THE ZONA INTERMEDIA OF THE ADRENAL CORTEX. A CORRELATION OF POSSIBLE FUNCTIONAL SIGNIFICANCE WITH DEVELOPMENT, MORPHOLOGY AND HISTOCHEMISTRY

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INTRODUCTION

In the course of their work on the effects of castration upon the adrenal cortex of the rat, Hall & Korenchevsky (1937) described a 'demarcation zone', 1–4 cells wide, between the zonae glomerulosa and fasciculata of the normal animal. The cells of this zone, they claimed, contained little or no lipid in contrast to those either side of it; in castrated rats, however, this fat-free zone became lipid positive.

Earlier, Reiss, Bálint, Oestreicher & Aronson (1936–7), in presenting a method of assay for ACTH in the rat, described a sudanophobe zone as the outer part of the z. fasciculata; this fat-free zone, broader in hypophysectomized animals, disappeared when these were treated with ACTH. This work was later to be confirmed by that of Simpson, Evans & Li (1943).

Tobin & Whitehead (1941–2) described a sudanophobe zone immediately internal to an outer fat-laden zone in the rat's adrenal cortex. In describing the cells of this sudanophobe zone in the rat, Greep & Deane (1947) used the term 'transitional zone', while Mitchell (1948) preferred to call it the zone of 'compression' because the number of cells per unit area was increased within it in comparison with the rest of the gland. Other authors, while recognizing the zone, have spoken of it as the outermost part of the z. fasciculata (Dalton, Mitchell, Jones & Peters, 1943–4), or included it in with the z. glomerulosa (Harrison & Cain, 1947). Nicander (1952) referred to it as the 'intermediate zone' since the component cells appeared to him intermediate in type between those of the zonae glomerulosa and fasciculata in a large number of the domestic animals. Yoffey & Baxter (1947), examing rat adrenals for birefringent material, reported a region of optical inactivity between the zonae glomerulosa and fasciculata. Furthermore, Harrison & Cain (1947), in the rat adrenal, found cholesterol in the zonae glomerulosa and fasciculata, but not in the sudanophobe zone.

Cain & Harrison (1950), in the rat, described the cytological features of the sudanophobe zone as similar but weaker than those of the z. glomerulosa, and commented that while there was an obvious reduction in cytoplasm in the former zone, the nuclei of both zones were the same size. They postulated that a cell movement from the sudanophobe zone was predominantly outwards to the z. glomerulosa through intermediate stages, while sharp cytological differences between the sudanophobe cells and those of the z. fasciculata suggested little inward passage from the sudanophobe zone. This idea of the sudanophobe zone in the rat being a generative region

is contradicted by the work of Mitchell (1948), who described a mitotically inert zone of the rat cortex between the z. glomerulosa and z. fasciculata. Cater & Stack-Dunne (1953) observed mitoses throughout the rat cortex, but with the lowest incidence in the sudanophobe region. Hoerr (1931), studying the reactions to injury by chloroform narcosis in the guinea-pig, noted an increased mitotic count principally in the z. fasciculata, with only a few mitoses in the z. glomerulosa; but it must be added that Hoerr did not recognize a sudanophobe or intermediate zone. Dalton *et al.* (1943–4) regarded the sudanophobe zone as a region of lipid release; according to them fat-laden cells of the z. glomerulosa, migrating centripetally, became depleted of lipid in this sudanophobe zone. This belief was largely based on the conception of a capsular or subcapsular origin for cortical cells which then migrated inwards to degenerate in the z. reticularis (Zwemer, 1933–4; Zwemer, Wotton & Norkus, 1938; Bachmann, 1939; Wotton & Zwemer, 1943).

The incidence of the sudanophobe or intermediate zone is the subject of some considerable controversy. Thus Feldman (1951) described it as variable and inconstant in the rat. Harrison & Cain (1947), also in the rat, found the zone present in immature animals of both sexes and usually present in mature males, but absent in adult females, and male castrates. Cain & Harrison (1950) later reported a sudanophobe zone in 123 out of a series of 154 rat adrenals (from immature animals) examined by the acid haematein method. Tobin & Whitehead 1941–2), while claiming the presence of a sudanophobe zone in the rat adrenal, reported its absence in the glands of the mouse, guinea-pig and rabbit. Nicander (1952) described an intermediate zone in a wide variety of the domestic animals, but claimed it to be sudanophobe only in the horse, dog and rat; according to him it was weakly sudanophile in the mouse, guinea-pig and in cattle, and definitely sudanophile in the cat and rabbit. Knouff & Hartman (1951), although they claim a zoning into glomerulosa, fasciculata and reticularis of the cortex in the brown pelican, do not mention a z. intermedia.

Mitchell (1948), describing the development of the rat adrenal, observed a 'zone of compression' just internal to the glomerulosa during the first postnatal week; by the second week the cells of this zone were basophilic and could be clearly seen interposed between glomerulosa and fasciculata.

Cain & Harrison (1950), in the rat, claimed that the sudanophobe zone was less vascular than the z. glomerulosa. Earlier, Popják (1944) had observed a hyperaemic zone between z. glomerulosa and z. fasciculata in rat adrenals, 24 hr. after leg crush injuries. However, Bennett & Kilham (1940), observing a narrowness of the capillaries in the z. fasciculata in the cat, believed this to be due to compression by the parenchymal cells of the zone which were turgid with lipid. Lever (1954), after intravital ink injections into the left ventricle in rats, demonstrated a zone of comparatively poor capillary filling corresponding to the z. intermedia plus a variable width of the outer fasciculata. He contends that the sharp outer edge of this zone corresponds to the external limit of the z. intermedia, while the inner edge, more irregular, probably corresponds to a succession of points where the intracellular lipids of the z. fasciculata are reduced in amount.

It is generally agreed that the sudanophobe zone in the rat becomes greatly widened following hypophysectomy. Lever (1954) demonstrated a linear increase with time in the extent of the intermediate or sudanophobe zone after hypophysectomy.

Following ACTH treatment in hypophysectomized animals, Reiss *et al.* (1936–7) and Simpson *et al.* (1943) described a re-establishment of the normal orderly arrangement of zones in the adrenal, a fact employed by them in their methods of assay of ACTH.

In this paper the term z. intermedia is used to indicate the anatomical location of a zone which may be present between the zonae glomerulosa and fasciculata. As will be shown later, it can be both absent or very clearly defined in the rat adrenal under different physiological conditions.

MATERIALS AND METHODS

A. *Comparative morphology*

In an attempt to find a homologous arrangement to the intermediate and sudanophobe zones described in some mammalian adrenals, the non-mammalian adrenal was studied in two of each of the following animals: the domestic fowl, missel-thrush, grass-snake, adder, crocodile, green lizard, salamander (*Salamandra maculosa*), common newt and frog (with the exception of the fowl, all were male animals). One adrenal was fixed in Susa and stained with haematoxylin and eosin, and the other was fixed in 4% neutral formaldehyde, freeze-cut, and stained with Sudan black for lipids. In the salamander and newt the diffuse arrangement of the glands along the ventrimedial borders of the kidneys makes separation difficult, and left and right adrenals were examined together.

In a short series of some readily available mammals (Tables 1 and 2), left-sided adrenals were stained with Sudan black for lipid and right-sided adrenals were stained with haematoxylin and eosin.

B. *Development and age changes in the rat's adrenal*

Forty-eight white Wistar rats (from the same stock as used for the histochemistry and experiments below) were examined. These included eight pregnant rats and the foetuses, two rats at birth, and at 1, 2, 3 and 4 days after birth; one rat at 5, 6, 7, 8, 10 and 14 days; a male and female rat at 3, 4, 5, 6, 7 and 8 weeks; male rats at 3, 4, 5, 6, 9 and 12 months. One adrenal from each rat was fixed in Baker's calcium-formol saline, and frozen sections cut for lipid studies. The other adrenal was fixed in Susa, paraffin embedded, and serial sections were stained with haematoxylin and eosin. For the mitotic counts, representative mid-sections were projected at a magnification $\times 100$ and drawn on squared paper. The sections were examined with a 2 mm. objective for mitotic figures, the positions of which were plotted on to the projection. The stage of each mitotic figure was also noted. Three adjacent sections were read, and the results of all three were recorded on the one projection to avoid errors of sampling.

