Ph. D. ANATOMY

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DISTRIBUTION OF CELL WEB-CONTAINING RETICULAR CELLS IN THE THYMUS AND SPLEEN OF THE RAT

"Cell-web"-containing reticular cells selectively stained by the tannic acid-phosphomolybdic acid-amido black (TPA) technique were identified and their distribution analyzed in the thymus and spleen.

In the cortex of the thymus, these cells formed a continuous layer on the inner surface of the capsule and around vessels, and constituted a loose network over the remaining of the cortex. In the medulla, two zones were distinguished: the "outer medulla" in which cell web-containing reticular cells were abundant and formed a dense network and the "inner medulla" in which no such cells were found.

In the spleen, cell web-containing reticular cells were abundant in the periarterial lymphatic sheaths and formed a distinct layer separating the white pulp from the marginal zone. They also formed a delicate layer separating the germinal center from the cortex of lymphatic nodules.

Thus, the thymus and spleen appeared to be compartmentalized by sheaths of TPA-stained reticular cells.

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by

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A thesis submitted to the Faculty of Graduate Studies and Research in partial fulfilment of the requirements for the degree of Doctor of Philosophy.

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April, 1968

A C K N O W L E D G E M E N T S

I wish to express my deepest thanks to Professor C.P. Leblond, Director of the Department of Anatomy, for the privilege of working in his Department.

My greatest debt is to Professor Y. Clermont, an accomplished teacher and faithful counselor, who guided my formation in the field of scientific research and directed this work with unfailing interest and patience.

I am very grateful to Miss R. Paradis for her skillful technical assistance in the preparation of histological material and to Dr. B. Kopriwa for the excellent radiautographs. I am indebted to Mrs. M. Oeltzschner for the quality of her drawings, and to Misses M. Paradis and N. Edson for the lay out of the plates.

Appreciation is acknowledged to Mr. T. Graham for the precision of his photographic work and for producing expert copies of the plates.

To my wife Céline who typed the manuscript, I wish to express all my gratitude for her patience and sustained encouragement.

This work was done with the financial assistance granted by the Banting Research Foundation and the National Cancer Institute of Canada.

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INTRODUCTION

PREFACE

Attempts to characterize the architecture of the thymus and spleen have been numerous. Questions concerning the open, the closed and combined concepts of splenic circulation were among the first ones to be asked and most investigators concurred that a knowledge of the architecture of the thymus and spleen was dependent upon an understanding of the distribution of blood vessels in them. Indeed, informative transillumination and electron microscopic studies showed that the structural organization of these lymphatic organs was dictated by their blood vessels. However, an understanding of the blood circulation through these organs did not suffice to elucidate all their structural peculiarities.

In recent years, several authors have justifiably focussed considerable attention upon structural characteristics of the thymus and spleen other than the circulation, such as the distribution of reticular fibers and reticular cells, in view of eventually understanding the functional implications of various parts of these organs. Many workers, using silver-impregnated sections prepared for light microscopy, have demonstrated that, while the thymus contains a relatively small amount of argyrophilic fibers closely associated with the blood

vessels, the supporting stroma of the spleen consists of a continuous meshwork of these fibers. This meshwork is connected to the vasal walls and apparently coincides with a meshwork of reticular cells to form a framework enmeshing lymphoid and other cellular elements.

Nevertheless, none of the research so far conducted at the light microscopic level on the distribution of argyrophilic fibers has provided an explanation for the zonation and the delineation of basic structural features of the thymus or the spleen. Owing to the inherent limitations of the light microscope and to the inability of the cytoplasm of reticular cells to stain with the dyes commonly used in histology, it has not even been possible to ascertain the relationships between the fibers and the reticular cells.

Recent electron microscopic studies have cleared up some of the points left unsolved by light microscopic investigations. They have determined more precisely the nature of the argyrophilic fibers, but have not added much to what was learned from light microscopic observations about their structural organization. Electron microscopic studies, moreover, have appraised more accurately, among other things, the connections between the fibers and the reticular cells.

More specifically, the electron microscope has clearly demonstrated that, in the thymus, the reticular cells of probably endodermal origin, often termed "reticular-epithelial cells", are generally not associated with argyrophilic fibers and are interconnected by their long cytoplasmic processes to form a continuous network enmes-

hing lymphoid elements in both the cortex and medulla of thymic lobules. In contradistinction, the reticular stroma of the spleen is composed of different types of reticular cells, most of which are interlocked and encompass the fibers of the reticular framework. It was also indicated that the fibers are not always connected with reticular cells, for there are areas in which the fibers are not wrapped in the cytoplasmic processes of these cells and are in contact at many points with blood or lymphoid elements.

Thus, it is evident, from these light and electron microscopic findings, that the structural peculiarities of the thymus and spleen cannot be fully understood by a knowledge of the distribution of blood vessels and argyrophilic fibers alone. Since reticular cells may be associated with neither blood vessels nor supporting fibers, a more precise appraisal of their distribution in the various regions and zones of these lymphatic organs may provide an answer to some architectural peculiarities. However, the electron microscope, which has extensively been used to observe details of these cells not visible in the light microscope, is admittedly a poor screening instrument owing to the limitations in sample size and does not permit one to have a panoramic view of their topographical arrangement throughout the argan. Therefore, despite the fact that the cytoplasm of reticular cells shows very little affinity for any stain and is indistinct and difficult to identify in ordinary histological preparations, the light microscope with its inherent limitation in resolution seems to be the instrument of choice.

The present work is a consideration of the topographical distribution of the reticular cells of the thymus and spleen of growing and adult rats, as it was revealed by the light microscopic study of serial sections of these organs in which the cytoplasm of most reticular cells was rendered clearly visible by a staining procedure, called the tannic acid-phosphomolybdic adie-amido black (TPA) technique. An accurate description of the spatial and temporal distribution of these cells led to a better understanding of some structural characteristics, such as the zonation of the organs, and therefore lay the morphological foundation for a study of specific functions of these intriguing organs.

REVIEW OF THE LITERATURE

No attempt will be made to review the literature exhaustively, and attention will be concentrated on the histological characteristics of the thymus and spleen which will be eventually related to the immediate object of the present research, i.e., the stellate "reticular" cells which with other connective tissue elements form the supporting framework of these two organs.

Morphological Features of the Thymus:

For a comprehensive description of the thymus, the classic references are those of Hammar (1921, 1936), Maximow (1932), Bargmann (1943) and Yoffee and collaborators (1956). This is an attempt

to present the state of our knowledge about this organ and in particular about reticular cells.

A) Histology.

(1) Topography

The thymus in the rat, as in most mammals, is situated in the anterior mediastinum of the thoracic cavity. In the fresh state, it is grayish pink in color and of soft consistency. It is roughly triangular in shape, with the base resting on the pericardium below; it is bounded posteriorly by the aortic arch and great vessels, and anteriorly by the sternum and its manubrium to which it is attached by delicate fibrous bridges containing fine blood channels.

The fully developed thymus consists of two lobes somewhat unequal in size and loosely joined along their medial border. Cranially, the conical, narrow, superior cervical portions of the thymus diverge from each other and extend a variable distance up into the neck on either side of the trachea.

(2) Architecture

According to the general description of the architecture of the rat thymus given by most authors, each thymic lobe is surrounded by a thin connective tissue capsule. It is divided into numerous lobules by connective tissue septa which extend into its parenchyma from the surrounding capsule. These interlobular septa, however, do not completely cut off the lobules. Thus, each lobule is a broad structure about 1mm. in diameter, which is incompletely delimited along its lateral margins by thin partitions of connective tissue

and whose parenchyma is in continuity with that of neighboring lobules.

In each of these lobules it is easy to recognize a peripheral region heavily infiltrated with lymphocytes, referred to as "cortex", and a central region with fewer lymphocytes, termed "medulla". The limit between these two regions, however, is not clear cut; Sainte-Marie and Leblond (1964a) observed no membrane or network of fibers between them. Thymic lobules may be so cut in sections that the medulla seems to exist in island formations surrounded by cortex. This appearance is, however, illusory for the medulla, since it is not completely severed by the interlobular septa, extends out into adjacent lobules. That is, each thymic lobe is composed of a single central medullary core which branches into many small lobules, so that the medulla is continuous in all parts of the lobe. Surrounding each distal arborization of the medulla is a cortical cap of lymphocytes which contracts in size and separates it from the connective tissue. The cortex is thus a continuous zone of cells surrounding the whole of the medullary tissue. According to Kindred (1942), in 80-day-old rats, the medulla accounts for 24% of the thymic parenchyma, and the cortex for the remaining 76%.

(3) Blood Vascularization

A review of injection studies of thymic circulation has been given by Bargmann (1943). Most authors have stated that the thymus has no hilum or main point of vascular attachment. According to Maximow and Bloom (1957), many small and inconstant arterial branches

of the internal mammary and inferior thyroid arteries pierced the capsule, branched in the interlobular septa, and penetrated the thymic cortex directly. Large venules arose in the medulla and combined into larger veins which emptied into the left innominate and thyroid vein. In the mouse, Smith et al. (1952) reported that the thymic lobes had a simple vascular pattern. The main thymic arteries ramified into cortical arterioles and capillaries. The capillaries were numerous and extended radially through the cortex and, at the cortico-medullary zone, drained into venules "in a pattern similar to a many-branched candelabra". The venules, in turn, joined into the veins of the septa. Similarly, in the rat, Sainte-Marie and Leblond (1964a) stated that the thymic cortex was characterized by few arterioles and capillaries, whereas many venules and capillaries were present in the medulla and at the cortico-medullary boundary.

Thus, for most workers, the pattern of arterial supply of the thymus is centripetal. Nevertheless, some authors still believe that the arterial pattern is centrifugal as in the spleen, lymph nodes, kidneys and brains. For example, quoting Weiss (1966a): "There are numerous arterial branches in the medulla. The blood supply of the cortex is derived from arterioles which run along the junction of cortex and medulla. From these arterioles, arterial capillaries run outward to the capsule. They return as venous capillaries to drain into venules running in company with the arterioles in the cortico-medullary junction. The venules drain into medullary veins, which

receive tributaries from the medulla as well."

It thus appears, from the above references, that the intrathymic blood vascularization is not fully understood and is still a controversial subject.

(4) Lymphatic pathways

Relatively few studies have been made of the lymphatic vascularization of the thymus. It is generally believed that the thymus, in contrast to lymph nodes, has no true afferent-efferent lymphatic vessels, but only small channels which run mainly in the interlobular septa and empty into the anterior mediastinal and tracheobronchial lymph nodes with apparently no direct connection to the thoracic duct or venous system (Maximow and Bloom, 1957; Weiss, 1966a). Sainte-Marie and Leblond (1964a) confirmed the existence of only rare dilated typical lymphatic vessels in the septa of the rat thymus.

However, Smith (1955), aside from these septal efferent channels, reported that she observed few sheath-like or tubular intrathymic lymphatic vessels in the medullary and cortico-medullary areas of the mouse thymus. These endothelium-lined vessels accompanied only the medullary veins and arteries to the hilum region and increased or decreased in diameter even outside the thymus. Moreover, in studies on the thymus of variously aged mice, Smith and Ireland (1941) described a double sheath of argyrophylic fibers, which they thought to be peculiar to the thymus, around the blood vessels of the thymic medulla. They also observed rows of lymphocytes in the spaces delimited by these two sheaths of fibers. In 8-9-week-old mice, they

noticed the double sheaths of fibers around both larger and smaller veins and, around the larger arteries, a third network of fibers which enclosed thymic cells. In still older animals, they observed three or more concentric layers of argyrophylic fibers around the medullary veins.

More recently, Sainte-Marie and Leblond (1958b, 1964a and 1964b) described a peculiar type of pathway consisting of spaces or channels sheathing venules and other blood vessels of the medulla and septa in the thymus of the rat. They referred to them as "perivascular lymphatic channels", and reported that a channel might not completely surround a blood vessel or that there might be two or more of them around a vessel. They described the outer wall of such channels as a thin, barely visible membrane, which stained with silver and PA-Schiff and which was at least partly endothelium-lined. Morecver, these authors observed figures of diapedesis of small lymphocytes in both the walls of the channels and those of the enclosed blood vessels. They thus concluded that "Lymphocytes which have entered the perivascular channels .... may reach the main circulation in 2 ways: 1) by diapedesis from these channels into the enclosed blood vessels and 2) by travelling along these channels to the main lymphatic circulation".

B) Morphogenesis and Histogenesis.

Before proceeding to a review of the cytological features of thymic reticular cells, it is desirable to report briefly on the

salient features of thymus morphogenesis and histogenesis relevant to this work. Thus, a discussion of the numerous classical studies concerning the embryology of the thymus by workers such as Beard (1902), Hammar (1905), Maximow (1909), Norris (1938), and others, important as they are to our present concepts, is beyond the scope of the current study and will not be attempted.

All embryologists are in agreement that the major portion of the thymus in mammals arises as paired hollow structures from the endodermal epithelium of the medial and ventral portion of the third branchial pouch in association with the parathyroid primordium. The thymic rudiment grows out first as a thick-walled cylinder, which elongates and thickens toward its free end and soon looses its lumen. Then, the thymus on each side migrates caudad and mediad and gradually becomes dissociated from the parathyroid primordium. The stalk connecting each developing thymic mass to the pharynx vanishes, leaving the proliferating terminal portion free in the presumptive mediastinal region. The two lobes of the thymus eventually come in contact with each other in the midline but never actually fuse (Hammar, 1909). Then, septa carrying invasive blood vessels lobulate the thymic lobes.

There has been much controversy concerning the histogenesis of the thymus, that is, the manner in which the embryonic epithelial thymic structure comes to assume its lymphoid appearance. Some workers, such as Norris (1938) and Downey (1948), followed the "substitution theory" developed largely through the studies of Hammar (1908)

and Maximow (1909) at a time when the doctrine of specificity of the germ layers was still prevalent. They contended that the reticular cells and Hassall's corpuscles were the only elements arisen from the original epithelium and that the small thymic cells were of mesodermal origin, having been derived from mesenchymal cells which had migrated into the epithelial rudiment of the thymus. Other investigators, such as Ackerman and Knouff (1959, 1964a,b,c, 1965), Auerbach (1960, 1961, 1964), Movat et al. (1963), Ruth (1961), Weakley et al. (1964), and others, following the "transformation theory" of Beard (1894, 1900), Bell (1906) and Stohr (1910), presented evidence that the thymic lymphocytes arose directly from the reticular epithelium.

Ontogenetically, the thymus is the first lymphocytic organ to develop in the embryo (Archer et al., 1964; Kelly, 1963). This undoubtedly argues for the local origin of lymphocytes appearing in the embryonic thymus. The controversy about the origin of thymic lymphocytes appears to have been settled by Auerbach (1961, 1964), who used tissue culture techniques and demonstrated that, when the 12-day-old embryonic thymus of the mouse was separated into its epithelial and mesenchymal components by the enzyme trypsin, within seven days the mesenchyme gave rise to the stromal elements of the thymus, while the epithelium was the main, if not the only, source of lymphocytes during embryogenesis of the thymus. Moreover, the author showed that thymic differentiation was the result of inductive tissue interaction between epithelium and mesenchyme.

More recent support for the epithelial origin of thymic lymphocytes is the work of Ackerman and Knouff (1965). These authors, in their study of the development of the thymus of the hamster, demonstrated that the endodermal-epithelial thymic primordium was separated from the mesenchyme by a well-developed, continuous basement membrane and that lymphocytes were observed first on the epithelial side. They distinguished two types of epithelial cells in the developing thymus: "undifferentiated epithelial" cells and "reticular-epithelial" cells. In early embryonic development, the "undifferentiated epithelial" cells were numerous and were characterized by a lightly basophilic cytoplasm; they contained no demonstrable glycogen. In contrast, the reticular-epithelial were filled with cytoplasmic glycogen particles.

According to the same authors, "the undifferentiated epithelial" cells underwent two distinct lines of differentiation during early development of the thymus, i.e., into lymphoblasts and into stellate reticular-epithelial cells which formed the organ parenchyma. They added: "If the reticular-epithelial cells forming the thymic parenchyma are involved in heteroplastic lymphocytopoiesis in the definitive thymus, the epithelial derivation of thymic lymphocytes is further indicated since these stellate cells also have been shown to be derived from the undifferentiated endodermal epithelial cells of the embryonic thymus". They believed that few, if any, "undifferentiated epithelial" cells remained in the thymus after birth.

