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A COMPARATIVE HISTOLOGICAL AND HISTOCHEMICAL STUDY OF THE ADRENAL GLANDS OF NATIVE RABBITS

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A COMPARATIVE HISTOLOGICAL AND HISTOCHEMICAL STUDY

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CHAPTER I

INTRODUCTION

In recent years, and particularly in the last decade, a great deal of research has been centered on the morphology and especially the function of the adrenal gland. The vast and voluminous literature in all fields of adrenal research clearly indicates a need for a better understanding of the processes involved in the biosynthesis and elaboration of hormones (Dorfman and Unger, '54), the physiological role (Hartman and Brownell, '49), and the morphological changes that accompany normal and pathological adrenal stress (Selye, '46).

In order to evaluate properly and integrate the physiological and morphological changes accompanying a variety of stresses, it becomes imperative that we acquire a clearer understanding of the normal parenchymal arrangement, cyto-architecture, and histochemistry in different mammalian species.

Differences among species seem to be both physiological and morphological, with some indication that they may extend down to the various strains of any one species or subspecies (Bourne, '49). The variations in the histology, cyto-architecture, and histochemistry in the various mammalian orders is quite apparent, but few, if any, comprehensive studies have been made to determine the degree of variation among families, genera, and species of any one mammalian order or group of closely related mammals (Bachman and Scharrer, '54).

Recent books by Bourne ('49), Hartman and Brownell ('49), and Bachman and Scharrer ('54), in which the literature has been admirably reviewed, clearly reveal our lack of knowledge in comparative adrenal histology.

Since the mammalian order Lagomorpha contains only two families and relatively few genera, it affords a splendid opportunity for a comparative histological study of the adrenal gland within a group of closely related mammals. A review of the literature indicates that the adrenal histology of Lepus cuniculus (the domesticated rabbit) is well-known, and this information will form the basis for many comparative remarks.

The purpose of this investigation will be to make a comparative study of the histology, cyto-architecture, and histochemistry of the adrenal gland of the following species within the order Lagomorpha: Ochotona princeps (Rocky Mountain pika), Lepus townsendii (mountain hare), Lepus americanus (snowshoe rabbit), Lepus californicus (desert jack rabbit), Sylvilagus nuttallii (cottontail rabbit), and Sylvilagus idahoensis (pigmy rabbit).

Although the nature of this study is for the most part morphological, it is hoped that by studying and comparing mammalian species which hitherto have not been investigated, we might broaden our general knowledge of the adrenal histology and make more compatible prevailing physiological and morphological views which might bring us to a closer understanding of the adrenal function.

CHAPTER II

MATERIALS AND METHODS

The observations made in this study have been based on the adrenal glands of approximately two hundred specimens of the following species within the order Lagomorpha: 39 Ochotona princeps (pika), 26 Lepus townsendii (mountain hare), 23 Lepus americanus (snowshoe rabbit), 63 Lepus californicus (jack rabbit), 37 Sylvilagus nuttallii (cotton-tail rabbit), and 6 Sylvilagus idahoensis (pigmy rabbit). The various species were identified by Doctors C. Lynn Hayward and D. Elden Beck, Department of Zoology, Brigham Young University.

In order to minimize possible sources of error, specimens for this study were selected only if they appeared mature and healthy. The rabbits were collected at monthly intervals over a period of two years, and only those specimens collected during January, February, and March appeared to be sexually active. Specimens from both sexes were selected for this study. All the native rabbits were shot, and those which did not die instantly were immediately killed by a sharp blow on the head. The adrenals were removed with minimum manipulation and immediately fixed by immersion. One gland, in toto, was fixed in Bouin's fluid. In order to minimize possible artifacts of fixation, the other gland was cut into two equal parts, and one half was fixed in Zenker-formal, the other in the following modification of Worchester's fixative:

Saturated mercuric chloride in 10% formalin	10 parts
Absolute ethyl alcohol	10 parts
Glacial acetic acid	1 part

After the gland had remained in Worchester's fixative for twenty-four hours, the tissue was transferred to 70% alcohol. A number of glands were also fixed in Severinhaus' modification of Champy's fixative; other glands were postchromated in a 3 per cent solution of potassium bichromate. Fresh adrenals or glands fixed in either buffered neutral formalin or Baker's calcium-formal were employed for the histochemical studies of the lipids.

Glands used for architectural reconstructions were embedded in paraffin, sectioned at 10 micra, and mounted serially. For critical cytological studies, glands were sectioned at 5 micra and the majority were mounted serially. Glands used for histochemical studies were frozen and sectioned or were embedded in polyethylene glycol wax after the method of Rinehart and Abu-Haj ('51). Using the latter technique, sections 2 to 5 micra were easily obtained.

Most sections were stained routinely in hematoxylin and eosin, Mallory's or Masson's stains, and were stained for elastic and reticular tissue using Gomori's technique ('50) and the coupled tetrazonium reaction (Pearse, '53).

For histochemical localization of lipids, the fluorochrome 3-4 benzpyrene used by Berg ('51) produced excellent results. Excellent preparations were obtained using the propolyene glycol-sudan or fettrot methods described by Chiffel and Putt ('51) for lipids.

Glass, plexiglass and cardboard were used to reconstruct the adrenal. When glass was used (Elias, '53), the refraction of the cut edges of the glass made it difficult to clearly visualize or measure the outlines of the models. By using plexiglass, the outer contour of the gland was cut out, the cut edges were polished, and an excellent transparent and exact reconstruction was obtained.

CHAPTER III

OBSERVATIONS

Ochotona Princeps

This species is the smallest of the mammals under consideration. It is classified under the mammalian order Lagomorpha and the family Ochotonidae. According to Hall ('46), the adult species weighs approximately 125 grams.

Pericapsular Tissue, Capsule, and Stroma

The adrenals of all specimens studied are embedded in adipose and areolar tissue, which condenses to form a dense membranous sheath that almost completely circumscribes the true capsule. Many nerves, ganglia containing binuclear ganglion cells, and branches of the principal adrenal vessels are located in this layer. These pericapsular elements intermingle with or penetrate the true capsule at many points.

The true capsule of *Ochotona princeps* is relatively thin, having an approximate thickness of 12-14 micra in the females; that of the males exceeds this thickness by 4-5 micra. Invariably, the outer portion of the capsule is composed of thickened and dense collagenous fibers, containing only few fibroblasts, little reticulum, and virtually no amorphous ground substances between fibers. Occasionally, a few smooth muscle elements are intermingled between connective tissue fibers, but, without exception, the muscular elements are components of small arteries. The inner portion of the capsule is more cellular, contains more fibroblasts, more reticular fibers, and a greater abundance of ground substance between these elements. From the internal capsular surface many reticular and fine collagenous fibers pass inward and contribute to the stromal network which supports and envelops the glandular parenchyma. Many collagen strands penetrate deeply into the cortex, forming incomplete septa. Their number and extent, however, are insufficient to partition adequately the gland into lobes or lobules.

Near the hilus of the gland the medulla comes to the surface and the capsule becomes attenuated, frequently not being discernible. At this point, the central vein emerges from the gland and empties into the main adrenal vein.

Vasculature

Within the connective tissue strands which are located between the convoluted glandular parenchyma of the traditional zona glomerulosa are thin-walled arterioles and basket-like capillary networks that surround the parenchymal masses. These basket-like capillary networks may be collapsed, becoming almost undiscernible, or they may be greatly engorged, altering considerably the appearance of the gland. Not all capillaries leading away from the subcapsular plexus contribute to the capillary network of the zona glomerulosa. Some penetrate as single vessels between the parenchymal continuum to empty directly into the sinusoids of the zona fasciculata. The sinusoids of the zona fasciculata are continuous with those of the zona reticularis. Occasionally, within the connective tissue strands that penetrate the cortex are found arteriae medullae. These vessels ramify near the cortico-medullary junction and are distributed within the medulla.

Innervation

Few nerve bundles are found within the connective tissue strands. In most instances they penetrate separately through the cortex and are distributed to blood vessels and, apparently, to groups of chromaffin cells. In the region of the hilus where the medulla comes to the surface of the gland, many nerves and nerve fibers pass through the attenuated capsule and immediately course between chromaffin cells and terminate near binuclear ganglion cells within the medulla. Many of the nerve fibers are myelinated and can be vividly demonstrated after sections have been stained by either sudan black "B" or fettrot, both being excellent lipid stains.

Small nerve fibrils and reticulum are argyophilic and possess almost identical staining properties. The close association of these tissue components with the chromaffin cells makes it extremely difficult to ascertain the precise manner by which the chromaffin cells are innervated. It is evident that large nerve plexuses terminate in the medulla and around large groups of chromaffin cells, but the smaller branches and nerve endings in the adrenal of *Ochotona princeps* are for the most part indistinguishable from the smaller reticular fibers of the medullary stroma.

Cortex

Zona Glomerulosa.--The organization of the cortical parenchyma and the disposition of the cells within the cortical components which will herein be described are not completely in agreement with the zonation introduced by Arnold (1866), but for the sake of convenience, clarity, and avoidance of entirely new terms, Arnold's cortical zonation will be adopted. The general cytology and histology of the mammalian adrenal are so well-known that they will not be described at length. Only a few statements on the cellular inclusion and disposition of the finer structures as they relate to the cortical continuum will be described.

The cellular organization of the adrenal cortex in Ochotona princeps may present a variable histological picture which is dependent upon two important factors: (1) the physiological status of the specimen at the time of death, and (2) the plane in which the gland is sectioned and prepared for histological examination.

The zona glomerulosa in this species is delineated by dense collagen interposed between the convoluted and intertwining glandular parenchyma. If a section is cut in a plane perpendicular to the surface of the gland (Plate I, Plane A, Sec. A), the zona glomerulosa appears to be composed of irregularly convoluted folds two or more cells thick. Viewed in this plane (which is almost exclusively used in descriptive histology of the adrenal), the convoluted folds appear to form loops, arches, clusters, solid plates, and many other cellular configurations. When the section is cut tangential to the capsule and parallel to the surface of the gland (Plate I, Plane B, Sec. A), transversing only through the outermost portion of the zona glomerulosa, the authentic nature of the parenchymal formations is easily depicted because each convoluted component is clearly delineated by collagen and/or capillary networks interposed between the collagenous stroma.

In this plane of section the organization of the parenchyma appears to be composed of many intertwining, branching, and anastomosing cordlike and/or platelike formations (Fig. 1).

In general, the basic width of each convolution is uniform and is composed of two columnar or pyramidal cells. The disposition of the cells within these cordlike formations is of some interest. Each plate is made up by two pyramidal or wedge-like cells whose apexes interlock near the center of the cellular plate (Fig. 1). Occasionally, one cell will span the thickness of the plate. The base of the cells is located near the periphery of the plate and is adjacent to the investing stroma. Generally, the nucleus is located at the base of the cell and occupies a peripheral position in relation to the center of an apparent solid cellular plate. In such an arrangement, the nucleus is proximal to the investing stroma and capillary network, but occasionally the plates in this region coil and intertwine to such an extent that a capillary is enclosed by a ring of radially arranged cortical cells. The polarity of the cells in regard to the vasculature consequently appears to be reversed.

By studying the zona glomerulosa in this plane, it can be unequivocally established that this zone in <u>Ochotona princeps</u> is a single continuum. After carefully reconstructing the zona glomerulosa of this species in three dimensions, it becomes apparent that isolated "glomeruli", "cords", "balls", "nests", and "clusters" that have been described by many authors (Torgersen, '40; Gruenwald and Konikov, '44) appear to represent only parenchymal components of a single whole. Small portions of the elevated cortical convolutions frequently project into the capsule, but rarely are isolated cortical cell aggregates seen completely circumscribed by the capsule.

In Ochotona princeps there is no indication that new cortical cells are derived from indifferent cells of the capsule. In reconstructions, if the capsule is stripped away from the cortex, the elevated cortical convolutions and general topography of the zona glomerulosa are reminiscent of the convolutions seen in the cerebral cortex (Plate I, Sec. A). Sections through Plane A, Plate I, indicate that the convolutions of the zona glomerulosa are continuous with the remainder of the cortex and represent integral components of a single cortical whole.

Zona Fasciculata.--When a gland is sectioned in transverse section perpendicularly to the capsule or gland surface, the zona fasciculata exhibits a radial pattern of parallel and centripetally converging cellular laminae or "sheets" separated by narrow and elongated tubular sinusoids interposed between the parenchyma. This zone is a concentric band in relation to the chromaffin tissue and completely circumscribes it except at the hilus, where the medulla is at the surface of the gland. Sexual differences are not very apparent, but this region appears to be well developed in females that are lactating or those with embryos.

The thicker double-celled parenchymal folds seen in the zona glomerulosa convert in this region into short interconnected laminae, one cell in thickness. This same zone when sectioned in a plane parallel to the surface of the gland presents a reticulated pattern, with hundreds of thin tubular sinusoids interposed between the interstices of the parenchymal network (Fig. 2). It is quite evident that the cellular disposition and arrangement within the parenchymal formations in this zone are entirely different from the convoluted folds of the zona glomerulosa. The sinusoids have irregular lumens, and are separated by a wall of parenchymal cells in such a manner that each polyhedral cell forming the parenchymal wall has at least one, and generally two, of its facets exposed to the vasculature (Fig. 2). The polyhedral cells that form the reticulated parenchyma of the zona fasciculata are readily identified because they stain lighter, contain more lipoid inclusions, and possess a centrally located nucleus.

After studying a three-dimensional reconstruction of this zone or region (Plate I) and reflecting on the nature of its embryological origin, it readily can be seen that though different parenchymal components are referred to as laminae or "sheets", they are not independent like sheets of paper, but are part of a single whole like septa of a sponge. Except for adjoining cellular abutment and bridging, the cellular facets are exposed to the vasculature interposed between the cellular laminae. If the vasculature is collapsed, the organization in this zone is almost indiscernible, and the entire cortex appears as a homogeneous mass of glandular cells.

Zona Reticularis.--The zona reticularis is at times difficult to discern or distinguish from the zona fasciculata, especially when the vasculature is collapsed. This zone, like the rest of the cortex, is a component part of the cortical continuum, and may present a different appearance depending upon the plane in which it is sectioned and on the physiological status of the specimen at the time of sacrifice. When the sinusoids are distended or engorged due to either active of passive hyperemia, it can be observed that the cellular plates anastomose more extensively and that each plate is only one cell thick. The stroma interposed between the cell surface and the sinusoids, if present, is indiscernible even after several histochemical and connective tissue stains are used in an effort to demonstrate the connective tissue elements. In this zone, where the parenchymal components are only one cell in thickness and are more anastomotic, the vasculature likewise becomes more anastomotic, more saccular, and no longer resembles the elongated and tubular-like vessels that characterize the vasculature of the zona fasciculata (Plate I, Sec. C).

The cortical tissue near the medulla intermingles freely with the chromaffin cells. By studying serial sections and three-dimensional reconstructions of this region (see Plate II), it can be demonstrated that the cortical elements within the medulla are thin, irregular-shaped projections one cell in thickness, and are intimately associated with the vasculature. These cortical elements are not "groups" or "clusters" of isolated cells within the medulla or "peninsulae of cortical tissue penetrating into the medulla" as Torgersen ('40) described them in the domesticated rabbit, but are integral components of a single cortical whole.

In some adrenals both the cellular elements of the stroma and the reticulo-endothelial system are disproportionately high, and, consequently, alter the "normal" histological appearance of the gland. Population cell counts of the cortical parenchyma, as well as cell size, vary in the different regions of the cortex. In the "normal" adrenal, the cortical cells are large, turgid, and closely packed. In the hyperemic glands the cortical cells decrease in size with a proportional increase of the connective tissue elements and reticulo-endothelial cells. The region affected most markedly is the medial half of the cortex, with the greatest alteration occuring in the region adjacent to the medulla. In a number of glands, the cellular elements of the stroma increase disproportionately, and appear to circumscribe each individual parenchymal cell; hence, the architecture of the zona reticularis and adjoining cortex appears to be composed of isolated cellular "columns", "cords", or "cell groups". However, examination of serial sections and three-dimensional reconstructions readily show that, although the parenchyma and stroma appear to be altered and the histological picture is modified, the basic architecture and the continuity of the parenchyma are not altered.

In the outer half of the cortex, little or no changes are effected. The zona glomerulosa appears to be the cortical region least affected in the hyperemic adrenal.

In some adrenals of <u>Ochotona princeps</u>, another modification which does not conform to the "normal" histological picture has been observed. This alteration is localized principally in the outer half of the cortex, and perhaps can best be described as "folliculation", possibly due to focal cellular alteration (autolysis or cytolisis), excessive engorgement of the vasculature, or interlaminar cavitation of the parenchymal folds. These spheroidal and parabolic follicular spaces, which measure up to 150 micra in diameter (see Fig. 3), may have an inner lining of what appears to be endothelial cells and may be engorged with blood. Other structures appear hollow and devoid of any visible endothelial lining, in which case the cortical cells appear to form the wall of the cavity. The wall of these "follicles" is invariably one cell thick and resembles the distended wall seen in follicles of an inactive thyroid gland. In almost all instances the outer periphery of the spheroidal cavitation is circumscribed by delicate reticulum and a cortical sinusoid. Although the "follicles" are focal and well localized, serial sections indicate that occasionally two of these structures can be continuous. In these native species it would be hazardous even to speculate on circumstances leading to these structural modifications, but from a physiological aspect it would be extremely valuable and of great interest to ascertain the types of stress that bring about the alterations noted here and to correlate them with the well-known modification described by Selye and his associates ('46).

Medulla

One of the most striking and distinctive features of the adrenal gland of Ochotona princeps is the medulla. In this species the arrangement of the chromaffin tissue is so clearly delineated that serial sections can be easily interpreted and its cellular organization can be easily reproduced. The ratio of medulla to cortex in Ochotona princeps is 1:11 by volume, and readily indicates the abundance of chromaffin ~~tissue in this native species. The most distinctive and conspicuous~~

feature of the medulla of Ochotona princeps is that the chromaffin tissue is well localized; nevertheless, it intermingles with the cortical elements.