C. *Histochemistry of rat's adrenal*

This was studied on rats treated as follows: normal (21), castrated 14 days (4), hypophysectomized (105), hypophysectomized and castrated (8). The hypophysectomized rats were studied at various times after operation: after 1 day (20), 2 days (18), 14 days (18), 19 days (1), 21 days (24), 24 days (5), 28 days (5), 35 days (1),

42 days (1), 49 days (1), 56 days (1), 67 days (4), 117 days (3). Half the hypophysectomized and castrated rats were examined after 14 days and half after 21 days. The two adrenals from each rat were separately treated to increase the range of histochemical study, which included the techniques listed below:

(1) *Lipids.* One hundred and thirty-three adrenals, fixed in Baker's calcium-formol saline, were cut with a freezing microtome; one section was stained with Scarlach R, one with Sudan black, and one examined unstained with the polarizing microscope.

(2) *Schultz's method.* Thick (25 μ) frozen sections of calcium-formol-saline-fixed material (103 adrenals) were examined by a modified Schultz's method for cholesterol.

(3) *Baker's acid-haematein method* (1946) was used on ten adrenals to demonstrate phospholipids.

(4) *Nile blue* (Cain, 1950). Ten adrenals were examined by this technique after post-chroming.

(5) *Plasmal reaction.* Ten adrenals were rapidly frozen and kept at $-20°$ C. until required. Unfixed frozen sections were obtained by using a knife cooled with CO_2 snow. They were dropped into the plasmal reagent (equal parts of (*a*) Schiff's solution half diluted with bisulphate water and (*b*) saturated 7% aqueous mercuric chloride solution). After washing with sulphurous acid and distilled water, they were mounted in glycerine jelly. Control sections were stained in Schiff's solution.

(6) *Nucleic acids.* Ten adrenals fixed in Susa were examined by the Feulgen method for desoxyribonucleic acid (Feulgen & Rossenbeck, 1924) and by pyronin-methyl green mixtures for ribose nucleic acid. Unfortunately, ribonuclease was not available.

(7) *Periodic acid-Schiff* (P.A.S.) *method* (McManus, 1948). Twelve adrenals fixed in ice-cold 80% ethanol were examined by this method. Freeze-dried vacuum embedded material was also examined.

(8) *Ascorbic acid.* Thirty adrenals were treated by the method of Barnet & Bourne (1941–2). Six rats hypophysectomized 24 hr. previously had the left adrenal removed and fixed in silver-acetic acid. The rats were then injected intravenously with a high, medium, or low dose of ACTH (Armour batch 84–85 H., 1·6, 0·1 or 0·006 μg./100 g. rat respectively). After 1 hr. the right adrenal was removed and placed immediately in the same fixative. In another experiment, nine hypophysectomized rats were injected with ACTH and 1 hr. later both adrenals were removed so that both the ascorbic acid and lipoid distribution could be compared with control adrenals (five hypophysectomized, and five normal rats).

(9) *Alkaline phosphatase.* Ten adrenals fixed in ice-cold 80% ethanol, and five adrenals freeze-dried and vacuum embedded, were examined by the Gomori technique (Danielli, 1946).

(10) *Acid phosphatase.* Six adrenals fixed in ice-cold absolute acetone and five freeze-dried adrenals were examined by the Gomori (1941) technique.

(11) *Esterase.* Six adrenals fixed in ice-cold absolute acetone, five freeze-dried adrenals, and two unfixed adrenals cut by the cold-knife method, were examined by the technique of Nachlas & Seligman (1948–9).

D. *Rat adrenal in various physiological and experimental conditions*

Rats used for studying (i) the effect on the adrenal of hypophysectomy, and (ii) the effect of ACTH on the adrenal ascorbic acid distribution, have been detailed above. The lipid distribution $1\frac{1}{2}$, 4, and 8 hr. after intravenous injection of ACTH (at three dose levels as above) was studied on eighteen rats hypophysectomized 2 days previously. In each case the left adrenal was removed as a control before the injection of ACTH. Twenty rats were used to study the effect of castration on the adrenal. In addition to histochemical examination of these adrenals as outlined above, routine histological examination was made of seventy-one adrenals, fixed in Susa and stained with haematoxylin and eosin.

RESULTS

A. *Comparative morphology*

(1) *Observations on some non-mammalian adrenals*

It is not claimed that a full examination of the adrenal cortical homologues was made, but such material as was available provided useful information.

The frog adrenal. The cortical elements are arranged in short irregular cords extending into the gland from the deep surface of the capsule (Pl. 1, fig. 1). The cells at the capsular end of the cords have an eosinophilic, homogeneous cytoplasm and a round to oval reticular nucleus with a nucleolus. The cells next encountered appear crowded and may constitute a zone of cell-compression. They have spindle-shaped nuclei, elongated at right angles to the axis of the cord, and their cytoplasm is scanty and eosinophilic. Immediately internal to these cells, the cord consists of larger cells with round reticular nuclei and foamy cytoplasm reminiscent of the spongiocyte cells in the mammalian outer z. fasciculata. These appearances in the frog are strikingly parallel to those seen in the outer cortex of certain mammals, e.g. the rat (Pl. 1, fig. 3). The presence of what appears to be a z. intermedia in a non-mammalian adrenal is of great interest. Lipoid studies on the frog adrenal (Pl. 1, fig. 2) are also suggestive of a zoning in the cortical cords. While the spongiocytes in the middle of the cortical cords have a high lipid content, the cells at both the outer and inner ends of the cords have a relatively low lipid content. In examination of lipoid preparations it should be remembered that the cells of the medullary homologues lie between the groups of cortical cells, contain no lipids, and may appear as unstained interruptions in a region of stained tissue.

Urodele adrenal. In the rather diffuse adrenals of the Urodeles no zoning could be observed in the loose masses of cortical cells, in either cytological or lipoid preparations.

Reptilian and Avian adrenals. In the reptilian gland there is no cytological zoning within the branching columns of cortical cells. While the adrenal of the domestic fowl has a cortical arrangement very similar to the reptilian gland, it must be added that pyknotic nuclei are more common in the central than in the peripheral portions of the cortical-cell cords. This is interesting in view of the work of Knouff & Hartmann (1951) who described zonae, glomerulosa, fasciculata, and reticularis in the adrenal of the brown pelican.

(2) *Observations on the zona intermedia of some mammalian adrenals*

(*a*) *Cytological comparisons.* In the following comparative study, a z. intermedia is judged to be present if there is interposed between the zonae glomerulosa and fasciculata a tissue layer, one or more cells thick, which is clearly unlike either of these zones. Differences of cell size, nuclear shape, and cytoplasmic staining and appearance, were collectively or individually, the distinctive criteria.

In view of the theory of a capsular origin for cortical cells and their centripetal migration to the z. reticularis (Zwemer, 1936; Zwemer *et al.* 1938; Wotton & Zwemer, 1943) the cellular arrangement of the capsule and the z. glomerulosa was studied. In some instances (cow, sheep, ox) there is no very sharp distinction between glomerulosa cells and those in the deeper layers of the capsule, while in others (horse, cat, mouse, rat and rabbit), the distinction is obvious.

The cell arrangement in the z. glomerulosa is very variable in different animals; thus in the horse, sheep and mouse, single columns or double columns with arched ends, are observed (Pl. 1, fig. 4), while in the cat and ox the cells are arranged in small clusters or rosettes (Pl. 1, fig. 5; Pl. 2, fig. 11). In other animals (pig, cow, guinea-pig) no one cell arrangement predominates and this can be described as diffuse.

All mammalian material was examined with particular reference to the intermediate region between the z. glomerulosa and the z. fasciculata, and the results of this comparative study of the z. intermedia are summarized in Table 1.

The z. intermedia is most commonly displayed as a region where the number of cells per unit area is greater than in other zones of the cortex (Pl. 1, figs. 5–7). This is probably not an invariable feature of the zone as is indicated by the example of the cow in Table 1. Again in some adrenals, notably those of the rat, sheep and ox, the cytoplasm of the z. intermedia is often markedly basophilic (Pl. 1, fig. 8), while in other animals (cat, guinea-pig, horse) this is not so.

The z. intermedia appears to constitute a region of cellular re-arrangement and change from the glomerulosal to the fasciculate patterns. In many animals the nuclei in the zonae glomerulosa and intermedia are similar, even though there may be cytoplasmic reduction in the latter zone. However, in the z. intermedia of the horse, rabbit and sometimes of the pregnant rat, a flattening is observed of both cell and nucleus, at right angles to the radius of the gland (Pl. 1, fig. 3). Flattened cells without nuclei in the plane of the section are also seen, suggesting a real spread of the cell cytoplasm (Pl. 1, fig. 9). These observations may indicate cell compression, as suggested by Mitchell (1948) in the rat, though he did not report nuclear flattening.