However, the problem of the origin of reticular-epithelial

cells and their role in lymphocytopoiesis seems not to be solved to the satisfaction of all, for Smith (1965), in her reinvestigation of the origin of the lymphocytes in the embryonic mouse thymus, strongly challenged the view held by Ackerman (1961, 1964) and Ackerman and Knouff (1965). She wrote: "Observations made in our study of the embryonic thymus in situ suggest that the 12-and 12½-day-old thymuses which Auerbach used as explants already possessed lymphocytes which had migrated earlier from the mesenchyme into the epithelium". She also noticed the penetration of the endodermal epithelium of the third branchial pouch of ten-day-old embryos by basophilic lymphoblasts; these cells were reported to bridge the epitheliomesenchymal border.

Thus, although many observations suggest an epithelial origin for thymic lymphocytes, pure morphology has not succeeded in proving it to the satisfaction of all. More sophisticated techniques, such as high resolution radioautography, may provide a satisfactory answer to this problem.

In a recent electron microscopic study of the developing thymus in the hamster, Weakley et al. (1964) described reticular cells of three types which they designated A, B, and C and which were thought to differentiate from the original endodermal epithelium of the thymic primordium through transitional forms. Type A was described as a typical, mature reticular-epithelial cell with an abundance of tonofilaments and desmosomes, a light, homogeneous nucleus, and a prominent nucleolus; type B was equated with a phagocytic

reticular cells which might also be secretory, and type C with a cell indistinguishable from a lymphocyte except for the presence of desmosomes, a feature arguing for the epithelial origin of thymic lymphocytes.

In a comparative study of the ultrastructural characteristics of the perinatal and adult thymus of the guinea pig, Izard (1965b) observed two types of reticular cells: reticular-epithelial cells and mesenchymal reticular cells. According to this author, there were no fundamental differences in the ultrastructure of the perinatal and adult thymus of the guinea pig. The reticular-epithelial cells had essentially the same appearance in both age groups. They were stellate in shape and were characterized by a light nucleus with a prominent nucleolus and by intracytoplasmic tonofilaments in association with desmosomes. The cytoplasmic processes in the perinatal thymus, however, contained a few fat droplets and numerous glycogen particles. The mesenchymal reticular cells were much less numerous, phagocytic and similar in all respects to those in adult.

More recently, Sanel (1967) made a light and electron microscopic study of epithelial and lymphoid cell differentiation during thymus histogenesis in mice. The author reported, in the 12-day-old thymuses, traces of the primitive pharyngeal duct with epithelial cells carrying cilia as well as microvilli on their free surfaces and "linked to those adjacent by intermittent desmosomes and terminal bars which bind them at the lumen". He noticed that, at thirteen days, fine filaments appeared to attach the encircling processes of

the surrounding stromal epithelial cells to lymphoblasts. Though he noted desmosomes between abutting stromal epithelial cells, he did not observe, unlike findings reported by Weakley et al. (1964), junctional specializations between lymphoid and epithelial cells. According to his observations, by fifteen days formation of a network of reticular cells began; the stromal epithelial cells were linked by desmosomes and formed a reticulum within the meshes of which lymphoid cells divided and matured; thus, at this stage, two types of epithelial cells were recognizable: reticulo-epithelial cells and unchanged primitive stromal epithelial cells of the thymic anlage.

In the final quarter of embryonic development, the same author recognized three species of epithelial cells: 1) columnar or conical stromal epithelial cells in acinar configuration and linked by junctional complexes at the luminal surface; 2) cortical reticular-epithelial cells linked by desmosomes and containing some short filaments; and 3) hypertrophied epithelial cells appearing in the medulla at eighteen days and containing bundles of fine filaments "haphazardly distributed either in paranuclear areas or at the cell periphery". As he noted transitional cell forms with some lymphoid characteristics, he suggested that earliest thymic lymphocytes might originate by transformation of the epithelial elements of the thymic anlage.

It was generally considered, in accordance with the hypothesis of Hammar (1905) which was later confirmed by Maximow (1909),

that the thymic medulla arose toward the end of the embryogenesis of the thymus. It was believed that, following the migration of lymphocytes from the deeper portions of the lobules toward their periphery, the reticular cells left behind in the deeper portions of the lobules hypertrophied and stained more lightly than those found in early embryonic development, thus forming an early medulla. The peripheral portions of the lobules into which lymphocytes had migrated therefore became the thymic cortex.

However, in a recent paper, Kostowiecki (1967) challenged this view. He reported that the early medulla arose in the adjacent mesenchymal septum of the developing thymus. According to this author, the first stage of medulla formation was a desquamation of epithelial cells from the thymic epithelial mass into an adjacent mesenchymal septum. This was followed by a migration of lymphocytes from this area toward the periphery of the developing thymus. The first or early medulla would thus be a mixture of epithelial and mesenchymal cells in which lymphocytes were absent. At slightly more advanced stages, the epithelial cells of the newly formed medulla would hypertrophy and then become transformed into reticulated cells as a result of a second invasion of lymphocytes. The same author stated that the cortex was, in contrast, exclusively of epithelial origin, developing directly from the epithelial cells of the thymic lobe. The cortical epithelial cells would later form a reticular network after giving rise locally to lymphocytes.

Lastly, investigators are accumulating structural and functional evidence that some reticular-epithelial cells of the embryonic thymus elaborate and secrete a hormonal substance. Ackerman and Knouff (1965) noticed the close association of the reticular-epithelial cells of the developing medulla with the vascular channels and their elaboration of cytoplasmic deposits of glycoprotein during late embryonic development of the hamster. They suggested that perhaps these medullary cells were involved in the elaboration and secretion of some substance, probably a carbohydrate-protein substance. They also felt that it was warranted to correlate the embryonic appearance of these cells and their cytoplasmic material with "the degree of lymphocyte-stimulatory activity of the thymus upon other lymphocytic tissues".

Similarly, Smith (1965) described two types of reticular cells in the developing medulla of the mouse thymus: esterase-rich inclusion cells with coarse granules embedded in a highly reactive cytoplasmic ground substance and reticular-epithelial cells occurring singly or as nests of cells, rich in esterase activity and containing fine cytoplasmic granules. She suggested that these cells might be secretory and contain the precursors of the humoral factor(s) of the thymus.

More recently, Clark (1966) presented cytological and histochemical evidence indicating that medullary reticular-epithelial cells of the prenatal thymus of the mouse manufacture and secrete a sulphated acid mucopolysaccharide. His examination of the thymus in fetal

and suckling mice indicated that "secretory activity may develop late in fetal life, accelerate after birth and moderate about two weeks after birth".

C) Cytology of Reticular Cells.

1) Light microscopic studies

It is generally agreed that the thymic parenchyma, in addition to lymphocytes, consists of two types of reticular cells: numerous reticular-epithelial cells and few mesenchymal reticular cells. By light microscopy it is usually very difficult to differentiate between reticular cells of endodermal-epithelial derivation and those of mesenchymal origin. Nevertheless, Downey (1948), in studies on the rabbit thymus, listed some morphological characteristics which, in well-fixed material, permitted a distinction between these two types of reticular cells. According to Downey (1948), the reticular-epithelial cell, in section, presented a round, oval, or grooved nucleus with a sharply defined spherical nucleolus, a thin nuclear membrane, and few scattered, small chromatin granules; its cytoplasm showed very little affinity for any stain and sometimes contained many small vacuoles. The author found the mesenchymal reticular cells to be identical to those of the lymph node. Their nuclei were more chromatic and had a heavier nuclear membrane and coarser chromatin particles than those of reticular-epithelial cells; their cytoplasmic processes occasionally contained vacuoles or phagocytosed nuclear debris.

However, Sainte-Marie and Leblond (1958a, 1958b, 1964a, 1964b),

in their investigation of the rat thymus, could not safely differentiate between the thymic reticular cells of endodermal-epithelial origin and those of mesenchymal nature. They stated that nearly all reticular cells in the thymic cortex and medulla seemed to belong to a single population of presumably epithelial cells, though they observed transitional cell forms exhibiting features intermediate between those of reticular cells and large lymphocytes. According to these investigators, the reticular cell of the thymic cortex was often in mitosis and had a distinct nucleus surrounded by a pale, indistinct cytoplasm. The round or oval nucleus appeared very light and was characterized by a loose network of fine chromatin threads and granules. The nuclear membrane was associated with fine chromatin dots. There was usually one, and sometimes 2-4 large oval nucleoli, "the center staining orange-red, the surface light blue" with the Dominici staining technique.

The above investigators also reported that a few reticular cells were phagocytic and contained inclusions usually in the form of nuclear debris. Although they did not attempt to classify reticular cells into distinct types, they mentioned that the reticular cells along the capsule and septa "had a pale nucleus whose shape varied from flat to hemispherical". In the medulla, however, according to the same authors, the reticular cells were similar to those of the cortex, but their cytoplasm was "more abundant and more clearly outlined", and their nuclei "less regular and often larger". Like in the cortex, they observed mitoses of reticular cells. They finally

reported that, unlike cortical reticular cells, those of the medulla frequently showed signs of nuclear and cytoplasmic degeneration, and that these medullary cells "seemed to fuse into a syncytium" and converged into Hassall's corpuscles.

Moreover, in the aging thymus of the mouse, Loewenthal and Smith (1952) gave a detailed description of the histochemical reactions of the "lipid-laden foamy cells" or "chromolipoid cells", often present in considerable numbers in the cortex. They believed them to be an expression of altered thymic metabolism accompanying thymic involution, because they were absent in the young thymus and increased in number with age. These cells were large, polymorphic, or rounded, and contained one or more usually eccentric nuclei. Their cytoplasm was filled with PAS-positive granules which were also stained with Sudan dyes and emitted a bright yellow autofluorescence in unstained paraffin or frozen sections. These chromolipoid cells, according to these authors, corresponded in size, location, and staining qualities to similar cells described by Hammar (1921) in the thymic cortex of aging humans.

More recently, Metcalf and Ishidate (1961, 1962) and Metcalf (1964) made a study of size, frequency and histochemical reactions of the chromolipoid cells in the thymus cortex of high-and low-leukemic strain mice. These authors reported that the nuclei of these cells resembled thymic reticular-epithelial cell nuclei rather than those of the lymphocyte series, and that their cytoplasm was voluminous and packed with PA-Schiff positive granules, hence the terms

"PAS-positive giant cells" or "PAS-positive reticulum cells" or "PAS cells" used to designate them. They also stated that these "cells appeared capable of phagocytosis, since they frequently contained pyknotic nuclear material, presumably lymphocytic in origin". According to these investigators, the PAS cells were usually subcapsular but were frequently randomly distributed throughout the cortex. They found more such cells in the high-leukemic thymic cortex than in the low-leukemic one. They suggested that, since the lymphocytes surrounding these PAS cells were more frequently in mitosis than lymphocytes located elsewhere in the thymus and since the PAS cells and associated mitoses occurred only in post-involutional thymus, they might be involved in the regulation of lymphocyte mitotic activity in the mouse thymus cortex.

In a recent study of the morphology of the preleukemic thymuses, Siegler (1964) offered similar observations concerning the PAS cells, which he thought to be reticular-epithelial cells. He speculated, after Toro (1961) and Lattes (1962), that these cortical cells were related to medullary reticular-epithelial cells which were postulated to form the Hassall's corpuscles. He further suggested the possibility that the cortical PAS cells, after ingesting lymphocyte nuclear fragments during lympholysis, would normally "enzymatically degrade these fragments, release the soluble components, and retain indigestible materials as liphchrome pigment". According to this author, when these cells contained significant amounts of this pigment, they migrated to the Hassall's corpuscles of the medulla, entered into

the central cavity of these bodies by the process of intrusion, and there their residual material was more completely autolyzed.

However, Sainte-Marie (1965) challenged some of the views held by the above workers concerning the "PAS cells". By means of fluorescent and bright field microscopy, he identified and studied the peculiar distribution of the "autofluorescent cells" of the lymphocytic tissues of the rat. The author revealed that these "autofluorescent cells" corresponded to the "lipid-laden foamy cells" described by Loewenthal and Smith (1952) and to the "PAS-positive reticulum cells" of Metcalf and Ishidate (1962). Unlike other investigators, he found these cells to be numerous at the cortico-medullary junction of each thymic lobule, scarce in the "perivascular channels" and rare in the cortex and medulla. Owing to their distribution and to their presence much before the onset of thymic involution, the author, unlike Loewenthal and Smith (1952), concluded that these cells were not specific thymic cells and were not arising from thymic involution. He finally suggested that these "autofluorescent cells" somewhow paralleled the development of postweaning growth and of immunity.

It appears, therefore, that only one type of reticular cells can be safely identified by light microscopy, presumably those of endodermal-epithelial origin which are known to degenerate in large numbers in the thymic medulla and to converge into nearby Hassall's corpuscles.

2) Electron microscopic studies

In their electron microscopic studies of the mouse thymus, Clark (1963) and Hoshino (1963) described two main types of reticular cells: reticular-epithelial cells and macrophages or mesenchymal reticular cells. According to these authors, the parenchyma of the thymus, in both cortex and medulla, was formed by a continuous meshwork of reticular-epithelial cells clearly distinguishable by characteristic cytological features from the macrophages or mesenchymal reticular cells that comprised the other type of large cell in the thymus. Numerous lymphocytes and relatively few mesenchymal reticular cells occupied the interstices of this epithelial reticulum. Around the periphery of each thymic lobule and around the intrathymic blood vessels, the reticular-epithelial cells formed a continuous sheet or barrier separated from the connective tissue by a basement membrane; they also completely enclosed the reticular fibers that pierced the thymic parenchyma.

According to the same authors, the reticular-epithelial cells of the cortex were sparse, thin and stellate; their slender cytoplasmic processes were distinguished by occasional bundles of fine tonofilaments and by desmosomes that connected adjacent cells in a continuous epithelium; the tonofilaments, however, were not always associated with desmosomes and tended to concentrate around the nucleus and at the periphery of the cell. These investigators also reported that, although the cortical reticular-epithelial cells contained a few lipoid inclusions, they did not appear to be phagocytic.

In the thymic medulla, Clark (1963) found the reticular-epithelial cells to be "numerous, voluminous, and arranged in clumps, solid cords, or nests resembling acini"; he observed no Hassall's corpuscles in the mouse thymus, but described two types of cytoplasmic inclusions, which might be considered secretory, in some medullary reticular-epithelial cells. Hoshino (1963), however, recognized two types of reticular-epithelial cells in the medulla of the mouse thymus: "reticular" and "hypertrophic" types. According to this worker, the cells of the reticular type were stellate in shape and often contained considerable amounts of tonofilaments in their cytoplasmic processes. Those of the hypertrophic type were large and round and seldom contained tonofilaments; they often underwent degeneration and were related to the formation of Hassall's corpuscles.

Moreover, Clark (1963, 1964b) reported that the perivascular spaces, referred to as "perivascular lymphatic channels" by Sainte-Marie and Leblond 1958b, 1964a, 1964b), contained collagenous fibers and no lymphatic endothelium, and appeared to be "limited only by the basement membranes of vascular endothelium and thymic epithelium".

Weiss (1963), Cowan and Sorenson (1964), and Blackburn and Miller (1967) have presented no discrepancies in their studies of the ultrastructure of the normal mouse thymus with the above-mentioned findings. Similarly, Izard (1966a) described two categories of reticular cells in the reticulum of the guinea pig thymus: numerous "desmosomal reticular cells" corresponding to the reticular-epithelial cells, and occasional "phagocytic reticular cells" with neither

desmosomes nor tonofilaments and corresponding to the mesenchymal reticular cells as proposed by Hoshino (1963). However, this author in a subsequent publication (1966b), unlike Clark (1963, 1964b) who did not recognize transitional cell forms between epithelial cells and lymphocytes or macrophages, reported the presence of desmosomes between medullary reticular-epithelial cells of the adult guinea pig thymus and lymphoid elements apparently corresponding to large lymphocytes. He suggested, after Sainte-Marie and Leblond (1964a, 1964b), that thymic reticular-epithelial cells might give rise to lymphocytes.

3) Nomenclature

As indicated in the above review of the cytology of thymic reticular cells, all investigators who used the light microscope were unable to define specifically whether or not a reticular cell in the thymus was of epithelial or mesenchymal nature, as can be done by electron microscopy. Some of them, therefore, have objected to the use of the term "epithelial" to refer to the great majority of thymic reticular cells. They consider the term "reticular-epithelial" to be confusing and to be justifiable only insofar as it openly conveys the difficulty, if not the impossibility, of distinguishing between reticular cells of endodermal-epithelial origin and those of mesenchymal nature.

