CHEMICAL PATHOLOGY

IN RELATION TO CLINICAL MEDICINE

The Adrenal Cortex

The Proceedings of a Symposium
organised by the
Association of Clinical Pathologists
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EDITED BY
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EDITORS' FOREWORD

This Symposium was sponsored by the Association of Clinical Pathologists in order to bring together laboratory workers and clinicians to discuss the theory and technique underlying biochemical tests of adrenocortical function, and their application to diagnosis and treatment. It is hoped that this will be the first of a series of such symposia.

We take this opportunity to thank, on behalf of the A.C.P., all the speakers who took so much trouble to make the Symposium a success and to supply a script for this publication. We own a particular debt of gratitude to Professor C.H.Gray for his skilful selection of speakers, for his firm and lucid chairmanship, and for his assistance in the scientific editing of this book; it is mainly to him that the success of the Symposium is due.

We also wish to acknowledge the assistance of the staff of the Royal Society of Medicine where the Symposium was held, and the co-operation of the publishers in getting the book so quickly through the press.

G.K.McGowan

M.Sandler

Chairman's Preface

This Symposium was intended to provide an up-to-date account of the chemical pathology of the adrenal cortex rather than a forum for discussion between experts in the field. For this reason Dr.McGowan has prepared a brief appendix to this published account which should provide a useful working knowledge of the chemistry of the steroids for those to whom this subject is not familiar. This, together with the glossary, should help to clarify the confusion which has arisen because of the varied terminologies in use in the steroid field, for instance the use of the trivial names compound F, hydrocortisone, cortisol and 17-hydroxycorticosterone for a single substance, the systematic name for which is 11β:17α:21-trihydroxypregn-3:17-dione. The term corticosteroid (rather than corticoid) has been used as a generic name to include all C_{21} steroids formed in the adrenal cortex, and their C_{21} metabolites.

Considerable care has been taken to ensure that when the term "free steroid" is used, no doubt is left as to whether it means unconjugated steroids or steroids which are not bound to protein. In general it is much better to use the terms "unconjugated"and "non-protein-bound".

There are still a number of routine laboratories that are hesitant to undertake estimations of steroids apart from 17-ketosteroids. It is difficult to understand why this should be since in this country, unlike the U.S.A., there has been little difficulty in obtaining satisfactory grades of sodium bismuthate for the Norymberski methods; nevertheless even here it is of advantage to make use of some form of quality control by the occasional analysis of urine before and after the addition of a known amount of cortisol. During the discussion several

workers reported difficulties in obtaining satisfactory results from the Morris method of separating 17-ketogenic steroids with an 11-oxygenated group from those without. Nevertheless, Dr. Eileen Hill found this investigation of considerable value in the differential diagnosis of congenital pseudohermaphroditism in children.

A survey of the literature and, indeed, perusal of this volume will reveal that there are, as yet, no generally agreed conditions of enzyme concentration or duration of action for the hydrolysis of steroid conjugates in urine. There are many uncontrolled variables in this procedure such as the concentration of non-steroid glucuronides which may compete for the enzyme and the degree of enzyme inactivation in the urine. All methods recommended in this symposium will give complete hydrolysis with most urines, but with some. and perhaps all, it is possible that steroid conjugates will not be completely hydrolysed in an occasional abnormal urine.

In the course of the discussion after some of the papers, it became apparent that blood to be analysed for adrenal corticosteroids was often collected at a time more convenient for the laboratory than optimal for diagnostic purposes. Thus, it is customary to collect samples at about 9 a.m. at a time when the blood level is usually high. While this may be appropriate in suspected hypo-adrenal states when a low value is expected, it may be less appropriate in suspected hyper-adrenal states or when the response of the adrenal to ACTH (cortico-tropin) is being investigated. Indeed, there is a case for paying more attention to the times at which blood is collected in this as in many other biochemical investigations.

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CHEMICAL PATHOLOGY

Anatomy and Physiology of the Adrenal Cortex

T. SYMINGTON

Introduction

The adrenal gland is composed of two distinct parts, cortex and medulla, and although they are in close proximity to one another in most animals, they differ in their embryological development and appear to have little in common. The cortex, but not the medulla, is indispensable to life. The medullary cells elaborate and store the hormones adrenaline and noradrenaline, in sufficient quantities to be demonstrable by histochemical techniques (Hillarp and Hokfelt, 1953; Kennedy, Symington and Woodger, 1960). The cortical cells on the other hand form steroid hormones but do not store them, so that little information is likely to be obtained from histochemical methods intended to demonstrate the steroid content of adrenocortical cells.

