Recent Experimental Work on the Mammalian Adrenal Cortex

C. P. Hickman, DePauw University, and R. R. Overman, Harvard University

For some time the authors of this paper have been investigating the effects on the mitochondria and other structures of the adrenal cortex in white mice when Collip's adrenotropic hormone is injected into them under control conditions. In connection with certain aspects of this work, and as a background for it, we are presenting this review of some of the recent experimental modifications of the mammalian adrenal cortex. In this account we wish to emphasize the general histological structure of the cortex and the prevailing views regarding its formation. Without a doubt much of the knowledge about the adrenal gland has been derived from the study of its physiological cytology. The very fact that the adrenals have had so many functions assigned to them in recent years implies great complexity in their physiological action. Although there seems to be a certain amount of uniformity in their structure among various mammals, it is not possible in our present state of knowledge to homologize all their details. There is considerable diversity in the histological components of the adrenal cortex, and it may be that such diversity of structures is associated somewhat with functional differences.

As a general thing, the functions of the adrenal cortex are even more shrouded in mystery than are some of the other endocrine glands. A list of symptoms displayed by adrenalectomized animals will give some idea of the ramifications of the action of the cortex. In recent years, it has been shown that animals without adrenals and without any type of replacement therapy show sodium excretion above normal, marked dehydration, rise in blood urea, retention of potassium in the blood, loss of glycogen storage, decrease in blood glucose, hypotension, pigmentation of the skin, susceptibility to toxins, gonadal disorders, lactation disturbances, and several other effects. In view of such varied functions, it seems easier and more to the point to enumerate the things this much-functioning gland does not do.

General histological structure of the mammalian adrenal cortex.—It is well known, of course, that the adrenal glands in mammals consist both structurally and functionally of two distinct separate parts, the cortex and the medulla. Each of these parts, it is also well known, has its own separate and distinct origin. The comparative anatomy of the two regions, as traced through the different vertebrate groups, is a very interesting one.

As a general feature, the adrenal cortex is formed of cords of cells, between which lie reticular tissue and capillaries. These cords are generally two cells wide. Since the shape and arrangement of these cords differ throughout their course, the cortex has been divided in a somewhat arbitrary manner into three zones: an outer or *zona glomeru-*

losa, a middle or *zona fasciculata*, and an inner or *zona reticularis*. As we shall point out later, we should not adhere to this zone division rigidly. It simply represents a convenient way of understanding the main features of the cortex.

There is considerable variation in cell types in the cortex among the different layers. In the *zona glomerulosa*, which is the narrowest of the three, the cells are columnar or sometimes pyramidal, arranged in ovoid groups or in small arcs which cap the next layer, the *zona fasciculata*. The nuclei of these cells stain deeply. Lipoid droplets are few in number.

The *zona fasciculata*, which makes up the widest part of the gland, as a usual thing, consists of larger cells chiefly polyhedral in shape. In the outer fasciculata region the cells stain rather lightly, and there are numerous lipoid droplets which, when dissolved away, give this region a spongy appearance. There are often two or more nuclei in each cell. In the inner part of the fasciculata the cells diminish in size and contain basophilic granules which stain deeply with iron hematoxylin. The cells of this region are arranged in cords.

The innermost zone, the *zona reticularis*, has its cells arranged in anastomosing cords. The outer cells of this region are similar to those in the fasciculata zone, except that they have fewer lipoid droplets. In other parts of the zone there are two distinct kinds of cells, light and dark. The light cells are more numerous than the dark ones and have rounded contours. The dark cells are smaller with a deeply staining cytoplasm and small shrunken (pyknotic) and hyperchromatic nuclei. Light cells have fewer mitochondria and lipoid droplets than the dark cells. The difference between the two kinds of cells seems to be a matter of difference in lipoid emulsification. Light cells have their protein material scattered among small lipoid droplets; dark cells have larger lipoid droplets, and the protein part, which stains darkly, here shows up to a better advantage. There are many transitional forms between the light and dark cells. There is also much cellular debris in the reticularis zone. The light and dark cells probably represent stages of degenerating, senescent cells.

