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A comparative histological study of the adrenal gland of native rabbits

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A COMPARATIVE HISTOLOGICAL STUDY

OF THE ADRENAL GLAND

OF NATIVE RABBITS

An Abstract of

A Thesis

Presented to

the Faculty of the Department of Zoology

Brigham Young University

In Partial Fulfillment

of the Requirements for the Degree

Master of Arts in Zoology

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This Thesis by I. Ernest Gonzalez is accepted in its present form by the Department of Zoology and Entomology as satisfying the Thesis requirements for the degree of Master of Arts.

May 1950 Signed

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INTRODUCTION

The purpose of this investigation is to compare the histological structure of the adrenal gland of the following species within the order Lagomorpha: Rocky Mountain pika (Ochotona princeps), mountain hare (Lepus townsendii), snowshoe rabbit (Lepus americanus), desert jack rabbit (Lepus californicus), cottontail rabbit (Sylvilagus nuttallii), and pigmy rabbit (Sylvilagus idahoensis).

In reviewing the literature on the adrenal gland, the writer has been unable to find a record of any histological investigations on members of the order Lagomorpha other than the domesticated rabbit. Bourne (1949) has recently published an extensive classified list of mammals and the authors who have described the mammalian adrenal glands. This list shows that extensive work has been done on the adrenal gland of the domesticated rabbit (Oryctolagus cuniculus), but mentions no work on other members of the order Lagomorpha.

Since many books and hundreds of articles have been written dealing with the adrenal gland, it may seem that another attempt to describe its anatomy would be unnecessary; however, the phylogenetic modifications of the gland and its variation among orders, families, and even species of mammals seem to offer an ample field for further investigation. This is especially true since a study of the literature as reviewed by Bourne (1949) reveals that many investigators are not fully agreed on the cytology, cellular arrangement, cell growth, innervation, and complete function of the adrenal gland in general.

METHODS AND MATERIALS

Approximately one hundred specimens of lagomorphs of various ages and sizes, and of both sexes, were collected over a period of nine months. These rabbits were collected in central Utah in Wasatch and Utah Counties, except two pigmy rabbits collected from Iron County, Utah, and two mountain hares collected from Pebble, Bannock County, Idaho. A classified list showing the weights, habitats, and life zones of these rabbits is given in Table I.

All animals except two were shot, and those which did not die instantly were killed by a sharp blow on the head. Two pigmy rabbits (which are scarce and difficult to collect in Utah) were trapped alive and killed with ether.

The adrenal glands were removed with minimum manipulation immediately after the rabbits were killed and were fixed by immersion. The common practice was to fix one gland in Bouin's fluid and the other in the following modification of Worcester's fixative:

Saturated mercuric chloride in 10% formalin	50 ml.
Absolute ethyl alcohol	50 ml.
Glacial acetic acid	5 ml.

After the glands had remained in Worcester's fixative for twenty-four, and never longer than forty-eight, hours, the fluid was decanted and replaced with 70% ethyl alcohol. Occasionally, organs were fixed in 10% formalin solution or in Bensley's formal-bichromate-sublimate mixture. After twenty-four hours in this fixative, the glands were transferred to a 3% potassium bichromate solution in which they could be left indefinitely if the solution was replaced every two weeks.

All glands were embedded in paraffin. The majority were sectioned at eight micra, although twenty-five pair of glands were sectioned at 12 micra and mounted serially.

Most sections were stained with Mallory's triple stain for connective tissue or with Harris's hematoxylin and eosine. Another stain which proved very satisfactory was Mallory's phospho-tungstic hematoxylin. Davenport's silver technique for nerve tissue and Masson's silver method proved to be effective for staining the cell bodies, but as stains for nerve fibers, they were far from satisfactory. This investigator finds that Guyer's (1936) description of the technique developed by Dr. Pearl E. Claus for sharp differential staining following Bouin's fixation gives splendid results. Dr. Claus suggests running slides from xylol to alcohol to water and then placing them in a 1% permangenate of potash solution until the sections turn brown, followed by 1% oxalic acid until the brown disappears, then in water again, followed by the stain desired.

TABLE I

LIST OF LAGOMORPHS UNDER CONSIDERATION*

Family	Genus	Species	Common Name	Approx. Wt. in Gms.	Habitat	Life Zone
Ochotonidae	Ochotona	princeps	Rocky Mountain Pika	120-129	Rock Slides	Canadian & Hudsonian
Leporidae	Lepus	townsendii	Mountain Hare	2179-3440	Sage Brush or Dense Brush	Transitional Canadian & Hudsonian
Leporidae	Lepus	americanus	Snowshoe Rabbit	746-760	Dense Brush	Canadian
Leporidae	Lepus	californicus	Desert Jack Rabbit	1120-1680	Sage Brush	Canadian Lower & Upper Sonoran
Leporidae	Sylvilagus	nuttallii	Cottontail Rabbit	786-963	Rocks and Dense Sage Brush	Transitional & Upper Sonoran
Leporidae	Sylvilagus	idahoensis	Pigmy Rabbit	404-430	Sage Brush	Upper Sonoran

*Compiled from Hall (1946).

OBSERVATIONS AND DISCUSSION

1. Gross Anatomical Morphology

Hoerr (1931), Zwemer (1936), Zwemer, Wotton and Norkus (1938), Gruenwald and Konikov (1944), and Hartman and Brownell (1949) have called attention to the variations in adrenal histological structure even among individuals of a single species of mammal. The present investigation shows that the morphological variation of the adrenal gland of each species under consideration is almost as extensive as the variation found within the different genera and families of the order Lagomorpha (Figs. 1-12).

The shape of the gland, both within a given species and within the order, varies greatly. It may be spheroidal, rod-like, elliptical, cylindrical, heart-shaped, oval, or pear-shaped. Generally, however, the gland is ovoid in shape and pale yellow in color.