From a study of Table 1 the following observations can be made: (*a*) In the rat, cat, dog, sheep, rabbit and horse where a definite z. intermedia is clearly present, the cell arrangement of the z. glomerulosa, in addition to being typical for each animal, is regular (Pl. 1, figs. 4–7). (*b*) Where the arrangement of the z. glomerulosa is diffuse, or irregular, then the z. intermedia is either, not detectable as in the pig, or not well demonstrated as in the cow and guinea-pig (Pl. 1, fig. 10). The ox and mouse adrenals do not fit into these generalizations. The ox adrenal, though it has a fairly obvious z. intermedia does not show a very regular arrangement in the z. glomerulosa (Pl. 2, fig. 11). In contrast, the mouse adrenal, though possessing

a regular form in the z. glomerulosa, does not have an obvious z. intermedia (Pl. 2, fig. 12). However, the cell count in the mouse adrenal shows an increase per unit area between 64 μ and 95 μ under the capsule, and the inner edge of the z. glomerulosa lies at 70 μ below the capsule (as measured with a micrometer eyepiece).

Table 1. *A comparison of the zona intermedia in some common mammals*

Column 2: the presence of a z. intermedia is indicated by a +. When the zone is particularly well developed this is shown by ++. Column 3: each haematoxylin and eosin section was projected on to $\frac{3}{4}$ in. squared paper at a magnification of $\times 576$ and nuclear counts were made for a variable number of squares from the capsule into the z. fasciculata. An increase in the cell count over a limited area in the outer cortex is indicated by +. (In the horse adrenal a variable amount of connective tissue between the columns of outer cortical cells rendered a cell count valueless.) Column 4: indicates the distance in μ beneath the capsule of the outer and inner margins of the square in which the maximum cell count was observed. Column 5: indicates the distance in μ from the capsule to the centre of the z. intermedia (mean of eight measurements on camera lucida tracings). When these values lie within the range indicated in column 4 there is a greater significance in a positive rise in the cell count. Column 7: + indicates a detectable amount of lipids; ++ indicates larger quantities of lipids.

Animals and number used (1)	Detected incidence of z. intermedia (2)	Cell count result (3)	Range in μ below capsule of maximum cell count (4)	Centre of z. intermedia below capsule in μ (5)	Arrangement of cells in z. glomerulosa (6)	Z. intermedia lipids present or absent (7)
Dog 1	++	+'ve	190 230	200	Regular	−
Horse 2	++	Unreadable		220	Regular	−
Rat 12	++	+'ve	64 95	80	Regular	−
Rabbit 4	++	+'ve	130 160	120	Regular	++
Sheep 2	++	+'ve	190 230	200	Regular	+
Ox 1	+	+'ve	130 160	150	Equivocal	+
Cat 3	+	+'ve	64 95	80	Regular	++
Guinea-pig 2	+	+'ve	34 64	40	Diffuse	+
Cow 2	+	−'ve	.	160	Diffuse	+
Mouse 2	−	+'ve	64 95	.	Regular	+
Pig 1	−	−'ve	.	.	Diffuse	.

(*b*) *Comparison of lipoid distribution in outer cortex.* Table 2 shows the lipoid distribution, as indicated by Sudan black, in the same range of domestic animals. It is noteworthy that in the rabbit and cat, where the z. intermedia is strongly sudanophilic, that the glomerulosal lipids are sparse (Pl. 2, fig. 13); while in the rat, dog and horse in which the z. intermedia is sudanophobe, the glomerulosal lipids are usually present in large quantity (Pl. 2, figs. 14, 21 and 22). Furthermore, in the cow, ox and sheep small quantities of lipid are equally present in the zonae glomerulosa and intermedia, while in the mouse and guinea-pig there is an upward lipoid gradient from the z. glomerulosa through the z. intermedia to the outer z. fasciculata. In the pig the z. glomerulosa appeared completely devoid of lipid in contrast to the heavy content of the z. fasciculata. It will be remembered that the z. intermedia was not detected in the pig adrenal.

Table 2. *A comparison of the lipoid distribution in the outer adrenal cortex of some common mammals*

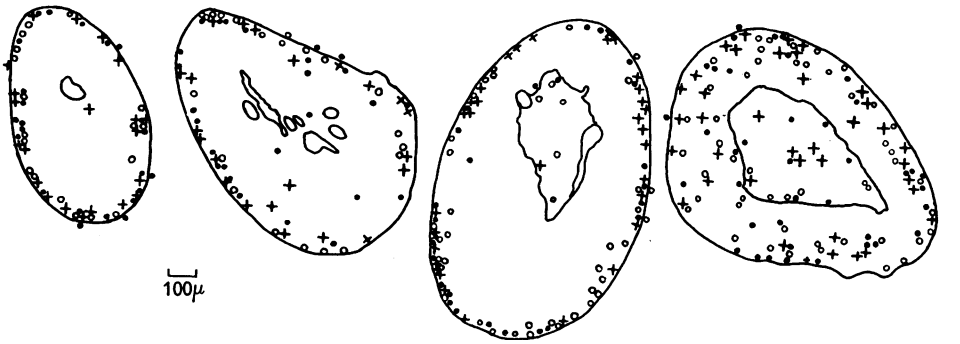
The lipids were visualized by staining with Sudan black. A single + indicates a definite but light lipoid distribution, while ++ indicates a heavy lipoid distribution. Absence of lipid is denoted by −. *Sparse* is a term used to denote an almost fat-free region which however contains a very few lipoid droplets.

Animal	Z. glomerulosa lipids	Z. intermedia lipids	Outer z. fasciculata lipids
Dog	++	−	++
Horse	++	−	++
Rat	++	−	++
Cat	Sparse	++	++
Rabbit	Sparse	++	++
Cow	+	+	++
Ox	+	+	++
Sheep	+	+	++
Guinea-pig	Sparse	+	++
Mouse	Sparse	+	++
Pig	−		++

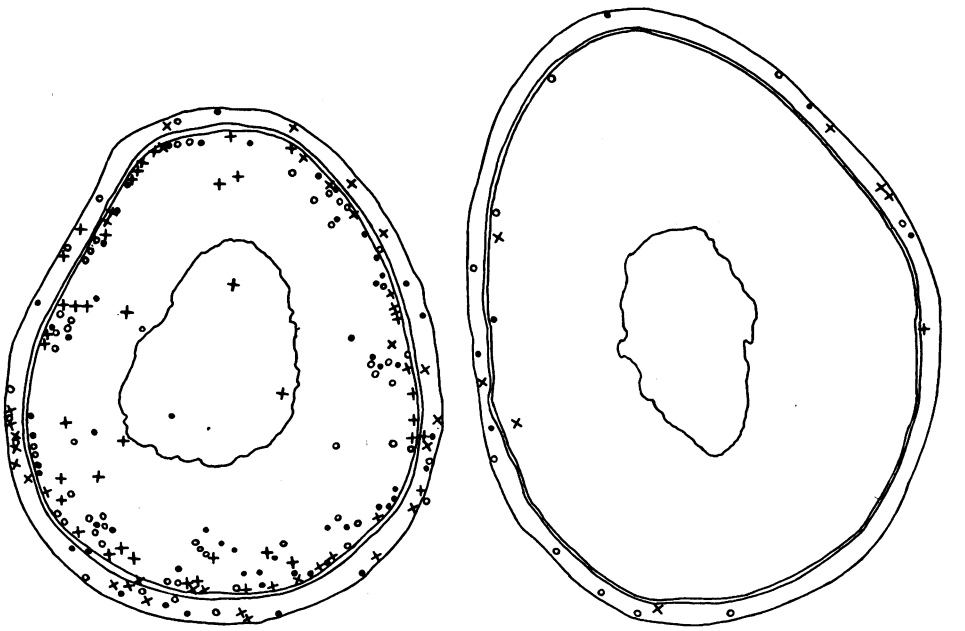
B. *Development and age changes in the rat's adrenal*