Connective tissue stains indicate that a homogeneous sheath of delicate collagen invests the medullary "cords" or "plates" and separates the vasculature and the cortical tissue from the chromaffin elements, thereby plainly demarking the limits of the medulla. Fibrous strands from the investing sheath penetrate and contribute to the formation of the stromal networks which envelope and support the parenchymal elements within the cellular cordlike formations. Within some of the fibrous strands are thin-walled capillary-like vessels. The principal venous tributaries are intimately associated with the cortical tissue, and the stroma is interposed between the anastomotic cellular platelike and/or cordlike formations that interconnect and anastomose extensively (see Plate II and Fig. 4).

The parenchyma around the periphery of the medulla appears to be organized into solid nodular formations, while the more medial portion (although it is part of the same continuum) exhibits a solid cordlike organization. The cellular cordlike and/or platelike formations are comprised of six to eight cell layers. In cross section the formations appear ovoid or circular. Cells in the central portion of the formations are polyhedral and closely packed, and the nucleus is located near the center of the cell. In favorable preparation the cells forming the outer or peripheral row appear to be wedge-like or columnar, and in most cells the nucleus is eccentric and located away from the peripheral contour of the cord.

The vascular polarity of the chromaffin cells with respect to the medullary venules observed by Henle (1866), Pfaundler (1892), and later described by Bennett ('41), whose observations dealt with the medulla of the cat, can also be seen in this species. It is interesting to note that in favorable preparations this polarity is exhibited by all the surface layer of the branching cords, whether or not the cell is intimately associated with sinusoids, venules, stroma, or cortical tissue.

Although the vascular polarity of the chromaffin cells is also characteristic of Ochotona princeps, it is important to stress that this orderly arrangement is not confined to the cells around the vasculature. The polarity and the columnar arrangement of the cells adjacent to the investing collagenous membrane confining the anastomotic medullary cord-like formations appear to be the basic disposition of all the peripheral cells. This same polarity is maintained irrespective of the type of structure adjacent to the chromaffin cell, i.e., cortical sinusoid, stroma, or cortical tissue (Fig. 4). Furthermore, serial sections and three-dimensional reconstructions offer conclusive evidence that the parenchyma of the adrenal medulla in this species is an orderly continuum made up of interconnected and branching cordlike formations that constitute a single whole. Hence the so-called "irregular cords", "whorls", "isolated cell groups", "islets of chromaffin cells", "chromaffin clusters" and many other related terms used in histology texts (Maximow and Bloom, '49; Ham, '50; Torgersen, '40; Roaf, '35) and many scientific papers may prove to be inadequate terms that have arisen from only two-dimensional observations. The arrangement of the medulla in Ochotona princeps and its three-dimensional isometric reconstruction can be seen in Plate II. It is important to recognize that the adrenal of Ochotona princeps is composed of two independent and orderly arranged continua differing considerably in their cyto-architecture and parenchymal organization.

In glands with considerable hyperemia and marked alteration of the cortical parenchyma and vasculature, only the investing collagenous membrane and a thin endothelium separate the chromaffin cells from the cortical sinusoids and venous tributaries of the central vein (see Fig. 5). Although intracellular turgor, capillary pressure, and stromal proliferation alter the cellular appearance, the polarity of the chromaffin cells and the basic organization of the parenchyma appear to remain unaltered.

Lepus Californicus

Pericapsular Tissue, Capsule, and Stroma

In Lepus californicus the adrenal has the shape of a slightly flattened paraboloid which measures about 6 mm. at its longest axis. As in most species, the gland is embedded in adipose and loose areolar tissue. Within the denser areolar tissue which ensheathes the gland, are located a number of pericapsular structures which include arteries, veins, nerve trunks, and many ganglia. The medulla is adjacent to the capsule at the hilus, and in this region the adrenal vein emerges from the gland. Small nerves and arteries pierce the attenuated capsule and penetrate directly into the chromaffin tissue.

In this species the capsule is composed of a dense fibrous outer layer approximately 45 micra in thickness and of a thinner but more cellular inner component. The outer layer contains many closely packed collagenous fibers, a few spindle-shaped fibroblasts, and is completely devoid of elastic fibers; while the inner portion is a loose arrangement of what appear to be delicate reticular and collagen fibers interposed among many large and discrete fibroblasts (Fig. 6). Although the inner portion of the capsule is extremely cellular, containing an abundance of round and spindle-shaped fibroblast nuclei that appear to intermingle with neighboring cortical cells, there is no positive indication that fibroblasts or other connective tissue elements differentiate or transform into definitive cortical cells. Seldom do the parenchymal folds project into the capsule, and rarely are isolated cortical cells intermingled within the capsule fibers (Fig. 6).

From the inner more cellular portion of the capsule, a delicate and loose network of collagenous and reticular fibers penetrates between the folds of convoluted cortical parenchyma. Denser connective tissue strands extend inward from the capsule into the substance of the cortex, binding a number of parenchymal folds. These strands are broad as they differentiate from the capsule, but become narrower and extremely attenuated as they traverse through the cortex. The collagen fibers of the capsule appear to be continuous with the supporting framework of the cortical parenchyma.

Vasculature

Many fine branches of the larger adrenal arteries ramify on the surface of the gland superficial to the capsule. Most of these vessels pierce the capsule and form a subcapsular plexus. From this plexus many capillaries pass into the cortex by first forming a capillary network around the folded parenchymal convolutions of the zona glomerulosa; then they continue inward between parenchymal elements to empty directly into the tubular sinusoids of the zona fasciculata. Other capillaries pass independently through the zona glomerulosa without ramifying to empty directly into the sinusoids of the zona fasciculata. Both types of capillaries converge radially toward the medulla. In the zona fasciculata these vessels change into irregular tubular sinusoids, anastomosing occasionally by cross-communications. These sinusoids acquire greater diameters, become more saccular and more anastomotic as they approach the cortico-medullary junction. Bordering on the medulla the sinusoids become dilated, and, as they penetrate this tissue, they become the first order of collecting radicles of the central vein.

The arteriae medullae in Lepus californicus are abundant, are of varied caliber, and arise from stem arteries of the subcapsular plexus or from a small superficial adrenal artery that may pierce the capsule and cortex without branching to end in a number of capillaries in the medulla. All arteriae medullae appear to be endarteries, and only through the medullary capillaries are they interconnected.

Innervation

Occasionally, nerve trunks penetrate the cortex and ramify in the medulla adjacent to the thin-walled vessels. Small rami course between chromaffin cells. These nerve fibrils are in close contact with the surface of the chromaffin cells, but the precise nature of their endings is not clearly apparent. Groups of large binuclear cells containing an abundance of fuchsinophilic cytoplasm and large vesicular nuclei often can be seen intermingled among chromaffin cells. These cells appear to be identical with the binuclear ganglion cells embedded in the areolar pericapsular tissue. Individual and/or groups of myelinated fibers are often seen in close proximity to these cells. The fact that the capsular ganglia and the cellular group within the medulla are consistently seen (if the gland is serially sectioned) suggests that these cells are not merely aberrant cells without an orderly function. These elements appear to be definitive nerve cell bodies of autonomic postganglionic nerves whose terminal fibers innervate blood vessels or possibly chromaffin cells.

The intimate association of the cortical vessels with nerve fibers and their endings denotes that these vessels are innervated by the autonomic system, but there is no conclusive evidence that might support innervation of individual cortical cells.

Cortex

Zona Glomerulosa.--A characteristic of the domesticated rabbit adrenal is an indistinct and very narrow zona glomerulosa. It is extremely interesting to find that in Lepus californicus, the zona glomerulosa is a wide and clearly delineated band of cells that appear to be organized into many "cords" or "glomeruli" that are sectioned transversely. However, a three-dimensional reconstruction of this region reveals that the apparent "glomeruli" and "cords" are only interconnecting parenchymal folds, constituting integral parts of a single cortical whole. The elongated cells within the folds take on a somewhat darker hue with most dyes, and can be easily differentiated from the lighter lipid-laden cells of the outer zona fasciculata. By comparison the approximate width of the zona glomerulosa in Lepus californicus measures 250 micra and represents one-fourth of the cortical width, while this zone in Ochotona princeps measures 80 micra and represents one-eighth of the total cortical width.

In this species the cortex does not completely circumscribe the medulla. In the region of the hilus the medulla comes to the surface of the gland and only a few scattered cortical cells are interposed between it and the attenuated capsule (Fig. 7). The outer zona glomerulosa is the first to be disrupted, and the same sequence is followed by the more medial cortical parenchyma.

The most distinctive characteristic of this species is the conspicuous width of the zona glomerulosa and the complex branching and anastomosing of the thin cordlike cellular formations comprising the zona glomerulosa.

Zona Fasciculata.--Perhaps the most uniform cellular arrangement of the cortex is seen in the zona fasciculata. In a section parallel to the tubular sinusoids, the parenchyma forms what appear to be single rows or columns of polyhedral cells separated by tubular sinusoids lined with elements of the reticulo-endothelial system. The radiating and converging cellular columns occasionally interconnect, maintaining an uninterrupted continuity. It is interesting that most of the prevailing descriptions of the adrenal morphology usually describe only the radial arrangement of the zona fasciculata. If an adrenal of

Lepus californicus is examined when the tubular sinusoids are cut in cross section, the cellular arrangement appears entirely different; and in such a section the parenchymal components seem to form a cellular reticulated continuum such as the one described in Ochotona princeps. The cellular arrangement of the adrenal in this plane of section resembles the architecture of a tubulosinusoidal liver recently described by Elias ('49). Sinusoids passing radially toward the medulla commonly bifurcate and less frequently intercommunicate by means of irregular cross-communications. Hence, the parenchyma of the zona fasciculata possesses an abundant blood supply, but due to sparse cross-communication between sinusoids, each individual sinusoid can supply only a limited area of cortical tissue.

An interesting observation is the intimacy of the parenchyma and the vasculature. The only barrier between parenchymal cells and the blood supply appears to be the endothelium of the sinusoid. Each polyhedral cell of the zona fasciculata has at least two or three of its facets exposed to the blood stream. The transition from two-cell-thick convoluted folds of the zona glomerulosa to a single cell arrangement in the zona fasciculata gives this zone a significant physiological advantage by increasing and exposing a greater cellular surface to the blood stream.

Zona Reticularis.--Without a doubt the zona reticularis is the most variable and poorly delineated zone in this species. The transition between this region and the zona fasciculata is so gradual that a line of demarcation is hard to establish, even in good preparations.

The principal difference between the zona reticularis and the zona fasciculata appears to be in the sacculation, dilatation, and greater girth of the sinusoids. With a pronounced increase in sinusoid ramification and cross-communication and a concordant increase of parenchymal interconnection, a greater physiological surface is exposed to the blood supply. Hence, the amount of parenchyma that each sinusoid can adequately supply with blood is increased even more in this zone due to extensive cross-communications of the sinusoids.

Cellular size, nuclear appearance, and lipoid inclusions may be employed to differentiate cells of the various cortical regions; however, in this species the transition of the differentiating cellular characteristics is so gradual in so many specimens, that only an arbitrary rather than a real line of demarcation can be assigned between cortical areas, and only individual discretion can be used in delimiting the zones. Consequently, in glands where the vasculature is collapsed, the basis for zonal differentiation is limited, and it is virtually impossible to distinguish the limits of the zona reticularis.

Medulla

When the medulla of <u>Lepus californicus</u> is first examined, the most striking feature is the abundance of chromaffin tissue. This species is comparable in size, body, and adrenal weight to the domesticated rabbit, but there is a great discrepancy between the corticomedullary ratio in the two species.

Other salient morphological features of this species are the confluent and interconnected medulla and the nature of the central vein as it emerges from the gland. A scanty and delicate collagen membrane ensheathes the interconnected parenchymal plates of the medulla; therefore, the boundaries of the chromaffin tissue are not as clearly delineated as in Ochotona princeps. However, the disposition of the peripheral cells within the cords is basically the same in the two species. In Lepus californicus the medulla is comprised of short interconnected platelike cellular formations exhibiting an indistinct complex interconnected pattern. The parenchymal pattern is not as clearly depicted as the cordlike cellular formation observed in Ochotona princeps.

In the latter species the disposition of the cells within these structures is of some interest and possibly of some significance. Most cells are tall, columnar, wedged or prismatic, but essentially they are similar and possess a distinct polarity in relation to the vasculature. In favorable preparations where the cordlike parenchymal formations are cut in cross section, they appear to be solid, nodular, ovoid, and, at times, circular. The cells which form these structures are columnar, and their nuclei are located near the center of the plate. In some instances the cells form a radiating pattern with their nuclei located near the center of the "cord", and the tapering parenchyma is directed peripherally toward the delicate investing stroma. Basically, the cell arrangement is uniform, and when differences are noted it is possibly due to the plane of the section. The radiating and columnar disposition of the cells around the collecting radicles of the central vein is evident, and its occurrence in other species has previously been described by Henle (1865) and Bennett ('41).

The cellular arrangement and the disposition of the cells is more difficult to depict in Lepus californicus than it is in Ochotona princeps, and this may be related to the following facts. The bulk of the medullary parenchyma in Ochotona princeps is organized into discrete and sharply demarked branching plates as cordlike formations. In Lepus californicus the medullary parenchyma is more confluent and is formed by many interconnected short platelike formations with limiting boundaries that are in many instances difficult to discern. The conspicuous cordlike formations seen in Ochotona princeps are separated by an abundance of cortical tissue and by numerous thin-walled collecting radicales of the central vein. In Lepus californicus the platelike parenchymal formations appear to be more confluent and tightly packed, with only an occasional projection of cortical cells separating these formations. The larger collecting radicals of the central vein interposed between the cordlike formations are fewer, possess thicker walls, and are not as intimately associated with the parenchyma as those seen in Ochotona princeps.

At the hilus the central vein emerges from the gland, and Fig. 7 illustrates the nature of this vessel. In this species this vessel has a wide lumen and extremely thin endothelial-lined wall, which is completely devoid of any muscles or collagenous coatings. As this vessel emerges from the gland, it is completely surrounded by a layer of closely packed prismatic or columnar chromaffin cells that are intimately associated with the endothelium of the vessel. In this species arteriovenous anastomoses at the cortico-medullary junction are not discernible.

Most of the chromaffin cells are monotonously consistent in character, differing only in the manner in which they are oriented. These cells appear to be prismatic or columnar, and any differences noted are usually due to the plane of the section and to the number of cells which are sectioned.

Various authors have pointed out similarities in the chromaffin tissue and nerve cells. It is not uncommon, but in fact the rule, to find aggregates of large binuclear ganglion cells intermingled among the chromaffin cells; however, in the native rabbits the two cells have entirely different cellular characteristics and are extremely divergent in their morphology.

Lepus Townsendii

Lepus townsendii is the largest of the species studied, and at times attains a weight of three and one-half kilos (Hall, '46). This native rabbit is classified under the family Leporidae and looks a great deal like *Lepus californicus*, although its common name is mountain hare.

Pericapsular Tissue, Capsule, and Stroma

This species has practically the same cellular organization and cyto-architecture that was described under *Lepus californicus*, but the denser supporting stroma affords a more vivid representation of the parenchymal organization. There is nothing particularly remarkable about the pericapsular tissue in this species. The capsule is thin, measuring approximately 15 micra in thickness. As in *Ochotona princeps* and *Lepus californicus*, this fibrous membranous envelope has a denser outer component and a more cellular inner portion. Occasionally, dense strands and sheaths of collagen penetrate deeply into the cortex, but their number and extent are insufficient to partition the gland into discrete lobes.

Vasculature

The nature of the pericapsular vessels can be seen in Fig. 9. This same figure also illustrates an exceptionally large muscular artery (arteriae medullae) which penetrates the cortex; however, not all arteriae medullae in this species are as large. It is interesting to note that 10 to 15 large arteriae medullae penetrate through the cortex, and appear to be endarteries that terminate in a spray of thin-walled arterioles at the cortico-medullary border or within the medulla. Generally, arteriae medullae penetrate the cortex singularly, but occasionally they are accompanied by nerve trunks.

The nature of the subcapsular plexus is apparent in Fig. 11. This particular section is cut tangential to the capsule, with less than 20 micra interposed between it and the capsule proper. It is obvious that the subcapsular capillary-like vessels circumscribe and form an elaborate network interposed between the parenchymal convolutions.

The narrow, elongated, and tubular nature of the vasculature in the zona fasciculata can be seen in Fig. 9. If these same sinusoids are cut in cross section (Fig. 10), the vessels of the zona fasciculata appear to be ovoid or irregular channels of small diameters not exceeding those of the neighboring polyhedral cells.

The morphology in this section suggests a progressive dilatation of the adrenal vasculature from a narrow capillary-like vessel (10 micra in diameter) seen in the subcapsular plexus to a venous-like vessel of the medulla, which exceeds 100 micra in diameter.

Cortex

Zona Glomerulosa.--The topography of the outer portion of the cortex is clearly depicted in Fig. 11. This section was taken from a cortical area just underlying the capsule, with only 20 micra interposed between it and the adjacent fibrous capsule. One is immediately impressed by the sharp boundaries formed by the convoluted cordlike parenchymal formations oriented parallel to the surface of the gland, but perhaps a more striking feature is the interconnected and uninterrupted continuity of the parenchyma. This parenchymal arrangement was seen in the two species that were previously described, and appears to be typical of the native rabbit adrenal. After studying the next underlying section, other salient features emerge. The parenchyma can still be seen to constitute a continuum; tangential and transverse sections of the cords are more numerous; and, most important, the parenchyma transforms from a double-layered cellular fold or cordlike formation to flatter cellular sheets. As a result of this transformation, a greater cellular surface is intimately exposed to the vasculature.

When the surface convolutions are cut in transverse sections, their shape is ovoid or circular. The disposition of the cells within the cordlike formation in this plane of section is of particular interest. The nuclei crowd toward the center of the formation, while the bulk of the wedge-like or prismatic cells radiates peripherally.

Hence, in appropriate histological preparations, the nucleus occupies an eccentric position within the cell and exhibits a polarity in relation to the periphery of the cellular cord or the vasculature.

Ordinarily, the highest concentration of lipoid inclusions are located in the peripheral portion of the cell and lie between the nucleus and vasculature. In the more laminar parenchymal sheets that ramify from the inner portion of the surface folds, a single cell spans the width of the sheet, the nucleus occupies a more central position within the cell, and the lipoid inclusions are evenly distributed throughout the cell.