The weight of the human adrenal

The adrenal glands lie in close relationship to the upper pole of the kidneys; the right is triangular in shape, the left rather more oblong. A perfectly normal adrenal is rarely found post mortem, but may be seen at operation when the gland is yellow in colour. On section (Fig. 1) the yellow outer zone of cortex is clearly marked; the inner zone is brown and corresponds to the zona reticularis. Frequently in post-mortem glands there is marked depletion of lipid and the whole cortex has the brown coloration of the zona reticularis. The medulla is pale and may be seen in the centre of the gland between the central vein and the zona reticularis. Sometimes islands of medullary tissue are present.

The human adrenal gland varies in weight at different ages and the female gland is slightly smaller than the male. In a comprehensive review, Bachman (1954) found the combined weight of the two glands at

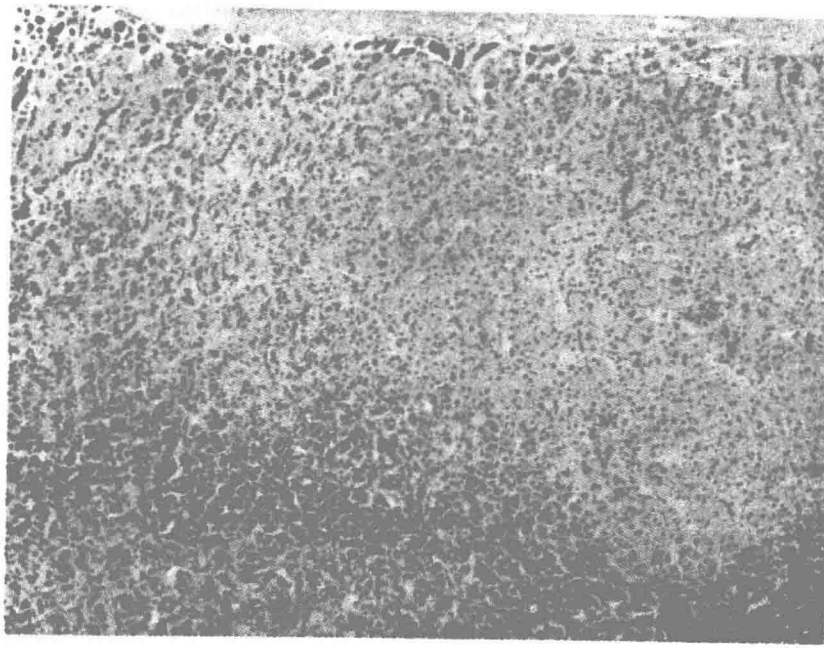

Figure 1. Cross section of the normal human adrenal cortex showing shallow, patchy glomerulosa, prominent fasciculata and dark-staining reticulata.

birth to average 6 g in the male and 5 g in the female. During the first year of life, as a result of atrophy of the foetal cortex, the weight of both male and female glands falls to 3 g. At the end of one year the glands increase in size, and by six to ten years of age have regained their birth weight. The glands continue to enlarge until the age of twenty when they reach their highest combined weight, averaging 13.5 g. This is maintained until the age of forty when there occurs a gradual small fall to about 13 g at the age of sixty to seventy years. The female glands are usually about 0.5 g lighter. Such figures are consistent with the writer's experience of apparently normal post-mortem adrenals, where the average combined weight of adult adrenals has been 13 g. Quinan and Berger (1933), in an excellent review, combined the results of fourteen workers and found a mean weight of 12.3 g for both adrenals, or a value slightly above 6 g for a single gland.

Such results are, however, at variance with those found when adrenals are obtained from apparently normal individuals, who died suddenly

from traumatic injury. The average weight of a single gland under these conditions varies from 4 to 4.5 g (Quinan and Berger, 1933; Williams, personal communication), while the average weight of normal adrenals removed from female patients at operation for breast cancer is 3.9 g (Symington and Forrest - unpublished). There is little doubt that, as will be seen later, the increase in weight of post-mortem glands is the result of stress changes, and accordingly it would appear that the average weight of the single normal adult adrenal gland is in the region of 3.9 g in the female and between 4 and 4.5 g in the male. A complete reappraisal of the age change is thus required but the difficulty in obtaining suitable material from the younger age groups is obvious.

Vasculature of the human adrenal cortex

The gland has a very rich vascular supply from three main arteries, the superior, middle and inferior suprarenal. The superior suprarenal usually arises from the inferior phrenic artery but may arise directly from the aorta. The middle suprarenal arises directly from the aorta and the inferior suprarenal from the renal artery. The three main vessels run along each of the three surfaces of the gland and from them arise from 20 to 50 small vessels so that the gland is completely encircled by arterial twigs, which penetrate the capsule of the gland to form a subcapsular plexus (Anson, Cauldwell, Pick and Beaton, 1947; Harrison and Asling, 1955; Clark, 1959). Capillary loops pass downwards between the cortical cells to enter the thin-walled vascular sinusoids of the zona reticularis, which are connected to the main central vein by small venules.

