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# The Human Adrenal Cortex

Pathology and Biology – An Integrated Approach

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All these things here collected are not mine,
But divers grapes make one kind of wine,
So I from many learned authors took
The various matters written in this book;
What's not mine own shall not by me be father'd,
The most part I in many years have gather'd.

John Taylor, the Water-Poet, 1580–1654

# Preface

We would like to take this opportunity of expressing our sincerest thanks to the many persons who have made adrenal tissue and related materials available to us for our work. Our especial gratitude is extended to Drs. J. J. Brown, A. Lever and J. I. S. Robertson of the M.R.C. Blood Pressure Unit, Glasgow, Dr. J. K. Grant, Royal Infirmary, Glasgow, Professor R. B. Welbourn and Dr. W. Kelly, Royal Postgraduate Medical School, Drs. D. B. Grant of Great Ormond Street, J. Ginsberg, Royal Free Hospital, D. C. Anderson, Hope Hospital, Salford, C. R. Edwards, St. Bartholomew's Hospital and Professor I. Doniach (formerly of the London Hospital) and Messrs. J.-C. Gazet, A. McKinna and P. Greening, Royal Marsden Hospital, London.

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Finally, we would like to extend our appreciation to Sir Thomas Symington, our former mentor, who initially stimulated our interest in the adrenal gland, and our many previous co-workers including Drs. A. M. MacKay, K. Grigor, P. Hornsby and E. Nice.

Sutton, September 1981

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Michael J. O'Hare

# Contents

# Chapter 1
# Introduction

We have been interested over the past two decades in aspects of both the functional biology and pathology of the human adrenal cortex. During this period, a substantial number of papers and monographs have been published by many investigators on various topics relevant to the animal and human adrenal glands and knowledge has been advanced in many respects.

Our purpose in compiling this book has been threefold. First, we have attempted to present an integrated approach to the pathology of the human adrenal gland, based in part on our personal experience, but also incorporating and summarising much of the recent data thereby providing the working pathologist with a single up-to-date volume.

During the past two decades several books have dealt with the adrenal cortex and its diseases from clinical (Soffer et al. 1961a; Mills 1964; Nelson 1980), multidisciplinary (Currie et al. 1962; Eisenstein 1967; Chester Jones and Henderson 1976, 1978, 1980) and comparative anatomical (Chester Jones 1957) viewpoints. However, only two books have been primarily directed towards aspects of diagnostic histopathology (Dhöm 1965; Symington 1969) and both are now well over a decade old. Consequently, as a second objective, we have attempted to produce a well-illustrated reference together with a comprehensive description of the underlying biology of the human cortex derived partly from our own experience. This should, we hope, enable the accurate pathological classification of adrenocortical disorders to be made on a rational basis.

Finally, we have endeavoured to provide a book where the biology, pathology, biochemistry and salient clinical features have been integrated in such a way as to interest the scientist as well as the clinician in the problems that remain to be solved, as we believe that such an integrated multidisciplinary approach will continue to prove of value in the future.

## Chapter 2
# Historical Aspects

Although the human adrenal gland was illustrated by Eustachius in 1563, the descriptions of Caspar Bartholinus the Elder (1611) were the first to receive significant attention because Eustachius' illustrations were immured in the Papal library and remained unknown until the early eighteenth century when they were republished by Lancisi.

For two centuries, debate took place as to whether the adrenal or suprarenal glands, as they were called by Riolan (1629), possessed excretory ducts and a central cavity. These imagined attributes, considered evidence for a supposed function of processing 'atrabilia' or black bile, gradually fell from favour and, by the beginning of the 19th century, their ductless nature and the probability that they formed some secretion which was returned to the blood stream was generally accepted (Sorkin 1957).

