RECENT ADVANCES
in
ENDOCRINOLOGY
(Cameron)

SEVENTH EDITION

By

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With 34 Illustrations

PREFACE TO THE SEVENTH EDITION

AFTER the death of Professor A. T. Cameron, the original author of this book, which he made so famous, I was invited by the publishers to write the present edition. I am fully aware of the responsibility which this entails, and the rather long interval of seven years since the appearance of the last edition is to some extent due to my diffidence in setting pen to paper to deal with a subject which is advancing almost more rapidly than I can write. Indeed, I am aware that some of the chapters which I wrote first, such as the one on the adrenal cortex, are to some extent out of date as the book becomes due for publication.

For various reasons I have decided to re-write the book completely. Professor Cameron was a Canadian biochemist and I am a British clinician, so I must inevitably view the subject of endocrinology from a rather different angle from the original author. Furthermore, I have decided not to attempt to write a comprehensive account of the whole of endocrinology. When Professor Cameron first wrote this book in 1933 there were practically no text-books of the subject written in the English language, and he performed a singularly valuable service and a prodigious task in covering the whole ground. To-day there are so many text-books of endocrinology that it is quite an accomplishment to have read even half of them. I have therefore chosen to deal with only certain aspects of endocrinology, and because I have completely omitted other aspects it does not mean that they have not been advancing recently. Moreover, I have treated the parts of the subject which I have chosen, purposely, in a rather different manner. In some chapters I have tried to present a detailed review, in others, such as the one which deals with clinical use of œstrogens, I have given an undocumented account based largely on my own personal experience.

In the chapter on the adrenal cortex, in addition to giving an account of the clinical effects of cortisone and ACTH, I have thought it necessary to deal rather fully with recent studies in the physiology and biochemistry of the gland, and I am grateful to Professor G. F. Marrian, F.R.S., for reading this portion of the chapter and making helpful suggestions.

I have introduced a chapter describing recent work on various aspects of carbohydrate metabolism, but for this purpose I have

made use chiefly of reviews by recognized experts, rather than providing a fully documented account of original work and experiments. The control of carbohydrate metabolism by endocrine factors provides an excellent illustration of the inseparable problems of endocrinology and metabolism.

My interest in Cushing's syndrome dates back to 1932, and for some years now at Guy's Hospital we have dealt as a team with cases of this condition. This team has consisted of a surgeon, Mr. F. N. Glover, M.S., F.R.C.S., a medical registrar, Dr. M. G. Thorne, M.D., M.R.C.P., my registrar, Dr. R. R. de Mowbray, D.M., M.R.C.P., and myself. I have, with his consent, freely used Dr. Thorne's M.D. thesis in compiling the chapter on this subject, and have consulted Mr. Glover concerning the details of the surgical technique employed by the Guy's team.

It has seemed to me that considerable advances have recently been made in our knowledge of the endocrine aspects of disorders of sex. This I believe is due largely, on the one hand, to the influence of such endocrinologist-pædiatricians as Lawson Wilkins and Nathan Talbot, who have given excellent and detailed accounts of such exceedingly rare conditions as pseudohermaphroditism and precocious puberty. In this country, Dr. Hugh Jolly, M.D., M.R.C.P., has conducted the most painstaking and comprehensive study of sexual precocity, and I am grateful to him for allowing me to make full use of his M.D. thesis, shortly to be published in book form, on this subject. On the other hand, the intensified interest in problems of male infertility, largely stimulated by such men as John Macleod and, following the introduction of the technique of testicular biopsy, of Warren Nelson, Robert Hotchkiss, Perry McCullagh and the Boston team headed by Fuller Albright, and containing Fred Simmons, has considerably clarified the understanding of the different types of testicular deficiency.

The distinction between virilism and simple hirsutism has always been difficult but much has recently been added to our knowledge of the causes of the extremely rare condition of virilism and it seemed timely to review this subject in some detail.