If the cordlike parenchymal formations are viewed in longitudinal sections, one immediately notices that the disposition of the cells within these formations has not changed.

In longitudinal section the nuclei form parallel rows with the longitudinal axis of the cord; in transverse section they follow the contour of the fold. Nevertheless, the central position of the nuclei in relation to the periphery of the cord remains constant.

It is extremely significant to be able to confirm that the width of the outer parenchymal folds, regardless of the plane of section, is spanned by two-columnar or prismatic-shaped cells, and to note that the cordlike parenchymal organization clearly changes to one-cell-thick laminae that ramify inward from the inner margin of the surface folds. In this species this precise region marks the inner limit of the zona glomerulosa and the outer limit of the zona fasciculata. Other readily observable changes are the increased number of sinusoidal channels, a tendency toward more polyhedral cells, the central position of the nucleus within the cell, and two or more facets of each cell are exposed to the vasculature. The authentic parenchymal organization and cellular disposition of the outer cortex can be depicted from different perspectives. However, one must keep the following in mind: the cordlike formations convolute, anastomose and interconnect extensively. Invariably they are sectioned obliquely; therefore, only in appropriate preparations and selected regions is the authentic organization of the cortex clearly demonstrable.

Zona Fasciculata.--The cellular organization and disposition of the vasculature in the zona fasciculata of this species is almost identical to that observed in *Lepus californicus*. It is important to note, however, that in this region it is the parenchyma that interconnects and not the vasculature that intercommunicates.

The cell shape in this zone is essentially polyhedral; the nucleus occupies a central position and exhibits no apparent polarity. Mitochondria and lipoid inclusions appear to be evenly dispersed throughout the cytoplasm. Each sinusoid is circumscribed by four or more cortical cells, and each polyhedral cell has at least two of its facets adjacent to the vasculature.

Predominately, all cells appear to be polyhedral, regardless of the plane in which they have been sectioned. This suggests hendecahedra- or tetrakaidecahedron-shaped cells.*

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*According to Duffy ('51), Lewis ('33), Marvin ('39) and Matzhe ('27), tetrakaidecahedrons, or 14 hedrons, more closely represent the shape of central cells in compact tissues.

These studies indicate that the parenchymal organization of the zona fasciculata in Ochotona princeps, Lepus californicus, and Lepus townsendii, viewed in a section cut parallel to the surface of the gland, is basically reticular.

Zona Reticularis.--The marked characteristics of this zone are an increase in parenchymal interconnections and a pronounced increase in vascular cross-communications. These features are responsible for the cancellous appearance of this zone. Consequently, a greater number of cellular facets come in contact with the blood supply. In this species a number of cells in the zona reticularis contact three sinusoids, and practically all cells contact two sinusoids; it is virtually impossible to locate a cell that is not in contact with the vasculature.

Medulla

The ratio of the cortex to medulla in this species is 12:1. The cordlike formations comprising the medulla are closely packed, with very little connective tissue or cotrical cells interposed between them. As a matter of fact, only where the vasculature is dilated can the cordlike formation be clearly depicted.

The cordlike formations observed in Lepus townsendii and Lepus californicus are not as massive as the formation observed in Ochotona princeps; notwithsatnding, the cellular organization in all these native species favors a branching and anastomosing cellular continuum.

Other salient features observed in the medulla of this species are the aggregates of binuclear ganglion cells which are invariably seen intermingled among the chromaffin cells (Fig. 12); these cells contain large vesicular nuclei and an abundance of fucshinophilic cytoplasm.

Lepus Americanus

This species is the smallest member of the Genus Lepus, and weighs approximately 750 grams (Hall, '46).

Pericapsular Tissue, Capsule, and Stroma

An interesting feature in this species is the thick adrenal capsule. This investing membrane is not uniform in thickness and varies in certain regions from 15 to 60 micra. Near the hilus of the gland it becomes filamentous, and, at times, difficult to discern. In many regions the inner portion of the capsule is noticeably more cellular, but this is not a constant feature. Small cell foci or groups of cortical cells appear to be isolated within the feltwork of the capsule (Fig. 14); however, examination of consecutive serial sections show that these cell foci are small outgrowths or extensions from the main cortical mass. In no instance did these outgrowths appear to be differentiating from indifferent capsule cells. An interesting characteristic of this species is the abundance of stromal tissue, particularly the many septa that penetrate the entire cortex. Although the septal sheaths are numerous in this species and incompletely partition the outer portion of the cortex, they are not extensive enough to completely partition this region into definitive lobes.

The supporting stroma is dense in the outer portion of the cortex, delicate in the zona fasciculata, and coarse in the zona reticularis.

Vasculature

Many small branches of the principal adrenal arteries penetrate the capsule and immediately branch into an array of small arterioles that ramify extensively and form a subcapsular capillary plexus. In some instances, an artery will penetrate the outermost portion of the cortex, course parallel to the capsule, give off small branches which contribute to the capillary network of the zona glomerulosa, and larger branches that penetrate the entire cortex to supply the medulla with arterial blood.

The sinusoids of the zona fasciculata are long, tubular, and radiate toward the medulla; in the zona reticularis, they intercommunicate and become saccular. The vascular pattern in *Lepus californicus*, *Lepus townsendii*, and *Lepus americanus* is almost identical.

Innervation

Many nerve trunks and nerve fibers penetrate the cortex in this species. Ganglion cells and nerve fibrils invariably intermingle with the chromaffin cell. The pattern of innervation in this species and in the other lagamorphs under consideration is essentially alike.

Cortex

Zona Glomerulosa.--The organization of the zona glomerulosa in this species is extremely complex and difficult to depict. In general, the disposition of the intertwining and convoluting surface folds mimic the configurations observed in *Lepus townsendii*, but are not as clearly outlined as the smoothly contoured, convoluted configuration observed in

Lepus townsendii. In *Lepus americanus* the zona glomerulosa constitutes a single cellular continuum comprised of short, solid, intertwining and interconnected cellular folds. The peripheral portion of the folds adjacent to the capsule are more massive, nodular, somewhat ovoid, and represent transverse sections of the intertwining cordlike formations oriented parallel to the surface of the gland. The surface cellular folds interconnect, branch, and anastomose more extensively in *Lepus americanus* than they do in *Lepus townsendii*. This becomes quite evident when comparable sections from the cortex of these two species are examined (Fig. 14 and Fig. 8).

Of particular interest is the difference in the disposition of the cells within the cordlike formation of these two species. In *Lepus townsendii* it was observed that the cells within the cords were somewhat columnar in shape and the nuclei crowded toward the center of the cord and exhibited a distinct polarity in relation to the periphery of the cord and to the vasculature. In *Lepus americanus* the prismatic cells are wedged and tightly fitted, but the polarity of the nucleus is just reversed in the majority of the cells and is located near the periphery of the cord and adjacent to the vasculature. The disposition of the cells in *Lepus americanus* conforms with that observed in *Lepus californicus* and *Ochotona princeps*.

Zona Fasciculata and Zona Reticularis.--The disposition and organization of the parenchyma in the zona fasciculata and zona reticularis conforms closely to that observed in *Lepus californicus* and *Lepus townsendii*.

Medulla

A surprising feature in this species is the significant and definite difference in the ratio of the cortical and chromaffin tissues. The amount of chromaffin tissue is high compared to the domesticated rabbit, but is not as abundant as in the other lagomorphs under consideration.

The organization of the parenchyma is practically identical, and closely parallels the cellular formations observed in Lepus californicus and Lepus townsendii; namely, a cellular continuum of tightly packed branching, anastomosing and interconnecting cordlike formations. In this peculiar parenchymal organization the formations are not regularly oriented in reference to the plane of section, and they loop, twist, and anastomose in an intricate manner. Transversely cut cords are generally the rule, regardless of the plane in which the gland has been sectioned.

Binuclear ganglion cells intermingle with chromaffin cells within the cordlike formations. There appears to be no remarkable difference in the basic organization of the medulla of Lepus californicus, Lepus townsendii and Lepus americanus. The outstanding difference within these species appears to be in the relative ratio of cortical and chromaffin tissue.

Sylvilagus Nuttallii

This species attains a weight of about 750 to 900 grams (Hall, '46), and is comparable in size to Lepus americanus. Both species are in the same family, but belong to different genera.

Pericapsular Tissue, Capsule, and Stroma

In most species the adrenal is embedded in a loose network of condensed areolar tissue connecting it to surrounding parts. Nerve trunks, ganglion cells, the main adrenal vessels and their branches ramify in this tissue as they approach the true capsule of the gland. It is of particular interest that in this species a large ganglion is located in close proximity to the gland. Very often nerve trunks can be seen leaving ~~this ganglion~~, and it is possible to trace these nerves to the adrenal, where they pierce the capsule, penetrate the cortex, and terminate at the junction of the cortex and medulla or within the medulla proper (Fig. 15).

Vasculature

Numerous branches from the main adrenal arteries ramify in the pericapsular tissue, pierce the capsule at many points, and subsequently break up into small arterioles and capillaries to form the subcapsular plexus. Few arterioles or capillaries course through the capsule as arteriae capsularis per se. For that matter, this type of vessel is not too frequently seen in any of the lagomorpha under consideration. Small muscular arteries consistently pierce the superior or inferior pole of the adrenal. These vessels branch and the smaller rami convert into a subcapsular plexus or ramify as capillaries in the zona glomerulosa. The larger rami become arteriae medullae.

In <u>Ochotona princeps</u> four or less small arteriae medullae penetrate the cortex; in all the other native rabbits ten or more large arteriae medullae supply the chromaffin cells.

Innervation

Of all the lagomorphs under consideration, the adrenal of *Sylvilagus nuttallii* appears to be the most highly innervated gland. A number of large nerve trunks penetrate the capsule and the cortex and terminate in the medulla (Fig. 15 and Fig. 16). Rami from these nerves form extensive plexuses throughout the medulla, appear to innervate the larger vessels, and undoubtedly innervate groups of and/or individual chromaffin cells. Approximately half of the nerve fibers within the large trunk appear to be myelinated, and many of the individual fibers between chromaffin cells are also myelinated. The chromaffin cells appear to be innervated by fine filamentous fibrils closely applied to the cell surface. If specialized nerve endings exist, they are not discernible in any of the histological preparations under study. It must be emphasized that it is extremely difficult to differentiate between fine filamentous nerve fibrils and delicate reticulum, even after a number of staining techniques are employed in an attempt to differentiate the two elements. In the adrenal, where reticulum is abundant and closely applied to the cell surface, one might easily identify reticulum as a nerve fibril.

Cortex

Zona Glomerulosa.--The organization of the cortical parenchyma just underlying the capsule is undoubtedly the most variable characterizing feature of the lagomorph adrenal. The cords and/or folds in this zone can be organized as extensive cellular, interconnected and convoluted folds like those observed in *Ochotona princeps* and *Lepus townsendii*; they can become extremely branching and interconnecting like those found in *Lepus americanus*; or they can be confined, anastomotic, branching cordlike formations like those seen in *Lepus californicus*. In addition, these folds, or cordlike formations, may be formed by a double row of prismatic or columnar cells wedged and tightly packed with the nuclei arranged in double rows near the center or axis of the fold and/or cordlike formation (*Lepus townsendii*). In other species (*Ochotona princeps*, *Lepus californicus* and *Lepus americanus*) the nuclei may be located near the periphery of the formation adjacent to the outer contour of the cellular cordlike formation and to the vasculature.

In *Sylvilagus nuttallii* this zone has a distinctive organization. The parenchymal formations anastomose, branch, loop, circle, and intertwine in all directions. This is especially noticeable in sections just underlying the capsule and cut parallel to the surface of the gland. Figures 16 and 17 present a clear and dramatic picture of this region. Notice the many different configurations described by the closely packed cordlike formations. In this species, one cell plainly spans the entire width of the clearly delineated formation. Although the cells are tightly packed within the twisted folds, they are well defined, uniform, and distinctly columnar. The nucleus is located precisely in the center of each cell, and occupies this position with monotonous consistency, so that in most sections, regardless of the plane of section, the nuclei form nearly perfectly outlined configurations that conform with the shape of the intertwining formations. Practically every cell in this zone contains minute, spheroidal fuchinuphilic granules which are distributed at random through the cytoplasm. (These inclusions also stain readily with Azophloxin.)

It is doubtful that these interesting inclusions are lipoid in nature or that they represent mitochondria, because they are most clearly discerned in glands that are fixed in Worchester's fixative and are processed as standard paraffin sections. The possible significance and interesting properties of these granules will be investigated in subsequent studies.

The orientation and disposition of the parenchyma within the zona glomerulosa, cut in transverse section vertical to the surface of the gland, are extremely interesting. In such sections (Fig. 18) the hairpin loops described by the interconnected convoluting parenchymal folds are plainly visible. Observe, however, that in this plane of section the parenchymal loops are confined to the outermost portion of the cortex, and that many of the branching and anastomosing cordlike formations (branching from the inner margin of the surface folds) straighten and become oriented with their long axes perpendicular to the surface of the gland. It is quite clear that the cordlike formations are integral components of a complex anastomotic continuum. This indicates that many of the surface intertwining parenchymal folds observed in Figure 18 send anastomotic plates toward the medulla. These parenchymal formations convert into more laminar units with their long axes oriented perpendicular to the surface of the gland. In longitudinal sections the inwardly directed cellular plates form a continuum presenting a radial pattern of parallel cell columns. Notice that in this species the single-cell column or plate is the basic parenchymal unit throughout the entire cortex. Two-cell-thick plates do not convert to single-cell plates (they did in the other species). Observe the tapering nature of the lamina and the smooth, gradual transformation of columnar cells to cuboidal and thence to polyhedral cells characterizing the the zona fasciculata. Note that the cells gradually acquire lipoid inclusions which are equally distributed through the cell.

Zona Fasciculata.--The convoluted and twisting parenchymal folds that characterize the zona glomerulosa straighten and are less convoluted as they branch toward the medulla. This cellular pattern is evident in Figure 18.

Population counts in the two areas indicate that the zona glomerulosa has more cells per unit volume than do the zona fasciculata or zona reticularis. This is surprising, since the width of the parenchymal lamina is reduced as it approaches the medulla. Two factors which can influence the cell population per unit area are (1) individual cells are more voluminous in the zona fasciculata, and (2) a greater volume per unit area of cortical tissue is occupied by the vasculature. In the zona reticularis, however, the individual cell volume is reduced and the cortical cell population count per unit area also decreases; conversely, the number of endothelial cell nuclei lining the sinusoids increases proportional to a decrease in parenchymal cell population. Since these features were also observed in the other native rabbits, these observations indicate that in the lagomorph adrenal the inner portion of the cortex is less cellular and has more vascularized tissue per unit volume. In the outer portion of the cortex, the cells are more numberous and constitute the greatest portion of the cortical tissue per unit area.

Zona Reticularis.--The cortical parenchyma adjacent to the medulla exhibits a great deal of variation in most species, and apparently this portion of the cortex is the first to respond to any physiological stress. In many glands the distended and irregular vasculature within the cellular interspaces give this zone a cancellous or spongy appearance similar to that observed in Ochotona princeps (Fig. 5); accordingly, the parenchyma is disposed as interconnected cancellated cellular plates. The thickness of the cellular plates is reduced to approximately 10 micra, with a concordant reduction in cell size. The width of this zone is extremely variable within the same species, and the limiting boundary between zona fasciculata and this zone is indistinct and irregular, but using some individual discretion an arbitrary line of demarcation can be adopted. In good preparation the cellular plates gradually taper as they approach the medulla. The vasculature in this zone clearly presents a more anastomotic and complex pattern. Individual cells appear to be smaller and not as laden with lipoid inclusions as are the spongiocytes of the zona fasciculata. Only a few nuclei appear to be pycnotic. These same features characterize this zone regardless of the plane in which it is sectioned.

It should be clearly understood that this cellular and vascular pattern is not always apparent in all the specimens within a single species. For instance, in many adrenals where the cortical vasculature is not distended, it is extremely difficult to recognize the zona reticularis (Fig. 20). The only apparent indication of this zone is a thin, irregular band of cells circumscribing the medulla. These cells stain with a darker hue and are almost devoid of lipoid inclusions; however, the large spongiocyte type of cell commonly seen in the zona fasciculata also extends to the cortico-medullary junction. There is no indication of pycnosis or cell degeneration in this zone. The tremendous variation seen in this portion of the cortex in the various species of native rabbits is readily apparent in Figures 5 and 20.

Medulla

In this species the ratio between the cortex and the medulla is 1:12. These figures conform to the ratios observed in the other native species under consideration, but deviate sharply from the ratio reported for the domesticated rabbit. From this study it becomes evident that the adrenal gland of native species consistently contains two to three times as much chromaffin tissue as does the adrenal of the domesticated species.

The cellular organization and disposition of the vessels in the medulla of this species seem to be identical with other native rabbits comprising the family Leporidae.

Most chromaffin cells in this species are extremely large polyhedrals and exhibit spheroidal vesicular nuclei. Around the large veins and smaller vessels, the cells are columnar in shape and exhibit a distinct polarity. The majority of these columnar cells are extremely long and voluminous. In some instances the cells are binuclear and measure 55 micra across their largest axis. Practically all the secretory granules and other cytoplasmic inclusions are interposed between the nucleus and the vasculature. A polarity in relation to the arterioles is not clearly discernible. Nearly every cell bulges into the lumen of the thin-walled venous channels; therefore, the lumen of these vessels is irregular and scalloped (Fig. 20). These observations are interesting in view of the fact that in Ochotona princeps the cellular polarity is not confined to the venous vasculature, since the chromaffin cell adjacent to the investing collagenous sheath exhibits a distinct polarity, regardless of the structure that might lie adjacent to the peripheral chromaffin cells (i.e., cortical sinusoid, cortical cells, a neighboring chromaffin plate, or thin-walled veins and venules).

It should be emphasized that the organization of the parenchyma is not determined by the disposition of the vasulature. Many chromaffin cells exhibit a peculiar orientation pattern around the small venules and larger vessels, but these cells comprise only a small portion of the total cellular chromaffin mass.