According to most authorities who have studied the problem, there is a growth from without inward in the cortex, and there is a corresponding continuity of cell types. It is the belief of most that there is a normal progression of the cell from the spindle, fibroblast-like cell of the capsule through the ovoid or prismoid shapes of *zona glomerulosa* and *zona fasciculata* to the small heterogenous, hyperchromatic cells of the reticularis zone. In the reticular region the presence of cellular debris would tend to show a breakdown of old cells whose function and shape have changed through the zonal progression from capsule to medulla. Most workers agree with Hoerr ('31) that the different functions of the cell types are independently derived or else are the different functional states of the same cell.

Most cases of mitosis are found in the fasciculata zone or in the border between the glomerulosa and the fasciculata. Usually only a few are found in the reticularis layer, and these are chiefly in young animals.

The adrenal X zone.—In some mammals, especially in mice, and possibly to some extent in the cat, rat, human, and a few other species, a so-called X zone has been described by many writers. This zone has often been included in the *zona reticularis* by most observers, but others regard it as a separate zone, although a transient one. It is not present in the adrenal of the adult male mouse although it first appears in both sexes at about three weeks of age. The cells of this zone are characterized by being small and free from lipoid droplets and staining darkly. In mice which are early castrated (before five days of age) the zone may develop to the extent of one-third or more of the entire cortex. In the female the X zone is normally larger and persists for a longer time, disappearing in that sex in the middle of the first pregnancy or later in the non-pregnant female. In the female its growth and development are unaffected by ovariectomy.

In mice castrated after sexual maturity, when the primary X zone has disappeared, a secondary X zone appears but disappears about 100 days later. It is thus seen that the spontaneous manifestation of the X zone is restricted to a limited portion of the life cycle. It has also been shown that castration changes in the mouse can be inhibited by injections of male hormones. Howard ('39) recently found that there is a correlation between the development of the seminal vesicles and that of the X zone. These and other experiments have given rise to the belief that gonadal hormones are produced in regions outside of the reproductive system, but in the course of development this function becomes concentrated in the gonad. However, in dwarf mice X zones fail to develop after castration. This may mean that the pituitary may also be involved in some way.

In rats, Howard ('38) has shown the presence of a zone, similar in most ways to the X zone of mice, which she calls the juvenile cortex. This zone appears well developed in rats of both sexes at three weeks of age and has almost disappeared in 40 days. This juvenile cortex shows no age differences and is little influenced by castration. The juvenile cortex is, for the most part, transformed into the adult reticular zone although some of its cells tend to persist in an isolated condition.

Man shares with mice in having the most X zone development, but in this case the zone degenerates soon after birth.

In the rabbit there are actually four zones in the adrenal cortex. The innermost interlocks with the medulla, but it is not known whether this inner zone may or may not be homologous with the X zone of the mouse.

Cytoplasmic inclusions of the different zones of the adrenal cortex.—Many investigators have described the mitochondria of the mammalian adrenal cortex, and, as a general thing, there is agreement among them regarding the shape and arrangement of these cytoplasmic inclusions. In the *zona glomerulosa*, where they are unusually abundant, the mitochondria are found chiefly in the form of threads and short rods; in rarer cases, they are in the form of granules. In some cases, these threads have beaded or clubbed ends. In the *zona fasciculata* they are found in the form of granules and are placed in the spaces between the lipoid inclusions. In the *zona reticularis* most of the mitochondria are in the form of rodlets which often clump together in a characteristic fashion. Although some writers have reported that the mitochondria are less abundant in this zone than in the others, this view has probably arisen from poor fixation. Nearly all workers agree that the *zona reticularis* is the most difficult part of the cortex to fix. Whenever good fixation is employed, the reticularis will show as many of the inclusions as the other zones. However, it should be pointed out that there is considerable variation in the distribution of mitochondria in the cortex of various mammals. In some cases, there is a marked tendency for them to be accumulated in clusters near the nucleus; in others they are scattered throughout the cytoplasm. Such differences cannot always be made accountable on the basis of differences in technique. The fact that mitochondria are more abundant in cells where there is little lipoid proves nothing, for they may here show up to greater advantage.

