THE HISTOLOGY OF THE ADRENAL GLAND
IN THE ALLIGATOR LIZARD, GERRHNOMOTUS MULTICARINATUS

by

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INTRODUCTION

The histology of the adrenal gland of the lizard has received relatively little attention. Brief descriptions have been made by Krause '22 and Radu '34. A search of the literature failed to disclose other studies. This study was made in effort to develop a more complete description of lizard adrenal gland histology.
MATERIALS AND METHODS

Adrenal glands were studied from both sexes of forty mature North American alligator lizards. These included Gerrhonotus multicarinatus scincicauda (Skilton), the Oregon alligator lizard; G. multicarinatus multi-carinatus (Blainville), the red-backed alligator lizard; G. multicarinatus webbii Baird, the San Diego alligator lizard. These represent all the subspecies of G. multicarinatus recorded for North America, Smith '46.

Local Oregon alligator lizards usually were killed within one week after capture. The other two subspecies were kept in captivity for periods varying from one to four months. Animals were maintained in as good a nutritional state as possible.

The lizards were anesthetized by intra-peritoneal injection of 15 mg. of sodium pentobarbital (1:100 solution in 0.9% saline) to facilitate the removal of the adrenals while the animals were alive. To remove the adrenals a ventral median longitudinal incision was made to expose the entire body cavity. The adrenals along with the gonads and mesenteries were cut from their attachments and placed in fixative. The total time elapsed from injection with barbituate to placing in fixative was in no case more than five minutes.
The following fixatives were used: Zenker-acetic, Zenker-formol, Bouin, Severinghaus and Wiesel's solution for chromaffin tissue, Krajian '40.

Two and one-half hours was found to be optimum fixation time with Zenker-acetic and Zenker-formol solutions at 32°C. If longer time was allowed, the tissues became difficult to section because of excessive hardening. Zenker-formol fixation provided better cellular detail than did Zenker-acetic fixation.

Following fixation the tissues were washed a minimum of five hours in running water. Complete dehydration was accomplished by five changes of dioxan at half-hour intervals.

For all Zenker fixed tissues, high melting point paraffin (56-58°C.) was used for infiltration and embedding. A minimum of five changes of paraffin at half-hour intervals provided complete infiltration. To embed the tissues the paraffin was heated to 60°C. After the tissues were oriented rapid cooling was accomplished with ice water to prevent air bubble formation.

Optimum time for Bouin fixation was found to be six hours at a temperature of 32°C. This was followed by storage of the tissues in 70% alcohol for fifteen days. Dehydration and embedding was the same as that used for
Zenker fixed tissues.

To demonstrate Golgi apparatus and mitochondria the Severinghaus technique was followed, Guyer '36. Optimum fixation time was found to be twenty-four hours. This was followed by incubation in 2% osmium tetroxide for three days at 37°C. Bleaching with hydrogen peroxide (20% aqueous) was necessary for Golgi studies because of excessive blackening. Dehydration and embedding was the same as before. This tissue was sectioned at 3 μ to 5 μ; this was facilitated by cooling the paraffin block and the microtome blade with CO₂.

The Wiesel method for differentiating chromaffin tissue proved satisfactory when saturated 70% alcoholic methylene blue was used in place of toluidin blue for staining the tissues.

Generally all tissues were sectioned serially, at thickness of 3 μ to 15 μ as required for the particular study.

Haupt adhesive was used to affix tissues to the slides in most of this work, Johansen '40. This method proved superior to Mayer's albumen when prolonged staining techniques were necessary and especially for the Foot-Menard reticular connectives tissue stain, Lillie '48.

General and special selective stains were employed. Iron hematoxylin and Harris' hematoxylin-eosin were used
for general studies. Van Giesen, Harris' hematoxylin-azure II-eosin, Lillie '48, and methylene blue were used for cytoplasmic secretory studies. Three connective tissue stains were used: for collagenous fibers Mallory triple stain; for reticular connective tissue the Foot-Menard method; and the MacCallum-Verhoff stain for elastic fibers, Krajian '40.

Chromaffin tissue differentiation was accomplished by the Wiesel methylene blue method. Osmium tetroxide-treated tissue was used for Golgi studies and Altman-Cowdry anilin acid fuchsin-methyl green for mitochondria, Guyer '36. The Pearson-O'Neill silver gelatine method provided good results for intrinsic innervation. Especially good results were obtained when the slides were counterstained with methylene blue.
OBSERVATIONS

Anatomical Relationships

The paired adrenal glands in *Gerrhonotus* are roughly ellipsoidal in shape, each located near the cephalic pole of the gonad. The surfaces of the adrenal are smoothly rounded with the cephalad and caudad poles narrowing to a nearly pointed form. Dorso-ventrally the shape is roughly rectangular.