2. Cortical and Medullary Arrangement

The medullary arrangement of the pika adrenal gland differs from that of the other lagomorphs examined. This tissue appears to be arranged in large and distinct lobed clusters. Cortical tissue projects between the medullary lobes, but is separated from the medullary tissue by a thin zone of reticular fibers (Fig. 14). Many cortical projections seem to have been severed or to have become separated from the general cortical mass, and appear as stellate cortical islets between medullary lobes (Fig. 14). The medulla of the other lagomorphs studied is generally found to be made up of small lobed clusters which merge to form a compact medullary mass. Cortical processes protruding into the medullary tissue are infrequent, and cortical islets within the medulla are rare.

In the majority of the glands examined, the medulla is completely surrounded by the cortical tissue, except in the region of the hilus, where the large medullary vein empties into the adrenolumbar vein. Thus, the medulla is not exactly centrally located, but is at the hilus against the surface of the gland (Figs. 1, 5, 8, 9). Glands of all the species are often seen with a considerable portion of the medulla not surrounded by the cortex (Figs. 10, 12).

Some sections of glands from lagomorphs other than the pika showed a peculiar medullary and cortical islet arrangement. Careful examination of the glands cut and stained serially showed this arrangement to be due to cross-sectioning of occasional cortical cell processes protruding in the medullary mass. In a number of glands, the medulla was in two masses, separated from each other by cortical tissue (Figs. 4, 6). In one instance, an odd arrangement was noticed where medullary tissue partially capped the cortical tissue (Fig. 3).

The great variation in a number of adrenal glands may be explained in part by Bourne's (1949) summary of Mitsukuri's (1882-83) findings on the adrenal development of domesticated rabbits, which is modified as follows:

a. The mammalian adrenal is composed of two parts, the cortex and the medulla, which are totally different in origin.

b. The cortical substance arises from the mesoblasts.

c. The medulla is derived from the peripheral part of the sympathetic system. At first, it migrates towards the cortical buds and eventually becomes enclosed by the cortical tissue.

It has been well established that the cortico-medullary arrangement results from migration of undifferentiated nerve cells into the solid cortical buds, and that their coalescence within builds up a continuous medulla. An incomplete coalescence and aberrant bodies of cortical tissue may be the factors governing, in part, the great variation in the adrenal morphology, and may, perhaps, explain cortico-medullary distortions.

3. Capsule and Framework

The capsule surrounding the gland is composed of dense connective tissue containing collagenous fibers, spindle-shaped fibroblast cells, and reticular fibers embracing nerve bundles and blood vessels. The capsule is extremely dense in the snowshoe rabbit and in the mountain hare. In these two species, undulating trabeculae extend from the capsule into the substance of the cortex and embrace from one to ten glomerular arches. These trabeculae are broad as they differentiate from the capsule, but diminish in width as they penetrate the gland. Frequently, they extend as deep as the <u>zona reticularis</u> (Fig. 15).

The capsule is not as dense in the cottontail or the pika. The trabeculae in these species have characteristic patterns. They are few in number, wedge shaped, short, and seldom penetrate deeper than the <u>zona glomerulosa</u> (Fig. 17). The framework of the glomerular arches seems to differentiate from a loose network of reticular fibers emanating from the subcapsular region. Glomerular arches of these species are well defined and surprisingly uniform. Reticula of the capsule and trabeculae appear to be continuous with the supporting framework of the gland parenchyma.

4. Cortex

The cell types studied in the normal lagomorph adrenal cortex will be described in the order in which they may be observed when studying the gland from the capsule toward the medulla.

Arnold's (1866) traditional zona glomerulosa, zona fasciculata, and zona reticularis are not clearly outlined in all glands. The zones are arranged concentrically, but are disrupted where the medulla comes to the surface of the gland. The relative width of these three zones varies somewhat from gland to gland. Age (Blumenthal, 1945), thermal environment (Bernstein, 1941), inanition (Hett, 1926), and sex (Bennett, 1940) are but a few of the factors governing this variation.

The cytological description will be confined to such cytological arrangements of the cortical and medullary tissue as are considered pertinent to, or differing in, the species under consideration.

A. Zona Glomerulosa

The snowshoe rabbit and mountain hare have distinct glomerular arches, separated by dense connective tissue. The cytoplasm of the cells making up the arch appears to be dense and finely granulated. The cells are elongated and almost columnar in shape. Their nuclei are round or oval, and contain scattered masses of chromatin and two or more deeply staining nucleoli. Nuclei of the glomerular zone are not very uniform in size.

While dense connective tissue forms the framework of the glomerular arches in the snowshoe and the mountain hare (Fig. 15), fine bundles of reticula make up the arch framework in the cottontail and pika (Fig. 17A, B). The cells within the glomerular arches of these species resemble those described in the snowshoe and mountain hare, but differ in having centrally located nuclei. Here, as in the snowshoe, the majority of the nuclei are oval, containing evenly distributed masses of chromatin and numerous large granules stained orange by Mallory's triple stain (Fig. 13). The cells are arranged in strands, and their centrally located nuclei form parallel rows that extend to the outer fasciculata. Many fibroblasts, described by Zwemer, Wotton, and Norkus (1938), in the cat were clearly observed between glomerular arches.

Cells found in the <u>zona glomerulosa</u> of the jack rabbit are practically identical to those described previously. In the jack rabbit, however, the glomerular arches are not so clearly defined.

The <u>zona glomerulosa</u> of the lagomorph adrenals so far examined shows definite epithelial arches (Fig. 17A, B). Remarkable indeed is the arrangement of the glomerular zone in the adrenal of the pigmy rabbit. It resembles the glomerular arrangement in the rhesus monkey and, somewhat, that of the human adrenal (Gruenwald and Konikov, 1944). The cords which form this layer are continuations of the fascicular cell cords (their distal ends), which are continuous from the subcapsular area to the medullary junction (Fig. 18A, B). In some glands which were apparently cut longitudinally, the glomerular cords appear to be slanting, and are oblique in relation to the medulla or the center of the gland (Fig. 13). Each cord appears to be separated by a minute blood capillary (Figs. 13, 18A, B).

The individual cells of the zona glomerulosa in the pigmy rabbit appear to be a densely packed mass of minute rods and granules. The cells are irregular in shape, but, for the most part, are elongated and columnar.