CHAPTER IV

DISCUSSION

Although the nature of this study is for the most part morphological, it is undertaken with the view first expressed by Virchow that every physiological process has its anatomical counterpart. Correlation of structural with functional changes is always difficult. Any attempts to read into the histological picture corresponding functional interpretation many times creates an aura of confusion; notwithstanding basic observations of the adrenal cellular architecture and parenchymal organization may throw light upon the physiological role of the adrenal by making more compatible prevailing physiological and morphological views which might bring us to a closer understanding of the adrenal function.

In comparative biological study it is a fact that the term "normal" is not explained by a limited and definite norm. This concept must, therefore, include considerable deviations from the average; thus, it might be prudent to consider the divergent possibilities for sources of error beforehand in order to avoid some pitfalls that might lead to erroneous interpretation.

It is evident that the adrenal glands in mammals have a similar structure: the cortex exhibits a peculiar tripartition, cortical cells possess considerable amounts of lipoids, and all mammalian species contain a medulla (Bourne, '49). But there is a certain structural and possibly also a functional difference between species. Many investigators have pointed out that age is an important factor, since the adrenal regresses immediately after birth and in old age (Blumenthal, '45; Hill, '30). Sex appears to be important because the adrenal seems to be modified by the sexual activity of the animal (Blumenthal, '45; Aschoff, '24). Dietetic conditions and weight are important factors, since inanition leads to peculiar changes in the cortical cells (Jackson, '19 and '25). Special complications such as infections, especially tuleremia in these native rabbits, could alter the norm. Thermal environment seems to alter the normal histology (Bernstein, '41). Agonal and postmortem alterations are extremely important because of the rapid autolysis, particularly in the medulla. Other sources of error could possibly be introduced by improper fixation and personal error in histological examination. Therefore, in order to minimize all possible sources of error, specimens for this study were collected at monthly intervals and over a period of two years. Only specimens that appeared mature and healthy were selected for this study.

In considering the periadrenal structures that were consistently observed in all the native rabbits under study, it should be emphasized that the glands in most instances were hastily removed in the field; consequently, these organs were not weighed or measured, nor were the surface relations or number of main vessels recorded. Occasionally, some pericapsular structures might have been removed by inadvertence, however the glands were trimmed so that a reasonable amount of periadrenal tissue remained in situ.

Bennett and Kilham ('40) report that after perfusing the vasculature of the cat adrenal with India ink, they were able to locate arteriovenous anastomosis in the adrenal pericapsular tissue. In this study such procedures were not possible; however, serial sections of many adrenals were carefully examined and arteriovenous anastomosis was never observed in the periadrenal tissue of the native rabbits.

Flint, in 1900, published an orderly description of the vasculature in the dog adrenal. For the sake of convenience and clarity, this same nomenclature has been used in the present investigation. One exception has been the term "sinusoid".

Minot (1900) coined the term "sinusoid" and used it to describe endothelial tubes of larger diameters than capillaries which had regular cylindrical forms. The sinusoids, he said, had irregular shapes and irregular connections. He found the capillaries embedded in connective tissue, while the sinusoids were fitted against the parenchyma of the organ. Minot suggested that these two vessels developed differently. He also pointed out that the nuclei of endothelial cells in the sinusoids were far apart; in true capillaries they were close together. He described the vessels in the adrenal as sinusoids. It has since been established that the cells lining the vascular channels of the adrenal cortex are phagocytic and capable of ingesting fine particulate dyes. This characteristic is another differentiating feature between capillaries and sinusoids. Since the vasculature of the adrenal cortex of all the native rabbits depicts the aforementioned characteristics, this term has been adopted in this investigation.

The vascular pattern and disposition of the vessels in the adrenal cortex of all the native rabbits under consideration is essentially the same. However, there is a marked difference in the vascular pattern of the vessels supplying the medulla and in the organization of the medullary parenchyma in species of the families Ochotonidae and Leporidae. In the single species of the family Ochotonidae (Ochotona princeps) only four or less small arteriae medullae penetrate the cortex and ramify at the cortico-medullary junction or within the medulla. In this species only the peripheral chromaffin cells within the cordlike cellular formations appear to touch or lie in close proximity to either the capillaries or the venous tributaries of the central vein. In all species within the family Leporidae, ten or more large arteriae medullae (Fig. 9) supply the medulla. Some of these arteries ramify at the cortico-medullary junction or within the medulla. Numerous capillaries ramify in the medulla and each chromaffin cell invariably touches or lies adjacent to a capillary and to a venous channel. Most chromaffin cells exhibit a true polarity in relation to the vasculature.

The larger venous channels of the medulla of all the lagomorphs are apparently devoid of smooth muscle coating and are intimately associated with the chromaffin cells.

It is of particular interest to find such a drastic variation in the structure of this venous channel among the various mammalian species, and after studying the classification for adrenal vessels proposed by Minot (1900) and Ferguson (1905), the question arises if such a vessel in Lepus californicus can be properly considered as a vein.

Other species from diverse mammalian orders, such as the spiney anteater and human beings (Bachman, '54) have highly developed medullary veins, with thick walls characterized by their longitudinal muscular ridges. This structural difference and relationship may be significant, and may be dependent on a specific pattern of development, differentiation, and function. These observations are of some interest after considering the following remarks by Velican ('47):

> After the involution of the androgenic zone near the cortico-medullary junction there develops during the first three postnatal years a large zone of arteriovenous anastomosis occuring between the cortico and medullary circulation. Moreover the common circulation and function of the two components which make up the adrenal can be interrupted by contraction of the pericytes around arteriovenous anastomosis. The degree of contraction is probably influenced by the amount of adrenaline and variation in blood pressure that prevails in the region of the central and peripheral veins, regulating the common or independent functions needed by the medulla and cortex of the organism and represents an important fact in the normal and pathological adrenal.

In spite of the extensive arterial ramification at the cortico-medullary junction in the species of the family Leporidae, no arterio-venous anastomoses were observed in these native rabbits.

The principal structures within the pericapsular tissue of these native rabbits are branches of main adrenal arteries, nerve trunks, small ganglia, small veins, and, of course, the adrenal vein after it emerged from the hilus of the gland. These structures have been repeatedly observed by many investigators, and appear to be present in many mammalian species.

LaCassagne and Nyka ('35), working with the domesticated rabbit, found accessory cortical bodies in 100 per cent of the specimens they examined. The site where these accessories were located was, however, in the region of the epididymis.

In the current study gross nodules of cortical tissue adhering to the principal glandular mass were evident, but in histological preparations they invariably turned out to be outgrowths of the adrenal cortex.

Many investigators have reported independent accessory capsulated or noncapsulated cortical bodies in the periadrenal tissue of other species, especially human beings. Denber ('49) described the tissue as organized, in spite of its apparent disordered and heterotropic growth.

The significance of this accessory tissue has been questioned, and one difference in the ability to endure or tolerate bilateral adrenalectomy was attributed by Stilling (1898) to the number of accessory cortical bodies found within different species. In recent years these encapsulated bodies have been much discussed by Broster and Vines ('33), Gruenwald ('46), and Sudds ('40). Goldzieher, in 1945, indicated that nonencapsulated cortical tissue proliferated loosely in the pericapsular tissue in certain types of pathological conditions (pemphigus vulgaris). Plaut ('45), in discussing this latter paper, stated that the picture of suprarenal cortex growing into the surrounding fat tissue was not a rare occurrence in his experience. Kovacs ('28) describes the infiltration of periadrenal fat by noncapsulated cortical masses differentiating between true accessory nodules. Denber ('49) found nonencapsulated cortical tissue within the pericapsular adipose tissue (human) in 72 out of 272 cases he studied (28 per cent). The 72 cases were reviewed for zonal composition, and in 34.7 per cent all the cells were of the glomerulosa type; cells of the glomerulosa and fasciculata types comprised 41.7 per cent; all three layers were present in 15.3 per cent; in 4 per cent the cell type could not be assigned; and in 16.7 per cent cells of the zona reticularis were present.

All of these observations are of extreme interest and point to pecularities among the various mammalian species, since at no time were nonencapsulated cortical masses observed in the periadrenal tissue of the native rabbits. Debner was unable to find any record reporting this type of tissue in other mammals, and points out ". . .that in all the animal work mention is made of meticulous trimming of the periadrenal fat". In this study his supposition of why this type of tissue has not been reported in other species is not valid, and must, therefore, indicate a pecularity among species.

The presence or absence of this type of nonencapsulated tissue might appear to be incidental, but a clue to the enigma of the manner by which the adrenal cortex regenerates might come forth by closely analyzing this type of tissue. This point will be further emphasized in the discussion.

Until recent years the thickness, nature and composition of the adrenal capsule was described in passing, but since Zwemer and coworkers ('38) categorically stated that this ensheathing membrane acted as a blastema for the cortical cells, a great deal of attention has been centered on this structure.

In the native species under consideration the capsule is well defined, and its nature and dimensions show little variation among these closely related mammals. A possible sex difference was noted in Ochotona princeps, where the adrenal capsule of the male appears to be thicker.

This unaccountable but consistently observed difference is minor and undoubtedly has no significance. The capsule was completely devoid of muscle and elastic tissue, and practically no nerve fibers were seen within the meshwork of this sheath. In all instances the capsule was composed of a dense collagenous outer feltwork and a looser but more cellular inner meshwork comprised of collagen, reticulum, and abundant spindle-shaped, turgid fibroblast nuclei.

Several authors have reported many differences in the composition of this capsule in other mammals. Kolmer ('18) found numerous smooth muscle fibers in the adrenal capsule of Rhinoceros unicornies, and Bachman ('39) reports pigment cells in Ovis aries. Elastic and nerve fibers intermingled with the collagenous fibers of the capsule have been found in other species (Bachman '54). These minor variations in different species are of an overall interest; however, in the native species under consideration, these elements were not present.

The inner portion of the adrenal capsule in the native rabbits is comprised of many spindle-shaped fibroblasts which lie adjacent to neighboring cortical cells. There is no positive indication that fibroblasts or other connective tissue elements differentiate or transform into definitive cortical cells. The parenchymal folds seldom project into the capsule and rarely are isolated cortical cells intermingled within the capsule fibers.

In recent years a disturbing trend has been developing to accept categorically the adrenal capsule as the cortical cell blastema. Undoubtedly, this trend has in part developed because many authors lump all the existing theories on adrenal regeneration together and speak of them collectively. For example, it is not uncommon to read (Turner, '39):

> That the cortical cells are proliferated from the peripheral glomerulosa and capsule and differentiate as they are pushed inwardly seems to be thoroughly established by the contributions of Mulon ('03), Goormaghtigh ('22), Hoerr ('31), Zwemer ('36), et al.

From this quotation one gains the impression that these authors postulate that capsule cells differentiate into cortical cells. This is obviously not the contention of all these authors. Furthermore, one cannot justifiably say that such a concept has been thoroughly established. For this reason, in the following discussions many authors will be quoted at length and verbatim in order to convey the precise findings of these investigators and to analyze the individual theories on regeneration in the light of the current findings. Zwemer, Wotton, and Norkus ('38) stated that "....New glandular cells are derived by a morphogenesis from indifferent cells of the capsule.... New capsular cells were constantly being formed from previously undifferentiated cells in the capsule."

Gottschow (1883), Mulon ('03), Bogomalez ('09), Graham ('16), Goormaghtigh ('21), and Hoerr ('31) had previously observed that a great number of mitotic figures were generally seen in the outer portion of the cortex underlying the capsule. Elias ('48) and other authors have supported Zwemer's concept of cellular differentiation. Bachman ('39) only supported a concept of a subcapsular blastema. In an attempt to correlate the theory of a capsular blastema with the excellent work of Graham ('16) and Hoerr ('31) (who had previously presented good evidence to support the theory that cortical regeneration took place principally in the zona glomerulosa or zona fasciculata and that cells migrated inward toward the zona reticularis, where they became senescent and finally degenerated), Salmon and Zwemer ('41) injected trypan blue into rats and noticed a progressive inward movement of blue cells; in 20 to 30 days the blue cells were located in the zona reticularis. These findings were vigorously and rightly attacked by Calmo and Forster ('43), MacPhail ('44), and Baxter ('46), who could not duplicate the experiment, and found that in high concentration (such as the one used by Salmon and Zwemer) of dye was peculiarly ingested by phagocytic cells and could also be found in all the cortical cells within 48 hours after the initial injection. These authors do not deny that cortical cells may possibly differentiate from a subcapsular blastema, but they do object to the inconclusive experiment of Salmon and Zwemer.

A number of papers have recently appeared dealing with autotransplantation and regeneration of the adrenal gland. Some enthusiastically support the theory of a capsular blastema; others are noncommittal; while still others postulate that the role of the capsule is insignificant. Ingle and Higgins ('39) indicate that bilateral adrenalectomy is necessary for subsequent regeneration of the autotransplant. If capsules were transplanted, they appeared as normal glands in 5 to 6 weeks. Williams ('45 and '47) studied adrenal autotransplants within transparent chambers installed in the ears of rabbits, and came to the following conclusions:

> Regeneration in adrenal cortex grafts begins in the glomerulosa region and possibly in the capsule. Zona fasciculata and zona reticularis in grafts are formed by centripetal displacement of cells from more superficial parts....
>
> The reticularis in grafts is a region of cell loss by death and degeneration and possibly by holocrine secretions.

~~Turner ('39) states:~~

> Notwithstanding the advantageous nutritional conditions in the anterior chamber the more differentiated cells of the reticularis and fasciculata degenerated, the new cortex being formed by proliferation from relatively undifferentiated cells of the glomerulosa and capsule.
>
> It is conclusively demonstrated that intra-ocular homotransplants of the cortices persist and undergo some degree of regeneration in the presence of both intact glands of the host in addition to one entire suprarenal graft.

Turner was also able to transplant successfully the medullary tissue if the period of nutritional interruptions was minimized.

Baker and Baillif ('39) made some interesting observations. These authors removed the right gland from rats and enucleated the left gland (according to the procedure of Ingle and Higgins). Their excellent description in part reads:

> The changes described in the capsular cell (Fig. 1) tend to produce a stratified appearance in the capsule as regeneration progresses. The innermost sheets of fibers become broken up, due to a centripetal migration of the former capsular cells. The process is probably not one of active movement but rather a change in the mitotic planes of the ingrowing cells. In early capsular hyperplasia the plane of mitosis is parallel to the inner surface of the capsule. As the process proceeds, the plane changes through an angle of 90° and the multiplying cells, as a result, begin to proliferate inward from the capsule. Later they fill the space left by removal of the inner portion of the gland. The cortex is thus replaced by cells derived from the substance of the capsule.

The findings of these authors are so interesting and pertinent to the forthcoming discussion that a partial summary of their observation will also be quoted:

> The cortical zones are seen to regenerate in the following manner:
>
> a. Narrow cords of cells appear as ingrowths from the capsule giving rise, by further ingrowth and multiplication, to the cell groups which are destined to become the zona glomerulosa. [This is precisely what one would expect to find if small cortical outgrowths were severed and remained in the meshwork of the fibrous capsule.]

b. From the inner margin of these cell groups, directed by the radically arranged connective tissue strands, new cords proliferate toward the center of the gland to form the zona fasciculata.

c. The inner ends of these cell cords show branching among the connective tissue in the center of the gland, and a zona reticularis is seen after 3 weeks.... Small but definitely marked islands of medullary tissue were noted in four regenerating glands removed after the twentieth day.

Mitotic cells were more frequently observed in the inner portion of the capsule by these authors. Their final conclusion was that cortical cells and their supporting tissues regenerate from the capsule of enucleated suprarenal glands.

Mitchell ('48), in a carefully controlled study, observed the postnatal development of the adrenal in rats. He used colchicine to detect mitosis, and came to the conclusion that only during the first week were mitotic counts high in the capsule. The author did not detect a gradual transition to cortical cells. He noticed a distinct inert layer separating the zona glomerulosa from the zona fasciculata (zone of compression). The mitotic counts reached their peak after 19 days of postnatal life; after that they declined, and, in the adult, they were scarce even after treatment with colchicine.

In discussing the escalator concept, Mitchell states:

When the mitotic activity of the adrenal cortex is examined in relation to the theory of cellular migration which, as we have seen in its widest sense postulates the continued translation of cells from capsule Zwemer and coworkers to the cortico-medullary boundary, [Gottschau and later Hoerr postulated an inward migration from the zona fasciculata toward the zona reticularis] it is clear that the results of the present investigation do not unequivocally support this theory. Mitotic activity in the capsule and subcapsular zone, although considerable during the first week of postnatal life, thereafter shows a progressive decline which leads to the inevitable conclusion that if capsular proliferation does contribute to the glomerulosa, after the first week, such contributions are unimportant.

Mitchell is in complete agreement that the greatest mitotic activity is in the outer part of the cortex, but does not believe that cells of the zona glomerulosa transform into cells of the zona fasciculata. On this point he states:

> The development of an additional region of high mitotic activity in the outer third of the fasciculata, separated from the proliferating glomerulosa by a zone of compressed, nondividing cells, suggests that the glomerulosa cells do not migrate into the fasciculata, but remain in situ.

His opinion about inward migration from the zona fasciculata is as follows:

> The occurrence of centripetal migration within the fasciculata itself, however, is strongly supported by the limitation of mitotic activity to its outer part, as well as by the polar contouring of the fasciculate cells and the presence of degeneration in the reticularis, facts which have previously been used by Hoerr ('31) to support this hypothesis.

About the cellular apposition hypothesis postulated by Gruenwald and Konikov ('44), Mitchell has some elucidating comments:

> The possibility that capsular cells are added to the glomerulosa by apposition of new layers of cells (Gruenwald and Konikov, '44) is not contradicted by my results; indeed in a few cases where layers of glomerulosa cells were oriented parallel to the capsule my findings appear to support this view. The intrinsic mitotic activity of the glomerulosa zone in the growing rat is so great, however, that normally this method of cell replacement can only be of secondary importance and must be restricted to the first week or two after birth. However, important mitotic proliferation in the capsule and subcapsular zone may be before birth, it is clear that after the glomerulosa has been differentiated, the subsequent contributions of the capsule to the cells of the cortex are insignificant.