The glands vary in size. In 40 specimens they were from 1 to 2.5 mm. in length and 0.5 to 1.5 mm. in breadth; in five specimens, however, they were so small as to be scarcely detectable grossly.

In both sexes the right adrenal lies between the dorsal body wall and the gonad which is ventro-lateral to the adrenal (Fig. 1). The retroperitoneal kidney lies caudad to the gonad. The anterior half of the right adrenal projects cephalad beyond the gonad almost to the caudal tip of the right lobe of the liver and lies ventro-lateral to the post caval vein which enters the liver in this region.

In both sexes the left adrenal and gonad lie slightly more caudad than the right adrenal and gonad. The anterior two-thirds of the left adrenal projects cephalad beyond the gonad. In the female the ovaries lie more caudad than
do the testes in the male.

Mesenteric attachments for the adrenals of both sexes are similar but differences are found for the right and left sides of the body. On the right side the adrenal is suspended from the dorsal body wall, slightly lateral to the midline, in the mesorchium of the male or the mesovarium of the female. The suspending mesentery is continuous with the mesentery of the right lobe of the liver. Within this mesentery the gonad lies ventral to the adrenal (Fig. 1). The mesorchium or mesovarium suspending the left adrenal from the dorsal body wall continues ventrally and caudally as the mesorectum and cephalically as the mesogaster.
Strona

The adrenal gland is enclosed in a thin connective tissue capsule intimately in contact for much of its surface with the supporting mesentery (Fig. 2). Collagenous, reticular and elastic fibers are present in the capsule.

Collagenous fibers, when stained with anilin blue with or without acid fuchsin, may be seen in the capsule as well as surrounding small groups of cells within the gland (Fig. 3). Fine collagenous fibers pass between the individual cells of the gland. Extending from the wall of the large ventro-lateral vein draining the organ a heavy network of collagenous fibers passes into the gland. Individual fibers follow the course of the vascular sinuses. Fibroblasts usually are found accompanying the fibrils in the walls of the blood vessels or in the walls of the vascular sinuses. Most of the individual fibrils have a fine wavy structure. Only a few straight fibrils were noted.

A striking picture is presented when the Foot-Menard method is used to demonstrate reticular connective tissue (Fig. 4). The capsule is rich in reticular fibers and heavy black bundles of these fibers extend inward and are distributed throughout the gland. These bundles definitely demarcate groups of cells in the chromaffin and
in the interrenal areas (Fig. 5). Extending from each heavy bundle are fine branching fibers passing among the individual cells and forming an intermeshed reticulum. The reticular connective tissue provides a much more extensive framework both around the groups of cells and among the individual cells than do the collagenous fibers.

Elastic fibers when stained by the Verhoeff-MacCallum method are seen as very fine short fibers in the capsule and the mesentery supporting the gland. Blood vessels show the typical arrangement of elastic fibers in the tunica adventitia. No elastic fibers were found elsewhere in the adrenal.

Melanophores are always present in the mesenteric support for the adrenal in the region adjacent to the dorsal body wall (Fig. 1). In some cases melanophores may be seen scattered throughout the entire mesentery.
The general structure of the alligator lizard adrenal is that of external chromaffin and internal interrenal tissues fused to form a single compound organ (Fig. 2). The chromaffin portion forms an incomplete capsule of variable thickness around the interrenal portion. Heaviest concentrations of chromaffin tissue are on the dorsal surface and at the caudal and cephalic poles of the gland (Fig. 9).

The histological structure of the chromaffin cells is different from that of the interrenal cells. Differentiation between the two is not difficult microscopically, although the chromaffin cells may extend deep into the interrenal portion and vice versa. Occasionally chromaffin cells are intermingled singly or in groups within the interrenal tissue in the interior of the gland.

The arrangement of the chromaffin cells is that of groups of low polygonal cells sometimes around a small blood sinus. Although these cell groups are not easily distinguishable upon examination of a single section, by study of serial sections this structure becomes evident. Both collagenous and reticular connective tissue fibers demarcate this arrangement. Close packing of the chromaffin cells causes distortions which gives rise to an unorganized
appearance.

In contrast, the interrenal tissue shows a more orderly pattern of cell arrangement. Here the tall polygonal cells are arranged radially around the numerous vascular sinuses (Fig. 6). This pattern is evident in most of the area of a single section cut a minimum 10 μ thick.

When the adrenal gland is fixed in solutions containing potassium dichromate the peripherally located chromaffin tissue is colored a brownish-yellow with numerous fine granules throughout the cytoplasm of the cells even in unstained preparations. The interrenal portion does not react in this manner; the cytoplasm remains nearly colorless.