The earliest stages of development studied were the adrenals of 1 and 1·8 g. embryos (approximately 16th and 18th days of pregnancy respectively). In these, mitotic figures are present in the gland capsule. Beneath the capsule there is a layer 1 to 3 cells thick containing numerous mitotic figures (Text-fig. 1). The cells of this layer have a small amount of basophilic cytoplasm containing a little lipid. The remainder of the adrenal cortex consists of a zone of larger lipid-laden cells (undifferentiated zona fasciculata) enclosing a central mass which is the foetal cortex. The cells of this foetal cortex are large, eosinophilic and fat-free (Pl. 2, figs. 15, 16). Mitotic figures are present in the outer part of the z. fasciculata in 1 and 1·8 g. embryos. In the 4·5 g. embryo (approximately 20th day of pregnancy) the outer zone of dark-staining cells beneath the capsule is more obvious, but the mitotic figures are more widely scattered and include some in the deeper layers of the cortex (Text-fig. 2). The foetal cortex is no longer seen, but the medulla is forming as a loose central mass by the aggregation of islands of chromaffin cells. At birth (5–6·5 g. rats) there is little lipid present in the outermost zone of the cortex, but by the 3rd day (9 g.) a lipid-containing z. glomerulosa and a lipid-free z. intermedia are recognizable (Pl. 2, figs. 17, 18; Text-fig. 3). The lipid-laden but undifferentiated z. fasciculata internal to the z. intermedia exhibits a fasciculate pattern at 7 days. At about the 10th day most of the lipid in the z. fasciculata is in its outer layers and there is a lipid-free zone bordering on the medulla (Pl. 2, fig. 19; Text-fig. 4). The z. reticularis is apparent by the 21st day and about this stage the z. intermedia appears narrower. The typical pattern of zones as seen in the adult is present by about the 35th day. Between the 28th and the 35th day the mitotic activity in the adrenal declines rapidly in this series (compare Text-figs. 5 and 6). There is no indication that the z. intermedia is the mitotic zone of the cortex; on the contrary, mitotic figures can be found in all zones and in the capsule. In Text-fig. 5 there is one mitosis in the capsule, forty-seven in z. glomerulosa, three in z. intermedia, ninety in the outer part of z. fasciculata, forty-nine in the inner part of z. fasciculata and four in the z. reticularis.

Age changes in the rat's adrenal. From the 2nd to the 4th month the z. intermedia becomes less distinct or may be altogether absent (Pl. 3, fig. 29). In the lipid preparations at this age, the zone is usually sudanophile (Pl. 2, fig. 20). However, by the 6th month the z. intermedia is again usually sudanophobe, and in addition can



Text-fig. 1. Text-fig. 2. Text-fig. 3. Text-fig. 4.



Text-fig. 5. Text-fig. 6.

Text-figs. 1–6. Mitotic activity in rat adrenal cortex at different ages. Each fig. represents the readings of three consecutive sections; key: 1st section, +; 2nd section, •; 3rd section, ○. Scale: 1 cm. = 280μ. Text-fig. 1, 1·8 g. foetus; Text-fig. 2, 4·5 g. foetus; Text-fig. 3, 9 g. male, 3 days old; Text-fig. 4, 14 g. male, 10 days old; Text-fig. 5, 54 g. male, 28 days old; Text-fig. 6, 57 g. male, 35 days old.

once more be identified in haematoxylin and eosin preparations by the small size of its cells. The z. intermedia becomes increasingly well defined with age (Pl. 2, figs. 21, 22) so that in the senile rat adrenal it is a region of marked cell flattening (Pl. 3, fig. 30). The above description applies particularly to the male rat. Our

observations (though limited) on the female rat indicate the presence of a z. intermedia in haematoxylin and eosin preparations from all age groups. Corresponding lipid studies indicate that the zone contains a few fine lipid droplets in the newly matured rat (2–6 months), but is lipid free in younger and older female rats.

C. *Histochemistry of the adult rat's adrenal*

Lipids. The z. intermedia is usually not stained (see above) by the Sudan stains Sharlach R and Sudan black, but when it is sudanophile the polarizing microscope shows no anisotropic lipid and the Schultz method for cholesterol is negative (Pl. 3, fig. 23). It must, however, be noted that neither of these tests is sufficiently sensitive to detect small quantities of cholesterol. The Baker method for phopholipids shows heavy staining in the z. fasciculata and some in the z. glomerulosa, but the z. intermedia is unstained. In the adrenal of the hypophysectomized rat the much wider z. intermedia or sudanophobe zone, forms a conspicuous unstained band in sections stained by the Schultz method (Pl. 3, fig. 24), and the Baker method (Pl. 3, fig. 25).

The plasmal reaction. Fresh unfixed frozen sections of adrenals, placed in a mixture of Schiff's reagent and mercuric chloride, gave a strongly positive plasmal reaction in the z. glomerulosa and z. intermedia (control sections placed in Schiff's solution were unstained). Similar results were obtained with the adrenals from rats hypophysectomized 14 days previously, in which these zones are relatively much wider. This finding must be accepted with caution. It is not easy to obtain satisfactory frozen sections of unfixed tissues cut by the cold-knife technique. It is possible that diffusion and smearing artefacts can occur in the unfixed tissues. On the other hand, it is equally possible that soluble materials may be leached out of the z. intermedia by aqueous fixatives or inactivated by formaldehyde. The phenylhydrazine reaction, introduced by Bennett (1940), for ketosteroids, probably stains the plasmals of the adrenal cortex (Gomori, 1942, 1952). Rogers & Williams (1947) calculated that the quantity of ketosteroids in the adrenal cortex is too small to be demonstrated histochemically. Pearse (1953), reviewing the subject, suggests that formalin fixation or auto-oxidation in air, liberates aldehydes from the unsaturated fats in the adrenal cortex, and that these aldehydes give both the plasmal and phenylhydrazine reactions. Because of these doubts concerning the reactions for ketosteroids none are described here.

Fixation artefacts. The possibility was considered that the small size of the cells in the z. intermedia and their sudanophobe nature might be due to the loss of soluble substances from their cytoplasm during fixation. In freeze-dried vacuum-embedded sections of the normal and hypophysectomized rats' adrenal, the cells of the z. intermedia are still small relative to those of the adjacent zones. Unfixed frozen sections, stained with Sudan black or examined with the polarizing microscope, show no definite evidence of lipids in the z. intermedia. Each of these methods is open to criticism, and all that can be said is that no positive evidence has been obtained to date to indicate that the z. intermedia is a fixation artefact. An intravital perfusion experiment was therefore performed, 5–8 ml. of 0·5% methylene blue in citrated normal saline being perfused into the femoral veins of rats. Death, due to circulatory failure, occurred in 10–20 min. and the adrenals were immediately

freeze-cut (without fixation) and photographed. Pl. 3, fig. 26, clearly indicates that the methylene blue has stained the z. glomerulosa and z. fasciculata but has not stained the z. intermedia. The experiment was repeated in the rabbit, in which the z. intermedia is found to be sudanophil; the zone was heavily stained by the methylene blue.

Pyronin-methyl green stain. The cells of the z. glomerulosa and z. intermedia stain with pyronin, and this confirms the basophilia of these cells seen after staining with haematoxylin and eosin. Study of the adrenals of rats hypophysectomized 2 weeks previously, confirms that the cells which stain strongly with pyronin are confined to the z. glomerulosa and intermedia (Pl. 1, fig. 8).

The periodic acid-Schiff (P.A.S. *method*). No more than traces of P.A.S. positive material are seen in adrenals of normal or hypophysectomized rats either in alcohol fixed or freeze-dried material.

Ascorbic acid. In both control, and ACTH-treated adrenals, the outer cells of the z. glomerulosa are practically free from the silver granules which indicate the presence of ascorbic acid. Intracellular silver granules are present in the inner part of the z. glomerulosa, but they are larger and more numerous in the z. intermedia (Pl. 3, fig. 27). In the cells of the z. fasciculata the silver granules are very large in the control adrenals, but after medium and large doses of ACTH become reduced in number and smaller in size. In the outer part of the z. fasciculata the capillaries show numerous fine silver granules lining their walls and in their lumina, presumably indicating that the ascorbic acid is leaving the cells (Pl. 3, fig. 28). This appearance occurs in some of the control adrenals in the z. reticularis and innermost portions of the z. fasciculata, but is only seen in the outer fasciculata after treatment with ACTH. The silver granules in the z. intermedia do not appear to be altered by treatment with ACTH in this experiment. Our findings are similar to, but not identical with, those of Deane & Morse (1948), and Greep & Deane (1949).

Enzymes. These histochemical reactions for acid phosphatase, alkaline phosphatase, and esterase, did not outline the z. intermedia and distinguish it from the adjacent zones. The staining for these enzymes is, however, rather stronger in the zonae glomerulosa and intermedia than in the z. fasciculata, but with these techniques only well-marked differences probably have any significance.

D. *Rat adrenal in various physiological and experimental conditions*

The zona intermedia in the male rat is more obvious than in the female rat of corresponding age, this observation concurs with that of Greep & Jones (1950). Castration of the male rat results in an adrenal simulating the female type. In both female and castrated male adrenals the z. intermedia can still be distinguished in haematoxylin and eosin preparations. Marked flattening of the cells and nuclei in the z. intermedia is frequently seen in adrenals from rats in late pregnancy (Pl. 1, figs. 3, 9).