Nevertheless, most authors (Clark, 1963, 1964b; Hoshino, 1963; Sainte-Marie and Leblond, 1964a; Weiss, 1963; Kohnen and Weiss, 1964; Izard, 1966a, 1966b; Cowan and Sorenson, 1964; Dukor et al., 1965; Blackburn and Miller, 1967; Sanel, 1967; and many others) are in favor

of the term "reticular-epithelial cells" to designate most stellate thymic cells for the following reasons: First, reticular-epithelial cells are believed to originate from the endoderm of the third pharyngeal pouch. Secondly, though they are ultrastructurally similar to macrophages or mesenchymal reticular cells, they are characteristically different since they do not appear to be phagocytic and display conspicuous epithelial characteristics, such as tonofilaments and desmosomes. Thirdly, wherever they border on connective tissue, there is a basement membrane. Fourthly and finally, they are judged to be "fixed" elements in contrast to mobile cells such as lymphocytes or macrophages, because through their desmosomal attachments they form a continuous epithelium.

D) Hassall's Corpuscles.

Considerable controversy has involved the nature and origin of Hassall's corpuscles, characteristic transitory structures of the thymus unique to its medulla. Hammar (1905, 1909) described Hassall's bodies as consisting of a core surrounded by a wall. According to this author, while the core was constituted by one and occasionally two enlarged reticular cells, the wall, in section, consisted of concentric layers of hypertrophied reticular cells, giving the corpuscles the appearance of an onion. Later, Hammar (1921, 1936) interpreted these structures as organizations derived from the endoderm and developed initially from one or two hypertrophied reticular cells which eventually impiged upon and fused with neighboring reticular cells of the thymic medulla. Dearth (1928) proposed an essentially similar theory, which was also favored by Sainte-Marie and Leblond (1958b, 1964a).

The view that Hassall's corpuscles represented obliterated blood capillaries and precapillary arterioles which had undergone endothelial hypertrophy and proliferation with peripheral addition of reticular cells was proposed by Jordan and Horsley (1927). However, Jordan and Looper (1928) conceded that the essential morphological factor in the formation of these corpuscles was the hypertrophy of reticular cells which might secondarily cause stenosis of adjacent blood vessels. Pertinent to this point of view are the observations of Kostowiecki (1930, 1938). This author believed that Hassall's corpuscles, to which he gave the name "Hassall's corpuscles of the second order", might develop concentrically around hypertrophied reticular cells and degenerating intramedullary capillaries and venules (1930) and around degenerating nerve fibers (1938). Kostowiecki (1962) also described thin-walled corpuscles characterized by invasion of lymphocytes and reticular macrophages and subsequently by the development of cavities containing cells and debris. In a recent study of human corpuscles, Kostowiecki (1964) observed that the primitive core of the corpuscle was usually a reticular macrophage, but that "the medium lymphocyte, the plasma cell, or even the basophile polymorphonuclear leukocyte" might occasionally provide a center for the developing corpuscle. Jaroslow (1967) has recently presented evidence favoring the theory of the vascular origin of these corpuscles.

According to Norris (1938), Hassall's corpuscles might develop from migration and proliferation of ectodermal remnants of the

cervical sinus associated with the third branchial pouch.

Nevertheless, the view to which most investigators subscribe is that stated by Kingsbury (1928). This author supported the idea that Hassall's corpuscles were epithelial in nature, that they developed from hypertrophied reticular-epithelial cells, but that their typical whorled configuration was a structural adaptation by reticular-epithelial cells which had grown in a confined area without available free surfaces and which could undergo necrosis and keratinization during their life history. Gilles (1944) and Smith and Parkhurst (1949) found a histological and histochemical resemblance between the concentric layers of reticular-epithelial cells forming the walls of Hassall's corpuscles and the strata of thick epidermis. This was also noticed by Dearth (1928).

Recent electron microscopic studies of thymic corpuscles (Palumbi and Millonig, 1960; Koka, 1960; Hoshino, 1963; and particularly Kohnen and Weiss, 1964, and Izard, 1965c, 1966a) appear to favor Kingsbury's theory. They revealed that the formation of Hassall's corpuscles in mouse, guinea pig, and cat thymuses was related to the transformation of medullary reticular-epithelial cells. These studies also showed that some of these cells underwent pyknosis and keratinization as the superficial cells of the epidermis by increase of the amount of intracytoplasmic tonofilaments attached to desmosomes and formation of dense bodies resembling keratohyalin granules. In addition, Izard (1965c) reported, that some Hassall's corpuscles contained fibroblasts and reticular fibers, which a delicate basement membrane

separated from the reticular-epithelial cells.

Little is known of the functional significance of Hassall's corpuscles, those transient, polymorphous structures which may consist of single hypertrophic reticular-epithelial cells or groups of these cells concentrically arranged about a central core of nuclear debris. They display considerable morphological variability: they are infrequent and small in mouse and rat thymuses (Harland, 1940; Clark, 1963), but they are numerous and large in guinea pigs and humans (Kohnen and Weiss, 1964; Izard, 1965c, 1966a; Kostowiecki, 1962). According to Flaum (1963), who observed these corpuscles in tissue culture, they are pulsatile and are capable of rotatory movements. Under certain experimental conditions, they can concentrate gamma globulin (Marshall and White, 1961; Gitlin et al., 1953).

E) Cysts and Duct-like Spaces.

Several types of cystic and duct-like spaces have been observed by many workers. Hammar (1921) reported the common presence of cysts in the centers of degenerating Hassall's corpuscles. Dearth (1928) described intercommunicating tubular cyst-like spaces produced by central degeneration in compact masses of reticular cells and by aggregation of Hassall's corpuscles. Spear (1938) observed two main types of thymic cysts: one type represented local degeneration of reticular cells, and the other, remnants of the thymopharyngeal duct. Subsequently, several investigators, such as Tesseraux (1959), observed in the thymus of most mammals occasional branching cysts lined by flat, cuboidal, columnar, mucus-secreting, or ciliated cells.

They varied considerably in number in different thymuses; they might be localized in the interlobular septa but were most frequently seen within the thymic lobule completely surrounded by thymic parenchyma. They ended blindly and might appear to be continuous with the thymic reticular-epithelial cells. Most authors believed that they arose from the failure of the primary thymic tubule to lose its lumen of from remains of tubules secondarily differentiated in the thymic epithelium as a result of some stimulus causing an increased rate of thymus growth during early embryonic development. Schambacher (1903) and Shier (1963) proposed that Hassall's corpuscles might develop from the epithelial lining of these tubular cysts.

In 1962 and 1963, Hoshino, in his electron microscopic study of the mouse thymus, reported the presence of obvious cytoplasmic inclusions in the cytoplasm of hypertrophic medullary epithelial cells. According to this author, these inclusions formed large collections of amorphous material, which stained with the PA-Schiff technique for polysaccharides. Such collections occurred both intracellularly and in extracellular spaces that might be lined by microvilli and cilia. In 1964, Kohnen and Weiss made similar observations and referred to these structures as "intracellular cysts" in contrast to "intercellular cysts", both of which were seen near Hassall's corpuscles. Thus, in addition to frankly epithelial cysts bounded by ciliated epithelium and probably representing remnants of the branchial duct, these authors showed two other types of cysts in the thymic medulla, namely "intracellular"and "intercellular" cysts.

Morphological Features of the Spleen:

The Roman anatomist, Galen, who regarded the thymus as a cushion serving to protect the great vessels of the thoracic cavity against the bony sternum, spoke of the spleen as an organ "full of mystery". The following report is a brief review of recent advances in the knowledge of the microscopic anatomy of this intriguing organ, based to a great extent on the comprehensive review by Weiss (1966b).

A) Histology.

1) Architecture

It is well established that there is considerable age and species variation in the architecture of the spleen, Krumbhaar (1926) stated: "Investigators of the relation of the spleen to various functions in animals should therefore take into account the age as well as the species of their experimental animals."

Textbooks of general histology uniformly agree to the statement that the spleen of the rat is a slightly curved, ribbonlike organ resting upon the greater curvature of the stomach and enclosed by a dense connective tissue capsule. The latter contains little smooth muscle fibers and numerous elastic fibers. Its outer surface is covered by a thin peritoneal coating of flattened to cuboidal mesothelial cells, which may possess microvilli forming a striated border. The splenic capsule is deeply indented at the points of entrance of each of the two or three main branches of the splenic artery.

From the inner surface of the capsule, trabeculae extend into the organ and subdivide it into intercommunicating compartments or "splenic lobules", which are ill-defined and rather arbitrary in extent. These trabeculae are composed of relatively more muscle and elastic fibers than the capsule.

The space between the capsule and trabeculae is filled with a reticular connective tissue consisting of a network of reticular fibers closely joined to a network of primitive reticular cells and phagocytic reticular cells or fixed macrophages. This reticular framework forms, together with the cells occupying its meshes, the "splenic stroma", or "splenic tissue", or again "splenic pulp" (Weiss, 1959, 1964, 1966b; Bloom and Fawcett, 1968). The splenic tissue is composed mostly of an atypical lymphatic tissue, referred to as the "red pulp", in which are scattered diffuse and nodular masses of typical lymphatic tissue, collectively designated as the "white pulp". Lying between the white pulp and the red pulp is a band of tissue looser than the white pulp and containing blood elements, termed the "marginal zone" (Weiss, 1966b; Bloom and Fawcett, 1968).

The white pulp forms a well-developed cylindrical sheath about the central artery and its terminal branches. This sheath is referred to as the "periarterial lymphatic sheath", a term more and more frequently used in current literature (Klemperer, 1938; Weiss, 1964, 1966b). Implanted on the periarterial lymphatic sheath are occasional spherical or ovoid, nodular structures, commonly designated as secondary

"lymphatic follicles" or "lymphatic nodules". These nodules may consist almost entirely of small lymphocytes or have a characteristic histological appearance which consists of an aggregate zone of large pale-staining cells, named "germinal center" by Flemming (1885), completely surrounded by a zone of dark-staining, closely packed cells, sometimes termed the "mantle zone" but more frequently referred to as the "corona" or "cortex". No delineating membrane or network of fibers is recognizable between cortex and germinal center.

The red pulp virtually consists of two vascular structures: "splenic venous sinuses" (or "sinusoids") separated by plates of vascular tissue, called "splenic cords" or "Billroth cords".

The marginal zone, that junctional vascular tissue separating the white pulp from the red pulp, has been called "Knotchenrandzone" by Weidenreich (1901), "perifollicular envelope" by Krumbhaar (1948), "border zone" or "outer zone of the follicle" by Andrew (1946), and "white pulp halo" by Baillif (1953). This zone merges gradually into the splenic cords (Weiss, 1966b; Bloom and Fawcett, 1968). Sinuses do not usually penetrate the zone (Snook, 1944, 1950, 1958).

2) Blood vascularization

The splenic circulation has been extensively studied by numerous investigators for centuries; nevertheless, the controversy over its nature still persists. This considerable disagreement stems mainly from the fact that the vascular connections between the terminal arterioles of the white pulp and the splenic venous sinuses, the so-called "intermediate circulation of the spleen", continues to defy

complete description by available means. It follows that the various theories of splenic circulation, including the open, the closed, and combined concepts, have found adherents. The points of contention may be attributable to two main factors: 1) as pointed out by Knisely (1936) in his historical transillumination studies, there is an altering arrangement in an individual spleen; 2) as believed by Snook (1950, 1958), who compared the spleens of 14 mammals and classified them into "sinusal" and "non-sinusal", and by Ohta (1957), there is a marked species difference. Nevertheless, for the purposes of the current study, only a brief review of the pattern of splenic circulation, as outlined by Snook (1944, 1950, 1958), Weiss (1957, 1963, 1966b) and Bloom and Fawcett (1968) will be presented.

In the spleens of most mammals, such as the guinea pig, the rat or the mouse, the arterial branches of the splenic artery, instead of being trabecular as in the human spleen, enter the white pulp directly from the hilum and radiate into the interior of the organ as "central arteries". As soon as the latter penetrate the splenic pulp, they become surrounded by periarterial lymphatic sheaths, which accompany them almost to the point where they break up into arterial capillaries. According to Weiss (1964, 1966b), the central artery, throughout its course within the white pulp, gave off numerous branches which tended to follow a radial pattern as they ran toward the periphery of the white pulp. Two sets of branches were received by the occasional lymphatic nodule: one set reached the germinal center and ramified into capillaries which ran to the periphery of the nodule; the other set supplied the peripheral region of the nodule.

Weiss (1966b) reported that the great majority of arterial capillaries of the white pulp terminated in the marginal zone. According to MacNeal et al. (1927), some capillaries might terminate in "olive or acorn-shaped ampullae" in the marginal zone of the guinea pig, rabbit, dog and human spleens. In the rat, Andrew (1946) described white pulp capillaries opening into an "intermediate sinus" frequently observed between the white pulp proper and the marginal zone and partially lined with endothelium. In a recent study of the rat spleen, Snook (1964) described this "intermediate sinus", which he called "marginal sinus", as a series of anastomosing vascular spaces lying between the lymphatic nodules of the white pulp and the marginal zone. He also presented evidence that the sinus was endothelium-lined on the nodular surface but the lining showed discontinuity along the lateral aspect, "thus allowing blood to pass freely into the interstices of the marginal zone". Weiss (1964) and Snook (1964) reported that a white pulp capillary might pass through the marginal zone, reach the red pulp, and curve back to empty into the outer edge of the marginal sinus.

The attenuated main stem of the central artery and some of its branches pass into the red pulp where they ramify into straight, nonanastomosing, slender vessels, designated as "penicilli", most of which are sheathed by phagocytic reticular cells in the dog and cat spleens, some in the human spleen, and none in the rabbit spleen (Mall, 1900; MacNeal et al., 1927; MacKenzie et al., 1941). Snook (1958), Weiss (1957, 1959, 1962, 1963, 1966b), Galindo and Freeman (1963)

and Roberts and Latta (1964) demonstrated that most of the terminal vessels ended in several ways in the splenic cords. According to these authors, a few arterial vessels terminated in the splenic venous sinuses which possessed gaps in their walls.

Blood from the sinuses eventually passes into the pulp veins, which in turn drain into trabecular veins.

3) Lymphatic pathways

Textbooks of human histology uniformly state that, in the human spleen, the lymphatic vessels are restricted to a capsular plexus with branches sometimes entering the thickest trabeculae, especially those in the vicinity of the hilum, and that they are not encountered in the splenic pulp (Weiss, 1966b; Bloom and Fawcett, 1968). However, several investigators have reached the conclusion that, in some mammals, capsular and trabecular lymphatic vessels must be connected in some way with the splenic pulp.

Indeed, Barcroft and Florey (1928), after ligating the splenic vein and introducing trypan blue into the splenic artery, were able to trace the dye directly to the cysterna chyli or to a nearly lymph node. Klemperer (1938) suspected the presence of deep lymphatic channels within the splenic pulp. In 1946, Snook, using silver-impregnated serial sections, presented convincing evidence that deep lymphatic vessels might occur in the splenic white pulp of some mammals, such as the guinea pig, mole, mouse, horse and monkey, but denied their existence in the dog, cat, cow, pig and rat. According to this author, in the guinea pig, mole and mouse deep lymphatic plexuses followed closely the course of the central artery and arterioles

and opened into lymphatic vessels at the splenic hilum. In the horse, he traced them into trabecular and capsular lymphatic plexuses. He pointed out that, unless the large numbers of lymphocytes removed by the deep lymphatics plexuses were taken into account, "comparisons of arterial and venous bloods alone would not give a true picture of splenic hemopoiesis in the animals listed above".

B) Cytology of Reticular Cells and Extracellular Reticulum.

1) Light microscopic studies

As the cytoplasm of reticular cells in lymphatic tissue is usually indistinct in classical histological preparations, few light microscopic studies have been undertaken on the cytological features and distribution of these cells in the spleen. Maximow and Bloom (1957) stated that investigations of the stroma of the splenic white pulp had led to the conclusion that it was "a network of reticular fibers closely joined to the primitive reticular cells and phagocytic reticular cells or fixed macrophages" holding, as in all lymphatic tissue, free lymphocytes of various sizes in its meshes.

In the periarterial lymphatic sheaths of rat and rabbit spleens, Weiss (1964), however, described by light microscopy several types of pale-staining reticular cells with abundant cytoplasm. According to this author, most reticular cells in the periarterial sheaths were large, branched cells which tended to be stellate in shape. Some reticular cells were elongate and might resemble fibroblasts. Only occasional reticular cells appeared phagocytic, for "the moderate number of phagocytes present in the lymphatic sheath .... were,

for the most part, free cells derived, perhaps, from monocytes sequestered from the blood". All these various types of reticular cells in the periarterial lymphatic sheath were fixed cells, and their extended cytoplasmic processes enclosed strands of "extracellular reticulum", a term introduced by the author to designate the splenic "fibrillar reticulum" or "reticular fibers" of the light microscope, which stained with silver and PAS.