Chromaffin cells

In the chromaffin cells the cytoplasm varies in intensity of staining especially in tissues fixed with Zenker solution or other chromium salt fixatives (Fig. 8). In these cells cytoplasmic granules are more concentrated in the areas staining more deeply while the more lightly stained cells have fewer granules.

With Mallory triple stain the majority of the peripheral cells color a deep orange-yellow, and a few light yellow cells are found intermingled with the orange-yellow
In the innermost region of the adrenal the chromaffin cells remain unstained. A similar variation is obtained with Van Gieson stain. With hematoxylin-azure II-eosin the outermost cells stain a deep orange-red while the innermost cells stain light pink. Iron hematoxylin produces a deep blue color in the peripheral cell with a decrease in intensity inward.

Zenker fixed tissues after post-chromation and subsequently treated with ferric chloride, iron hematoxylin produces a sharply defined yellow-green color in the chromaffin cells (Vulpian reaction), Grollman '36 (Fig.2). Harris' hematoxylin-eosin does not differentially stain the different chromaffin cells.

Unstained chromaffin cells fixed by the Wiesel method followed by post-chromation for three days have brownish-yellow granules evenly distributed throughout the yellow cytoplasm. Wiesel fixed tissues stained with methylene blue-eosin show brilliant green peripheral chromaffin cells and deep blue inner chromaffin cells.

Tissues treated with osmium tetroxide for three days after Zenker-formol fixation show minute brown granules in the cytoplasm of the outer cells, with fewer granules in the inner chromaffin cells.

The nucleus of chromaffin cells is relatively large.
and round or oval in shape (Fig. 11). Usually it is located in the center of the cell. Nucleoli vary from one to three in number. These stain bright red with anilin acid fuchsin and deep blue when stained with iron hematoxylin. Occasionally other unidentifiable granules are seen in the nucleus. In chromaffin cells showing variation in cytoplasmic staining there is little evidence of changes in nuclear staining.

Mitochondria are present as very short rods which are difficult to distinguish from secretion granules. In most cells they appear to be scattered throughout the cytoplasm. Mitochondria stain bright red with anilin acid fuchsin in contrast to the brownish-red of the secretion granules (Fig. 11).

Golgi bodies are found as scattered blackened particles throughout the cytoplasm and sometimes as definitely localized structures in the region of the nuclei. The localized Golgi bodies have a varied appearance. They may be in the form of many small circles lying over the nucleus, or as an irregular network lying over or beside the nucleus. Definite polarity could not be established in the sections studied.

Interrenal cells

Cytoplasmic staining intensity of the interrenal cells
varies little when fixed with Zenker solution. This is in contrast to the varied cytoplasmic staining shown by the chromaffin portion of the gland.

Zenker-formol fixed tissues treated with osmium tetroxide for three days show distinct rounded black droplets distributed uniformly within the interrenal cells (Fig. 7). In Zenker-formol fixed preparations the outer portion of these droplets often stain deeply with cytoplasmic stains such as orange G, methyl green or iron hematoxylin. The inner portion of the droplets stains very faintly leaving what appears to be a reticulum. The droplets may represent interrenal secretion.

The location of the nuclei is varied. In some the nuclei are located in the center, nearly filling the breadth of the narrow cells (Fig. 6). In cases where the cells are arranged in rows along vascular sinuses the nuclei appear to be aligned close to the lumen.

The shape of the nuclei varies. Some are spherical while others are elongated, the latter occurring in narrow cells and evidently elongated by compression.

Nucleoli vary in number from one to seven when seen in sections cut at 10 μ. These are best demonstrated with iron hematoxylin or anilin acid fuschin.

Mitochondria in the interrenal cells are in the form
of short rods and granules, the latter sometimes appearing to be joined by fine filaments (Fig. 12). In all cells studied the mitochondria appear either to be localized close to the nucleus or widely scattered throughout the cytoplasm.

Golgi bodies may be identified in tissues fixed by the Severinghaus method. They are present; both in a dispersed granular form and as a heavy localized network near the nucleus. Definite polarity could not be established in these preparations.
Blood Supply

Only a limited study of the blood supply of the lizard adrenal has been made. The following observations are based on examination of ten sets of serial sections of the complete gland and surrounding structures.

The arterial blood supply is by way of small arteries lying within the surrounding mesenteries. (Fig. 3). These small arteries penetrate the capsule to a position directly under the capsule but just outside the parenchymal cells of the gland. They may pass along the surface of the parenchymal cells for a considerable distance before dividing into arterioles. Arterioles are found on or near the surface but not deep in the organ (Fig. 2).