B. Zona Fasciculata

The rows of fascicles in the snowshoe and the mountain hare are not conspicuous. They are buckled and twisted. Cells and nuclei of the outer fasciculata stain lighter and are more nearly round and larger than those of the zona glomerulosa (Fig. 13). Careful examination of these turgid and spongy looking cells reveals that a fine fibral network encloses evenly distributed and uniform minute vacuoles. Hoerr (1931), Zwemer, Wotton and Norkus (1938), and Bennett

(1940) postulate that in the guinea pig, human, and cat adrenal similar vacuoles are osmophilic and are those of secretion-bearing lipoids.

Cells of the inner _zona fasciculata_ in the snowshoe rabbit are distended, but are not as large or uniform as those observed in the outer and, especially, the central fasciculata. The outline of individual fascicular cells may be clearly observed. The cell morphology seems to conform with the reticular framework of the zone. In some instances, it looks as if individual cells are enclosed by extremely fine reticular fibrils which weave irregularly across from one side of the fascicle to the other.

A clear and vivid arrangement of the fascicular columns can be observed in the jack rabbit, cottontail and pika (Fig. 17A, B). Most spectacular is the columnar arrangement in the pigmy rabbit. In this species, the _zona reticularis_ is absent and the fascicular cords extend to the medullary junction (Fig. 18A, B). Individual cells of all lagomorphs examined clearly exhibit the characteristic turgid, spongy cell of the _zona fasciculata_ previously described in the snowshoe and the mountain hare.

C. Zona Reticularis

Only one of the species examined, the snowshoe rabbit, can be said to possess distinctly a _zona reticularis_. In this species, the connective framework stains beautifully with Mallory's triple stain. The <u>zona reticularis</u> can be recognized immediately by its net-like, or honeycomb, appearance. Each cell is clearly enclosed by heavy connective tissue (Fig. 16). Cells of this region are not as large as those observed in the <u>zona fasciculata</u>. Many cells have a spongy appearance, but the vacuoles within vary womewhat in size and distribution. Scores of cells with pyknotic nuclei and in various stages of degeneration can be observed (Fig. 20).

In all other species examined, the <u>zona reticularis</u> is so indefinite that it is, in reality, indistinguishable from what appears to be a slightly compressed <u>zona fasciculata</u> (Fig. 17A, B). Many blood vessels, varying in size, clearly anastomos in this region, and in many glands distort the typical cellular arrangement. Individual cells of this region appear to be surrounded by fine reticular fibers. Many cells appear to be degenerating, as manifest by their shrunken and deformed shapes, as well as their pyknotic nuclei (Fig. 20).

D. Discussion of the Cortical Cells

Zwemer, Wotton and Norkus (1938) present an adequate description of the stages in the history of the cortical adrenal cells in the cat. These authors maintain that gland cells in the adult adrenal cortex may be derived from certain indifferent cells found in the capsule, and that these capsule cells lose their long processes, differentiate into subcapsular cells, multiply, accrue lipoid, and become large outer fascicular cells. An elaborated secretion is discharged into the capillaries by these cells, which gradually become smaller and finally degenerate at the medullary border.

Gruenwald and Konikov (1944) presented evidence supporting Zwemer and co-workers. Bennett (1940) proposed four zones in accordance with the supposed actual functional state of the cells in the respective zones. He termed them "presecretory," "secretory," "postsecretory," and "senescent." Bennett has correlated cell appearance with histochemical findings. His zoning does not coincide with that of Arnold's (1866), which is based on the arrangement and configuration of the cell columns.

The present investigation of the cortical cells reveals that all cell stages described by Zwemer, Wotton and Norkus in the cat can also be observed in the lagomorphs under consideration. The physical appearance of the respective glomerular, fascicular, and reticular cell within the lagomorph adrenal is uniform throughout all of the specimens examined. The contour of the cell is somewhat altered by the surrounding reticular framework. Cell categories described by Bennett, rather than cell contours, serve as excellent criteria in zoning the cortex of these lagomorphs.

5. Innervation of the Adrenal

During the course of this investigation, ganglia of varying size have been frequently found embedded in the capsule or adjacent to or near the adrenal gland (Figs. 3, 22). Clusters of ganglion cells are infrequently found in the medulla. When observed, they contain from 7 to 21 cell bodies (Figs. 21, 24). The majority of these cell bodies have two nuclei (Figs. 23, 24). Kuntz (1934) reports that this binuclear condition also exists in the autonomic ganglia of various members of the order Rodentia, especially in young animals. The reason for this binuclear condition is not clear. Swinyard (1937) found that in some cat adrenals as many as 12 ganglion cells were located within the medullary tissue. In other cats he examined, he found no ganglion cells within the adrenal medulla. He attributes this irregularity to the variation in adrenals of the same species.

In lagomorph glands cut and mounted serially, nerves can be traced leaving the ganglia and penetrating the capsule, where, invariably, they are lost among the reticular fibers. Other nerves penetrate the gland and diminish in size as they traverse through the cortex (Fig. 19). Some nerves disperse their fibrils at the cortico-medullary junction, but others penetrate the medulla and can be seen to end near the walls of medullary blood vessels.

Due to their analogous affinity for stains, reticular and nerve fibers are practically indistinguishable from each other. There is meager evidence supporting innervation of the blood vessels and capillaries of the cortex; more conclusive evidence supports the idea of innervation of the medullary blood vessels (Fig. 19). It appears that the individual cortical cell is not innervated; however, it must be emphasized that the writer found that neither the silver method of Davenport nor that of Masson for nerve tissue is totally reliable for differentiating reticular and nerve tissues.

SUMMARY AND CONCLUSIONS

A review of the literature shows that extensive work has been done on the morphology and histology of the adrenal gland of the domesticated rabbit, but it appears that little or no work has been done on other members of the order Lagomorpha.

This investigation shows that the gross morphological variations of the adrenal gland of each species of native lagomorphs examined is almost as extensive as the variation found within the different genera and families of the order Lagomorpha. There are fewer histological variations within a given species, but more extensive variations within genera and families of this order.