Moreover, the same author noticed that the endothelial and adventitial cells of arterial channels in the periarterial lymphatic sheaths had the same morphological appearance as reticular cells. He also observed that, as arterial vessels ran out toward the central artery, they became slimmer and that their wall might be composed of only three fixed elements: endothelial and adventitial cells separated by a basement membrane, which he included as part of the extracellular reticulum. He showed that the adventitial cells might "branch away from the vessel into the surrounding white pulp and, with the reticular cells and extracellular reticulum of the white pulp, form the meshwork within which lie lymphocytes and other free cells". He finally reported that the reticular cells with their associated extracellular reticulum tended to run circumferentially about the central artery, forming "several concentric circumferential tiers", the most peripheral of which constituted a rim separating the white pulp from the marginal zone.

Little is known of the distribution and light microscopic appearance of reticular cells and associated fibers in the lymphatic

nodules of the spleen. Bloom and Fawcett (1968) stated: "In the center of the lymphatic nodules of the spleen, as in the nodules of lymph nodes, the framework (reticular cells and fibers) consists of thin, scattered threads, while at the periphery it is coarser and much denser." Weiss (1964) reported that secondary lymphatic nodules were uncommon and small in the spleens of rabbits and rats and might or might not contain a germinal center surrounded by a mantle of principally small lymphocytes. According to Nossal et al. (1968), "primary follicles (nodules) simply represent rounded aggregations of small lymphocytes and reticular cells, with few if any blast cells, and are not demarcated sharply from the surrounding cortex. .... Secondary follicles (nodules) represent the end result of the deposition of an appropriate antigen in the primary follicle. They consist of a pyroninophilic germinal center composed of rapidly-dividing lymphoid cells and characteristics 'tingible body' macrophages; and of a cuff of surrounding small lymphocytes, of varying degrees of prominence."

Snook (1964) reported a dense reticular fiber network at the periphery of the lymphatic nodules of the rat spleen alongside the marginal zone. Such a network was called the "capsule of the follicle" by MacNeal (1929) and the "rind of collagenous fibers" by Krumbhaar (1948). In his studies on the perifollicular region of the rat spleen, Snook (1964) found that many "metalophil" cells, referred to as "reticulum cells" by Marshall (1956), "were aggregated to form a dense ring around the margin of the white pulp at the level of the

marginal sinus". According to this author, these "marginal metalophils" tended to be rounded with blunt processes in contrast to the highly branched metalophils, that is, the fixed reticular cells of the splenic white pulp. He also noted that the pale oval nuclei of these cells corresponded to those of reticular cells. Since the marginal metalophils of only a few rats reacted positively to tests for iron, he suggested that they represented potential phagocytes which may be mobilized quickly into active macrophages. Recently, Pettersen et al. (1967) confirmed Snook's findings in the rat spleen and suggested that the initiation of the antibody-forming process took place in the lymphatic nodules "where marginal zone cells came into close contact with the marginal metalophils".

In the marginal zone, which was separated from the white pulp proper by the marginal sinus, Weiss (1964) found by light microscopy that the reticular cells were also associated with strands of extracellular reticulum and had long, slender processes which broke up the zone into large communicating vascular compartments. He also observed that the reticular cells at the boundary of the periarterial lymphatic sheath might send processes out into the marginal zone. Snook (1964) reported that the network of reticular fibers in the marginal zone was more delicate than and continuous with that at the periphery of the white pulp.

In the red pulp, according to Maximow and Bloom (1957), the reticular cells were organized as venous sinuses and as cellular cords separating these sinuses. In sinal blood vessels reticular

cells were more or less flattened and, with the underlying basement membrane, comprised the wall of the vessels. In the cords, however, the reticular cells were stellate in shape.

In his light microscopic studies of the red pulp in the rat, the rabbit and man, Weiss (1957, 1959) showed a system of multiple vascular channels in the red pulp lined by a wall consisting basically of a reticular cell, a homogeneous basement membrane (or "extracellular reticulum", as he called it), and another reticular cell. He felt that reticular cells in the red pulp were organized as sinuses, and as cords separating the sinuses. He believed that "cords" were "collapsed sinuses". In a later report, however, he (1962a) discounted the idea of sinuses and cordal channels being identical, feeling rather that "cords" were lined by one type of reticular cell and "collapsed sinuses" by another type.

Moreover, Weiss (1957, 1959) reported that the cord areas in the red pulp of the rat spleen were broad and predominated over the sinuses, a large number of which had a patent lumen and were branching vessels. He pointed out that the stellate reticular cells lining the cords were active phagocytes, while the reticulo-endothelial cells of the patent sinuses were spindle-shaped and seldom showed evidence of phagocytosis. He also observed that the sinal cells rested on a fenestrated basement membrane or "sinal reticulum", which stained with the PA-Schiff technique and which on surface view had the form of a grid.

Lastly, "basal plates" were reported by Mangubi-Kudrjawtzewa

(1909) in the cytoplasm of the spindle-shaped reticulo-endothelial cells lining the splenic venous sinuses; they were found to occupy the portion of the cytoplasm of sinal cells which was in contact with the fenestrated basement membrane. These so-called basal plates accounted for the basal striations seen by light microscopy. In 1960, Leblond, Puchtler and Clermont were able to stain these structures with the tannic acid-phosphomolybdic acid-amido black (TPA) technique. According to these investigators, the basal plates occurring in the sinal reticulo-endothelial cells were related to the cytoplasmic "web", a term they used to designate all intracytoplasmic fibrils of presumably the keratin-myosin group of proteins which stained strongly with TPA. They found this "basal web" to be homogeneous, unattached to desmosomes or other junctional sites, and confined to the territory of only one reticulo-endothelial cell.

2) Electron microscopic studies

Since 1962 investigators have undertaken excellent pioneer electron microscopic studies on the reticular cells of the spleen. In their investigation of the white pulp of the mouse spleen, Galindo and Imaeda (1962) described two types of reticular cells in the stroma of the white pulp designated "fixed reticular cell type A" (FRCA) and "fixed reticular cell type B" (FRCB). The FRCA were fusiform with elongate cytoplasmic extensions, had a dense cytoplasm and contained large quantities of free cytoplasmic ribosomes. The FRCB were oval or elongate with fewer free ribosomes. Both types of fixed reticular cells were intimately associated with the extracellular reticulum, which was found, in accord with Weiss (1957, 1962b) and Stoeckenius

(1959), to be an amorphous substance in which was embedded a fibrillar element. The structural pattern of the FRCA suggested to these workers that they were less differentiated than the FRCB and, in consequence, might potentially transform into FRCB and/or macrophages which were often found in close association with the extracellular reticulum. These authors recorded no cellular types corresponding to the "primitive reticular cells", described by light microscopy (Bloom and Fawcett, 1968).

In his electron microscopic study of the periarterial lymphatic sheath in rabbit and rat spleens, Weiss (1964) distinguished different types of reticular cells. According to this author, most reticular cells in the periarterial lymphatic sheath of the white pulp were large, branched cells intimately associated with the extracellular reticulum; they contained characteristic vacuoles with material similar to the amorphous substance of the reticulum and dense cytoplasmic material aligned in that portion of the cytoplasm lying alongside the reticulum. Some reticular cells lacked the dense cytoplasmic substance and cytoplasmic vacuoles; they were often encountered in the central white pulp close to large arterial vessels; they occasionally assumed the appearance of fibroblasts and were fixed to an extracellular reticulum rich collagenous fibrils. A few fixed reticular cells had an electron dense cytoplasm and corresponded to the fixed reticular cells type A (FRCA) observed by Galindo and Imaeda (1962) in the white pulp of the mouse. Finally, occasional fixed

reticular cells were phagocytic but often lacked the characteristic dense basal substance of most reticular cells.

The same author also reported that the extracellular reticulum, together with the reticular cells tended to have a circumferential pattern, especially at the periphery of the periarterial lymphatic sheath. Moreover, he noted a similarity between endothelial and adventitial cells in the wall of the arterial capillaries of the white pulp; these two cell types were found to contain vacuoles and dense basal material similar to that present in reticular cells.

There is little information on the ultrastructure and distribution of reticular cells in the uncommon secondary lymphatic nodules of the spleen. In his recent report on the ultrastructure of germinal centers in normal mouse spleens, Swartzendruber (1967) described an extensive reticular framework consisting of two types of reticular cells, namely, reticular cells with electron dense materials in their elongated cytoplasmic processes and "dark" reticular cells or "transitional" cells linked by desmosomes. He pointed out that the "transitional" cells seemed to be undifferentiated cells belonging to the reticular framework "that must not be ignored as precursors of rapidly proliferating cells in germinal centers after antigenic stimulation".

In the marginal zone, Weiss (1964) reported that the reticular cells had long, slender cytoplasmic processes covering some of the strands of the extracellular reticulum which seldom contained collagenous

fibrils and which was often bare of reticular cell cover.

In recent years the electron microscope has made possible more detailed descriptions of the reticular cells in the splenic red pulp. Weiss (1962b, 1963, 1966b) described two main types of reticular cells in the red pulp of rabbit spleens, namely, "sinal" and "cordal" reticular cells. Sinal cells or reticulo-endothelial cells lining the splenic venous sinuses were elongate reticular cells with tapered ends, attached to the fenestrated basement membrane, but free of attachment to adjacent cells. They were similar in their cytologic components to the reticular cells in the white pulp, marginal zone and splenic cords but differed in having a spindle form and not a branched or stellate one. According to this author (1963), both sinal and cordal reticular cells were characterized by the presence of an electron dense substance which, in some instances, assumed a filamentous appearance in the part of their cytoplasm lying in close physical relation to the sinal basement membrane or cordal extracellular reticulum. The author believed that these cytoplasmic filaments were related to the "cell web" described by Leblond, Puchtler and Clermont (1960) in a wide variety of cells stained by successive treatment with tannic acid, phosphomolybdic acid amido black. He also thought that these filaments were similar to the cytoplasmic fibrils which he sometimes observed in other reticular cells and in the endothelial cells of splenic arterial capillaries (Weiss, 1962b).

Galindo and Freeman (1963) confirmed most of the findings of Weiss (1962b) in several mammalian spleens but found two morphologically

discernible types of reticulo-endothelial cells along the sinal basement membrane. One type of reticulo-endothelial cell had little or no phagocytic activity and resembled the fixed reticular type B (FRCB) of the white pulp as described by Galindo and Imaeda (1962). The other type of reticulo-endothelial cell was phagocytic and corresponded to the fixed macrophages of the white pulp (Galindo and Imaeda, 1962). The same investigators also described three categories of fibrillar elements which might be embedded in the homogeneous substance of the extracellular reticulum: 1) fibrils with an axial periodicity as mature collagen fibrils, 2) fibrils of similar diameter but without axial periodicity, and 3) undulating fibrils about 70 Å in diameter without axial periodicity. They noticed, however, that the fibrillar component of the reticulum varied in amounts in different areas of the splenic pulp.

Unlike Weiss (1962b, 1963, 1966b), Roberts and Latta (1964) distinguished three forms of reticular cells in the red pulp of the rabbit spleen, which were similar to those described by Galindo and Imaeda (1962) in the white pulp. According to these workers, "reticular cell type I" was a primitive, multipotential type of reticular cell which could be seen as a free or fixed-lining cell and which was probably the stem cell of the other two types; "reticular cell type II" was intermediate in the series, whereas "reticular cell type III" was the most differentiated of the three varieties of cells. These authors observed that similar or dissimilar cells might line a basement membrane on the same or opposing sides, i.e., that the three forms of

reticular cells occurred randomly in the sinus walls and cords.

However, according to a recent report by Thomas (1967) on the microscopic and submicroscopic structure of sinuses in rabbit and dog spleens, the sinus wall was described as consisting of an inner layer of sinal cells, an intermediate layer of basement membrane, and an outer layer of cord-limiting cells with elongate cytoplasmic extensions. She reported that sinal and cord-limiting cells were morphologically alike and appeared to be reticular cells with little phagocytic activity. She also noticed that sinal cells were often contiguous and did not always alternate with patent apertures.

ORIENTATION OF THE PRESENT STUDY

The primary aim of the present work was to give an accurate description of the spatial distribution of reticular cells in the thymus and spleen. This was made possible and was greatly facilitated by the staining, with the tannic acid-phosphomolybdic acid-amido black (TPA) technique, of fibrils in the cytoplasm of most reticular cells, which could thus be readily identified. This technique was used by Leblond et al. (1960) to demonstrate intracytoplasmic protein fibrils of various configurations in many cell types (including myofibrils in muscle cells and tonofibrils in stratified squamous epithelial cells). It was also necessary

to use a large number of histological and histochemical staining procedures to correlate the results obtained with classical histological findings.

This description contributed to clarify several basic structural aspects of these two intriguing lymphatic organs.

MATERIALS AND METHODS

INTRODUCTION

It is well established that the thymus, spleen and lymph nodes reach their maximal weight at different ages before a gradual decline sets in (Reinhardt, 1943 and 1946). Therefore, to effectively study the distribution of cell web-containing reticular cells in these lymphoid organs, it was imperative to adopt procedures which not only permitted the characterization and rapid identification of these cells, but which also took into account the age as well as the species of the experimental animals. A systematic approach made it necessary to investigate first how these reticular cells were distributed in space in young adult and adult animals and secondly, how such a pattern came about with time. It was the examination of the spatial distribution and the temporal pattern of the cell web-containing reticular cells which prompted the adoption of the following methods.

ANIMALS

Species:

Male albino rats of the Sherman strain, descended from the

Rockland Farms (New City, New York) colony, were routinely used throughout this work. Use was also made of some female rats of the same strain. In addition, the embryos and the neonatal male offspring of pregnant Holtzman albino rats provided material for investigations.

Animal Maintenance:

The animals were maintained in the animal quarters of the Department of Anatomy where a quiet, climate-controlled environment was provided to avoid minor stresses or any other adverse effect on the extremely sensitive thymo-lymphatic system. The temperature was kept at a constant 80°F, while a lighting cycle of 12 hours light and 12 hours dark was used.

Animal Breeding:

Pregnant Holtzman albino rats were obtained from Holtzman Company in Madison, Wisconsin, where the breeding of rats was rigidly controlled. The animals were allowed to mate freely during the night, and the females were examined the following morning for vaginal sperm. The presence of sperm was detected by vaginal smears within 12 hours after mating. Pregnant females were then shipped to Montreal where some were sacrificed at different time intervals, while others were allowed to bear their litters. Also studied were a few litters from Sherman rats bred in the animal quarters of the Department of Anatomy.

Ages Selected for Study:

For the young adult and adult series, the selection of ages was based upon the comparative study made by Reinhardt (1946) on the growth of the thymus, spleen and lymph nodes in the normal rat. This author reported that the thymus of male and female rats enlarged rapidly from the time of birth to reach its maximum size at about 70 days of age (or after attainment of a body weight of about 200 gm) and retained this size for some time before involution set in. He found that the spleen of male and female rats attained its maximum average size at about 100 days of age (or after attainment of a body weight of approximately 200 gm in the female and 300 gm in the male). He also stated that cervical lymph nodes reached their maximum weight at about 100 days of age and mesenteric nodes at about 70 days. Guided by these data, male rats weighing between 175 and 500 gm were selected for investigation. Also studied were three female rats with a body weight ranging from 130 to 160 gm.

For the embryonic and post-natal series, fetal rats from 13 to 20 days of age, newborn and 1-to 7-day-old postnatal rats were used in this study. Table 1 furnishes details of the groups studied.