The subject of hormones and cancer is at the moment very topical, in view of the heroic methods of total adrenalectomy and hypophysectomy which the surgeons have recently added to their therapeutic arsenal. Standing as we do, at the present moment, in the midst of these exciting developments, the chapter on this subject may have a rather conservative flavour. This chapter is

based on a clinical lecture given by me at Guy's Hospital and subsequently published in the *Guy's Hospital Gazette*, to the Editor of which I am grateful for permission to make use of the article. I am also grateful to Sir Charles Dodds for reading the manuscript of this chapter.

The final chapter represents an attempt to summarize the present position with regard to the management of thyrotoxicosis. Enough evidence has now accumulated concerning the relative efficacy of surgery, anti-thyroid drug treatment and radio-active iodine therapy to make it possible to evaluate the problem in some detail.

The Clinical Endocrinology Committee of the Medical Research Council has embarked on the policy of suggesting standard methods of hormone estimation. So far they have dealt only with 17-ketosteroid hormone estimations, and the method advised by them is reprinted in Appendix I.

Finally, I am grateful for the cordial permaission which various authors have given me to reproduce illustrations from their papers or books. Dr. R. R. de Mowbray, recently my registrar, has helped me considerably in the early stages of the proof-reading, and Dr. Peter Hall, M.R.C.P., my present registrar, has not only read the page proofs with me, but is responsible for the arduous task of checking the references and compiling the index, for which I express my gratitude. My task has been facilitated throughout by the friendly and sympathetic attitude of my publishers.

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LONDON, W.1.

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CHAPTER I

THE ADRENAL CORTEX

Histology

THE adrenal cortex is divided into three main zones, the outer or zona glomerulosa, the middle or zona fasciculata, and the inner, or zona reticularis. Two other zones have been described, a transition zone which in certain circumstances lies between the zona glomerulosa and zona fasciculata, and an " X," or androgenic zone which lies between or replaces the zona reticularis and the medulla.

Two principal theories exist concerning the significance of these zones. The first (Ingle, D. I., 1950; Greep and Deane, 1949) suggests that the zona glomerulosa consists of immature cells which gradually mature and, as they do so, sink deeper into the cortex, so that the cells of the outer part of the zona fasciculata contain the precursors of the steroid hormones secreted by the cortex, probably in the form of cholesterol. The hormones are synthesized in the inner fasciculate region and discharged from the reticular zone. According to this hypothesis the changing histological picture merely represents the stages of maturation and degeneration of the cells which accompany the elaboration and discharge of " the " hormone of the cortex. Radioactive studies with labelled adrenocorticotrophic hormone (ACTH) however show that the zona reticularis is the site of main activity, the most active area being a transitional zone between the fasciculata and reticularis. The labelled ACTH is localized in the zona reticularis.

According to the second hypothesis, each zone is responsible for individual functions of the gland. Thus the zona glomerulosa is concerned with elaboration of the factor or factors which control electrolyte metabolism and are represented by those compounds, such as the synthetic substance deoxycorticosterone (DOC), which lack a hydroxyl or oxygen group at the 11-carbon atom. The fasciculate and reticular zones are, according to this theory, concerned with the synthesis, probably from cholesterol, of the 11-oxygenated corticosteroids, such as cortisone and hydrocortisone (17-hydroxycorticosterone or compound F of Kendall), and their subsequent secretion. The X-zone when it exists, and

otherwise the inner layers of the zona reticularis, is devoted to the elaboration of adrenal androgens.

The study of the functional activity of the adrenal cortex is complicated, from a histochemical point of view, by the lack of storage of biologically active hormones in the gland. The gland secretes its hormones continuously, and if endogenous synthesis were to cease, whereas secretion were allowed to continue at the normal rate, it is calculated that the cortex would be deprived of all its hormones within six to twelve seconds. This necessity for constant synthesis accounts for the large blood flow through the cortex and its high oxygen consumption.

