THE EFFECT OF HYPOPHYSECTOMY ON THE ADRENAL GLAND OF THE HAMSTER (MESOCRICETUS AURATUS) [1, 2]

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TWENTY-EIGHT FIGURES

The effect of hypophysectomy on the histology of the adrenals has not been studied in the hamster. In several other species, the cells of the medulla show little histological change (White, '33; McPhail, '35; Crooke and Gilmour, '38; Reese and Moon, '38). Houssay and Mazzocco ('33) reported that the concentration of epinephrine is not decreased in the medulla of the dog after hypophysectomy. On the other hand, Smith ('30) observed that the volume of the medulla of long-term hypophysectomized rats is 50% less than that of the adrenals of normal animals; he concluded that the medulla of young hypophysectomized animals does not develop at a normal rate. Cutuly ('36) also found that the calculated weight of the medulla of young female rats was 42% less than that of control animals 30 days after hypophysectomy.

Nevertheless, the adrenals of all species which have been studied exhibit a profound decrease in weight. This is accompanied by a reduction in thickness of the cortex and in the size of its individual parenchymal cells. Smith ('30) found the total volume of the cortex in the female rat to be

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reduced by 80%. Observations in other species are in general agreement with these findings in the rat. This atrophy, which involves primarily the zona fasciculata and zona reticularis, is associated with a diminution in size and number of mitochondria in the rat (Deane and Greep, '46) and mouse (Miller, '50), and with changes in form of the Golgi apparatus (Reese and Moon, '38; Greep and Deane, '49).

Lipid droplets are present in the cortical cells of almost all mammals (Bourne, '49) and show significant alterations after hypophysectomy. In normal glands the droplets are sudanophilic and stain with reagents used for the demonstration of aldehydes, including leucofuchsin (Schiff reaction), 2-4-dinitrophenylhydrazine and 2-hydroxy-3-naphthoic acid hydrazide. They fluoresce in ultraviolet light, are birefringent and associated with positive reactions for cholesterol. All of these tests are negative after prior extraction with acetone. On the basis of these and other observations, it is believed that the lipid droplets contain neutral fats, unsaturated fatty acids, cholesterol and its esters, and lipid aldehydes, the exact identity and origin of the latter not being fully understood (Gomori, '52b). The hormones secreted by the cortex are ketosteroids, and since they possess chemical and physical properties which give positive reactions to one or more of the above named reagents, Bennett ('40) and Dempsey ('48) have suggested that glandular cells which are positive to all of these tests contain ketosteroids in addition to precursors and metabolically related compounds. Other investigators hold that the presence of autofluorescent, birefringent and Schiff-positive material does not indicate that a gland contains steroid hormones (Knouff, Brown and Schneider, '41; Albert and Leblond, '46; Claesson and Hillarp, '47; Skelton, Fortier and Selye, '49; Boscott and Mandl, '49; Feldman, '50; Gomori, '52b). Regardless of the chemical meaning of these reactions, all of them are reduced in the zona fasciculata of the rat after hypophysectomy (Deane and Greep, '46).

In contrast to the clear-cut involution of the zona fasciculata which occurs in all forms after hypophysectomy, there is disagreement concerning the extent of pituitary control over the zona glomerulosa. Swann ('40) and Deane and Greep ('46) believe that this zone functions autonomously in the rat and secretes a hormone which regulates electrolyte metabolism. After hypophysectomy, it becomes thicker and retains its fluorescence, birefringence and sudanophilia (Simpson, Evans and Li, '43; Deane and Greep, '46), alkaline phosphatase activity (Dempsey, Greep and Deane, '49) and ascorbic acid (Deane and Morse, '48). When pituitary corticotropin is administered (Bergner and Deane, '48), or its endogenous secretion accelerated by feeding vitamin-deficient diets (Deane and McKibbin, '46; Deane and Shaw, '47; Olson and Deane, '49), lipid is depleted from the zona fasciculata but not from the zona glomerulosa. At higher doses of corticotropin, birefringent material may be increased in the latter region (Weaver and Nelson, '43).

The autonomy concept of the zona glomerulosa as studied in the rat is supported by the observation that after hypophysectomy it remains histologically normal in the dog (Houssay and Sammartino, '33), mouse (Jones, '50) and guinea pig (Schweizer and Long, '50). However, Jones ('50) and Schweizer and Long ('50) observed that the lipid content of this zone in the mouse and guinea pig, respectively, is reduced soon after hypophysectomy. Lane and de Bodo ('52) reported that the zona glomerulosa of the dog undergoes an atrophy and a loss of lipid equal to that exhibited by the zona fasciculata. Therefore, it remains unsettled whether the apparent freedom from pituitary control exhibited by the zona glomerulosa of the rat is peculiar to this species or is characteristic of others as well.

Investigation of the adrenal cortex of the hamster after hypophysectomy shows promise of aiding in the solution of these problems because it differs from that of other laboratory animals in the following important respects. (A) The usual sex difference in adrenal weight is reversed; from the

time of sexual maturity the adrenal of the male enlarges until it may outweigh that of the female by 65% (Kupperman and Greenblatt, '47). (B) Histochemically demonstrable cholesterol and sudanophilic lipid are absent from the normal adrenal (Peczenik, '44; Alpert, '50; Wexler, '51). (C) Cholesterol and sudanophilic lipid appear in the cortex of hamsters infected with Leishmania donovani (Leathem and Stauber, '52) or chronically treated with diethylstilbestrol (Koneff, Simpson and Evans, '46; Alpert, '50). (D) The role of the adrenal hormones in maintenance of life appears to differ from that in other species. Progesterone is more effective than desoxycorticosterone acetate in maintaining the adrenalectomized hamster (Snyder and Wyman, '51a). Saline drinking water does not affect the serum sodium or prolong life after adrenalectomy; serum sodium remains normal after adrenalectomy but urinary potassium is decreased and serum potassium is increased. The retention of potassium is not alleviated by desoxycorticosterone acetate (Snyder and Wyman, '51b).

MATERIAL AND METHODS

Thirty-two male and 30 female hamsters *(Mesocricetus auratus),* averaging 75–90 gm in weight, were hypophysectomized by the parapharyngeal approach. The technique described by Thompson ('32) for the rat was used with only minor necessary modifications. Bleeding from the dural sinuses was the major obstacle to a clear view of the hypophysis. In the absence of excessive bleeding, recovery was rapid and no special care of the animals was necessary. Littermate controls were used in the majority of cases. All animals received Purina Laboratory Chow and water ad libitum, with a weekly supplement of lettuce, oranges and cod liver oil. Frequent observations were made of the general physical condition, activity, and status of the hair and costovertebral spot. The external genitalia, testes and vagina were observed daily in several groups of experimental animals.

Autopsy was performed 30 and 60 days later under ether anesthesia. The right adrenal was dissected out and the ani-

mal exsanguinated by cutting the renal vessels. Then the other adrenal was removed, trimmed of fat, and the two glands were placed in weighing bottles filled with fixing fluid. The difference in weight of the bottle and fluid before and after addition of the adrenal is the wet weight of the gland. This weighing procedure is necessary to avoid the cytological artifacts produced by excessive handling and rapid drying of an organ as small and friable as the adrenal of the hamster. Other organs were weighed after being freed of connective tissue and fat.

After fixation in Bouin's fluid, the head was decalcified with 5% trichloracetic acid, trimmed to the area of the pituitary capsule and surrounding bone, embedded and sectioned serially at 10 μ. These preparations were studied microscopically in order to determine the completeness of pituitary ablation. The hypophyses, pituitary capsules and surrounding bone of 4 control males and 4 females were prepared in the same manner. All sections were stained by the trichrome technique of Masson ('28).

The volumes of the hypophyseal fragments remaining in 9 females, 30 days after hypophysectomy, and in 10 males, 60 days after hypophysectomy, were determined by making camera lucida drawings (× 150) of the fragments of pars distalis in every 5th section of the serially sectioned pituitary capsules. The areas of these drawings were measured with a planimeter and the true volume calculated. In order to express these fragments in terms of per cent of the total hypophysis, the volumes of the pars distalis, pars intermedia and pars nervosa of 4 control males and 4 females were measured in the same manner except that a lower magnification (× 66) was used in making the drawings and the area of every 10th section was measured. The sum of these determinations represents the total volume of the gland.

The following procedures were used in preparing adrenals for histological study: fixation in Bouin's fluid and staining with hematoxylin and eosin or by the Masson technique; fixation in formol-Zenker, post chromation in 3% potassium di-

chromate and staining with phosphotungstic acid hematoxylin; or fixation in Regaud's fluid (two days), post-chromation for two days, embedding in celloidin and paraffin and staining with iron hematoxylin, Sudan black B, Sudan IV or with the Altmann acid fuchsin technique for mitochondria as modified by Severinghaus ('33). The glands were sectioned at 3 to 7 μ. Adrenals of several control animals were fixed in picro-alcohol-formalin and stained with the periodic acid-leuco-fuchsin method for polysaccharides (Hotchkiss, '48).

Five to 8 μ frozen sections of adrenals fixed in 10% formalin were used for several lipid stains including Sudan black B, Sudan IV, Nile blue sulfate, the Schultz ('24) test for cholesterol, the Schiff reaction, 2-4-dinitrophenylhydrazine (Albert and Leblond, '46) and the Ashbel-Seligman technique (Ashbel and Seligman, '49; Seligman and Ashbel, '52). The latter three reactions demonstrate aldehydes; with these procedures other sections of each gland were extracted with absolute acetone, chloroform or pyridine at room temperature for periods up to 5 days, before or after staining, in order to test the solubility of the reactive material. With all stains for lipids, sections of adrenals of normal rats and dogs were mounted on the same slide with sections of the glands from hamsters. The reactions observed in these "control" rat and dog sections served to corroborate the results of other investigators and also to insure that the prescribed staining conditions were present. For example, the validity of the observation of sudanophobia in the adrenal of the hamster was strengthened by the presence of sudanophilic lipid in the sections of the adrenals of rats and dogs which were mounted on the same slide. Phospholipids were demonstrated by the acid hematein method of Baker ('46), one adrenal being fixed in calcium formaldehyde and the contralateral gland in weak Bouin's fluid for the pyridine extraction test. Several control adrenals were fixed in formol-Zenker-osmic acid to demonstrate osmiophilic lipid. Other sections of both fresh and formalin-fixed adrenals were mounted unstained and examined with the polarizing microscope for birefringence.