Sacrifice of Animals and Removal of Organs:

For the young adult and adult series, a rat was weighed and then placed in a glass jar containing cotton wool soaked in ether. Once the animal was anesthetized, the skin was removed from the chest and abdomen of the animal. First, cervical lymph nodes were excised

TABLE 1

AGE, NUMBER AND AVERAGE WEIGHT OF NORMAL ANIMALS STUDIED

Age in Embryonic Days	No. of Animals	Average Body Weight in Grams
13	24	0.184
14	19	0.285
15	20	0.457
16	26	0.812
17	14	1.325
18	13	2.237
19	11	4.725
20	5	6.330

and immediately placed in the fixative. Then, the abdominal cavity was opened and the spleen as well as some mesenteric lymph nodes were removed and immersed in the fixative with a minimum of handling. Finally, dissection of the thymus was carried out according to the routine employed by Sainte-Marie (1962), excepting that the step involving the superficial fixation in situ of the thymus and other pleural viscera by the injection of 5 ml of fixative through an intercostal space into the thoracic cavity (before the latter was opened) was omitted. This procedure consisted in making two incisions on both sides of the sternum, parallel and well lateral to it, and in cutting out the entire anterior thoracic wall in one firm plate. The plate was then separated from the diaphram and lifted very carefully by the xiphoid process of the sternum, severing with a fine pair of scissors the connective tissue strands binding the upper ventral part of the thymus to the back of the sternum. After removal of the plate, the trachea and esophagus were grasped with forceps in the neck region and cut just below the level of the thyroid gland. They were then carefully pulled down, while an incision was made between the esophagus and the posterior body wall to liberate the various thoracic viscera. After cutting away with scissors the portion of the heart and lungs projecting below the thymus, the remaining tissue was placed in a large volume of fixative. This procedure was used to prevent any stretching and deformation of the fresh thymus whose topography is believed to be sensitive to even the most moderate direct handling.

For the embryonic series, the pregnant rat was anesthetized with ether, its abdominal cavity opened, and the uterus containing embryos excised and placed in isotonic saline. The mother was then sacrificed. One by one, the embryos were removed from the uterus, freed of their membranes, and weighed on a gram-a-matic balance. The embryo was then hemisected, and its upper half placed in the fixative. As intracytoplasmic fibrils were not visible in the reticular cells of the spleen and lymph nodes before birth, only the embryonic thymus was investigated.

For the newborn and post-natal series, young animals less than one week of age were decapitated. The thymus and spleen were removed from the thoracic cavity and abdominal cavity respectively, and immediately placed in fixative.

HISTOLOGICAL PROCESSING

Introduction:

The tannic acid-phosphomolybdic acid-amido black (TPA) technique was the main staining procedure used in this investigation. This method has been extensively employed to demonstrate intracytoplasmic fibrils of various configurations in a wide variety of cells (Puchtler and Leblond, 1958; Leblond et al., 1960; Kallenbach, 1963).

However, previous histological studies have shown that most fixatives have the disadvantage of inhibiting or interfering with the TPA reaction (Puchtler and Leblond, personal communication; Puchtler

and Sweat, 1962; Kallenbach, 1963). Of all the fixatives tested, only Carnoy's fluid insures maximum TPA staining results. Electron microscopy aldehyde fixatives, such as glutaraldehyde and acrolein, give a fairly strong and specific mitochondrial and nucleolar stain with TPA, but leave unstained the cell web, terminal bars or other organelles (Pereira, 1965; Clermont and Pereira, 1966). Moreover, it seems that the patterns of precipitated protein and the affinity of tissue protein components for TPA are dependent not only upon the fixative used, but also upon the mode of washing and embedding. The so-called "double embedding technique" proved to be the tissue processing method of choice for the TPA staining procedure (Pereira, 1965).

Therefore, all the tissues used in the present study were fixed in Carnoy's fixative for periods of time varying with the age of the experimental animal, embedded according to the "double embedding technique", and stained either with TPA alone, PA-Schiff-hematoxylin alone, TPA in conjunction with PA-Schiff, PA-Schiff-toluidine blue, hematoxylin and eosin, iron hematoxylin or with a silver technique.

Fixation of Tissue:

Thymuses, spleens and lymph nodes were fixed by immersion in an excess of freshly prepared Carnoy's solution No. 2 (Romeis, 1948). Its composition is as follows:

60 volumes ethanol (anhydrous)

30 volumes chloroform (Baker Analyzed Reagent)

10 volumes glacial acetic acid (Baker Analyzed Reagent).

All tissues were fixed at room temperature. Embryonic tissues were fixed for 12 hours, postnatal tissues up to 18 hours, and older material progressively longer until the maximum of 24 hours was reached.

Trimming of Tissue:

After a preliminary time of 2 hours in the fixative, the tissues were hard enough to be trimmed. The cervical-thoracic region of the embryos was trimmed. The spleens were sliced with a sharp razor blade into 3 mm pieces, each of which included a branch of the splenic artery where it penetrated the organ, while the large lymph nodes were hemisected. Excessive amounts of heart, lung and trachea about the thymus were removed with a fine pair of scissors, whereas lymph nodes, portions of trachea and esophagus and large blood vessels were left attached to the thymus which was untouched during the whole process. Subsequently, all the tissues were placed in a new volume of fixative in which they remained until the end of fixation. After fixation, the thymi were hemisected transversely; all the tissues were then bagged in cheese cloth and either stored in 70% alcohol or embedded immediately.

Embedding of Tissue:

Following fixation, the tissues were infiltrated and embedded according to the following method, referred to as the "double

embedding technique" (Pereira, 1965):

Dehydrate in ascending strengths of alcohol (70,80,95 and 100%), only if stored in 70% alcohol (a procedure which is not recommended since the tissue is already dehydrated following fixation in Carnoy's fluid).

Clear in a 1:1 mixture of alcohol and methyl benzoate (Fisher Reagent) for 30 minutes and in methyl benzoate (Fisher Reagent) alone for another 30 minutes.

Treat with a 2% solution of nitrocellulose (or celloidin) dissolved in methyl benzoate (Fisher Reagent) for 24 hours in a closed air-tight container to prevent evaporation.

Transfer to a 10% solution of nitrocellulose dissolved in methyl benzoate (Fisher Reagent) for 24 hours (also in a tightly stoppered container).

Pass through two changes of benzene for about 45 minutes each (total 1½ hours).

Transfer to a 1:1 mixture of paraffin and benzene at a temperature a few degrees above the melting point of the paraffin being employed (which is 50-52° C), for 30 minutes.

Infiltrate with two changes of paraffin (M.P., 50-52° C) in a vacuum oven heated at 54-56° C, for about 45 minutes each (total 1½hours).

Embed in paraffin (M.P., 58-60° C).

The "double embedding technique" is thus a combined low viscosity nitrocellulose (or celloidin) infiltration and paraffin

infiltration and embedding. It is the tissue processing method of choice for the TPA staining procedure for three main reasons.

First, it reduces artifacts, for the celloidin, since it is not removed, supports and holds together the fragile structures of the tissue until the time that the section is mounted on the slide. Indeed, in this process, the tissue is infiltrated with a celloidin-methyl benzoate solution, using two strengths, namely thin celloidin (2%) and thick celloidin (10%). Since no heat is required for this step, high strengths of celloidin are used and methyl benzoate shrinks and hardens tissues less than other solvents (like xylene or toluene), shrinkage is negligible and the consistency of the tissue remains unaffected.

Secondly, this technique improves the texture of the final paraffin block, allowing 2 mu sections to be cut adequately. Following clearing with benzene, the tissue is transferred to a 1:1 mixture of benzene and paraffin (M.P., 50-52° C) in a vacuum oven for a total of only 1½ hours. In this step, practically all the solvent is gradually evaporated without the formation of air bubbles and without prolonged treatment in molten paraffin (which tends to cause shrinkage and hardening of tissues). As a result of this, when the tissue is subsequently enclosed in a fairly hard paraffin (M.P., 58-60° C), a consistent, uniform degree of hardness is attained throughout the block, which greatly facilitates sectioning.

Thirdly, this mode of embedding appears to preserve or affect, as an increased TPA staining intensity was achieved, the pattern of

precipitated protein. Puchtler and Leblond (personal communication) found that dioxane as the sole medium between Carnoy's fluid and paraffin gave poor results with the TPA stain. TPA staining tests performed with tissues infiltrated only with paraffin following clearing in dioxane resulted in a weak and diffuse overall staining of TPA positive structures and in tissue damage (Pereira, 1965). The damage, though variable, most frequently showed itself as holes or cracks in the specimen, as if an explosion of some sort had taken place during succeeding steps of the processing method. Although the exact cause of this pale staining and "explosion damage" is not yet fully understood, it seems that the simple paraffin embedding technique involving clearing in dioxane affects the pattern of precipitated protein so that the affinity of tissue protein constituents for TPA is decreased. It also appears that paraffin alone does not have sufficient strength to "set" the tissue protein components.

Sectioning of Tissue:

For the young adult and adult series, more than 2000, 2 and 4 mu, serial sections were prepared. For the embryonic series, the cervical-thoracic region of the embryos was serially sectioned. For the post-natal series, serial sections from the paraffin blocks were also made. In addition, from the paraffin blocks at each age, several slides of both 2 and 4 mu sections were prepared. Sections were mounted routinely on albumin-glycerin coated glass slides and allowed

to dry at 45° C, every adjacent section being mounted on a different slide.

The TPA Stain:

To demonstrate cell web fibrils in the cytoplasm of reticular cells, sections were stained with the TPA technique as follows (Kallenbach, 1963; Kallenbach et al., 1965):

Hydrate sections through two changes of xylol (5 minutes each), two changes of 95% alcohol (1 minute each), and one change each of 70 and 50% alcohol (1 minute each) to distilled water.

Mordant sections in a freshly prepared 5% aquenous solution of tannic acid for 10 minutes.

Rinse briefly in distilled water (for 5 seconds with agitation).

Mordant in a freshly prepared 1% aqueous solution of phosphomolybdic acid for 10 minutes.

Rinse briefly in distilled water (for 5 seconds with agitation).

Dehydrate in one change each of 95% alcohol, absolute alcohol, methanol-glacial acetic acid 9:1 for 5 seconds each (with agitation).

Stain in a saturated solution of amido black 10B (Chroma Gesselschaft, Stuttgart) in methanol-glacial acetic acid 9:1 for 1 minute.

Wash in three changes of methanol-glacial acetic acid 9:1 for 2 minutes each.

Clear in three changes of xylol (3 minutes each).

Mount in balsam.

Some observations.

Paraffin sections stained with the TPA technique showed bluish-green TPA positive sites and brownish-yellow TPA negative sites. With the exception of intraluminal fibrin threads and hemoglobin droplets (Fig. 2), the TPA technique did not stain extracellular material but did stain the nuclei of connective tissue cells (Fig. 17).

In any given TPA-stained paraffin section, the nuclei of epithelial cells and of cell-web containing reticular cells were invariably intensely stained, while nuclear staining was light or totally lacking in lymphocytes and other cell types (Figs. 15-27).

In the cytoplasm of cells, the ergastoplasm and the mitochondria were often stained to some extent with TPA. However, all intracytoplasmic fibrils of presumably the keratin-myosin-epidermin group of proteins (including the myofibrils of muscle cells) were avidly TPA stained (Figs. 1, 2, 21, 53, 76, 78, 79). Moreover, the TPA technique stained intensely intercellular attachment sites (desmosomes, half-desmosomes and terminal bars) on which cell web fibrils often inserted (Figs. 64 and 46). It also stained the so-called "Russell bodies" in the cytoplasm of some plasma cells in the red pulp of the spleen (Fig. 3). Lastly, it readily demonstrated bundles of microtubules which assumed a fibrillar appearance under the cell membrane of red blood cells and blood platelets as well as in cilia and mitotic

spindles (Figs. 27, 50, 51, 53, 79, 100, 101). Also avidly TPA positive were the points of insertion of certain microtubules, such as basal bodies and centrioles (Figs. 50, 51, 53).

Choice of Other Stains:

In addition to the TPA staining method, other procedures were employed to relate the TPA staining results to classical histological findings. Of all the stains tested, it was felt that only the following procedures furnished useful additional information.

The hematoxylin-eosin stain.

In certain series, an occasional section was saved out and stained with hematoxylin and eosin for routine studies. The section was treated with Harris's hematoxylin for 3 to 4 minutes, rinsed in distilled water, and counterstained by dipping in 1% eosin Y.

The PA-Schiff-hematoxylin stain.

In the embryonic and post-natal series as well as in some of the adult series, every third section was stained with the PA-Schiff-hematoxylin technique. The section was oxidized for 15 minutes in periodic acid and stained in Schiff reagent for also 15 minutes. It was then washed in three changes of sulfite water for 2 minutes each, counterstained with Harris's hematoxylin for 3 to 4 minutes, dehydrated and mounted in balsam. This staining procedure was used to demonstrate intracellular PA-Schiff reactive sites as well as extracellular reticulum. To determine if the PA-Schiff reactive sites in the embryonic series were glycogen-containing, some test slides were

incubated at 40° C with saliva prior to treatment with the Schiff technique. In this procedure, glycogen was digested by the alpha-amylase of the saliva, and therefore glycogen-containing sites were no longer reactive to the Schiff stain.

The TPA-PA-Schiff stain.

Some TPA-stained series were counterstained with PA-Schiff. This double staining technique was used to relate TPA positive sites to both intra-and extra-cellular PA-Schiff reactive sites.

The PA-Schiff-toluidine blue stain.

Following treatment with periodic acid and Schiff reagent, sections were occasionally counterstained with a 0.5% solution of toluidine blue in water (Pearse, 1954, p. 432).

The iron hematoxylin stain.

Finally, some test slides were subjected to Regaud's iron hematoxylin stain, which usually tends to stain (depending on the degree of differentiation) cell membranes, nuclear and connective tissue components in addition to give results identical to those of the TPA stain. (Kallenbach, 1963; Pereira, 1965). Following deparaffinization, the sections were mordanted in 5% iron alum for 24 hours. After thorough washing in water, they were stained for 24 hours in an alcoholic hematoxylin solution; the sections were considered to be adequately stained when, on being taken out of the hematoxylin, they were a homogeneous jet-black and microscopically showed no cellular detail. This was followed by differentiation in 1% iron alum, controlling the results with a microscope. Lastly, the sections were washed in running water for 30 to 60 minutes, cleared and mounted.

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The results of the iron hematoxylin stain are often identical to those of the TPA stain, though the former (depending on the degree of differentiation)usually tends to stain cell membranes and connective tissue components (Kallenbach, 1963; Pereira, 1965). Surprisingly, iron hematoxylin failed to stain cell web fibrils in the reticular cells of the thymus, spleen and lymph nodes. It therefore appears that the intracytoplasmic fibrils of these reticular cells are stereochemically different from cell web fibrils found elsewhere, such as the tonofibrils of stratified squamous epithelial cells, the circumferential web of liver parenchymal cells, the cell web of renal and intestinal epithelial cells, and so forth.

Silver reticulum stains.

In all the series, for each single section stained with TPA, an adjacent section was impregnated for reticular fibers by the method outlined by Gridley, a variant of Maresch's Bielschowsky silver technique. After treatment with 0.5% periodic acid for 15 minutes, the section was sensitized with a 2% solution of silver nitrate for 30 minutes and then stained in Maresch's Bielschowsky silver solution for 15 minutes (Lillie, 1954, p. 334). Following washing in distilled water, the section was subjected to 30% formalin for 3 minutes, 0.5% gold chloride for 5 minutes and 5% sodium thiosulfate for 3 minutes. The section was then counterstained with Harris's hematoxylin, cleared in xylene and mounted in balsam. This technique gave optimal results with Carnoy-fixed tissues.

Some observations

It is generally assumed that fibers which are argyrophilic in paraffin sections treated with Gridley's silver reticulum stain represent reticular fibers (Lillie, 1954, p. 334). However, no evidence has ever been presented that this staining method is specific for these fibers.

When Carnoy-fixed paraffin sections of thymus, spleen and lymph nodes were treated with Gridley's silver method, reticular fibers, including ring fibers surrounding splenic venous sinuses, were colored black, while collagen fibers had a brownish color and elastic fibers were unstained. Furthermore, this technique was found to stain all renal basement membranes, including those of the glomeruli which are known to be associated with neither reticular nor collagen fibers (Figs. 8 and 10).

In contradistinction, Bielschowsky's silver reticulum method failed to reveal the ring fibers around splenic venous sinuses and the basement membranes of renal glomeruli and tubules (Figs. 9 and 11). However, in all other respects, this staining procedure gave results identical to those of Gridley's silver reticulum stain.

This therefore led to the conclusion that Gridley's silver method did stain basement membranes and was not specific for reticular fibers, while Bielschowsky's method stained the latter selectively. Since the black fibers found after treatment with Gridley's technique represented reticular and/or basement membranes, all argyrophilic fibers seen with this technique will be referred to as "extracellular reticulum" fibers.

The periodic acid-sodium bisulfite-resorcin-fuchsin stain.

Because the staining properties of ring fibers surrounding splenic venous sinuses were very similar to those of basement membranes, it seemed advisable to ascertain the identity of these fibers. Carnoy-fixed paraffin sections of spleen and kidney were treated consecutively with 0.5% aqueous periodic acid for 5 minutes and sodium bisulfite overnight. They were then stained with resorcin-fuchsin for 4 hours according to Puchtler and Sweat (1964b).