It must be emphasized that not all morphological and histological structures found in the adrenal gland of rabbits are the same. What may appear to be a typical histological structure or arrangement in one species of rabbit is not necessarily the typical structure or arrangement in all species.

1. The adrenal gland of the lagomorph is generally an ovoid organ. Its variations are rod-like, elliptical, cylindrical, heart-shaped, oval, or pear-shaped.

2. The medullary arrangement of the pika is characteristic and can be used in identifying it from other lagomorph species. The tissue is grouped in small lobed clusters which are separated by fine reticular tissue from the cortical cells. This is a variation from the common lagomorph adrenal, where the medulla is found to be a continuous body or, occasionally, two such bodies.

3. The common occurrence in the lagomorph adrenal is one in which the medulla is almost completely surrounded by the cortical tissue. Infrequent divergencies noted are partial capping of the cortex by medullary tissue and the presence of medullary islands within the cortical tissue. An incomplete coalescence of the medullary tissues during the embryological development of the gland may, perhaps, explain cortico-medullary distortions.

4. Trabeculae in the mountain hare and the snowshoe rabbit are dense, embrace one to ten glomerular arches, and frequently extend to the _zona reticularis_. Trabeculae in the cottontail and pika are wedge shaped, short, few in number, and seldom penetrate deeper than the _zona glomerulosa_.

5. The _zona glomerulosa_ of the lagomorph adrenal shows definite epithelial arches. The pigmy rabbit is the exception. In this species, cords and not arches form this layer. The glomerular arrangement of the pigmy rabbit is not

a typical lagomorph arrangement, but resembles the glomerular arrangement in the rhesus monkey and, somewhat, that of the human adrenal.

6. The fascicular columns can be observed in all lagomorphs. Most spectacular is the cord arrangement in the pigmy rabbit. The cords in this species extend to the medullary junction.

7. The snowshoe rabbit possesses a distinct _zona reticularis_. This zone in all other species examined is so indefinite that it is, in reality, indistinguishable from what appears to be a slightly compressed _zona fasciculata_. There appears to be no reticularis in the pigmy rabbit.

8. The physical appearance of the respective glomerular, fascicular, and reticular cells of the lagomorph adrenal is uniform. The contour of the cell appears to be determined by the surrounding reticular framework.

9. There is meager evidence supporting innervation of the blood vessels and capillaries of the cortex; more conclusive evidence supports the idea of innervation of the medullary blood vessels. It appears that the individual cortical cell is not innervated.

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PLATES

PLATE 1

Explanation of Figures

1-3	Adrenal glands of the rocky mountain pika.
4-6	Adrenal glands of the cottontail rabbit.
7-9	Adrenal glands of the pigmy rabbit.
10-11	Adrenal glands of the mountain hare.
12	Adrenal gland of a snowshoe rabbit.

Structures within the adrenal gland.

A Adrenal Cortex
B Adrenal Medulla

Structures near the adrenal gland.

C Ganglion

Plate I

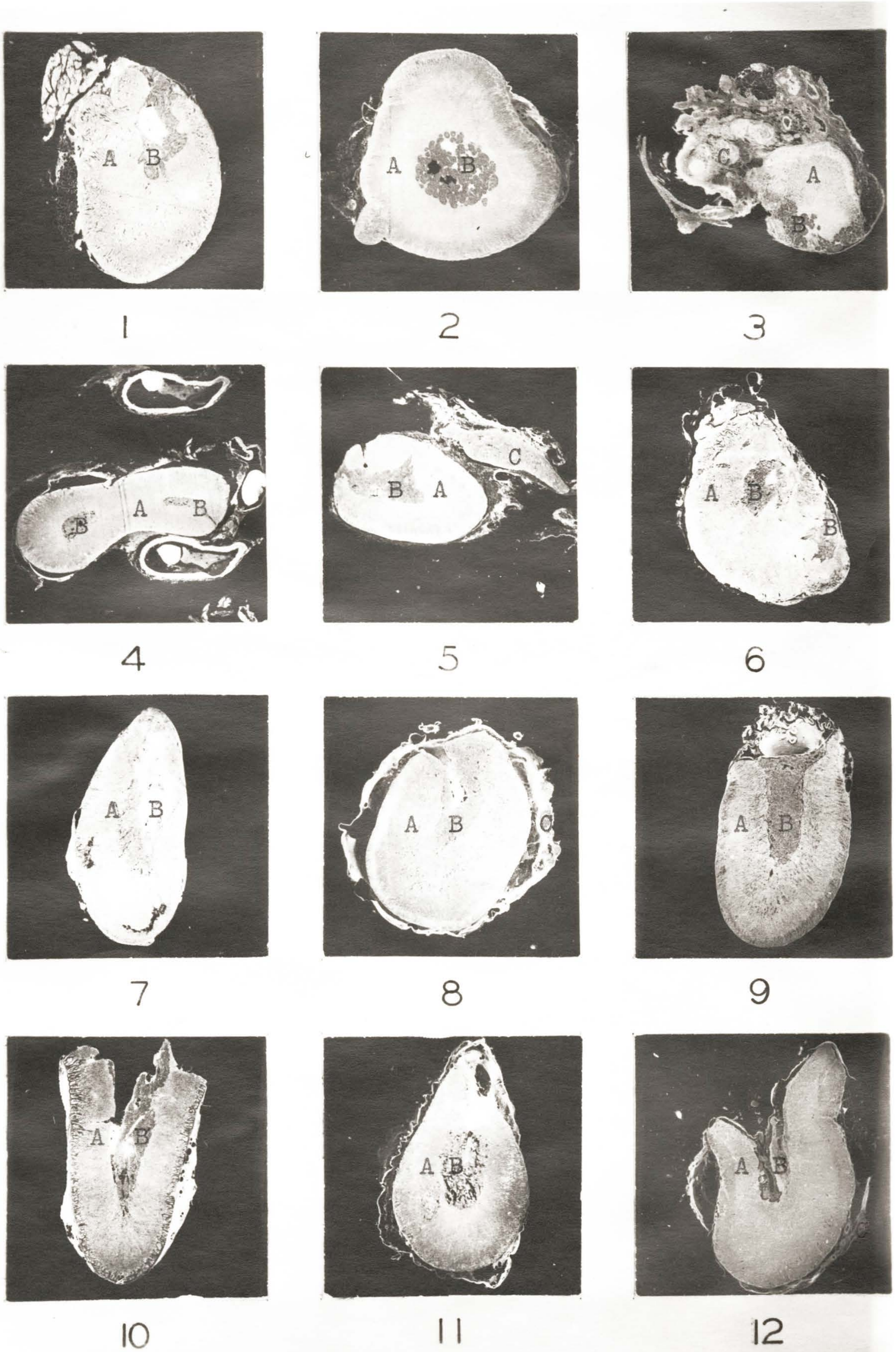
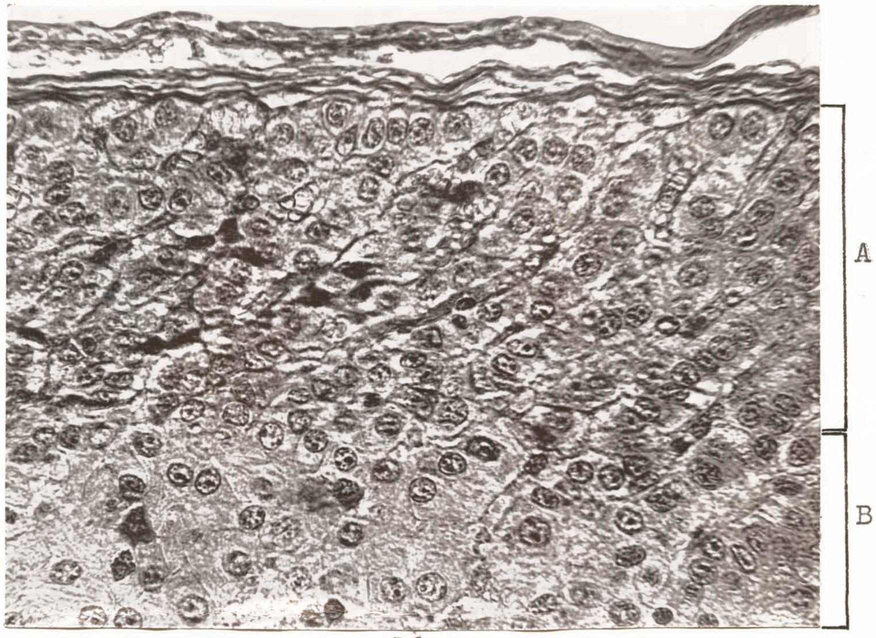


