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HISTOLOGY OF THE ADRENAL GLAND OF THE LIZARD
ANOLIS CAROLINENSIS.

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HISTOLOGY OF THE ADRENAL GLAND OF THE
LIZARD ANOLIS CAROLINENSIS

DISSERTATION

Presented in Partial Fulfillment of the Requirements for
the Degree Doctor of Philosophy in the Graduate
School of The Ohio State University

By

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* * * * *

The Ohio State University
1970

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INTRODUCTION

A survey of the literature associated with the comparative histology of the adrenal gland indicates that less is known of reptiles than of any other vertebrate group (Jones, 1957; Hartman and Brownell, 1949; Andrew, 1959; and Patt and Patt, 1969). Although studies have included certain reptilian species, very little has been published on the adrenals of Anolis carolinensis, the American "chameleon."¹ Hartman and Brownell (1949) describe the gross anatomy of the adrenals of Anolis. Histologically, they state only that "the chromaffin cells lie mostly on the dorsal side of the interrenal tissue, penetrating very little..."² Other than descriptions of the general orientation of the two cell types, a search of the available literature failed to disclose a description of the histology of the adrenal gland of A. carolinensis.

¹Anolis, a member of the Iguanidae, is often sold at circuses and at pet shops for its ability to "change colors." Anolis is not a true chameleon as all members of the Chamaelontidae have prehensile tails, opposable toes, and are found only in the Old World (Storer, 1951).

²Chromaffin cells are the homologues of the mammalian adrenal medulla and the interrenal cells are the homologues of the mammalian adrenal cortex. In the evolutionary ascent of vertebrates, the two types of tissue gradually progress from separate masses in cyclostomes, to mingling of the two cell types in amphibians, and finally to the cortical and medullary portions of the single gland found in mammals.

The adrenal gland of this species will be histologically studied before and after removal of the hypophysis. Hypophysectomy will illustrate the physiological relationship of the two glands. These studies will thus contribute to the knowledge of adrenal gland comparative histology and endocrinology.

Historical Summary

According to Grollman (1936), the adrenals were not recognized as specific organs by the ancients. Probably as a result of their small size and yellowish, fat-like consistency, the adrenals were overlooked as parts of the perirenal fat. Eustachius first clearly described the adrenals of man in 1563. During the next 300 years many functions were erroneously applied to the adrenals. The modern study of the physiology of adrenals began when Thomas Addison published in 1855 one of the classics of medical literature. Other researchers described the effects of an extract of the adrenal medulla and their work led to the isolation and identification of epinephrine, the first hormone to be crystallized, identified, and synthesized. The adrenal cortex, being vital to the organism, was not studied until later, and Grollman, writing in 1936, states that "although the function of the gland is still a mystery and the product elaborated by the cortex still unidentified, much progress has been made."

The adrenal medulla, or its homologue, is now known to produce two catecholamines--epinephrine and norepinephrine. These two hormones are produced in different proportions and have different actions, depending on the species. The catecholamines are sympathomimetic and increase heart rate, cardiac output, oxygen consumption, basal metabolic rate, blood sugar level, production of ACTH, and blood pressure (Patt and Patt, 1969).

The adrenal cortex, or its homologue, secretes three general types of hormones. In man, the zona glomerulosa secretes mineralocorticoids which influence electrolyte and water balance; the zona fasciculata secretes glucocorticoids which influence the metabolism of carbohydrates, fats, and proteins; and the zona reticularis secretes androgens and estrogens which influence secondary sex characteristics. Such distinct zonation is not found in many of the lower vertebrates; furthermore, a particular chemical configuration that acts predominantly as a glucocorticoid in one species may exhibit salt regulation as its major activity in other species (Patt and Patt, 1969).

METHODS AND MATERIALS

Forty-nine animals were used for this study, thirty-seven males and twelve females. Four were used for the description of gross structures, twenty-five were used to describe normal histological detail, thirteen were hypophysectomized for experimental studies, four were used for electron microscope studies, and three were used for sham operations. The ages of the animals were unknown, but overall size and gonadal development indicated that all were adults. All animals used in this study appeared to be in good health. Two terraria were used to house the animals, the environmental conditions of each being as nearly the same as possible. One terrarium was used only for post-operative hypophysectomized animals. In all instances, animals were given light ether anesthesia before surgery.

Histological Studies

Tissues to be used for light microscopy were routinely fixed in Formol-sublimate-acetic acid FSA after Movat (1955). Regaud's fixative was used for Altmann's method of illustrating mitochondria (Humason, 1967). Tissues were dehydrated, cleared in methyl salicylate, and embedded in Paraplast and then Tissuemat. Sections used for general orientation of the head and body were decalcified with

lactic-nitric acid and cut at 8 to 12 μ (micrometers). Sections of adrenal gland were cut at 4 to 6 μ . Five stains were used including Harris' hematoxylin (Humason, 1967), Weigert's hematoxylin (Humason, 1967), Azan modification of Mallory Heidenhains's (Humason, 1967), Periodic acid - Schiff/iron hematoxylin/picro-indigo carmine (Wismar, unpublished), and Altmann's (Humason, 1967). The hematoxylin stains are standards for histological studies, Azan illustrates connective tissue, PAS illustrates a number of carbohydrate and carbohydrate-protein structures, and Altmann shows mitochondria.

The presence of norepinephrine was illustrated by a potassium iodate technique (Thompson, 1966). The adrenal gland was infiltrated with gelatin (Thompson, 1966) and sections were cut on a freezing microtome at a thickness of 20 μ . Routine paraffin sections were also used to illustrate the presence of norepinephrine via the potassium iodate technique.

Tissue for electron microscopy was processed according to the method of Hayes (1970). Tissue was fixed in a cold solution of 1 per cent glutaraldehyde buffered at pH 7.4 with phosphate buffer for 2 hours. Tissue was then transferred to cold phosphate buffer at pH 7.4 for 8 hours and post-fixed in 1 per cent osmium tetroxide for 1 hour. Following dehydration in a graded series of ethanol solutions, tissue was placed in propylene oxide and embedded

in Epon 812. Sections were cut on a Porter-Blum ultra-microtome following staining with uranyl acetate and lead citrate. The specimens were examined and photographed on a RCA EMU-3F electron microscope.

A Zeiss Universal microscope with attached 35 mm camera was used for the light microscope studies.

Hypophysectomies

A small hole was made into the palate with a micro-dissecting needle and a $\frac{1}{4}$ dental burr in the region indicated in Figure 20. A pipette, drawn to a small tip, was connected to a vacuum pump and the gland was aspirated at 15 pounds negative pressure. My first attempts at hypophysectomy involved entrance through the palate according to a line drawing of Kleinholz (1938). He illustrates that approximately 75 per cent of the hypophysis is dorsal to the cartilagenous bar with the remaining 25 per cent dorsal to a bony protuberance. As can be determined by Figure 17, approximately 50 per cent of the gland is dorsal to the white bony protuberance which Kleinholz describes. The author usually removed a portion of the cartilagenous bar and also a portion of the bone to provide a clear view of the hypophysis.

Of the thirteen hypophysectomized animals, three were kept alive for nine days. The other animals were sacrificed from one to seven days after hypophysectomy.

RESULTS

Gross Anatomy of Adrenal Glands

The adrenal glands of Anolis are closely associated with the gonads which are located near the dorsal body wall bounded on the cephalic end by a lobe of the liver and on the caudal end by the kidneys (Figs. 1 and 2). The adrenals are located dorsomedial to the gonads (Figs. 3 and 4) near the vena cava and within the mesorchium or mesovarium. The gross anatomy of Anolis parallels closely that of other reptiles as reported by Jones (1957).