Some observations

This method failed to stain reticular fibers in the organs investigated but resulted in a purplish black coloration of the elastica interna of arteries and a black coloration of elastic fibers in the walls of blood vessels and in the capsule and trabeculae of the spleen (Figs. 4 and 5). In addition, this technique stained black all renal basement membranes, including those of the glomeruli, and, most important of all, the ring fibers around splenic venous sinuses and some fine fibrillar elements which were intimately associated with the coarse reticular fibers of the splenic white pulp (Figs. 6 and 7).

After counterstaining with Van Gieson's picro-fuchsin, the black fibers, which were elastic fibers and/or basement membranes, stood out in sharp contrast to the red reticular and collagen fibers. This differential staining method showed that ring fibers as well as the delicate fibers in the stroma of the splenic white pulp were not identical to reticular fibers.

Verhoeff's stain in combination with Masson's trichrome stain.

This double staining technique was used to determine the true identity of the ring fibers around splenic venous sinuses and of the delicate fibrillar elements which were found in close association with the coarse reticular fibers of the splenic white pulp, both of which stained black with the method of Puchtler and Sweat (1964), indicating that they represented elastic fibers and/or basement membranes. This technique resulted in a blue coloration of collagen fibers and a blue-black to black coloration of elastic fibers. Not only were the elastic fibers in the walls of blood vessels and in the splenic capsule and trabeculae stained, but also the delicate fibers associated with the coarse reticular fibers of the splenic white pulp were intensely colored and thus exhibited staining properties identical to those of elastic fibers.

However, this double staining technique failed to reveal the ring fibers around splenic venous sinuses. The latter, therefore, did not represent elastic fibers and were true basement membranes. These observations confirmed the findings of Lillie (1951, 1952) and Puchtler and Sweat (1964a, 1964b) that the staining properties of ring fibers were very similar to those of basement membranes. They also showed that some fine elastic fibers were intimately associated with the coarse reticular fibers of the splenic white pulp.

The study of the spatial distribution and temporal pattern of the cell web-containing reticular cells in the thymus, spleen and lymph nodes was greatly facilitated by the compiled observations made with these various staining procedures.

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Radioautography:

Clark (1963), in his electron microscopic investigation of the mouse thymus, described reticular-epithelial cells rich in cytoplasmic tonofilaments, but could not observe mitotic figures of these cells. It therefore seemed of interest to see if these cells were capable of incorporating radioactive thymidine and of undergoing mitosis.

To test this possibility, three young adult male Sherman albino rats with a body weight of about 200 gm received a subcutaneous injection of 2 muc of ^{3}H-thymidine per gm of body weight at some time between 9:00 A.M. and 11:00 A.M. The specific activity of the tritiated thymidine used was 1.9 c/mM (Schwartz Laboratories, New York). The animals were sacrificed $2\frac{1}{2}$ hours after the ^{3}H-thymidine injection.

The thymuses were then removed and processed as described previously, with fixation in Carnoy's fluid for 24 hours. Some 2 mu serial sections of thymus were made, and every adjacent section was mounted on a different slide. All the sections were stained with TPA. Every second slide was treated with two coats of celloidin to preserve the staining and then processed for radioautography according to the technique of Kopriwa and Leblond (1962). In a dark room the slides were first dipped into Kodak NTB2 emulsion. Following exposure in the dark for 30 days, the slides were developed and mounted in balsam. Extremely little background fog was produced, and the staining intensity of cell web structures remained unaffected.

Fig. 1 Spleen. A trabecula in the splenic red pulp (RP) can be seen to contain a large number of intensely stained smooth muscle cells (arrow).

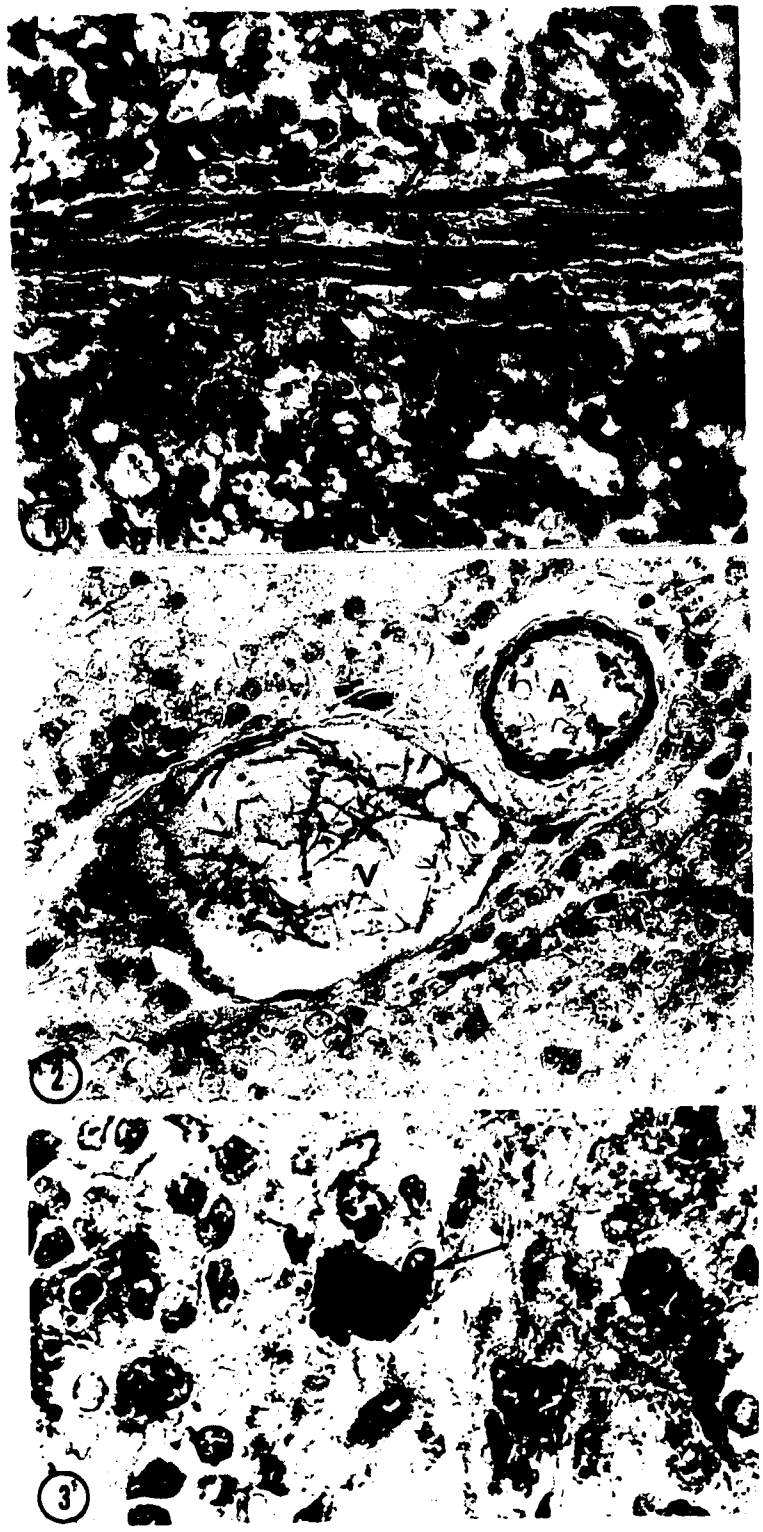
Carnoy fixation and TPA (x600).

Fig. 2 Thymus. A vein (V) and an artery (A) may be seen in an interlobular septum with unstained collagenous bundles. A network of fibrin threads is visible in the lumen of the vein (V).

Carnoy fixation and TPA (x570).

Fig. 3 Spleen. The arrow points to the nucleus of a plasma cell in a Billroth cord of the red pulp. Note the well-stained "Russell bodies" in the cytoplasm of the cell.

Carnoy fixation and TPA (x1260).


A
V

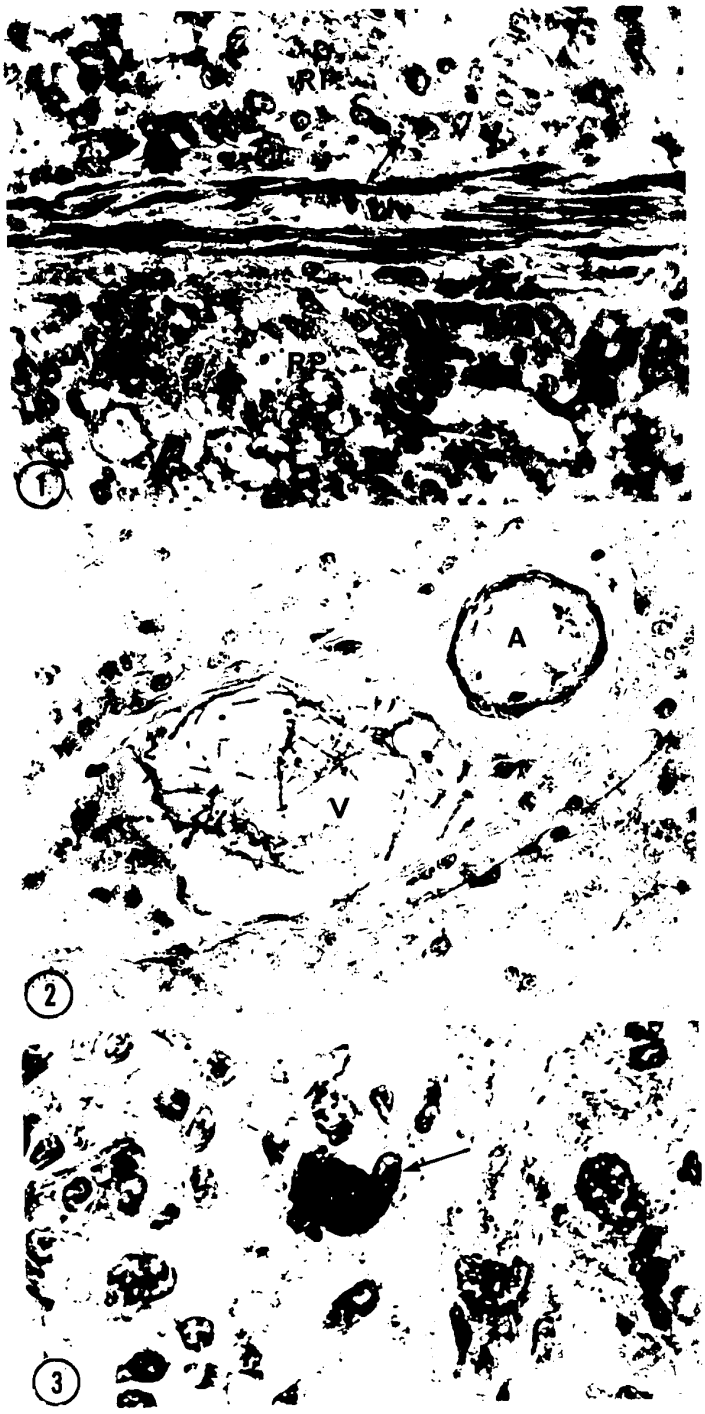
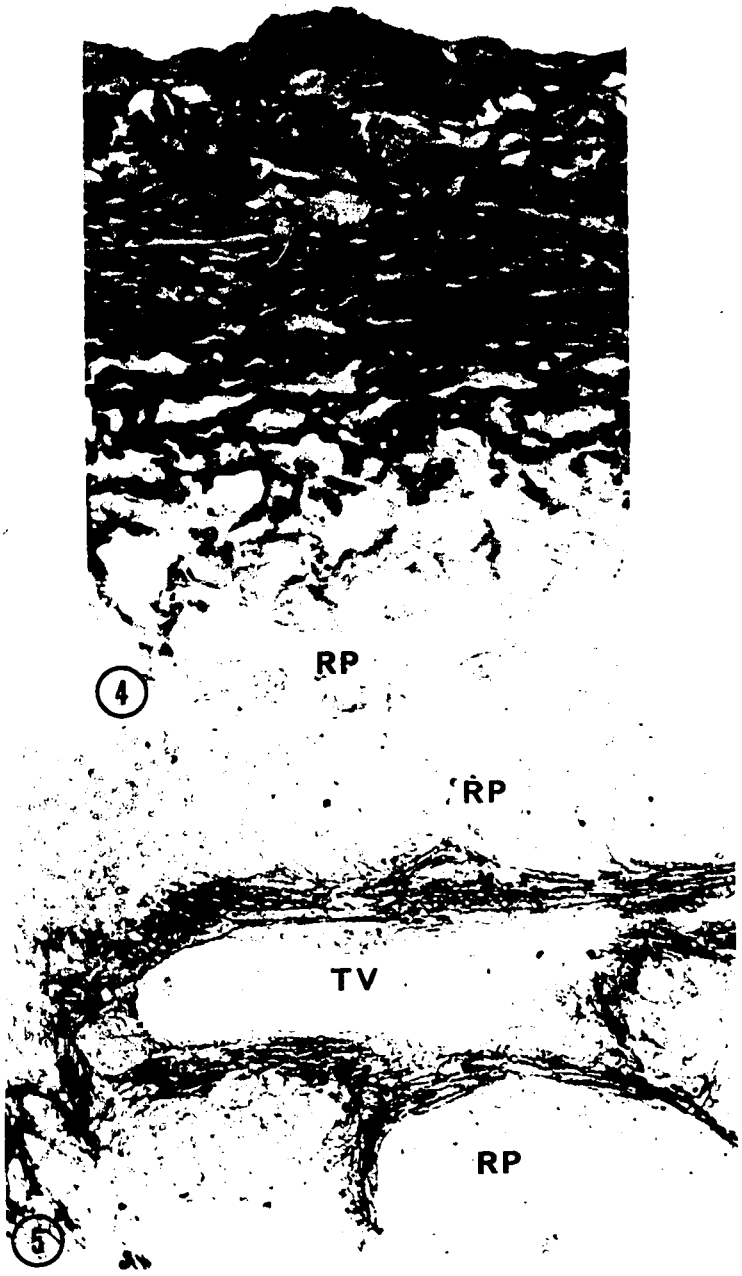

RP
RP
1
A
V
2
3

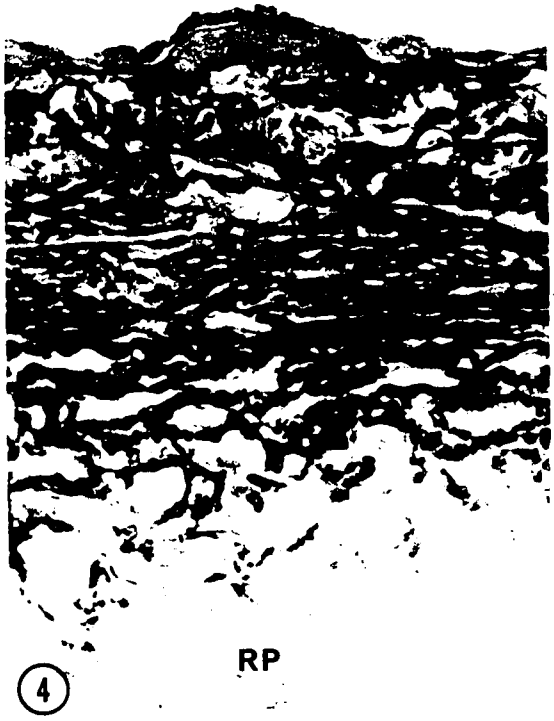
Fig. 4 Spleen. Above the red pulp (RP) is a portion of the thick splenic capsule. Note that elastic fibers form a network between the unstained collagenous bundles. In the deep layers of the capsule, near the red pulp, is located the network of the thickest elastic fibers.

Carnoy fixation and PA-sodium bisulfite-resorcin-fuchsin stain (x1120).

Fig. 5 Spleen. A trabecula in the red pulp (RP) contains a trabecular vein (TV) and a large number of elastic fibers.

Carnoy fixation and PA-sodium bisulfite-resorcin-fuchsin stain (x170).