In the present investigation hundreds of glands and thousands of sections have been studied, and special attention has been devoted to the problem of cellular regeneration and apposition of the cortical cells in the native rabbits. There is no positive proof to indicate that indifferent capsule cells differentiate to definitive cortical cells. From these studies it is impossible to support the concept that cortical cells differentiate from undifferentiated capsule cells.

In view of the current findings, the author would suggest that many cell outgrowths from the zona glomerulosa project into the fibrous capsule, and that possibly these cells proliferated and differentiated as cortical cells. The capsular fibroblasts undoubtedly proliferated, but their contribution is, perhaps, toward forming the supporting stroma. It must be emphasized, however, that in this study different species were studied and only the glands from normal adult specimens were selected. Cellular differentiation from the capsule might occur just during stress. If the capsule is indeed a cortical cell blastema, it is definitely not evident in normal native rabbits; if it functions in this capacity (as a blastema) only during acute stress, then it is difficult to understand why much of the evidence used to support the escalator concept has been observed in a score of adult normal species. Surely such basic phenomena as cellular regeneration are not confined to a few selected mammals, for it has been observed in many mammalian orders.

In the native rabbits the line of demarcation between cortical cells and capsular tissue is extremely sharp, and not in a single instance was there any indication that fibroblasts or any other connective tissue cells gradually differentiated into cortical cells. Small cortical cell masses often projected into the capsule, but the cells comprising them were always fully differentiated. When sections were stained with the flurochrome 3-4 benzpyrene, the cells in these outgrowths fluoresced as strongly as did the cells in the zona glomerulosa. Small lipoid granules were often seen in the fibroblasts of the capsule, but they never fluoresced like the cortical cell outgrowths. It might be prudent to point out at this time that sudan black B and fettrot apparently do not indicate the total lipoid content of the cell, since adjacent sections stained with the caffeine-benzpyrene technique of Berg ('51) indicate that cells in the zona glomerulosa and a thin band of cells adjacent to the medulla contained significant amounts of masked or bound lipid that did not stain with sudan or fettrot stains. These observations are based on the premise that even bound lipids fluoresce after they have been treated with 3-4 benzpyrene. There was never any indication that a gradual transition from fibroblast-like cells to cortical cells occurred.

The current findings support the work of Mitchell ('48) in many respects. This author, in his study of the postnatal development of the adrenal, was not able to detect any signs of capsular proliferation or cellular differentiation after the first week of postnatal life, indicating that the capsule contributed cells to the zona glomerulosa. He found mitotic figures scarce in glands from adult rats, even after the animals were treated with colchicine. The absence of mitotic cells certainly concurs with the author's observations on the native rabbit adrenals. Mitchell, however, did not think he could contradict the work of Gruenwald and Konikov ('44), and felt that, in a few cases, his findings appeared to support the views of these authors.

Unless the structural difference in mammalian adrenals is far beyond the limits of normal expectation, it is the opinion of this writer that the interpretations of Gruenwald and Konikov ('44) are somewhat questionable and their work should be reviewed. In fairness to these authors, their observations on the domesticated rabbit were limited, and their final opinion on this particular species was inconclusive, so one could not preclude that regeneration by cellular apposition does occur in some species and not in others. However, this inconsistency in such a fundamental phenomenon as cellular regeneration appears to be contrary to reasonable expectation. This is especially true since the entire gamut of figures and plates (especially those of the guinea pig) that these authors submit in support of their hypothesis can be reproduced almost exactly from the serial section of any one native rabbit adrenal. Furthermore, by depicting the zona glomerulosa and the entire cortex as a cellular continuum, the interpretation of Gruenwald and Konikov becomes questionable. The work of these authors has been quoted so extensively as evidence that complements the concept of a capsule blastema and cellular apposition, that in this author's opinion their evidence should be reviewed and analyzed, keeping in mind that the cortex is a cellular continuum. Gruenwald and Konikov state:

> Our findings in the adrenal of man and rhesus monkey have fully confirmed the mentioned concept of Zwemer, Wotton and Norkus concerning the mechanism of incorporation of newly differentiated cortical cords in the zona glomerulosa. However, indications of another mechanism were also seen in human material. This latter process has been studied in other mammals and these will be described first.

Since the guinea pig was the first animal they described and is often referred to in their discussion, their observations on this species are quoted at length:

> In the guinea pig the zona glomerulosa is somewhat more conspicuous than in man. End to end junction of newly formed cortical cords with those of the old cortex is not a typical occurrence in this species. The adrenals show considerable variability in the structure of their zona glomerulosa and its relation to what we believe to be newly formed cortical tissue. The various forms can readily be

> arranged in a series covering the process of differentiation of cortical tissue in the capsule, and the formation of a new zona glomerulosa by that tissue. Various phases may be seen on sections of one adrenal; in other words, different areas of the adrenal surface show, at the same time, different stages of replacement of cortical tissue.

It is the opinion of this writer that if dense connective tissue strands from the capsule separate the intertwining and branching surface folds of the cortical continuum, one can observe what might appear to be stages of cortical replacement. The observations of the above authors continue: "The earliest phase of new formation has previously been illustrated by Gruenwald ('42, fig. 13): small groups of cortical cells appear between the fiber bundles of the capsule". The study of the native rabbit indicates that the cortical cell groups are small cellular outgrowths invariably traced to the principal cortical mass. The discussion of Gruenwald and Konikov reads:

> The following states are illustrated by the present figures 4 to 7 which show the outlines of the cortical cords by silver impregnation of the lattice fibers. The first of these figures shows the cortical tissue in the capsule in a form which appears on section as solid cords. Examination of serial sections reveals that the actual form is that of irregular plates parallel to the surface organ. [With this observation the writer fully concurs.] Figures 5 and 8 show one of these plates much thicker than in the previous stage.

Similar cellular plates in the native rabbit adrenal turned out to be tangential or longitudinal sections of the intertwining surface folds. The author proposes that the cellular plates depicted in figures 5 and 8 referred to above are not in the capsule; dense fibrous strands from the capsule simply project down between the parenchymal folds.

Resuming Gruenwald and Konikov's observations:

> It is not always easy to distinguish sections of these plates from longitudinal sections of contracted small arteries. However,

> the decision can be made by following the structure in serial sections if, particularly with silver impregnation, single sections should leave any doubt. The large plates of cortical tissue eventually break up into many approximately spherical balls (fig. 6). It is obvious at this stage, particularly when the cytoplasm is adequately stained, that the underlying cortex has lost the structure of a zona glomerulosa, and contains a zona fasciculata reaching up to the aforementioned cell balls (fig. 9).

After analyzing the excellent illustration to which Gruenwald and Konikov refer, the author would suggest that the so-called spherical cellular balls are merely cross sections of the surface folds or cord-like cellular formations that run parallel to the surface of the gland. The zona fasciculata does not reach up to the aforementioned cell balls; the cellular plates branching from the inner margin of the surface parenchymal folds are merely directed downward into the cortex and away from the capsule. This was a common occurrence in the native rabbit adrenal. In these native species the collagenous strands extend down from the capsule and incompletely separate the intertwining parenchyma. Often this supporting tissue appears to circumscribe completely a short interconnecting plate and it might appear as a cellular ball, but invariably they turn out to be only integral components of a single whole. This was evident in the foregoing observation of the native rabbits. As a matter of fact, several of these integrants are illustrated in Plate I. It is clear that these cellular balls are only integral components of a single whole.

Gruenwald and Konikov continue their discussion:

> In preparations in which the separating fibrils are not stained, the layer of newly formed cell balls might, at this stage, appear to be the zona glomerulosa. The transformation of the old zona glomerulosa has already begun during the formations of cortical cell plates in the capsule (fig. 8). In figure 7, a stage is shown in which the

cell balls of figure 6 have connected with the old cortex, and present a typical zona glomerulosa.

This stage again is a matter of critical interpretation, keeping in mind that the cortex is indeed a cellular continuum. The figure to which these authors refer (7) illustrates perfectly that the surface folds and/or cordlike cellular formations are oriented parallel to the surface of the gland. It also depicts beautifully that from the inner surface of these cellular formations branching and interconnected plates are directed inward toward the medulla and away from the capsule. These plates are oriented perpendicular to the surface of the gland. Gruenwald and Konikov further state:

> In contrast to the mechanism described by Zwemer, Wotten and Norkus (and illustrated by the present figures 17 and 18), one finds in guinea pigs that a new formation of an entire layer of cortical tissue becomes secondarily apposed to the old cortex and replaces the zona glomerulosa over an area covering many cortical cords.

In this instance an intertwining surface fold could easily be cut longitudinally, and the ramifying plates branching from its inner surface present a radial pattern. This same cellular organization was repeatedly observed in the native rabbit adrenals, and in these species the zona glomerulosa always proved to be a continuum.

In summary of their observations, Gruenwald and Konikov state:

> A hypothesis is proposed according to which the replacement of functioning cortical cells take place by three mechanisms: 1. mitotic division at the junction of zona glomerulosa and fasciculata. 2. mobilization of cells from reserve store represented by the zona glomerulosa. 3. replacement of depleted stores by apposition from the capsule.

The first mechanism need not be at variance with the observations made in the native rabbit adrenals.

The third concept is simply not tenable in the native rabbit adrenal, since no positive evidence was ever found to indicate that such cellular masses arise in this manner. In fact, in the native rabbit adrenal, there is some evidence against such a regenerative concept. This statement is based on the following facts: (1) the so-called cellular "foci" observed within the fibrous meshwork of the capsule invariably proved to be integral components of a single cortical whole or cellular continuum; (2) a gradual transition of capsule cells converting to cortical cells was never observed in thousands of sections from native rabbit adrenals; (3) cells within the cellular "foci" stained like differentiated cortical cells; and (4) the lipoid cellular inclusions within the cells of the capsule "foci" appeared to be identical with the inclusions observed in the cells of the zona glomerulosa, and were conspicuously different from the lipoid inclusions of the neighboring connective tissue cells.

The second mechanism cannot be contradicted, and in one respect might receive some support from the current investigation; however, the evidence is only circumstantial or suggestive. It will be recalled that in Sylvilagus nuttallii and Lepus townsendii the straight, tapering, and inward-directed cellular plates exhibited a vivid and gradual transition from elongated and often columnar cells in zona glomerulosa to a cuboidal or polyhedral shape in the zona fasciculata. The cells within the zona reticularis were smaller and were not so heavily laden with lipoid inclusions. This gradual transition in the cortical cells might indeed indicate that the glomerular cells do convert to inner cortical cells. However, proving categorically that these cells migrate inwardly is something else.

Mitchell, who was previously quoted, feels that a zone of compressed nondividing cells separate the zona glomerulosa from the zona fasciculata, and that glomerular cells do not migrate into the fasciculata, but remain in situ. This area of compression was occasionally observed in the native rabbit adrenal, but was definitely not a constant feature in these species. Conversely, in some glands, this region was extremely cellular, the population cell counts were higher than in any other portion of the cortex. Cellular compression and high cell population counts appeared to be normal variations in the native rabbit adrenal, since most adrenals exhibited a smooth and gradual cellular transition; namely, a gradual decrease in the thickness of the inward-directed cellular plates, with a concomitant transition from elongated or columnar glomerulosa-type cortical cells to polyhedral fasciculata-type cortical cells. It must be emphasized that in some glands (Lepus californicus) this transition was not always observed, and in Sylvilagus nuttallii the majority of the cortical cells within the narrow zona reticularis exhibited the turgid appearance of the spongiocytes that characterize the zona fasciculata.

During the early course of the current investigation, it became evident that both the adrenal cortex and the adrenal medulla were, in effect, orderly continua. It was first noted that in sections cut perpendicular to the surface of the gland the zona glomerulosa appeared to be made up of many cellular "loops", "arches", "balls", and other often-described configurations. However, sections cut parallel to the surface of the gland exhibited an entirely different organization. This zone appeared to be composed of many convoluted, intertwining, branching, and anastomosing cellular folds and/or cordlike formations. The cellular configurations presented a pattern similar to the convolutions seen in cerebellar tissue. This appearance is due to the strands of connective tissue which penetrated the cortex from the capsule. Ingrowths from the collagenous capsule and small vessels were interposed between the intertwining folds. When this portion of the cortex was reconstructed (Plate I), the entire zona glomerulosa could be seen as a unit. The many cellular folds were continuous integral components of a single whole. The cellular "loops", "arches", "balls" and solid plates previously observed proved to be only tangential or cross sections of the surface intertwining and convoluting surface folds. From the surface folds numerous small cellular outgrowths often projected into the meshwork of the capsule. From the inner margin of these surface folds, many branching and interconnected plates extended inward toward the medulla and away from the capsule. This basic organizational pattern was observed in all the native rabbit adrenals. Individual variations were observed between species, but they were principally restricted to the disposition of the cells within the cords and to the length, shape, and number of anastomosing and interconnected cellular integrants.

In analyzing these findings an interesting correlation can be established with the observations of Baker and Baillif ('39). These investigators, while studying the regeneration of the adrenal in rats, noted that in early capsular hyperplasia the plane of mitosis was parallel to the inner surface of the capsule and that as the process proceeded the plane changed through an angle of 90 degrees. As the cells proliferated they extended inward from the capsule. These findings complement the foregoing concept of a surface continuum of intertwining folds oriented parallel to the surface of the gland. A change in the direction of growth and inward proliferation is in harmony with the present findings dealing with the branching cellular plates that extend inward from the inner margin of the surface cellular folds.

Gruenwald and Konikov ('44) studied a limited number of embryonic adrenals, and their findings can be well correlated with the present concept of a single cortical whole. They state:

> Only scattered observations can be reported concerning embryonic stages of the structures under consideration, that is, the zona glomerulosa and adjoining levels. The early embryonic adrenal cortex is nonepithelial: each cell is surrounded by a network of lattice fibers (Gruenwald). The epithelial zona glomerulosa appears later by a structural change in the peripheral layers of cortical cells. With the customary staining methods it can then be distinguished from the remaining portions of the cortex by its much smaller cells. Coincident with the appearance of this difference in cell size is a disappearance of the lattice fibers within the cords of the zona glomerulosa (fig. 23). This fact as well as the outlines of the cords themselves can best be visualized by silver impregnation. The usual methods will not show the arrangement of the cells of this zone in cords, but just a continuous cell mass. The zona glomerulosa appears relatively late in the development of the organ, in human embryos of the fourth month, and in cat embryos of more than 70 mm. Not in all instances is this layer distinctly bounded against the capsule; this suggests that transitions may occur here, but not by formation of definite cords as in adult animals, so that the mechanism cannot be followed in detail by the study of sections. The very early zona glomerulosa may show no subdivision in cords, and thus resemble the condition shown in figure 13 in a kitten by the presence of a continuous layer of cortical tissue. This was seen in cat and pig embryos. Just what role the appearance of cavities in the zona glomerulosa of human (and some other) embryos plays in the shaping of the structural units of this layer (Hett), cannot be decided. The final result is essentially similar in species with and without cavities in their embryonic zona glomerulosa.

Unless the cellular organization in mammalian adrenals varies greatly--and such a variation was not observed in the native rabbits--, it is the opinion of this author that the adrenal gland is not comprised of isolated cellular groups (i.e., "glomeruli", "cell balls", "clusters", etc.), but that it persists as a single continuum even in the adult species. The cortical continuum in embryonic adrenals is without a doubt easier to depict. When it becomes fully differentiated it is very complex and difficult to discern.

Concerning the cellular organization of the zona glomerulosa, as great a variation was observed between the genera of the family Leporidae as between species of the two families within the order Lagomorpha. In Ochotona princeps the intertwining and convoluting cellular, cordlike, and/or platelike formations are comprised of two-cell-layer plates. This cellular pattern is also observed in three species of the genus Lepus, namely, Lepus californicus, Lepus townsendii, and Lepus americanus. However, in Sylvilagus nuttallii (genus Sylvilagus) a single elongated cell spans the entire width of the cellular platelike formation (Fig. 17). This cellular organization is somewhat significant because each pole of the cell is adjacent to the vasculature, and consequently the cells possess a greater physiological advantage over two-cell-layer formations observed in all the other species under consideration. This advantage is effected because a greater cellular surface is exposed to the blood stream.

Variation in the cellular organization of the zona glomerulosa in the species where the formations are two cell layers in thickness are of less consequence and are not restricted to different genera or species.

The cords and/or folds in this zone can be organized as extensive cellular, interconnected and convoluted folds like those observed in

Ochotona princeps and Lepus townsendii; they can become extremely branching and interconnecting like those found in Lepus americanus; or they can be confined, anastomotic, branching cordlike formations like those seen in Lepus californicus. In addition, these folds or cordlike formations may be formed by a double row of prismatic or columnar cells wedged and tightly packed with the nuclei arranged in double rows near the center or axis of the fold and/or cordlike formation (Lepus townsendii). In other species (Ochotona princeps, Lepus californicus, and Lepus americanus) the nuclei may be located near the periphery of the formation adjacent to the outer contour of the cellular cordlike formation and to the vasculature.

The cellular organization in the zona fasciculata proved to be stable and similar in all the native rabbits. In the preceeding histological observations, viewed from a single perspective, this zone appeared to be comprised of many cellular columns arranged parallel to each other and separated by irregular thin-walled sinusoids. The parenchyma is disposed in a radial pattern, with the parallel cellular columns converging centripetally and oriented perpendicular to the surface of the gland.(Fig. 18). This cellular pattern has been repeatedly observed in many mammalian species; however, this is not the only pattern that this zone exhibits. In sections cut parallel to the surface of the gland, this zone appears to be comprised of many short, interconnected cellular plates. The interstices between the polyhedral cells are permeated by numerous irregular sinusoids (Fig. 19). Of particular interest is the organization of the parenchyma, which interconnects extensively while the sinusoids form a spray between the cellular continuum. It is important to recognize that it is the cellular parenchyma that interconnects more extensively and not the vasculature that intercommunicates.

In the past the cellular pattern as observed from this latter perspective has not been fully recognized. From this perspective the zona fasciculata resembles the cellular architecture of the tubulo-sinusoidal liver recently described by Elias ('49).

It is essential to examine the parenchymal organization of the adrenal cortex from perspectives at right angles or perpendicular to each other in order to fully visualize the true nature of this cellular continuum. Plate I exhibits the basic structure of the tripartate cortex in the native rabbits.

