THE ADRENAL STEROIDS

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The widespread biological, biochemical and medical interest now being taken in the adrenal glands is due to the realization that adrenocortical hormones are concerned in a fundamental way with the metabolism of the entire organism. Although many people may be unable to accept Selye's concept of the general adaptation syndrome in its entirety, his work has helped to emphasize that the adrenal glands are not only of interest in certain endocrine conditions, but that they play an important role in maintaining or modifying the ability of the organism to withstand the injurious effects of a wide variety of stressful agents, ranging from infections and trauma to changes in environmental temperature and deep X-irradiation. The preservation of homeostasis depends on many factors, of which adrenocortical activity is only one. The importance of the adrenal glands in this respect is shown by the increased secretion of adrenal hormones after almost any kind of stimulus and by the lack of flexibility in various metabolic and physiological processes when the adrenal glands are removed or not functioning. Thus patients with Addison's disease have a relatively constant high urinary output of sodium and chloride but are unable to make the normal compensatory adjustments when given a high or low salt intake.

It is now established that the adrenal cortex secretes several different hormones. For a proper understanding of their physiological actions it is necessary for the clinician to know something of their chemical structure, just as it is necessary for him to subdivide white blood cells by their morphological appearances into granulocytes, lymphocytes and monocytes. The clinician has learnt to associate a granulocytosis with certain pyogenic infections; in the future we may learn to associate an increase or decrease in one particular steroid with some particular syndrome.

Chemistry of Adrenal Steroids

All the hormones isolated from the adrenal

cortex of animals and man are steroids containing the reduced cyclopentenophenanthrene nucleus (I) in which the carbon atoms are numbered as shown in formula (II). Most steroids have methyl groups ($-\text{CH}_3$) attached to C-10 and C-13, the position of such groups being indicated by a short line. Steroids may be called by several different names. Throughout this paper the common, trivial or even colloquial name will be used in preference to the systematic chemical name which is often cumbersome. For example, cortisone (x) is the common name for 17-hydroxy-11-dehydrocorticosterone. The systematic chemical name is pregn-4-ene-17 (α): 21 diol-3:11:20 trione. The name 17-hydroxy-11-dehydrocorticosterone shows the relationship of cortisone to corticosterone (VII)—a hydroxyl ($-\text{OH}$) group is present at the C-17 position, and the substituent group at C-11 is dehydrogenated from $-\text{OH}$ to $=\text{O}$. The use of the systematic chemical name provides information of the molecular structure without reference to corticosterone. Thus 4-ene (or Δ^4 as it is sometimes written) signifies a double bond ($\text{C}=\text{C}$) situated at the fourth carbon atom; 17 (α): 21 diol signifies two hydroxyl ($-\text{OH}$) groups attached to C-17 and C-21, that at C-17 being in the α -position (i.e. below the plane of the steroid nucleus); 3:11:20 trione indicates three ketone ($\text{C}=\text{O}$) groups at C-3, C-11 and C-20.

The biological activity and the chemical properties of adrenal steroids are dependent on the substituent groups, and the compounds can be divided according to their chemical structure and physiological actions into three main groups.

1. Steroids of the pregnane group which contain 21 carbon atoms (III). The most biologically active members have a ketone group ($\text{C}=\text{O}$) at C-3 with an adjacent olefinic grouping ($\text{C}=\text{C}$) between C-4 and C-5, a hydroxyl group ($-\text{OH}$) at C-21 and another ketone group at C-20 (III); thus the side-chain attached at C-17 contains a primary α -ketol grouping. These hormones have been given the colloquial name 'corticosteroids'

and 'corticoids.' They can be further subdivided into (a) those with a ketone or hydroxyl group at C-11, which exert their main influence on carbohydrate and protein metabolism (so-called glucocorticoid or 11-oxygenated steroids), and (b) those with no substituent group at C-11 (IX), (XII) which mainly influence the metabolism of electrolytes (so-called mineralocorticoids). As will be shown later this is only a relative differentiation not an absolute one.

2. Steroids of the C₁₉ group which have no side-chain attached at C-17. Many of these are androgenic steroids, which in addition to such androgenic properties as promoting the growth of axillary hair, have an important action in stimulating protein anabolism. Adrenosterone (IV) is an example of such a compound.

3. Estrogenic compounds, such as oestrone (V), which are phenolic since ring A is aromatic and carries a hydroxyl group at C-3.

