration of oestrogen, especially when there is a family history of breast cancer.



of, in all, 35 rabbit units (about 17.5 mg.) of progesterone. In a second case similar success was obtained, using between 30 and 40 mg. of oestradiol benzoate, followed by five injections each of 6 mg. of progesterone. (Loeser (120) has estimated that the human ovary secretes per month the equivalent of 25 to 30 mg. of oestradiol, and 20 to 25 mg. of progesterone.)

Kaufmann has treated a fairly large number of cases of secondary amenorrhoea, and succeeded in establishing true menstruation in most of them, by close simulation of the normal stimuli of the cycle. Thus, 6.25 mg. of oestradiol in solution were injected on each of the first, fourth, eighth, eleventh and fifteenth days of a month, and then 3.5 mg. of progesterone on each of the nineteenth, twentieth, twenty-first, twenty-second and twenty-third days. True menstrual bleeding commenced in most cases on the second day after the last injection of progesterone. After artificial menstruation has been so induced for three consecutive months, if treatment was stopped complete regular spontaneous menstruation often occurred for several months.

Oestrogenic therapy during the ninth, and combined oestrogenic and progesterone therapy during the tenth month of pregnancy is stated to increase lactation considerably in women whose lactation has been inadequate in previous pregnancies. Progesterone alone, and A-P-L are said to be without effect (53). With this claim, however, may be contrasted the statement of Foss and Philipps (61) that oestrogenic therapy (of the order of 0.5 mg. of oestradiol, spread over two to six days) will inhibit lactation.

**Androgens** are of value in properly selected cases of endocrine disorders in both male and female patients.

A logical use of androgens would seem to be for replacement therapy in sexual senility. This assumes that the androgenic hormone is produced throughout adult life, but in decreasing amount with age. Such a view has, however, been criticized by Moore (145) and Rowe (176), who considered that subjective criteria of hypogonadism in the male are misleading and cannot be depended on. Results from earlier methods of overcoming sexual senility in man, such as testicular implants (Voronoff's method (212)) and ligation of the vas deferens (Steinach's procedure) are peculiarly open to the criticism of a re-eroteriza-



tion through the psychotherapy induced by the procedure. Yet animal experiments seem to indicate that these procedures produce some definite result, which cannot be explained by psychotherapy.

The present availability of crystalline androgens has permitted treatment less open to criticism, and therefore more definite conclusions are available. Crystalline testosterone and its esters are the most powerful androgenic compounds known, and are available commercially, though still very costly.

As judged by the capon-comb test, testosterone propionate is two-thirds as active as testosterone, but its activity is vastly more prolonged (126). It is, of the various esters, the most powerful and longest-acting. Administration of testosterone with beef tallow, or palmitic or stearic acids, also prolongs its action (cf. 46, 85, 54, 141).

Probably subcutaneous implants of crystalline tablets of testosterone or its esters will finally prove to be the administration method of choice (cf. 85, 141, 95AA).

McCullagh *et al.* (127) devised a method of determining definite hypogonadism by estimating the androgen in blood and urine, and recently (128) he has reported that severe prepuberal hypogonadal cases treated by injection of testosterone propionate show, in order, penile erections, increase of pubic and axillary hair, growth of penis, lesser growth of scrotum, and still lesser growth of prostate. It is doubtful if the testes increase in size. Nocturnal emissions occur, and the volume of semen increases, but it contains no spermatozoa unless they were present before treatment started. The larynx grows and the voice becomes lower. Facial acne occurs, and the beard grows. Epiphyseal closure is not produced when treatment is limited to testosterone. No definite change is produced in basal metabolism, though in some patients a low rate was increased to normal.

McCullagh reports further that in functional hypogonadism in the adult the treatment relieves nervous and sexual symptoms, while in castrated males it can abolish nervous and vasomotor symptoms and impotence. His dosage varied considerably in different cases, from 2.5 mg. to 20 mg. or even more, three times a week or still oftener.

A similar result is reported by Foss (61) in the case of a post-



puberal eunuch (due to war injury at age of nineteen). Foss considers that an initial dosage of 100 to 140 mg. of testosterone propionate, with subsequently about 40 mg. weekly, is probably adequate in such cases. Prolonged treatment leads to no dangerous results (95AA).

Such observations as those of Zuckerman (231) and Papanicolaou (162) indicate that androgenic and oestrogenic therapy may each have appropriate place in the treatment of the opposite sex. The former observed that injection of testosterone propionate inhibits the experimental production of prostatic enlargement in immature rhesus monkeys which can be induced by injections of oestrone, while the latter found that testosterone propionate inhibited the menstrual cycle of a normal young woman, who menstruated spontaneously twenty-eight days after cessation of the treatment.

Mazer and Mazer (140) have obtained good results with testosterone in treating dysfunctional uterine bleeding at the menopausal age. They give 2.5 to 25 mg. intramuscularly three times per week for two to nine weeks. Larger doses can cause temporary amenorrhoea through inhibition of the gonadotrophic function of the anterior pituitary, and may also induce facial hair growth and masculinization of the voice. The stated dosage does not disturb the menstrual rhythm or cause atrophy of the endometrium, and presumably in such dosage the testosterone acts directly on the endometrium. Claims of benefit with testosterone have been made in treatment of cases of dysmenorrhoea (177), while Kurzrok (109) has recommended its use (30 to 50 mg. of the propionate, weekly) in treatment of the menopause.<sup>1</sup>

Nathanson and Towne (152) find that in treatment of women castrates with testosterone there is a correlation between the symptomatic relief and laboratory findings, with a definite

<sup>1</sup> A clue to safe dosage with women is given in the results of a recent study by Geist (69A) on a series of twenty-five women with normal menstrual cycles. He found that doses of 500 mg. of testosterone propionate per month produced temporary suppression of menstruation, hypoplasia and atrophy of the endometrium, and evidence of oestrogenic deficiency in the vaginal smear. All cases returned to normal after cessation of treatment. Still larger doses sometimes produced hoarseness, hirsuties, acne, and enlargement of the clitoris, though in almost all cases there was spontaneous regression after the treatment was discontinued. Doses of 200 mg. per month produced none of the above effects.



increase in oestrogen excretion, and a decrease in the excretion of the follicle-stimulating hormone.

**Gonadotrophic Therapy.** This theoretically includes use of the gonadotrophic hormones of the anterior pituitary, A-P-L from placenta or urine of pregnancy, and the gonadotrophic hormone from pregnant mares' serum. None of these have yet been obtained pure, nor can the degree of impurity of commercial products yet be estimated.

Hamblen (80) has critically reviewed the results of gonadotrophic therapy, and his conclusions are rather unfavourable. He stresses the confliction of reports in the literature, and the unreliability of subjective symptomatology as a criterion of improvement, and recapitulates the objective observations possible (cf. p. 296).

Analysing the observations of his own group of clinicians and of others, he concludes that no definite proof has been adduced that administration of gonadotrophic hormones (from whatever source) can call forth complete ovarian response (growth of follicles, rupture of follicles, and formation of corpora lutea) in patients whose ovaries are not responding spontaneously to their own endogenous pituitary hormones. There is some evidence that such treatment may increase an ovarian response previously present in slight degree.

He concludes further that study of endometrial specimens taken after such therapy has afforded no evidence that treatment with gonadotrophic hormones can alter the ovarian-endometrial disturbance of patients who bleed from oestrogenic endometria. The treatment will not convert an anovulatory to an ovulatory cycle. (As Fluhmann (60) points out, in such cases gonadotrophic treatment early in the cycle seems logical. Unfortunately, after ovulation has once occurred, there is never evidence of permanence of anovulatory cycles, so that recurrence of ovulation can never be definitely ascribed to treatment.)

Schaefer (182), in a somewhat earlier review, does not quite draw such negative conclusions. Fluhmann (60) says that A-P-L is beneficial in metropathia haemorrhagia in young women.

A-P-L is of no special value in the treatment of acne in women (219). Good results have been claimed from its use in



cases of migraine probably associated with a slight degree of hypopituitarism (144).

*Treatment of Undescended Testicle.* Numerous clinicians during the past decade have reported favourable results following the use of A-P-L in cases of undescended testicle (*e.g.*, 183, 76, 1, 198); the hormone of pregnant mare's serum is also said to be effective (108). In the past two or three years a number of more critical papers have appeared.

Thompson (203) considers that A-P-L is valuable both before and after surgery. In all cases in which, in his series, it was used successfully without surgery, descent occurred within nine weeks of the commencement of treatment. Since it produces genital growth and even premature puberty, prolonged treatment should be avoided. (He notes that it is useful in hypogenitalism of the Fröhlich type.)

Mimpress (143) records results of the use of A-P-L in twenty cases, of which nineteen showed resulting hypertrophy of the external genitalia, but descent of the testes occurred only in six. He believes that this treatment should be limited to bilateral cases with subnormal genital development, and fears possibility of either subsequent atrophy or precocious sexual development.

Browne (12) remarks that the "treatment will not bring down any testes that would not have descended without it, though it will hurry the descent. Whether this acceleration is worth the risk of certain disquieting possibilities is a matter of opinion."

Wilson (220) states that under three years of age the diagnosis of uncomplicated non-descent is uncertain. Spontaneous descent may be awaited till ten years of age, but, unless the testis lies in the superior scrotal position, waiting should not be persisted in beyond that age. Hormonal therapy may be employed before operative treatment as a means of determining whether spontaneous descent is possible, but should only be used in the pre-pubertal period. He fears the production of an artificial puberty, and prefers to delay hormonal treatment until the age of ten. Hormonal treatment is unlikely to be successful with the unilateral cryptorchid. In his own series he obtained complete descent in five and improved condition in two out of seventeen cases of average age eight years, but



considers that descent only occurred in those cases which would normally have undergone spontaneous descent at a later date. He considers that operative treatment can be safely carried out after three years of age, and should be completed before puberty, the optimal age for operation being from eight to eleven years.

**X-ray treatment** may be in a sense endocrine, in so far as it be designed to depress undue endocrine stimulation. Irradiation of ovaries and uterus is potentially dangerous during their functional life. It is said to be possibly useful in controlling uterine haemorrhage at or near the menopause. Claims have been made that irradiation of the pituitary is of value in controlling dysmenorrhoea and post-menopausal symptoms. Fluhmann has discussed the subject in the conservative manner that it needs (60).

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## CHAPTER VIII

### THE PITUITARY GLAND

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#### Introduction

THE pituitary body, like the adrenals, is built up of two unrelated parts, composed of different types of tissue, of which one is typically glandular, the other related to nervous tissue. The two parts come together in foetal life. The embryology and histology have frequently been fully described (217, 251, 53). The following brief statements are taken chiefly from Bailey's description (13).

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)  $\times$  6 mm. (dorsiventrally)  $\times$  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" (59).

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* (or *neuralis*), 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* (or *glandularis*), 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 (189) states that the *pars intermedia* is practically absent in the adult human pituitary, but Brander (30) finds that it is extremely variable in extent and arrangement.

The pituitary of the adult human female is larger than that of the adult male, due to a larger anterior lobe. It is larger in negroes than in whites. At least in some proportion of cases pregnancy increases the size of the anterior lobe. It decreases in size in man after middle age, but less noticeably so in woman. In both sexes the *pars neuralis* and *pars intermedia* increase in size with age (190).

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.)<sup>1</sup>

<sup>1</sup> Engelbach (76) 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



In the monkey and probably in man the pars glandularis is supplied by (i) afferent arteries and (ii) afferent (not efferent) portal veins which originate in the region of the stalk from a plexus which surrounds and also penetrates the infundibular stem. The terminal parts of these arteries and veins unite to form sinusoids. The veins from the pars glandularis pass from the lateral pole to the cavernous sinuses. The blood supply of the remaining parts of the pituitary is almost independent of that of the anterior lobe, the vessels entering and leaving by the posterior pole of the pars neuralis. The blood supplies of the hypothalamus and the pituitary are not linked, so that hormones from the pituitary body do not have immediate access to centres in the hypothalamus (261, 248).

Little is known of the origin of nerves controlling the anterior pituitary, though evidence of such control is accumulating. (For example, it is known that light affects the secretion of gonadotropic hormone in birds and in the ferret, while strong diffuse electrical stimulation causes ovulation in the rabbit (248).) 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 (13). But a large proportion of the anterior lobe seems to be practically devoid of nerve fibres so that Rasmussen (191) rather doubts whether the fibres present have a secretory function and thinks that they are associated with the vascular system.

A tract of nerve fibres originates in the paired supraoptic nuclei of the hypothalamus, and descend 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" (13). In the human pituitary this tract contains at least 50,000 fibres (191). The function and morphological maintenance of the pars neuralis depend on a normal flow of stimuli to it from the supraoptic nuclei. If they are destroyed, the neural lobe atrophies and ceases to produce its hormones (248).

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)."







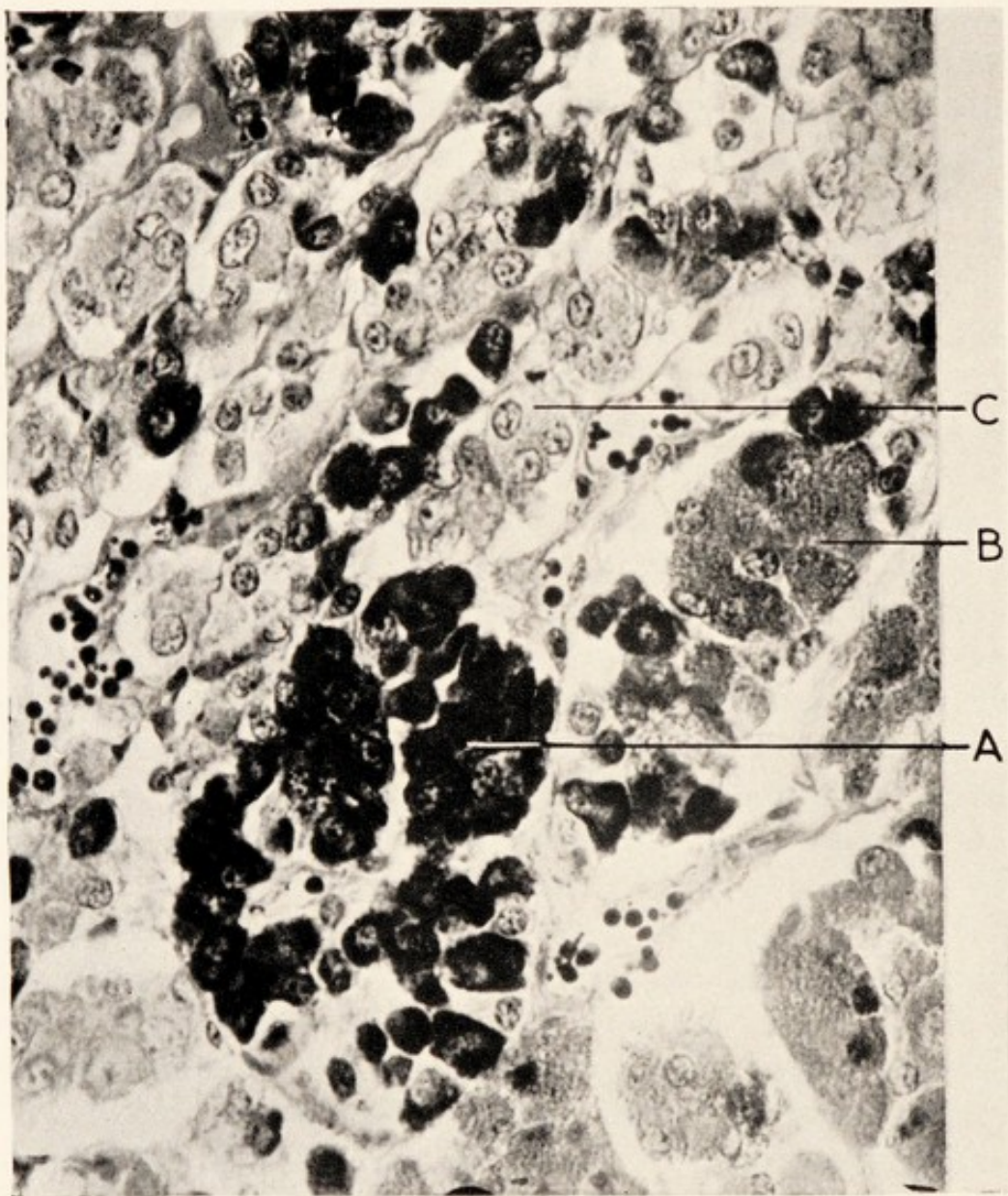


FIG. 42. Section of anterior lobe of human pituitary, stained with aniline blue and acid-fuchsin. *A*, acidophile cells, dark-staining and clustered; *B*, basophile cells (intermediate shade); *C*, chromophobe cells (faint shade).  $\times 400$ . (Photomicrograph by Professor William Boyd.)



While there is some experimental evidence that in cold-blooded animals the pars intermedia is under nerve control (248), Rasmussen states (191) that in the human pituitary large areas of this portion of the pituitary seem to be devoid of nerve fibres, though at least a few fibres end on its cells (while a negligible number pass through it from the neural lobe and end just within the anterior lobe).

It is stated that lymphatic vessels have not been demonstrated in the pituitary.

Microscopically, the anterior portion consists of columns of cells separated from one another by large vascular sinuses and some connective tissue. Two groups are differentiated as *chromophile* and *chromophobe* by the different intensities of their staining reactions. The deeper staining properties of the former are due to granules in their cytoplasm. These granules are of two types. From the presumption that their staining reactions are restricted to acid and basic dyes respectively, they are usually termed *acidophile* and *basophile*. Since they do not show such restricted staining properties, Bailey termed them *alpha* and *beta* cells respectively. It is generally considered that no cell contains more than one type of granule.