One effect of hypophysectomy, as is well known, is an increase in the width of the z. intermedia and the z. glomerulosa. A major part of this change is undoubtedly due to the shrinkage in size of the adrenal (most marked in z. reticularis and z. fasciculata) which greatly reduced the surface area of the ellipsoid over which the peripheral zones are spread. Until the 3rd week after hypophysectomy the z. intermedia

still shows a greater number of cells per unit area than the other parts of the cortex, but later than this, shrinkage of cells throughout the cortex results in homogeneity of cell size in the outer three zones (Lever, 1954).

The changes following hypophysectomy plus castration are essentially those which follow hypophysectomy alone. Thus after 2 weeks, the adrenal of the *castrated* hypophysectomized rat appears identical with that of the hypophysectomized rat (Pl. 1, fig. 8).

In the hypophysectomized rat treated with ACTH, the changes in ascorbic acid distribution (see section C) can be demonstrated within the hour. Changes in the lipid distribution are more difficult to assess. Rats hypophysectomized 48 hr. previously showed a slight depletion of adrenal lipids 4½ and 8 hr. after high dosage with ACTH. Changes were not observed in the z. intermedia. Reiss *et al.* (1936–7) and Simpson *et al.* (1943) describe the re-establishment of the normal orderly arrangement of zones in the adrenal after treating hypophysectomized animals with ACTH. Cater & Stack-Dunne (1953), however, found that the adrenals of rats hypophysectomized 2 weeks previously and treated for 4 days with ACTH, or growth hormone, could be distinguished from the adrenals of normal rats. The glands from such treated animals do not show a sudanophobe zone in lipid preparations, nor is the z. intermedia apparent in haematoxylin and eosin preparations.

DISCUSSION

From the present findings a well-defined z. intermedia is apparently associated with a z. glomerulosa of regular form (columnar or rosette), while in adrenals where the z. glomerulosa is a diffuse cell collection, as in the cow (Pl. 1, fig. 10), the z. intermedia is ill defined. A possible suggestion is that the z. intermedia interposed as it is, between glomerulosa and fasciculata (each with their distinctive cell groupings), constitutes a region of cellular re-arrangement. Thus it would not be well defined in glands in which the cells of the outer layers of the cortex are diffusely grouped.

Cell zoning in the frog adrenal cortical tissue was originally suggested by Stilling (1898): in the cortical cell columns just internal to the rounded peripheral cells, he observed cell flattening at right angles to the column axis: he strikingly described these flat cells as 'stacked like logs'. At variance with both Stilling's description, and the present observations, is the account of the frog adrenal by Sluiter, Mighorst & van Oordt (1949); they refer only to one type of 'interrenal' cell which they claim is filled with lipid. Grynfeltt (1904) observed less lipid in the peripheral than in the deeper cortical cells of the frog adrenal; and Bulliard, Maillet & Droz (1953) reported that after ACTH treatment in the frog, lipid depletion was maximal in the peripheral cortical cells.

The presence within the cortical cell columns of the frog adrenal of a region of flattened cells and nuclei, akin to those of the mammalian z. intermedia, suggests that this zone is not only a property of the compact concentrically layered adrenal cortex. Mitchell (1948) claimed that the flattening of cells in the rat z. intermedia is due to compression, but he was not specific as to the cause of the compression. Cell flattening and smallness of cell size though usual, are nevertheless not invariable features of a z. intermedia, as already indicated. It is a significant fact that in the pregnant rat adrenal, in which the z. fasciculata is wider than normal, there is often

marked flattening of cells between the zonae glomerulosa and fasciculata (Pl. 1, figs. 3, 9). A hypertrophy of the z. fasciculata relative to the other zones is likely to increase the tension within the gland, and cell flattening may be an expression of this. Fluctuations of cell lipid content and hence cell size in the z. fasciculata of the normal rat adrenal have been claimed as physiological (Cain & Harrison, 1950). If this is true, then the presence of real cell flattening in the z. intermedia may be conditional on the degree of cellular distension in the z. fasciculata.

The view that an increased number of cells per unit area in the z. intermedia may be due to excessive mitotic activity within the zone, is probably untenable. Mitoses in the zone, though present, are few in number (Cater & Stack-Dunne, 1953). The possibility that the z. intermedia may be the product of rapid cell division at the inner edge of the z. glomerulosa or outer edge of the z. fasciculata must be considered, particularly as these are the regions of maximum mitotic activity in the cortex (Mitchell, 1948; Cater & Stack-Dunne, 1953). If an inward passage of cells from the z. glomerulosa is responsible for the presence of a z. intermedia, this would support the popular theory of a centripetal migration of cortical cells.

There is no doubt that the cells of the z. intermedia are subject to the influence of the anterior pituitary. It is interesting to note that when hypophysectomized rats are treated with ACTH the fat-free cells of the z. intermedia become filled with fat, and if treatment is continued they become indistinguishable from the lipid-laden z. fasciculata cells. In this connexion it should be restated that the z. intermedia is usually not clearly identifiable, either histologically or histochemically, in rat adrenals of both sexes between the 6th and 26th weeks (approximately). During this time the animal reaches sexual maturity. After the 26th week, and increasingly with old age, the zone is more clearly defined. It is suggested that the level of anterior pituitary activity is a factor controlling the presence or absence of cells constituting a z. intermedia. Greep & Jones (1950), observing the effects of androgens and oestrogens on the adrenals of intact and gonadectomized rats of both sexes, conclude that androgens probably favour the formation of a z. intermedia ('transitional zone') but are not an essential condition, since the zone is present in the adrenals of untreated spayed females.

The present findings on lipid distribution in the adrenals of the common domestic animals largely confirm those of Nicander (1952). A rigid claim that the z. intermedia is invariably fat-free or fat-filled in any animal is probably unwise, as our experiments on the rat have indicated. The presence or absence of lipid in the zone may depend, among other things, on the level of pituitary stimulation of the adrenal.

The z. intermedia, in normal adult male rats, constitutes a zone of capillary compression with a sharp outer edge corresponding to the inner limit of the z. glomerulosa (Lever, Cater & Stack-Dunne, 1953; Lever, 1954). Clearly this capillary compression is directly proportional to the degree of cell packing within the z. intermedia. In rats hypophysectomized several weeks previously, the adrenal cortex with a high degree of cell packing is much less vascular than normal. The question arises as to whether the action of the anterior pituitary on the cells of the outer layers of the cortex does in fact alter the degree of compression in the z. intermedia and thus exercise some control over the capillary blood flow. In this connexion it must be

mentioned that the *arteriae corticis* (Flint, 1900) arising from the subcapsular arterial plexus in the rat, most commonly join the basic capillary bed in the outer z. fasciculata and the z. glomerulosa (Gersh & Grollman, 1941; Lever, 1952). Fine nerves, with occasional boutons, have been described in relation to vessel walls in the outer layers of the rat adrenal cortex (Lever, 1952). It is probable that some, at any rate, of these nerves provide a vaso-motor control over the *arteriae corticis*. The suggestion is made that in the rat at any rate, sudden alterations in the blood flow through the outer layers of the cortex may be effected by alterations in the calibre of the *arteriae corticis*, while a basic and slower control is exercised by the degree of capillary compression within the z. intermedia and outer z. fasciculata. This last form of control is probably under the influence of the anterior pituitary.

SUMMARY

1. The comparative morphology of the adrenal cortex is described in certain Amphibia, reptiles, birds and common domestic and laboratory mammals, with special reference to the presence or absence of the z. intermedia.

(*a*) The frog adrenal shows some zoning; in other amphibians, reptiles and birds there is none.

(*b*) In the rat, cat, dog, sheep, rabbit and horse, in which the z. glomerulosa has a regular form, the z. intermedia is well defined. In the guinea-pig, mouse, cow and pig it is not readily seen. The zone is usually sudanophobe in the rat, dog and horse, but contains lipids in the other mammals studied.

2. In the developing rat's adrenal the z. intermedia is present by the 3rd day after birth. It is prominent and sudanophobe up to the 6th week, but between 6 weeks and 6 months it is less prominent and may contain lipids. After the 6th month it becomes increasingly prominent as the rat ages. At no stage of development is there any concentration of mitotic figures in the z. intermedia. The z. glomerulosa and the outer part of the z. fasciculata appear to be the chief sites of cell division. Between the 28th day and the 35th day mitotic activity becomes much reduced.