Histochemical Studies. The impossibility of identifying the actual hormones *in situ* makes it difficult to determine which of the two theories concerning the histological structure of the cortex is most likely to be true. Probably both of them are gross oversimplifications of the actual facts. It is to some extent possible to identify some of the hormone precursors in the gland. Both ascorbic acid and cholesterol are stored in the cortex in considerable concentration in certain circumstances. Various histochemical methods are employed to identify the lipoid substances in the cortical cells. The Schultz reaction identifies chromogenic steroids. Sudanophilic and Schultz-positive cells almost certainly contain cholesterol. These are probably also Schiff-positive and birefringent. In the normal cortex most of the lipoid material is found in the outer fasciculata and zona glomerulosa. The inner layers of the zona fasciculata and the zona reticularis contain considerably less sudanophilic material. The lipoid droplets in the zona glomerulosa and the outer fasciculata are also found to contain ketosteroids, whereas in the deeper layer of the fasciculata and in the reticularis triglycerides are found. Administration of ACTH leads to reduction of lipoid in all three zones with narrowing of the glomerulosa and hypertrophy of the fasciculata and reticularis. This suggests increase of synthesis and secretory activity. Cortisone, which inhibits the activity of the cortex, on administration leads to atrophy of the two inner layers, whereas the zona glomerulosa hypertrophies. Administration of deoxycorticosterone acetate (DCA) suppresses the secretory activity of the zona glomerulosa. Following hypophysectomy, in the rat and the dog, the zona glomerulosa remains normal in size or even hypertrophies.

These histochemical studies therefore suggest (1) that the zona glomerulosa fulfils a different function from the rest of the cortex,

and (2) that steroid hormones, such as the 11-oxycorticosteroids, are synthesized from cholesterol in the outer layer of the zona fasciculata and are eventually secreted by the inner layer of the fasciculata and by the zona reticularis.

The significance of the transition zone between the glomerulosa and fasciculata is still left in some doubt. It is a sudanophobic zone, and therefore presumably does not contain lipoid material. It is suggested that it is capable of proliferating in either direction, and both the glomerulosa and fasciculata show relatively high degrees of mitosis, though mitoses are not absent from the reticularis. The transition zone is present only when there is a low level of cortical activity and when the gland is diminished in size. This zone, for instance, is absent in intact female and castrate male rats and in all œstrogen-treated animals of this species, and present in spayed female and in immature rats and in all androgen-treated animals whether male or female (Greep and Jones, 1950). These observations support the belief that œstrogens stimulate the secretion of ACTH and androgens inhibit it. The X-zone is found only in fœtal adrenals and disappears rapidly after birth. It is probable however that the post-natal zona reticularis is derived from cells of the X-zone which fail to undergo involution. It has been suggested that the X-zone and the zona reticularis are concerned with the elaboration of adrenocortical sex hormones, especially androgens. Enlargement of the reticular zone has been observed in cases of adrenal virilism. On the other hand, histochemical studies have failed to identify much sudanophilic material (containing ketosteroids) at any time in the zona reticularis and, as has been observed above, the lipoid material of this area is mainly in the form of triglycerides.

Cholesterol as a Source of Adrenocortical Steroids

It has been assumed so far that the cortical hormones are derived from lipoid precursors identified in the cells of the zona fasciculata and probably consisting mainly of cholesterol. Other evidence seems to support the belief that cholesterol and its esters may take part in the formation of cortical hormones. Administration of ACTH to normal individuals leads to a sharp decrease in the total serum cholesterol, 85 per cent. of this decrease being due to disappearance of the esterified cholesterol fraction, whereas free cholesterol remains relatively unchanged. Cessation of ACTH administration results in a return of the total serum cholesterol to its base line. Patients with Addison's disease

show no significant alteration in the serum cholesterol level. These observations suggest that ACTH depletes the cholesterol store of the adrenal cortex in the process of forcing the production of large amounts of cortical steroids, and that cholesterol, and especially cholesterol esters, are removed from the circulating blood to restore these depleted reserves (Conn, Vogel, Louis and Fajans, 1950). The perfusion studies of Hechter, Zaffaroni and Pincus, described in some detail below, strongly support the belief that the cholesterol is the precursor of the adrenocortical hormones and that ACTH plays an important part in its " mobilization " (see p. 14).