Fig. 42 reproduces an excellent photomicrograph, kindly furnished me by Professor William Boyd, and which well illustrates the relative distribution of the three types of cells. Koneff (139) describes an adaptation of the Mallory-Azan staining method which he claims gives tinctorial differentiation and shows good cytological detail. By this procedure basophile cells stain blue, acidophile cells orange red, and chromophobe cells are colourless or light gray.

According to Rasmussen (188), the distribution of these three types of cell in the anterior pituitary of man is :

	Extreme Values.	Mean Values.
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. Basophiles, as will be evident, are more numerous in man than in woman. With increasing age chromophobes increase and acidophiles decrease in men; basophiles increase in women (190).

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. 76, 26, 74). While there is evidence for the existence of the latter, Rasmussen has been unable to identify specific "pregnancy cells." The enlargement of the pituitary during pregnancy is not due to hyperplasia of any one of the three types of cells. Special "pregnancy cells" do not occur in the guinea-pig (138). The changes following gonadectomy in the experimental animal which result in appearance of "castration cells" can be corrected by administration of oestrogens or androgens (248). Rasmussen considers that so-called "pregnancy cells" are really highly active acidophile cells (190).

There is evidence that adrenal cortical deficiency is accompanied by a marked reduction in the percentage of basophile cells. When large doses of oestrogens or androgens are injected into normal animals basophile cells are depleted of their granules (248).

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 (59) 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 (215). 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 (215).

There is still, however, no complete agreement as to the precise relationship between the chromophobe and chromophile cells (cf. 248).

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 (31). 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.

An early suggestion that the hormones of the posterior pituitary were elaborated in the pars intermedia and transferred to the nervous portion for secretion (cf. 84) has been discarded. A later suggestion that the basophile cells from the pars intermedia streaming to the pars nervosa there degenerate to hyaline material (so-called Herring bodies) and liberate its essential hormones (cf. *e.g.*, 59, 61), excessive production being associated with hypertension and with eclampsia, cannot be upheld (cf. 163, 18, 26, 190), and, indeed, Gersh has shown that the Herring bodies are merely artefacts, protein material changed to hyaline by fixative, and not at all associated with the hormones of the posterior pituitary (108).



The pituicytes, being peculiar to the neural lobe, are frequently assumed to be the cells responsible for its hormones vasopressin and oxytocin (see p. 334). Griffith has adduced evidence from tissue culture experiments that the pituicytes form vasopressin (111). Gersh (109) has furnished evidence of possible glandular nature of what he terms the "parenchymatous cells" of the posterior pituitary, and deduces on histological grounds that they probably produce vasopressin. These cells are practically identical with Bucy's pituicytes.

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 (59).

The first important observation bearing upon the function of the posterior pituitary was that of Oliver and Schafer in 1895. They showed that extract of the gland, when injected intravenously into animals, produced a marked and prolonged rise of blood pressure. Shortly afterwards Howell proved that this effect is due to extract of the posterior lobe, while Dale found that this extract caused contraction of uterine 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 (39) 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**

Extracts of the posterior pituitary gland such as "puitritin" possess three outstanding properties: (i) ability to raise blood pressure (pressor activity); (ii) ability to produce uterine contraction (oxytotic activity); and (iii) power to produce diuresis or anti-diuresis under differing conditions (renal activity).

The first important advance in the isolation of the hormones of the posterior pituitary was made by Kamm and his associates in 1928 (137, 32). The posterior lobes were sharply separated from fresh beef pituitaries, desiccated with acetone, and extracted with 0.25 per cent. acetic acid. The extract was concentrated at low temperature and proteins (with the hormones) were salted out with sodium chloride or ammonium sulphate. The precipitate was treated with anhydrous acetic acid, which extracted the hormones, with but little protein. The acid extract was fractionated by repeated treatments with acetone, ether, and petroleum ether (the ether filtrate contained the oxytotic principle). After twenty such fractionations two hormones were obtained, relatively free from each other. Two hundred beef pituitaries gave 50 grams fresh (8 grams desiccated) posterior lobe material, which yielded 0.05 gram pressor principle and 0.015 gram oxytotic principle. When samples of the two were mixed in these proportions, dissolved in acidified water, and diluted to correspond with an extract of the original glandular material, the physiological properties of the two solutions were found to be indistinguishable, indicating that the processes employed in separating the hormones had affected neither of them.

The final fractions were white, stable powders, water-soluble,



and basic. The pressor fraction was, as regards its pressor effect, eighty times as powerful as the then international standard preparation of powdered pituitary. The pressor hormone was termed *beta-hypophamine*, and, pharmaceutically, *vasopressin* and *pitressin*. The oxytocic fraction was more than 150 times as powerful as the international standard, and was termed *alpha-hypophamine* (pharmaceutically, *oxytocin* and *pitocin*). Subsequently the international standard has been defined in terms of both pressor and oxytocic activity.

The pressor principle is responsible for the diuretic-antidiuretic action of pituitary extracts (137, 104). In normal animals, not under anaesthesia, the predominant effect is suppression of flow of urine (33). The beneficial effects produced on patients with diabetes insipidus are due to this principle, oxytocin being without effect (104, 229), and it was early 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 (34). Adolph (2) 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 (104) are due to pitressin.

Zinc salts prolong the anti-diuretic activity of vasopressin (67).

Stehle and Fraser (231) have also prepared concentrated preparations of the posterior pituitary hormones, by fractioning methyl alcohol solutions with ethyl acetate. Stehle and Trister (232) have hydrolyzed such preparations. They isolated from the hydrolysate of the pressor substance arginine, proline, cystine, and tyrosine, and obtained evidence of the presence of isoleucine. Histidine, hydroxyproline, glycine and methionine were absent, and tryptophane was probably absent. The hydrolysate of the oxytocic preparation contained the same amino-acids, except that leucine replaced isoleucine. (For earlier studies, in general agreement with these results, cf. 112, 211, 249.)

The two hormones possess different electrophoretic migration rates, the pressor principle migrating towards the cathode cell



much the faster of the two, and these differences are exhibited even in the mechanically expressed juice from fresh posterior pituitary tissue (250).

While neither hormone has yet been obtained in definitely pure state, the results quoted above suggest strongly that each is a polypeptide, probably built up from relatively few amino-acids.

Certain posterior pituitary preparations produce an antagonism to adrenaline-hyperglycaemia, when injected into various species of animals. The available data do not allow this effect to be ascribed to known pituitary hormones (cf. 174).

### The Posterior Pituitary as an Endocrine Gland

The two hormones undoubtedly possess specific pharmacological effects, but until very recently there was no indisputable evidence that they could be truly considered hormones, secreted from the gland, and performing physiological functions. Such evidence is now available for vasopressin, while strongly suggestive evidence is available for oxytocin.

The work of Gilman and Goodman in 1936-37 (248) has shown that the tubular reabsorption of water from the glomerular filtrate in the kidneys depends upon the action of vasopressin. If the rate of excretion of water is likely to lead to dehydration, the rate of secretion of vasopressin is increased, the rate of tubular reabsorption becomes greater, and water is conserved.

As has been pointed out, the neural lobe is under nervous control, receiving stimuli through the supraoptic nuclei of the hypothalamus. This has been amply demonstrated by the work of Fisher, Ingram and Ranson and others (248, 243) on cats and monkeys, in which it has been shown that if this nervous control be lost (as by complete severance of the pars nervosa from the hypothalamus), the kidneys lose their power to concentrate urine, so that polyuria and polydipsia result—an experimental diabetes insipidus—while there is concurrently a retrograde degeneration of the supraoptic nuclei. It also explains how suitable afferent stimuli can inhibit secretion of urine after renal denervation.

Conversely, it has been shown that dehydrated rats and cats excrete an anti-diuretic substance, unless they have been hypophysectomized, or the supraoptic-hypophyseal tracts have



been interrupted (248). Further, dehydration causes definite cytological changes in the pituicytes of the posterior pituitary of the rat (109).

Lim and his colleagues have shown that during the pressor response following stimulation of the central end of the vagus the jugular venous blood contains, in the normal animal, an oxytocic substance, and an anti-diuretic substance. (These are not found, under like conditions, in the hypophysectomized animal.) Repeated vagus stimulation results in a lessening of the pressor response; it is re-established by rest. There is a parallel exhaustion and reappearance of secretory granules in the pituicytes (243).

Haterius and Ferguson (116), by stimulating rabbits in the region of the infundibular stalk several hours post-partum, produced an increase in uterine activity (both increased frequency and amplitude of contraction) closely resembling the action of oxytocin. This result persisted after spinal transection, section of the splanchnic nerves, and vagotomy, but not after blockage of the pituitary stalk. It seems legitimate to conclude that the posterior pituitary actually secretes oxytocin.

### 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.

Tumours of the anterior pituitary may damage the posterior lobe, even to the extent of almost complete obliteration (cf. Fig. 43, p. 343). There is some evidence of a resulting decrease in blood pressure (59). 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.

*Diabetes insipidus* is the most outstanding abnormal condition which is presumably associated with hypofunction of the posterior lobe. 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 "puitritin." 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 "puitritin" 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 (39) (cf. also Bailey and Bremer (14)). Delayed adiposity and genital atrophy can occur in these cases.

Maddock, and later Mahoney and Sheehan (160), found that application of silver clips at various levels of the hypophyseal stalk in various animals (though not in the monkey) produced a marked and enduring polyuria. Such results at first tended to be interpreted as due to a damming back of some hormone, believed normally to pass to the ventricles from the posterior lobe by this channel, though Cushing pointed out (59) that in such experiments the nerve impulses to the posterior lobe are interrupted, and this has proved to be the actual cause of the polyuria.<sup>1</sup>

As already recorded, interruption of the nerve tracts between the supraoptic nuclei and the posterior pituitary results in a

<sup>1</sup> Dandy (64A) has recently reported a case of persistent polyuria and polydipsia of over eleven years' duration, which followed transection of the hypophyseal stalk in a girl of seventeen during an operation for presumed tumour. Anterior pituitary functions have not been interfered with, and normal pregnancy has occurred.



persistent polyuria and a consequent polydipsia, the essential features of clinical diabetes insipidus.

On the other hand, complete ablation of the whole pituitary gland usually results in only transient polyuria, and the persistent polyuria produced experimentally can be relieved by ablation of the anterior lobe of the pituitary. Thus some hormone of the anterior pituitary must be functioning normally for the development of true diabetes insipidus (precisely which hormone has still to be determined). The earlier views of Hann (1918) and Richter (195, 196) have thus been confirmed by the recent work of Fisher and Ranson and others (248, cf. also 66).

An interesting case of diabetes insipidus, which adds further confirmation, has been reported by Bernstein (21). At autopsy it was found to be a case of primary bronchogenic carcinoma, with metastases involving the supraoptico-hypophyseal system.

Good results have been reported in treatment of diabetes insipidus following administration of posterior lobe pituitary powder intranasally (162, 40, 161).

Daily administration of amidopyrone (pyramidon), alternated every fourth day with pitressin, is said to produce good results in the treatment of diabetes insipidus (136).

According to Teel and Reid (240) concentrates from the urines of pre-eclamptic and eclamptic patients during the phase of acute water retention have a marked anti-diuretic effect on cats, the action resembling that of vasopressin.

*Hyperfunction of the Posterior Pituitary (?)*. Jones (135) has reported a case which presented hypertension, hyperchromic macrocytic anaemia (which responded to liver therapy), achlorhydria, and depressed carbohydrate tolerance. Noble *et al.* (176) obtained from the urine of this patient an extract exhibiting pressor and anti-diuretic activity of vasopressin type. As the patient improved clinically the excretion of pressor-substance decreased. Dodds and his co-workers (see below) have shown that injection of vasopressin can produce achlorhydria, sometimes followed by anaemia. It was hence thought possible that the patient's condition might be due to hyperfunction of the posterior pituitary.

*Pituitary headache*, 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 (76).

*Gastro-intestinal Ulcers*. It has recently been suggested that undue secretion of the posterior pituitary may be a factor in



the causation of these 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 "puitrin" cause in man (presumably through stimulation of a "parasympathetic centre") an increase in gastric motility, hyper-tonus, and hypersecretion, leading to retching and vomiting (the vomit ultimately containing occult blood), Cushing considered 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 believed that while all ulcerative processes, under all conditions, cannot be so accounted for, yet the majority can (63). (Cf. 120.)

Dodds (65) found that extracts of the posterior pituitary, injected subcutaneously or given orally to laboratory animals, produce 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. It is due to vasopressin (214). Van Dyke (248) remarks that it is unlikely that the large doses of vasopressin used by Dodds to produce gastric lesions are of significance in considering the etiology of clinical lesions.

### The Pars Intermedia

Extracts of the posterior pituitary gland such as "puitrin" cause a dispersion of the black pigment granules in the epidermal melanophores of the frog and other amphibia. This effect is not due to a hormone of the posterior lobe; separation of the pars intermedia from the pars neuralis is not easily effected.



The pars intermedia of the pituitary of fish, amphibia, birds and mammals (including man) elaborates this hormone. Certain fishes, such as the stickleback (*Gastrosteus aculeatus*), bitterling (*Rhodeus amarus*), and the small carp-like *Phoxinus laevis*, develop at the time of spawning a brilliant red colour in the ventral part of the body, and especially in the region of the fins (247). According to Zondek and Krohn (263) this is due to a hormone which they term "intermedin," and which is present in an aqueous extract of the pars intermedia. Intermedin is probably identical with the melanophore-dispersing hormone present throughout vertebrates, but essential proof of this is still wanting.

Geiling showed that cultures of the pars intermedia of the mouse and rat, while they had no effect on blood pressure (showing that vasopressin was absent), had a marked melanophore-dispersing effect when injected into frogs (107).

In the porpoise (260) and the whale (245) and probably in marine mammals generally, the pars intermedia is absent; the melanophore-dispersion hormone is formed by the anterior lobe of the whale (245). Geiling has studied the pituitary of the finback and sperm whales very carefully (106), 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 gonadotrophic, thyrotrophic, and adrenotrophic principles (cf. pp. 378, 383, 388) but a very low prolactin content.

While in amphibia the melanophore-dispersion hormone produces effects which suggest a possible physiological function, its function in birds and mammals is not at present definitely known. However, O'Donovan and Collip recently prepared extracts from the pituitary containing what they have termed "the specific metabolic principle" and they have shown that this hormone is most richly present in extracts of the pars intermedia and in "pituitary colloid" (known to be rich in



the melanophore hormone), while its chemical properties, as well as its distribution, closely resemble those of the melanophore hormone, with which it may therefore be identical (243).

This hormone appears to depress carbohydrate oxidation and to increase combustion of fat. Collip and his associates have shown that it produces ketonaemia in normal and in adrenalectomized rats, decreases total body fat, but increases liver fat in fasted mice, increases glycosuria and ketonuria in the hypophysectomized-depancreatized (Houssay) dog (see p. 394), maintains liver and muscle glycogen (the so-called "glycostatic effect") and renders rabbits resistant to insulin (a "glycotrophic" effect).

It increases the oxygen consumption and depresses the respiratory quotient of rabbits and of man.

It is present in almost any pituitary extract. A very active preparation of melanophore hormone prepared by Stehle (230) is rich in it.

An extract of pituitary glands, from which the thyrotrophic and gonadotrophic principles have been precipitated by alcohol yields an excellent preparation by concentrating the alcoholic filtrate to the aqueous phase, extracting with ether, and saturating the aqueous residue with ammonium sulphate, repeating the latter process, and effecting final purification by alcoholic and then isoelectric precipitations. The product is rich in metabolic and melanophore factor, and almost free from other pituitary hormones.

Active extracts have been prepared from ox, sheep and pig pituitaries. The hormone is thermostable, resistant to alkali and pepsin, and destroyed by trypsin, in all these respects resembling the melanophore hormone. It dialyses through cellophane. (Cf. 177, 27, 175, 187.)

It is important to remember the properties of this hormone, in considering the apparently involved relationship of pituitary hormones to carbohydrate and fat metabolism (cf. p. 393).

### **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 implanta-



tion and injection experiments in animals, although biochemical knowledge of the endocrine principles is making rapid progress. It is convenient to give some account of the diseases associated with the anterior lobe before dealing with experimental data.

These diseases, or at least the most important of them, are :

(A) Hypofunctional conditions (and mixed syndromes)—

- (i) Pituitary cachexia. General hypofunction of the anterior lobe, usually due to actual destruction of the glandular elements.
- (ii) Pure anterior lobe deficiency, possibly a true hypoplasia, but probably merely one form of—
- (iii) The Lorain-Levi, Fröhlich, and Lawrence-Moon-Biedl syndromes, associated with hypofunction of the beta cells.

(B) Hyperfunctional conditions (and mixed syndromes)—

- (i) Gigantism, a functional disturbance in childhood and adolescence, associated with hyperplasia or tumours of the alpha cells.
- (ii) Acromegaly, associated with tumours of the alpha cells.
- (iii) Cushing's pituitary basophilism, associated with tumours of the beta cells.
- (iv) Amenorrhoea and disturbances of vision, associated with tumours of the chromophobe cells, which cause pressure effects.

It is perhaps of 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 several distinct endocrine compounds, which (i) control growth, (ii) control the gonads, (iii) control the thyroid, (iv) possibly control the islets of Langerhans, (v) control the adrenal cortex, (vi) control the secretion of milk, and (perhaps) (vii) control fat metabolism. The precise number of hormones concerned in this wide control of the organism will be discussed later (see p. 396). It is to be remembered that no function can be associated with the chromophobe cells beyond being precursors of the others, so that all hormones of the



anterior pituitary must be produced in either the acidophile or the basophile cells.