3. A histochemical study of the rat's adrenal does not suggest that the z. intermedia is a fixation artefact. The zone is usually free from lipids, and cholesterol, is not outlined by special phosphatase or esterase activity, but the cytoplasm of its cells is stained with pyronin. Ascorbic acid is still present in the cells of the z. intermedia 1 hr. after treatment with ACTH but ascorbic acid is leaving the cells of the z. fasciculata and entering the capillaries.

4. The changes in the z. intermedia are described after hypophysectomy, castration, and treatment with ACTH.

5. The role of z. intermedia in the adrenal cortex is discussed with special reference to the capillary circulation.

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EXPLANATION OF PLATES

PLATE 1

Fig. 1. Frog adrenal: 7 μ haematoxylin and eosin preparation, $\times 450$. From left to right note: (i) the capsule; (ii) glomerulosal groups of cells with round nuclei; (iii) a layer of flattened cells with spindle-shaped nuclei; (iv) a spongiocyte layer of vacuolated cells. A few medullary cells are seen as darkly stained areas intermixed with the spongiocytes.

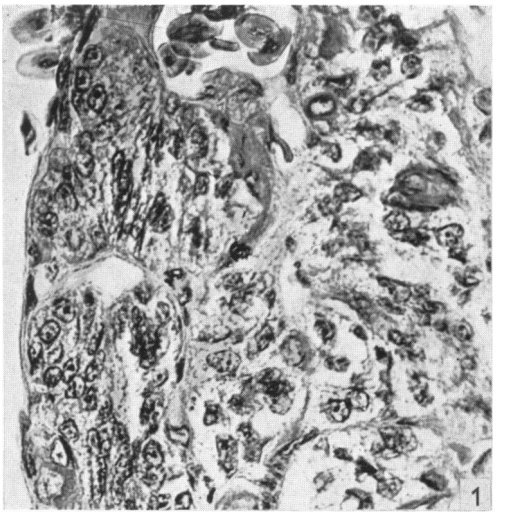
Fig. 2. Frog adrenal: 25 μ, gelatine-embedded frozen section stained with Scharlach R, $\times 450$. Note major lipid distribution in spongiocyte layer (right), with outer part of cortical columns comparatively lipid-free. Refer to fig. 1.

Fig. 3. Pregnant rat adrenal: 7 μ haematoxylin and eosin preparation, $\times 230$. From left to right note: (i) the capsule; (ii) the z. glomerulosa; (iii) a layer of flattened cells, some showing flattened nuclei; (iv) the spongiocytes of the outer z. fasciculata. Compare with fig. 1.

Fig. 4. Horse adrenal: 10 μ haematoxylin and eosin preparation, $\times 230$. From left to right, note: (i) long arched columns of the z. glomerulosa with cells and nuclei at right angles to the long axis of column; (ii) the z. intermedia seen as a layer of cellular re-arrangement; (iii) the polygonal cells with rounded nuclei of the outer z. fasciculata.

Fig. 5. Cat adrenal: 7 μ haematoxylin and eosin preparation, $\times 230$. From right to left, note: (i) the capsule; (ii) a rosette form of z. glomerulosa; (iii) the z. intermedia apparent as a layer of smaller packed cells; (iv) the spongiocytes of the outer z. fasciculata.