The gross distinction between the cortex and medulla was noted by Cuvier in 1805 although Huschke (1845) was the first to apply these terms to the adrenal. Ecker (1846) gave the first detailed histological description of the human adrenal gland and Arnold (1831) was the first to study their embryology but erroneously believed that they were derived from the Wolffian bodies. A relationship between the gonads and adrenals was also suggested on comparative grounds by Meckel (1806). Thomas Addison (1855) provided the first concrete evidence of the vital nature of the adrenal gland describing accurately the symptoms of adrenal insufficiency and their association with destruction of the gland by a variety of infective diseases and neoplastic lesions. In his own words, he 'stumbled upon' these symptoms, which he believed usually included idiopathic anaemia, while searching for the cause of pernicious anaemia, although, in fact, the two diseases (i.e. Addison's disease and Addison's anaemia) are seen together only in a minority of cases.

Early experimental adrenal research was subject to misinterpretation and controversy. In a much quoted study, Brown-Séquard (1856) demonstrated by extirpation that the adrenals were essential to life. His experiments were prompted specifically by Addison's pathophysiological observations; they were, however, poorly controlled and the death of his animals was probably due as much to shock and sepsis as to true adrenal insufficiency. The failure of many subsequent experiments by other workers to give a consistent picture led to many decades of fruitless debate. The root of the problem lay not only in a failure to distinguish between medullary and cortical functions, but also in the widely varying frequencies with which accessory cortical tissues occur in various animals (noted in man by Marchand in 1883). Final proof that the cortex and not the medulla was the vital tissue was not obtained until the studies of Wheeler and Vincent (1917) and Houssay and Lewis (1923).

The second half of the nineteenth century had, therefore, seen little further progress in adrenocortical physiology; nevertheless, the structural relationships in the tissue and its development were further defined. Thus, the zonation of the cortex was recognised by Harley (1858) and Arnold (1866) coined the terms zona glomerulosa, zona fasciculata and zona reticularis (Fig. 2.1). The independent origin of the medulla and its homology with ganglionic elements was described by Balfour (1878) and