PLATE II

Explanation of Figures

13 Portion of the adrenal gland. Longitudinal section of the adrenal gland of a pigmy rabbit. A, <u>zona glomerulosa</u>; B, <u>zona fasciculata</u>.

14 The lobed arrangement of the medulla of a rocky mountain pika. A, medullary lobes; B, cortical tissue between medullary lobes; C, blood vessel within the medulla; D, cortical tissue.



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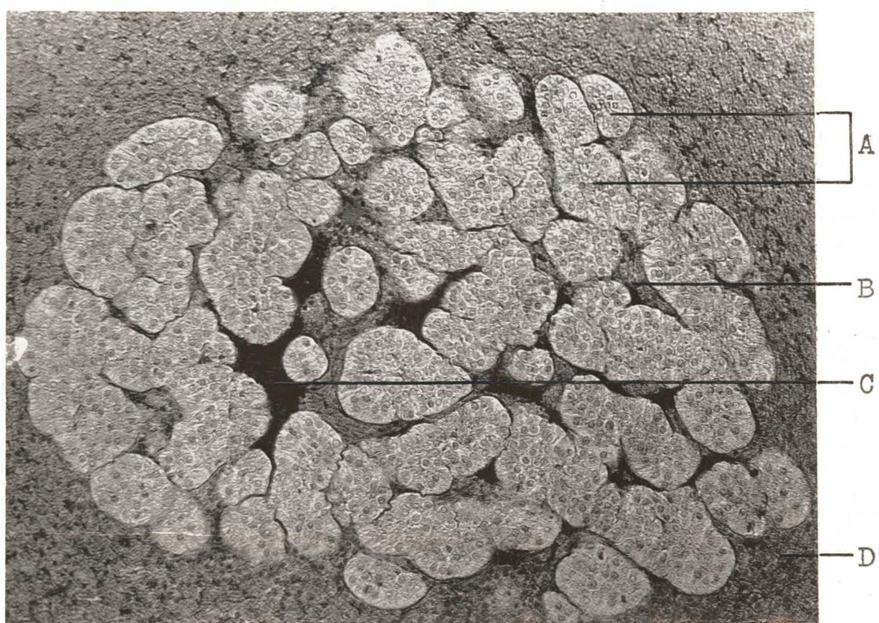

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PLATE III

Explanation of Figures

15 Adrenal cortex of a snowshoe rabbit. A, zona glomerulosa; B, zona fasciculata; C, zona reticularis; D, trabeculae extending from the capsule to the zona reticularis.

16 A section of the medulla and zona reticularis of the adrenal of a snowshoe rabbit. A, zona reticularis; B, small and densely packed medullary lobes; C, blood vessel within the medulla.