Normal Histology of Adrenal Glands

The normal histology of the adrenal is illustrated in Figure 5. No significant difference exists between adrenal histology of the male and female (Figs. 6 and 7). Chromaffin cells are found along the entire dorsal surface and over both poles of the gland. Numerous cords and islets of chromaffin tissue are scattered throughout the interrenal tissue. Connective tissue of the surrounding capsule penetrates and surrounds the chromaffin tissue and separates the chromaffin tissue from interrenal tissue. The interrenal tissue is composed of anastomosing cords of cells which are separated into lobules by blood sinuses.

Chromaffin tissue

Two cell types are found in chromaffin tissue of Anolis. In one type, the nucleus stains dark with hematoxylin (Fig. 8) and red with the Azan stain (Fig. 9) and has many mitochondria distributed throughout the cytoplasm (Fig. 10). In the second cell type, the nucleus does not take hematoxylin but stains blue with the Azan stain and the cytoplasm contains fewer mitochondria. The potassium iodate technique also illustrates two cell types, about half of which secrete norepinephrine. (Figs. 11 and 12). Both cell types are spherical to oval in shape and vary from 10 to 20 μ in diameter. The nuclei are 5 to 6 μ in diameter, generally centrally located in the cells, and contain one to three nucleoli. The two cell types are distributed in small groups throughout the chromaffin tissue.

Interrenal tissue

Interrenal cells are arranged into lobules that are separated by connective tissue and blood vessels (Figs. 6, 7, and 14). With the staining methods used, only one cell type is evident. The cell boundaries are difficult to differentiate because of the high degree of vacuolation, but the average cell diameter approximates 12 μ . The nuclei average 5 μ in diameter, are spherical to oval in shape, and contain one or more prominent nucleoli (Fig. 14). Within

each lobule the cells are highly organized and liposomes of several cells may be arranged in a straight line (Figs. 13 - 15). Mitochondria, as visualized by the Altmann stain, are aligned along and between the liposomes (Fig. 15). The close relationship of mitochondria and liposomes is also illustrated via electron microscopy (Fig. 16).

Gross Anatomy of the Hypophysis

The hypophysis is located dorsal to the palate in an area bounded cephalically by a cartilagenous bar and caudally by an arched bone (Fig. 17). The area is comparable to the mammalian sella turcica. The gland is separated by a cleft into an anterior lobe, which is located ventrolaterally (Figs. 17 and 18), and a posterior and an intermediate lobe, which are attached via the infundibulum to the hypothalamus (Fig. 17). The gross location of the hypophysis is illustrated in Figures 20 and 21.

Effect of Hypophysectomy on Adrenals

There is no apparent change in the histology of the adrenal one day after hypophysectomy. The remainder of the experimental animals show increasing histological change with increased postoperative life.

Nine days after hypophysectomy the chromaffin tissue shows no apparent change. Interrenal tissue, however, is markedly altered (Figs. 22 and 23). The cytoplasmic component of most cells is greatly reduced, as evidenced by the

close proximity of adjacent nuclei, and there is a loss of liposomes. Nuclei hypertrophy to an average diameter of 8 μ . As the result of the apparent loss of basophilic nuclear material, the nucleoli are more evident. The nuclear membrane of some cells is indented and is suggestive of degenerating changes.

The completeness of hypophysectomies was verified by histological sectioning of the head (Fig. 19).

Sham operations did not alter the adrenal gland.

DISCUSSION

The histology of adrenal glands of Anolis carolinensis has not been previously described. The results of this work indicate that the adrenals are similar in most respects to other reptiles which have been described, but with some exceptions.

Normal Histology

Chromaffin tissue

The present study indicates that two cell types of chromaffin tissue are randomly distributed in Anolis. Miller (1952) studied the Yucca night lizard, Xantusia vigilis, and describes two cell types, "one peripheral and the other central or adjacent to the interrenal tissue." Miller further states that the "outer zone cells have very fine granules which do not stain subsequent to chromation or osmication, while the inner or central zone cells have larger granules which after chromation or osmication are strongly acidophilic."

Wright and Jones (1957) describe the adrenals of the West African lizard, Agama agama. They also describe a zonation of two cell types and further correlate the outer zone with the production of norepinephrine and the inner zone and islets with the production of epinephrine.

In Anolis the two cell types are not zoned but form small groups which are randomly mixed. One cell type secretes norepinephrine (Figs. 11 and 12) while it is assumed that the other type secretes epinephrine. Further work will be required to correlate the production of the two catecholamines by these two cell types.

Interrenal tissue

The interrenal tissue of Anolis is similar to that reported for other reptiles. However, Jones (1957) reports that atrophic cells are scattered among the normal cells in captive green and West African lizards. Wright (1957) also found a narrow area of atrophic cells lying at the periphery of the cortex against the chromaffin tissue. Jones (1957) reports similar findings in Gerrhonotus and in Philodryas. Miller (1952), on the other hand, found no such atrophic area in the adrenal of Xantusia, either from freshly caught or starved organisms. I also found no atrophic area in Anolis.

Fat vacuoles (liposomes) in reptilian adrenals have been described by several authors (Jones, 1957; Wright and Jones, 1957; and Miller, 1952). Their data indicate that the lipid includes cholesterol, the precursor of all interrenal hormones, as tested via the Schultz modification of the Liebermann-Burchardt reaction and via difitonide precipitation. Gist and deRoos (1966) studied the steroids

of the alligator adrenal gland in vitro. Through the use of ultraviolet light photographs of chromatograms, they were able to demonstrate the presence of corticosterone, aldosterone, and two unidentified components. Further work will be required to determine the specific hormones found in Anolis.

The author finds no description of liposomes which in any way suggests the linear arrangement found in Anolis via light microscopy. Further study of the ultrastructure of the adrenals will be required to elucidate this arrangement. The small sampling of tissue used in the present electron microscope study indicates that the linear arrangement of the liposomes may be confined within a single cell (Fig. 16). Idelman (1970) describes the ultrastructure of the mammalian adrenal cortex and also reports a comparative study which includes birds, amphibians, and fishes. He does not include reptiles, as is seemingly common in comparative studies of the adrenal gland.

Adrenals of Hypophysectomized Animals

Chromaffin tissue

Chromaffin cells of Anolis do not appear to change following hypophysectomy. Wright and Jones (1957) and Miller (1952) report the same for Agama and for Xantusia.

Interrenal tissue

The present study indicated a depletion of liposomes

9 days after hypophysectomy. Wright and Jones (1957) report that large sudanophilic, Schultz-positive, lipid droplets abound in Agama 30 days after hypophysectomy. Miller (1957) found that interrenal tissue of completely hypophysectomized Xantusia of 7 days "showed partial loss of lipid, decrease in cell size, and pycnosis in some areas. In this group there was a tendency for some cells to remain well packed with lipid, while adjacent cells were completely evacuated." Jones (1957) reports that hypophysectomy of the snake, Natrix natrix, for 30 days decreases interrenal weight by 58 per cent. He found that some cells had reduced cytoplasm and pycnotic nuclei, others had normal nuclei and were heavily vacuolated, and various intermediate types were also found.

The great variation reported for adrenal lipid vacuoles can not be explained at this time. Species may vary in their response, there may be seasonal differences or sex differences, and the variation may be a function of the time interval elapsed between hypophysectomy and sacrifice of the animal.