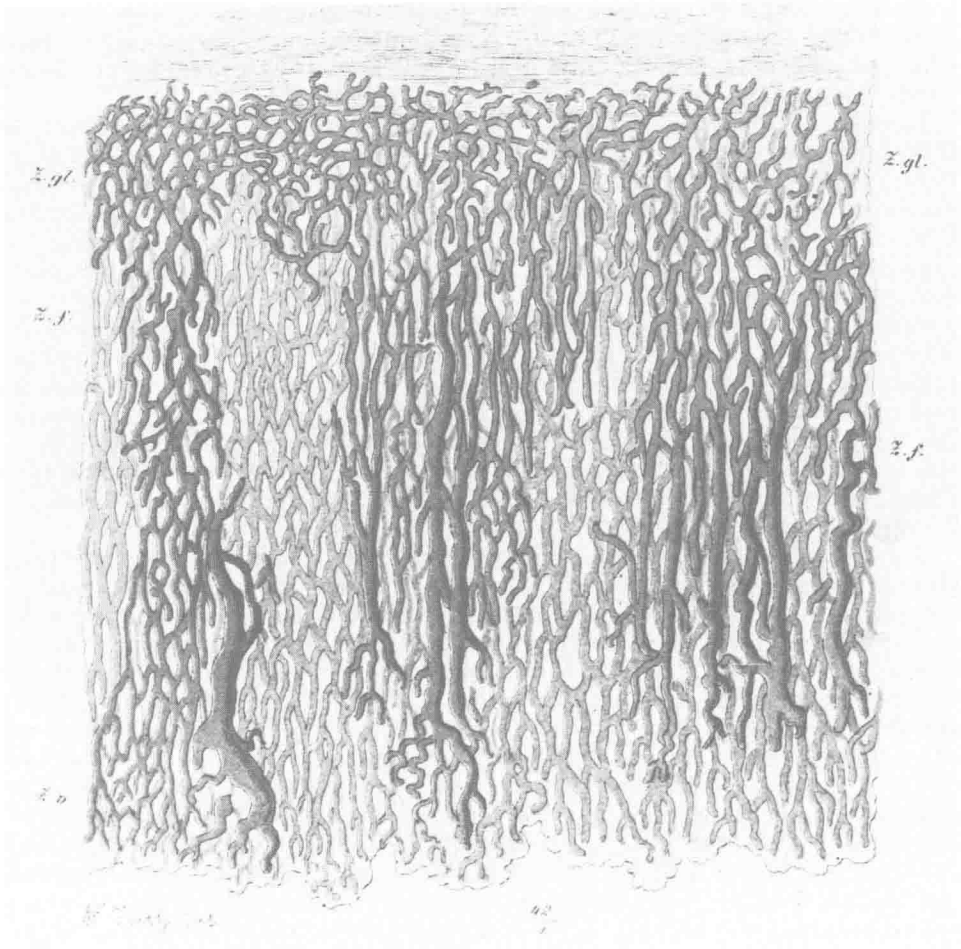

**Fig. 2.1.** The zonation of the adrenal cortex as illustrated by Arnold (1866).

Mitsukuri (1882), although these observations had been pre-empted to some extent by Leydig (1852) and Köllicker (1854) with their descriptions of the gland in the German literature. Balfour was, however, the first to describe correctly the development of the cortex from coelomic mesothelium dorsal to and separate from the Wolffian body, and dynamic relationships between the constituent cells were first suggested by Gottschau (1883) who originated the concept of centripetal adrenal cell migration.

Experimental interest in the adrenal cortex during the years 1890–1920 was largely overshadowed by the medulla, its relation to the sympathetic nervous system and the general concepts of reflex nervous action that were then fashionable. Thus the endocrine secretions of the gland were by and large identified with adrenaline, which had been isolated and characterised in 1901. Many attempts to prepare adrenal extracts active in Addison's disease had been made during this period, but most met with little objective success (Adams 1903). Reasons for the 80-year gap between Addison's description of adrenal insufficiency and the success of Swingle and Pfiffner (1930) in preparing active cortical extracts are not, however, hard to find. Contemporary studies of other endocrine tissues had been facilitated by factors such as

the water-soluble nature of their products, the relatively large quantities of hormone stored within the tissue, and in several cases identification of specific target tissues which served as assay systems. None of these criteria could be fulfilled by the adrenal cortex.

Early studies of adrenal pathology (e.g. Woolley 1903) did not assist materially in the identification of cortical hormones and their effects although Bulloch and Sequeira (1905) subsequently described a series of adrenal tumours and hyperplasias which elicited precocious or inappropriate development of secondary sex characteristics. The term 'suprarenal virilism' or 'adrenogenital syndrome' describing these effects is usually attributed to Gallais (1912a,b); the first detailed description of such a case was that of DeCrecchio (1865). The problems of adrenocortical pathology at this time were compounded by several factors. The rare medullary and even rarer cortical tumours were not clearly distinguished from one another until the turn of the century, and the presence of symptoms such as hypertension with both types of tumour added to the confusion. Furthermore, the erroneous identification of renal clear cell adenocarcinomas (hypernephromas) as so-called adrenal-rest tumours by Grawitz (1883) did little to clarify the situation. Although Grawitz's theory was rejected by Stoerk in 1908 the distinction between these tumours and true adrenocortical lesions continued to cause problems for many years.

Evidence for the higher control of adrenal cortex began to accumulate in the early years of the 20th century although it was largely ignored in contemporary studies. Atrophy of the adrenals in anencephalic fetuses had been noted by Morgagni as early as 1719 but it remained for Ascoli and Legnani (1912) to provide the first experimental evidence of pituitary control with their observation of adrenocortical atrophy in hypophysectomised dogs. Simmonds (1919) noted a similar effect in man. It was Smith (1930) with his hypophysectomised rat assay who laid the foundation for the characterisation of the 'adrenotrophic hormone' as a separate principle by Collip et al. (1933), its subsequent isolation by Li et al. (1943) and by Sayers et al. (1943), its sequencing by Bell and co-workers (1954) and finally its total synthesis by Schwyzer and Seiber in 1963. The role of the adrenal gland in relation to stress was repeatedly emphasised by Selye (1946), who suggested that derangement of the pituitary-adrenal axis was involved in a wide variety of pathogenetic mechanisms. Along with these controversial theories Selye coined the terms 'mineralocorticoid' and 'glucocorticoid'.