PLATE IV

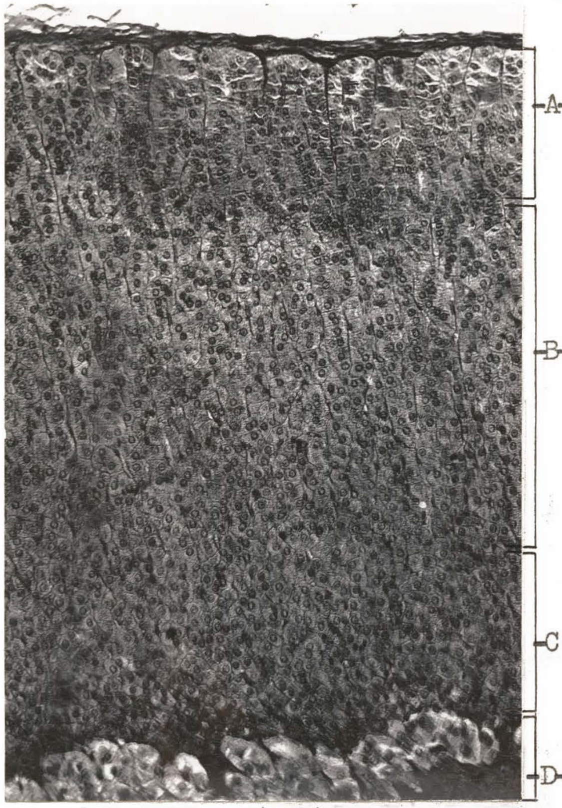
Explanation of Figures

17A Adrenal cortex of a cottontail rabbit. A, zona glomerulosa; B, zona fasciculata; C, zona reticularis; D, medulla; E, glomerular arch; F, short and wedge-shaped trabeculae.

17B A diagrammatic drawing of 17A, showing typical cortical arrangement of the native lagomorphs.

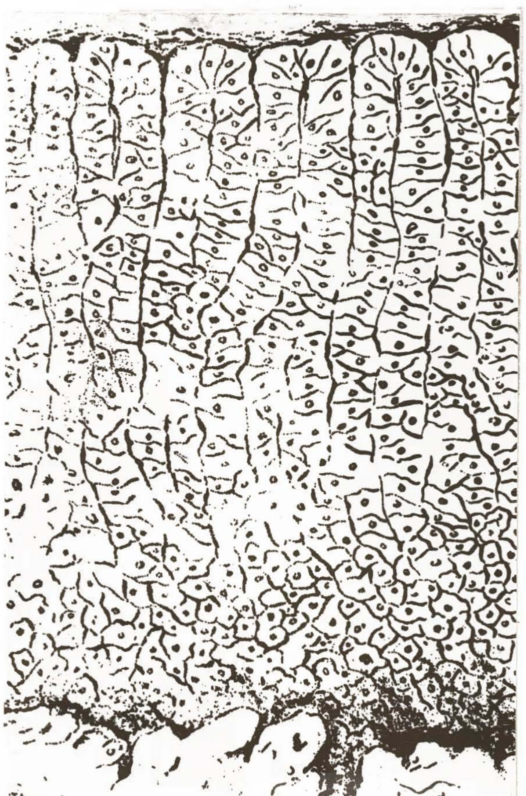
18A Adrenal cortex of a pigmy rabbit. A, zona glomerulosa; B, zona fasciculata; C, medulla; D, fascicular cords; E, blood capillaries.

18B A diagrammatic drawing of 18A, showing the peculiar cortical arrangement of a pigmy rabbit.



17 A

18 A



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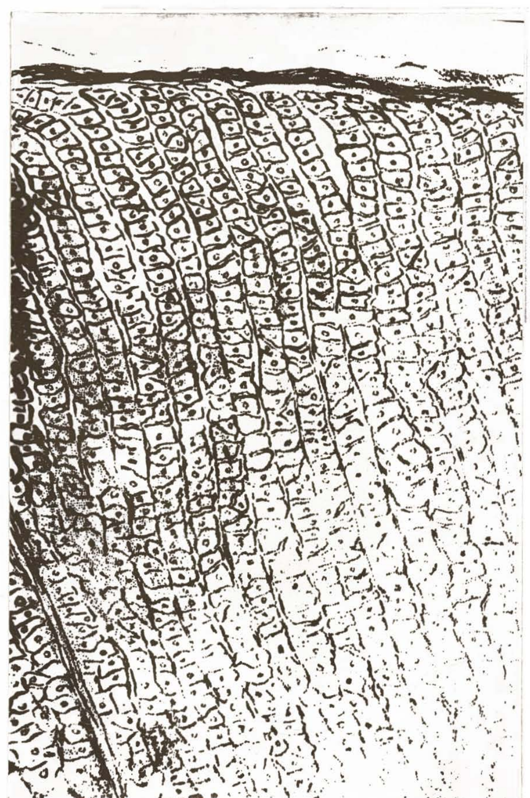


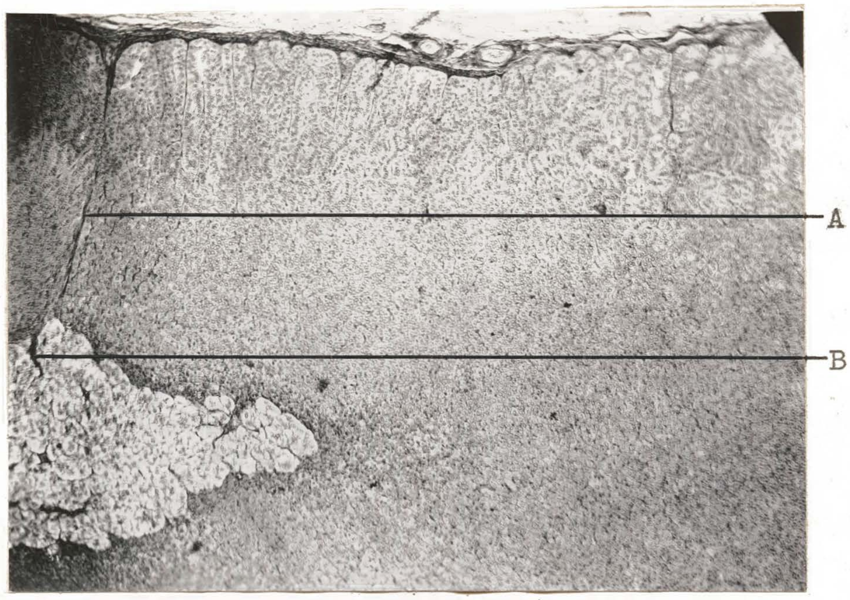
18 B

PLATE V

Explanation of Figures

19 Adrenal of a cottontail rabbit. Note a small nerve which penetrates the adrenal cortex and terminates near a blood vessel located within the medulla. A, nerve; B, blood vessel.