The venous system in the human adrenal is distinct from that in other animals. A central vein with prominent longitudinal muscle runs through the middle of the gland in its longitudinal axis (Fig.2). In the centre of the gland (C) the muscle is concentric, but away from the centre of the gland (A) it is composed of eccentric longitudinal bundles between which pass the small venules draining the cortex. Here the non-muscular side of the vein has a thin wall and no venules enter the lumen of the central vein through this. At the periphery of the gland (B), some vessels have eccentric musc. bundles, and a few have a

thin layer of concentric muscle lying under the endothelium. A main drainage vein runs from the central vein to join the renal vein but in addition, accessory veins connect the central vein with the exterior and provide an alternative drainage channel (Fig.2).

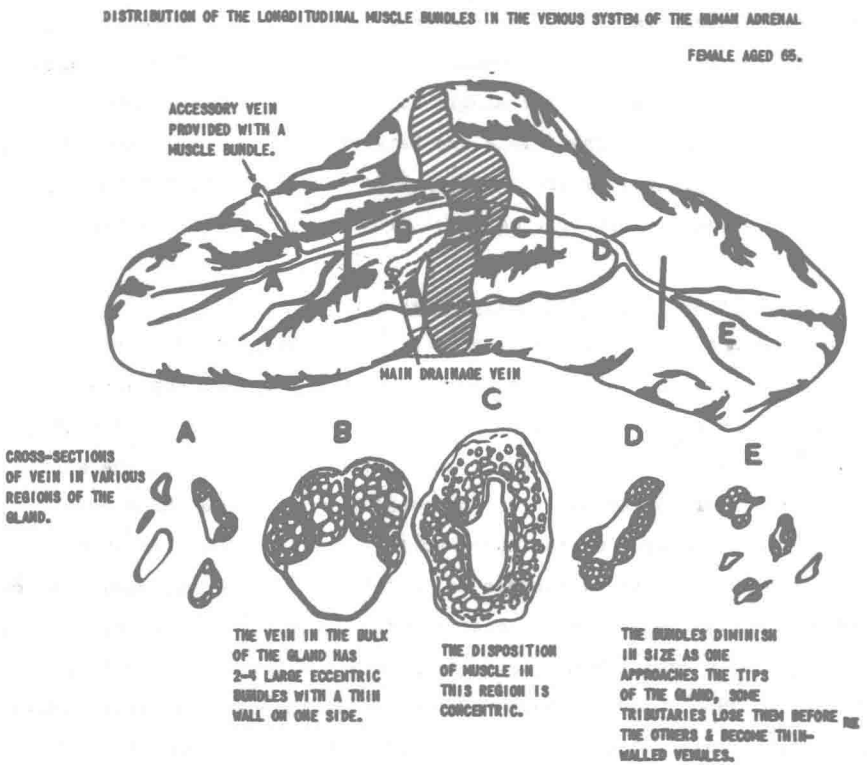

Figure 2. Human adrenal gland. Model of gland showing disposition of longitudinal muscle bundles in the central vein. Note accessory veins.

Innervation of the human adrenal gland

The adrenal medulla is richly innervated and preganglionic fibres end around the cells (phaeochromocytes). There does not appear to be any innervation of the cells of the human cortex, and any nerve fibrils present appear to be related to the thin-walled blood vessels. The longitudinal muscles in the adrenal vein are innervated from post-ganglionic fibres, possibly of sympathetic origin which may arise in

the cable of the splanchnic nerve, the coeliac ganglion, and in ganglia in the capsule of the gland and sometimes in the muscle of the vein. The longitudinal fibres projecting into the lumen of the vein are thus abundantly supplied by minute nerve terminals (Sheppard, 1922).

Hormones formed by the human adrenal cortex

A number of steroid hormones are formed by the adrenal cortex, the most important of which are the C_{21} steroids, cortisol, corticosterone and aldosterone. Cortisol and corticosterone are produced by the reticularis-fasciculata zone, while recent evidence (Ayres, Gould, Simpson and Tait, 1956; Giroud, Stachenko and Venning, 1956; Giroud, Stachenko and Piletta, 1958) indicates that aldosterone is formed by the zona glomerulosa. The adreno-cortical cells also form C_{19} compounds or androgens (including dehydroepiandrosterone and androstenedione) and opinions vary as to the site of production of these hormones. Jones (1957) and others believe that the sex hormones (C_{18} oestrogens and C_{19} androgens) are formed in the zona reticularis. Symington (1960, 1961) however holds that all the hormones (C_{21}, C_{19} and C_{18}) with the exception of aldosterone are formed for normal daily requirements in the cells of the zona reticularis and that, in conditions of stress or after ACTH stimulation, the steroid precursors in the cells of the zona fasciculata come into use. The answer to the problem must await further work. The evidence that the C_{18} hormones are formed in the adrenal is largely indirect; the amount formed there must be very small.