In the past not all authors have been fully agreed on the nature and composition of the zona reticularis of the domesticated rabbit. Mitsu Kuri (1882) described a regular zona reticularis. Roaf ('35) was hesitant about the nature of this zone, and comments as follows: "It is definitely not fasciculata and is here called reticular, in accordance with popular usage, though this term does not always describe the arrangement well." This same author introduced another zone, composed of a variable amount of darkly staining cells arranged in small groups around the medulla, and termed this region the interlocking zone. In his opinion, this zone was homologous with the X-zone of the mouse; however, the interlocking zone persisted into adult life while the X-zone degenerated.

Hoerr ('31) stated that the rabbit adrenal contained no reticularis, and that typical fasciculate cells come right up to the medulla.

In 1940 Torgersen suggested that the adrenal cortex histology in the domesticated rabbit differed little from the description given in textbooks.

In the preceeding observations of the native rabbit adrenal, it was noted that the zona reticularis was, in effect, an irregular zone exhibiting some interesting features; however, the limiting boundaries of this zone were generally difficult to discern. Although a great deal is known about the structure of this zone, some salient features about its structure in the native rabbit adrenal should be discussed, keeping in mind a cellular continuum.

This zone is interesting and variable, for in this region of the cortex the cellular plates twist, buckle, and anastomose with a concomitant sacculation and intercommunication of the vasculature.

The vasculature in this zone appears to form a true network or plexus, and, in this respect differes from the vascular and parenchymal arrangement of the zona fasciculata, where the vascular channels are disposed in a spray. Consequently, the zona reticularis presents the same cellular pattern, regardless of the plane in which the gland is sectioned, and differs from the zona glomerulosa and zona fasciculata, where the disposition of the parenchyma exhibits different cellular patterns when viewed from different perspectives.

In hyperemic glands, the zona reticularis appears as a cancellous mass comprised of interconnected cancellate cellular plates.*

*From the nature of its origin it should be readily understood that though the different integral parts of the cortical parenchyma are referred to as cellular plates or sheets they are not independent like sheets of paper but are integrants of a single whole like septa of a sponge.

In the adult native rabbit adrenal a zone comparable or homologous with the X-zone of the mouse was never evident. The interlocking zone described by Roaf ('35) was occasionally observed, but since this band of darker staining cells was not a constant feature, this zonation is not adequate for the adrenal cortex of native rabbits. Adjacent to the medulla, the cortical cells are more fuchsinophilic. They sometimes contain pigment, and, in many instances, are not heavily laden with lipoid inclusions; however, these are variable features, for one can often find spongiocyte cells adjacent to the medulla. Degenerative changes and pycnotic nuclei are often seen in the zona reticularis, notwithstanding, these same changes are often seen in other parts of the cortex.

Hoerr was not able to detect a zona reticularis in the domesticated rabbit. At this time it might be prudent to point out that if the vasculature is collapsed, this region is indeed difficult to discern (Fig. 20). However, in favorable preparations and in hyperemic glands this zone is more clearly apparent (Fig. 5). The width and extent of this zone is perhaps the most variable feature in the native rabbit adrenal; nevertheless, the zone is invariably present.

Bennett ('40) suggested that the cortex be partitioned into four different zones. The outermost layer he called "presecretory" because the cells contained little lipoid material. The second zone, being usually rich in lipids, he designated as "secretory". Underlying this zone is the "postsecretory" zone varying in width and lipoid content. A fourth and innermost layer located adjacent to the medulla he termed "senescent"; it contained less lipids than the secretory, but more than the postsecretory zone. Frequently, glands presented these cellular characteristics; however, these features were too inconsistent to partition adequately the gland into these zones.

In Ochotona princeps, Lepus californicus, Lepus townsendii, and to a lesser extent the other native rabbits, large follicle-like structures were noted. These parenchymal modifications which did not conform to the normal histological picture were localized principally in the outer half of the cortex, and perhaps may best be described as "follicles" or "pseudofollicles". It is difficult to correlate structural with functional changes, and in the words of Rogoff, "Premature attempts to read into the histological picture corresponding functional interpretations necessarily react in bringing even apparently genuine morphological distinctions under suspicion". It may be that these parabolic cavitations are merely locally distended sinusoids in which the blood has pooled. This, however, does not explain the nature of hollow structures devoid of blood and of any apparent endothelial lining, in which case the cortical cells appear to form the wall of the "follicle" (Fig. 3 and 6). The delicate processes of one or more connective tissue elements are often seen within the follicle, and there is some indication that some tissue fluid was contained within these structures. Cellular alterations (autolysis or cytolysis) might possibly produce such a parenchymal dearrangement, but these modifications in the cellular organization are well localized and do not appear to be postmortal changes or artifacts due to improper fixation.

The eliciting factors and the significance of the foregoing parenchymal modifications are difficult to assess; notwithstanding, these alterations are commonly seen in the adrenal of native rabbits.

Many adrenals showed marked changes in the reticulum and parenchyma of the zona reticularis. Such alterations are known to occur in other species, and have been ascribed to increase in age (Blumenthal, '45). Since this area is so liable to many types of stress and fluctuates so much within a species, it is important that these stromal alterations and concomitant parenchymal changes be noted; however, such detailed data and information are not readily available in these native species, and this type of correlation is limited in this study.

In recent years several authors have correlated structural with functional changes. For instance Deane and Greep ('46) have indicated that the zona glomerulosa produces salt-regulating hormones and that the zona fasciculata produces carbohydrate-regulating hormones. Grollman ('36) indicates that the zona reticularis and/or the androgenic zone synthesize male and female-like hormones. The findings of these authors are significant, and should be elaborated upon, but from this study not much can be said that might amend or foster their findings.

From the observations of the adrenal medulla of the native rabbit, several interesting features emerge. The adrenal medulla of Ochotona princeps (family Ochotonidae) presents a characteristic pattern which is not observed so clearly in the other native rabbits. In this species the topography of the medulla more closely resembles the medullary pattern of the rat adrenal than it does the medullary pattern of the other native rabbits under consideration. Nevertheless, the basic cellular organization is the same in all the native rabbits.

The branching and anastomosing cordlike and/or platelike cellular formations comprising the adrenal medulla of Ochotona princeps are clearly depicted because they are separated by cortical tissue, by a homogeneous collagenous investing membrane, and by the vasculature. Because the medullary components are so clearly outlined in this species, they can be reconstructed without difficulty. When the medulla was reconstructed it was, in effect, a single whole comprised of many branching and anastomosing cordlike and/or platelike cellular formations. The individual medullary integrants are comprised of 6- to 8- layered cells. In transverse section many of the cordlike formations are ovoid and exhibit a radial cellular arrangement resembling tubes of exocrine glands prior to canalization. The disposition of the cells within the parenchymal formations is of special interest. Cells nearest to the central axis of the cellular formations are tightly packed and are polyhedral, while the cells adjacent to the peripheral investing membrane are columnar and exhibit a radial pattern. In these peripheral columnar cells, the nucleus is eccentric and is located away from the investing membrane; in the central polyhedral cells, it is located near the center of the cell.

The basic arrangement of the chromaffin cells about the medullary veins has been known for almost a century. Henle (1865) was perhaps the first to observe the columnar shape and radial arrangement of the cells around the venous collecting radicals of the adrenal medulla.

Bennett has wisely cautioned against the use of the terms "irregularly arranged cords of the medulla" and "medullary whorls", which are suggestive of an irregular cellular arrangement. He has also carefully re-emphasized the vascular polarity and orderly arrangement of the cells around the medullary veins. The aforementioned author did not find an epithelium-like arrangement of the cells around the medullary arteries or capillaries. His observations are in full accord with the arrangement noted in most native rabbit adrenals. Bennett and Kilham ('40) stated that in the cat adrenal the number, size, and complexity of the venous vessels provide the medullary cells with an ample framework on which to arrange themselves, so that every medullary cell touches or lies close to one of the veins or its branches.

In the native rabbits comprising the family Leporidae, the medulla is made up of cellular, platelike formations of two- or three-cell layers; each medullary cell appears to touch or lie in close proximity to a vein or its tributary. The polarity of the cell in relation to the veins is quite apparent, but its relationship to the arteries and capillaries is not clearly observed.

Concerning Ochotona princeps (family Ochotonidae), where the cordlike formations are six or more cell layers thick, the polyhedral cells near the central axis of the formation do not appear to be in contact with the vasculature. The disposition of the peripheral columnar cells adjacent to the enveloping collagenous sheath is remarkably consistent. As a result the cells exhibit a polarity irrespective of the structure they contact, i.e., cortical sinusoid, stroma, cortical tissue, or the medullary vasculature. Although the peripheral cells adjacent to the investing sheath exhibit a polarity, the more central polyhedral chromaffin cells are not endowed with a true polarity. This pecularity or variation among the native rabbits can, perhaps, be related to different vascular patterns among these species.

In species of the family Leporidae many large arteriae medullae penetrate the cortex and branch at the cortico-medullary junction and within the medulla where they ramify as capillaries. According to Bennett and Kilham ('40), this same arterial pattern has been observed in the cat. Harrison ('51) observed the same arterial pattern in the domesticated rabbit and in the cat. Recently, Lever ('52) reported this pattern in the cat, but in the rat only four or less arteriae medullae were found.

In the single species of the family Ochotonidae (Ochotona princeps), the medullary arterial pattern appears to be identical to the pattern observed in the rat. It is stimulating to find such a similarity in the general topography, arterial pattern, and disposition of the medullary continua of Ochotona princeps and the rat; whereas the topography, vasculature, and disposition of the medulla among the species of the family Leporidae are similar to the cat adrenal. Hence, the vasculature of the medulla comprises perhaps the most real and divergent feature observed in the native rabbit adrenal. Associated with this difference in vascular pattern is the varied disposition and organization of the chromaffin cells comprising the medulla. The observations of Bennett and Kilham ('40) dealing with the vasculature and cellular organization of the cat adrenal do not precisely describe the cellular organization seen in Ochotona princeps. Whereas these authors observe that in the cat the chromaffin cells arrange themselves around the veins and their tributaries and are endowed with a true polarity with respect to the arterioles and medullary vein, in Ochotona princeps all the chromaffin cells do not touch or lie next to the vasculature. In this native rabbit the platelike and/or cordlike cellular formations are six or more cell layers thick, and the polyhedral cells near the central axis of the cellular formations are not endowed with a true vascular polarity; hence, the vasculature in this species does not appear to provide the framework on which the chromaffin cells arrange themselves. The vasculature merely permeates the interspaces between the platelike and/or cordlike cellular formation. In Lepus californicus, Lepus townsendii, Lepus americanus, and Sylvilagus nuttallii the vascular pattern and cellular disposition closely follow the cellular arrangement observed by Bennett and Kilham ('40) in the cat adrenal, except that the cellular polarity in relation to the arterioles and capillaries is not as clearly depicted in the native rabbit adrenal.

From the present study little can be added to the existing knowledge of adrenal innervation. In Ochotona princeps, Lepus californicus, Lepus townsendii, and Lepus americanus only small ganglia containing large binuclear ganglion cells were generally found in the pericapsular tissue. However, in Sylvilagus nuttallii only one large massive ganglion was consistently found adjacent to the capsule. Furthermore, large nerve trunks leaving this ganglion were traced to the richly innervated medulla. The presence and size of ganglia found in the pericapsular tissue is apparently a species characteristic, since many workers have confirmed and others have failed to locate these ganglia in various mammalian species. MacFarland and Davenport ('41) studied the adrenals of many mammalian species, and found that large ganglia were present in the dog and cat; only small ganglia were observed adjacent to the human, monkey, and cow adrenals; and no ganglia could be found around the rat or mouse adrenal. The type of cell comprising the ganglia apparently is not identical in all species. To the best of the author's knowledge, binuclear ganglion cells have not been described in the pericapsular tissue of many mammals. Kuntz ('34), however, mentions that binuclear cells are often seen in the autonomic ganglia of young rodents. The significance of these interesting cells is not clear and has not been widely discussed.

Concerning the nerve trunks that penetrate the parenchyma of the gland (Fig. 16), nearly half of the fibers comprising these nerves are myelinated. Consequently, many of these myelinated fibers are easily traced to the binuclear ganglion cells embedded or intermingled among the chromaffin cells. This might indicate a possible synapse between preganglionic and postganglionic fibers, which might, in turn, innervate the chromaffin cells. The bulk of the experimental evidence-- Swinyard ('37), Hoshi ('27), Willard ('36), Hollinshead ('36), Young ('39), Bennett ('40), and Denber ('49) -- indicates that preganglionic sympathetic fibers innervate the chromaffin cells, and that both myelinated and nonmyelinated fibers innervate the vasculature. The prevailing concept is that only preganglionic fibers innervate the chromaffin cells, and such evidence cannot be disregarded from the current study.

CHAPTER V

SUMMARY

A. Observations

The results of the last ten or fifteen years' investigations and the current findings of the adrenal have shown that the histology of the adrenal gland varies considerably with physiological conditions, and one must therefore use great care in defining histological features as characteristic of a particular group of animals. Notwithstanding, some real variations do exist in the cellular and vascular patterns of families, genera, and species of any one mammalian order or group of closely related species.

In the native rabbit the adrenal is embedded in a loose network of areolar and fatty tissue connecting it to surrounding organs. Nerve trunks, ganglion cells, the main adrenal vessels and their branches ramify in this tissue as they approach the true capsule of the gland. At no time were capsulated or noncapsulated accessory bodies observed adjacent or near to the adrenal. Arteriovenous anastomoses were not evident in the periadrenal tissue of the native rabbits.

In Sylvilagus nuttallii a large ganglion containing large binuclear cells is consistently observed in close proximity to the adrenal capsule. Large nerve trunks penetrate the adrenal gland and can be traced from this ganglion to the medulla. Approximately 50 per cent of the fibers comprising the nerve trunks are myelinated. Within the medulla the nerve trunks ramify to form a plexus. Many nerve fibers intermingle among large binuclear ganglion cells within the medulla and among the chromaffin cells. Delicate nerve endings appear to be closely applied to the chromaffin cells. However, it is difficult to differentiate between fine nerve fibrils and delicate reticular connective tissue fibers. Myelinated and nonmyelinated nerve fibers appear to innervate the vasculature within the medulla.

Numerous branches from the main adrenal arteries ramify in the pericapsular tissue, pierce the capsule at many points, and subsequently break up into small arterioles and capillaries to form the subcapsular plexus. Small muscular arteries consistently pierce the superior or inferior pole of the adrenal. These vessels branch, and their smaller rami convert into a subcapsular plexus or ramify as capillaries in the zona glomerulosa. Small muscular arteriae medullae penetrate the substance of the gland and terminate near the cortico-medullary junction or within the medulla. All arteriae medullae appear to be end arteries, and only through the medullary capillaries are they interconnected.

A real variation exists in the vascular pattern of the arteriae medullae within the order Lagamorpha. In *Ochotona princeps* (family Ochotonidae) four or less small arteriae medullae penetrate the gland. The arterial supply to the medulla is limited and all chromaffin cells do not touch or lie adjacent to arterioles or capillaries. In *Lepus californicus*, *Lepus townsendii*, *Lepus americanus*, and *Sylvilagus nuttallii* (family Leporidae) ten or more large arteriae medullae penetrate the gland. In these species the medulla is adequately supplied with arterial blood, and most chromaffin cells appear to exhibit a true polarity in relation to the vasculature.

Most of the vascular channels within the zona fasciculata are tubular and form a radial pattern of parallel elongated tubes if this zone is sectioned transversely and vertically to the surface of the gland. These same tubular vessels sectioned transversely to their long axis appear as small thin-walled irregular sinusoids between the interstices of the turgid polyhedral cells. As these sinusoidal channels approach the medulla they acquire a larger girth. In the zona fasciculata these sinusoids are disposed as a spray with limited intercommunications. In the zona reticularis they sacculate and intercommunicate extensively. These same features characterize this region irrespective of the plane of section. These thin-walled sinusoids are continuous with the collecting radicles of the medulla.

The venous vessels of the native rabbit adrenal medulla are practically devoid of any muscular or fibrous coatings. Large chromaffin cells bulge into the lumen of these vessels, and in transverse section the lumen appears scalloped. The central vein has a very thin fibrous coating as it emerges from the hilus.

The organization of the cortical parenchyma underlying the capsule appears to be the most variable characterizing feature of the adrenal cortex. This zone is comprised of many intertwining, branching, and anastomosing platelike and/or cordlike cellular formations; these cellular integrants are not isolated or dependent from each other, but constitute integral components of a single cortical whole. These cellular formations are two cell layers in thickness in Ochotona princeps, Lepus townsendii, Lepus americanus, and Lepus californicus. In the two former species the formations convolute and branching formations are not difficult to discern. In Lepus americanus they are extremely branching and interconnecting, and consequently they are difficult to depict. The formations are confined, anastomotic, and branching in Lepus californicus, but they are not as massive as the formations seen in the foregoing species. In addition these cellular plates or cordlike formations may be formed by a double layer of prismatic or columnar cells wedged and tightly packed, with their nuclei arranged in double rows in the central axis of the plate or cordlike formations (Lepus townsendii). In Ochotona princeps, Lepus californicus and Lepus americanus the nucleus is generally located at the base of the cell near the periphery of the formation and adjacent to the vasculature. The tapering apices of the cells interlock in the central axis of the cellular cordlike and/or platelike formations. In Sylvilagus nuttallii this zone has a distinctive organization. The cellular formations anastomose, branch, loop, and intertwine extensively. This is especially noticeable in sections just underlying the capsule and cut parallel to the surface of the gland. However, in this species, a single elongated columnar cell spans the width of the cellular platelike formation. The cellular platelike formations are broad (30 to 35 micra) adjacent to the capsule and gradually taper as they approach the medulla. Hence, species within the genus Lepus possess two-cell-layer, cordlike formations and species of the genus Sylvilagus possess one-cell-layer, platelike formations. The latter cellular organization has a greater surface area exposed to the blood supply, and consequently may possess a significant physiological advantage.