Alkaline phosphatase activity was demonstrated by the method of Gomori ('39), using chilled 80% alcohol as a fixative. Sections were incubated at 37°C. with sodium glycerophosphate for 3 and 24 hours at pH 9.2.

The relative involution of the cortex and medulla was determined by measuring the area of each region with a planimeter on tracings of projected sections (× 90). Sections of greatest area were selected from serially-sectioned, Bouin-fixed glands.

OBSERVATIONS

The normal adrenal cortex

General histology. The adrenal of the hamster possesses a capsule composed primarily of fibroblasts and collagenous fibers. The capsule varies regionally in thickness, often consisting of only a few thin fibers and small, flattened fibroblasts. Elsewhere on a gland it is thicker and may be differentiated into an outer layer of dense connective tissue and an inner layer of loose connective tissue in which cells predominate. Delicate strands extend from the capsule between the clusters of glandular cells which compose the zona glomerulosa. More prominent sheaths of connective tissue accompany the vessels and nerves which pass into the cortex. Frequent nodules of parenchymal cells are found in the capsule and range in size from small groups of three or 4 cells to nodules measuring 300 μ in diameter. The larger ones appear to lose their connective tissue investment and become continuous with the cortical parenchyma. In preparations fixed in Bouin's fluid and stained with the Masson procedure, the cytoplasm of cells in smaller nodules stains lightly and is vacuolated. Nuclei vary widely in size and shape. The staining properties and appearance of these cells are similar to those of the cells of the zona glomerulosa (fig. 4). Larger nodules are often differentiated into two zones, a peripheral one composed of glomerulosa-like cells and an inner one consisting of larger, more deeply stained cells. These latter cells resemble those of the zona fasciculata since their cyto-

plasm is finely granular and non-vacuolated, and their nuclei are uniformly spherical.

The zona glomerulosa is made up of a narrow band of cells which are arranged in clusters and cords at the outer border of the gland (figs. 2, 4). Frequently the zona fasciculata extends almost to the capsule with only a few, flattened cells of the zona glomerulosa intervening. In Bouin-fixed glands the membranes of the cells in the zona glomerulosa are indistinct. With all stains used, the cytoplasm is delicate, vacuolated and more lightly stained than that of the cells in the zona fasciculata and zona reticularis (figs. 2, 4). When fixed in Regaud's fluid and stained with iron hematoxylin, the intracellular vacuoles are more clearly defined and tend to be located in that portion of the cell adjacent to a sinusoid. The nuclei exhibit a wide range of sizes and shapes. In those locations where the capsule is differentiated into dense and loose layers, the cells of the zona glomerulosa often seem to have arisen from the capsular connective tissue.

Between the zona glomerulosa and the zona fasciculata is a narrow and often inconspicuous band of small cells, flattened in a plane parallel to the capsule, which is designated as a transitional zone (fig. 25). This term is suggested because the component cells possess characteristics which blend with those of both neighboring zones. The term "sudanophobic" cannot be applied to this region as it is in the rat, because the cortex of the hamster contains no free sudanophilic lipid. The nuclei of these cells are generally smaller and darker than those of the zona glomerulosa and zona fasciculata. The presence of transitional cells is more apparent in areas where the constituent cells of the zona glomerulosa and zona fasciculata are joined in a continuous column.

The zona fasciculata comprises most of the cortex and is composed of radially arranged columns of cells separated by narrow sinusoids. The cells of the outer portion of the zone are somewhat larger than those in the inner half. As compared with other forms, the absence of cytoplasmic vacuolation in the zona fasciculata and zona reticularis is perhaps

the most striking feature. When 3 to 4 μ sections are stained by the Masson procedure employing light green as one constituent dye, the cytoplasm contains discrete green bodies which vary slightly in size in different cells (fig. 27). The nucleus often lies in the part of the cell which borders a sinusoid. A clear juxtanuclear network is present in some cells and is believed to be the negative image of the Golgi apparatus (fig. 27).

The zona reticularis is not delineated sharply from the zona fasciculata. A sex difference does exist in the zona reticularis. In the female it consists of small, closely packed cells with densely arranged nuclei. Many cells possess coarse, eosinophilic cytoplasm and pycnotic nuclei or lipochrome pigment; others are vacuolated. A layer of connective tissue which is generally present at the cortico-medullary boundary increases in thickness with age. In the male, the cells of the zona reticularis are larger, with abundant cytoplasm and vesicular nuclei. The innermost cells are associated more intimately with the medulla than in the female, the intervening connective tissue being sparse. Regressive changes are less frequently observed than in the female.

Nerve fibers are particularly abundant in the hilar periadrenal connective tissue, from where they spread into the capsule or penetrate immediately into the cortex, usually extending into the medulla.

Liposomes. When adrenals are fixed in Regaud's fluid and post-chromated the zona fasciculata and zona reticularis stain intensely with iron hematoxylin, Altmann's acid fuchsin and Sudan black B (figs. 18, 19). In contrast, the zona glomerulosa stains lightly. The structures revealed in the cortical cells by these procedures are liposomes and mitochondria.

The liposomes found in the cells of the zona fasciculata and zona reticularis are small, spheroid bodies which, after fixation in Bouin's or Regaud's fluid, resist extraction in alcohol. When stained with iron hematoxylin or acid fuchsin the sections can be dehydrated and cleared without destaining the liposomes; after Sudan black B, equally precise results are

obtained but sections cannot be dehydrated since the dye is removed by alcohol. On the basis of these properties, liposomes of the cortex of the hamster appear to be morphologically similar to liposomes described in the rat by Greep and Deane ('49). To others the term "liposome" has a different connotation, being used to denote any of the demonstrable lipid material in the adrenal cortex of the guinea pig (Hoerr, '36b) or lipid droplets which are removed by fat solvents (da Costa, '51).

The zona glomerulosa and small capsular nodules are devoid of liposomes. The walls of the vacuoles revealed in the cells of the zona glomerulosa by Regaud fixation stain intensely with Sudan black B, appearing in the form of crescents and rings (fig. 22). There are, in addition, small uniformly colored droplets. With iron hematoxylin or acid fuchsin, the crescents and rings are not stained (fig. 25).

Small liposomes are present in the cells of the transitional zone (fig. 25). The cells of the zona fasciculata contain intensely stained liposomes which are larger than those of the transitional zone. They are distributed throughout the cytoplasm except for a clear triangular or crescentic juxtanuclear area (fig. 23). Considerable variation in size and number exists from cell to cell and to a lesser degree within individual cells. In the female, liposomes are found throughout the entire zona fasciculata and zona reticularis. In the male, discrete liposomes occur in the outer half of the zona fasciculata (fig. 19). In the inner part of the zona fasciculata and in the zona reticularis the cytoplasm stains diffusely with iron hematoxylin; larger lightly-stained liposomes are present in some cells.

Mitochondria. After fixation in Regaud's fluid and staining with iron hematoxylin, the mitochondria are distributed randomly (fig. 23). They are smaller than liposomes, rather constant in diameter and have the shape of granules, short rods or filaments. The granular and rod-like mitochondria are often arranged in short chains. The smallest spherical body considered to be a liposome is about twice the diameter

of the granular mitochondria. No staining procedure has been found which will differentiate mitochondria from liposomes or selectively demonstrate one without the other. Therefore, size, shape and arrangement are the only criteria available for the differentiation of liposomes from mitochondria.

Since liposomes are not present in the zona glomerulosa, mitochondria are seen readily. The small, granular mitochondria have no regular positional relationship to the vacuoles in these cells. The mitochondria in capsular nodules are often more prominent than those in the zona glomerulosa.

In the transitional zone and outermost part of the zona fasciculata the differentiation between mitochondria and liposomes is difficult since the liposomes are small and the mitochondria are usually granular. In the mid-portion of the zona fasciculata the mitochondria are frequently rod-shaped and arranged in short chains. In the inner half of the cortex of the female mitochondria are fewer, but similar in size and shape to those in the outer portion of the zona fasciculata. In the male, mitochondria are likewise reduced in number and difficult to distinguish clearly.

Lipid. Frozen sections of formalin-fixed adrenals of both males and females do not stain with Sudan black B or Sudan IV. Because occasional multivacuolated cells appear in the zona fasciculata of Bouin-fixed sections stained with the Masson procedure, special care was taken to determine whether these vacuoles contain sudanophilic lipid as described by Peczenik ('44). No Sudan-colored droplets were found. The appearance of sudanophilic pigment under experimental conditions as described by Alpert ('50) was not observed in my animals. With Nile blue sulfate, the zona fasciculata and zona reticularis are uniformly blue; the zona glomerulosa is pale or colorless. The Schultz reaction for cholesterol is negative. Birefringence is absent from sections of both fresh and formalin-fixed glands. In contrast to these negative results with the hamster, the "control" sections of adrenals of the dog and rat mounted on the same slide present positive reactions with Sudan black B, Sudan IV, Nile blue sulfate and

the Schultz procedure; they exhibit birefringence when examined with the polarizing microscope. The distribution of sudanophilia, cholesterol and birefringence as seen in the adrenals from rat and dog agrees with the descriptions of Simpson, Evans and Li ('43), Deane and Greep ('46) and others for the rat and Houssay and Sammartino ('33), Lane and de Bodo ('52) and others for the dog.

The zona fasciculata and zona reticularis of the hamster color intensely and uniformly with the Schiff reagent; the zona glomerulosa stains weakly (fig. 10). Prior treatment with mercuric chloride does not alter this result. With 2-4-dinitrophenylhydrazine there is produced a pale yellow color throughout the entire cortex. With 2-hydroxy-3-naphthoic acid hydrazide the zona fasciculata and zona reticularis are stained blue with the former zone being somewhat darker, the zona glomerulosa is either faint or not stained (fig. 14), and darkly stained liposomes are revealed in many cells. These bodies are not seen in sections treated with either the Schiff reagent or with 2-4-dinitrophenylhydrazine. Sections of the adrenals from the rat or dog stain intensely in the zona glomerulosa and outer fasciculata and less vividly in the inner zona fasciculata and zona reticularis.