The biological effects of adrenocortical steroids

The C_{21} steroids (cortisol, cortisone and corticosterone) are particularly active in carbohydrate metabolism and relatively inactive in electrolyte metabolism; they are therefore called 'glucocorticoids. Deoxycorticosterone (DOC) has a weak action on carbohydrate metabolism but exerts a pronounced effect on electrolyte and water metabolism, and is therefore referred to as a 'mineralocorticoid. Aldosterone is probably the principal mineralocorticoid, although it has a small effect on carbohydrate metabolism.

The biological potency of the adrenal C_{19} androgens is weak when compared with testosterone which comes from the testis. However, it would appear that the development of sexual hair, seborrhoea, increased

growth and excretion of 17-oxosteroids (ketosteroids) in young girls at puberty is due to this group of steroids.

Some observations on the methods used to investigate the adrenal cortex

It is impossible in a brief survey to discuss the various technical staining methods which have been used in adrenal investigations. A summary of some of the methods is given in Table 1 and it can be seen

TABLE 1

Human Adrenal Investigations

Methods available

1. Conventional and Histochemical Techniques (Post-mortem)
   (a) Haematoxylin and eosin; Sudan; Schultz; phospholipins.
   (b) Non-specific enzyme methods:
       acid and alkaline phosphatase; dehydrogenase.
   (c) 'Specific' stains for carbonyl groups:
       phenylhydrazine; NAH (Camber, 1949; Ashbel and Seligman, 1949).

2. Correlation of Conventional Methods with Function (Operation)
   (a) 11, 17 and 21-hydroxylases and possibly other enzymes.
   (b) Adrenal vein effluent - cortisol, corticosterone.
   (c) Steroid biosynthesis - in vitro (Saffran and Schally, 1955).
   (d) Incubation with radioactive precursors.
   (e) Electron microscopy.

3. Microchemical and Histochemical Approach (Operation)
   Macro-analytical techniques scaled down 500-1000 times.

that three distinct lines of investigation are available depending on whether the adrenal is removed from a human being at autopsy or removed from an animal or human at operation. When the gland is fresh all three methods can be used but only the first is available for human glands removed post-mortem.

The use of haematoxylin and eosin in association with Sudan stains for lipid can provide valuable information about adrenal structure and

has contributed much to our knowledge of the comparative morphology of the gland, of the peculiar arrangement of longitudinal muscle in human adrenal veins, as well as of the complex histological patterns which occur in the human gland in response to stress. The 'non-specific' enzyme stains for alkaline and acid phosphatase and non-specific esterase, when used in conjunction with the above methods, have focussed attention on the zona reticularis (Symington, Currie, Curran and Davidson, 1955; Yoffey, 1955). This zone, far from being senescent as was believed originally, is very rich in those enzymes and it is now believed (Symington, 1960, 1961) to be the site of formation of steroid hormones (cortisol, corticosterone) for daily requirements.

The nature of the lipids in the cortex has been a subject of considerable interest since it was noted that the droplets possess peculiar properties when compared with ordinary fat. They are bi-refringent when examined under polarized light, give a positive Schultz reaction and precipitate with digitonin. Since these optical and staining properties are possessed by cholesterol esters and cholesterol, it became clear that such substances were present in abundance in cortical lipid droplets with other sudanophilic unsaturated lipids.

While much work has been done on other lipid components of the adrenal cortex, in recent years most interest has centred round attempts to demonstrate steroid hormones in the cells of the adrenal cortex. The subject is comprehensively reviewed by Deane and Seligman (1953). The basis of the so-called 'ketosteroid' or carbonyl reaction is that steroid hormones elaborated by the adrenal gland possess ketonic groups and the α-ketol side-chain ($CO.CH_2OH$) attached to C-17 confers strong reducing properties similar to those of reducing sugars. Bennett (1940) using 100 μ sections of the adrenal, stained them with phenylhydrazine and found yellow hydrazones in the cells of the cortex. He believed that the lipid droplets contained ketonic steroids in addition to cholesterol and its esters. Gomori (1942) disagreed with these conclusions and believed that the carbonyl grouping demonstrated by phenylhydrazine was due to plasmals; these are fatty aldehydes derived from the phospholipid precursor plasmalogen, which reacts with mercuric chloride in the Schiff reagent to give the "plasmal reaction". Gomori pointed out