The Control of Adrenocortical Activity

ACTH. ACTH is undoubtedly of considerable importance in controlling adrenocortical activity. In so doing it behaves like the two other groups of " trophic " hormones of the pituitary, the thyrotrophic hormone and the gonadotrophic hormones. Thus administration of ACTH leads to enlargement of the gland and acceleration of the rate of secretion of its hormones, with depletion of the stores of the hormone precursors, such as cholesterol and ascorbic acid. On the other hand, hypophysectomy leads to atrophy of the adrenal cortex, though in some species, such as the mouse, the rat, the guinea-pig and the dog, the atrophy is confined to the fasciculate and reticular zones, and the zona glomerulosa may even hypertrophy. In these species electrolyte metabolism is less seriously impaired in hypophysectomized than in adrenalectomized animals, whereas carbohydrate, fat and protein metabolism are affected to the same extent in both hypophysectomized and adrenalectomized animals. Furthermore, administration of DCA both to intact and hypophysectomized rats induces atrophy of the cells of the zona glomerulosa, indicating that exogenously administered steroid substitutes for the endogenous product of the cells of this zone and leads to " disuse atrophy." This suggests that, in these species, the zona glomerulosa is independent of pituitary control, and is concerned with elaboration of a hormone similar to the synthetic compound DOC which controls electrolyte metabolism. There is, however, considerable species variation, and these studies by no means prove that the zona glomerulosa is independent of pituitary control in the human species, nor that it secretes an electrolyte-controlling factor. Indeed, administration of ACTH in man leads among other things to sodium and chloride retention, though this may be due to the increased production of

cortisone and hydrocortisone. Nevertheless it is a clinical impression that in panhypopituitarism (Simmonds's disease) there is not the same difficulty in regulating salt excretion as in cases of Addison's disease. The difference between man and mouse may be one of degree rather than of kind.

The Nature of the Adrenocorticotrophic Hormone. In 1948, proteins homogeneous in sedimentation, electrophoresis and solubility studies (i.e. " pure " proteins) were isolated from sheep pituitaries by Li, Evans and Simpson (1948), and from pig pituitaries by Sayers, White and Long (1948). The physicochemical properties of these proteins were similar and both had a molecular weight of 20,000. Sixty per cent. of the protein could be digested with pepsin without losing its biological activity, though this was destroyed by trypsin and other enzymes. Thus by digesting 1 gm. of ACTH protein with 40 mgm. of crystalline pepsin, dissolved in 100 ml. of 0·1 M acetic acid at 37·4° C. for seventeen hours, a compound of peptides soluble in trichloracetic acid can be produced, in which the average peptide chain length varies from 7 to 9. The molecular weight is 1,200. Analysis by means of paper partition chromatography identifies at least six distinct spots. One was found to be about twice as active as the original peptide mixture. Well-defined clinical effects are obtained by using a daily dose of only 13 mgm. of this ACTH peptide mixture, Li (1950).

Cortis-Jones *et al.* (1950) submitted beef ACTH concentrates to *ultrafiltration* and were able to prepare a polypeptide mixture twice as active as homogenous pig ACTH, and from this mixture a polypeptide (ACTP) was isolated and found to be ten times as active as homogenous pig ACTH (Morris and Morris, 1950). The activity of these ACTH preparations has been estimated by two procedures. The first is the ascorbic acid depletion method of Sayers (Sayers, M. A., Sayers, G., and Woodbury, 1948), which depends on the percentage depletion of adrenal ascorbic acid in the hypophysectomized rat. This is the most sensitive method of biological assay, as little as 0·5 mgm. of pig ACTH leading to a 20 per cent. depletion. The second is the adrenal repair, or " adrenal weight " method, which assays the material to be tested by estimating the increase in adrenal weight produced in rats hypophysectomized ten to fourteen days previously, so that the weight of their adrenals has diminished to 25 to 30 per cent. of those of the controls. It had been assumed that either of these methods of assay accurately reflected the potency of any prepara-