In the search for the active principles of the adrenal cortex, 1930 was undoubtedly a turning point. In that year Swingle and Pfiffner published a detailed summary of their studies of active cortical extracts, relying on a time-consuming and difficult assay based on the survival time of adrenalectomised cats and dogs. Knowledge of the tissue-specific effects of putative cortical secretions was at that time largely non-existent, although Houssay had suspected that they were related to carbohydrate metabolism and an involvement in the regulation of kidney functions had been suggested by Marshall and Davis (1916).

In the late 1920s, however, considerable progress had been made in the development of a reliable survival-time assay of cortical activity, notably by Rogoff and Stewart (1928), and using these assays several groups soon claimed preparation of active cortical extracts. They included Rogoff and Stewart (1928) themselves with 'interrenalin' and Hartman and his colleagues (1928) with their adrenaline-free 'cortin'. All these studies were, however, superseded by Swingle and Pfiffner at Princeton, to whom the preparation of the first really potent cortical extract can be credited. Their material, which was prepared by organic solvent extraction, could be freed from residual adrenaline without loss of activity and supported adrenalectomised animals indefinitely, rather than merely postponing their demise. Dramatic remissions with this extract in cases of Addison's disease were obtained during 1930–32 by

Rowntree and Greene at the Mayo Clinic and by Harrop and Weinstein at Johns Hopkins Hospital.

The vast quantities of adrenal tissue used in these studies (usually of bovine origin) clearly precluded the routine clinical use of such extracts and no progress had been made at this time in identifying the chemical nature of the adrenocortical hormone(s). Szent-Györgi had isolated adrenal ascorbic acid in 1928 but it was not until 1934 when Kendall at the Mayo Clinic announced the preparation of a crystalline cortical extract with a molecular weight of 350 and a tentative empirical formula of $C_{20}H_{30}O_5$ that the road to the routine therapeutic use of cortical hormones was opened. During the period 1934–1938, his group, that of Wintersteiner and Pfiffner (1935) and Reichstein (1936) in Basel succeeded brilliantly in attaining these objectives. Using vast quantities of adrenal tissue (batches of 3000 lb were not uncommon) and methods of solvent extraction, countercurrent distribution and fractional crystallisation, the major products of the cortex were prepared. Compound A (11-dehydrocorticosterone) and and Compound E (cortisone) were characterised in 1936, Compound B (corticosterone) and Compound F (cortisol) in 1937, and Substance S (11-deoxycortisol) and Substance Q (deoxycorticosterone) in 1938. Chemical studies showed that these were all steroids with 21 carbon atoms, i.e., $C_{21}$-compounds. Their relationship to the then newly characterised sex steroids was readily apparent from their chemical degradation to steroids with 19 carbon atoms such as adrenosterone (Substance G). It was suspected from their structure that they might all be synthesised from cholesterol, although direct proof was lacking until the work of Hechter (1951).

These studies did not immediately resolve the problem of the identity of the active cortical hormone, although belief in a unitary hormone was gradually eroded by the multiplicity of products isolated from the gland. The precise role of individual compounds remained in doubt and Gaunt (1975) has recently published an entertaining personal account of this exciting era of research. Not only was it apparent that various steroids possessed widely differing levels of life-sustaining 'cortin' activity but it was also realised that the yields of various steroids obtained by the laborious processes then in use did not necessarily reflect tissue levels. In fact, it was not until 1953 that Bush and Sandberg showed that cortisol was the major free plasma steroid in man.

Returning to the chronological sequence, however, by 1940 a battery of tests of steroid bioactivity were now available. They included disturbance of electrolyte balance (Loeb), nitrogen balance (Harrop), hepatic gluconeogenesis (Britton and Silvette) and asthenia and work tests (e.g. Everse-de Fremery and Ingle tests). These assays revealed that a large part of the activity in cortical extracts, notably its salt-retaining activity, resided in an 'amorphous fraction' that had thus far resisted crystallisation and characterisation (Kendall 1941).

The objective of complete synthesis of the active steroids already isolated was nevertheless pursued, and in 1948 Sarett at Merck eventually announced the synthesis of cortisone in quantities sufficient for clinical use, in itself a major feat of organic synthesis. Cortisone was, in fact, largely ignored when first isolated as it was only weakly active as a 'life-sustaining' steroid in adrenalectomised animals; its potency was only revealed when it was tested for specific 'glucocorticoid' activity.