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,

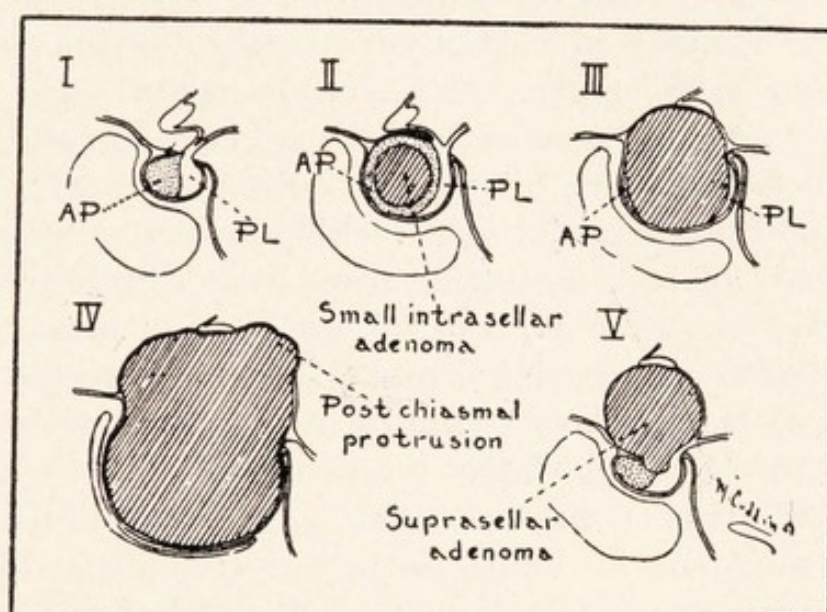


FIG. 43. A series of drawings to illustrate the mechanical effects of an expanding pituitary adenoma. I. The normal pituitary gland and optic chiasm. II. A small intrasellar adenoma with only a slight expansion of the sellar. III. A larger adenoma beginning to stretch the chiasm—a little anterior lobe 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.)

so that X-ray examination reveals them. Some idea of the changes accompanying tumours of different sizes is given by the diagrams in Fig. 43.

**Pituitary Cachexia.** This condition, in which there is pituitary deficiency in marked but varying degree, must be differentiated from *anorexia nervosa*, though the differential diagnosis may be extremely difficult (cf. 197) and rests mainly on the discovery of an initial psychological factor (cf. Spence, 207). The extreme type is met with in *Simmonds' disease*, which term, according to Sheehan (219), should be confined to



cases in which the disease results from post-partum necrosis of the pituitary. Milder types have been termed by v. Bergmann *pituitary emaciation*, and, according to Spence (207), are less uncommon than Simmonds' disease.

Anorexia nervosa occurs chiefly in girls and young women, usually unmarried, and usually but not always psycho-neurotic, manifestations of endocrine dysfunction being secondary (207). Sheldon (207) states that cases of anorexia nervosa, Simmonds' disease (in the limited sense of Sheehan) and pure chronic starvation all show loss of weight, amenorrhoea, lowered basal metabolism, changes in carbohydrate metabolism, subnormal temperature, slow pulse, and hypotension. In anorexia nervosa and chronic starvation there may be an increased hair-growth (in females, on trunk, limbs and face); this does not occur in Simmonds' disease. Sheldon considers that the lowered intake of food (resulting from loss of appetite due to a mental cause) results in nervous inhibition of pituitary hormone activity, whence the parallelism in these conditions. Spence (207) suggests that lack of vitamins may be the essential factor of the starvation which affects the pituitary.

Richardson (197) considers that a diagnosis of pituitary cachexia should not be made until persistent attempts fail to diagnose a neurosis or to relieve it, and until efforts have been made to increase intake of food and vitamins and to ascertain the effects of such increased intake. He reports that in several cases of anorexia nervosa no benefit was obtained from pituitary therapy.

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. 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 correctly insisted



that the primary etiological factor in this case and in two somewhat similar cases which he subsequently reported was the destruction of the anterior pituitary.

Sheehan (219), basing his conclusions on a detailed analysis of fifty-one published cases, together with information derived from over seventy others, states, as already mentioned, that most cases of true Simmonds' disease are the late effects of post-partum necrosis of the anterior pituitary body, and that the original necrosis follows a delivery which is invariably complicated by collapse, usually as a result of severe haemorrhage. From his review of cases he presents the following composite description (stating that individual cases present exceptions to most of the conditions mentioned).

During the puerperium there is complete absence of lactation, and sometimes a hypoglycaemia. Then the uterus becomes superinvolted and the external genitalia atrophy. Menstruation does not return, libido is absent, there is a gradual loss of axillary and pubic hair. The patient becomes apathetic and dull, very sensitive to cold, and may show a myxoedematous or a prematurely senile condition. The basal metabolism drops to about — 25 per cent. The body weight is usually but little altered. The blood pressure tends to be rather low. Hypochromic anaemia is present. Blood cholesterol may be slightly raised, blood sugar slightly low. Sugar tolerance curves show a delayed fall.

After ten, twenty, or even thirty years the patient may become more typically myxoedematous,<sup>1</sup> or may develop mental changes with some weight loss, but *severe emaciation is uncommon*. At this stage the anaemia may become hyperchromic, and the basal metabolism fall to — 35 per cent., but the blood pressure is usually normal.

Finally, usually through some intermittent illness or a phase of severe anorexia, the patient lapses into coma (with hypoglycaemia usually present) and dies.

At post-mortem examination the anterior pituitary is replaced by scar tissue, the adrenal cortex is atrophied, the thyroid fibrosed, the ovaries and uterus shrunken, and the viscera small.

<sup>1</sup> Castleman and Hertz (41) have reported a case clinically diagnosed as myxoedema, and only proved to be Simmonds' disease at post-mortem.



Sheehan states that the results of pituitary substitution therapy are not yet very satisfactory, but—a somewhat curious finding considering the non-return of menstruation—that if a subsequent pregnancy results, this cures the patient, presumably through hypertrophy of remaining pituitary tissue. (It would seem more plausible to suggest that hypertrophy of remaining pituitary tissue prevented the onward progress of the disease,

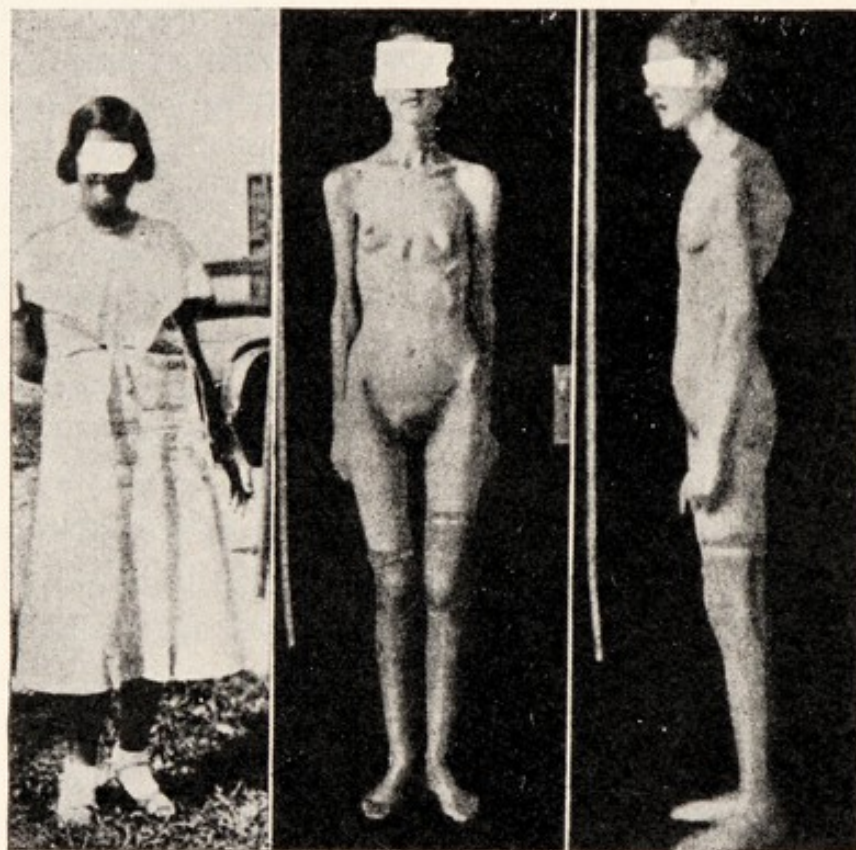


FIG. 44. Case of pituitary cachexia. *Left*: patient in July, 1933, aged twelve, weight 142 lb. About seven weeks before onset of illness. *Centre and right*: patient in June, 1934, aged thirteen, weight 85 lb. About nine months after onset of illness. (From Dunn, *J. Nerv. Mental Dis.*, 1936, lxxxiii, 166.)

caused restoration of ovulation, and so permitted possibility of a further pregnancy.)

Sheehan believes that "true" Simmonds' disease is relatively common, but frequently remains undiagnosed, but that, on the other hand, many cases reported under this title are in reality cases of pituitary cachexia or of anorexia nervosa.

Such a limitation of cause as Sheehan suggests seems unnecessarily artificial, since it is obviously admitted that



pituitary hypofunction can arise from other causes than post-partum necrosis of that gland. It is further most probable that varying grades of damage to the anterior lobe arising from various causes will present a graded syndrome which, as far as women are concerned, will not differ whether the primary cause be a post-partum haemorrhage into the anterior pituitary or not. Hence the syndrome-picture given in an earlier review by Calder (36) may well be compared with that of Sheehan. (It will be noted that the two descriptions differ in some essential points.)

Calder reviewed seventy cases in the literature (eighteen males, forty-seven females, five of unrecorded sex) and presented the following conclusions: Emaciation develops sooner or later and is a striking and characteristic feature. Falling of the teeth and hair (particularly axillary and pubic hair), trophic changes in the nails, and thickening and loss of lustre of the skin combine to give the patient an 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. Basal metabolism, when it has been measured, is low; blood pressure is invariably low. In women menstruation ceases and sterility ensues. In men there results sexual weakness which may amount to complete impotence. In both sexes desire ceases. Many patients display peculiar forms of pathological sleep. Coma frequently precedes death. Without exception autopsy reveals destruction of the anterior pituitary. In about half the cases autopsied the glandular elements were replaced by

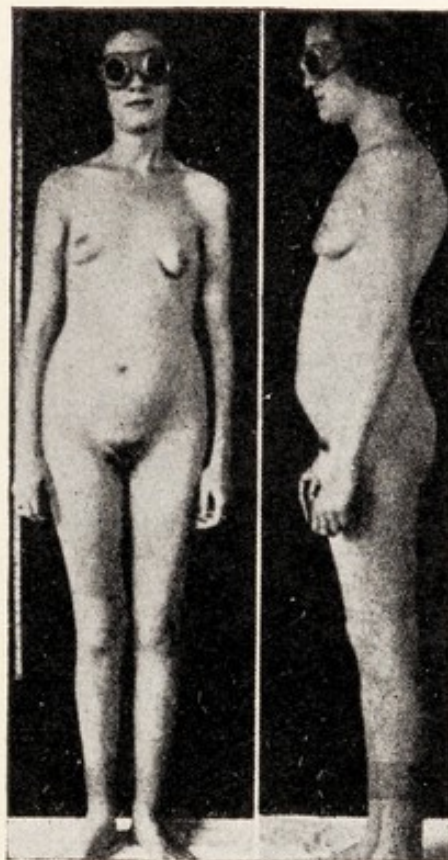


FIG. 45. The same patient as in Fig. 44. In October, 1934, after twenty-two weeks of combined anterior pituitary and oestrogenic therapy. (From Dunn, *loc. cit.*)



scar tissue indicating healed injury. Calder considers that probably no one pathological process is responsible for all cases. Many of the symptoms resemble those of Addison's disease, a chief point of differentiation being the pigmentation of the skin generally present in the latter. Since autopsy shows adrenal involvement, the asthenia, low blood pressure, and subnormal temperature may be ascribed, as Calder points out, to secondary involvement of the adrenal cortex.

In a number of cases of pituitary cachexia beneficial results have been reported to follow continued injection of anterior pituitary extracts (cf. 76, 194, 37, 157); an example is shown in Figs. 44 and 45. Other cases so treated have not responded. At least some of the latter may well have been cases of anorexia nervosa.



FIG. 46. Comparison of a pituitary dwarf girl at the age of  $9\frac{1}{2}$  years with a normal boy of the same age. (From Engelbach, *Endocrinology*, 1932, xvi, 11.)

The mild cases of "pituitary emaciation" described by v. Bergmann are stated by him to have responded to pituitary treatment (20). (Stephens has also described a syndrome of mild hypopituitarism in a group of cases (233).)

It is probable that there is no true line of demarcation between pituitary cachexia and the Lorain-Levi syndrome (see p. 350). The close relationship of the two is illustrated by a case described by Hart and Lisa (115). A man aged thirty-seven, with Lorain infantilism, a subnormal temperature, low blood pressure, and who had been suffering from severe frontal headaches of two months' duration, was found at post-mortem to have a massive cyst of the pituitary which had completely obliterated the anterior lobe (and had spread upwards and forwards into brain tissue), a completely inactive thyroid, and underdeveloped testes.

**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 (75) defined 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 considered 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 pituitary cachexia corresponds to myxoedema.

The following appears to be a classical case of this condition, as put forward by Engelbach himself (75). But it possibly should merely be considered as the childhood form of the Lorain-Levi syndrome.

A girl, aged nine and a half years, exhibited marked physical underdevelopment and diminished appetite, conditions present from birth. During the first two years she was overweight. She could sit alone at six months. She did not walk until four years of age. Growth-rate was retarded from the first year, and growth ceased after the sixth year. Her mentality was good, and she was physically active. She had suffered practically no illnesses.

Her height when examined was  $35\frac{1}{2}$  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. 46 and 47). The

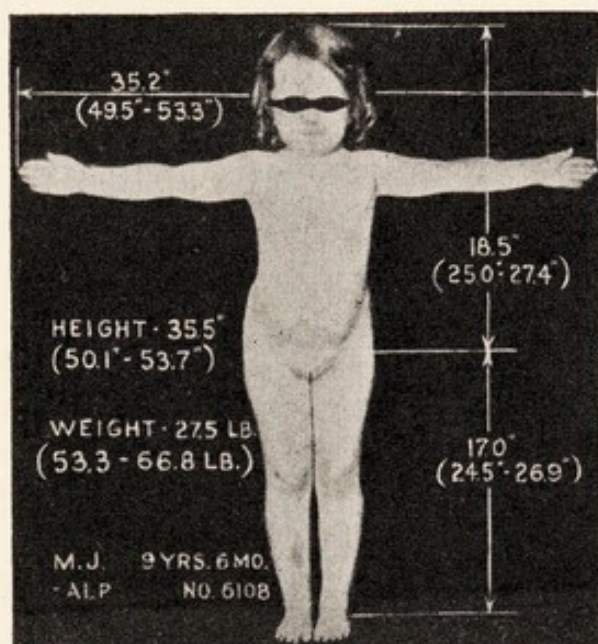


FIG. 47. Comparison of the actual body measurements of the pituitary dwarf of Fig. 46 with the normal maximum and minimum measurements for her age. (From Engelbach, *Endocrinology*, loc. cit.)



head was large in proportion to the body. The sella turcica was normal for the size of the head.

Under treatment with a purified extract of the pituitary growth principle (cf. p. 369) 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 freed from the gonadotrophic hormones.) 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 (220). Equally good results have been reported in a number of other cases exhibiting retarded growth due to pituitary deficiency (77, 220, 208, 69).

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 hormone 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 considered that heredity is the prime causative factor of the Lorain-Levi syndrome, with infections and intoxications playing a secondary, excitatory rôle. Early



recognition is very desirable in order that treatment may be instituted while the retarded osseous development is still capable of modification.

Biedl (23) 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 under-development of the genitalia is usually noticeable before adolescence (75). 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 (75).

Engelbach held 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 may well be that the adiposity is due to hypofunction of the pars intermedia. Obviously chromophobe tumours within the sella turcica or extra-sellar tumours such as cranio-pharyngiomas can provide the pressure effects necessary to depress the functions of the chromophile cells of both pars anterior and



intermedia. If the condition arises without tumour growth, then hypoplasia of both these 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 (45). 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 (193; cf. also 227).

**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. The conditions present in the Laurence-Moon-Biedl syndrome obviously require more than pituitary correction.

Lawrence and Harrison (143) report a good response to pituitary treatment in a boy who, at sixteen years of age, was 59 $\frac{3}{4}$  inches tall, 87 lb. in weight, and exhibited sexual infantilism and dwarfism of the eunuchoid type. After two and a half years' treatment he had gained 6 $\frac{1}{2}$  inches in height and showed normal sex development (cf. also 236, 170).

**Renal Rickets and Dwarfism.** Chown (42) has reported a case which he believes can be attributed to pituitary malfunction.

**Cranial Dysplasias of Pituitary Origin.** Mortimer and his colleagues (172, 173) have made prolonged roentgenographic studies of crania in hypophysectomized dogs and rats, as a result of which he suggests that in human cases of hyperplasia or hypoplasia of the paranasal sinuses among the causal conditions may be (i) transmitted inherent pituitary disability, or (ii) disturbed pituitary function arising during post-natal growth (but possibly a late manifestation of (i)).

**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 (24). The majority of cases are males. Engelbach's description seems complete, although it is doubtful



FIG. 48. 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.)