In the native rabbit adrenal the zona fasciculata and zona reticularis constitute a single parenchymal whole continuous with the zona glomerulosa. From the inner margin of the surface, double-layered cordlike formations, single cell plates ramify and are inwardly directed so that in sections cut perpendicular to the surface of the gland the zona fasciculata presents a radial pattern of parallel cell columns. This precise cellular pattern constitutes the basic cellular organization in the zona fasciculata of all the lagamorphs under study. This cellular pattern has often been described in the past, and hitherto has been the only cellular arrangement discussed in the principal histology texts and in other studies of the adrenal histology. When this zone is viewed from a different perspective, in a plane where the sinusoids have been cut in transverse section, the cellular continuum presents a complex reticulated pattern of interconnected and branching cellular plates. In this perspective the cellular plates interconnect extensively, and the sinusoids merely permeate the interstices between the interconnected cellular plates. In such a cellular arrangement the sinusoids appear to separate the cortical cells, and, accordingly, the cells form walls around the individual sinusoids. Each sinusoid then appears to be surrounded by a wall of cortical cells which is common to two or more vessels. From this perspective the cellular organization of the zona fasciculata resembles the cellular pattern of a tubulosinusoidal liver (Elias, '48). Hitherto this particular cellular organization has not been thoroughly discussed or exemplified. Elias ('53) and Pauly and

Elias ('54), however, have published short abstracts indicating that the adrenal cortex of human beings is in effect a continuum.

In the zona reticularis the cellular plates buckle and twist. In good preparations the most conspicuous changes are a sacculation and increased girth of the sinusoids, and extensive interconnection of the cellular plates with a concomitant increase in the cross-communication between the sinusoids. In this cortical region adjacent to the medulla the vasculature appears to form a true plexus. The cellular plates decrease in dimension and the cells appear smaller. In many glands the distended and irregular vasculature within the cellular interspaces give this zone a cancellous or spongy appearance; accordingly, the parenchyma is disposed as cancellated cellular plates. Unlike the zona glomerulosa or zona fasciculata, these same features characterize this zone regardless of the plane in which it is sectioned.

The cellular organization and disposition of the vasculature in the zona fasciculata and zona reticularis appear to be very constant within this small group of closely related mammals. Individual variations within a species seem to be as pronounced as variations within families, or genera, of this mammalian order.

The medulla in all the native rabbits under consideration is comprised of anastomotic and branching cellular platelike or cordlike formations which constitute a single whole. However, the cellular organization and disposition of the cells are different in species of the families Ochotonidae and Leporidae. In Ochotona princeps (family Ochotonidae), where the cordlike formations are six or more cell layers thick, the polyhedral cells near the central axis of the cord do not appear to be directly in contact with the vasculature. The disposition of the peripheral cells adjacent to the collagenous sheath, which invests the cellular platelike formations, is remarkably consistent. As a result the cells exhibit a polarity irrespective of the structure they contact, i.e., cortical sinusoids, stroma, cortical tissue, or medullary veins and their tributaries. The central polyhedral cells are not endowed with a true polarity. In *Lepus californicus*, *Lepus townsendii*, *Lepus americanus*, and *Sylvilagus nuttallii* (family Leporidae) the cellular formations are comprised of only two or three cell layers; each medullary cell appears to touch or lie in close proximity to a vein or its tributary. The polarity of the cells in relation to the veins is quite apparent, but their relationship to the arteries and capillaries is not too evident. The disposition of the cells and vasculature in *Ochotona princeps* represents a real variation between species of the order Lagomorpha. In the native rabbits the vasculature does not provide the framework on which the chromaffin cells arrange themselves. The vasculature merely permeates the interspaces between the platelike and/or cordlike cellular formations.

B. Relationship to Other Studies

As a result of this investigation, and in view of other comparative studies, it is evident that the histology of the adrenal varies considerably with physiological conditions, and therefore one must use great care in defining histological features or peculiarities as unequivocal characteristics of a particular group of animals.

The hitherto undescribed adrenal histology of six native rabbits is discussed. This gland is remarkably consistent in its cellular organization. It is comprised of two individual cellular continua: the cortex and the medulla.

Cortical accessory bodies are not present in the periadrenal tissue. In other species this type of tissue has often been discussed.

Ganglia containing large binuclear cells are located in the periadrenal tissue. A difference in their size and number was noted between the various species, but apparently this is a common and variable occurrence among different mammalian orders (MacFarland and Davenport, '41). The nature of the binuclear cells comprising the ganglia in the periadrenal tissue appears to be peculiar to rodents and lagomorphs.

Arteriovenous anastomoses have been observed in other mammalian species (Bennett and Kilham, '40) and between the cortico-medullary circulation (Velican, '47), but in the native rabbit adrenal these structures were not discernible.

The native rabbit adrenal contains about four times more chromaffin tissue than does the domesticated rabbit adrenal. Kolmer ('18) reported the cortico-medullary ratio for the domesticated rabbit as 40:1; the ratio in the native rabbit adrenal is approximately 10:1.

One native species (Ochotona princeps) possesses a distinctive characterizing feature; the vascular pattern in this species resembles the vasculature of the rat adrenal (Harrison, '51; Lever, '52), and is divergent from the vascular pattern observed in the other lagomorphs which resembles the vasculature of the cat. The cellular organization and disposition of the cells within the medullary continuum of this species does not conform to the medullary pattern of the cat adrenal described by Bennett and Kilham ('40). In Ochotona princeps, every chromaffin cell is not endowed with a true polarity.

Bearing in mind that in this study only adrenals from adult and healthy native rabbits were examined, while in other studies different species of all ages were studied, the following comparative observations as they relate to other species may in part require emendations and additions.

In the native rabbits under consideration there is no positive proof to indicate that indifferent capsule cells differentiate into definitive cortical cells. Zwemer and coworkers ('38) indicate that this type of cellular differentiation takes place in other species. The isolated cellular groups of "foci" of cortical cells observed in the capsule, which theoretically become incorporated into the cortex (Zwemer, et al, '38; Gruenwald and Konikov, '44), merit further study. In native rabbit adrenals these cellular groups can be considered with just as much justification as cellular outgrowths and integral components of a single cortical continuum, for they are sharply demarked from the fibroblast and other connective tissue elements. There is no evidence of cellular differentiation, the cellular groups are comprised of cells exhibiting the same cellular characteristic as the cells of the zona glomerulosa, and the lipoid fluorescence in these two cellular groups appears to be identical but drastically different from the connective tissue element of the capsule.

The concept of mass cellular apposition postulated by Gruenwald and Konikov ('44), and observed in a number of adult mammalian species, does not appear to be valid in the native rabbit adrenal. Bearing in mind that the adrenal cortex is in effect a single whole, their interpretations are not entirely tenable or unequivocal.

CHAPTER VI

CONCLUSIONS

1. Variation in the adrenal histology of six species of native rabbits hitherto undescribed was studied.
2. Graphic and model reconstructions indicate that the cortical and medullary parenchyma each comprise independent epitheliod continua.
3. In *Ochotona princeps* (family Ochotonidae) *Lepus californicus*, *Lepus townsendii*, and *Lepus americanus* (family Leporidae) the outer cortical portion is comprised of interconnected convoluted folds and/or cellular cordlike formations which convert to a one-cell-thick laminar continuum that progressively interconnects as it converges toward the medulla.
4. Minor variations in the disposition of the cells within the cellular formation of the zona glomerulosa are present among the various species of native rabbits.
5. In *Sylvilagus nuttallii* (family Leporidae) a single, elongated columnar cell spans the width of the cellular platelike formations. In this cellular organization each pole of the cell is adjacent to the vasculature.
6. In all the native rabbits under consideration the zona fasciculata and the zona reticularis constitute a single parenchymal whole continuous with the zona glomerulosa.

7. From the inner margin of the convoluting cordlike formations, single cell plates ramify and are inwardly directed so that in sections cut perpendicular to the surface of the gland the zona fasciculata presents a radial pattern of parallel cellular columns or plates. In a plane of section where the sinusoids have been cut in transverse section, the cellular continuum presents a complex reticulated pattern of interconnected and branching cellular plates.
8. In the zona reticularis the cellular plates buckle, twist, and interconnect more extensively. With the increased interconnection of the cellular plates there is a concomitant increase in the cross-communication between the sinusoids. Unlike the zona glomerulosa or zona fasciculata, these same features characterize this zone regardless of the plane in which the gland is sectioned.
9. Although the different integral parts of the cortical parenchyma are referred to as cellular plates or sheets, they are not independent like sheets of paper, but are integrants of a single whole like the septa of a sponge.
10. The disposition of the cells within the medullary parenchyma is markedly different in species of the families Ochotonidae and Leporidae.
11. In <u>Ochotona princeps</u> the platelike and/or cordlike formations are massive and are comprised of 6- to 8-cell layers. All the chromaffin cells within the formations are not endowed with a vascular polarity.
12. In all species of the family Leporidae the cellular formations interconnect more extensively and are not nearly as massive. They

are comprised of two or less cell layers. Most chromaffin cells exhibit a true vascular polarity.

13. Differences in the cellular organization of the medulla in these species may possibly be correlated with the difference noted in the arteriole supply to the medulla. In Ochotona princeps four or less small arteriae medullae supply the chromaffin cells. In species of the family Leporidae ten or more large arteriae medulla penetrate the cortex and ramify at the cortico-medullary junction or within the medulla. Many thin-walled capillaries are interposed between the cellular formations.

14. All arteriae medullae appear to be endarteries, and only through the medullary capillaries are they interconnected.

15. The ratio of cortex to medulla in the native rabbits is approximately 10:1.

16. Follicle-like cellular structures were observed in the zona glomerulosa of all native rabbits under consideration.

17. There is no positive evidence to indicate that indifferent capsule cells differentiate into definitive cortical cells.

18. Isolated cortical cell groups within the capsule invariably turned out to be transverse sections of the surface cordlike cellular formations, and therefore integral components of a single cortical whole.

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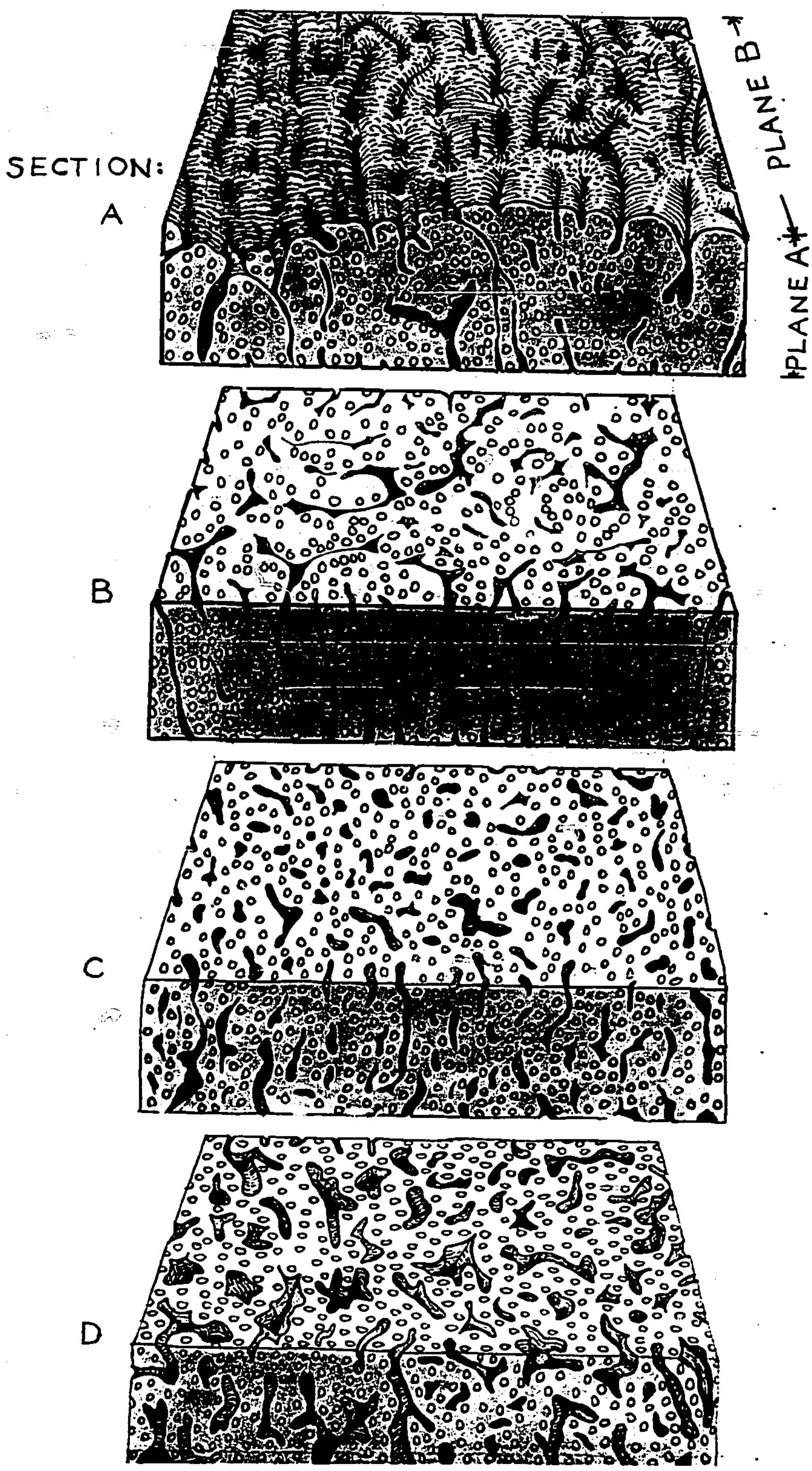
PLATE I

Graphic model of the adrenal cortex of *Ochotona princeps*. 250 X.

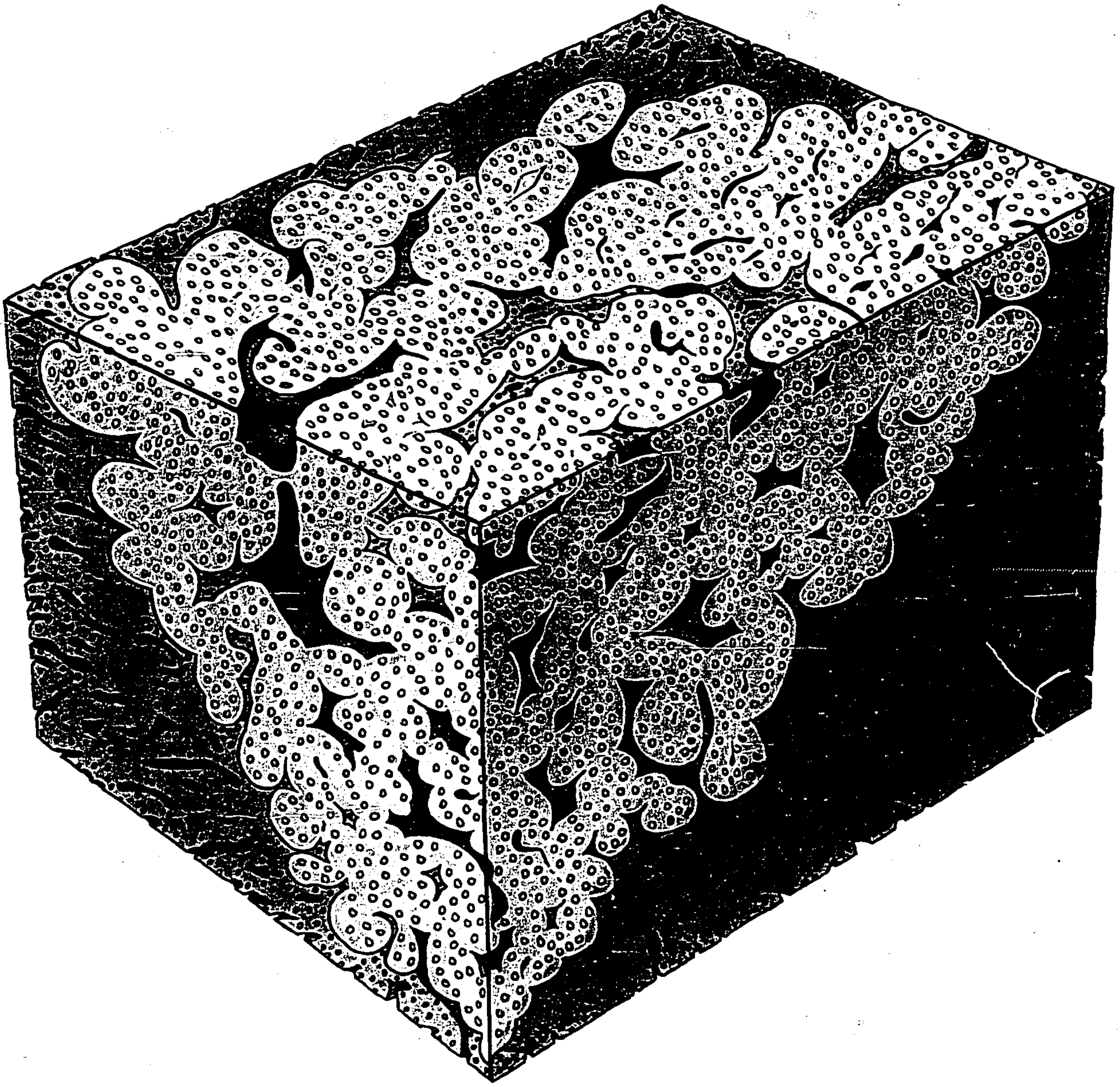


SECTION:
A
PLANE B
PLANE A
B
C
D

PLATE II

Graphic isometric model of the adrenal medulla of *Ochotona princeps*. 250 X.

PLATE II



ABBREVIATIONS

B. - Bouin's fixation
C. - Cortex
F. - 10% neutral formalin fixation
G. - Goldner modification of Masson's stain
H. - Hematoxylin-eosin-orange G stain
M. - Medulla
Mal. - Mallory connective tissue stain
Mas. - Masson trichrom stain
N. - Nerve
W. - Worchester fixation

EXPLANATION OF PLATES

All photomicrographs were taken with 35 mm. Exacta VX, plus X film. Magnification at film was 50; enlarged in printing to 150 X. All figures are unretouched photomicrographs of native rabbit adrenal gland sections.