Adenohypophysial-Adrenocortical Axis

There may be an adenohypophysial-adrenocortical relationship in reptiles that is similar to the type found in mammals. Wright and Jones (1957) report that hypophysectomy is followed by adrenal atrophy, as is substantiated by my

work. They also state that unilateral adrenalectomy produces contralateral adrenal hypertrophy and that the injection of bovine ACTH restores atrophied adrenals to normal histological appearance. Miller reports similar findings (1952). Thus the naturally occurring reptilian hypophysial agent which influences interrenal tissue may be similar to mammalian ACTH.

In man, ACTH principally affects the zona fasciculata (Leeson and Leeson, 1970) and thus principally affects glucocorticosteroidogenesis. The interrenal tissue of lower vertebrates, however, apparently is not divided into zones. It should therefore be of interest to the comparative anatomist or comparative endocrinologist to determine whether the apparently undifferentiated interrenal tissue of Anolis contains more than one cell type or whether all of the cells have the potentiality to secrete any of the adrenocortical hormones.

Since the hypophysectomized Anolis is seemingly able to maintain a relatively stable electrolyte and water balance, and since electrolyte imbalance does follow adrenalectomy (Wright and Jones, 1957), and since all interrenal hormones are formed from cholesterol, I hypothesize that in the absence of ACTH the metabolic pathways of cholesterol are shunted toward the mineralocorticoids at the expense of glucocorticoids. Such a mechanism would account for

Wright's observation (1957) that normal cells (producing mineralocorticoids) may be found adjacent to atrophic cells (which formerly produced glucocorticoids). The quantity of mineralocorticoids required to maintain homeostasis in the presence of hypophysectomy may account for the decrease of lipid material in Anolis. Histochemical and physiological studies will be required to clarify this problem.

SUMMARY

The histology of the adrenal glands of Anolis carolinensis has not been previously described. The glands are described grossly and histologically with references to the similarities and dissimilarities that exist between Anolis and reports published on other reptiles.

Chromaffin tissue, the homologue of the mammalian adrenal medulla, contains two cell types which are believed by some authors to be responsible for the production of epinephrine and norepinephrine. These two cell types are scattered randomly throughout the chromaffin tissue of Anolis in contrast to the more circumscribed areas reported for other reptiles.

Interrenal tissue, the homologue of the mammalian adrenal cortex, is composed of anastomosing cords of cells with no apparent differentiation of cell types--in contrast to zonation found in mammals. The cytoplasm of interrenal cells contains many liposomes which are presumably filled with cholesterol or adrenocortical hormones derived from cholesterol. It was noted that in Anolis these liposomes are arranged in straight parallel lines which may extend over a distance of several cells. This configuration of liposomes has not been reported in other reptilian species.

Animals were hypophysectomized and were sacrificed up to nine days later. Hypophysectomy caused no apparent change in chromaffin tissue, but caused a marked change in interrenal tissue. Interrenal cells displayed degenerative change and there was a large loss of lipid material from the cytoplasm.

The adeno-hypophysial-adrenocortical axis was discussed and an hypothesis was proposed to account for a possible mechanism of ACTH in reptiles and to account for the loss of lipid material after hypophysectomy.

The precise location of the hypophysis on Anolis was described, which modified a description previously reported.

Many areas were delineated for additional research; areas that involved more detailed descriptions of reptilian adrenals to help complete the file of the comparative anatomist, histochemical studies which would indicate more precisely the function of various cells found in the adrenals, and studies which would more accurately describe the adeno-hypophysial-adrenocortical axis and the function of ACTH in reptiles.

PLATE I

Figures 1 to 3 are gross photographs of Anolis carolinensis. X7 (scale = 2 mm)

Explanation of Figures

1. Dorsal body wall of a male. The peritoneum is darkly pigmented and difficult to distinguish from the black background. The two testes (T) are shown near the midline. A lobe of the liver (L) is characteristically attached to the right gonad. The ductus deferens (D) and kidneys (K) are shown caudal to the testes.
2. Dorsal body wall of a female. The yellowish ovaries (O) are shown against the dark peritoneum. The liver (L) is attached to the right ovary. Blood vessels (V), including the vena cava and aorta, are seen dorsal to the ovaries.
3. Adrenals attached to gonads. The paired adrenal glands (A) are seen on the dorsal aspect of the testes (T). The ductus deferens (D) is lateral to each adrenal.

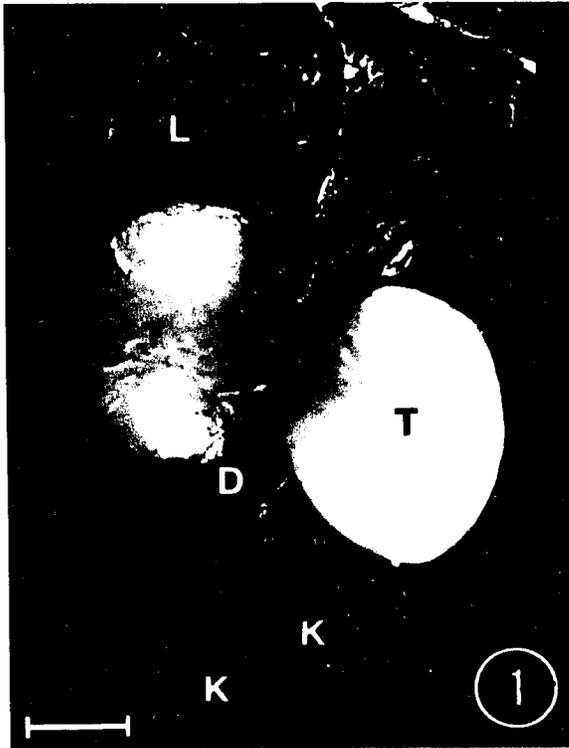


PLATE II

Explanation of Figures

4. Cross section through the body wall of a male Anolis. The adrenals (A) are located near the midline between the spinal cord (S) and the testes (T) close to the bifurcation of the vena cava (V). Weigert's H & E. X210 (scale = 0.1 mm)
5. Cross section of adrenal gland. Chromaffin tissue (C) surrounds the dorsal surface and caps both poles of the gland. Cords and islets of chromaffin tissue penetrate deeply into the interrenal tissue (I). The interrenal tissue is divided by connective tissue and blood sinuses into lobules. Connective tissue also separates the adrenal from the ductus deferens (D). Weigert's H & E after chromation. X625 (scale = 25 μ)