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 been recorded by Behrens and Barr (19), whose observations extended over eighteen months. Somewhat against Engelbach's views, the

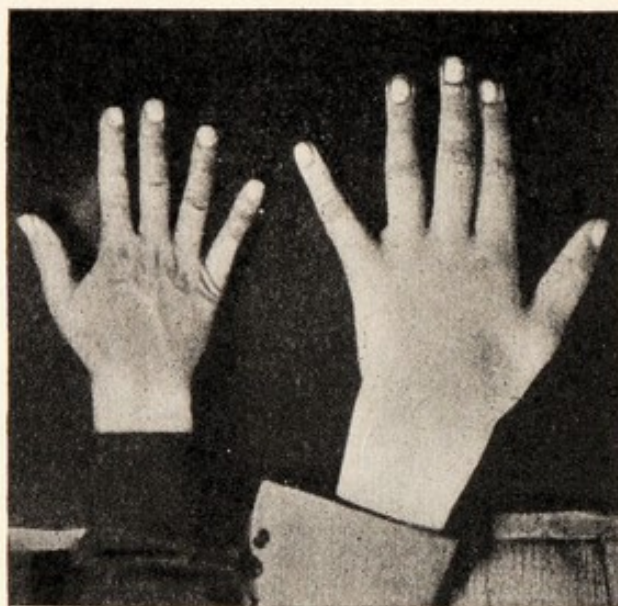


FIG. 49. Hand of patient (Fig. 48) compared with that of a man 6 feet in height. Noteworthy are the long, lightly tapering fingers and the delicate, fine skin. (From Behrens and Barr, *ibid.*, p. 124.)

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. . . .

"His expression and appearance are best shown by the photographs. Notable is the wide spacing between the eyes and the complete absence of mandibular prognathism. There is some spreading of the upper teeth. The skin was moist, delicate, and of fine texture, but the hands and feet tended to be cold and slightly cyanotic. He had no hair on his face, and the hair on his body was scanty. His father reported that

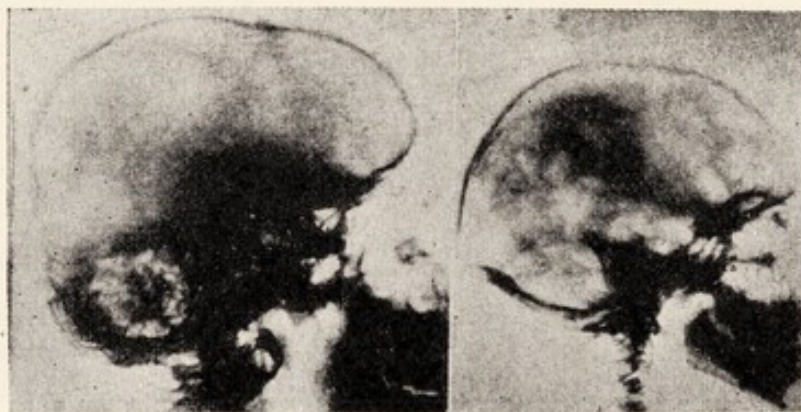


FIG. 50. X-ray photograph of skull of patient (Fig. 48) at age of eleven, compared with that of a normal boy of the same age. There is an extraordinary development of the mastoid air cells. The sella turcica measured 2.5 cm. anterior-posteriorly; it has been outlined with dots to indicate its extent. (From Behrens and Barr, *ibid.*, p. 122.)

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. 48 to 51.

"The X-ray examination revealed in the bones of the face and maxilla a moderate tendency to prognathic development. The mastoids showed extraordinary development of pneumatic structure. . . . The sella was of extreme size, measuring 2.5 cm. in its anterior-posterior diameter. The floor of the sella showed a loss of continuity, being broken by a tubular structure which extended downward and forward from the



sella and reached almost to the posterior wall of the pharynx, where there was an indefinite soft tissue shadow encroaching upon the lumen of the pharynx itself." It was thought that there was evidence of a persistent Rathke's pouch. X-ray photographs of the hands showed no abnormality in the state of the epiphyses or degree of calcification, as compared with a normal boy of the same age.

He was seen again at the time of his thirteenth birthday. Measurements at the two examinations were :—

Age.	11 yrs. 11 months.	13 yrs.
Weight . . . . .	112.3 kg.	126.4 kg.
Height (bare feet) . . . . .	208.0 cm.	219.0 cm.
Sitting height . . . . .	103.5 "	—
Arm spread . . . . .	203.5 "	215.0 "
Head circumference . . . . .	65.5 "	—
Chest circumference . . . . .	104.5 "	107.5 "
Length of hand . . . . .	22.0 "	23.5 "
Length of foot . . . . .	37.0 "	38.5 "

During the interval between these examinations he had shown good progress at school, had lost much of his bashfulness, and displayed general interest and co-operation. His physical strength had been maintained. "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. 51 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."

A further report on this case has been made by Humberd (130). At the age of eighteen years and three months his height had



increased to  $99\frac{1}{4}$  inches. This unusually authenticated giant is frequently featured in the American pictorial press.

Engelbach (75) 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.

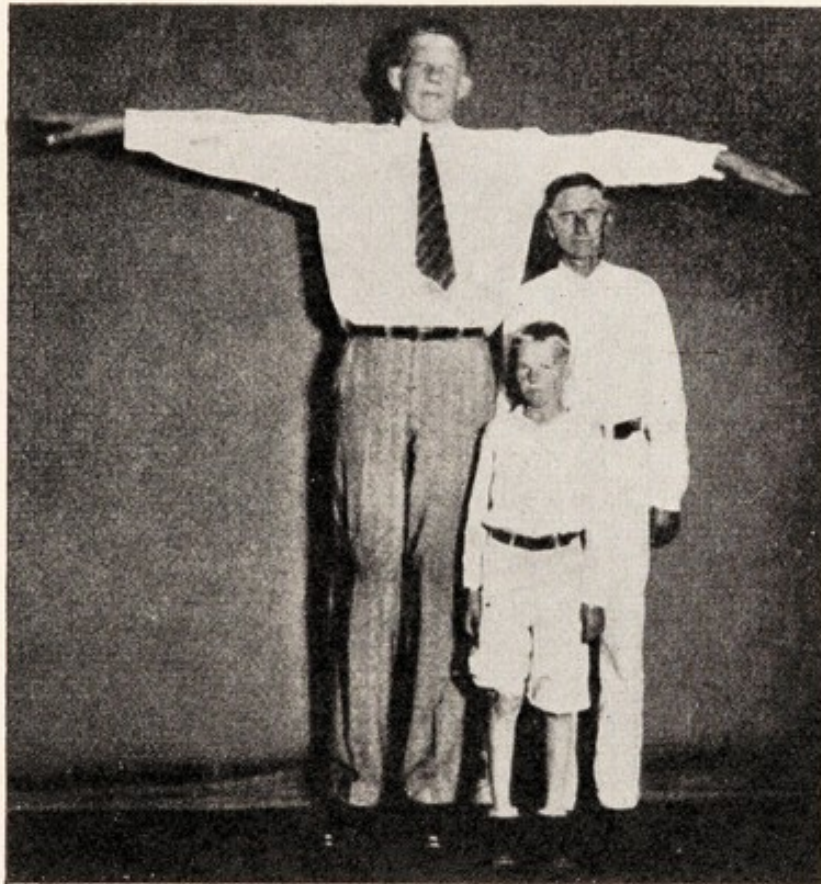


FIG. 51. The patient at the age of  $13\frac{1}{2}$ , shown standing with his nine-year-old brother, and his father, whose height is 5 feet 11 inches. (From Behrens and Barr, *ibid.*, p. 125.)

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.

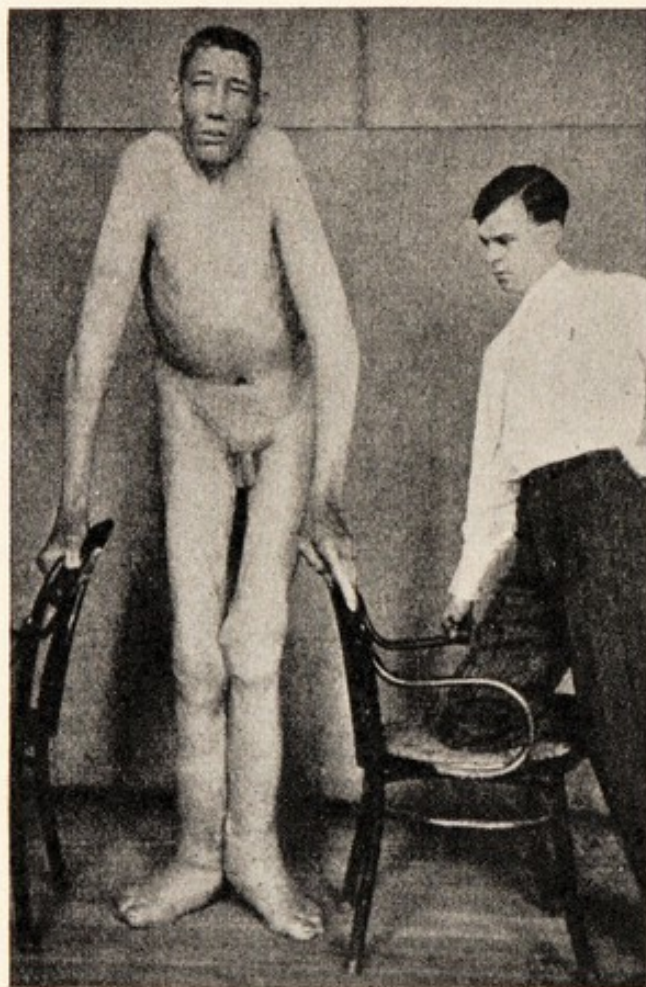


FIG. 52. A case of gigantism. Final stage. Note the narrow chest, large joints, hypotrichosis, and the large size of the hands compared with that of the normal person of height 68 inches. (From Cushing, "The Pituitary Body and its Disorders," Lippincott, 1912, Case XXXII.)

The sella turcica showed no evidence of proliferation or erosion, measuring  $13 \times 12$  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 (62), a man aged thirty-six, "an extraordinary prototype of the folk-lore giant—overcome by his own size." His appearance is shown in Figs. 52 and 53. 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.<sup>1</sup> 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,



FIG. 53. The same patient as in Fig. 52. Exhibiting a maxillary, rather than the mandibular prognathism of the acromegalic. (From Cushing, *loc. cit.*)

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.

<sup>1</sup> Humberd (131) adduces reasons for belief that the height of this patient was only 7 feet 3 inches, and has critically reviewed the heights attributed to various giants in the older literature. However, Behrens and Barr's patient has definitely proved that growth can exceed 8 feet, as Humberd (130) himself has recorded.



Fig. 52). 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. 53). X-ray of the skull showed a relatively shallow sella turcica,  $2.7 \times 1.7$  cm. (anterior-posterior  $\times$  depth measurements). There were huge maxillary and frontal sinuses.

He exhibited a high carbohydrate tolerance.

He died six months later. Autopsy showed diminutive adrenals, fibrosed testes with almost complete disappearance of spermatogenous cells, and a small and fibrosed pancreas. The pituitary gland was largely represented by a cyst. Cushing commented on the pituitary condition: "As regards the hypophysis itself, it is fair to assume that there was originally an extreme functional hyperplasia of the pars anterior with subsequent cystic degeneration. These hyperplasias are capable of various transformations—here a degenerative one."

These giants are usually believed to die young and childless. However, they occasionally reach middle age. The giant Chang is said to have died at fifty-one, and Palozzi, reported by Levi and Franchini in 1909, at sixty-six (62).

A possibility of successful treatment is seen in results quoted by Cushing (60). 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 hypertrophy. The gradual onset of the facial hypertrophy is beautifully shown in the photograph of Cushing's case XXX (62), reproduced in Fig. 54. 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. 54 such enlargement was not present.

There may be some degree of gigantism, depending on the



FIG. 54. 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.)

age of onset.<sup>1</sup> 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 (64).

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

<sup>1</sup> Mortimer believes that acromegalic cranial changes can appear early in the second decade (cf. 205A).



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 of the function of the pars intermedia.

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 (148, 141).<sup>1</sup>

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 (60), 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 in 1912 described a syndrome associated with an adenoma of the basophile cells of the anterior pituitary (58, 60). 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 hypertension, abdominal pains, fatigability and ultimately extreme weakness. (Cf. 179.)

A number of such cases found in the literature, and some

<sup>1</sup> "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 (15).



under his own observation, including one predicted clinically by Teel (237), 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 (185) is shown in Fig. 55.

It is fully quoted by Cushing (58), 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

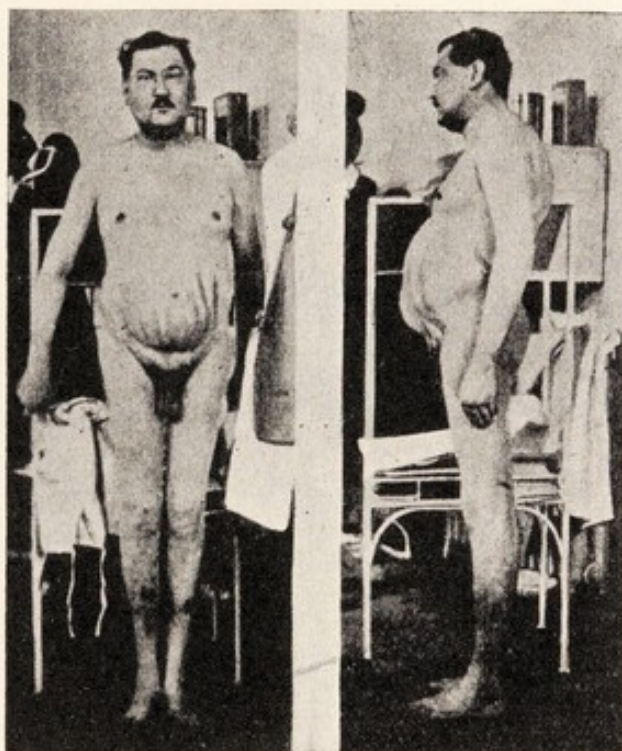


FIG. 55. Dr. Raab's patient with verified basophilic adenoma of the pituitary. (From Cushing, *Bull. Johns Hopkins Hosp.*, 1932, 1, 137.)



in Fig. 56, 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.

Since Cushing described the syndrome numerous cases have been reported (cf., *e.g.*, 28, 169, 55, 235). Rasmussen and Nelson described two cases of basophile adenoma originating from the pars intermedia; the first showed no symptoms definitely attributable to the tumour, but the second exhibited

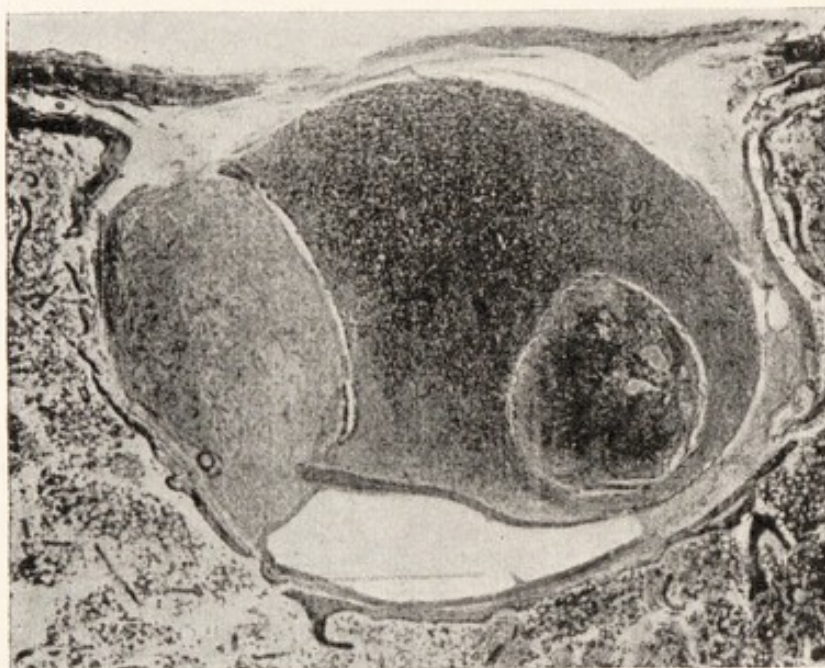


FIG. 56. Cross section of the pituitary from a case of pituitary basophilism. (From Bishop and Close, *Guy's Hospital Reports*, 1932, lxxxii, 143.)

adiposity, striae, hirsuties, high blood pressure, and a florid face (192).

Furtado and Morato have reported an unusual case (101); in a boy aged seventeen the syndrome was associated with an enlarged sella turcica, marked optic atrophy, and hemianopsia. At operation a large tumour the size of a walnut was found between the two optic nerves, and most of it was removed. Twelve hours after operation an epileptic seizure occurred, and death followed. The tumour apparently contained acidophile, basophile and chromophobe cells. Hill (119) has reported a case of the syndrome in which no endocrine tumour of any kind could be found at autopsy.



It is doubtful whether any true differentiation can be made between the syndrome associated with a basophile adenoma of the pituitary and that associated with an adenoma (or malignant tumour) of the adrenal cortex (cf. p. 228). Both should probably be termed "Cushing's disease." The syndromes of the two, especially in women, are practically identical (cf. 17, 97, 145, 253). In each type, whether or not a pituitary basophile adenoma is present, Crooke has shown (56) that the basophile cells exhibit a characteristic hyaline change. Since the pituitary controls the adrenal cortex through a hormone which is probably elaborated in the basophile cells (cf. p. 391) the primary dysfunction in all cases of the disease may well lie in the pituitary, whether or not an adenoma be present there.