tion of ACTH, though the ascorbic acid depletion method was generally employed because it is so sensitive, and was thought to reflect more faithfully the clinical effectiveness of various commercial preparations. It has now been shown by Stack-Dunne and Young (1951) that there are *two different pituitary* factors, both of which may be described as "ACTH," and that they differ considerably in their behaviour towards ascorbic acid depletion and adrenal weight. They have shown, for instance, that growth hormone is rich in the adrenal weight factor (A.W. factor). When hypophysectomized animals are treated with this factor the histology of the gland returns to normal and lipoid is deposited in the transition or sudanophobe area of the outer fasiculata. This factor also stimulates mitotic activity. On the other hand, a preparation poor in A.W. factor but rich in ascorbic acid depleting substance (A.A. factor) will not restore the lipoid-staining material to the same extent, and the transition zone remains; nor has it any influence on mitosis. Thus it is possible that the A.W. factor promotes the deposition of cholesterol and synthesis of its intermediates leading to the production of adrenocortical steroid hormones, whereas the A.A. factor is associated with the mechanism of release of the newly produced hormones from the gland. These workers have separated, by means of an ion exchange column, from the protein hormone prepared by the method of Sayers, White and Long, a major component which is almost devoid of A.A. activity, and a minor fraction which appears to be a basic substance and is highly active, and they therefore conclude that ACTH (A.A.) is a basic substance, probably a peptide, associated in the gland with a slightly acidic carrier protein.

Bruce, Parkes and Perry (1952) have pointed out that the ascorbic acid depletion test is based on the acute effects of ACTH, whereas the adrenal weight test depends on the chronic effects of the hormone. Both are carried out on hypophysectomized animals and are therefore laborious and require considerable technical skill. The Mill Hill group have therefore introduced an assay method which depends on the hypotrophy of the thymus of the nestling rat injected with a suspension of ACTH in arachis oil containing 5 per cent. beeswax. Injections are given thrice daily, and the infantile intact animal is chosen because it is unaffected by "stress" before the eighth day of life. This assay method is not as sensitive as the other procedures which have been mentioned, but is quite adequate for routine assay of bulk preparations.

Purification of ACTH Extracts. Payne, Raben and Astwood (1950) had shown that it was possible to achieve a ten-fold concentration of ACTH by adsorption of the crude glacial acetic acid extract of pig anterior pituitary powder on a fifty-fold weight of powdered cellulose, and suggested that the cellulose, by virtue of its constituent carboxyl groups, acted as a cation-exchange medium. More recently Astwood and his colleagues (1951) have shown that oxidized cellulose possesses a much larger capacity and is more selective for the active fraction, and by its use they have prepared a highly potent extract, forty times as active as the starting material, and eighty times as active as the Armour's standard preparation La-I-A. Doses of 0·3 to 1·5 mgm. daily were found to be fully effective in the treatment of patients suffering from rheumatic and allergic disorders.

"Stress." Not only does endogenous ACTH or the exogenous administration of a potent pituitary extract stimulate adrenocortical function, but innumerable conditions which increase the metabolic demands of the whole body or some of its organs will stimulate adrenocortical activity. Some examples of such "stressor" agents are exposure to cold, heating, burning, trauma, infection, anæsthetics and drugs. It has been shown that stress can lead to the outpouring of adrenocortical hormones in animals with an intact pituitary, and therefore it is clear that its pathway of action is *via* the pituitary, giving rise to secretion of ACTH. Indeed Selye points out that when the pituitary is stimulated by stress there is a "shift in pituitary hormone production" in the direction of ACTH and away from the gonadotrophic hormones, the growth hormone and the lactogenic hormone. Stress, however, not only stimulates the pituitary to produce ACTH. It also leads to a number of other events. One is to initiate the "alarm reaction," which is the first step in the development of Selye's general adaptation syndrome. Another is to cause acceleration in the utilization of circulating cortical steroids by the tissues, leading temporarily to a lowered level of cortical hormones in the blood stream. Yet another is to stimulate the adrenal medulla to produce an emergency ration of adrenaline, and finally to stimulate certain hypothalamic nuclei. Each of these events plays a part in the control of the adrenal cortex and must therefore be considered.