Numerous capillaries are seen penetrating the chromaffin and interrenal tissues. At times the arterioles appear to open directly into the vascular sinuses.

Within both the interrenal and chromaffin portions of the gland are numerous vascular sinuses of variable size (Fig. 3). Venous drainage of the entire organ is by way of these vascular sinuses, some passing to the periphery of the organ and others coalescing to form one or more larger central sinuses. These central sinuses drain into the ventro-lateral post-caval vein of the right adrenal or the large ventro-lateral vein of the left adrenal which then joins the post-caval vein (Fig. 3).
The sinuses which drain to the periphery usually follow a course just under the capsule before making their way to the post-caval vein.
Innervation

At the caudal end of the adrenal gland a large aggregation of sympathetic ganglion cells is found among the chromaffin cells (Fig. 9). Similar ganglionic cells are also found in small groups or as single cells scattered throughout the peripheral chromaffin tissue. These are frequently found concentrated in the region of the small arteries. The ganglionic cells are clearly stained by methylene blue.

The Pearson-O'Neill silver gelatine method with methylene blue counterstain sharply outlines the distribution pattern of nerve fibers of the adrenal (Fig. 10).

From the ganglionic cells bundles of nerve fibers pass to the capsule of the gland; others follow the course of the blood vessels and are distributed throughout the entire organ.

Both the chromaffin and interrenal tissues are innervated by these fibers from the capsule and the blood vessels. Passing inward from the capsule heavy fibers surround groups of chromaffin cells with single fibers extending among the individual cells. The few nerve endings seen appear to be free-endings on the surface of the cells.

Bundles of nerve fibers clearly mark the groups of radially arranged interrenal cells (Fig. 10). Here too,
single fibers appear to pass among the cells. Nerve endings were occasionally seen as fine filamentous terminations on the cells. More often the fine intercellular fibers appear to pass to the blood sinuses around which the interrenal cells are arranged.
DISCUSSION

Previous histological studies of the lizard adrenal gland by Krause '21 and Radu '34 present only a partial description of the organ. The gross histological structure of reptilian adrenals described by Selye '47 as irregularly intermingled chromaffin and interrenal cells does not apply to the alligator lizard Gerrhonotus multicarinatus. In all specimens studied the chromaffin cells form an incomplete capsule around the interrenal mass. Heaviest concentrations of chromaffin cells are on the dorsal surface, the cephalic and the caudal poles of the gland. Only slight intermingling of chromaffin and interrenal cells occurs, although in some regions one portion may extend deep into the other. The arrangement of peripheral chromaffin cells forming an incomplete capsule around the interrenal cells is the reverse of that found in mammalian adrenals; in the latter chromaffin cells form the medulla and the interrenal cells the cortex. In birds the two portions of the gland are irregularly intermingled.

Embryologically the adrenal gland originates from two anlagen; the chromaffin portion from migrant neural crest cells, the interrenal from mesoderm. Chromaffin cells are so named because of their specific brown granular chromophilic reaction to fixatives containing
chromium salts. Interrenal cells derive their name from the fact that in Elasmobranchs and fishes these cells lie in masses between (or within) the kidneys, Dornfeld '45. These structures are homologous to the interrenal cells of the compound adrenal of higher vertebrates.

Variations in the cytoplasmic staining of chromaffin cells are evident in tissues fixed with Zenker-formol. It is of interest to note that the deeper staining chromaffin cells usually are more concentrated on the periphery of the adrenal and close to arterioles. Lighter staining cells are found toward the inner limits of the chromaffin region. These variations in staining reaction may indicate difference in secretory activity of the chromaffin cells.

The possibility of the differences in staining being due to incomplete penetration of the chromium salts has been minimized by lengthening the fixing time of six specimens to nine hours as well as bisecting the organ before fixation. In these tissues the same selective differences in staining of the chromaffin cells were present.

Interrenal cells show little variation in cytoplasmic staining reaction. Secretory activity of these cells is shown by the reduction of osmium tetroxide by evenly distributed droplets throughout the cytoplasm, coloring these
black. Frequently in Zenker-formol fixed tissues the secretion droplets are outlined by cytoplasmic stains leaving nearly colorless vacuoles throughout the cytoplasm.

The stroma of the gland consists of both collagenous and reticular connective tissues. The arrangement of both chromaffin and interrenal cell groups is outlined by these connective tissue fibers. Among individual cells both types of connective tissue fibers are seen. Elastic fibers are found only in the tunica adventitia of the blood vessels.