In the adrenal cortex the Golgi apparatus appears chiefly in the form of a network of filaments with varicosities. Around this network there are often small spheres. There is also a fairly close relationship between the Golgi apparatus and the cell vacuoles.

Lipoid inclusions of the different zones.—Lipoid inclusions of some form are found in all the cortex zones. They are especially abundant in the fasciculata cells, and, when the lipoid is dissolved out, the cells present a peculiar vacuolated cytoplasm. From this appearance the cells here are called spongiocytes. The *zona reticularis* contains much less lipoid, and the light cells found in some contain little or none. Zalesky ('36) distinguishes two varieties of lipsomes in the cortex of the guinea pig. The smaller ones, which he calls microliposomes, are 0.4 to 2.5 microns in diameter. The larger are known as macroliposomes and are from 3.5 to 20 microns in diameter. The macroliposomes may be intercellular although usually they are intracellular. They do not stain as deeply with osmic acid as do the microliposomes and are often dissolved out completely with alcoholic dehydration and infiltration with paraffin. A chemical difference between the two is evident. The macroliposomes are confined chiefly to the fasciculata zone; the microliposomes have a much wider distribution.

Experimental modifications of the histological structure of the adrenal cortex.—Investigations on the experimental modifications of the mammalian adrenal cortex have been numerous in recent years. Naturally, most of this investigation has centered upon the gland with the purpose of determining its varied functions. The problem of the exact role of the adrenal cortex has been attacked from many angles. In the space of this paper it is impossible to report on all of this investigation. We wish to point out some of the most striking work recently performed on the adrenal cortex and to indicate the main conclusions to be drawn from this work. In spite of contradictory points here and there, there is much of a definite character that seems to be well established.

The experimental modifications of the cortex has mainly involved two methods: (1) the effects produced in the cortex by the removal of other endocrine glands and (2) the effects produced by the injection of substances such as hormones and toxins as well as other things.

Considering the interrelations of the endocrine glands, it is quite logical that much of our knowledge of the adrenal cortex has arisen from the removal of other glands, or glandectomy, noting the effects of the removal. Hypophysectomy has been one of the most common. There is almost general agreement that such operations lead to marked atrophy of the adrenal cortex. Reese and Moon ('38) have shown that many changes in the cortex begin a few hours after this operation. This is especially the case with the Golgi apparatus which remains in a shrunken form for 300 to 400 days after hypophysectomy. Cutuly ('36), working on the same animals, rats, observed that the greatest atrophy occurred 30 days after the operation. Crooke and Gilmour ('38) observed in immature rats that this degeneration is most evident in the reticular zone where the cells were reduced in number and there was a marked infiltration of lymphocytes as well as an increased pigmentation. The lipoid content in the same zone was also reduced.

Simmons and Whitehead ('37) reveal that in cases of unilateral adrenalectomy the cortex of the unmolested adrenal shows a decrease in the fat density in the outer zone of the gland but an increase in the inner part. As regards gonadectomy and its effects on the cortex, Zalesky ('36) found that the operation performed before sexual maturity induces no permanent hypertrophy or atrophy in the guinea pig adrenal but does cause some increase in the lipoids of the cortex.

It has long been known that animals dying from adrenal deficiency show symptoms similar to those in severe toxemia. Moreover, animals which survive double adrenalectomy are more susceptible to various toxins than are the normal controls. Deanesly ('31) found that the injection of certain bacilli, morphine, and other toxins appears to have the same effect on the cortical zones as that of thyroxin. These changes are associated with enlargement of the cortex and a considerable decrease in the cortical fats and lipoids. Schmidt and Schmidt ('37) discovered that ingestion of thyroxin resulted in an increase of mitotic activity, but in this case the scene of the mitoses was confined chiefly to the *zona fasciculata* instead of being also in the *zona glomerulosa* as was the case with control animals.