The Long Adrenaline Hypothesis. Long (1950) showed that intravenous infusion of adrenaline, and conditions of stress leading to discharge of adrenaline from the medulla, did not result in a fall

in the level of circulating eosinophils or to a depletion of adrenal ascorbic acid in hypophysectomized animals. He therefore postulated that adrenaline had no direct action on the adrenal

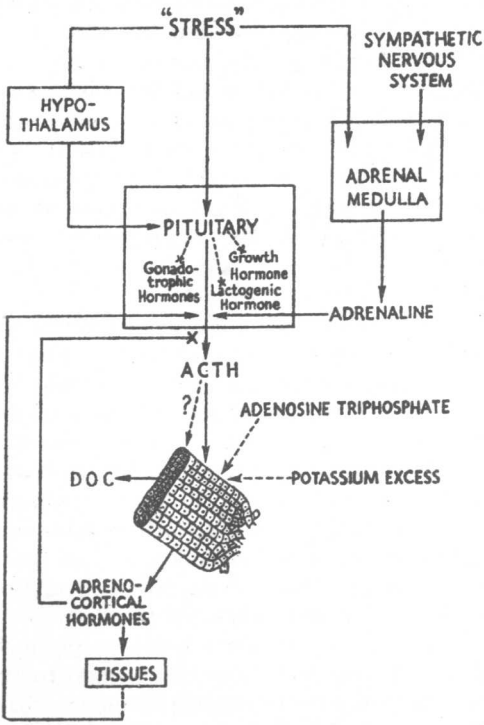

FIG. 1. The control of adrenocortical activity. Stress may act on the pituitary or through the hypothalamus to stimulate the secretion of ACTH at the expense of other pituitary hormones. ACTH stimulates the zona fasciculata and zona reticularis, though possibly not the zona glomerulosa, which may secrete DOC independently. The level of adrenocortical hormones in the blood controls the rate of production of ACTH. Rapid utilization of cortical hormones by the tissues lowers the blood level of cortical hormones and thus stimulates ACTH production. Adrenaline may also stimulate ACTH production, and adenosine triphosphate or potassium excess may stimulate the adrenal cortex.

cortex but must operate through the pituitary to release ACTH. Any interference with this reflex pathway will cause failure of discharge of cortical hormones, as demonstrated by diminution in the level of circulating eosinophils. Thus, following removal of

the adrenal medulla, spinal section at the level of the third or fourth dorsal vertebra, or appropriate diencephalic lesions, there is no decline of eosinophils after exposure of the animal to cold for four hours. A small subcutaneous injection of 10 per cent. sodium chloride constitutes a painful stimulus which produces a marked and persistent fall in eosinophils. If this stimulus is applied above the level of the section in rats, in which the spinal cord has been transected at the level of the third or fourth dorsal vertebra, no fall of eosinophils occurs. Nevertheless, all the sensory pathways above the lesion are unaffected, and therefore it should be possible for reflex activation of any hypothalamic centres to take place, and if this were the pathway of stimulation of ACTH release one would expect that the pain impulse would travel along it. If, however, the impulse could stimulate ACTH release only after secretion of adrenaline by the medulla, the appropriate pathway has been cut by the cord transection, and one would therefore not expect the fall in eosinophils to take place. As the result of his studies, Long has postulated that there are at least two components in the regulatory mechanism for the secretion of ACTH. One is entirely humoral and depends on the relative blood levels of ACTH and adrenocortical hormones. It is probably by means of this constant reciprocal stimulation and inhibition that the basic level of adrenocortical activity is maintained. The other is a rapidly acting emergency mechanism initiated by the release of adrenaline from the adrenal medulla, and is brought into play as the result of various stressor stimuli.

The Sayers Tissue Utilization Hypothesis. Sayers (1950) has laid emphasis on the reciprocal relationship that is a characteristic feature of the control of the secretion of the trophic hormones of the pituitary by the output of hormones from the target glands, the ovary, thyroid and adrenal cortex. He suggests that one of the first reactions to a stressor stimulus is increased rate of utilization of cortical hormones by the peripheral tissues ; and that this leads to immediate exhibition of the characteristic responses of the organism to stress, such as gluconeogenesis, mobilization of protein, etc. The immediate utilization of these hormones results in a lowering of the level of circulating cortical hormone, and this in turn gives rise to release of ACTH from the pituitary. Long agrees that this mechanism would explain the prolonged response to stress, for so long as the condition of stress persists, cortical hormones will be utilized by the tissues at increased rates, and continued outpouring of ACTH will occur. Harris is sceptical of

this hypothesis, and enquires why this accelerated utilization of cortical hormones should not lead to increased entrance of these hormones from the systemic blood into the pituitary cells themselves, thereby inhibiting rather than accelerating the release of ACTH. Sayers and Long, however, are in more complete agreement with one another, for Long suggests that increased utilization by the tissues is not only a response to stress—and therefore an emergency mechanism—but that the reciprocal concentrations of ACTH and adrenocortical hormones serve to regulate the constant and basic rate of ACTH release. Sayers, in his turn, suggests that adrenaline may act by promoting the utilization of adrenocortical hormones by the tissues.