RP
4
RP
TV
RP
5


RP
4

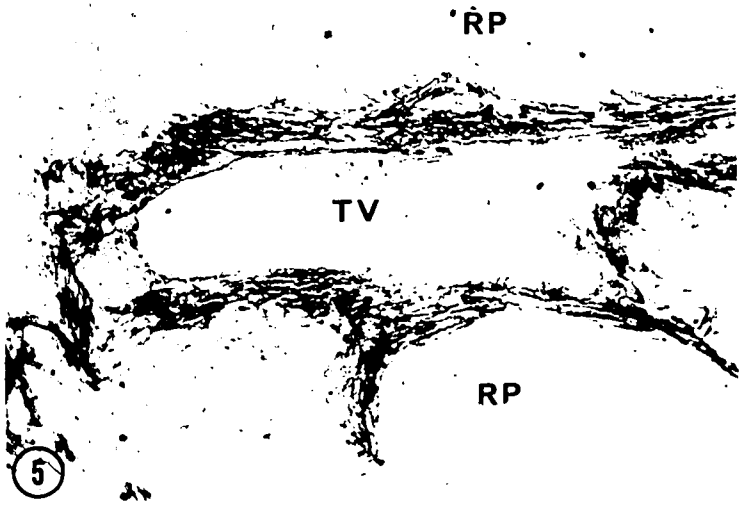

RP
TV
RP
5

Fig. 6 Spleen. A central arteriole (a) in a periarterial lymphatic sheath of the white pulp is surrounded by concentric layers of pale-staining coarse collagenous fibers containing darkly stained elastic fibrils (arrows).

Carnoy fixation and PA-sodium bisulfite-resorcin-fuchsin-Van Gieson's picro-fuchsin stain (x1120).

Fig. 7 Spleen. Ring fibers of the fenestrated basement membrane surrounding a venous sinus (S) are intensely stained. The nucleus of a reticulo-endothelial cell is visible at right angles to the ring fibers. In cross sections ring fibers appear as dark dots (arrow).

Carnoy fixation and PA-sodium bisulfite-resorcin-fuchsin-Van Gieson's picro-fuchsin stain (x1120).

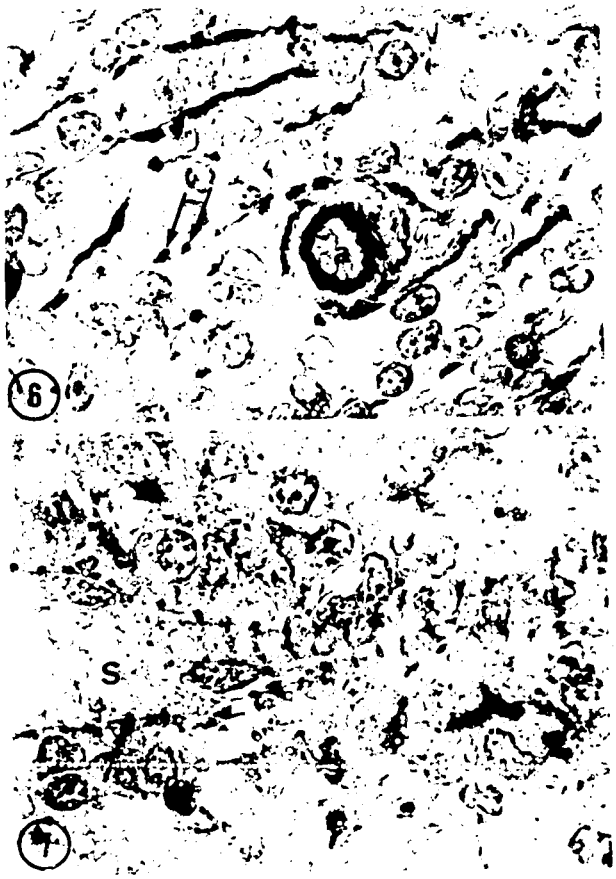

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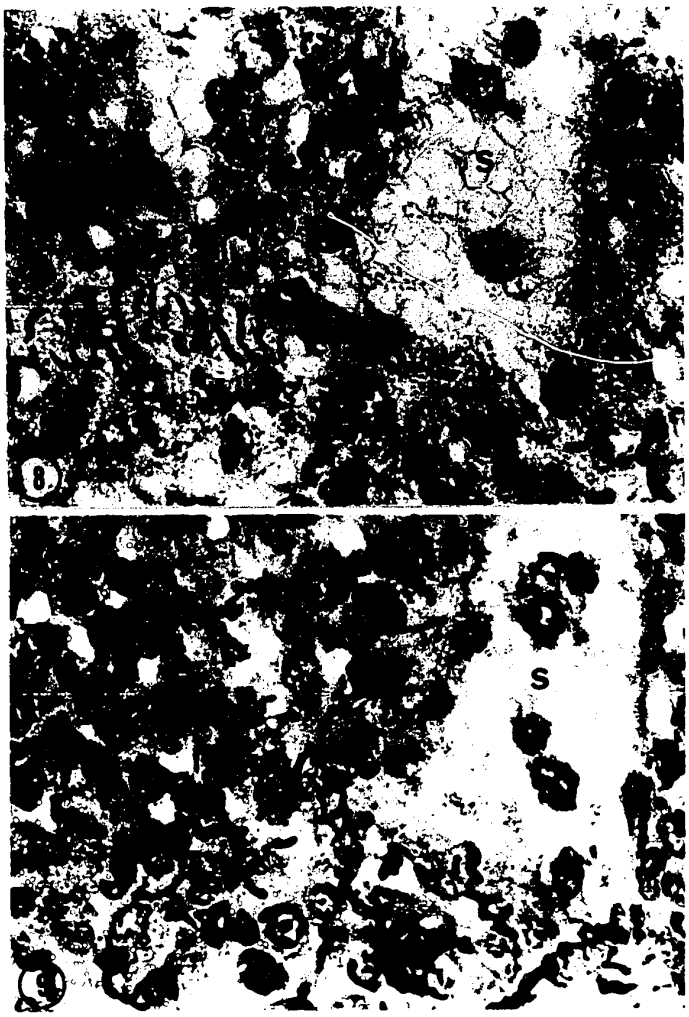
Fig. 8 Spleen. Ring fibers (center left) of the fenestrated basement membrane surrounding a venous sinus (S) are intensely stained. In cross sections the ring fibers appear as dark dots (arrow).

Carnoy fixation and Gridley's silver reticulum stain (x1120).

Fig. 9 Spleen. Same venous sinus (S) as in Fig. 8. Note that the ring fibers of the fenestrated basement membrane surrounding the sinus are unstained. The reticulum fibers are intensely stained.

Carnoy fixation and Bielschowsky's silver reticulum stain (x1120).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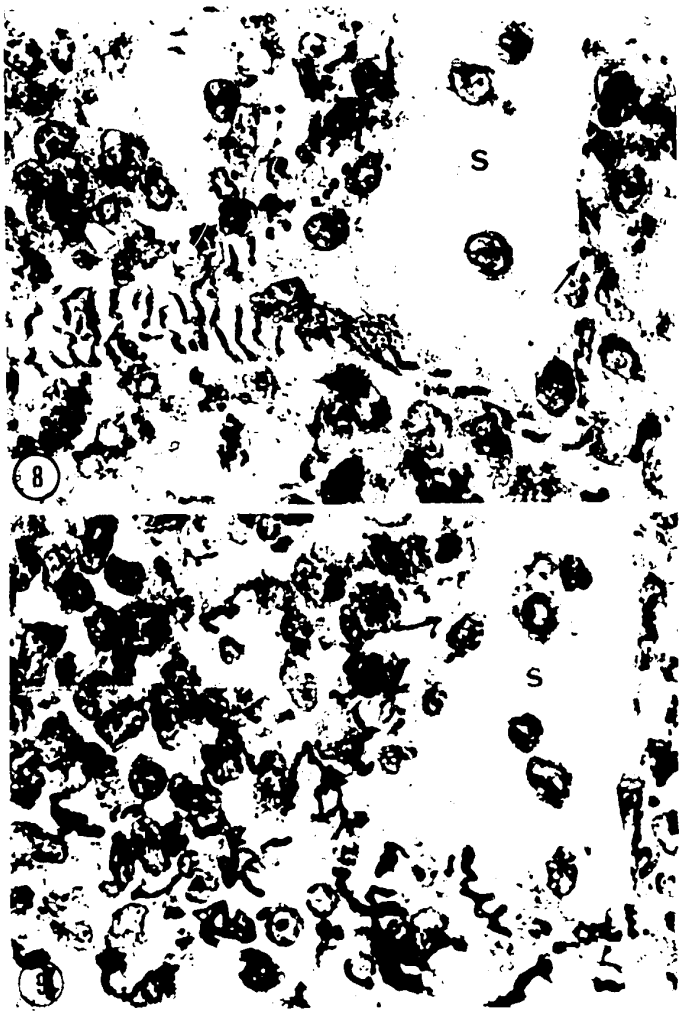
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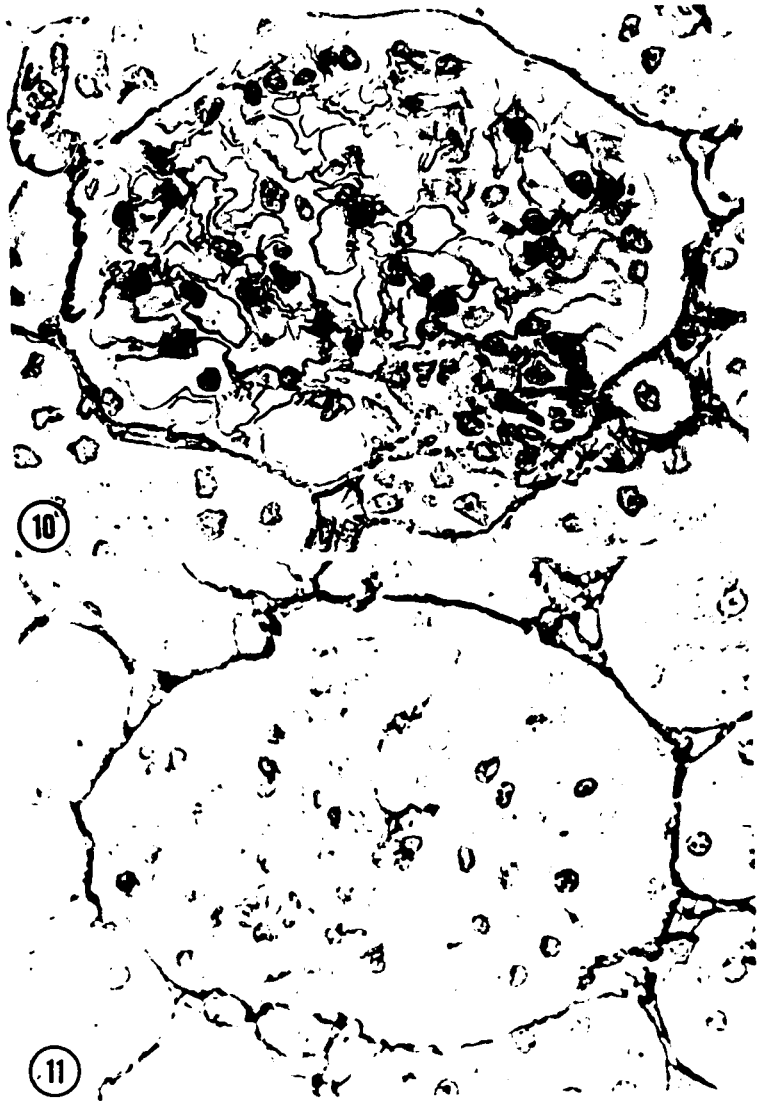
Fig. 10 Kidney. Basement membranes of glomerulus, Bowman's capsule and tubules are intensely stained.

Carnoy fixation and Gridley's silver reticulum stain (x600).

Fig. 11 Kidney. Reticular fibers and basement membranes of Bowman's capsule and tubules are intensely stained. Glomerular basement membranes remain unstained.

Carnoy fixation and Bielschowsky's silver reticulum stain (x600).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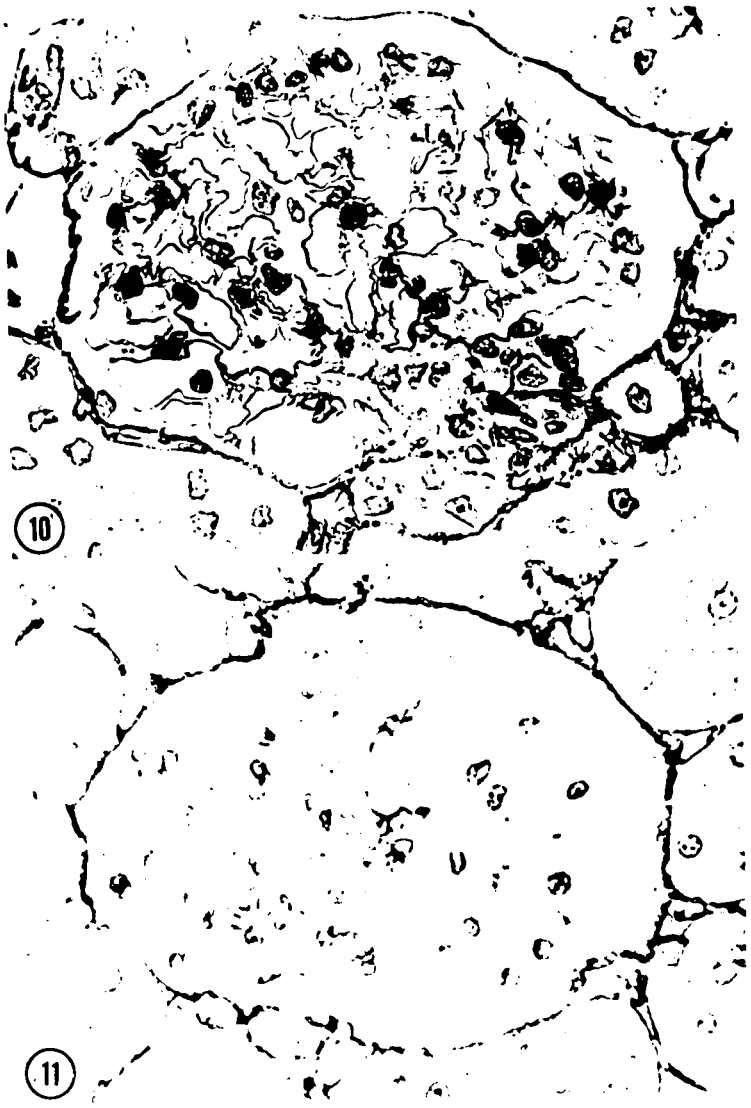
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RESULTS

DISTRIBUTION OF RETICULAR-EPITHELIAL CELLS IN THE ADULT RAT THYMUS

Before entering on a detailed description of the adult thymus as seen in TPA-stained preparations, it would be useful to review briefly the general architecture of the organ and at the same time define the terms used.

General Architecture of the Thymus:

A) Lobular structure and zonation.

Each thymic lobule was a broad structure, roughly pyramidal in outline and almost completely delimited along its lateral margins only by thin partitions of loose connective tissue, referred to as "interlobular septa", which never totally severed the lobule so that the parenchyma of one lobule was connected to that of neighboring ones by an axial pedicle or stem. The septa did not normally penetrate the thymic parenchyma except for the thin connective sheaths or "intralobular septula" accompanying intrathymic blood vessels (Fig. 12).

In classical histological preparations, under low power, each lobule comprised two clearly defined regions: a peripheral

dark area or "cortex" surrounding a central paler area or "medulla" (Fig. 13). However, under high power, the limit between these two regions was not clear cut and was recognizable only by the greater concentration of lymphocytes in the cortex than in the medulla.

B) Blood and lymphatic vascularization.

When the anterior wall of the thorax was cut out in one firm plate and lifted very carefully by the xiphoid process of the sternum, it was readily observed that the blood supply of the rat thymus was derived mainly from branches of the internal mammary arteries which were seen to pierce the lateral aspects of the thymus at different levels, although the inferior thyroid arteries also contributed some twigs to the apical portion of the organ.

Each main arterial branch with its accompanying vein, nerve and lymphatic vessel coursed between the chain of mediastinal lymph nodes and a thymic lobe and pierced the capsule on the lateral aspect of the lobe to run in an interlobular septum. Such a connective tissue area, corresponding to the point of entry of a lobar artery and to the point of exit of a lobar vein, constituted one of the major "lobar hila".

From the lobar artery, as it was revealed by the study of serial sections of thymus, small interlobular arteries were given off. These arterial branches were never encountered in the dense connective tissue capsule. They were preferably distributed in the interlobular septa according to two different vascular patterns. The majority of interlobular arteries broke up into terminal arte-

rioles, characterized by a single layer of smooth muscle cells (Fig. 56), which in turn ramified into precapillary sphincters without an internal elastic lamina (Fig. 57). The latter gave rise to numerous capillaries which penetrated the thymic cortex.