Treatment of cases of basophile adenomata seems almost hopeless, although application of X-ray therapy in early cases may have some benefit (60). It is fortunate, therefore, since good results are obtained by surgical removal of adrenal cortical tumours (cf. p. 228) that Cushing's disease in the child is almost always associated with an adrenal cortical tumour, and is frequently so associated in the adult woman, although this association is rare in adult man (57).

Differentiation as between pituitary and adrenal adenoma is not easy. In rare cases the presence of an adrenal tumour can be recognized directly by palpation, or by X-ray photography after perirenal insufflation of air (35, 53A). Crooke and Callow have recently produced evidence (57) that in the cases with adrenal cortical tumour there is marked increased excretion of urinary androgens and that such an increase does not occur in cases of pituitary basophile tumours. Test of their procedures should yield results of interest.

**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 (15). 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 (59, 117). Careful X-ray therapy gives good results in some proportion of cases (113).

The ocular signs involved through such pressure include perimeter defects and optic disc changes, diplopia and strabismus. The general intracranial pressure signs include deep-seated headache, projectile vomiting, choked disc and photophobia.

Patients with chromophobe adenomas usually exhibit a lowered basal metabolic rate (64).

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 actual number of hormones elaborated in the acidophile and basophile cells of the anterior pituitary is at present a matter of opinion. Claims for new hormones steadily appear. Well-established claims for the existence of others are being challenged. None has yet been obtained in indisputably pure condition. All are proteins, perhaps chemically closely related, and certainly separable with difficulty. Some or all of those now supposed to exist may prove to be artificial products of larger-moleculed hormones, to be in fact pseudo-hormones



produced from these larger-moleculed compounds by the chemical and physical procedures used for isolation. Some discussion of this problem is given at the end of this chapter.

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. 344). 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 (217, 76). It seems doubtful if these differing views are more than a difference of opinion as to the cause of death.

In the earlier experiments the usual results of complete extirpation, following an initial latent period, were fall in body temperature, slow respiration and pulse, limp musculature, coma, and death. Houssay noted polyuria in young pups and oliguria in adult dogs. 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 (217).

Section of the stalk leads to somewhat parallel changes which are probably traceable to interference with the blood supply of the anterior pituitary (70). Smith (222) 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 (223) and Allen (5) 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 (50) 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. 388). 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 (213).

Thus from deficiency of pituitary hormones, produced by extirpation, there is evidence that these hormones 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.<sup>1</sup> The effects of excess of the 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 which have proved much more sensitive than normal animals to the action of potent extracts.

There is now definite evidence that various extracts prepared from the anterior pituitary control general body growth, the

<sup>1</sup> Cf., however, Collip's recent experiments, p. 393.



gonads, the thyroid, certain other endocrine glands, and the production of milk. It is generally supposed (but is not proved) that each separate effect is caused by a separate hormone, and it is therefore convenient to consider these supposed hormones in turn.

**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

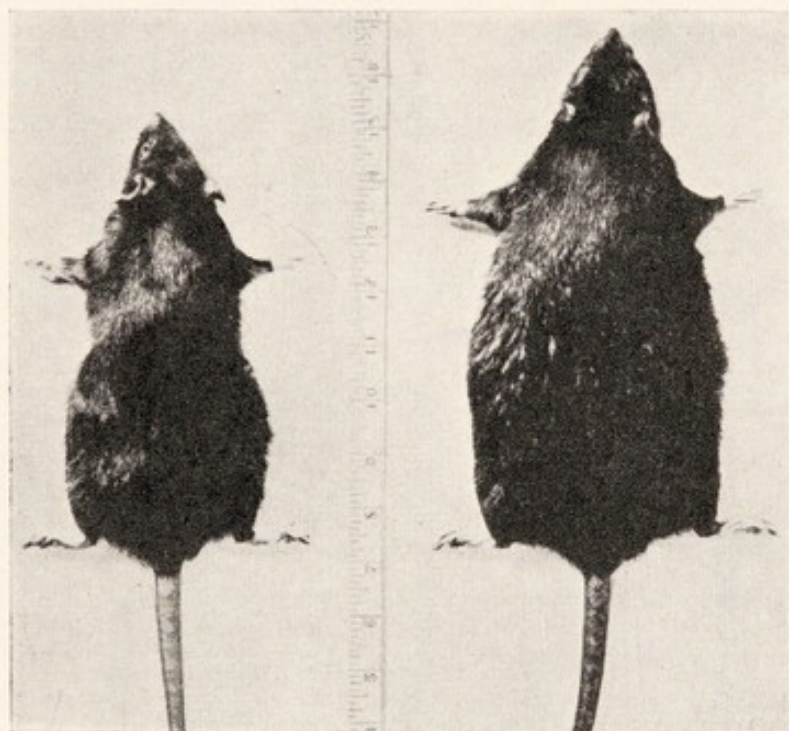


FIG. 57. Photograph at time of autopsy (at somewhat over 400 days of age) of two female rats whose growth curves are shown in Fig. 58. The rat on the right received daily intraperitoneal injections of anterior lobe extract for over a year. The rat on the left is the untreated litter-mate control. (From Evans, *Harvey Lectures*, 1923-24, p. 212.)

into rats produced gigantism (81). Their first results were accidental, observed in an attempt to modify the vaginal smear response in rats (cf. p. 256) by injections of endocrine extracts.

Saline extracts of anterior bovine pituitaries were injected daily into rats for prolonged periods, 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. 57-59. Under such treatment female rats have reached a weight of over 700 grams, as compared with controls of



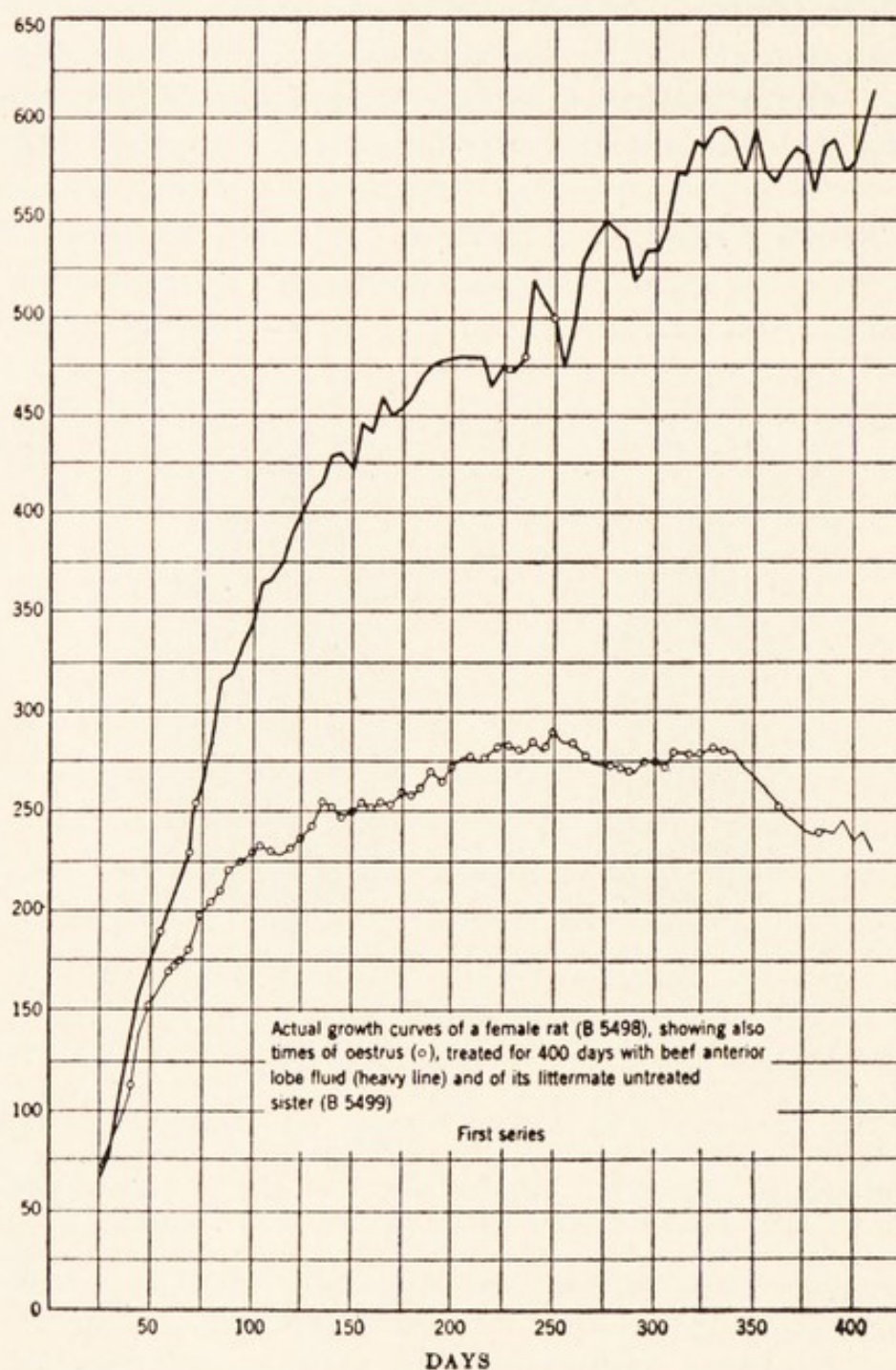


FIG. 58. Actual growth curves of the two female rats shown in Fig. 57. That of the treated rat is given in heavy line. (From Evans, *loc. cit.*)

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 (82).

In the earlier experiments growth of the ovaries and maturation of ova were impaired or inhibited, but this result was



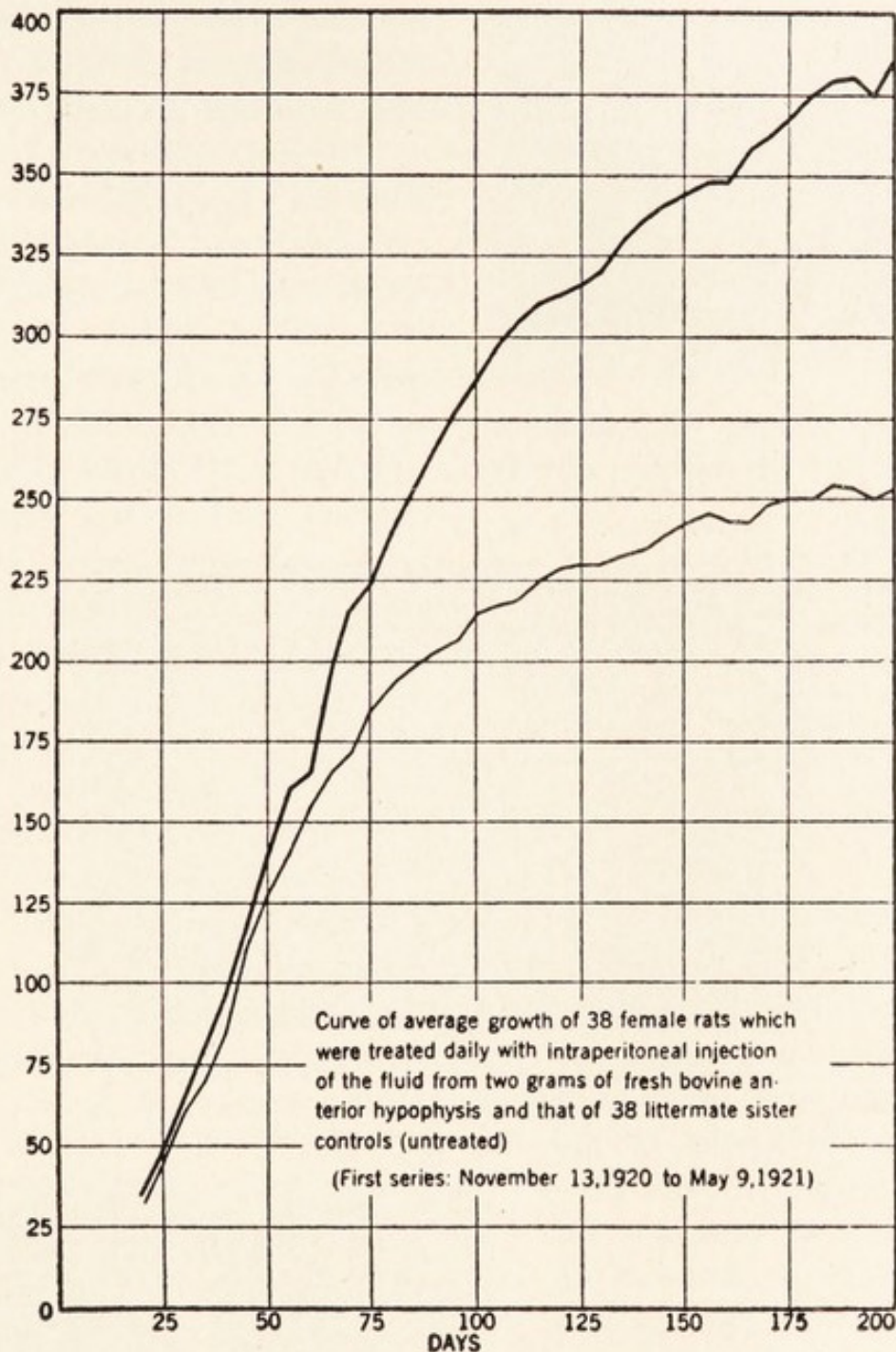


FIG. 59. Curve (thick line) of average growth of thirty-eight female rats which were treated daily with intraperitoneal injections of the fluid from 2 grams of fresh bovine anterior pituitary, contrasted with the curve (thin line) of thirty-eight untreated litter-mate sister controls. (From Evans, *loc. cit.*)

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





FIG. 60. 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.)

An experiment on bull-dogs was carried through to the death of the experimental animal, and the details have been published in full (183, 238). It shows perfectly the gigantism, ultimately an enfeebled gigantism, produced by prolonged and marked hyper-pituitarism.

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. 60-63.

After three and a half months' treatment the lower jaw and skull in

acid-aqueous extracts have no effect on growth but a marked effect on the gonads (84).

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 (184). Nitrogen-retention, and prolonged diminution of blood non-protein nitrogen, was produced in dogs (239).



FIG. 61. The same two animals eight months later. Treated animal on right. (From Putnam, Benedict and Teel, *ibid.*, p. 1710.)



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

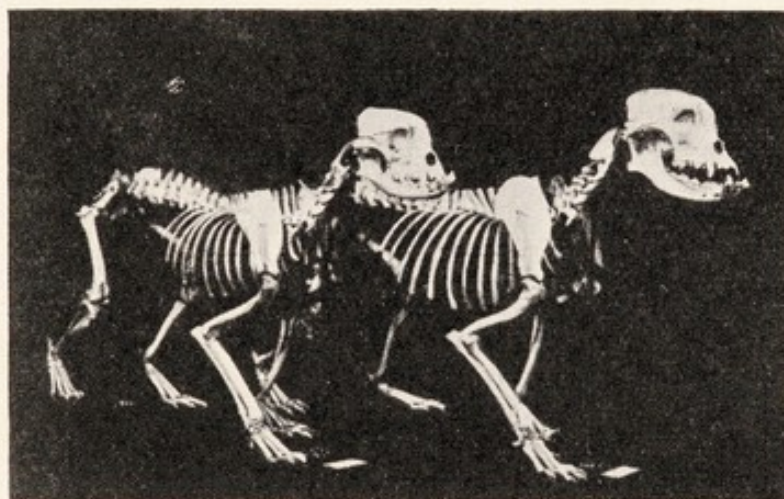


FIG. 62. Skeletons of the treated and untreated animals at the end of the fourteen months' experiment. Treated animal on right. (From Teel and Cushing, *Endocrinology*, 1930, xiv, 158.)

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, dispro-



portionately 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. 62. 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. 63.

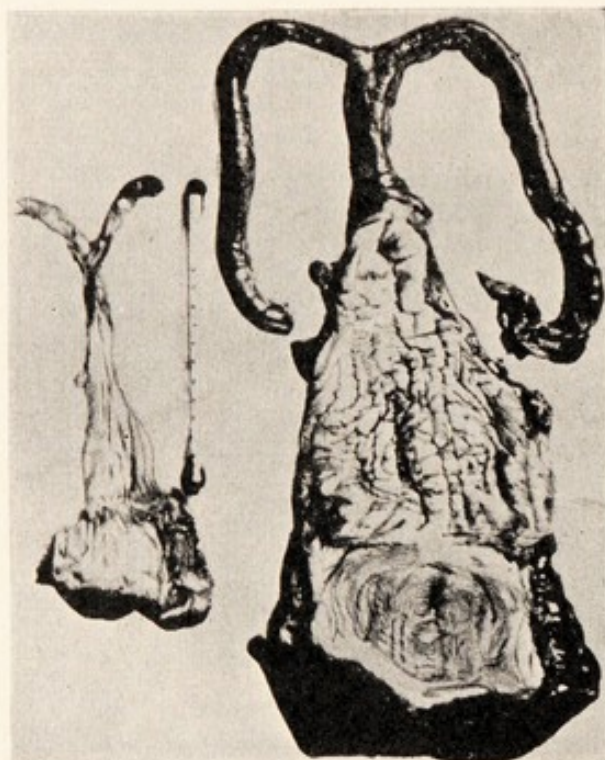


FIG. 63. Vaginae, uteri and left ovaries of the two animals. The vaginae have been laid open by an incision along the anterior wall. Note the rugosity and thickness of the specimen from the treated animal (right). (From Putnam, Benedict and Teel, *loc. cit.*, p. 1719.)