The Harris Hypothalamic Hypothesis. Harris (1951), however, impressed with the prominent part played by the hypothalamus in controlling the secretion of gonadotrophic hormones, determined to investigate the possibility of a similar mechanism for the regulation of ACTH secretion. Using the remote control technique in which a stimulating electrode unit is buried under the animal's scalp with the tip of the electrode placed with great accuracy to impinge directly upon the desired hypothalamic nucleus, and the primary coil enlarged to surround the cage containing the animal, he eliminated the possibility of subjecting the animal to concurrent emotional stress during periods of stimulation. By varying the position of the stimulating electrode, it was found that only stimulation of the posterior region of the tuber cinereum or mamillary body resulted in lymphopœnia (as a sign of ACTH secretion and adrenocortical hormone release). On the other hand, if the zona tuberalis (the part of the anterior pituitary gland traversed by the hypophysial portal vessels) was destroyed in the rabbit, or if the posterior part of the tuber cinereum or mamillary body was damaged in other animals, the lymphopœnic response to stress was abolished. From this it was concluded, in the animals studied, that a stress stimulus followed a pathway through the hypothalamus *via* the hypophysial portal vessels to release ACTH from the pituitary. Recent studies by Harris and Jacobsohn (1952) on pituitary grafts have shown that if the graft is placed in the region of the tuber cinereum in hypophysectomized rats it will become revascularized by the hypophysial portal vessels, and the adrenal glands will return to normal size and histological appearance. If the grafts are placed elsewhere in the subarachnoid space, though they may become richly vascularized (but not by the hypophysial portal system), the adrenal glands remain atro-

phied. Harris feels that this provides strong evidence in favour of the hypothalamus playing a prominent part even in the constant day-to-day control of ACTH secretion and maintenance of adrenocortical activity. He furthermore believes that the blood level of adrenocortical hormones exerts a fine adjustment to the rate of ACTH output.

Other Factors. Vogt (1950) has conducted studies on the perfused isolated adrenal gland in the dog. A number of physiologically occurring substances were introduced into the perfusing fluid, and two were found to increase the rate of hormone production from the isolated gland. They were:

Adenosine-triphosphate. This gives rise to a short burst of increased secretion of cortical hormones. It is known that substances carrying high energy phosphate bonds take part in the metabolism of most cells, and studies of the uptake of the phosphorus isotope P^{32} have shown that it is utilized by the cells of the adrenal gland and that its uptake is accelerated by ACTH. These experiments suggest that phosphates play a part in the synthesis of cortical hormones, and under normal conditions they are probably synthesized *in situ* within the gland cells. Reiss and Halkerston (1950) have observed that, as the result of stress, the ascorbic acid depletion and increased production of corticoids is accompanied by increased phosphorylation (increased uptake of P^{32}), and that this is due to ACTH stimulation, for it does not occur in hypophysectomized rats.

Increased Potassium Content. An increase in the potassium content of the perfusing fluid also leads to acceleration of cortical hormone output. The increase is of a magnitude which would be expected to occur only in adrenalectomized animals, and it is not known whether a small increase, within the physiological range, would provide such a stimulus. If this were the case the adjustment of the rate of cortical secretion to meet the demands of electrolyte metabolism might be entirely independent of pituitary activity, and this would support the view that electrolyte metabolism is governed by the activities of the zona glomerulosa which is not subject to ACTH control. But, quite possibly, the increase in potassium concentration is an emergency mechanism and does not stimulate cortical hormone secretion within normal physiological levels.

Apart from these observations, however, it was found that the isolated perfused adrenal gland secretes appreciable amounts of hormone, even when it is perfused with blood containing no