Lipid solubility tests were performed by dehydrating frozen sections of formalin-fixed glands through graded alcohols and placing them in absolute acetone at room temperature for periods of one-half hour to 5 days. Sections of glands from hamsters continue to stain with the Schiff reagent, 2-4-dinitrophenylhydrazine and with 2-hydroxy-3-naphthoic acid hydrazide, the Schiff reagent being the least effective after prolonged extraction. Cholesterol, sudanophilic and birefringent lipids are removed from sections of the control glands of rats and dogs by a 30-minute extraction.

Non-deparaffinized sections of glands fixed in formol-Zenker-osmic acid were examined immediately after sectioning to insure that any rapidly fading structures were not overlooked (Hoerr, '36a); others were deparaffinized and stained with Altmann's acid fuchsin. Both preparations re-

veal the presence of intensely black osmiophilic droplets in the cells of the zona glomerulosa (fig. 26) which vary in size within single cells and in number in different cells. The vacuoles previously described in Bouin- and Regaud-fixed preparations are accounted for by the solution of these droplets because the vacuoles and osmiophilic droplets are of identical size and distribution. The remainder of the cortex contains no osmiophilic droplets.

Phospholipids, as revealed by the acid hematein technique (Baker, '46), are present. Considerable variation occurs in the intensity of staining and in the morphology of the intracellular bodies. Similar irregularities are apparent in the observations reported by Cain and Harrison ('50) on the adrenal cortex of the rat. The zona glomerulosa of the hamster usually stains intensely. Especially prominent are the large lipid droplets which, after fixation in formol-Zenker-osmic acid, are osmiophilic. All of the droplet or only the periphery make take up the hematein. In addition, a few plaques with an attached granule, similar to the "discharge bodies" described by Cain and Harrison ('50) in the rat, are observed frequently. These structures are of irregular shape and usually only the rim is stained. Smaller free granules, which may be mitochondria, are demonstrated also.

The plaques are present in the transitional zone and zona fasciculata, being much more numerous than in the zona glomerulosa. They are encountered most frequently in the outer portion of the zona fasciculata. Occasional bodies which are stained uniformly appear to be liposomes. Plaques may be partially stained liposomes but this relationship is not clear.

Alkaline phosphatase. The capsule exhibits considerable alkaline phosphatase activity, which is especially intense in the connective tissue surrounding capsular nodules. The zona glomerulosa is generally negative in both sexes. However, the activity of the remainder of the cortical parenchyma is strikingly different in the two sexes. In the male, after incubation for three hours, alkaline phosphatase activity is present in the zona fasciculata and zona reticularis (fig. 6), with the

outer half of the zona fasciculata exhibiting considerable variation. In the female, the cortex stains less intensely after three hours of incubation, the disposition of cobalt sulfide being restricted to the cells of the zona reticularis and to some of the nuclei of the inner portion of the zona fasciculata (fig. 8).

Polysaccharides. The connective tissue of the capsule and of the cortico-medullary boundary, after fixation in picro-alcohol-formalin, stains lightly with periodic acid-leucofuchsin. The parenchymal cells of the cortex are unstained.

The normal adrenal medulla

The architecture of the medulla of the hamster is similar to that in other mammals. The parenchymal cells are columnar and arranged in clusters and cords (figs. 2, 4). In Bouin-fixed glands stained by the Masson technique, the cytoplasm of most cells is finely granular, non-vacuolated and stains a reddish brown. Along the cortico-medullary boundary are frequent clusters of pale-staining cells whose cytoplasm contains relatively large vacuoles. Kolmer ('18) observed a pattern of alternating light and dark cells in the medulla of the European hamster *(Mesocricetus frumentarius)* and considered these cells to be in different states of secretory activity.

After post-chromation of glands fixed in solutions containing potassium dichromate (Regaud's fluid), the medulla exhibits a pale yellow-brown color. This reaction is not intensified by treatment of the sections with Fontana's ammoniacal silver solution for periods of two to 6 hours, a procedure which intensifies the chromaffin reaction in the cat (Bennett, '41).

After fixation in Regaud's fluid and staining with iron hematoxylin or acid fuchsin, granular mitochondria are demonstrated. They are considerably smaller than those of the cortical cells and lie randomly in the cytoplasm. All tests for lipids which were applied to frozen sections of formalin-fixed glands fail to stain the medulla except that fixation in

formol-Zenker-osmic acid reveals small osmiophilic droplets in isolated cells. With the Baker technique for the demonstration of phospholipids, the mitochondria and cytoplasm are stained blue-black. Alkaline phosphatase activity is absent from sections incubated for three hours (figs. 6, 8). Polysac-

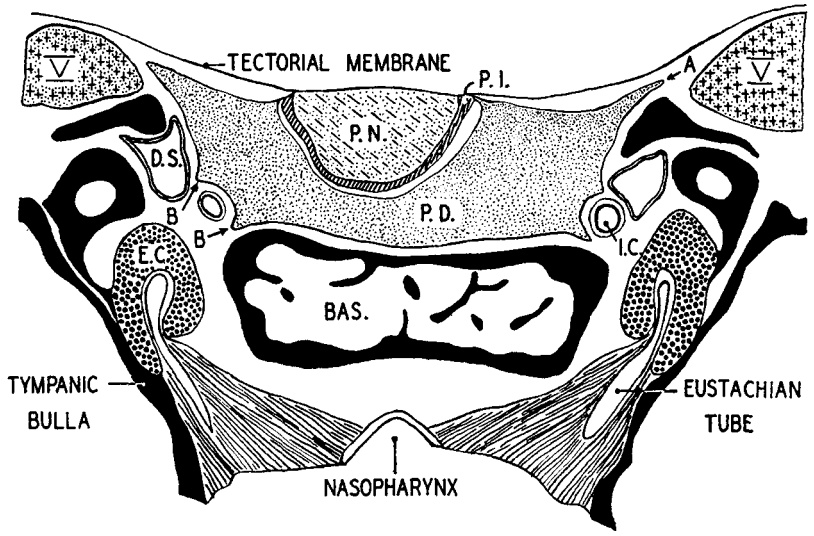

Fig. 1 Diagram of a frontal section of the hypophysis of the hamster showing its anatomical relations. In other sections the process of pars distalis at A extends laterally to come in contact with the sheath of the trigeminal nerve; the processes at B extend to positions lateral or ventral to the internal carotid artery. Legend: D.S., dural sinus; P.N., P.I., P.D., pars nervosa, pars intermedia and pars distalis, respectively, of the hypophysis; I.C., internal carotid artery; Bas., basisphenoid; V., trigeminal nerve; and E.C., cartilage of Eustachian tube.

charides, as demonstrated by the periodic acid-leucofuchsin technique, are likewise absent from the medullary parenchyma.

Anatomy and volume of the hypophysis

The anatomy of the pituitary region in the hamster is similar to that of the rat. The hypophysis is situated in a shallow depression on the body of the basisphenoid and occipital bones and is invested completely by a thin connective

tissue membrane (pituitary capsule). Ventrally the capsule is fused with the periosteum; dorsally it separates the gland from the hypothalamus and in this region is known as the tectorial membrane (fig. 1). In young hamsters approximately two-thirds of the hypophysis lies anterior to the cartilaginous junction between the basisphenoid and occipital bones but in older animals it lies completely anterior to it. As seen during the operation, this junction and the mid-line ridge of the occipital bone are not as conspicuous as in the rat.

Complete operative removal of the hypophysis is somewhat more difficult in the hamster than in the rat because processes of the pars distalis extend far forward and laterad. When traced in frontal sections, the antero-lateral processes indicated in figure 1 are seen to approach the dorsal surface of the trigeminal nerve. They are aspirated with extreme difficulty. Other portions of the pars distalis are applied closely to the connective tissue surrounding the internal carotid arteries and associated dural sinuses, sometimes partially enveloping the arteries (fig. 1). Also, the hypophysis is connected extensively to the surrounding capsule by bridges of connective tissue, the infundibulum and pars nervosa, especially, being firmly attached to the tectorial membrane. This observation is verified by removal of the hypophysis from its capsule at autopsy. In contrast to the rat hypophysis, which is "shelled out" of its capsule easily, the hypophysis of the hamster tends to remains fixed and usually separates into several pieces upon excision.

The pars distalis of the male makes up 69.9% of the hypophysis (table 1); pars intermedia, 9.3%; and pars nervosa, 20.3%. For the female the corresponding figures are 76.4%, 8.3% and 15.3%, respectively. The female hypophysis is approximately 27% larger than the male hypophysis because the volume of the pars distalis is 24% greater in this sex (table 1). In the rat (Donaldson, '15; Jackson, '17; Stein, '33, '34; Brolin, '40), rabbit (Hammar, '32) and man (Ras-

mussen, '28, '34) the hypophysis is also larger in the female due to the greater size of the pars distalis.

Completeness of hypophysectomy and histology of the hypophyseal fragments

Hypophysectomy is considered complete when all of the glandular tissue lying beneath the tectorial membrane is removed as determined by microscopic examination of the pituitary capsules. Most of the pieces left in the capsules are

TABLE 1

The volume of the hypophysis of normal hamsters

ANIMAL NO.	TOTAL ($\times 10^{-3}$ cm^3)	PER CENT OF TOTAL VOLUME		
		Pars distalis	Pars intermedia	Pars nervosa
Males				
194	1.18	70.2	10.0	19.8
123	1.48	68.8	9.3	21.8
170	1.23	70.8	8.2	20.7
114	1.32	69.5	9.5	19.8
Avg.	1.32	69.9	9.3	20.3
Females				
143	1.62	78.4	8.8	12.8
172	1.91	78.9	7.7	13.4
138	1.77	75.4	8.8	15.8
119	2.01	73.0	7.8	19.2
Avg.	1.83	76.4	8.3	15.3

portions of the antero-lateral extensions of the pars distalis and of those associated with the internal carotid artery. In addition, variable amounts of pars intermedia and neurohypophysis are found near the site of penetration of the infundibular stalk through the tectorial membrane.