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 co-workers (86) 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. 64.

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  $31\frac{1}{2}$  weeks of these injections the hypophysectomized animal was larger than its control.

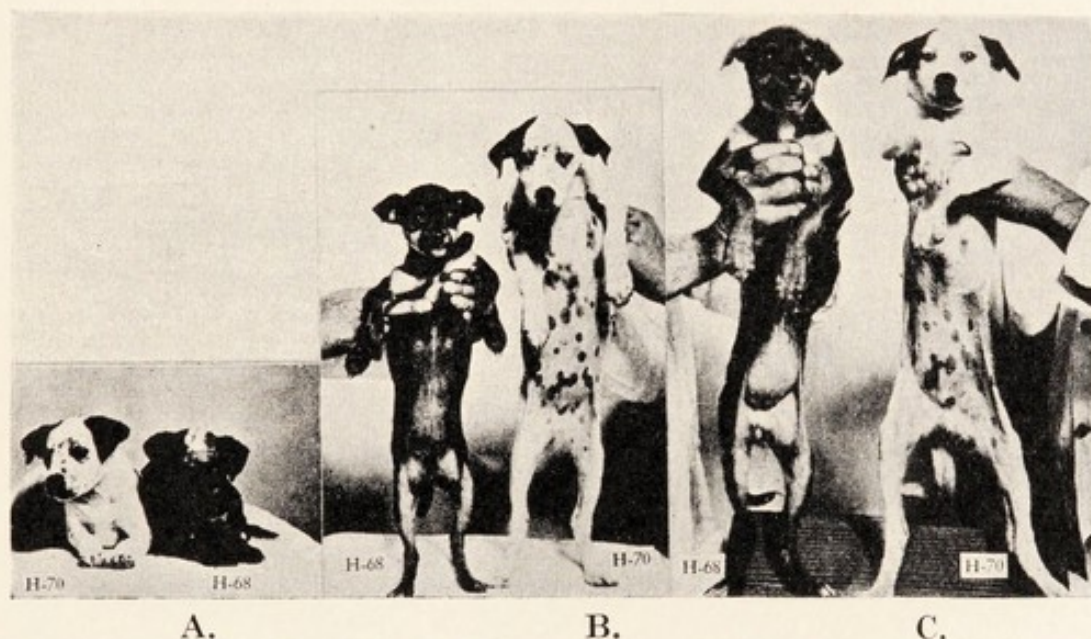


FIG. 64. A. Puppy bitch H-68 was hypophysectomized at eight weeks of age; H-70, litter-mate male control. B. Appearance four weeks later. Injection of growth extract commenced. C. Appearance after two months of injections. (From Evans, *et al.*, *Memoirs of the University of California*, 1933, xi.)

(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 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 (252).

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 controls growth; it seems natural to suppose that a specific hormone is responsible for this control.

Restoration of normal growth has been demonstrated following pituitary implants into hypophysectomized (dwarf) tadpoles (4), and into a strain of dwarf mice (225).

**The Achondroplastic Dwarf.** Although achondroplasia is usually considered to be associated with the germ-plasm itself, from time to

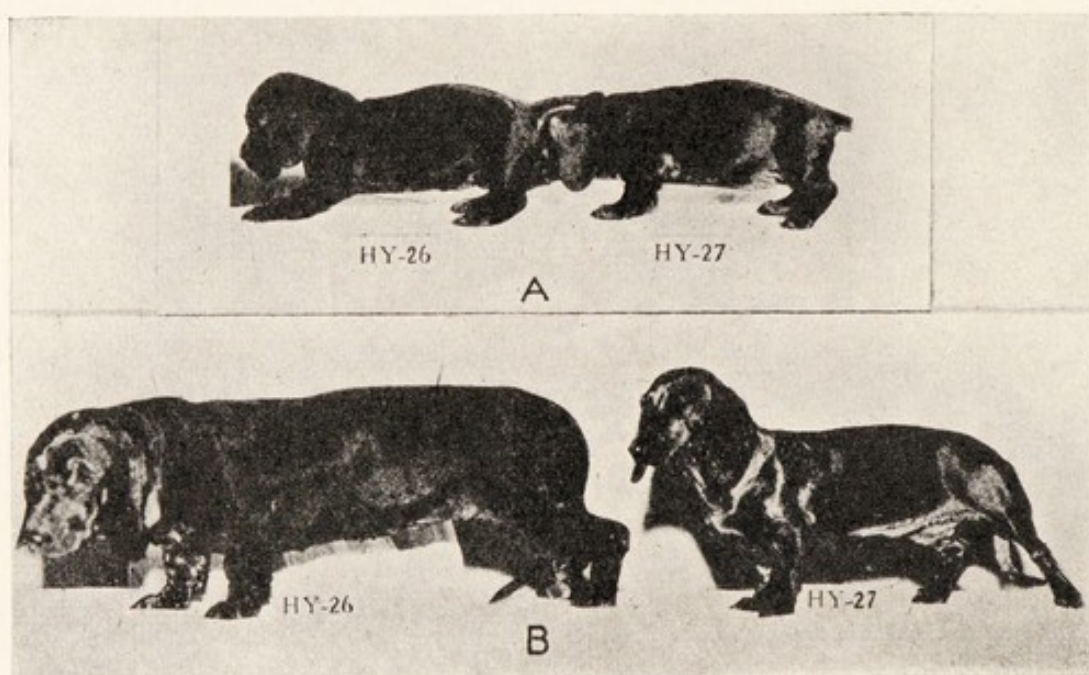


FIG. 65. A. Litter-mate female dachshunds six weeks old. B. The same animals eleven and a half months old. HY-26 had been injected with the growth principle for thirty-five weeks. Note the elongated body, huge head, and redundant skin. (From Evans *et al.*, *Memoirs of the University of California*, 1933, xi.)

time the theory is advanced that the pituitary may be a causal factor. Evans and his associates (86) 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 injected daily a pituitary growth-stimulating preparation, freed from gonad-stimulating hormones, 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. 65.



*Preparation and Properties of Growth-promoting Extracts.*

Numerous investigators have attempted to prepare concentrated growth-promoting extracts (cf., *e.g.*, 86, 246, 51, 114). Within recent years, owing to the claims of Riddle and others that the growth effects were not due to a specific factor, but to a summation of effects of other pituitary hormones, efforts have been especially directed to obtain preparations which will solely promote growth, without producing other known effects of pituitary hormones. Most methods start by extraction with alkali, in which the growth-promoting material is soluble.

Dingemanse, Freud *et al.* (cf. 98) claim to have obtained a highly purified preparation by adsorption on norite, elution with liquid phenol, and isoelectric precipitation. The product was labile to heat, acid, and alkali, was destroyed by pepsin and trypsin, and was dialysable; its freedom from other hormones has not been established.

Evans and his colleagues (87) have described a method of fractionating alkaline extract of anterior pituitary by use of ammonium sulphate, which, they claim, effects a concentration of growth hormone and a lessened concentration of thyrotrophic hormone; they detail additional methods for removal of most of the latter and of the lactogenic hormone. In a later paper (168) they have shown that the growth factor is unaffected by cysteine, whereas the lactogenic hormone is precipitated and the thyrotrophic and gonadotrophic hormones are inactivated through cysteine reduction. They have therefore added this treatment to the method of purification of the growth factor.

Evans has shown further (88) by experiments on hypophysectomized rats that the presence of the thyroid is unnecessary for promotion of growth by such a purified extract, but is necessary for the production of its maximal effect.

As already stated, Riddle believes that growth effects are incidental to the combined actions of other hormones such as the lactogenic and thyrotrophic factors (cf., *e.g.*, 200).

Van Dyke (248) has recently commented that until further evidence is provided it is hazardous to affirm or to deny either that a specific growth hormone can be prepared, or that a suitable combination of other pituitary hormones can imitate the recognized growth-promoting effect of a crude alkaline extract of anterior pituitary tissue. He further points out



that there is general agreement that the pituitary is the most important regulator of growth. The problem still requiring solution is to determine what factor or factors is actually responsible for that regulation.

It seems to be generally established that the factor or factors concerned affect protein metabolism. The store of reserve protein is increased and the exogenous catabolism of amino-acids is decreased (cf. 83).

Freud *et al.* (99) state that the growth-promoting factor

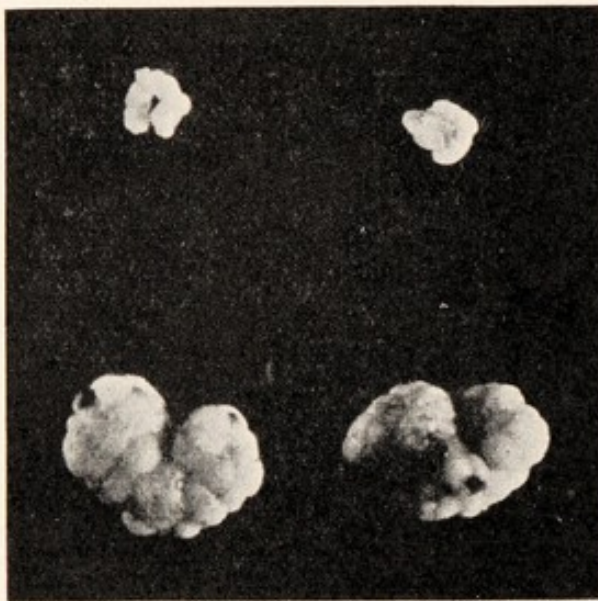


FIG. 66. Above. Ovaries of littermate control rat. Weight, 21 mg. Below. Ovaries of experimental rat after thirty implantations of fresh rat pituitary gland over eighteen days. Weight, 340 mg. (From Collip, *Proc. California Acad. Med.*, 1930.)

might well be termed the chondrotrophic hormone, since its point of action is the proliferating cartilage. In experiments on rats they find that the epiphyses are closed soon after hypophysectomy, and that once this epiphyseal closure is completed it cannot be reversed by treatment with growth hormone. Such treatment, commenced immediately after hypophysectomy, prevents closure and normal growth continues.

**The Gonadotrophic 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. 367, 368).

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.

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. 66 and 67. The uterus corresponds in weight to that of normal animals maturing at normal time, and structurally the uterus and vagina are typical of the adult animal.

The genital system of the immature male is not so definitely affected. The testes show a more variable response; the secondary sex organs are increased in weight and in physiological activity.

Similar treatment applied to the adult female rat leads to ovarian hypertrophy and superovulation. The male exhibits no demonstrable response.

The secondary sex responses are not shown in spayed and castrated animals. The gonadal degradation following extirpation of the pituitary ceases following pituitary implantation and the gonads are restored to normal condition.

Pituitary implants from both immature and senile animals are active.

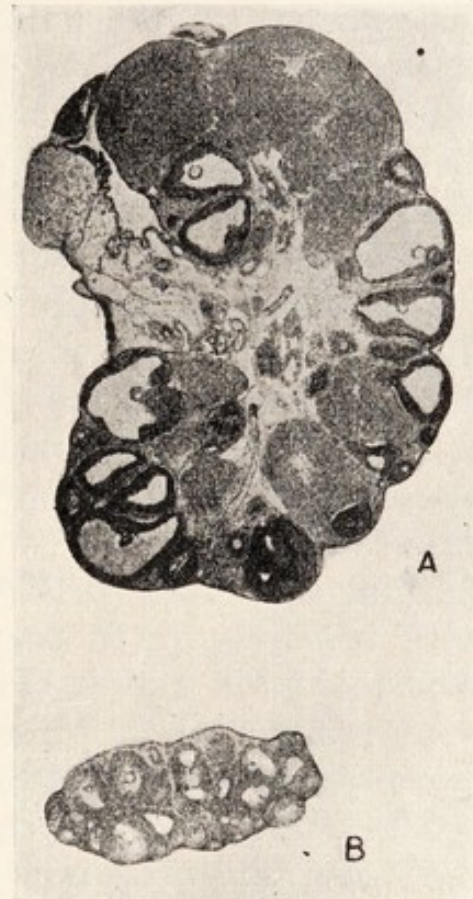


FIG. 67. A. Cross section of ovary of rat. Sexual maturity induced on the twenty-seventh day of life, following four daily transplantations of anterior pituitary lobe of the rabbit. B. Ovary of untreated, litter-mate control. (From Smith and Engle, *Am. J. Anat.*, 1927-28, xl, 188.)



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 (78).

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 (84).

In addition to the work just quoted, the experiments of Cushing and Teel (p. 374 and Fig. 63), and of Evans (p. 369), with impure growth preparations, afford additional proof of gonadotrophic action, while Collip's and Evans' later work with purer growth principle preparations indicates that the two principles are not identical.

The evidence associating the gonadotrophic principles with the basophile cells is strong, but not yet final. The chief clinical evidence available is that from studies of pituitary basophilism (p. 362). 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 gonadotrophic principle (25); similar changes occur in the rat (74). This is in line with the evidence just quoted that pituitaries from castrated rats and rabbits are richer than usual in gonadotrophic material (cf. 226).

Zondek, using implantation methods in mice (264), finds that the hormones 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.

Evidence has already been mentioned, which indicates that



the elaboration of gonadotrophic hormones is under nervous control, and that stimuli can be set up through the action of light (cf. p. 252), and some experimental work has been published which suggests that the paths of such stimuli are through the subthalamus by way of the accessory optic tracts (43).

Zondek postulated two gonadotrophic hormones, prolan A and B, both produced in the pituitary. The first is gametokinetic or gamogenic, stimulating male germ cells and ovarian follicles, and is now generally termed the follicle-stimulating hormone (FSH). The second stimulates the theca-cells of the ovaries to become theca-lutein cells, and can stimulate the interstitial cells of the testes. This is now usually termed the luteinizing hormone (LH).

The urine of women after the menopause or ovariectomy contains FSH only; the pituitary of long-castrated horses contains FSH, and at most but a trace of LH. Preparations from such sources stimulate ovarian follicles in female hypophysectomized rats, without luteinization; in immature rats, mice and monkeys, following stimulation of the follicles, there is a delayed luteinization due to the LH of their own pituitaries. Such FSH preparations also restore to normal the seminiferous tubules of the atrophied testes of hypophysectomized male rats (224, 52).

These pituitary gonadotrophic hormones must be carefully distinguished from the chorionic gonadotrophic hormone of woman (APL) which resembles LH in its action, and the corresponding hormone present in the blood of pregnant mares, whose action resembles that of a combination of FSH and LH (cf. Chapter VII, p. 289).

Additional evidence of the chemical difference between these has been recently furnished by Fraenkel-Conrat, Simpson and Evans (96), who have shown that cysteine completely inactivates the pituitary gonadotrophic hormones, but has no effect on the placental hormones of woman or mare.

Several other pituitary gonadotrophic hormones have been postulated. These include a hormone for the specific stimulation of ovulation, and a synergic factor postulated to account for an apparent enhancement of the action of APL by addition of low dosage of pituitary gonadotrophic preparations. Smith



considers the evidence for existence of these is inadequate (224). Various substances, including certain metallic ions, augment the effect of gonadotrophic preparations (cf. 259).

Evans (89) claimed to have separated a fraction which repaired the interstitial tissue of the hypophysectomized male and female rat, and which was not identical with FSH or with LH and which he in consequence termed the "interstitial cell-stimulating hormone," ICSH. This is now generally considered to be identical with the luteinizing hormone (cf. 243). Nor is there any conclusive evidence for the separate existence of a so-called "antagonist" or of an "activator," which have both been postulated. Thus Evans (90), reporting on the biological behaviour of purified FSH and ICSH as studied in normal and hypophysectomized immature female rats, states that (a) synergism (activation), (b) luteinization, and (c) antagonism can all be produced by the simultaneous injection of these two factors under certain specified conditions (cf. also 133). It therefore seems desirable to retain the customary terminology of the two pituitary gonadotrophic hormones, FSH and LH.

*Preparation and Properties.* Within recent years several methods have been published which the writers claim permit satisfactory separation of the two hormones.

Jensen, Simpson, Tolksdorf and Evans (134), starting with 40 per cent. ethyl alcohol as extractant (it extracts practically all the FSH, and 90 per cent. of the LH), precipitate the active material in the extract by increasing the alcoholic concentration to 85 per cent., extract the precipitate with dilute acetic acid at pH 4, and then adjust to pH 5 and add ammonium sulphate to half saturation. The LH is precipitated; FSH remains in solution. Further similar treatment effects at least a 90 per cent. separation, while the potency of the final products is more than 100 times that of the original pituitary powder.

Fevold (92) had previously published a somewhat similar procedure based on separation at pH 4.2 in presence of ammonium sulphate. Rinderknecht and Williams (205) have reported that earlier methods of Evans and of Fevold did not give in their hands a satisfactory separation, by that by modifying these (to a very complex procedure) they succeeded in obtaining a relatively pure FSH, free from LH.



The moderately pure preparations obtained by such methods have permitted more definite differentiation between the two hormones. Thus Fevold (93) finds that FSH is precipitated and inactivated by picrolonic, picric and flavianic acids (while LH is not inactivated); the activity of FSH is restored when the acids are removed from the precipitate.

Abramowitz and Hisaw (1) state that crystalline trypsin and chymotrypsin completely destroy FSH, LH (and APL), but that commercial papain (while completely destroying APL) only partially destroys FSH, and has no action on LH (cf. also 41A).

Evans, Fraenkel-Conrat, Simpson and Li (91) have sought to characterize the two pituitary hormones by their carbohydrate and glucosamine content. FSH is much richer in both than is LH (and also than any other pituitary hormone fractions tested). The maximum figures they found for FSH were 13.1 per cent. for carbohydrate and 8.1 per cent. for glucosamine.