Cortisone was not the first chemically synthesised adrenal steroid to be used therapeutically. Both 11-dehydrocorticosterone and deoxycorticosterone (acetate) had been previously employed by Thorn and his associates (1942) in clinical trials in Addison's disease, but with only limited success. The widely publicised and dramatically successful use of cortisone by Hench and Kendall in rheumatoid arthritis in 1949, however, saw the culmination of a project initiated nearly 20 years earlier, albeit in a clinical context that had hardly been envisaged at the outset.

The most notable advance in understanding the pathophysiology of the adrenal cortex that took place during this era of chemical steroid research was the description, by Harvey Cushing in 1932, of symptoms of adrenal hyperfunction found in association with basophilic pituitary adenomas. Up to that time many clinical manifestations of cortical hyperfunction had escaped general recognition, with the exception of the adrenogenital syndrome to which we have already referred. Occasional cases of apparent endocrine imbalance of unknown aetiology had, however, surfaced from time to time.

Cushing's unique contribution was to describe both the precise physical changes and the pituitary alterations in a series of such previously obscure 'polyglandular syndromes'. Although he acknowledged that the adrenocortical hyperplasia noted in some cases might be responsible for some of the systemic manifestations of pituitary basophilism, his primary concern was to link all these effects to the pituitary tumours, which he considered their root cause. Bauer (1936) was probably the first to champion the role of the adrenal cortex in the aetiology of Cushing's syndrome, which he termed 'interrenalism', and he drew attention to the fact that an identical syndrome could be observed in many cases where no pituitary changes were demonstrable, thus distinguishing between what came to be known as Cushing's disease proper and Cushing's syndrome.

At this time no techniques for measuring directly physiological quantities of adrenal steroids, let alone adrenocorticotrophic hormone, existed. Proof of the excessive quantities of adrenal steroids secreted in Cushing's syndrome was obtained by Anderson and Haymaker (1937) when they prolonged the life of adrenalectomised rats with extracts of patients' sera. Further evidence of the inverse relationship between physiological changes in Cushing's syndrome and Addison's disease was forthcoming from studies such as those of McQuarrie, Johnson and Ziegler (1937) on electrolyte balance, and in 1938 Haymaker distinguished clearly for the first time between the concept of excessive adrenal androgen secretion as responsible for the adrenogenital syndrome and excess 'cortin' secretion in Cushing's syndrome. The latter was not finally identified until Mason and Sprague (1948) isolated cortisol in increased amounts from the urine of a patient with Cushing's syndrome. The 'adrenal theory' of Cushing's syndrome had, however, gained strength for many years previous to this finding, notably as propounded by Fuller Albright (1943), even to the point where the pituitary changes were deemed secondary in nature.

The clinical recognition of both the adrenogenital syndrome and Cushing's syndrome had preceded the isolation of the relevant hormones by several years. This was not, however, the case with the last member of the classic triad of hypercortical states, hyperaldosteronism. This was probably due in part at least to the fact that its symptoms, notably hypertension, overlapped those of many other disease states. By the late 1940's it was evident that an unidentified cortical steroid was a key factor in hydromineral regulation, notably the retention and excretion of sodium. There was, furthermore, good circumstantial evidence from animal studies that it originated in the zona glomerulosa, as suggested by Swann (1940). A salt requirement of adrenalectomised animals had been noted as long ago as 1927 by Marine and Baumann, but it was the studies of Loeb (1932) on electrolyte balance in Addison's disease that clinched the case and drew attention to the probable role of adrenal hormones in salt and water metabolism. Deoxycorticosterone (Substance Q) was potent in causing salt and water retention and for over a decade, therefore, it remained the most likely candidate for the adrenal 'mineralocorticoid'. Doubts as to its true physiological role persisted on account of the very limited amounts of this steroid in the gland. By the end of the 1940's several groups had begun to reinvestigate the 'amorphous fraction' of cortical extracts with the aim of finding a new mineralocorticoid. The prize fell to Simpson and Tait at