The volumes of the fragments of pars distalis remaining in 19 of the 62 hypophysectomized animals were measured and expressed as per cent of the total gland. In 9 females they range from 0.3 to 1.5% and in 10 males, from 0.2 to 4.1%.

The completeness of hypophysectomy in the remaining 43 hamsters was estimated by subjective comparison with the volumes of these pieces. No pituitary tissue is left in 4 males and two females. The fragments left in 34 capsules range from approximately 0.1 to 2.0% and in three, from 4 to 6%. A remainder of 10% of the pituitary gland of the rat is sufficient to maintain normal histology in the adrenal cortex, but loss of body weight occurs (Smith, '30).

Thirty and 60 days after hypophysectomy the pituitary capsule is filled with granulation tissue and the original opening drilled through the basisphenoid is being closed by intramembraneous bone formation. The remnants of pars distalis consist of small clusters and cords of cells separated by sinusoids which are more narrow than normal. Since small acidophils and basophils are observed rarely, reversion of chromophils to chromophobes is suggested. This is not due to degranulation by the decalcifying agent because the acidophils in hypophyses of control animals which were treated similarly stain a vivid red and basophils are easily recognized by their large size and granular cytoplasm. In hypophysectomized guinea pigs chromophobes and basophils of hypophyseal fragments are reported to increase in number (Schweizer and Long, '50). Fragments of pars intermedia were observed in only 4 instances; the cells appear histologically similar to those in control animals.

The effects of hypophysectomy

General observations. As compared with other species, the hamster withstands hypophysectomy remarkably well. In contrast to the rat, the hamster remains in good physical condition, showing no apparent change in the pelage. The hypophysectomized hamster is as active and aggressive as his non-operated control. Respiratory infection did not occur.

The pigmented costovertebral spot of the male undergoes gradual regression, at 15 days after hypophysectomy being reduced significantly in size and at 60 days having lost al-

most all of its pigment. The testes retract into the peritoneal cavity and cannot be palpated after 12 to 15 days. The scrotum becomes wrinkled and loses its pigment. The penis is smaller, with the great involution of the skin often creating the appearance of priapism. In the female, the costovertebral spot is normally smaller and less heavily pigmented than in the male; 15 to 20 days after hypophysectomy, it is reduced in size and depigmented. The lips of the vagina become dry and thin.

Weight of the body and adrenal glands. The body weight decreases 5 to 10 gm during the first few days after hypophysectomy, but in both sexes the weight at autopsy surpasses that prior to operation (table 2). The rate of increase in body weight after hypophysectomy is most rapid during the first 30 days. However, the net gain is approximately 50% less than that of the control animals. Hyperphagia or other signs of hypothalamic damage were not observed.

Thirty days after hypophysectomy the adrenal of the male weighs 55% less than that of the controls, and at 60 days 53% less (table 2). Comparable figures for the male rat 30 days after hypophysectomy are: 62% (Cutuly, '36), 56% (Deane and Greep, '46) and 61% (Houssay, H. E. J., '47). In the female, at 30 days the gland weighs 42% less than in the controls and at 60 days, 40% less. Comparable figures for the female rat are: 70% (Cutuly, '36) and 61% (Houssay, H. E. J., '47). With respect to the hamster 30 days after the operation, the mean weight of the gland is 4.1 mg as compared with 9.1 mg for the control and in the female, 3.5 mg and 6.0 mg, respectively. Peczenik ('44), Kupperman and Greenblatt ('47) and Keyes ('49) also found the adrenal of the female to be lighter than that of the male.

Reduction in volume of the cortex, as indicated by measurement of the area of sections through the thickest part of the gland, accounts for the major portion of the weight lost by the glands after hypophysectomy. In both sexes the mean cortical area 60 days after hypophysectomy is slightly less than that at 30 days. This indicates that a continuous slow

TABLE 2

The effect of hypophysectomy on the weight of the body and adrenal glands

TREATMENT	NO. HAMSTERS	DAYS AFTER HYP.[1]	MEAN BODY WEIGHT (GM)		MEAN ADRENAL WT. (MG)	ADRENAL WT. (MG) / BODY WT. (GM) × 10
			Initial	Final		
Males						
Control	15	..	87.4 ± 2.6[2]	99.1 ± 2.8	9.1 ± 0.37	0.94 ± .04
Hyp.	15	30	87.8 ± 2.6	92.4 ± 3.7	4.1 ± 0.21	0.50 ± .02
Control	10	..	74.5 ± 3.0	94.0 ± 4.3	9.9 ± 0.28	1.04 ± .03
Hyp.	10	60	78.4 ± 2.7	84.3 ± 3.1	4.7 ± 0.19	0.50 ± .03
Females						
Control	11	..	86.8 ± 3.7	105.0 ± 4.5	6.0 ± 0.23	0.56 ± .05
Hyp.	16	30	86.1 ± 2.8	98.1 ± 2.6	3.5 ± 0.16	0.38 ± .02
Control	6	..	75.1 ± 2.7	99.1 ± 3.3	6.9 ± 0.29	0.56 ± .02
Hyp.	6	60	77.1 ± 2.3	91.1 ± 3.4	4.1 ± 0.13	0.45 ± .02

[1] Hyp. = Hypophysectomy.

[2] Standard error = $\frac{\text{Standard deviation}}{\sqrt{N}}$.

involution of the cortex ensues after pituitary ablation, although most of the loss occurs during the first 30 days.

The mean cross-sectional area of the medulla of most groups (table 3) is not decreased after hypophysectomy. However, in females at 60 days after hypophysectomy a significant reduction is indicated. Smith ('30) noted a similar reduction in medullary volume of three rats which were hypophysectomized for much longer periods of time (104 to 442 days).

TABLE 3

The effect of hypophysectomy on the cross-sectional area of the adrenal cortex and medulla

TREATMENT	NO. HAMSTERS	DAYS AFTER HYP.[1]	MEAN CROSS-SECTIONAL AREA, MM²	
			Cortex	Medulla
Males				
Control	9	..	2.79 ± 0.14 [2]	0.68 ± 0.03
Hyp.	8	30	1.64 ± 0.23	0.61 ± 0.03
Control	10	..	2.85 ± 0.08	0.57 ± 0.02
Hyp.	10	60	1.58 ± 0.07	0.65 ± 0.04
Females				
Control	8	..	1.98 ± 0.08	0.67 ± 0.03
Hyp.	8	30	1.32 ± 0.10	0.57 ± 0.05
Control	3	..	2.21 ± 0.33	0.79 ± 0.15
Hyp.	3	60	1.12 ± 0.08	0.42 ± 0.03

[1] Hyp. = Hypophysectomy.

[2] Standard error = $\frac{\text{Standard deviation}}{\sqrt{N}}$.

Correlation of the weights of the adrenal glands with the volumes of fragments of pars distalis remaining in the pituitary capsules gives no indication of effective secretion of corticotropin. In the case of the three animals which retain the largest pieces of pituitary tissue, the adrenals weigh 2.4, 5.6 and 4.4 mg as compared with 5.4, 11.2 and 9.2 mg for their respective controls. The reduction in weight is approximately 50% in each case.

General histology. The histological changes which occur in the adrenal after hypophysectomy are similar in both sexes.

Since only slight differences exist between the glands 30 and 60 days after hypophysectomy, the following description applies to both post-operative periods.

The capsule is not thickened after hypophysectomy and areas differentiated into outer dense and inner loose layers are observed as frequently as in control animals. The incidence and histology of capsular nodules are not changed.

The thickness and architecture of the zona glomerulosa are not disturbed by hypophysectomy (figs. 3, 5, 20). Its cells do not appear to be reduced in size although their boundaries are indistinct. The transitional zone is slightly wider after hypophysectomy; the line of demarcation between it and the adjacent zones is not as sharply defined as in the controls. The zone can be distinguished by its small cells with lightly stained cytoplasm and by its small, compact and densely arranged nuclei (figs. 3, 5).

The cells of the zona fasciculata undergo extensive atrophy and the cords of cells become somewhat distorted and compressed (fig. 3). The cross-sectional area of individual cells is approximately one-half that of control animals. Their nuclei remain spherical and vesicular (fig. 28). When fixed in Bouin's fluid and stained with either eosin or by the Masson technique, the cytoplasm of the cells in the zona fasciculata is coarsely granular as is true of the normal gland (figs. 27, 28). At least some of this granularity is due to the presence of liposomes. However, the cytoplasm as a whole is stained less intensely (figs. 3, 5).

Coiled, juxtanuclear bodies, which are seen rarely in control glands, appear in the cytoplasm of many cells of the zona fasciculata. They are observed most clearly in glands fixed in Bouin's fluid and stained with eosin or the Masson technique. With the latter (fig. 28), they are grayish, flattened spheres within which coiled elements are indistinctly visible. These bodies vary in size and are located most frequently adjacent to the nucleus. They are more conspicuous in the male than in the female and are most numerous 60 days after hypophysectomy.

The zona reticularis cannot be identified 30 days after hypophysectomy (figs. 2 to 5) and sexes can no longer be distinguished on this basis. Pigment increases in the inner layers of the cortex as does the number of multivacuolated cells throughout the zona fasciculata. There is no significant cellular degeneration.

Liposomes. In order to obtain preparations adequate for comparison of liposomes and mitochondria, slides bearing sections from control and hypophysectomized hamsters were run, back to back, through all stages of the staining procedure. After iron hematoxylin, liposomes are a brilliant black in control animals and gray-black after hypophysectomy. This difference in affinity for stain is not as marked with acid fuchsin or Sudan black B. As in the controls, no liposomes are found in the zona glomerulosa. However, the cells of the zona fasciculata, especially in its middle portion, are filled with liposomes which are larger than in the controls (fig. 24). This enlargement of liposomes bears no relation to the amount of hypophyseal tissue left in the animal or to the duration of time elapsing after hypophysectomy.