**The Thyrotrophic Hormone.** *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 (242).

When the pituitary is extirpated in frog tadpoles development of the thyroid ceases (p. 368). 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 (244) or adult toads (129) provoke hyperfunction of their thyroids. The thyroid atrophies following hypophysectomy in young rats (50).

Attention was focussed on the possible existence of a thyrotrophic principle by the independent discovery by Loeb (149) and Aron (12) 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. Collip (52) gives a full bibliography.

Schockaert has carried out very accurate studies on the duck (209, 210). 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. 44).

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 (128).

The decrease in iodine content of the thyroid under thyro-



trophic stimulation is paralleled by decrease in thyroxine content, indicating a definite discharge of the thyroid hormone (95); furthermore, the active iodine fraction of the blood is increased (110, 46, 265). According to Loeser, the thyrotrophic hormone even controls the taking up of iodine by the thyroid (150). Administration of potassium iodide along with thyrotrophic hormone may prevent release of the thyroid hormone, but does not prevent production of cellular proliferation of the thyroid (8).

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. 374).

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 (64).

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 (94) 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. (64). (Cf. also (29).)

In acromegaly, on the other hand, the rates are either normal or high (29). Cushing and Davidoff (64) 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 (123).

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 hormone of the anterior pituitary controls the output of the thyroid hormone. 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 (165). It has been shown by a number of investigators (228, 244, 149) that acetic acid extracts of anterior pituitary contain the thyrotrophic 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 (100) who states that sodium iodide can temporarily inhibit the effect on the basal metabolic rate.)

Marine (165) 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 thyrotrophic 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 thyrotrophic concentrate (71). Hence the action is direct. This direct action is also shown by the fact that the thyrotrophic hormone stimulates thyroid transplants as rapidly as non-transplanted thyroid. The action must take place through the blood stream, and not through a nervous mechanism (164, 125, 140).

Administration of pituitary extracts containing the thyrotrophic hormone 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 (241, 212, 218).

*Preparation and Properties of the Thyrotrophic Principle.* Most of the experimental work with this hormone 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 (7). They commence with the filtrate and washings from the calcium phosphate precipitate formed in an alkaline extract of anterior pituitary during their preparation of the growth principle. 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 adrenotrophic hormone, but has no growth-promoting properties.



This product is readily soluble in water and dilute acids and alkali, but is insoluble in lipid 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. 104), while it increases the creatine output just as does the administration of thyroxine (181, 182), and depletes the liver of the guinea-pig of glycogen, another thyroid effect (72). It decreases the serum cholesterol content in rats and dogs (180). When the extract is administered to hypophysectomized rats, it prevents the atrophy of the thyroid which usually follows such operation (7).

All known thyroid effects are produced, so far as the material has been tested for them. Rowland and Parkes (206) have described a method of assay.

**The Lactogenic Hormone, Prolactin.** The existence of this hormone has been firmly established by Riddle and his colleagues, and by other groups of workers who have suggested other names, such as *galactin* (102, 159), and *mammotropin* (155).

*Evidence for the Existence of the Hormone.* 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 (202). Crude extracts of anterior pituitary produce copious lactation in virgin and in dry goats (79), in virgin heifers, and in normal bitches (154). That lactation depends on pituitary function is shown by the fact that when lactating rats are hypophysectomized lactation stops (50), 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 (122).

*Preparation and Properties of Prolactin.* Bates and Riddle (16) extract pituitary tissue with 60 to 70 per cent. aqueous ethyl alcohol. The alcohol content is raised in the extract, and the pH adjusted to 6.0. The gonadotrophic, thyrotrophic, and



lactogenic principles are all precipitated. Separation of prolactin from the others is effected by taking advantage of its insolubility between *pH* 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 gonadotrophic or thyrotrophic principle, and that it can be considered as approximately pure.

White, Catchpole and Long (257), by dissolving a prolactin concentrate in 13 per cent. acetic acid, and adding a little pyridine, and repeating the procedure many times with the cloudy precipitate which resulted, finally obtained crystals which were believed to be pure prolactin, apparently a protein. This work still lacks confirmation.

Ketene, which acetylates amino-groups, rapidly destroys the activity of prolactin, indicating that amino-groups are essential to its activity (147).

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, which perhaps sets up nervous stimuli leading to production of more prolactin. There is some evidence that the oestrogenic compounds depress the formation of prolactin during pregnancy (171).

Prolactin is without influence on the immature mammary gland, and is only effective following adequate oestrogenic stimulation (103, 6, 122). A fairly large amount of it seems to be secreted daily in the milk of lactating women (155).

The physiological actions of prolactin have been extensively studied by Riddle and his colleagues (203). They have found excellent evidence associating it with four (not unrelated) 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 (204).

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) (158).

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 calorogenic action; the mechanism is unknown (199).

The methods used for isolation of prolactin, when applied to other tissues and body fluids, indicate that a lactogenic substance is present in liver, placental extract, milk and urine (186). Such a substance has been detected in the urine of new-born babies, and may well be responsible for the production of "witches' milk" in the infantile breast (156).

Riddle summarized the chief results of work on prolactin (198, 201) 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 (142, 255, 234, 137A). 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" (80).

**The Adrenotrophic Hormone.**<sup>1</sup> *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 inter-relationship.

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 adrenotrophic hormone of the anterior pituitary is found in the work of Evans, Houssay, Anselmino and Collip. Evans (86) 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 gonadotrophic principle was not involved, but could not exclude the growth principle.

Houssay (127) 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

<sup>1</sup> This compound is concerned with the adrenal cortex and not with the medulla. Hence the term "adrenotrophic" is too inclusive, and "interrenotrophic" has been suggested instead. Since mammals do not possess an interrenal gland, this term is also open to criticism. "Adrenocorticotrophic" has also been used.



pituitary, thyroid and gonads, and after section of the splanchnic nerves.

Anselmino and Hoffmann (9) use as a biological test for the hormone 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 hormone can be separated by ultra-filtration (through 8 per cent. acetic acid collodion) from the gonadotrophic, thyrotrophic growth, lactogenic and other hormones. It is present in the acid ultra-filtrate, which only contains in addition the diabetogenic hormone. The substance is water soluble, but insoluble in lipid solvents. It is precipitated from aqueous solution by excess of alcohol or acetone. Its properties suggest a relatively small molecule.

Collip (52) 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 thyrotrophic extracts the adrenals were frequently restored to normal along with the thyroid, but as the purity of the thyrotrophic preparations increased the effect on the adrenals became less. Hence he tested extracts from the alcoholic mother liquors from which most of the thyrotrophic principle had been removed (cf. p. 387), and found that they had excellent adrenotrophic activity. From a 75 per cent. acetone soluble fraction he obtained on concentrating in the aqueous phase at *pH* 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 thyrotrophic 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, and Collip considers it as pure a preparation of any pituitary principle as he has been able to obtain (49).

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 adrenotrophic factor, and limited to



those cases in which destruction of adrenal cortical tissue is not complete (258).

**Other Suggested Anterior Pituitary Hormones.** Claims have been made that a *parathyrotrophic hormone* is produced (10), and various evidence appears to lend some support (cf., *e.g.*, 216, 118, 3), but no recent work supports the view that a special hormone of the pituitary specifically controls the parathyroids.

Collip has very recently shown that an 83 per cent. alcoholic extract of pituitary tissue, rapidly prepared and concentrated, and kept at low temperature till used, when fed orally to hypophysectomized rats had a trophic effect on the so-called "dark cells" of the adrenal medulla, which may relate to some function not associated with production of adrenine; he suggests that a "medullotrophic principle" of the anterior pituitary is responsible for the results observed (47).

This extract also produces mild hypoglycaemic effects in normal fasted monkeys and rabbits, and has proved beneficial in at least one case of human diabetes. It has no effect on carbohydrate metabolism in absence of the pancreas.

**The Pituitary-Adrenal-Pancreas Control of Metabolism.** Numerous clinical and experimental facts indicate that the pituitary exerts great control over carbohydrate and fat metabolism, though the extent to which this control is exerted directly by pituitary hormones, or indirectly by their control of other endocrine glands, is still not clear. Some of the recognized outstanding facts follow.

The acromegalic frequently exhibits a lowered carbohydrate tolerance and may show glycosuria (cf. p. 361). Some proportion exhibit a true diabetes mellitus, from which they may recover either spontaneously (54), or after removal of a pituitary tumour (73).

Antagonistic action between the pituitary and the islets of Langerhans seems to exist in most vertebrates. It has been demonstrated for the dog and toad (126, 105), cat (151), fish (178), various batrachians and a snake (128). Houssay first showed that when the anterior pituitary is removed in the toad subsequent pancreatectomy does not produce diabetes, but pituitary implants can then produce it. On the other hand, the hypophysectomized dog is much more sensitive to insulin than a normal dog.



In the dog, after removal of the whole pituitary, pancreatectomy only causes a mild diabetes, and the animal can survive for a long time. Such a dog, minus pituitary and pancreas, and now usually termed the "Houssay dog," though not completely free from the symptoms which follow pancreatectomy, may live for many months without specific treatment, but finally becomes more and more undernourished, extremely cachectic, and dies. This Houssay dog shows an exaggerated hyperglycaemic response to a meal, and may exhibit glycosuria, with, however, but little ketonuria. It is very sensitive both to insulin and to certain "diabetogenic" pituitary extracts. It can be balanced by careful combined treatment with insulin and suitable pituitary extracts, and then resembles the pancreatectomized dog balanced with insulin, though with a higher respiratory quotient. It can store liver glycogen. (Cf. 52, 22, 221.)<sup>1</sup>

Long (152, 153) has shown that the adrenalectomized, depancreatized cat and dog, if just maintained with adrenal cortical extracts, presents a somewhat similar picture, suggesting that at least part of the pituitary effect is mediated through the adrenals. In further work on rats he has strengthened the evidence that pituitary participation in carbohydrate metabolism needs the presence of functioning adrenal cortical tissue, though the pituitary hormones may not necessarily act through the adrenal cortex in this connection (cf. 83, 243).

The hypophysectomized animal is very susceptible to prolonged fasting, which may lead to a fatal hypoglycaemia, though the fed animal has a normal blood sugar. In human hypopituitary states hypoglycaemia may be present (52).

After hypophysectomy there is increased oxidation of carbohydrate, and diminished formation of glucose from amino-acids (the latter possibly being traceable to the induced hypothyroidism) (243).

Alkaline extracts of anterior pituitary are diabetogenic. Such extracts, when injected along with insulin, lessen its effect. Similar extracts can be prepared from urine, especially diabetic urine (52, 167), though their identity with a pituitary factor is not proved (cf. 254).

<sup>1</sup> An apparent case of the Houssay phenomenon in man has been reported (139A).



It has long been known that alkaline extracts of the pituitary when injected into rats induce ketonuria (Burn and Ling, 1928) and ketonaemia (Hoffmann and Anselmino, 1931), and a special hormone has been postulated, variously termed "fat metabolism hormone," "orophysin," and the "ketogenic hormone" (52). Extracts supposedly rich in this hormone have been prepared (11, 52). Claims have also been made for "lipoitrin," supposed to increase blood lipides, and a "pancreatrophic hormone," supposed to control the islets of Langerhans (cf. 52). There is no convincing evidence for these.

Young has published important results within the past two or three years. He has shown that if crude saline extracts of fresh pituitary tissue are injected daily into dogs, cats, or rabbits, on the third or fourth day the animals exhibit hyperglycaemia, polyuria, glycosuria, and ketonuria. These subside and disappear within two or three days, but if the amount injected is increased every third day a continuous diabetes results, and, when a sufficiently severe state has been produced, it will persist in dogs even after cessation of the injections. Such animals do not develop excessive loss of weight, and may survive for several months without insulin treatment. A high carbohydrate diet, however, causes rapid loss of weight. The islets of Langerhans are definitely affected, the degenerative changes indicating exhaustion and loss of function (262, 166).

Young's work has been adequately confirmed (38, 68).

The type of action produced by Young's crude extracts is not produced by purified or heat-treated extracts (124), indicating that the purification and heat have destroyed the hormone responsible for them.

The somewhat confused mass of data, of which only part has been recorded above, does not as yet permit a complete and simple interpretation. It must be borne in mind, however, that O'Donovan and Collip's metabolism factor of the pars intermedia depresses oxidation of carbohydrate, and increases combustion of fat, producing ketonaemia, decreasing total body fat, increasing liver fat, maintaining liver and muscle glycogen, and increasing resistance to insulin (cf. p. 341), so that this single hormone is at once ketogenic, glycostatic, and glycotrophic.

Thomson and Collip in a recent review (243) point out that



pituitary extracts might produce hyperglycaemia and glycosuria in three ways, (i) by depression of islet tissue (to an extent that may become irreversible), (ii) by stimulation of the adrenal cortex, and (iii) by inhibition of carbohydrate metabolism.

The third method is a function of the metabolism factor, and is probably exerted directly on the liver. The second procedure is produced by the adrenotrophic factor. The first procedure may be the result of an exhaustion from continuous hyperglycaemic stimulation produced by the metabolism factor (with possible aid from the adrenotrophic and thyrotrophic factors), or may be due to a specific hormone of the pituitary which directly stimulates the islets of Langerhans. But there is as yet no convincing evidence for supposing the existence of such a hormone.

Thus the control of metabolism by the pituitary may well be traceable solely to the three recognized hormones, the thyrotrophic, adrenotrophic, and especially the metabolism hormone of O'Donovan and Collip.

### The Actual Number of Pituitary Hormones

As indicating the confusion at present existing, allied perhaps with an undue optimism, a recent writer stated that the anterior pituitary probably develops thirteen hormones, while the posterior (or more probably the intermediate) lobe develops nine (256).

It is interesting to record the opinions of a few more critical writers. Jensen and Tolksdorf (132) in a recent review pointed out that different investigators have seriously postulated the following hormones of the anterior pituitary: (A) Gonadotrophic hormones, (1) follicle stimulating, (2) luteinizing, (3) interstitial cell stimulating, (4) the antagonist, (5) the synergist, (6) the activator; (B) the thyrotrophic hormone; (C) the adrenotrophic hormone; (D) the lactogenic hormone; (E) the growth hormone; (F) metabolic hormones, (1) diabotogenic, (2) ketogenic, (3) pancreatrophic, (4) glycotrophic, and (5) glycostatic. It will be seen that in all some fifteen hormones are here suggested. Jensen and Tolksdorf, examining the available evidence, conclude that at present there is only reasonable support for the existence of the following: (a) the



follicle-stimulating hormone, (b) the interstitial-cell-stimulating hormone (which, they think, is identical with the luteinizing and thyrotrophic hormones), (c) prolactin, and (d) the adrenotrophic hormone (which, they think, produces the glycotrophic effect). Thus they reduce the total to four.

Riddle was one of the first to challenge the gradually increasing list of postulates, and insists that the growth effects of pituitary extracts are due to the combined effects of prolactin and one or more of the other factors (200).

Collip (48), writing in 1936, stated, "My considered opinion is that the normal living gland (anterior lobe) produces probably not more than three hormones. I believe, for example, that one prosthetic group of a single protein molecule represents the growth hormone activity, another prosthetic group the adrenotrophic effect, and still another prolactin or the mammary secretagogue action. . . . I am in agreement with Evans that extracts can be so processed that it can be shown that the prolactin effect is due to a different substance than that responsible for the growth effect, but . . . I believe that each of these effects, together with the adrenotrophic effect, is due to a different constituent of a protein molecule, itself a single substance in the living gland."

Diseased states at present afford the best clue to the association of individual hormones with acidophile or basophile cells. The acidophile tumours of acromegaly (with its occasional hyperthyroidism) associate the growth and thyrotrophic hormones with acidophile cells. The basophile tumours and hyaline changes of basophile cells in Cushing's disease associate these cells with the adrenotrophic hormone (though Rasmussen (190) considers that they are degenerating rather than actively secreting), while other evidence, already quoted (cf. p. 380), also associates the gonadotrophic hormones, or at least the follicle-stimulating hormone with these cells.

At present it seems possible, conservatively, to make the following statements.

The posterior pituitary lobe produces, in its pituicyte cells, two hormones, oxytocin and vasopressin.

The pars intermedia produces a melanophore-dispersion hormone, probably identical with the "intermedin" which affects fishes, and possibly identical with O'Donovan and



Collip's metabolism factor. In animals possessing no pars intermedia its function devolves on the anterior lobe.

The anterior lobe produces (1) and (2), two gonadotrophic hormones, follicle-stimulating and luteinizing, one (cf. 190) or both of which are produced in the basophile cells, (3) a thyrotrophic hormone, probably produced by the acidophile cells, (4) an adrenotrophic hormone, probably produced by the basophile cells, and (5) a lactogenic hormone. Further, the growth effects produced by pituitary extracts are probably due to a specific hormone, but the final proof for this is still wanting. In any case these growth effects are associated with the activities of the acidophile cells.

It is by no means certain, however, that the five or six hormones so listed actually are produced as such by the two types of cells in the anterior lobe. Collip's surmise seems very possible, and the functioning cells may in reality only produce two or three hormones, combining the above effects, those present in concentrated extracts and exhibiting separate activities being only artefacts produced during their preparation.

### Clinical Use of Anterior Pituitary Preparations

Clinical trials of the concentrated extracts now available have given some good results (cf. pp. 348, 350, 352, 391, 393).

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 (184, 144, 146, 85). (Cf., however, p. 393.)

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## CHAPTER IX

### SOME ACTUAL AND PRESUMPTIVE HORMONES

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#### Introduction

THE literature contains a large number of suggestions that certain phenomena indicate the existence of new endocrine compounds. There is no appearance of any falling off in the number of such suggestions through the development of more critical tendencies.