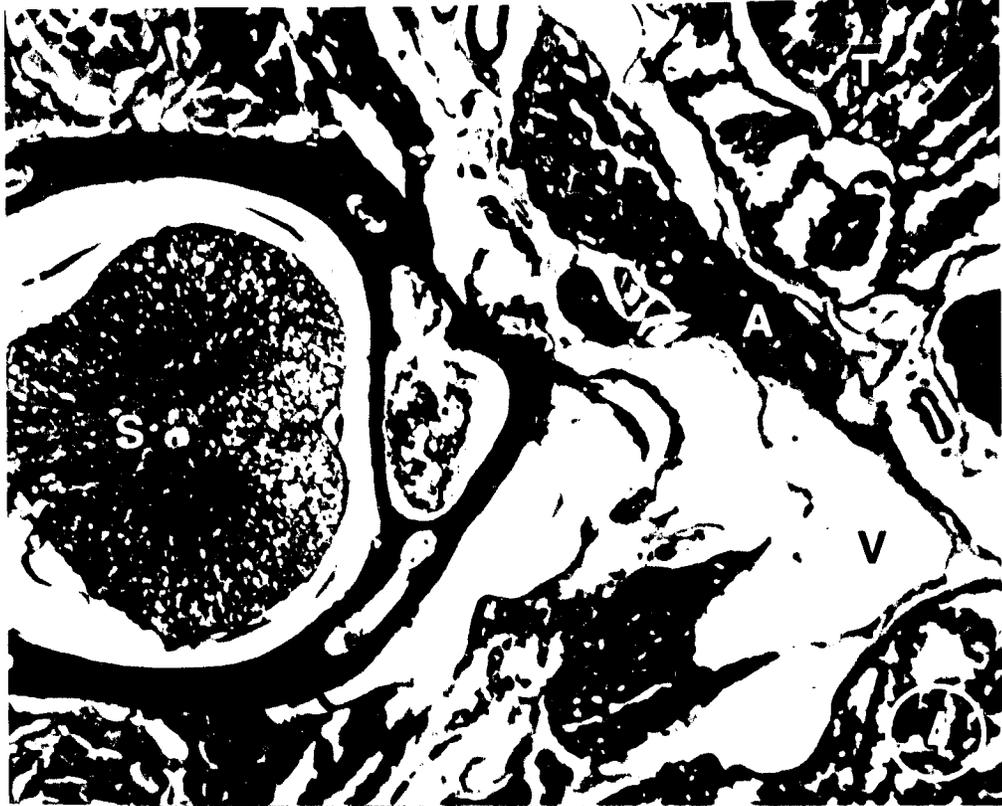


PLATE III

Figures 6 and 7 are longitudinal sections of the adrenal gland. Weigert's H & E after chromation.

Explanation of Figures

6. Adrenal of male. Chromaffin tissue (C) surrounds and penetrates the interrenal tissue (I). The presence of erythrocytes (E) and connective tissue (dark arrows) illustrates the division of interrenal tissue into lobules. X950 (scale = 25 μ)
7. Adrenal of female. The arrangement of chromaffin tissue (C) and interrenal tissue (I) is similar to that found in the male (Fig. 6). Erythrocytes (E) and connective tissue (CT) are shown. The ovary (O) lies adjacent to the adrenal. X1400 (scale = 25 μ)

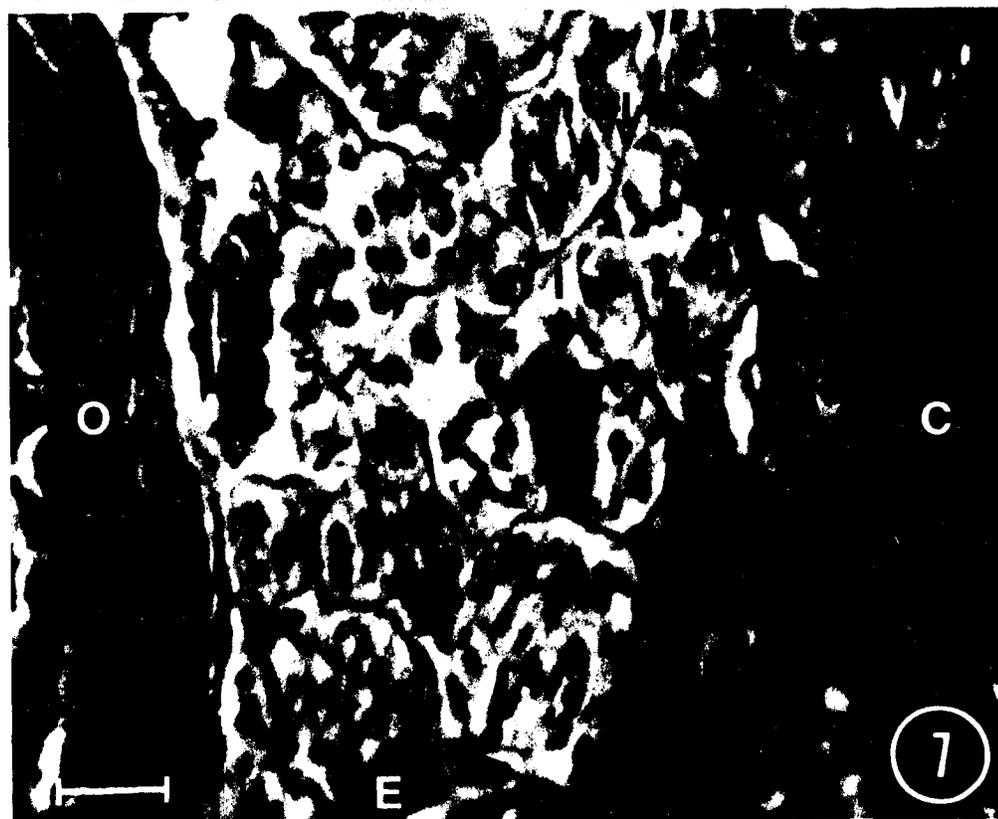
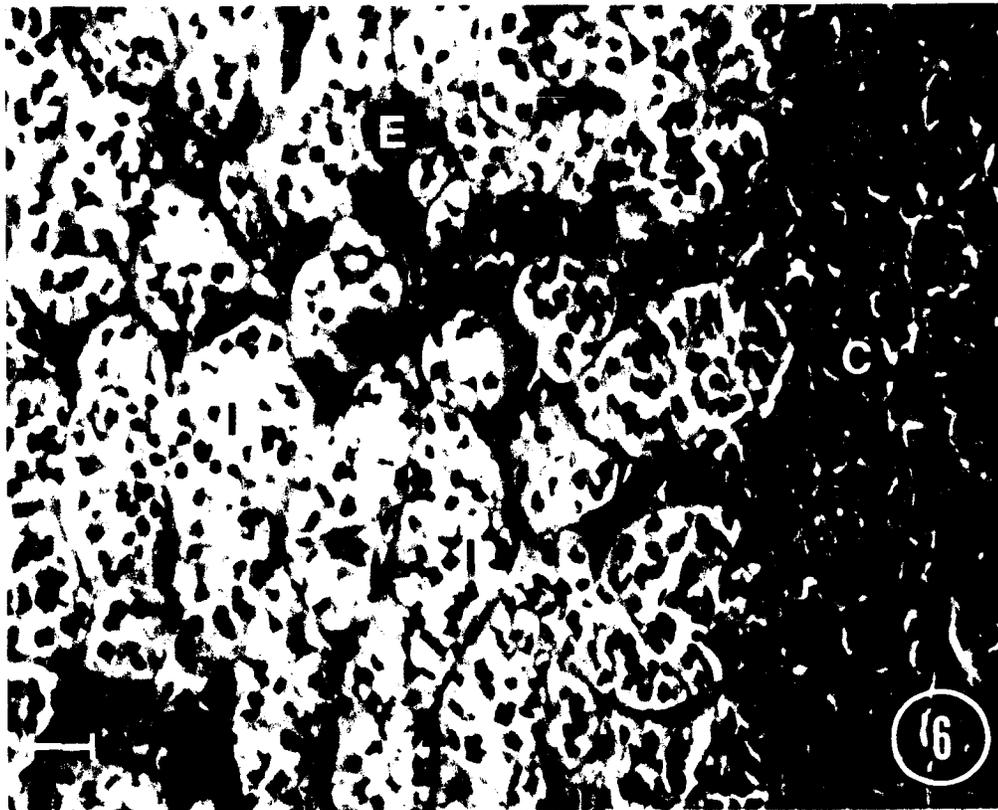


PLATE IV

Figures 8 to 10 illustrate chromaffin tissue.
X4,780 (scale = 10 μ)

Explanation of Figures

8. Chromaffin tissue. With the use of Weigert's H & E stain, some nuclei stain darkly (light arrows) and some stain lightly (dark arrows).
9. Chromaffin tissue. With the use of Azan stain, some nuclei stain red (dark arrows) and some stain blue (light arrows).
10. Chromaffin tissue. With the use of Altmann's stain, mitochondria are evident as more darkly staining granules (arrow).