Mitochondria. Sections from control and hypophysectomized hamsters stained on the same slide with acid fuchsin show the mitochondria of the zona glomerulosa and small capsular nodules to be unaffected by hypophysectomy in 12 of 17 preparations. The mitochondria of the remaining 5 cases are colored more intensely than in non-operated animals, but their number is not increased significantly. Although this increased affinity for stain may be of questionable significance, it should be noted that Miller ('50) observed an increase in the number of mitochondria accompanied by a decrease in lipid droplets in the outer portion of the zona glomerulosa of the mouse after hypophysectomy. These changes may indicate accelerated secretory activity. Mitochondria are reduced profoundly in number in the zona fasciculata (figs. 23, 24), especially in those cells which contain the largest liposomes.

Lipid. Sudanophilic lipid, cholesterol or birefringence do not appear in the cortex of either sex. The staining of the cortex with Nile blue sulfate, leucofuchsin, 2-4-dinitrophenylhydrazine and 2-hydroxy-3-naphthoic acid hydrazide shows no change in intensity or distribution (figs. 11, 12, 15, 16). With the latter reagent, large deeply stained liposomes are revealed against a colorless or faintly blue cytoplasm, as is true of the controls. Extraction in acetone at room temperature does not alter these results (figs. 13, 17).

Phospholipids were demonstrated by the acid hematein technique in 18 animals. The staining of the zona glomerulosa and of the medulla is not significantly different from that in the controls. In the zona fasciculata, the plaques and spheroid bodies as delineated with acid hematein are still present. The spheroid bodies are quite prominent in the inner part of the cortex of a few animals. As pointed out previously, at least some of these intracellular structures are probably liposomes. Thus, the acid hematein-stained bodies and liposomes as revealed by the staining of Regaud-fixed glands with iron hematoxylin or Sudan black B are both retained after hypophysectomy.

Alkaline phosphatase. Hypophysectomy causes a marked decrease in alkaline phosphatase activity. The effect is maximal in both sexes after 30 days, when only occasional small areas near the cortico-medullary boundary may stain lightly (figs. 6 to 9). The alkaline phosphatase activity of the capsule usually remains unchanged, being particularly intense in the connective tissue around capsular nodules.

DISCUSSION

Lipids in the normal adrenal gland

The results of my study agree with the current concept that the adrenal cortex of the hamster contains little, if any, sudanophilic lipid. However, failure of Sudan dyes to stain frozen sections of formalin-fixed tissues is insufficient evidence for considering an organ to be lipid-poor. Since the

Sudan dyes stain lipids only by dissolving in them, lipids which are masked or bound will not be colored (Lison, '36). By applying appropriate techniques, it is shown that lipids are present in the adrenal cortex of the hamster in significant amounts. Exclusive of those contained in mitochondria, lipids in the hamster fall roughly into two groups: (A) in the transitional zone, zona fasciculata and zona reticularis where they appear to be associated chiefly with the liposomes, and (B) in the zona glomerulosa.

Transitional zone, zona fasciculata and zona reticularis. The liposomes are regarded as a lipoprotein complex, in which the lipid component is masked and is probably a phospholipid. In my study, demonstration of lipid in the zona fasciculata and zona reticularis was dependent upon oxidation by potassium dichromate during or after fixation. If this procedure is omitted, sudanophilia is not evident. After chromation, lipids of the liposomes may be demonstrated by iron hematoxylin, acid fuchsin or Sudan black B. It is generally believed that potassium dichromate produces a variety of lipid oxidation products which form insoluble complexes with chromium (Cain, '50; Gomori, '52b). The presence of positive lipid reactions after chromation demonstrates that the lipid material has been freed from a masked or bound form (Lison, '36). Fixation in Regaud's fluid with subsequent chromation, as used in this study, is equivalent to Ciaccio's method I for the demonstration of masked lipids, the liberation of which he termed "lipophanerosis" (Ciaccio, '26). Masked lipids may be demonstrated in the nuclei of leucocytes by staining with Sudan black B after the lipid portion of a lipoprotein complex is released by brief treatment with carboxylic acids (Ackerman, '52).

The lipid portion of the liposome is probably a phospholipid because its stains with Sudan black B and, at times, with the acid hematein procedure of Baker ('46). Sudan black B is unique among the Sudan dyes for its capacity to reveal phospholipids. Although it stains triglycerides and fatty acids as well, these are probably not present in the material under

consideration because nothing is delineated by Sudan IV. The absence of osmiophilia in the liposomes further suggests that triglycerides and free, unsaturated fatty acids are not present. The acid hematein technique is regarded as specific for phospholipids. In some instances, it stains the liposomes in the adrenal cortex of the hamster but this is not a constant outcome. Using Baker's acid hematein technique, Alpert ('50) reports that phospholipids are present throughout the adrenal cortex of the hamster as fine granules. In my material, the most frequently observed acid hematein-positive objects in the cells of the zona fasciculata and zona reticularis are plaques resembling the "discharge bodies" of Cain and Harrison ('50) and sparse, more uniformly stained structures which may be liposomes.

The presence of large amounts of phospholipids in liposomes and mitochondria throughout the zona fasciculata and zona reticularis may explain the results obtained with the reagents which demonstrate aldehydes, including leucofuchsin, 2-4-dinitrophenylhydrazine and 2-hydroxy-3-naphthoic acid hydrazide. These reagents react with a wide variety of preformed aldehydes, acetal-phosphatides and aldehydes produced by the oxidation of unsaturated fatty acids (Danielli, '49; Gomori, '52a, '52b). The fatty acids of phospholipids are generally highly unsaturated (Wittcoff, '51; Gomori, '52b), and the intense Schiff reaction produced at sites where phospholipids are known to be present is evidence of the oxidation of their unsaturated fatty acids (Gomori, '52b). The aldehydes in the adrenal cortex of the hamster stained by these reagents are insoluble in acetone. Other possible sources of aldehydes, including plasmalogen, free unsaturated fatty acids, ketones and ketosteroids are soluble in acetone and would have been removed by extraction.

In lipid complexes of animal tissues, lipid is almost always bound to protein, whereas in plant tissues it is usually joined to carbohydrate (Chargaff, '44; Wittcoff, '51). The presence of protein in liposomes is suggested by their affinity for eosin, aniline blue and light green after fixation in Bouin's

fluid. The affinity of liposomes for these acid dyes might be explained on the basis of the presence of basic radicals associated with the precipitated protein. After fixation in Bouin's fluid, they do not stain with iron hematoxylin, acid fuchsin or Sudan black B.

Zona glomerulosa. Exclusive of the lipid contained in the mitochondria and in plaques as revealed by the Baker acid hematein technique, the lipid of the zona glomerulosa is collected in droplets which are peripherally located in cell clusters (fig. 26). These droplets have the following unique characteristics: (A) They are apparently soluble in 10% formalin since after fixation in this fluid they are not stained by Sudan dyes. Some free phospholipids react similarly. (B) After fixation in Regaud's fluid and chromation in 3% potassium dichromate, the droplets are stained with Sudan black B suggesting that a phospholipid has been rendered insoluble by dichromate oxidation. Phospholipids chromate faster than other lipids and, therefore, color more intensely with Sudan black B (Gomori, '52b). (C) The lipid is at least partially immobilized by calcium ions and is probably a phospholipid since it then stains with acid hematein. (D) It is osmiophilic, being blackened by fixation in formol-Zenker-osmic acid. The reduction of osmic acid may be accomplished by the presence of double bonds in the unsaturated fatty acid moiety of phospholipids. Phospholipids are second only to neutral fats in the rapidity with which they reduce osmic acid (Gatenby and Cowdry, '28). Thus, the lipid droplets of the zona glomerulosa differ from the liposomes of the zona fasciculata and zona reticularis by being osmiophilic and soluble in 10% formalin. The major similarity is the presence of phospholipid in both structures.

The effects of hypophysectomy

General histology. Following hypophysectomy the zona glomerulosa of the hamster maintains its normal histology and proportions. The failure of this zone to become relatively

thicker concurrently with a reduction in the total volume of the gland may indicate a minor degree of involution though this could not be detected by direct observation. Thus, the hamster may be similar to the rat in not requiring pituitary secretions for maintenance of the zona glomerulosa. In the rat the cross-sectional area of cells of the zona glomerulosa decreases approximately 40% after hypophysectomy, but this involution may be offset by formation of new cells from the capsule (Deane and Greep, '46; Greep and Deane, '47). Greep and Deane ('47) believe that the zona glomerulosa regulates electrolyte metabolism in the rat. No data are available concerning electrolyte balance in the hypophysectomized hamster and, in this species, the effect of adrenal cortical secretions is different from that in other forms (Snyder and Wyman, '51b). Therefore, a specific function cannot be attributed to the zona glomerulosa of the hamster.

The transitional zone appears to widen after hypophysectomy, when identified on the basis of cellular size and shape and the presence of small liposomes. However, it never occupies more than a small fraction of the cortex with more than half being composed of the zona fasciculata. This conclusion differs somewhat from the interpretation of Deane and Greep ('46) in the rat. Using the absence of lipid droplets as an identifying characteristic, these authors describe a progressive widening of the transitional zone, with the zona fasciculata and zone reticularis being severely involuted and remaining only as a narrow region of small, lipid-containing cells at two months after hypophysectomy.

The most marked effect of hypophysectomy occurs in the zona fasciculata and zona reticularis. These changes include reduction in size of parenchymal cells and in cytoplasmic chromophilia, disruption of the orderly arrangement of the cell columns and eventual disappearance of the zona reticularis. These modifications are, in general, similar to those described previously for the rat, mouse, dog, guinea pig and rabbit.

The significance of the darkly stained juxtanuclear bodies which appear in so many cells of the zona fasciculata is not clear. Their position suggests that they may represent some component of the Golgi apparatus. Reese and Moon ('38) state that in the hypophysectomized rat the Golgi apparatus shrinks to a small juxtanuclear cap without any radial extensions. The literature contains no description of an intracellular structure in the adrenal gland of any species which responds to hypophysectomy by an increase in size and which might be comparable to the juxtanuclear body in hypophysectomized hamsters.