The vascular system consists of subcapsular arterioles which may pass along the surface of the parenchymal cells before penetrating to lie within the gland. The arterioles give rise to numerous capillaries which supply the entire gland. Frequently arterioles appear to open directly into the vascular sinuses.

Venous drainage of both chromaffin and interrenal tissues is by way of small vascular sinuses which coalesce to form larger sinuses to enter the large ventrolateral vein draining the entire organ. Frequently the plasma in the venous sinuses stains the same color as adjacent chromaffin cells. Plasma in sinuses of the interrenal tissues does not stain in this manner. This staining reaction of the chromaffin cells may indicate
the secretory pathway.

Innervation of the gland is through bundles of nerve fibers which arise from sympathetic ganglion cells found in the chromaffin tissue. These fibers pass by way of the capsule and vascular sinuses to both the interrenal and chromaffin cells.
The adrenal gland of the alligator lizard *Gerrhonotus multicarinatus*, is a compound organ consisting of chromaffin and interrenal tissues. The chromaffin tissue forms an incomplete capsule of variable thickness around the interrenal tissue.

Chromaffin cells treated with chromium salt fixatives are characterized by minute chromophilic granules in the cytoplasm. Gradation in cytoplasmic staining is evident, some cells staining more deeply than others. Chromaffin cells are low polygonal in shape having large spherical nuclei.

Interrenal cells treated with chromium salt fixatives show a uniform non-specific staining reaction. When stained with osmium tetroxide, Zenker-formol fixed interrenal cells show large cytoplasmic secretion droplets. Interrenal cells are tall polygonal in shape and have small rounded or elongated nuclei.

The stroma consists of collagenous and reticular connective tissue fibers, the latter being more extensive than the former. No elastic fibers are found except in the tunica adventitia of the blood vessels and in the capsule of the organ.

The entire adrenal is highly vascularized. The vascular system consists of arterioles, capillaries and venous
sinuses. Innervation is by way of nerve fibers originating from sympathetic ganglion cells found among the peripheral chromaffin tissue. These nerve fibers pass to the chromaffin and interrenal cells.
### ABBREVIATIONS

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<tr>
<td>ad</td>
<td>adrenal</td>
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<td>c</td>
<td>capsule</td>
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<td>ch t</td>
<td>chromaffin tissue</td>
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<td>interrenal tissue</td>
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<td>mesentery</td>
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<td>t</td>
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<tr>
<td>vlv</td>
<td>ventro-lateral vein</td>
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<td>vascular sinus</td>
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Plate I

Figure 1  Anatomical relationships of adrenal to testis. Ovary lies in same relative position in the female. Transverse section, x 50, iron hematoxylin.

Figure 2  Differential stain showing chromaffin cells forming an incomplete capsule around interrenal cell mass. Note subcapsular arterioles. Transverse section, x 70, iron hematoxylin (Vulpian reaction).
Plate I

Fig. 1

Fig. 2
Plate II

Figure 3  Cross section of adrenal showing collagenous connective tissue. Note vascular sinus draining into ventro-lateral vein. x 70, anilin blue-orange G.

Figure 4  Cross section near cephalic pole of adrenal with reticular connective tissue fibers stained by Foot-Menard method. x 70
Plate III

Figure 5  Reticular connective tissue fibers outlining interrenal cells. Note fibrils among individual cells. x 1500, Foot-Manard stain.

Figure 6  Tall polygonal interrenal cells arranged around a small vascular sinus. x 1000, methyl green stain.
Plate III

Fig. 5

Fig. 6
Plate IV

Figure 7  Osmophilic secretion droplets within interrenal cells. x 1000, osmium tetroxide-iron hematoxylin.

Figure 8  Differential staining of chromaffin cells forming a deep staining dorsal border of the gland. Lighter staining interrenal cells make up larger portion of the adrenal. Note vascular sinuses, subcapsular arteries and arterioles. x 70, Van Giesen stain.
Plate V

Figure 9  Caudal end of adrenal gland showing sympathetic ganglion cells among chromaffin cells. x 100, Mallory triple stain.

Figure 10  Nerve fibers among chromaffin and interrenal cells. x 1500, Pearson-O'Neill silver gelatine stain.
Plate V

Fig. 9

Fig. 10
Figure 11 Chromaffin cell mitochondria in form of short rods and granules. Deeper staining cells have heavy concentration of secretion granules which conceal mitochondria. x 1500, anilin acid fuchsin-methyl green.

Figure 12 Interrenal cells showing mitochondria much the same as preceding figure. Deeper staining cells show secretion droplets which are surrounded by mitochondria. x 1500, anilin acid fuchsin-methyl green.
BIBLIOGRAPHY