PLATE III

Explanation of Figures

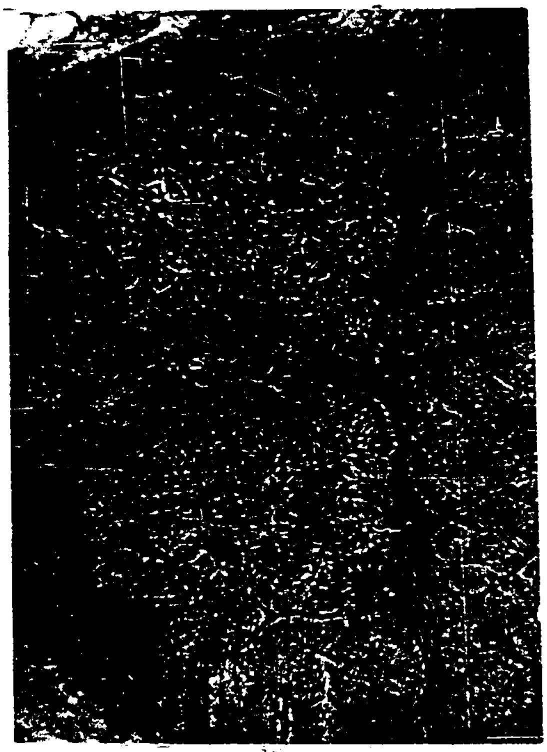
Figure 1. A tangential section through the zona glomerulosa of *Ochotona princeps*. Note the convoluting and branching cord-like cellular formations. W., Mal.

Figure 2. A transverse section through the zona fasciculata of *Ochotona princeps*. In this plane of section the sinusoids are cut transversely and present an over-all reticulated pattern. B., Mal.

Figure 3. Adrenal cortex of *Ochotona princeps*. The zona glomerulosa in left lower field appears as a homogeneous mass of cell. Note the "follicle" like structures in this zone. The zona fasciculata in upper right field is freely permeated by irregular sinusoids.

Figure 4. Adrenal medulla of *Ochotona princeps*. Note the massive cordlike cellular formation comprised of six or more cell layers and the polarity of the chromaffin cells in relation to the periphery of the cordlike formation. W., Mal.

PLATE III



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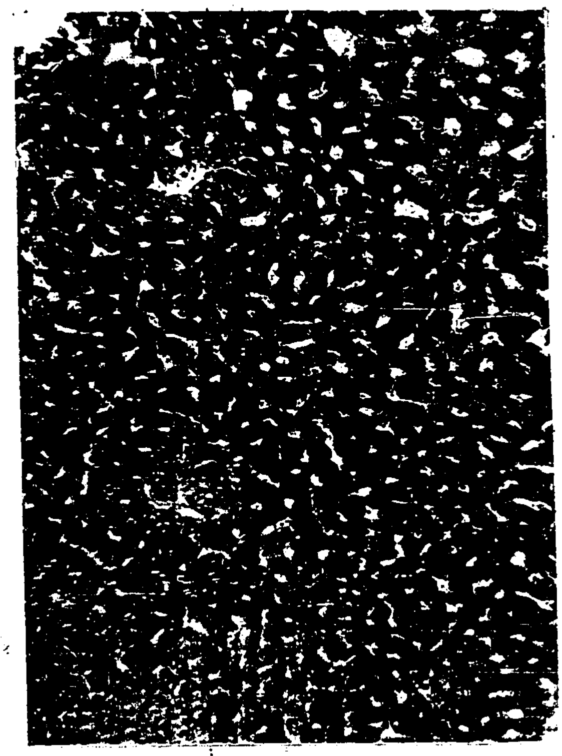

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PLATE IV

Explanation of Figures

Figure 5. Transverse section of a hyperemic adrenal of *Ochotona princeps*. The sinusoids of the zona reticularis are saccular and engorged. Notice that the polarity of the chromaffin cells is not altered. W., Mal.

Figure 6. Adrenal cortex of *Lepus californicus*. The outer portion of the capsule is dense; the inner component is more cellular. Note the "follicle" like structures at the junction of the zona glomerulosa and zona fasciculata. B., G.

Figure 7. Medulla of *Lepus californicus*. At the hilus of the adrenal the medulla is at the surface of the gland. The central vein emerges from the gland at this point. Generally, the cortex does not completely circumscribe the medulla. Observe the orientation of the chromaffin cells adjacent to the central vein. W. G.

Figure 8. Adrenal cortex of *Lepus californicus* in transverse section. Note the wide zona glomerulosa and the intricate branching and twisting cordlike cellular formations comprising this zone. B., Mal.

PLATE IV

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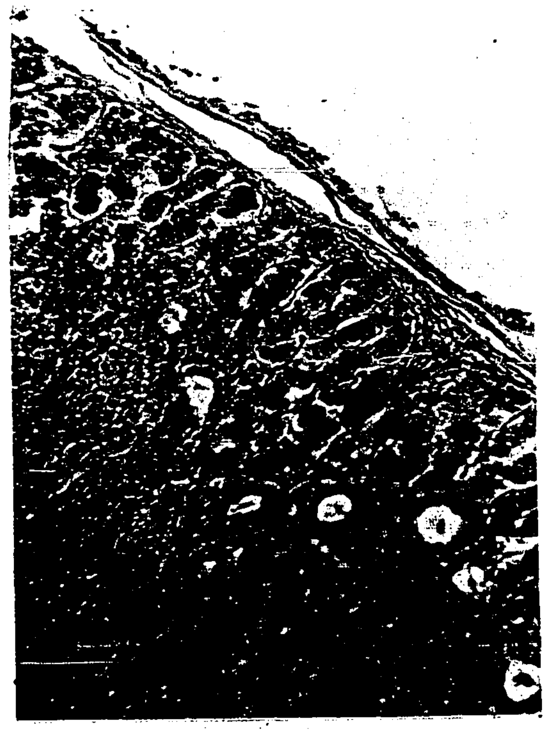

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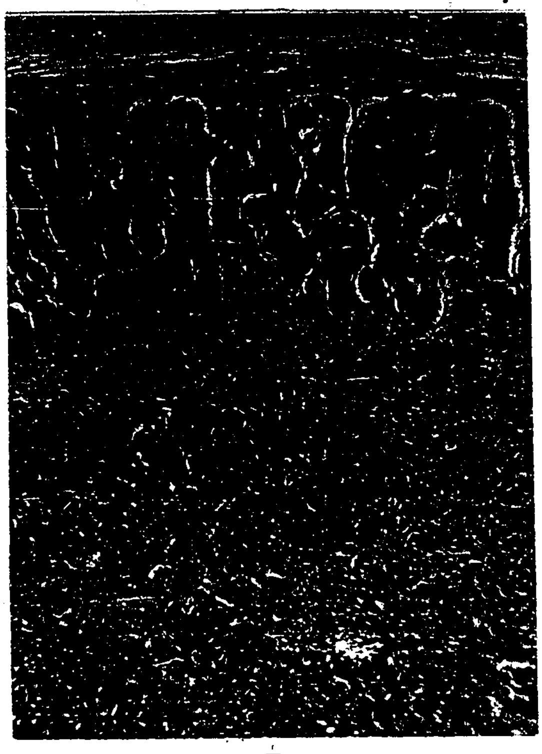

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PLATE V

Explanation of Figures

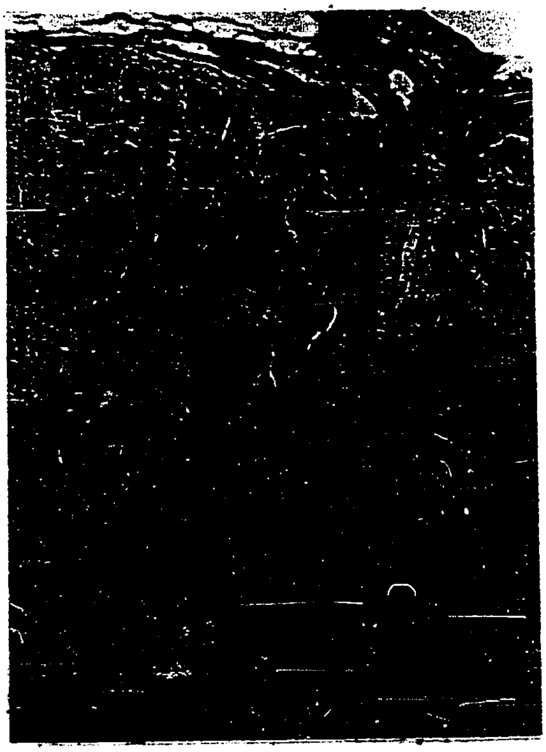
Figure 9. Adrenal cortex of *Lepus townsendii* in transverse section. Note the caliber of the arteriae medullae that penetrate the cortex. The sinusoids of the zona fasciculata in this plane of section appear long and tubular. B., Mal.

Figure 10. Tangential section of the zona fasciculata of *Lepus townsendii*. This zone has been sectioned in a plane parallel to the surface of the gland. The sinusoids permeating the zona fasciculata are cut in transverse section. W., H.

Figure 11. Tangential subcapsular section of the adrenal cortex. Note the nature of the intertwining and convoluting cellular formations comprising the zona glomerulosa in *Lepus townsendii*. W., Mas.

Figure 12. The cortex and medulla of *Ochotona princeps*. In the upper left field, binuclear ganglion cells are intermingled among the chromaffin cells. Notice the orientation of these cells relative to the venous channels. B., Mal.

PLATE V



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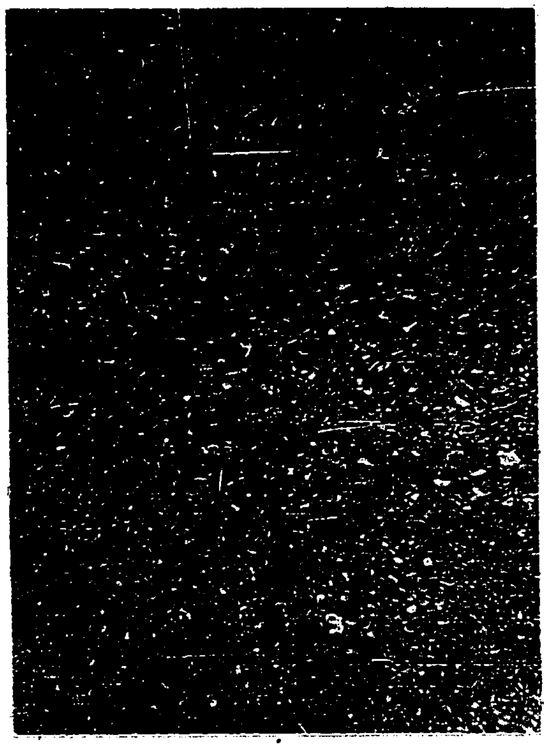

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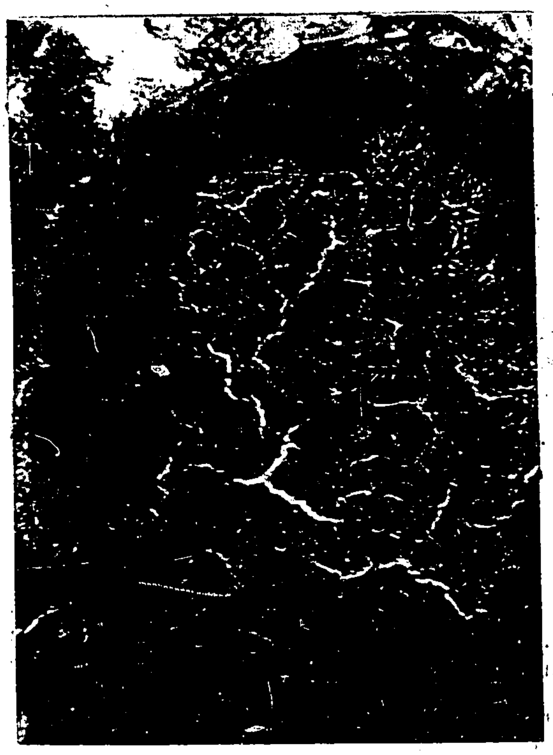

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PLATE VI

Explanation of Figures

Figure 13. Adrenal medulla of *Lepus townsendii*. Notice the one-cell-thick platelike and/or cordlike formations comprising the medulla and the saccular sinusoids of the zona reticularis. B., Mas.

Figure 14. Adrenal capsule and zona glomerulosa of *Lepus americanus*. Small cell foci or groups of cortical cells appear to be isolated within the fibrous meshwork of the capsule. B., Mal.

Figure 15. Adrenal cortex and medulla of *Sylvilagus nuttallii*. Notice the nerve (N.) in the upper central portion of the photomicrograph which has penetrated the cortex (C.). This nerve was traced to the medulla (M.) where it ramified extensively. B., Mal.

Figure 16. Large nerve trunks penetrate the adrenal cortex of *Sylvilagus nuttallii*. In this photomicrograph two of these nerve trunks can be depicted. Notice the single-cell layered cordlike formation of the zona glomerulosa. B., Mal.

PLATE VI

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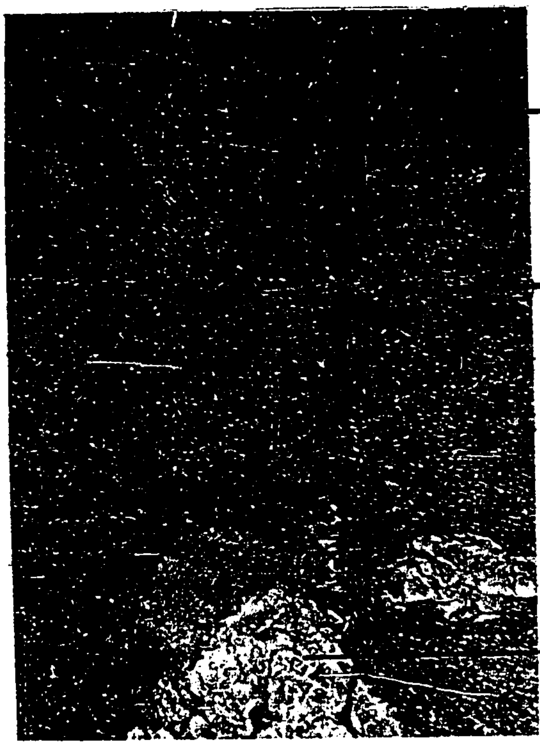

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PLATE VII

Explanation of Figures

Figure 17. Tangential section of the zona glomerulosa of Sylvilagus nuttallii. Notice the intricate manner in which the cordlike formations twist, branch and convolute. In this species a single cell spans the width of the cordlike formation. B., Mal.

Figure 18. This section of the adrenal cortex and the section observed in Figure 17 were taken from the same gland. This, however, is a transverse section of the gland. Notice that in this perspective the intertwining cordlike formations are cut transversely and that the "hairpin" loops are confined to the outermost portion of the cortex. The parenchymal epithelial plates of the zona glomerulosa extend centrally in unbroken continuity toward the medulla, and thus give parenchymal structures to the zona fascicularis which have a general radial arrangement. B., G.

Figure 19. Tangential section of the zona fasciculata of Sylvilagus nuttallii. The irregular sinusoids are sectioned transversely and permeate the interstices between the polyhedral cells. Notice that the parenchyma interconnects extensively; however, the sinusoids less frequently intercommunicate. B., Mal.

Figure 20. Adrenal medulla and cortex of Sylvilagus nuttallii. Observe the large size of the chromaffin cells. Around the larger veins and smaller vessels the cells are columnar in shape and the nucleus exhibits a distinct polarity. The large cells bulge into the thin-walled vessels which exhibit a scalloped lumen. The zona reticularis is often very difficult to depict. In this preparation only a narrow band of closely packed and dark staining cells circumscribe the medulla. B., Mal.

PLATE VII

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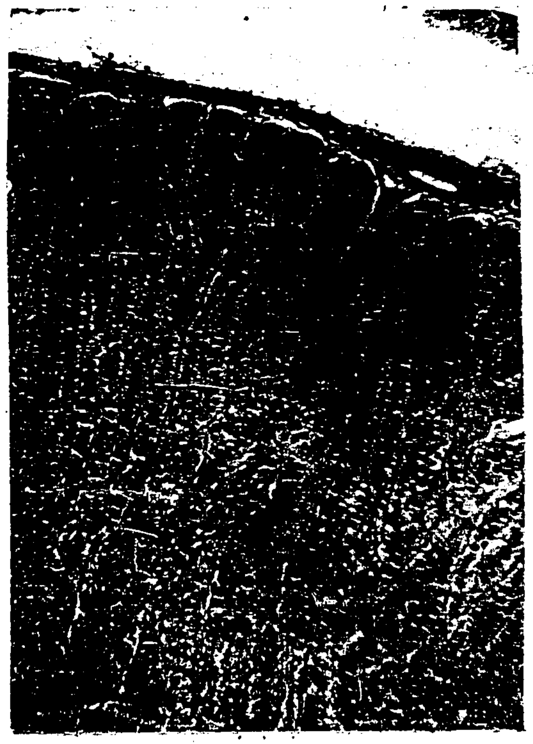

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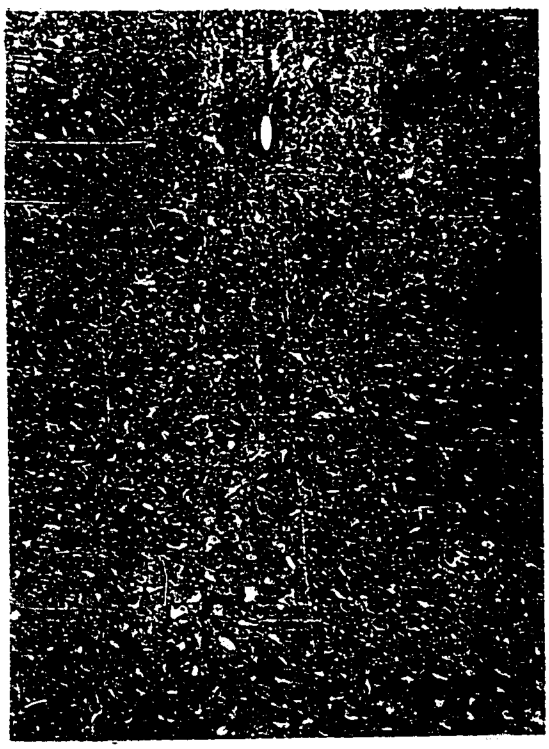

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