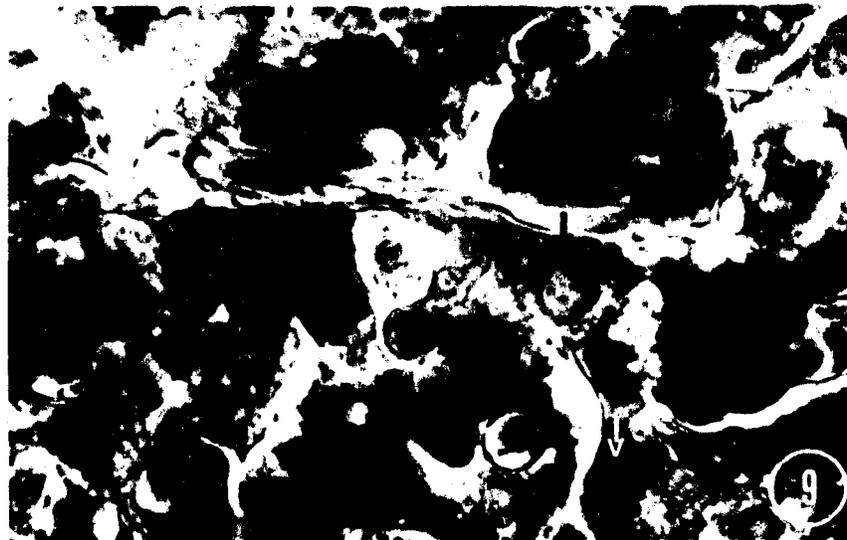
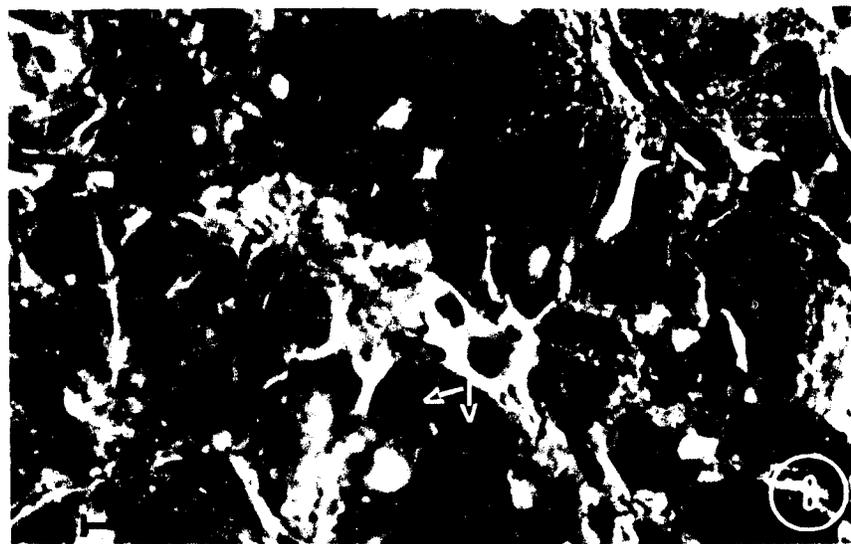


PLATE V

Figures 11 and 12 were treated with potassium iodate which demonstrates cells producing norepinephrine.

Explanation of Figures

11. Chromaffin tissue. Cells producing norepinephrine are dark (light arrows) while cells believed to be producing epinephrine are much lighter (dark arrows). Frozen section. Potassium iodate and hematoxylin. X1,325 (scale = 35 μ)
12. Chromaffin tissue. Cells producing norepinephrine contain very dark granules (light arrows) while cells believed to be producing epinephrine contain no granules. Paraffin section. Potassium iodate and H & E. X4,780 (scale = 10 μ)

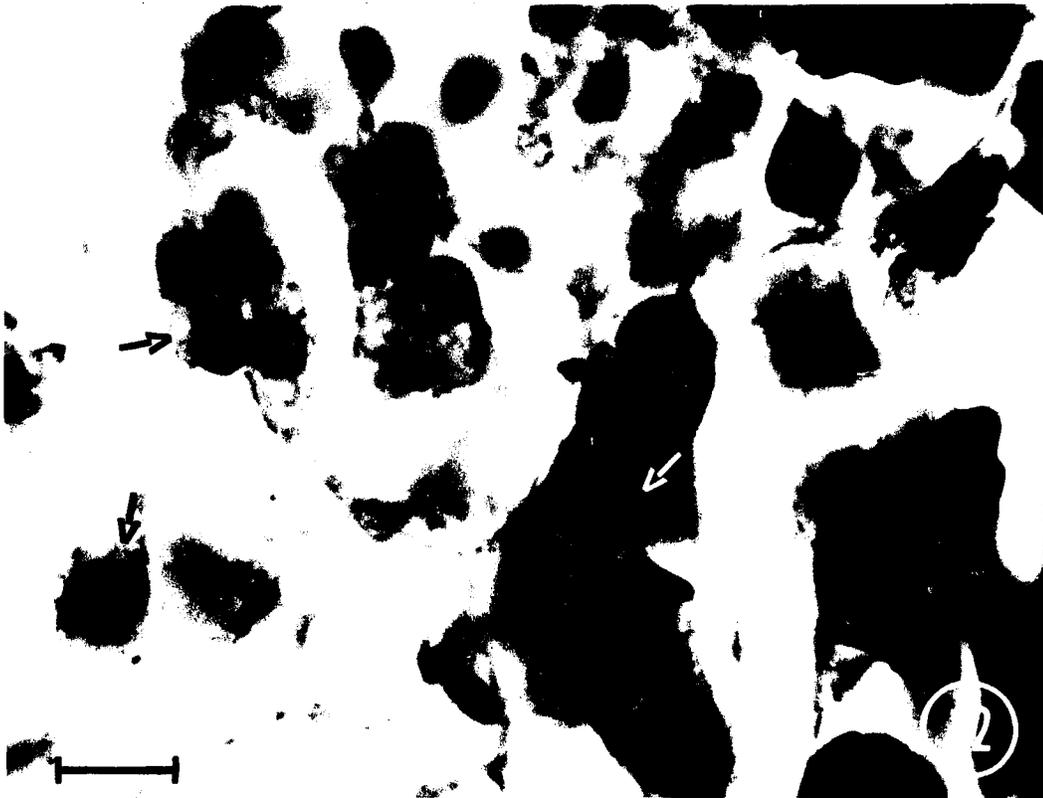
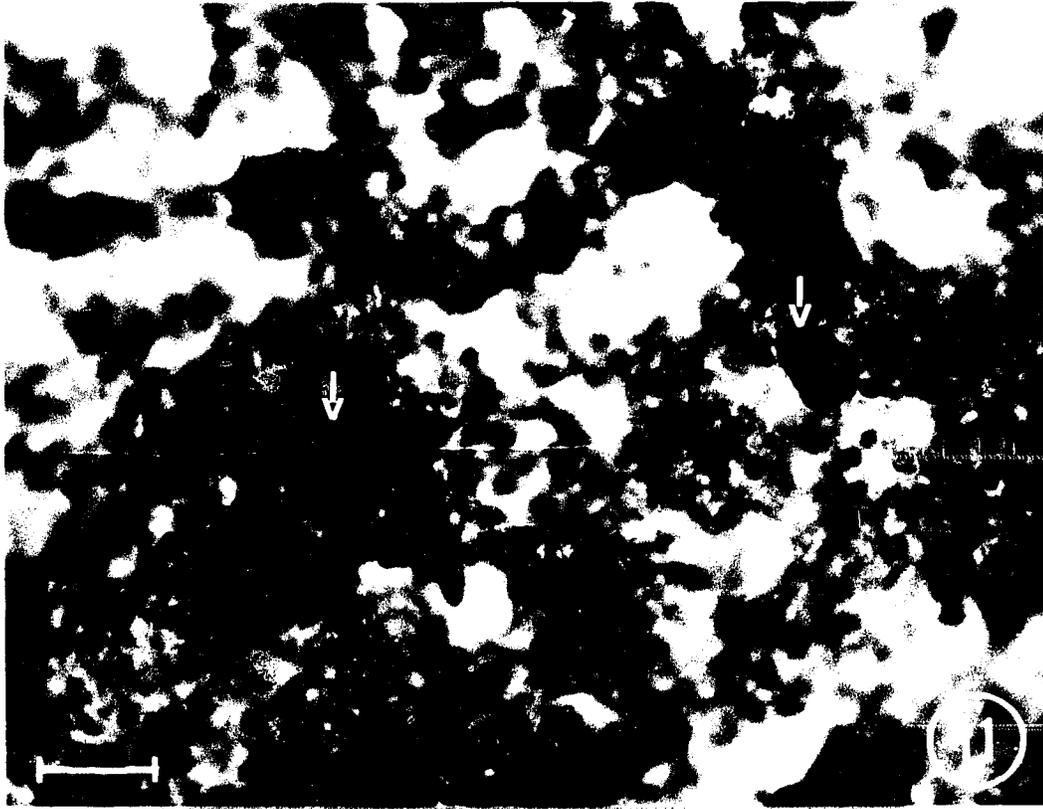