CORTICOSTEROIDS

Twenty-eight crystalline compounds have been isolated by complex chemical methods from adrenal glands by three groups of workers, Reichstein in Switzerland, Pfiffner and Wintersteiner, and Kendall in the United States. Only six of these (VII-XII) have been found capable of maintaining life in adrenalectomized animals, although the amorphous fraction, which remains after removing the crystalline compounds, is more active

in this respect weight for weight than any of the pure substances. The hormones active in maintaining life are all α -ketols. Their metabolic activity can be related to their chemical structure. Compounds with a hydroxyl group at C-17 and a ketone or hydroxyl group at C-11, such as cortisone (X)—Kendall's compound E—and 17-hydroxycorticosterone (VIII)—Kendall's compound F or 'hydrocortisone'—have significant effects on carbohydrate and protein metabolism. In contrast, deoxycorticosterone (IX)—DOC—which has neither a hydroxyl group at C-17 nor a hydroxyl or ketone group at C-11 exerts its most striking effect on the reabsorption of sodium by the renal tubules and augments the excretion of potassium. Corticosterone (VII)—Kendall's compound B—which has a hydroxyl group at C-11 but no hydroxyl group at C-17, and 11-dehydrocorticosterone (XI)—Kendall's compound A—which has a ketone group at C-11 but no hydroxyl group at C-17, are intermediate in their metabolic effects between cortisone and compound F on one hand and deoxycorticosterone on the other.

The question has arisen as to whether the many steroids which have been isolated are in fact true secretory products of the adrenal cortex or whether some of them are artifacts produced by the chemical methods used in their isolation. The answer to this is being provided by chromatographic analysis of adrenal venous and peripheral venous blood from man and animals. Zaffaroni

and his colleagues in America and Bush in this country have found by paper partition chromatography, a method unlikely to give rise to artifacts, that a number of different α -ketols are secreted together with the reduction products of hormonally active corticosteroids (Zaffaroni and Burton, 1951; Reich *et al.*, 1950; Bush, 1952). By perfusing animal and human adrenal glands, Pincus and his colleagues have isolated chromatographically a number of different α -ketols from the perfusate and have tentatively postulated the pathway by which the adrenal steroids are synthesized *in vivo* (Hechter *et al.*, 1951). From these studies it appears that the adrenals secrete a number of different corticosteroids but the two which appear in the greatest amount are 17-hydroxycorticosterone (compound F) and corticosterone (compound B).

Metabolic Effects of Corticosteroids

Caution is necessary when interpreting the action of adrenocortical hormones, because the changes induced depend on the particular hormone given and the state of the patient being studied. Large doses of 11-deoxycorticosterone, which may produce little sodium retention in normal subjects, will cause hypertension, oedema and pulmonary congestion in patients with Addison's disease. It is also important to distinguish between the effects of these hormones when given in an amount likely to be present in body fluids under normal circumstances (physiological dosage) and the effect when given in larger amounts (pharmacological dosage). For example, small doses of adrenocortical extract promote nitrogen retention and growth in adrenalectomized animals whereas larger doses produce a negative nitrogen balance and inhibit growth (Ingle and Prestrud, 1949).

Adrenal steroids appear to influence the behaviour of almost every part of the organism, but the fundamental mechanism by which they affect metabolic processes is still unknown. Kept under strictly controlled conditions, adrenalectomized animals survive and even breed. Thus it seems that by their presence or absence adrenal steroids neither initiate nor stop any metabolic reaction but rather they modify the rate at which existing reactions proceed. Whether this effect is achieved by influencing enzymic activity or by increasing the permeability of cells, and thus increasing the availability of specific substrates, is still uncertain. Little would be achieved by reviewing here the large volume of experimental data on the metabolic effects of the adrenal steroids. It is sufficient to mention a few observations of particular clinical interest.

Nitrogen metabolism. In large dosage cortisone causes a negative nitrogen balance in normal and

adrenalectomized animals either by promoting the breakdown of protein or by preventing its synthesis. This loss of nitrogen has been observed in patients with rheumatoid arthritis, treated with cortisone, but this undesirable effect can to some extent be offset by increasing the patient's intake of protein and this often occurs spontaneously as the patient's appetite improves under treatment. The osteoporosis and cutaneous striae in Cushing's syndrome are probably related to excess secretion of glucocorticoids.