20 A section of the zona reticularis of a desert jack rabbit. Note the abundance of pyknotic nuclei in this zone.



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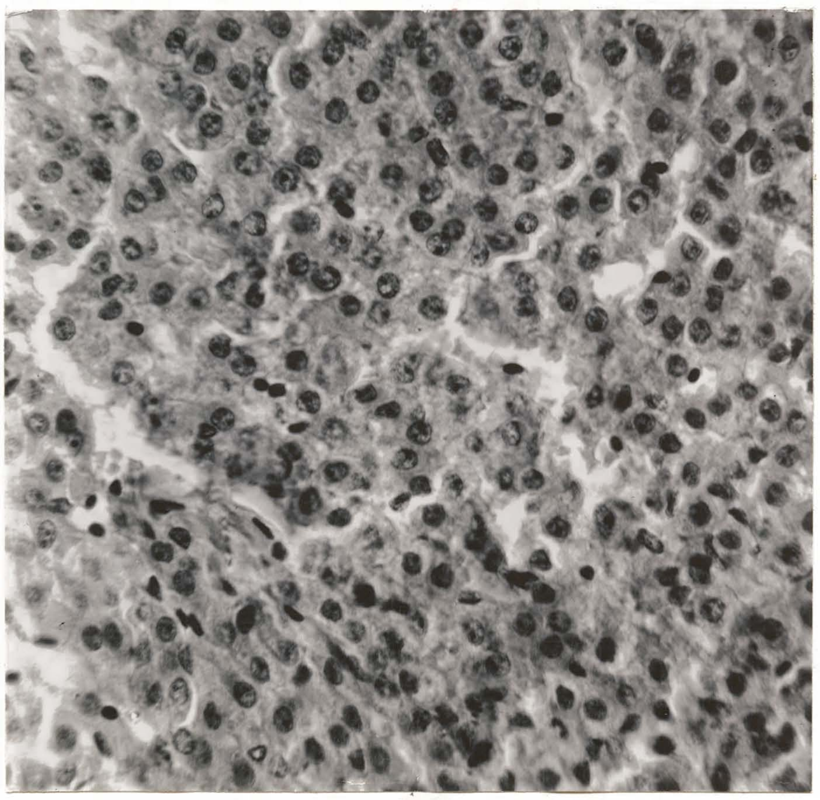

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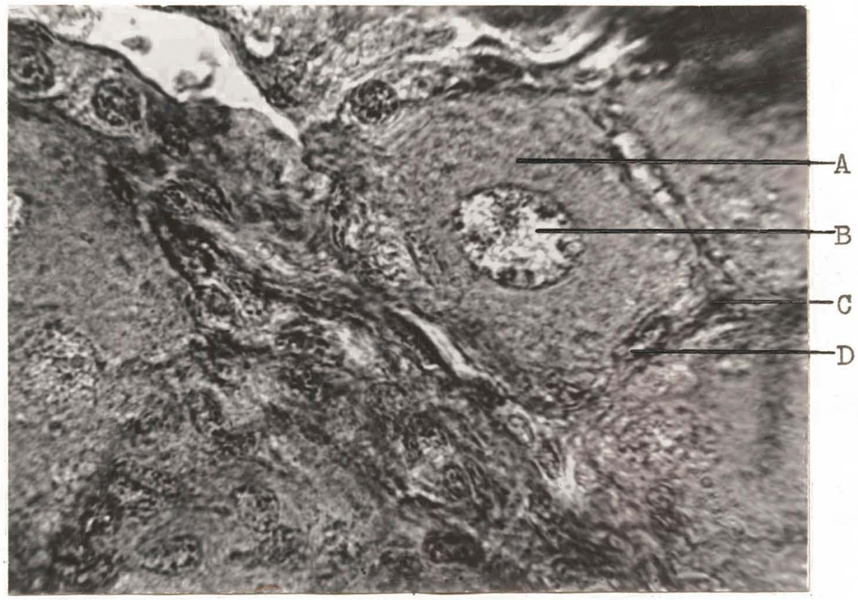
PLATE VI

Explanation of Figures

21 High magnification of a nerve cell described in figure 24. A, nerve cell; B, nucleus of the nerve cell; C, nerve fiber; D, satelite cell.

22 Ganglion near the adrenal gland of a cottontail rabbit. Note hundreds of cell bodies within this exceptionally large ganglion. Some nerves leaving this ganglion were traced in different serial sections made of the gland and were found to terminate near blood vessels located within the medulla (see figure 19). An enlargement of the nerve cell bodies of this ganglion is shown in figure 23. A, ganglion; B, fatty tissue between ganglion and adrenal gland; C, adrenal cortex.



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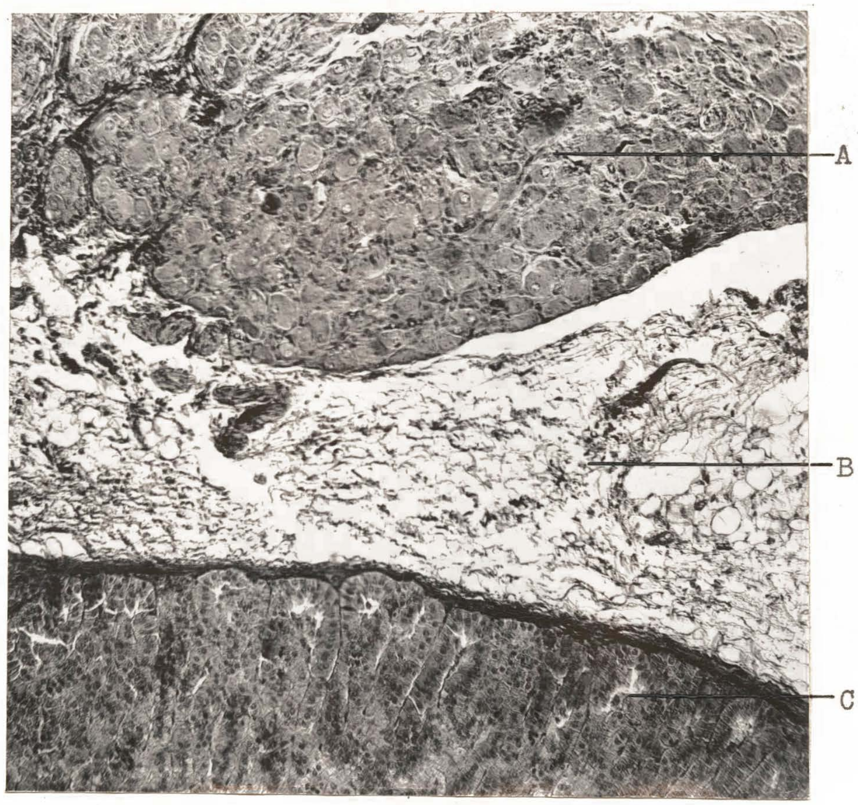