PLATE VI

Figures 13 to 15 are of interrenal tissue. The figures well illustrate the lobular arrangement of the cells. Note the linear arrangement of liposomes (arrows). X4,780 (scale = 10 μ)

Explanation of Figures

13. Interrenal tissue. Liposomes (arrow) are evident. H & E.
14. Interrenal tissue. Lobules, nucleoli within nuclei (N) liposomes (dark arrows), and connective tissue (CT) are well illustrated. Azan stain.
15. Interrenal tissue. Mitochondria (M) are seen lining the liposomes. Connective tissue (CT) and lobules are shown. Altmann stain.

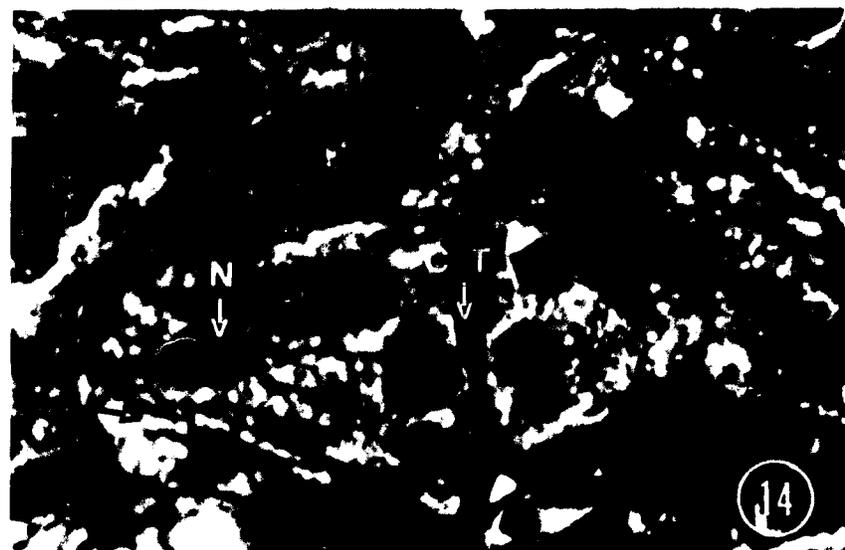
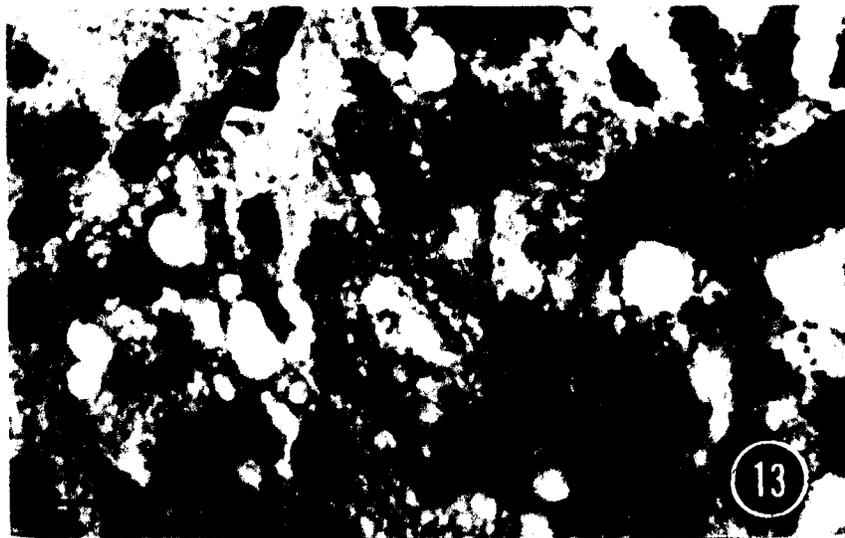


PLATE VII

Explanation of Figure

16. Electron micrograph of chromaffin and interrenal cells. Chromaffin cells are shown with a nucleus (N) and many catecholamine granules (G). Connective tissue cells (CT) are seen at the interface between chromaffin and interrenal cells. Many liposomes (L) and mitochondria (M) are found in the interrenal cells. X6,290