Of all the hormones 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 Hormones 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 1928, when J. Mellanby made active preparations by extracting pig's duodenal mucosa with absolute alcohol, and subsequent fractionation with acetone and precipitation with acetic acid. Bile salts were initially added to the alcoholic extract, secretin being precipitated with the bile acids on acidification. Subsequently use of bile salts was omitted. By his final method Mellanby obtained from 1 kg. of fresh tissue 20 mg. of a white amorphous powder, slightly soluble in water, soluble in dilute alkali and aqueous alcohol, and insoluble in lipide solvents. It appeared to be a polypeptide,



containing sulphur but no phosphorus, it did not dialyse through collodion, and its activity was rapidly destroyed by tryptic digestion (31).

Ågren and Wilander in 1933 obtained a similar preparation which was active when injected into cats in dosage of 0.005 mg. per kg. (2); this was then obtained in crystalline form, and found to be a polypeptide with a molecular weight of about 5,000 and one atom of sulphur to the molecule (17).

Greengard and Ivy (15) have isolated secretin as a crystalline picrolonate, which can be recrystallized without loss of activity. They consider that it has a simpler molecule than earlier work suggested. One hundred pieces of the first 6 feet of hog's intestine yielded 0.5 gm. of the picrolonate, which by acid hydrolysis gave 0.08 gm. of free secretin (of which 0.014 mg. is equivalent to one Ivy "dog-unit"). Their free secretin gives negative ninhydrin, Hopkins-Cole, and Millon's tests, and a doubtful biuret reaction. It is very weakly basic, and appears to contain no free amino or carboxy groups. It is completely inactivated by treatment with potassium permanganate.

The discrepancy between the results by these different schools of investigators obviously needs clarification.

Scott and Still (37) 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 (32) has reviewed the early work on gastrin. Ivy (21), 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 his results at first suggested that gastrin might be histamine (34). Babkin has recently reviewed the experimental evidence concerning the chemical phase of gastric digestion (3). His pupil MacIntosh (29) can find no evidence that the histamine content of blood is increased during digestion (while it is less than the histamine content of the gastric juice), and discusses the possibility that histamine merely mediates the secretory action of the vagus on the parietal cells of the gastric mucosa. Komarov (25) claims to have extracted from the pyloric mucosa a protein-like substance with secretagogue effect on the fundic glands of the stomach; this material is free from histamine. It is present in lesser amount in the duodenal mucosa, and its presence is believed to support the "gastrin" theory. Babkin believes that histamine plays a part in the first or nervous phase of gastric secretion, while "gastrin" probably plays a rôle in the second phase (3).

Babkin has shown that histamine stimulates the parietal cells of the gastric glands (and can inhibit the activity of the peptic cells in appropriate concentration). Its diagnostic clinical use is limited to determining the ability of the stomach to produce hydrochloric acid. Babkin has shown further that subcutaneous or intravenous injection of insulin provokes a copious secretion of gastric juice in animals and man, only a little less acid than that produced by histamine, but rich in pepsin and mucus. Insulin administration therefore stimulates both parietal and peptic cells, and does this presumably through production of a hypoglycaemia which stimulates appropriate brain centres to cause the needed vagal stimuli (3.)

**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 (23) 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 (38). (Cf. also 40, 20.) Ågren has prepared a highly active extract, free from secretin (1).

**Enterogastrone.** An extract has been prepared from the upper intestinal mucosa, which inhibits gastric motility and secretion, and is believed by Ivy to contain a specific hormone which he terms enterogastrone. It is considered to be liberated from the intestinal



mucosa when neutral fat and glucose come into contact with the mucosa. Urine also appears to contain this substance (14, 22).

**The Insulinotrophic Principle of the Duodenum.** Heller (18) 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 (39). Laughton and Macallum prepared an extract from the duodenal mucosa freed from protein and peptone, and still showing the activity described by Heller (28). 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 pH 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 (8).

La Barre and his colleagues (27) 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.

**Villikin** is said to be an endocrine excitant for the intestinal villi (24).

*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 (cf. 4A). 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 (7) 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 Hormones

**Haberlandt's "Heart-hormone."** Haberlandt has published numerous papers (16), in which he claims that a specific "heart-hormone" exists, which will stimulate the non-beating (frog's) heart to movement. Oppenheimer (33) 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 Kisthinos made an acidified-alcoholic extract and termed it *angioxyl* (6, 13). Kraut and Frey's extract is termed by them *kallikrein* (26), while Santenise has termed his preparation *vagotonine* (36, 12); the term *padutin* is also used for this substance. *Carotidin*, from the carotid gland, may be similar (5). According to Bischoff and Elliott (4) *kallikrein* is of colloidal protein nature and somewhat unstable. They consider that its physiological significance is questionable.

**Renin.** Vasopressor extracts of the cortex of the kidney were first prepared by Tigerstedt and Bergmann in 1898, and the active constituent termed by them *renin*. Some evidence has been adduced that the ischaemic kidney secretes this compound directly into the circulation, to produce a permanent hypertension, independent of adrenal action. The literature has been reviewed by Fasciolo, Houssay and Taquini (10), and by Helmer and Page (19). The



latter have obtained a very active preparation from pig kidney cortex. After initial acetone extraction, the residue is treated with 2 per cent. sodium chloride solution, the renin dissolving. After further treatment they have obtained material which, injected in dosage of 0.027 mg. nitrogen (of the substance) per kg., raises the blood pressure of dogs 30 mm. (mercury), and is three times as active in cats. It contains guanidine and pentose groups, adrenaline is absent, and the pressor effect is not abolished by ergotamine, as is that of adrenaline.

It is as yet doubtful whether this substance should be regarded as a hormone. Munoz (31A) has put forward evidence that it is to be regarded as an enzyme. He finds that blood from an animal whose blood pressure has been increased by compression of the renal artery, or injection of venous blood from the kidney, contains a pressor substance "hypertensin," which is not precipitated by addition of three volumes of acetone to this blood serum, is insoluble in ether but soluble in glacial acetic acid, and is only destroyed by boiling for three hours in normal hydrochloric acid. The same substance is formed by incubating renin with blood serum or its pseudo-globulin fraction for fifteen minutes at 37° C., and differs from adrenaline, tyramine, and pitressin. He thinks that renin is a proteolytic enzyme of papain type, which liberates hypertensin from a blood plasma protein belonging to the pseudo-globulin fraction.

**A Liver Detoxicant.** Claims have been made for a specific detoxicant in the liver, *yakriton* (37).

**"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

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#### Introduction

CUSHING has written (11) "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 usually wasteful, unnecessary, and unscientific.

### Pituitary Interrelationships

These are of outstanding importance. While Cushing's dictum (11) 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 *thyrotrophic 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 (44). 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 hormone. Marine considers that the thyroid hormone affects the pituitary as strikingly as the thyrotrophic hormone affects the thyroid (31).

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 (5).

It is very doubtful if observations of this nature are sufficient to justify certain differentiations such as Engelbach (14), for example, has suggested: "Interhormonic action exists between the thyroid and pituitary glands resulting in the clinical entities of their combined disorders, thyropituitarism and pituitarothyroidism."

With our present knowledge, it seems safest to regard all cases in which both thyroid and pituitary are involved as due primarily to pituitary insufficiency, and as requiring pituitary-replacement therapy, which must be by injection of potent extracts of the correct hormone or hormones 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 Chapters VII and VIII. It will be



recalled that the gonadotrophic principles stimulate the ovaries to maturation and formation of corpora lutea, and the concomitant production of oestradiol, and, in the male, stimulate 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.

**The Anterior Pituitary and the Adrenal Cortex.** There is definite evidence that the adrenal cortex is directly under the control of the adrenotrophic 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 Islets of Langerhans.** The relationship has been discussed in Chapter VIII (cf. p. 393).

### 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 indicated 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 Islets of Langerhans.** Potential interrelationships have been discussed in Chapters V and VIII.

**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. 85), because the diminution of response to various stimuli necessitates the use of less oxygen, and the heart muscle has a greater opportunity to recover (30).

### **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 hormone 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 (23), 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 (3) 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 simultaneous occurrence of spontaneous myxoedema and diabetes mellitus is very rare. Carey, Arey and Norris (6) list nineteen authentic cases.

**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 (37). Menstrual disturbances are frequent accompaniments of thyroid disorders in women; an uncontrolled rhythm is often recorded and seems especially characteristic (34). Administration of thyroid is often beneficial in such cases with a low basal metabolic rate but no specific hypothyroid symptoms (19).

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, 25).

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 hormone (cf. p. 9). Many of the other phenomena can be regarded as incidental developments following changes in the degree of the thyroid control of general oxidative processes throughout the organism.

### 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 (18). Definite lesions of the parathyroids are produced (22).



### 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 nicotinic acid is involved in the former condition (33).

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 (39).

### 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 (46) and by Joslin and Lahey (24).

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. 416). 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. Foster and Lowrie report on the study of 42 such cases in a recent paper (16).



In rare instances hypothyroidism and diabetes mellitus are associated (46, 34).

Rowe and Lawrence (35) 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, interrelationship cannot be considered as excluded, their results illustrate the great rarity of true pluriglandular conditions.

Rowe has summed up the matter still more recently (34): "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."

### Antihormones

*A Presumptive Anti-thyroid Compound.* Early therapeutic treatment of hyperthyroid conditions included use of the serum of thyroidectomized animals. Such preparations as "anti-thyreoidin Möbius" had a long vogue, but ultimately more critical appraisal led to disuse (cf. 15). More recently Blum prepared a concentrate "Katechin" from dried blood, believed to contain an anti-thyroid compound, and sold under the name of "Tyronorman." Good results have been claimed for it in treatment of Graves' disease (20, 4), though its value is doubtful (48).

*An Anti-thyrotrophic Compound.* Soon after the discovery that certain pituitary extracts possess thyrotrophic activity, it was observed that chronic treatment with such extracts rapidly ceases to affect the thyroid (cf., *e.g.*, 27, 28, 43) suggesting that some protective action gradually set up an immunity (cf. 17, 41). Detailed studies of the phenomenon were made by Collip and Anderson (9), who showed that chronic treatment of normal or hypophysectomized rats or guinea-pigs with a purified thyro-



trophic preparation produced an initial rise in basal metabolism, which after two or three weeks fell to normal, and then to levels much below normal; further treatment, even with larger doses, then produced no apparent effect, though the thyroids of such animals were still hyperplastic, while the animals themselves still responded to thyroxine.

Chronic injection of a mare with an active thyrotrophic preparation yielded a serum with anti-thyrotrophic potency, which definitely inhibited the action of the thyrotrophic preparation on normal or hypophysectomized rats.

*Anti-gonadotrophic Compounds.* Collip and his co-workers showed that chronic overdosage with the follicle-stimulating hormone of the pituitary, or with A-P-L of pregnancy-urine similarly led to production of anti-compounds (36, 7).

*Present Views on the Nature of the Antihormones.* Numerous investigators suggested that the anti-reactions set up were really of immunological type (cf., e.g., 13, 29, 38, 42). In most of the experiments such a possibility obviously exists, since protein-preparations from animals of one species produced the effect following injection into animals of a different species.

Marrian and Butler (32), reviewing the subject in 1937, stated that in their opinion the direct and positive evidence bearing on the potential physiological significance of anti-hormones could be summarized as follows:

Supporting the view that antihormone formation is a normal physiological function, resulting in a delicate balance between a hormone and its antihormone (as suggested originally by Collip) are two cardinal observations, (a) rats develop immunity to the gonadotrophic effect of rat pituitary implants (7), and (b) an immune serum may be developed in sheep by the injection of sheep pituitary gonadotrophic preparations (8).

Supporting the view that antihormones are antibodies formed in response to administration of foreign protein are also two cardinal observations, (a) the power of beef pituitary thyrotrophic extracts to induce antihormone formation in the guinea-pig depends on the method of their preparation (45), and (b) inactivated human pregnancy-urine gonadotrophic preparation (A-P-L) is as effective in inducing antihormone formation as are active preparations (42).

Marrian and Butler concluded that the evidence indicated



that the antihormone formation to pituitary gonadotrophic stimulation was a physiological response, while the anti-thyrotrophic hormone and the anti-compound to A-P-L were merely antigens.

Since they wrote, the evidence in favour of the antibody view seems to have become stronger. For example, Spence, Scowen and Rowlands (40) could demonstrate no antihormone formation in blood serum of a patient given pituitary gonadotrophic preparations for several months. Thompson treated two ewes with an alkaline extract of whole sheep pituitary daily for six months, and could discover no anti-gonadotrophic substance in their sera (47 ; cf. 26). Katzman (cf. 47), in confirmation of earlier work of Smith in 1930, could find no evidence that rats develop an immunity to the gonadotrophic effect of rat-pituitary implants.

Yet Anderson and Evans (1) found that injection of rat pituitary extract into rats led to production of an anti-thyrotrophic substance in their serum, though Cutting (12) found that the freshness of the glands determined whether or not an anti-effect developed.

It must not be forgotten, in considering this problem, that it is quite likely that the majority of pituitary preparations used in all experimental work at present are artefacts (cf. p. 398), containing proteins which are potent as hormones, but altered from those actually formed by the pituitary, and therefore through this alteration potentially foreign proteins capable of eliciting antigen formation. It must also be remembered, even if antihormones are merely antibodies whose formation is due to foreign proteins, that their effects must still be considered in interpretation of all experimental work in which their formation is possible.

A review of the subject by Collip, Thomson, and Selye, which has just appeared (10), concludes with the statement that it is not yet possible to say whether these antihormones are or are not antibodies.

### 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 hormones 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) lactation and maternal behaviour; (vi) general growth of all tissues; (vii) fat metabolism; and (viii) 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.

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 (21) has presented a conservative statement of possibilities in this direction.

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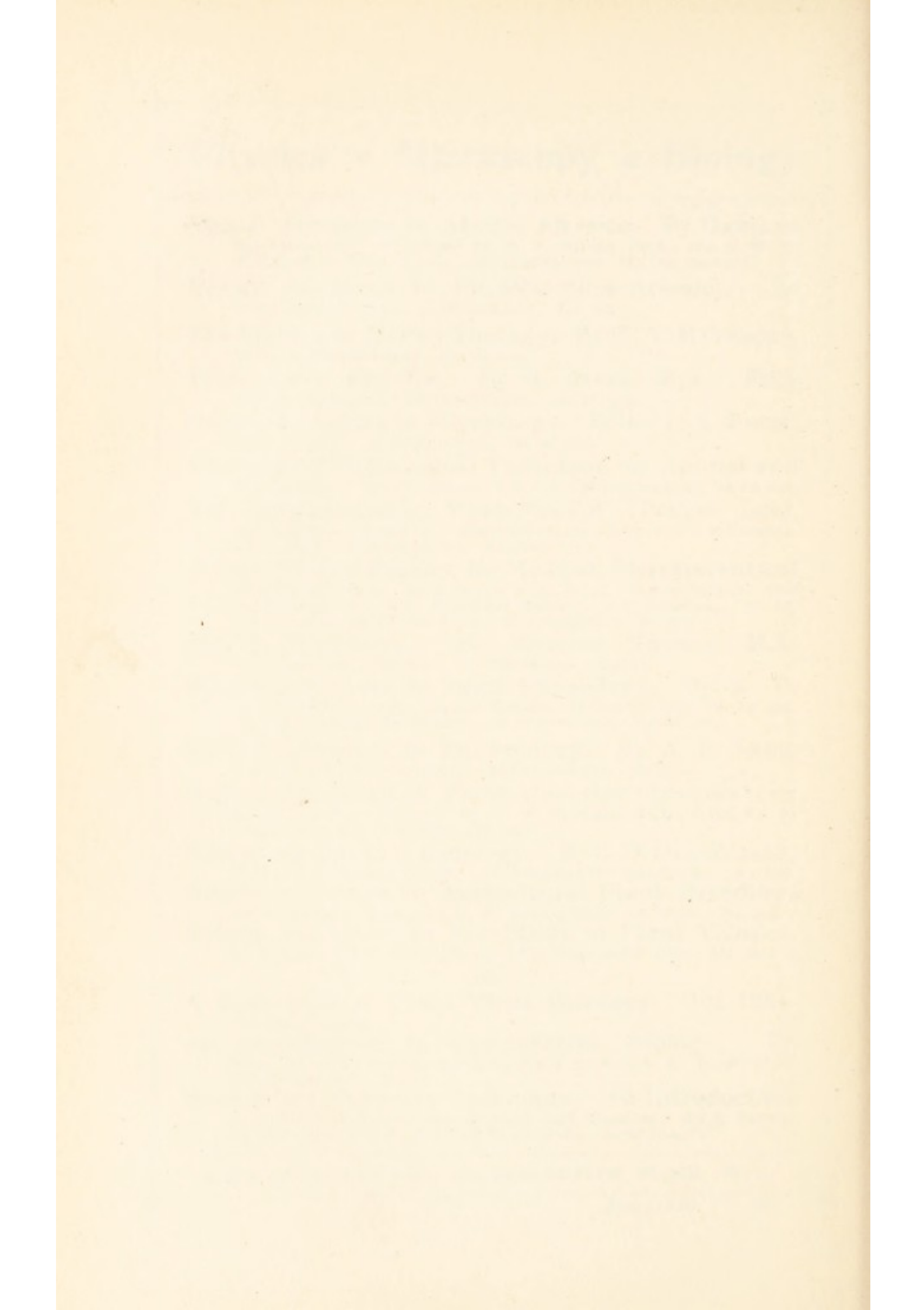
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