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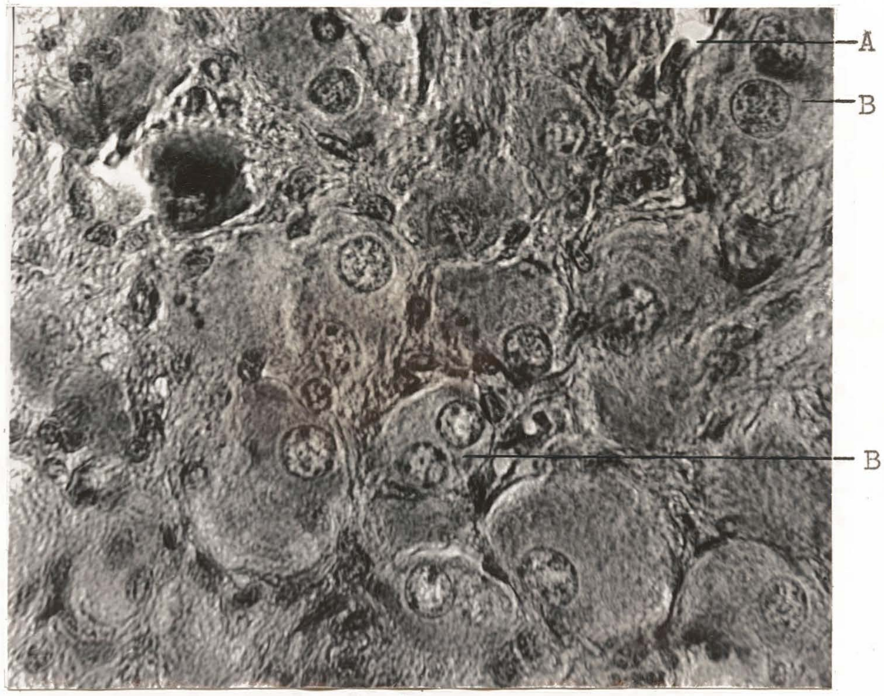
PLATE VII

Explanation of Figures

23 High magnification of nerve cell bodies described in figure 22. A, blood capillary; B, binuclear cell.

24 A cluster of nerve cells found within the adrenal medulla of a mountain hare. A, blood vessel; B, nerve cell body; C, medullary tissue.



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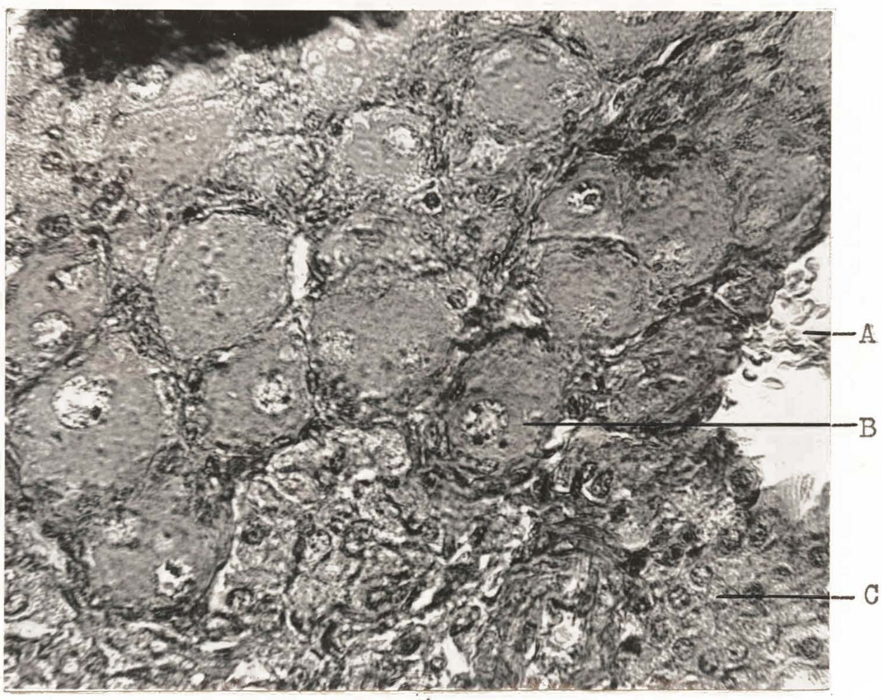

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ABSTRACT

A review of the literature shows that extensive work has been done on the morphology and histology of the adrenal gland of the domesticated rabbit, but it appears that little or no work has been done on other members of the order Lagomorpha.

Approximately one hundred specimens of rabbits of various ages and sizes, and of both sexes, were collected over a period of nine months. All adrenal glands were fixed by immersion and were embedded in paraffin. The majority of the glands were sectioned at eight or twelve micra, and were stained with Mallory's triple stain.

This investigation shows that the gross morphological variations of the adrenal gland of each species of native rabbits examined is almost as extensive as the variation found within the different genera and families of the order Lagomorpha. There are fewer histological variations within a given species, but more extensive variations within genera and families of this order. It must be emphasized that not all morphological and histological structures found in the adrenal gland of all rabbits are the same. What may appear to be a typical histological structure or arrangement in one species of rabbit is not necessarily the typical structure or arrangement in all species.