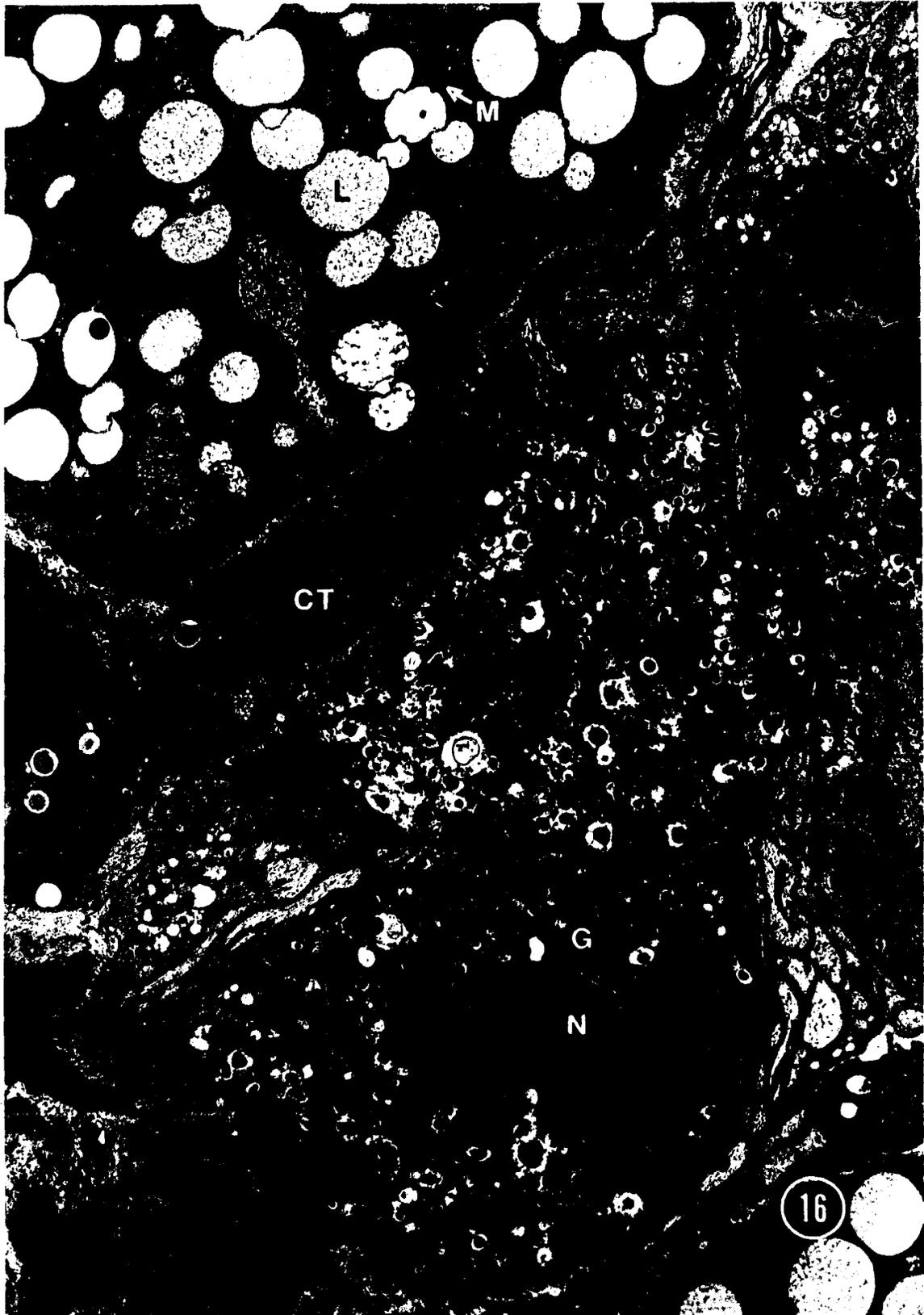


PLATE VIII

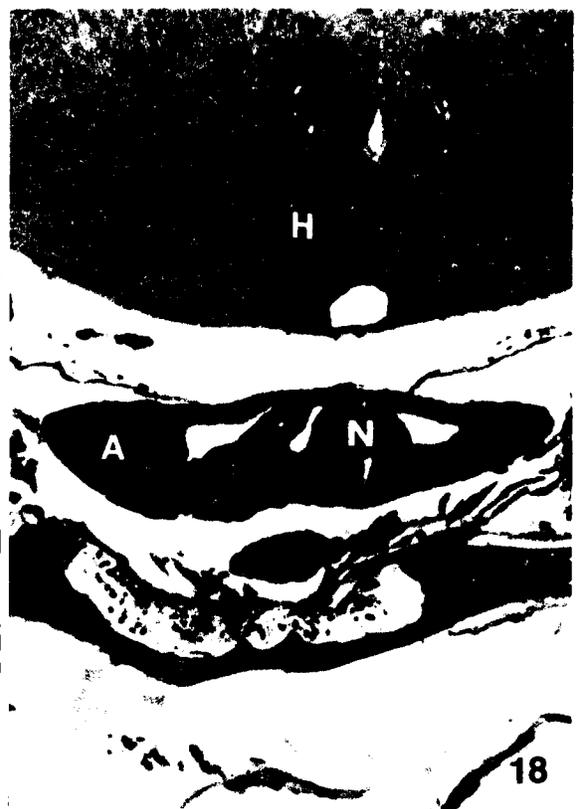
Figures 17 to 19 illustrate the hypophysis of Anolis.

Explanation of Figures

17. Mid-sagittal section of normal animal. The hypophysis is shown dorsal to a cartilagenous bar (C) and a bony protuberance (B) of the palate. The adenohypophysis (A) is shown ventral to the neurohypophysis (N), and the latter is connected to the hypothalamus (H). PAS stain. X175 (scale = 0.1 mm)
18. Transverse section of normal animal. The adenohypophysis (A) is shown lateral and ventral to the neurohypophysis (N). This section was taken posterior to the infundibulum and thus does not show the connection to the hypothalamus (H). Weigert's H & E. X230
19. Mid-sagittal section of hypophysectomized animal. On this animal, entrance to the hypophysis was gained by cutting through the cartilagenous bar (C). A small portion of the neurohypophysis (N) was not removed from this animal. The area is filled with many blood cells (B) as the result of surgery. Harris' H & E. X175



17



18



19

PLATE IX

Figures 20 and 21 are gross photographs of the head of Anolis. X9 (scale = 2 mm)

Explanation of Figures

20. Dorsal aspect of palate. The area bounded by () marks the junction of the cartilagenous bar and bone and is the location best suited for removal before hypophysectomy.
21. Ventral aspect of dissected head. An area comparable to the mammalian sella turcica (S) is shown posterior to the orbits.