Fig. 6. 12-month-old rat adrenal: 7 μ haematoxylin and eosin preparation, $\times 230$. From right to left, note: (i) the capsule; (ii) the z. glomerulosa of regular form; (iii) an increased number of cells per unit area is well seen in the z. intermedia; (iv) the spongiocytes of the z. fasciculata.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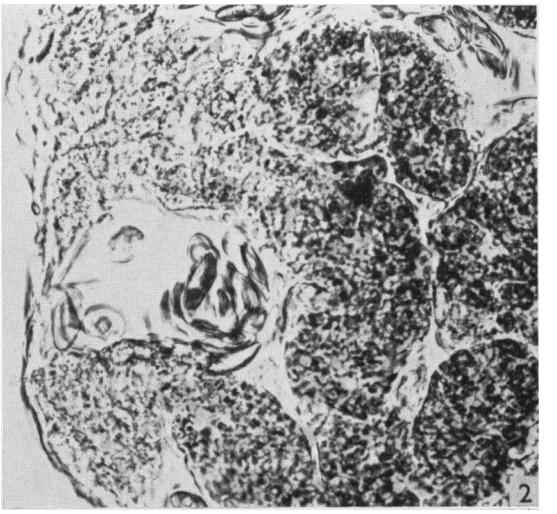
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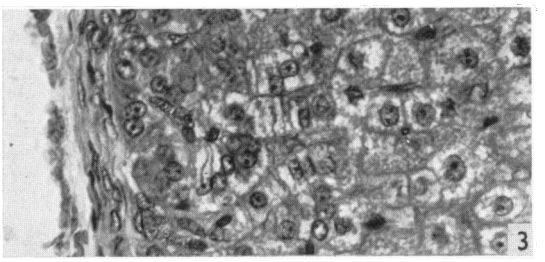
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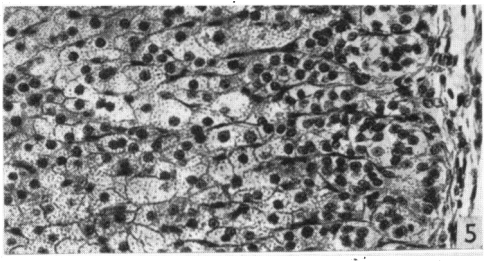
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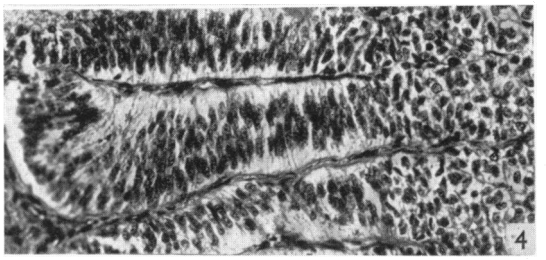
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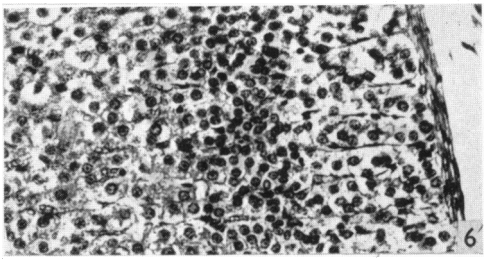
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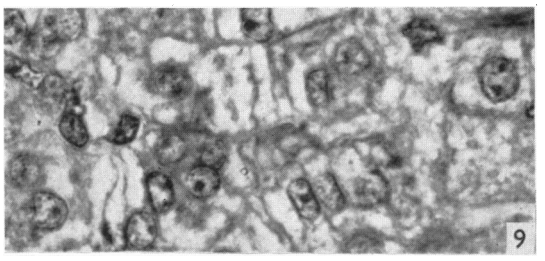
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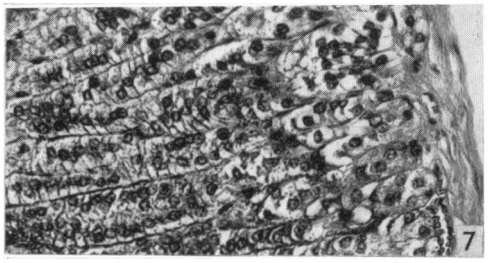
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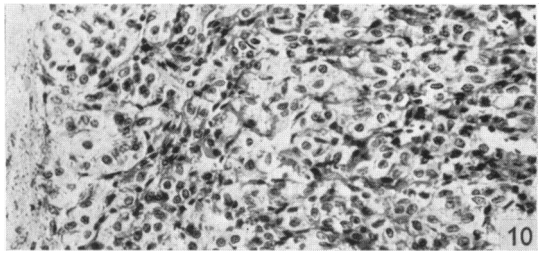
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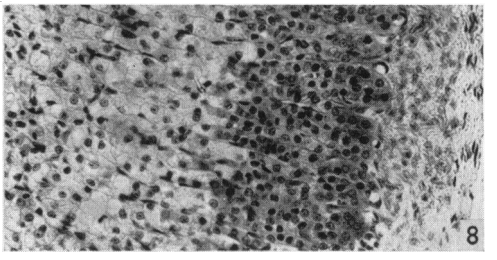
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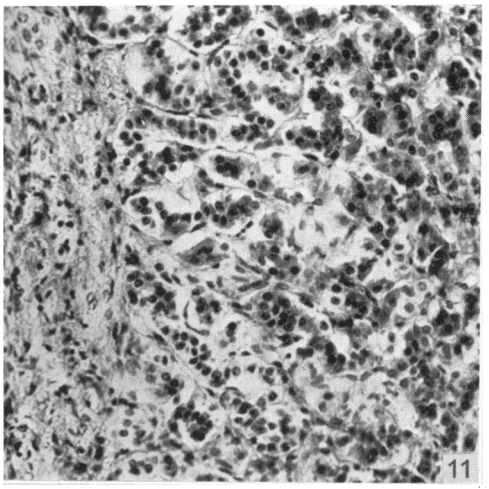
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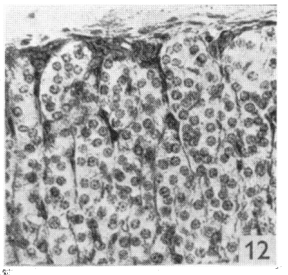
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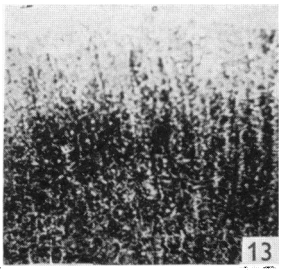
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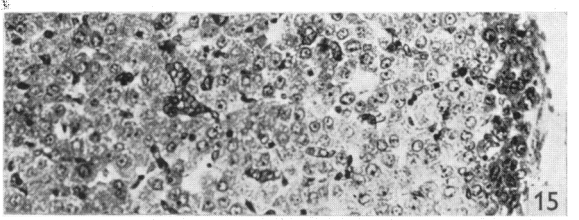
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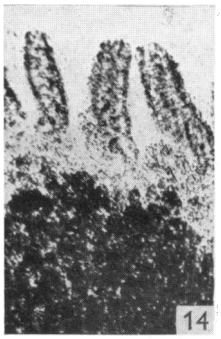
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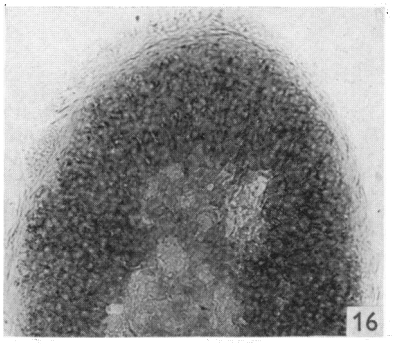
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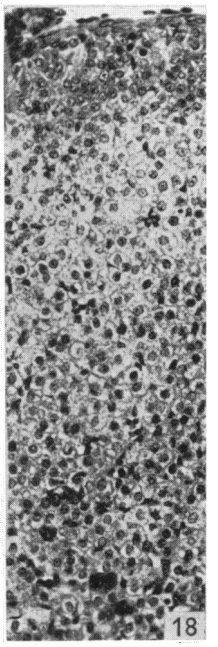
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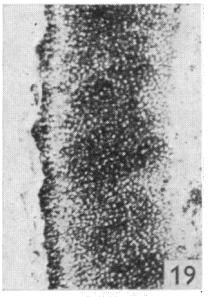
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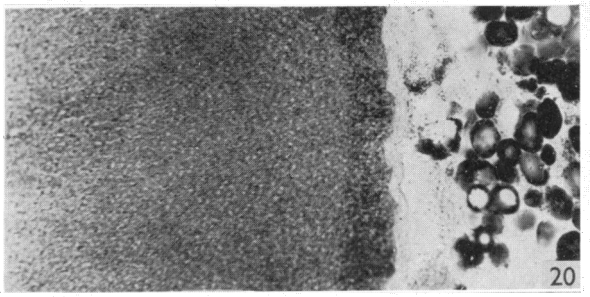
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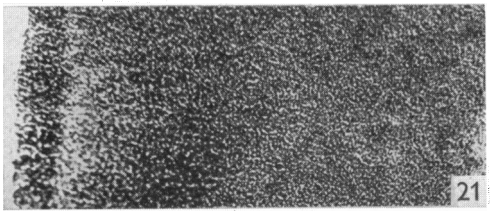
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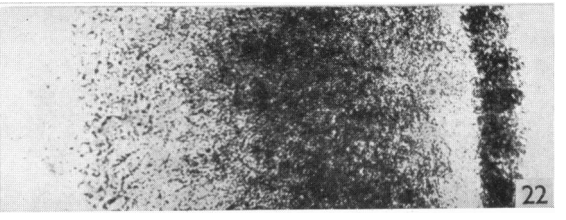
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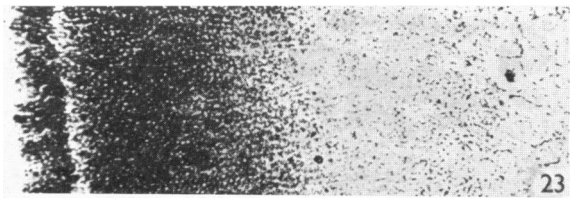
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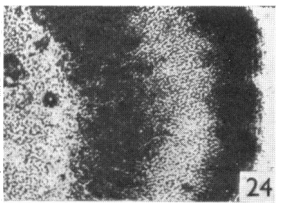
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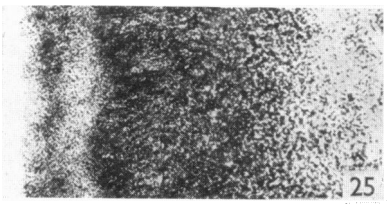
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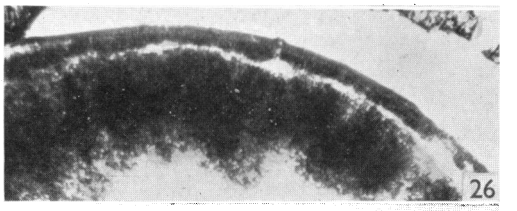
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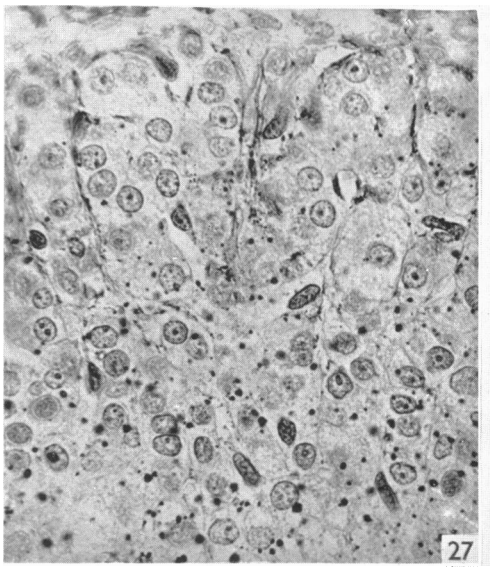
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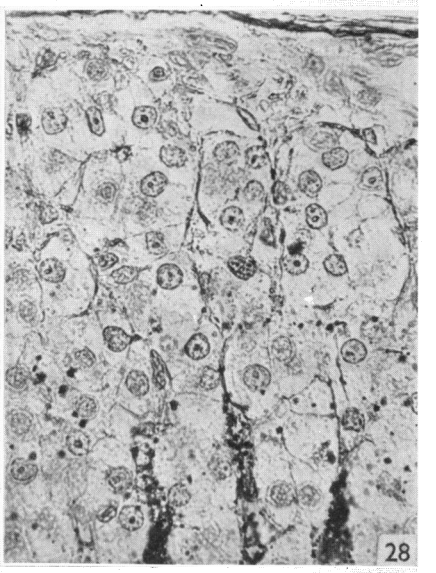
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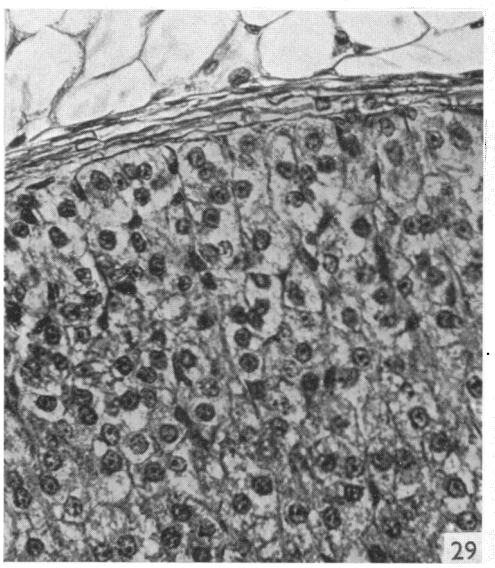
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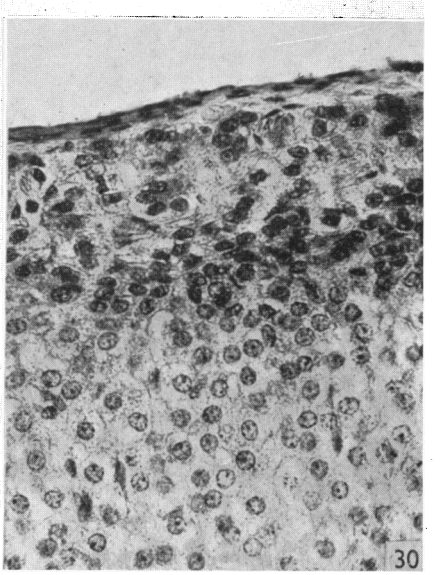
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Fig. 7. Rabbit adrenal: 7 μ haematoxylin and eosin preparation, $\times 230$. From right to left, note: (i) a columnar z. glomerulosa; (ii) well-marked cell flattening and packing in z. intermedia which is broad and irregular in extent; (iii) the outer z. fasciculata.