Carbohydrate metabolism. Adrenalectomized animals and patients with Addison's disease show a tendency to hypoglycaemia which is particularly evident after prolonged fasting. Cortisone is able to prevent this abnormality. In normal force-fed rats large doses of cortisone cause hyperglycaemia and glycosuria. The impaired glucose tolerance, hyperglycaemia, glycosuria and insulin resistance seen in Cushing's syndrome are attributable to overproduction of glucocorticoids. A diabetic-like state may be induced in normal subjects during cortisone treatment, but the abnormality is only temporary, and normal carbohydrate metabolism is restored on cessation of treatment. The hyperglycaemia is mainly due to decreased peripheral utilization of glucose and only in part to increased gluconeogenesis from protein breakdown. The glycosuria, to some extent, is due to lowering of the renal threshold for glucose, and in some instances may not be associated with significant hyperglycaemia.

Electrolyte metabolism. It has been known for many years that patients with Addison's disease usually have a low serum sodium concentration and may show evidence of dehydration. It is still uncertain whether in health sodium balance is controlled by one or a combination of the corticosteroids which have so far been isolated. Compounds E and F may cause sodium retention but their action is weak, and cortisone alone may fail to maintain sodium balance in Addisonian patients (Salassa *et al.*, 1950). Corticosterone has a stronger action but less than that of 11-deoxycorticosterone which finds its principal use in the correction of electrolyte abnormalities in Addison's disease. Although deoxycorticosterone has been isolated from adrenal gland extracts (Reichstein and von Euw, 1938; Zaffaroni and Burton, 1951) and from the perfusate of beef adrenal glands stimulated with ACTH (Hechter *et al.*, 1951), its presence has not yet been demonstrated in human blood or in urine after acid (Burton *et al.*, 1951) or enzymic hydrolysis (Bayliss, unpublished observations). Thus it remains problematical whether deoxycorticosterone is a naturally occurring substance in man or indeed whether a specific salt-retaining hormone is elaborated, but the claim of

Tait *et al.* (1952) to have isolated in human urine a substance, having strong salt-retaining properties, which is not deoxycorticosterone or any hitherto recognized steroid, lends support to such a hypothesis.

Deoxycorticosterone and, in large doses, cortisone may induce a negative potassium balance and produce electrolyte changes similar to those seen in Cushing's syndrome, namely hypochloraemic, hypokalaemic alkalosis. In rare instances during cortisone therapy it may be necessary to restrict the sodium and supplement the potassium intake.

Effect on mesenchymal tissue. Tissues derived from the primitive mesoderm—connective tissue, synovia, vascular tissue, lymphocytes, lymphoid tissue and eosinophils—are influenced by glucocorticoids, and it was in modifying the responses of synovial tissue in rheumatoid arthritis and connective tissue in the collagen diseases that cortisone first came into therapeutic use.

INDICES OF ADRENOCORTICAL FUNCTION

Many methods have been proposed for assessing adrenocortical activity; some of them not applicable to man and many too laborious for routine use. Most are indirect.

Alterations in the Adrenal Cortex

The functional activity of an organ may not be reflected in its histological appearance but increased activity of the adrenal glands is associated with an early decrease in the ascorbic acid and cholesterol content of the cortical cells and a later increase in weight. Such changes are of great value in assessing increased adrenal activity in experimental animals but are of no value in clinical medicine.

Alterations in the Organism

Various clinical tests, based on disordered metabolism in adrenal insufficiency, have been used in the diagnosis of Addison's disease. In the Kepler test (Robinson *et al.*, 1941), for example, the ability of the kidneys to excrete water, sodium and urea is assessed. Such clinical tests are relatively non-specific and insensitive, and are of no value in assessing adrenocortical activity when the pituitary-adrenal system is intact.

Hills *et al.* (1948) have shown that the number of circulating eosinophils is related to the activity of the pituitary-adrenal system, and this has led to the development of a test of adrenocortical activity following stimulation of the gland with ACTH (Thorn *et al.*, 1948). Absence of an eosinopenia following administration of ACTH indicates adrenocortical insufficiency. Stressful agents of many kinds cause an eosinopenia in normal

subjects. Provided the proper technique is employed, the eosinophil test is reliable and of considerable value in clinical medicine.