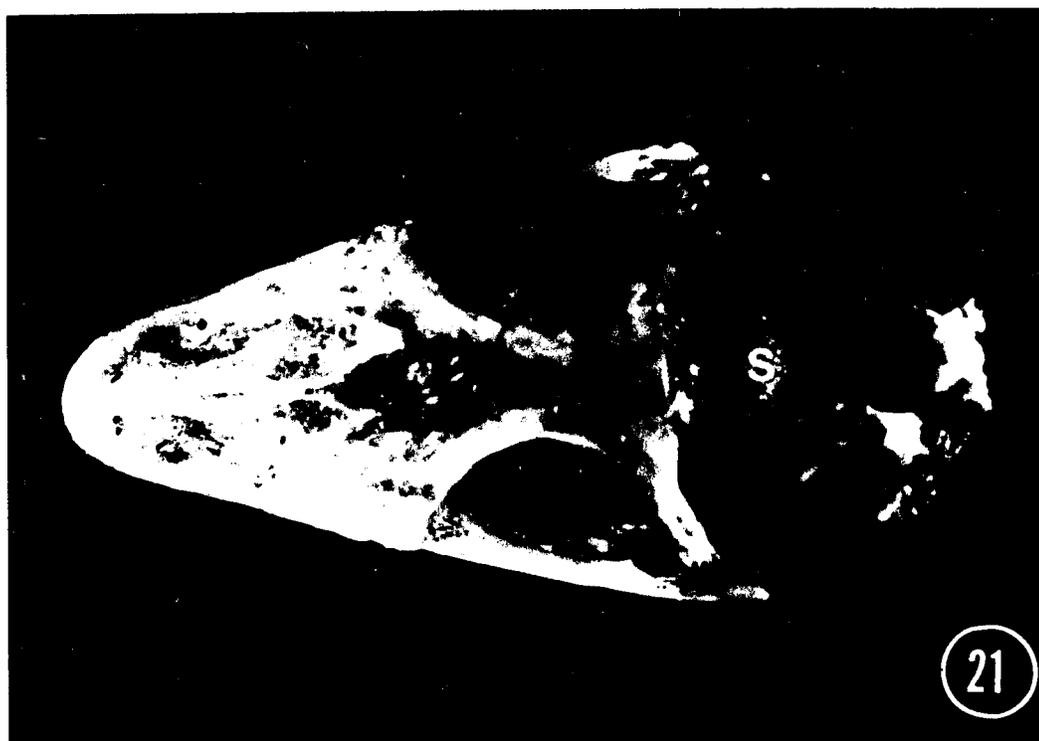
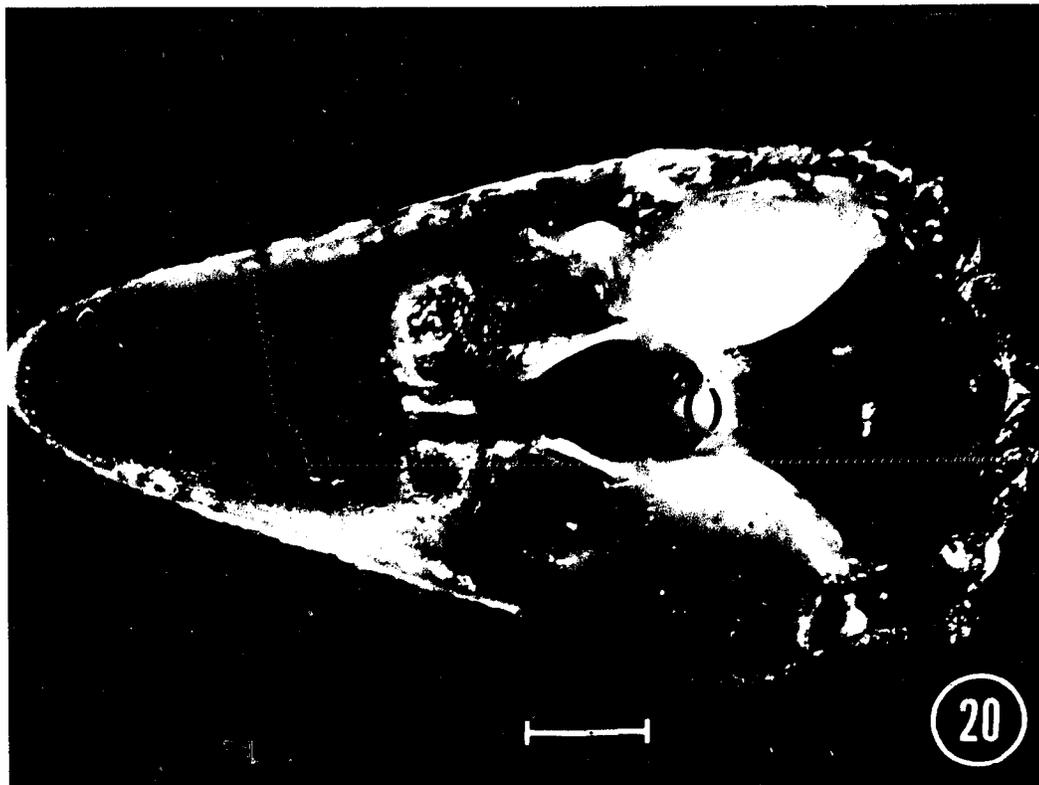
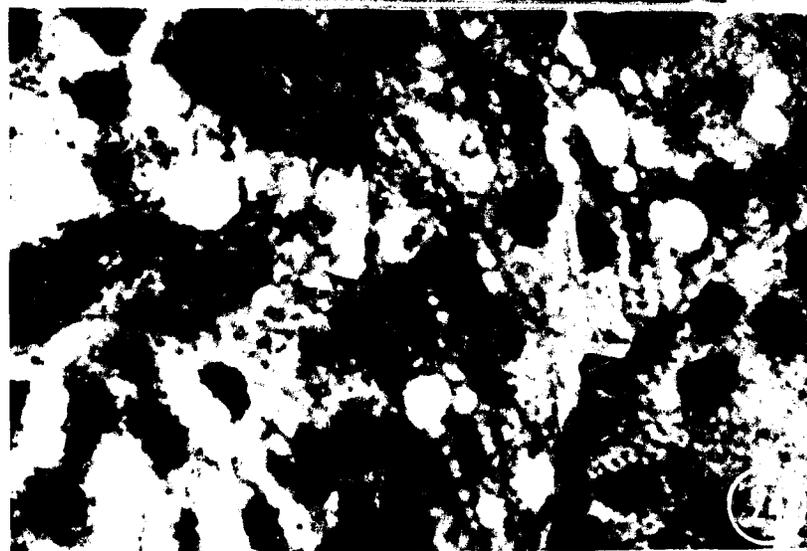
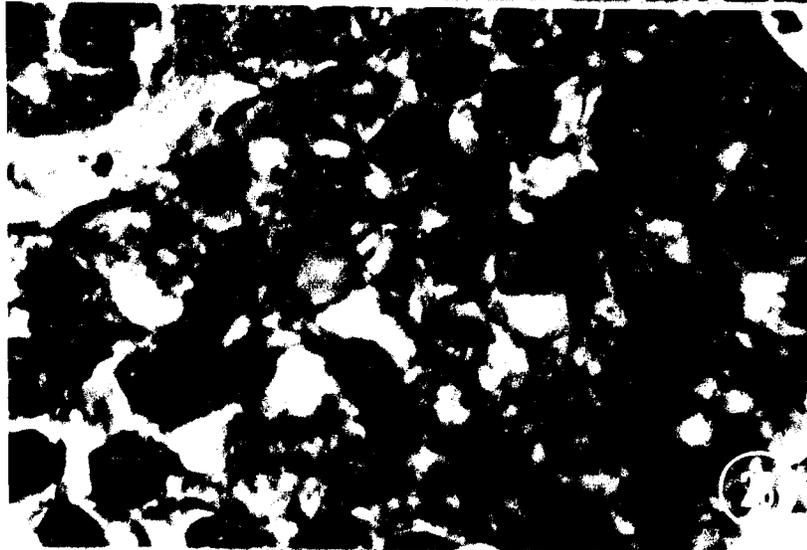
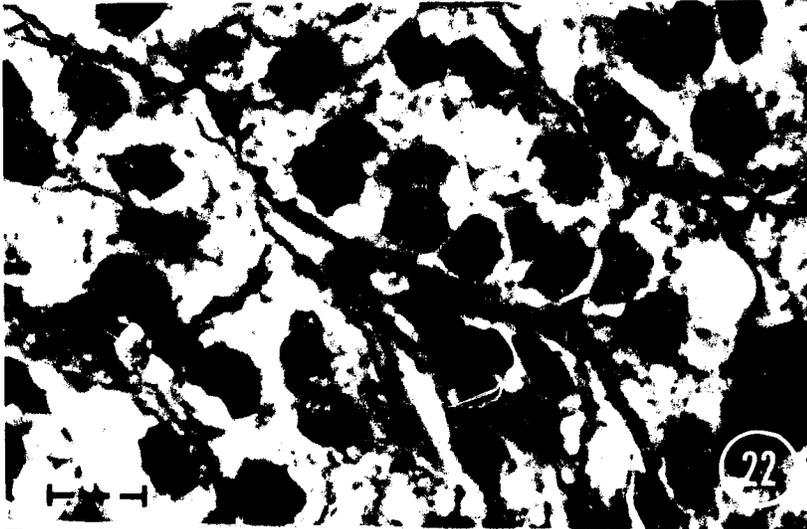


PLATE X

Figures 22 and 23 illustrate interrenal tissue of hypophysectomized animals. Figure 24 illustrates the same tissue of a normal animal. H & E. X4,780 (scale = 10 μ)

Explanation of Figures

22. Interrenal tissue of experimental animal (number 11 - nine days after hypophysectomy). The cytoplasm of most cells is greatly reduced, there is a loss of lipid material, the nuclei are hypertrophied, there is an apparent loss of basophilic material, and the nuclear membrane of some cells is indented. Most cells appear to be degenerating.
23. Interrenal tissue of experimental animal (number 12 - nine days after hypophysectomy). Degenerating changes similar to that of Figure 22 are illustrated.
24. Interrenal tissue of normal animal. This section illustrates the difference between interrenal tissue of normal and hypophysectomized animals (Figs. 22 and 23).



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