A marked atrophy of the cortex is produced when large amounts of cortin are administered to an animal. Ingle ('38) points out that, when this is injected into a normal animal, the adrenal cortex decreases. If the hypophysis has been removed, however, and the animal receives the adrenotropic hormone, there is no apparent effect of cortin on the adrenal cortex. Reese and Moon ('38) state that the injection of the adrenotropic hormone causes hypertrophy of the Golgi apparatus in the cortex of the normal male rat. When this hormone is injected into the hypophysectomized animal, however, the hormone not only maintains the normal appearance of the apparatus but also causes it to hypertrophy. Many other investigators confirm this.

In many of these experimental modifications one thing is quite noticeable. Of the three zones, the cells of the reticularis are unusually sensitive to diseases and toxins. This may be due to the fact that these cells are senescent or dying and receive an enormous blood supply which brings them into contact with a large quantity of the noxious agencies.

A preliminary account of the authors' work on the adrenal cortex of the albino mouse.—Our own work has centered mainly upon one or two aspects of the physiological cytology of the adrenal cortex, especially upon cytoplasmic organelles. The theory has long been known in biology that there is a relation between certain cytoplasmic inclusions and the functions of the cell. Most of the attention in this respect has been directed to the organs of exocrine secretion where it has been possible to stimulate them to their optimum rate. In this way it is possible to follow the cells in their secretory cycles.

Only recently have the organs of endocrine secretions been so studied since little has been known of their secretory rate, their intrinsic secretory cycle, methods of bringing about optimum secretory activity, and their secretory products. But with the recent purification and the fairly accurate assay of endocrine material, the authors are endeavoring to repeat, using the adrenal cortex, the work of earlier exocrine cytologists.

The mouse adrenal was chosen as the optimum tissue, owing to its small size and the ease of its fixation in the entire condition. Cell structures are very sensitive to manipulation, and fixation of the much larger adrenals of the common laboratory animals is not feasible for most of this cytological work.

Experimental animals (adult albino mice) were given one Collip rat unit of the adrenotropic factor. The substance used for this was Ayerst and McKenna's growth complex, which contains growth substance as well as the adrenotropic factor. Since adult animals were used throughout, the growth factor can be ruled out as affecting our results. Controls were given a similar volume of standard saline solution.

Our results, to date, show, among other things, that the adrenal weights of the mouse bear no absolute constant relation to the body weight but may vary in the normal animal from 0.01% to 0.035% of the body weight. In all cases of experimental animals, the weights increased up to more than 0.06% by the fifth day following administration of the hormone. The average actual weight of adrenal material in the normal mouse is 5 mg. Actual weights increased in some cases as much as three times the normal by the fifth day after injection and in all cases had at least doubled by that time.

Gross histological examination reveals that the cortex had increased greatly in width in relation to the width of the entire gland. The increase seems to be confined mainly to the *zona reticularis* and *zona fasciculata*.[1]

Weight increases seem to be due to this augmented activity as well as to the appearance of more lipoids in the gland.

The glands were fixed according to the formalin-bichromate and other techniques, and observations to date reveal that there is an in-

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[1]Work on this point, using colchicine to determine the mitotic index of these regions, is being done at the Biological Laboratories, Harvard University, by the junior author.

crease in mitochondria in the reticularis and fasciculata zones, that there is an increase in the rod-like forms of mitochondria, that the granular forms appear to be swelled, and that the mitochondria is seen for the most part on the capillary side of the cell.

Further work to extend these preliminary findings is under way.

Conclusion.—At the present time we may briefly conclude that the mammalian adrenal cortex is a gland of continuous change as regards its histological and cytological make-up. It may be considered a complex of cells, which in their migration from capsule to medulla undergo a progressive change of cell types more or less localized into different zones. These cell types reveal differences in cytological details, as lipoids, cell inclusions of various kinds, size relations, etc., which are correlated with the stages in the life history of the cell. Moreover, the experimental modifications induced by such agencies as injections of toxins, and hormones, and by glandectomy are manifested chiefly in an accelerated or retarded differentiation of these cell relations and characters. But the picture is far from complete, and there are yet lacking many details which must be filled in before we can arrive at a sound morphological and functional interpretation of the adrenal cortex.

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