Fig. 8. Rat adrenal 2 weeks after hypophysectomy: 6 μ section, $\times 230$. Stained by the methyl green-pyronin method. Marked basophilia of the zonae glomerulosa and intermedia contrasts with the palely stained thickened capsule to the right, and the z. fasciculata to the left.

Fig. 9. High-power view ($\times 820$) of the pregnant rat adrenal in fig. 3. Note: (i) cell flattening in the z. intermedia; (ii) in some cells of the z. intermedia nuclei are flattened; in others the plane of section does not include a nucleus; (iii) the spongiocytes of the outer z. fasciculata are seen to the right.

Fig. 10. Cow adrenal: 10 μ haematoxylin and eosin preparation, $\times 230$. From left to right, note: (i) the capsule; (ii) an irregular form to the z. glomerulosa; (iii) there are no very definite outlines to the z. intermedia but differences in cell grouping suggest its presence; (iv) the outer z. fasciculata.

Plate 2

Fig. 11. Ox adrenal: 10 μ haematoxylin and eosin preparation, $\times 230$. From left to right, note: (i) the capsule; (ii) loose cell grouping in the z. glomerulosa; (iii) a z. intermedia of smaller cells with basophilic cytoplasm; (iv) the outermost z. fasciculata.

Fig. 12. Mouse adrenal: 7 μ haematoxylin and eosin preparation, $\times 230$. From above downwards, note: (i) the thin capsule; (ii) rounded cell clusters in the z. glomerulosa; (iii) the z. intermedia is difficult to define although there is a suggestion of cell packing between the zonae glomerulosa and fasciculata.

Fig. 13. Rabbit adrenal: 25 μ gelatine-embedded frozen section stained with Sudan black, $\times 90$. The zonae intermedia and fasciculata contain lipid while the z. glomerulosa, above, is almost lipid-free.

Fig. 14. Horse adrenal: 25 μ gelatine-embedded frozen section stained with Sudan black, $\times 90$. A sudanophobe z. intermedia is interposed between a lipid-containing z. glomerulosa (above) and a lipid-laden z. fasciculata (below).

Fig. 15. Adrenal of a 1·8 g. rat embryo: 6 μ haematoxylin and eosin preparation, $\times 230$. From right to left, note: (i) a thin cellular capsule with a layer of dark-staining cells immediately deep to it; (ii) a broader zone of vacuolated pale cells which in frozen preparations contain lipid; (iii) the large lipid-free eosinophilic cells of the foetal cortex. Within the foetal cortex is an irregular darkly stained clump of chromaffin (medullary) cells.

Fig. 16. Adrenal of a 1 g. rat embryo: 25 μ gelatine-embedded frozen section stained with Sudan black, $\times 90$. Two major regions are seen: (i) an outer zone consisting of the lipid-containing anlage of the adult cortex; and (ii) an inner patchy zone with little or no lipoid staining, comprising the foetal cortex (see fig. 15).

Fig. 17. Adrenal of a 3-day rat: 25 μ gelatine-embedded frozen section stained with Sudan black, $\times 90$. The first signs of a fat-free z. intermedia appear at this age. The outer edge of the lipid laden z. fasciculata marks its inner limit. See fig. 18.

Fig. 18. Adrenal of a 3-day rat: 6 μ haematoxylin and eosin preparation, $\times 230$. From above downwards note: (i) the capsule and deep to it an outer darkly stained zone which is differentiating into z. glomerulosa and z. intermedia. In the z. intermedia an increased number of cells per unit area is noticeable; (ii) the z. fasciculata comprises the remainder of the cortex; the pale spongy cells of the outer z. fasciculata are lipid-laden in frozen preparations.

Fig. 19. Adrenal of 10-day rat: 25 μ gelatine-embedded frozen section stained with Sudan black, $\times 90$. From left to right, note: (i) the lipid-containing z. glomerulosa; (ii) a lipid-free z. intermedia; (iii) the outer z. fasciculata is lipid-laden, while the inner part of the z. fasciculata, bordering the unstained medulla, is almost fat-free.

Fig. 20. Adrenal of a 4-month rat: 25 μ gelatine-embedded, frozen section, stained with Sudan black, $\times 90$. The lipid-laden z. glomerulosa, on the right, contrasts with the fine lipoid staining in the zonae intermedia and fasciculata.

Fig. 21. Adrenal of a 9-month rat: 25 μ gelatine-embedded frozen section, stained with Sudan black, $\times 90$. Note the fat-free z. intermedia, or sudanophobe zone. Compare the lipid distribution with that in fig. 20.

Fig. 22. Adrenal of a 1-year rat: 25 μ gelatine-embedded frozen section stained with Sudan black, $\times 90$. The z. intermedia is seen as a wide sudanophobe layer internal to the lipid-laden z. glomerulosa on the right.

PLATE 3

Fig. 23. Adrenal of rat hypophysectomized 24 hr. previously: 25 μ, frozen section, stained by the Schultz method, $\times 90$. The z. intermedia stands out as a clear band between the Schultz-positive zonae glomerulosa and fasciculata.

Fig. 24. Schultz preparation (as for fig. 23) of adrenal of rat hypophysectomized 3 weeks previously, $\times 90$. The z. intermedia, unstained, is broader than in fig. 23.

Fig. 25. Adrenal of rat hypophysectomized 2 weeks previously: 25 μ frozen section, stained by the Baker acid haematein method, $\times 90$. From left to right note: (i) an irregularly stained z. glomerulosa; (ii) an unstained z. intermedia; (iii) a heavily stained outer z. fasciculata; (iv) the inner fasciculata and the reticularis contain small amounts of stained material.

Fig. 26. Fresh frozen section at 25 μ of rat adrenal, stained intravitally by methylene blue, $\times 25$. The photograph was taken 5 min. after death. From above downwards, note: (i) stained capsule and z. glomerulosa; (ii) a thin but definite unstained z. intermedia; (iii) a stained z. fasciculata; (iv) a narrow patchily stained z. reticularis bordering on an unstained medulla.

Figs. 27 and 28. From 6 μ sections of rat adrenals, $\times 450$; ascorbic acid is demonstrated as black granules by the acetic acid-silver nitrate method, and nuclei are counterstained with neutral red. Fig. 27 (the control) shows the ascorbic distribution in the left adrenal removed from a rat 24 hr. after hypophysectomy. In the upper part of the print, the ascorbic acid content in the deep layers of the capsule and the z. glomerulosa, is low, while internal to this in the z. intermedia, the granules are larger and more numerous. The outermost cells of the z. fasciculata with large silver granules are seen at the bottom of the print. The silver granules do not noticeably lie within vessel walls. Fig. 28 shows the ascorbic distribution in the right adrenal of the same rat 1 hr. after a medium dose of ACTH (Armour, 84–85 H. 0·1 μg. intravenously). Cell depletion of ascorbic acid silver granules in the outer z. fasciculata is associated with a crowding of these into the vessel lumina. At this stage (1 hr. after ACTH) some silver granules are still present in the z. intermedia.

Fig. 29. 2-month-old male rat adrenal: 7 μ haematoxylin and eosin preparation, $\times 400$. There is no obvious z. intermedia between glomerulosa and fasciculata at this age.

Fig. 30. 2½-year-old rat adrenal: 7 μ haematoxylin and eosin preparation, $\times 400$. A very well defined and basophilic z. intermedia exhibits marked cellular and nuclear flattening, which is also a feature of the deeper glomerulosa cells; the z. glomerulosa is patchily basophilic.