Urinary Steroids

Although little is known about the individual fate of the steroids elaborated in the adrenal cortex, there are substances in the urine which are derived from corticosteroids (urinary corticosteroids or corticoids), from androgenic steroids (17-ketosteroids) and other steroids the origin of which is less certain (Marrian, 1952; Butler and Marrian, 1937, 1938). The activity of the gland appears to be reflected in the excretion of these compounds.

17-ketosteroids. The best known group of excretory products are the neutral 17-ketosteroids, in which a ketone group is attached to C-17 (vi). The term 17-ketosteroid is usually, as here, used to denote neutral, non-phenolic 17-ketosteroids which are mainly, but not exclusively, the metabolites of androgenic hormones. Oestrone (v) is a 17-ketosteroid but since it has a phenolic ring it is acidic and is removed from urinary extracts when they are washed with alkali. In females practically all the 17-ketosteroids are of adrenal origin; in males a proportion is derived from hormones secreted by the testes. Children up to the age of 8 excrete very little 17-ketosteroid. Adult females excrete 5 to 15 mg. and adult males 8 to 22 mg. per 24 hours. The range in health is very variable, and a value cannot be considered abnormal unless it is twice the upper or half the lower limit of normal (see Butt *et al.*, 1950; Mason and Engstrom, 1950). In females with Addison's or Simmonds' disease the excretion is often negligible, but in males with Addison's disease amounts up to 5 mg. per 24 hours may be found in the urine, presumably due to continued testicular activity. In the adrenogenital syndrome and after ACTH therapy in subjects with normal adrenals the excretion of 17-ketosteroids is increased. A proportion of the neutral 17-ketosteroids is probably derived from α -ketol corticosteroids because 17-ketosteroid excretion may be increased during cortisone treatment of Addisonian patients, and because the structure of some compounds suggests their origin from corticosteroids. In patients with intact adrenals, however, cortisone may decrease the excretion of 17-ketosteroids—an effect attributable to suppression of endogenous ACTH production.

17-ketosteroids may be subdivided into α and β fractions depending on whether the hydroxyl group at C-3 is below (α -fraction) or above (β -fraction) the plane of the steroid molecule. The α -17-ketosteroids are derived from both the adrenals and the testes, and constitute the greater part of the total 17-ketosteroid output in normal urine. The β -fraction originates solely from

the adrenal cortex and normally comprises less than 15 per cent. of the total. In adrenocortical carcinoma the proportion of β -ketosteroids excreted may be increased up to 30 per cent., whereas in simple adrenal hyperplasia the β -total ratio remains normal although the total amount of 17-ketosteroid excreted may be increased (Butt *et al.*, 1948; Haslam and Klyne, 1952).

Urinary corticosteroids. Increased adrenal secretion of corticosteroids is not necessarily reflected in an increased urinary excretion of 17-ketosteroids, and patients with Cushing's syndrome in which increased amounts of compound F and corticosterone are found in the blood (Bush, 1952) do not always have a raised 17-ketosteroid output. Compound F and cortisone and other corticosteroids have been isolated from urine, together with their reduction products in which the ketonic groups and the double bond between C-4 and C-5 have been reduced (Schneider, 1950, 1952; Burton *et al.*, 1951).

Numerous biological and chemical methods have been evolved for estimating these urinary corticosteroids, but only a few have been generally adopted and none is suitable for routine use. Perhaps the best known are the bioassay procedure of Venning *et al.* (1946) in which use is

made of the ability of 11-oxygenated compounds to deposit glycogen in the liver of adrenalectomized mice; methods in which the reducing power of steroids with a primary α -ketol grouping is measured (Talbot *et al.*, 1945; Heard and Sobel, 1946; Robinson, 1951); and methods which estimate the amount of formaldehyde liberated when certain steroids are oxidized with periodic acid (Lowenstein *et al.*, 1946; Daughaday *et al.*, 1948). Many corticosteroids are excreted as conjugates, and their hydrolysis by chemical and enzymic methods (Marrian, 1951; Bayliss, 1952) is not sufficiently well understood to give confidence in the methods at present available.

Blood Corticosteroids

Until recently only biological methods were available for estimating corticosteroids in blood (Vogt, 1948; Paschkis *et al.*, 1949), and these were not practicable for routine use. The development of chemical methods will provide means for direct determination of adrenocortical activity, and such methods are now under trial with promising results (Nelson and Samuels, 1952; Butt and Crooke, 1952; Bayliss and Steinbeck, unpublished observations).

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