Liposomes and mitochondria. Although the general histological changes which appear in the adrenal of the hamster following hypophysectomy are similar to those in other species, the response of intracellular bodies is markedly different. The cortical liposomes of the hamster increase in size after hypophysectomy. In contrast, the morphologically similar liposomes of the rat and mouse disappear after hypophystomy (Deane and Greep, '46; Miller, '50) and during pantothenic acid deficiency (Deane and McKibbin, '46). Pantothenic acid deficiency is accompanied by hyperactivity of the adrenal cortex. It appears, therefore, that the liposomes of the hamster and those of the rat play different roles in the secretory processes. How the enlarged liposomes in the hamster are to be interpreted in terms of the histological involution and apparent hypofunction induced by hypophysectomy is not known. Additional knowledge of the physiology of the adrenal cortex in this species and of the behavior of the liposomes in varying states of adrenal cortical activity is necessary before their physiological role can be assessed.

Hypophysectomy does not alter significantly the appearance or number of mitochondria in the zona glomerulosa. In the zona fasciculata they are reduced in number, a finding which is in accord with the belief that these organelles reflect the secretory status of adrenal cortical cells (Greep and Deane, '49).

Lipid. As determined by chemical analysis, the adrenal of the normal hamster contains 0 to ½% cholesterol (Agate, '52). This small amount cannot be detected by the Schultz modification of the Liebermann-Burchardt test for cholesterol and its oxidative derivatives (Reiner, '52) as employed in my study. Alpert ('50) has suggested that the absence of histochemically demonstrable cholesterol from the adrenal cortex in the hamster may result from failure to store secretory precursors in the gland and that the hormones are released as rapidly as synthesized. The cortical atrophy which follows hypophysectomy is not accompanied by the appearance of demonstrable lipid or cholesterol. The low cholesterol concentration in the cortex poses interesting questions concerning the secretory capacity of the adrenal cortex of the hamster. In the rat the large stores of cholesterol and its esters are thought to represent secretory antecedents, "held in readiness for periods of acceleration of cortical hormone synthesis" (Sayers, '50). Physiological conditions which produce a sudden need for increased amounts of cortical hormones cause a rapid depletion of adrenal cholesterol. In view of this and other evidence indicating that cholesterol is a possible precursor of adrenal hormones (Sayers et al., '44, '45; Conn et al., '50), one wonders how the hamster's adrenal cortex is capable of synthesizing large amounts of hormone rapidly in time of need with so little cholesterol stored in it.

In contrast to this low concentration of cholesterol in the normal gland, prolonged exposure of the hamster to stressors results in the appearance of considerable amounts of cholesterol. The Schultz histochemical test reveals cholesterol in the cortex after Leishmania infection (Leathem and Stauber, '52) and stilbestrol administration (Peczenik, '44; Koneff, Simpson and Evans, '46; Alpert, '50). Using the Sperry-Schoenheimer method for chemical analysis, Agate ('52) found a cholesterol concentration of 4 to 5% in the adrenal glands of hamsters bearing transplanted mouse sarcoma 180. In the rat the re-accumulation of cholesterol during prolonged

stressful conditions is characteristic of the stage of resistance (Selye, '50). The cholesterol which accumulates in the adrenal cortex of the hamster, however, is associated with degenerative changes and an apparent collapse of the secretory capacity of the gland (Alpert, '50; Leathem and Stauber, '52).

The absence of an altered staining capacity with leucofuchsin, 2-4-dinitrophenylhydrazine and 2-hydroxy-3-naphthoic acid hydrazide after hypophysectomy contrasts with the marked decrease observed in the hypophysectomized rat, where involution of the zona fasciculata is accompanied by a complete loss of aldehydes or of substances which may liberate them (Deane and Greep, '46; Deane, '50). Since the liposomes of the zona fasciculata of the hypophysectomized hamster enlarge and do not disappear, the persistence of acetone-insoluble reactive material after hypophysectomy may indicate, further, that the unsaturated fatty acids of the phospholipid component in liposomes is the source of this abundant aldehyde material.

Dempsey ('48) postulates that the presence of ketosteroids is indicated by a combination of characteristics including sudanophilia, ultraviolet fluorescence, birefringence, staining with reagents which demonstrate aldehydes, presence of cholesterol and of acetone-soluble lipid. If these criteria are valid and are applied to the hamster, one must assume either that the hormones secreted are not ketosteroids or that they are not stored in the gland.

Alkaline phosphatase. In the hamster, as in the Swiss mouse (Elftman, '47) and rat (Dempsey, Greep and Deane, '49), cortical alkaline phosphatase activity is greater in the male than in the female. The distribution of this enzyme in the hamster differs from that obsrved in the rat. In the rat the zona glomerulosa and the endothelium of the sinusoids throughout the cortex exhibit considerable enzyme activity (Dempsey, Greep and Deane, '49). Under the conditions used for study of the hamster, the zona glomerulosa exhibits no enzyme activity and the sinusoidal endothelium is positive

only where adjacent parenchymal cells also stain. Leathem and Stauber ('52) relate the increased alkaline phosphatase activity in the adrenal of the hamster infected with Leishmania to phospholipid metabolism. This suggestion is significant in view of the large amounts of phospholipids contained in the cortex of this species. The adrenals of cattle (Ault and Brown, '34), guinea pigs (Oleson and Bloor, '41; Knouff, Brown and Schneider, '41) and rabbits (MacLachlan, Hodge and Whitehead, '41) possess concentrations of phospholipids equalled only by brain and spinal cord (Wittcoff, '51). In general, large amounts of phospholipids are found in organs which secrete steroid hormones (ovary, corpus luteum, placenta) whereas other endocrine glands, such as the thyroid, contain less than one-twentieth as much (Wittcoff, '51). Additional knowledge of the significance of phospholipids in these endocrine glands is needed.

SUMMARY

The adrenal gland of the normal hamster is in general similar to that of other species with respect to zonation and histology. The gland differs, however, from that of other species in several respects: it is heavier in the male than in the female, it contains no acetone-soluble lipid, is negative to the Schultz test for cholesterol and lacks birefringence. These observations indicate a paucity of triglycerides and free or esterified cholesterol. The cells of the zona glomerulosa do contain a lipid which appears to be composed chiefly of free phospholipid. It appears as vacuoles after fixation in Regaud's fluid and is demonstrated by post-chromation and staining with Sudan black B, by the Baker acid hematein procedure for phospholipids and by blackening as a result of fixation in formol-Zenker-osmic acid. Droplets of this lipid are located at the vascular pole of the cells and vary in size within individual cells and in number from cell to cell. The lipid of the zona fasciculata and zona reticularis is histochemically different from that in the zona glomerulosa. It is insoluble in acetone and is stained intensely by reagents which demon-

strate aldehydes including leucofuchsin, 2-4-dinitrophenylhydrazine and 2-hydroxy-3-naphthoic acid hydrazide. Some of this lipid is present in the form of acetone-insoluble liposomes which appear to be a lipoprotein complex, in which the lipid portion is a phospholipid. The phospholipid is revealed by oxidation with potassium dichromate and subsequent staining with Sudan black B, iron hematoxylin or acid fuchsin.

After hypophysectomy the adrenal exhibits changes which are similar to those observed in other species and some which are uniquely different. Thirty days post-operatively the absolute weight of the gland of the male is decreased by 55% and that of the female by 42%; at 60 days the decrease is 53% and 40%, respectively. The zona glomerulosa remains histologically normal. The cells of the zona fasciculata are involuted greatly. Sudanophilic lipid, cholesterol and birefringence do not appear and reagents for the demonstration of aldehydes stain as intensely as before hypophysectomy. The liposomes of the zona fasciculata are enlarged. The mitochondria of the zona fasciculata are reduced in number. Alkaline phosphatase activity persists in the capsule of the adrenal but disappears almost completely from the parenchymal cells of the cortex. The medulla is unchanged after hypophysectomy.

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PLATES

PLATE 1

EXPLANATION OF FIGURES

The adrenals for figures 2 to 5 were fixed in Bouin's fluid, sectioned at 5 μ and stained with the Masson ('28) technique. Those for figures 6 to 9 were prepared by the Gomori ('39) procedure for alkaline phosphatase with incubation for three hours at pH 9.2. The cortico-medullary boundary is delineated by black lines in figures 6 to 9. $\times$ 100.

2 Male. The narrow zona glomerulosa stains less intensely than the zona fasciculata. The transitional zone is not clearly demarcated from the zona glomerulosa and the zona fasciculata. A narrow, poorly defined zona reticularis is present next to the medulla.

3 Male, 30 days after hypophysectomy. The zona glomerulosa is not increased in width. A widened transitional zone is indicated by the smaller nuclei between the zona glomerulosa and zona fasciculata. The cells of the zona fasciculata are smaller, less intensely stained and the columnar arrangement of the cells in the zona fasciculata is distorted. A zona reticularis is absent. The medulla is unchanged.

4 Control female. A small nodule of glandular cells is present in the capsule. The zona glomerulosa, transitional zone and zona fasciculata are similar to those of the male. The zona reticularis consists of smaller cells with compact nuclei adjacent to the medulla.

5 Female, 30 days after hypophysectomy. The capsule is not thickened. Changes in the zona glomerulosa, transitional zone and zona fasciculata are similar to those of figure 3. The zona reticularis is absent.

6 Control male. Alkaline phosphatase activity is present in the entire zona fasciculata and less of it in the zona reticularis. The zona glomerulosa and medulla are negtive.

7 Male, 30 days after hypophysectomy. Alkaline phosphatase activity is retained by the capsule of the gland and slightly by a few cells at the cortico-medullary boundary.

8 Control female. Alkaline phosphatase activity is present in the inner part of the zona fasciculata and in the zona reticularis.

9 Female, 30 days after hypophysectomy. Alkaline phosphatase in the capsule is not changed significantly and is almost gone from the remainder of the gland.

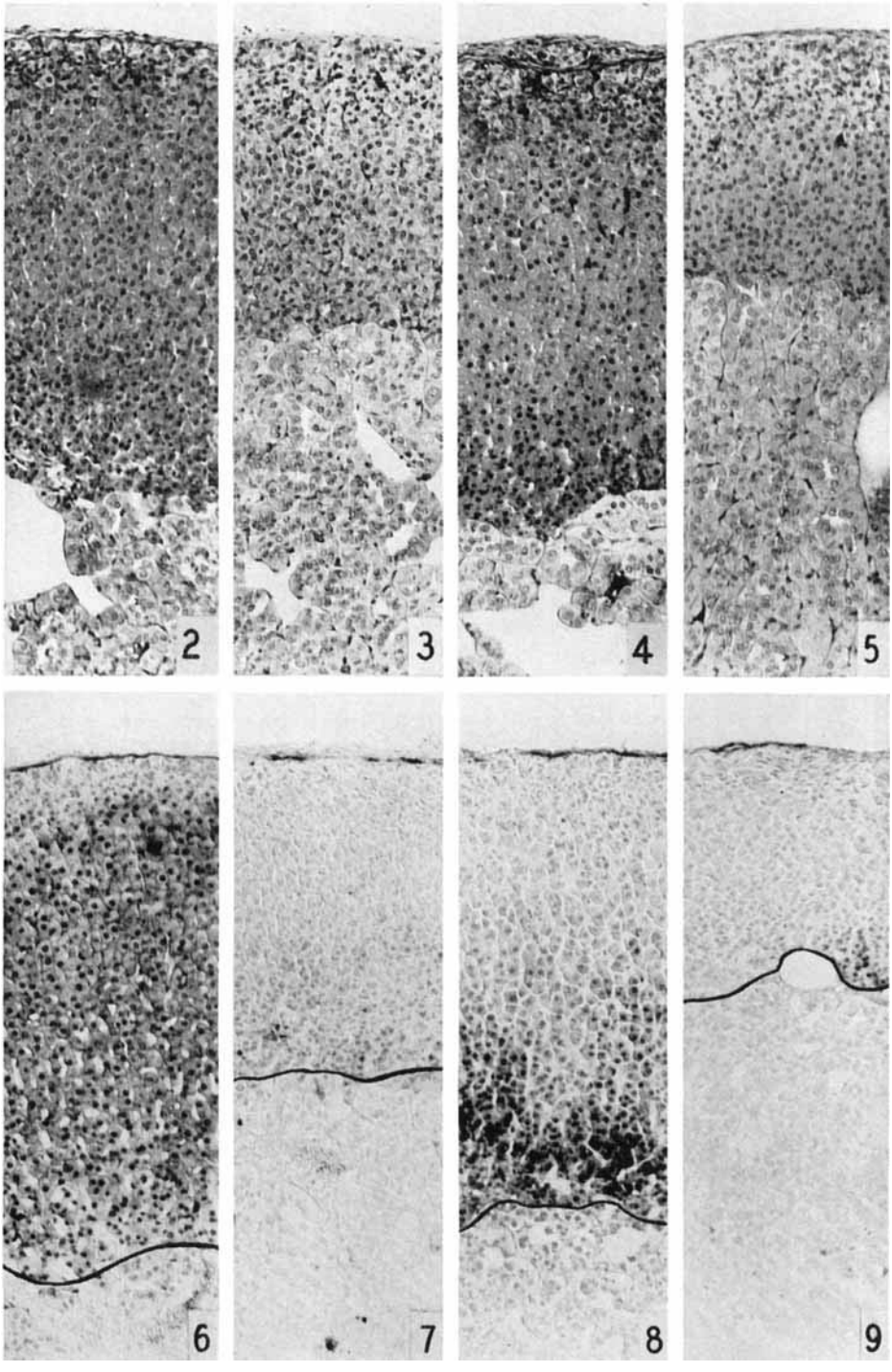
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PLATE 2

EXPLANATION OF FIGURES

The adrenal glands for figures 10 to 17 were fixed in 10% formalin and sectioned at 5 to 8 μ on the freezing microtome. Those for figures 10 to 13 were stained with leucofuchsin without prior treatment with mercuric chloride. Those for figures 14 to 17 were treated with 2-hydroxy-3-naphthoic acid hydrazide according to the method of Ashbel and Seligman. $\times$ 100.

10 Control male. The zona fasciculata and zona reticularis are stained intensely, the zona glomerulosa faintly and the medulla not at all.

11 Male, 30 days after hypophysectomy. The intensity of the stain is not significantly different from that of the control (fig. 10).

12 Female, 30 days after hypophysectomy. The intensity of the stain is similar to that of the hypophysectomized male (fig. 11).

13 A section of the same adrenal gland shown in figure 12, but extracted with acetone for three days prior to staining. The Schiff-positive material is not removed by acetone.

14 Control female. The Ashbel-Seligman reaction is intense and uniformly distributed throughout the zona fasciculata. The zona reticularis is slightly less intense. The zona glomerulosa and the medulla are not stained.

15 Female, 30 days after hypophysectomy. The reaction is similar to that of the control (fig. 14).

16 Male, 30 days after hypophysectomy. The Ashbel-Seligman reaction is similar to that of the hypophysectomized female (fig. 15).

17 A section of the same adrenal gland as shown in figure 16, but extracted with acetone for 5 days prior to staining. The material stained by the Ashbel-Seligman reaction is not removed by the acetone.

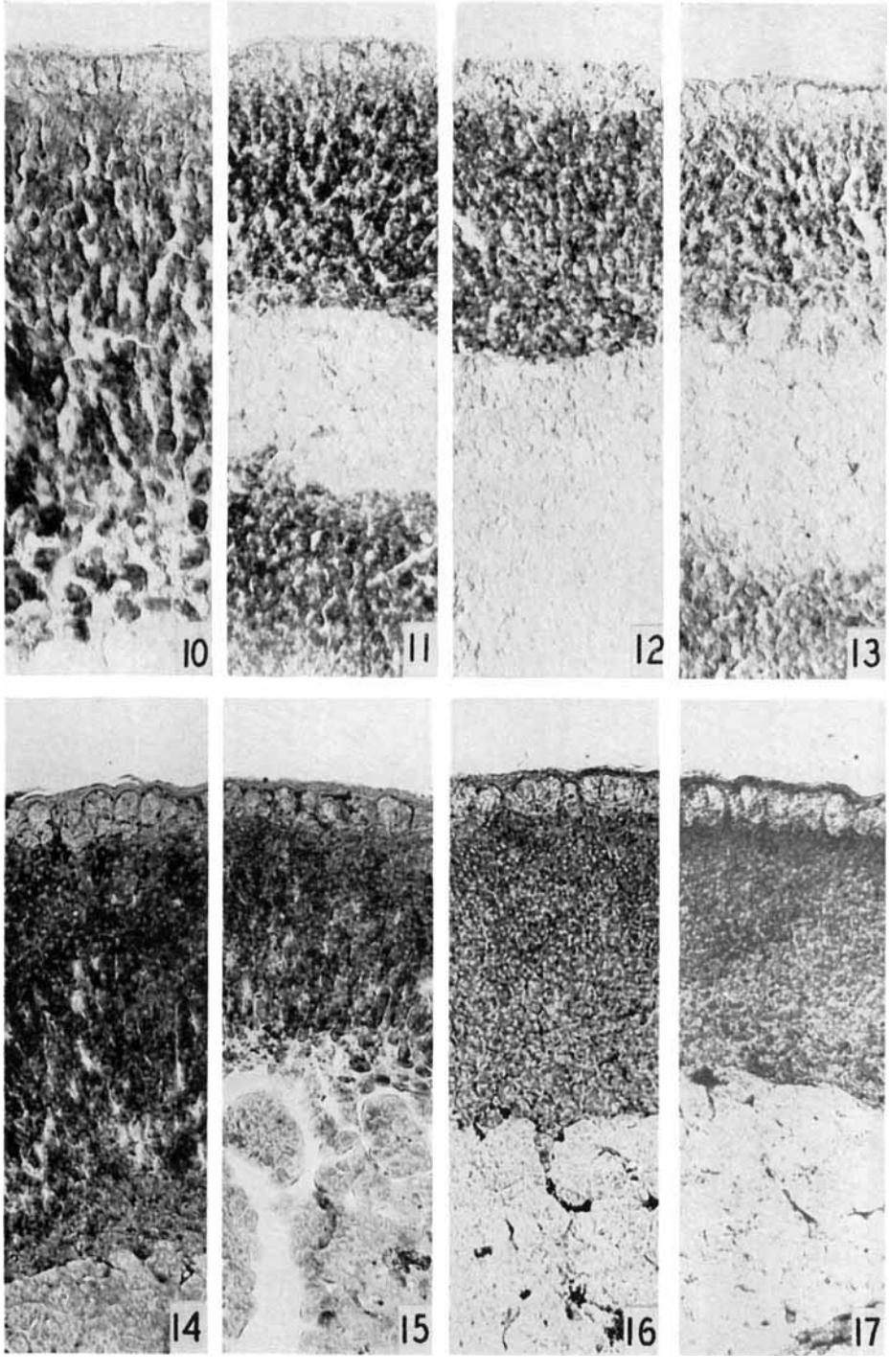
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PLATE 3

EXPLANATION OF FIGURES

The adrenal glands for figures 18 to 24 were fixed in Regaud's fluid, post-chromated and sectioned at 3 μ. Those for figures 19 to 21, 23 and 24 were stained with iron hematoxylin. Those for figures 18 and 22 were stained with Sudan black B and mounted in glychrogel.

18 Control male. The cells of the zona fasciculata and zona reticularis are colored by Sudan black B; the zona glomerulosa is colored faintly and all nuclei are negative. × 100.

19 Control male. With iron hematoxylin, the zona glomerulosa is demarcated sharply from the outer half of the zona fasciculata which stains intensely. The inner half of the zona fasciculata and the zona reticularis are stained diffusely. × 100.

20 Male, 30 days after hypophysectomy. The capsule and the zona glomerulosa are unchanged as compared with figure 19. The outer half of the zona fasciculata continues to stain but the diffuse staining of the inner half is reduced. × 100.

21 Female, 30 days after hypophysectomy. The affinity for iron hematoxylin is similar to that of the hypophysectomized male (fig. 20). × 100.

22 Zona glomerulosa and outer zona fasciculata of a control male. Lipid droplets of the zona glomerulosa are colored by Sudan black B either uniformly or as crescents or rings. The cells of the transitional zone and outer zona fasciculata are stained more uniformly. Nuclei are not colored. × 560.

23 Middle zona fasciculata, control male. Several cells show variation in size of the liposomes (L) and the relative numbers of granular and filamentous mitochondria (M). At (G) is a large, clear juxtanuclear area with several radial, canalicular extensions. × 1320.

24 Middle zona fasciculata, male, 30 days after hypophysectomy. Only a few pale-staining filamentous mitochondria are present among the enlarged liposomes. × 1320.

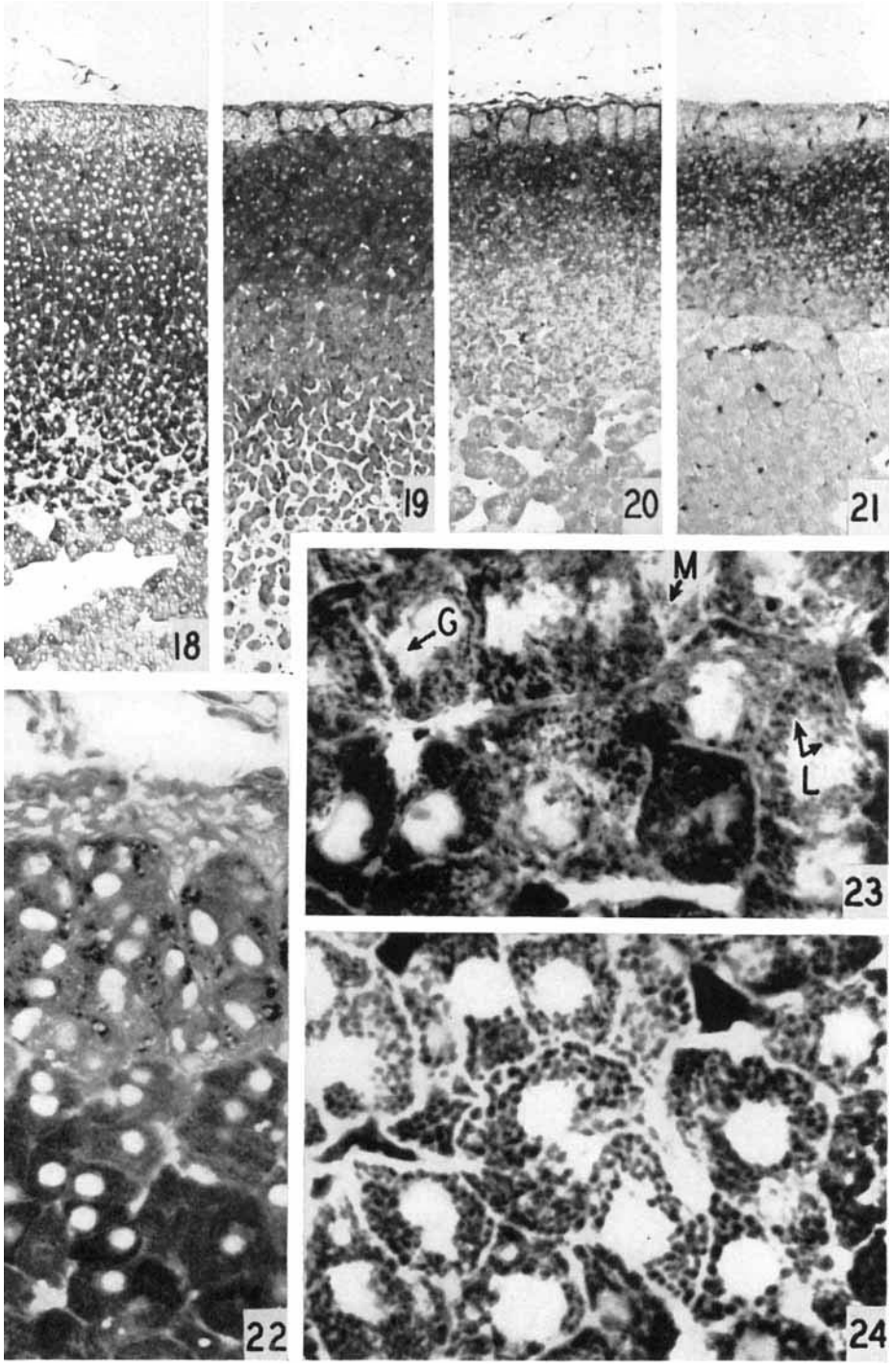
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PLATE 4

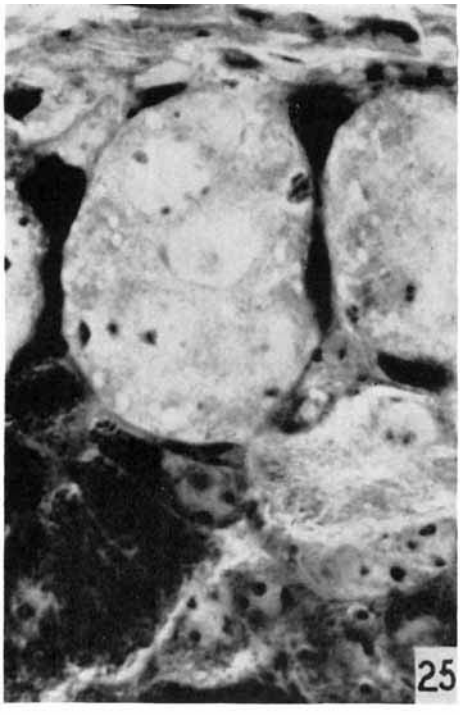
EXPLANATION OF FIGURES

25 Zona glomerulosa, control male. The cytoplasmic vacuoles are located at the periphery of the cluster near the sinusoids (black). At the lower left, the change to flattened, darkly-stained cells of the transitional zone is abrupt; at the lower right, this transition is more gradual. Regaud's fluid, iron hematoxylin, 4 μ. $\times$ 1150.

26 Zona glomerulosa, control male. The osmiophilic lipid droplets are large and correspond to the vacuoles of figure 25. The smaller, faint granules are mitochondria. Formol-Zenker-osmic acid, Altmann's acid fuchsin and Masson, 4 μ. $\times$ 1150.

27 Middle zona fasciculata, control male. At least some of the granules of the cytoplasm are believed to be liposomes. The negative image of the Golgi apparatus is visible in several cells (G). Bouin's fluid, Masson stain, 4 μ. $\times$ 1320.

28 Middle zona fasciculata, male, 30 days after hypophysectomy. Several of the coiled, juxtanuclear bodies are seen in several planes (arrows). Parenchymal cells are smaller than in figure 27, and liposomes are still present; the nuclei are large. Technique as in figure 27.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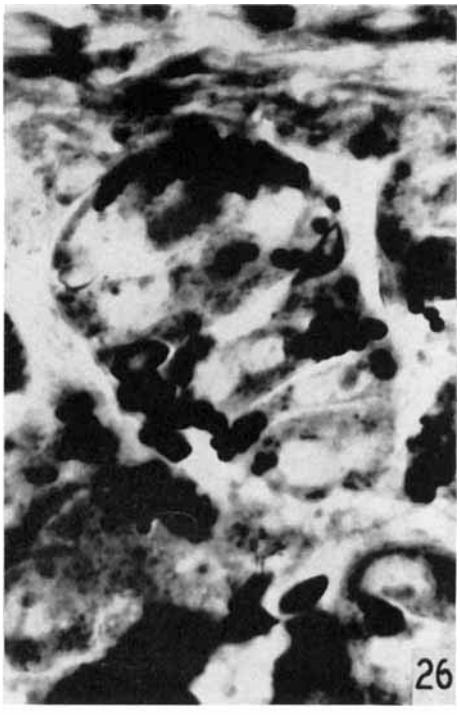
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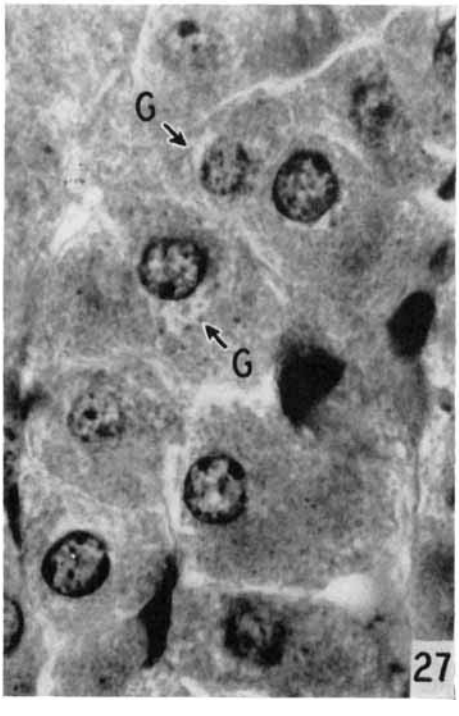
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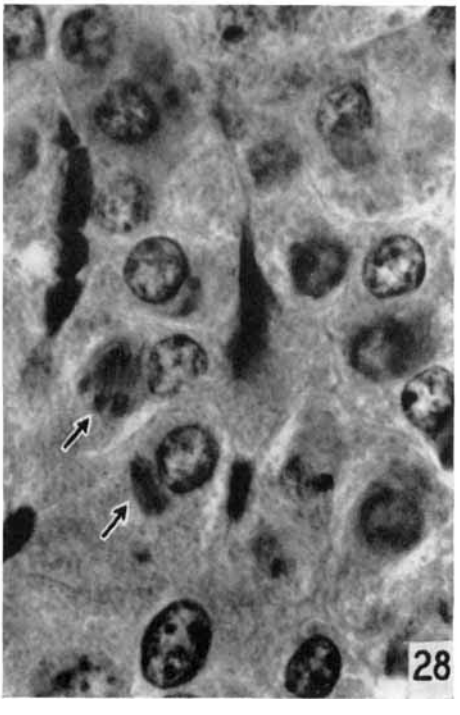
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