Some of the cortical capillaries traversed singly the whole thickness of the cortex and the external portion of the medulla in a radial fashion to drain into post-capillary venules situated in the deeper parts of the medulla (Fig. 58). Others crossed singly the cortex radially and, in the cortico-medullary zone, broke up into two arcuate capillaries which ran along this zone for some distance before traversing the medulla to pass over into more deeply located medullary post-capillary venules (Fig. 61). Still other cortical capillaries, soon after penetrating the cortical parenchyma, ramified into capillary networks (Fig. 59), which joined into post-capillary venules that had extended into the periphery of the medulla and the cortico-medullary boundary (Fig. 60), each being surrounded by a perivascular connective tissue space.

However, as shown in Figure 61, a few small interlobular arteries coursed into interlobular septa which were continuous with the medulla and penetrated the innermost part of the latter before breaking up successively into terminal arterioles, precapillary sphincters and venous capillaries. The latter in turn drained into "inner medullary" post-capillary venules and were, therefore, confined to the territory of the deeper portions of the medulla.

All the post-capillary venules eventually converged to form inner medullary veins, which in turn joined lobular veins. Each lobular vein left the thymic lobule by coursing into an interlobular septum with a lobular artery to then drain into an interlobular vein. The latter thereafter passed over into a lobar vein.

In summary then, the cortex was characterized by a network of numerous radiating capillaries, the outer medulla by a preponderance of post-capillary venules (each with a perivascular space) and fewer capillaries, and the inner medulla by a preponderance of veins and fewer arteries and capillaries.

Lymphatic capillaries were not found in the parenchyma of the cortex and the outer zone of the medulla. However, they were of frequent occurrence in the inner zone of the medullary region where they originated. They then converged to form larger vessels. Each large lymphatic vessel coursed, in company with a lobular artery and vein, into an interlobular septum, thence passed over into a lobar lymphatic vessel in one of the major lobar hila.

Spatial Distribution of Reticular-epithelial Cells in the Cortex:

In TPA preparations, under low power, the cortex of each thymic lobule was lightly stained and was delimited peripherally by a TPA-stained line which ran along the inner side of the capsule, septa and septula. Throughout the cortical parenchyma intensely TPA-positive elements were widely scattered (Fig. 14).

However, the TPA-stained line seen around the periphery of the cortical parenchyma was found, under higher power, to correspond to a continuous layer of flattened reticular-epithelial cells. Such cells were interconnected by their elongate cytoplasmic processes and formed a continuous layer along the inner side of the capsule, the two surfaces of the interlobular septa and of the intralobular septula accompanying cortical capillaries (Figs. 15-18).

Most reticular-epithelial cells of the flattened type had a flattened or low triangular nucleus. The latter contained one or two round or oval, deeply TPA-positive nucleoli; the nucleoplasm was pale and contained a few scattered small chromatin granules; the nuclear membrane was clearly visible, owing to the aggregation of TPA-stained material on its outer surface. The cytoplasm of these cells (with usually very little affinity for most stains) was stretched into extensions which their intensely TPA-stained tonofibrils rendered distinct (Figs. 16-18).

Some of the reticular-epithelial cells of this type possessed a larger, triangular or oval nucleus, which protruded into the cortical parenchyma (Fig. 17). They were often found in pairs in which the two nuclei were contiguous.

The reticular-epithelial cells of the flattened category also constituted a continuous sheet investing perivascular connective tissue spaces surrounding lobular veins traversing the cortex singly (Fig. 19) or in company with a lobular artery and typical lymphatic

vessels(Fig. 16). Occasionally, they delimited compartments or "cortical pockets" occupied mainly by small lymphocytes and located at the periphery of the lobule right along the interlobular septa (Fig. 21). When traced through serial sections, such pockets were found to end blindly. They also formed a continuous lining along the occasional pericystic spaces.

In all these locations, the flattened reticular-epithelial cells, excepting those outlining the portions of the small peripheral compartments which were contiguous with the cortical parenchyma, always rested upon a thin, uninterrupted membrane or extracellular reticulum fiber, which stained with silver and PA-Schiff. The cortical stroma itself was formed by a small amount of argyrophilic fibers, most of which were associated with cortical capillaries. Thus, the flattened reticular-epithelial cells formed a continuous layer resting upon a thin, uninterrupted extracellular reticulum fiber and clearly demarcating the cortical parenchyma from the neighboring connective tissue (be it infiltrated with lymphocytes or not). Figures of diapedesis were very seldom observed across the epithelial sheet.

In the intervening space, the reticular-epithelial cells were stellate in shape. They represented the avidly TPA-stained elements seen, under low power (Fig. 14), in the cortical parenchyma. The nucleus of the cells of the stellate type contained 2 to 4 intensely TPA-positive, sharply defined nucleoli; the nuclear membrane was smooth and avidly stained with TPA (Fig. 20). The stellate appearance of these cells was due to their slender, elongate cytoplasmic

processes which their TPA-stained tonofibrils rendered visible. However the amount of demonstrable tonofibrils contained within each cytoplasmic extension was so scanty that the latter could not be seen throughout its length (Fig. 20). Like the flattened reticular-epithelial cells, those of the stellate variety were often found in pairs in which the two nuclei were in close contact with each other (Figs. 18 and 20).

In the cortical parenchyma the stellate reticular-epithelial cells were connected by their slender cytoplasmic extensions, not only to each other but also to those of the flattened type (Figs. 17 and 20). They constituted a loose network containing densely packed lymphoid cells in its meshes (Figs. 15, 20, 34).

Spatial Distribution of Reticular-epithelial Cells in the Medulla:

In TPA preparations, under low power, two zones could be readily identified in the medulla: a peripheral dark zone (for which the term "outer medulla" was proposed) and a central paler zone (referred to as "inner medulla"). The outer medulla was characterized by an abundance of avidly TPA-stained elements in its parenchyma, while the inner medulla was completely devoid of such elements and clearly delimited from the outer medulla by a TPA-positive line (Figs. 14, 22-24).

The cytological features and spatial distribution of the reticular-epithelial cells in both the outer and inner medulla will now be described.

A) Outer medulla.

The TPA-stained elements seen, under low power, in the outer medulla (Figs. 14, 22-24) corresponded to five types of reticular-epithelial cells that could be identified by their cytological characteristics: flattened, irregularly stellate, multibranched, hypertrophic and degenerating types.

The flattened reticular-epithelial cells of the outer medulla were morphologically identical to those of the cortex. As in the cortical area, they formed a continuous layer wherever they bordered upon tissue, namely along the intralobular septula accompanying outer medullary capillaries and on the outer surface of the perivascular connective tissue spaces surrounding post-capillary venules, lobular blood and lymphatic vessels as well as occasional pericystic spaces in this zone (Figs. 25-27, 32, 43, 51). They also formed a continuous sheet at the limit between outer and inner medulla (Figs. 22-24, 34). Like in the cortex, this layer of flattened epithelial cells rested upon a continuous extracellular reticulum membrane or fiber, which stained with silver and PAS (Figs. 28 and 44), except at the boundary between outer and inner medulla where this fiber was found to be discontinuous, or even absent (Figs. 35-38). It is noteworthy that the outer medulla contained more argyrophilic fibers than the cortex (Figs. 36 and 38).

The reticular-epithelial cells of the irregularly stellate type were the most abundant reticular cells of the outer medulla. They differed from the stellate reticular cells in that they were

more irregular in form, their nucleus was usually elongated and seldom contained a few ill-defined nucleoli, their cytoplasm was more voluminous and was sent into extended cytoplasmic processes particularly rich in tonofibrils running parallel to the long axis of the cell or cytoplasmic processes (Figs. 24-26).

Not infrequently, some widely scattered, reticular-epithelial cells of the irregularly stellate category appeared to contain more TPA-positive tonofibrils around their nucleus and in their cytoplasmic processes (Fig. 32). Occasionally, the cytoplasm of such cells was sent into numerous ramifying branches and was so rich in TPA-staining material that all other cytoplasmic components were obliterated. As a result, the whole "multibranched" cell, with its dendrite-like processes, assumed the appearance of a large neuroglial cell impregnated with silver.

In the parenchyma of the outer medulla, the reticular-epithelial cells of the irregularly stellate type, together with the occasional multibranched one, adhered tightly to each other by their branching cytoplasmic processes rich in tonofibrils and constituted a dense meshwork, in the small interstices of which loosely packed lymphoid elements accumulated (Figs. 22-26). At the cortico-medullary junction, they were connected to the more regularly stellate epithelial cells of the cortex (Fig. 34). No membrane or network of fibers were observed between cortex and outer medulla. These epithelial cells were also linked by their cytoplasmic extensions to the flattened reticular-epithelial cells of this zone (Figs. 25-27).

In the cellular reticulum, or "cytoreticulum", of the outer medulla, reticular-epithelial cells of the hypertrophic type were often observed. They were relatively large and rounded with a few blunt cytoplasmic processes. Their nucleus was large and usually oval in shape and contained 1 to 3 distinct nucleoli. In the nucleoplasm, the chromatin granules were more clumped. Their cytoplasm was plump and was characterized by the presence of numerous small PAS-positive granules of uniform size and of a scanty amount of TPA-staining tonofibrils which formed a thin rim under the cell limit (see Fig. 30). Despite these cytological alterations, these hypertrophic cells remained contiguous with the other cells of the outer medullary network, adhering tightly to them by their few short cytoplasmic extensions (Fig. 30).

Moreover, the reticular-epithelial cells of the hypertrophic type were often seen to undergo degeneration. Indeed, they were often found in association with reticular-epithelial cells in different stages of the degenerative process. In TPA-PAS preparations, the nucleus of a degenerating reticular-epithelial cell first enlarged and then became vacuolated. Concomitantly, the PAS-positive cytoplasmic granules increased in number and eventually coalesced to form a single large, PAS-positive, colloid-like inclusion or "intracellular cyst" (Fig. 29). The latter progressively augmented in size until it filled the entire cell, thus obliterating the nucleus and transforming the whole cell into an amorphous mass or "intercellular cyst" reacting positively to the PAS technique (Fig. 30).

Such hyaline degenerative changes usually occurred simultaneously in one or more contiguous hypertrophic reticular-epithelial cells. These degenerating epithelial cells appeared to be transformed eventually into the primary and central elements of Hassall's corpuscles in formation. Such newly formed corpuscles were oval structures consisting of one or two hyalinized reticular-epithelial cells surrounded by a group of flattened epithelial cells massed together in a more or less irregular fashion (Fig. 31). The reticular-epithelial cells near the hyaline central core showed signs of deposits of numerous TPA-positive tonofibrils closely associated with keratohyalin-like granules; their nuclei were pale, displayed fine chromatin granules along the nuclear membrane, and contained one or two nucleoli (Fig. 31). Some of these deep cells had lost their nuclei and had become transformed into avidly TPA-stained fibrillar areas. The peripheral epithelial cells of these corpuscles were spindle-shaped and possessed large nuclei with finely dispersed chromatin and one or two nucleoli; the most superficial ones were connected by their cytoplasmic processes rich in tonofibrils to the other cells of the outer medullary cytoreticulum (Fig. 31).

In well-developed Hassall's corpuscles, the central core of the Hassall's corpuscle had enlarged and contained cellular debris as well as nuclear outlines of reticular-epithelial cells and lymphocytes, suspended in a PAS-positive, colloid-like substance (Figs. 32 and 33). More spindle-shaped peripheral epithelial cells had been added from the cellular network and had molded themselves to the

sides of the central core with their long axis conforming to the circumference of a circle, thus producing a lamellar structure and yielding a concentric pattern.(Figs. 32 and 33). The peripheral cells near the central core presented evidence of degeneration (deposits and fragmentation of tonofibrils, accumulation of keratohyalin-like granules), while the most superficial ones often extended cytoplasmic processes which diverged from the main body to become continuous with one or more cells of the outer medullary network. It is noteworthy that deposits of tonofibrils increased as one progressed from the center of the corpuscle toward its periphery (Figs. 32 and 33).

As new epithelial cells were added to the periphery of the corpuscle, its central portion became more compact, so that often it had the appearance of a solid homogeneous, PAS-positive hyaline mass, without any remnant of cellular detail. No argyrophilic fibers or fibroblasts were found in association with Hassall's corpuscles at any stage of development.

B) Inner medulla.

In TPA preparations, no true reticular-epithelial cells with TPA-positive tonofibrils were recorded in the inner medulla (Figs. 14, 22-24). This zone, however, was clearly demarcated from the outer medulla by a continuous layer of flattened reticular-epithelial cells. It was characterized by the presence of numerous collagen and reticular fibers, which stained negatively with TPA and were associated with some fibrocyte nuclei. It was pale and was more heavily infiltrated with lymphocytic elements than the outer medulla (Figs. 35-39).

Furthermore, it contained numerous mast cells, whose cytoplasmic granules were rendered visible by counterstaining TPA-stained sections with toluidine blue or PA-Schiff.

Of extremely rare occurrence in the inner medulla were multinucleate giant cells (Fig. 52). The latter were found exclusively in the inner medulla and contained 10 to 14 nuclei disposed in a circle and giving a doughnut appearance to the cell. These nuclei were round to oval in shape and were characterized by a few scattered, small chromatin granules, a sharphy defined spherical or oval prominent nucleolus, and a thick intensely TPA-stained nuclear membrane. Their voluminous cytoplasm was unstained with TPA and appeared to be completely devoid of visible fibrillar elements.

The inner medulla was richly vascularized. It was characterized by a preponderance of post-capillary venules and veins and fewer arteries and capillaries. It also contained plexuses of typical efferent lymphatic vessels in close association with the arterial channels (Figs. 39, 40, 61).

In silver preparations, under low power, the inner medulla could be easily distinguished from the other zones of the thymic lobule (Figs. 35-39). First, it was dark owing to the fact that it was more heavily infiltrated with lymphocytes than the outer medulla, but the concentration of lymphocytic elements was slightly lower than that in the cortex. It followed that the inner medulla was not as darkly stained as the cortex. Secondly, the inner medulla contained much more argyrophilic fibers than the outer medulla and the cortex. In addition, this inner zone of the medullary

region was not delimited, in silver preparations, from the outer medulla by a continuous argyrophilic membrane or extracellular reticulum fibers.

When followed in serial sections in TPA or silver preparations, the inner medulla was found to be continuous with the perivascular spaces surrounding post-capillary venules in the outer medulla. These perivascular spaces contained the same cellular and connective tissue elements as the inner medulla itself. They were always separated from the outer medullary parenchyma by a continuous layer of flattened reticular-epithelial cells, which blended imperceptibly with the layer demarcating outer from inner medulla. Unlike the latter, the layer delimiting perivascular spaces rested upon a continuous membrane, which stained with silver or PAS (Fig. 28). As the perivascular spaces were nothing but a prolongation of the inner medulla around the post-capillary venules of the outer medulla, the continuous PAS-positive membrane or argyrophilic extracellular reticulum fiber was missing where the perivascular spaces passed into the inner medulla (Figs. 35-38).

The study of serial sections of thymus also revealed that the inner medulla extended through the outer medulla and cortex of the thymic lobule, to become continuous with an interlobular septum, in the form of a sheath or perivascular space around lobular blood and lymphatic vessels traversing these two zones (Figs. 41-44). Like the outer medullary perivascular spaces, those surrounding lobular blood and lymphatic channels were delimited by a layer of flattened reticular-epithelial cells resting upon a continuous argyrophilic

membrane. However, the reticular fibers within these spaces tended to be disposed in concentric layers separated by rows of lymphocytes (Figs. 42-44).

In summary then, the inner medulla is essentially a very irregular connective tissue space, which is richly vascularized and characterized by an abundance of argyrophilic fibers. It is heavily infiltrated with lymphocytes and is continuous with the perivascular spaces surrounding post-capillary venules in the outer medulla and lobular blood and lymphatic vessels as well as occasional cystic and duct-like spaces in their course through the outer medulla and cortex. It is coutinuous with the interlobular septa and completely devoid of true reticular-epithelial cells, but is clearly demarcated from the other zones of the thymic lobule by a continuous layer of flattened reticular-epithelial cells, which may or may not rest upon a continuous membrane or extracellular reticulum fiber.

Cystic and Duct-like Spaces in the Thymus:

If there were no true reticular-epithelial cells in the inner medulla, solid nests or masses of epithelial cells with TPA-stained fibrils were not infrequently encountered in this zone (Fig. 45). However, when such epithelial nests or masses were traced through serial sections, they were found to be tangentially cut branching cystic and duct-like spaces, which ended blindly (Figs. 46 and 47).

Some of these cystic structures occurred in close proximity to the boundary between outer and inner medulla, so that they appeared to be continuous with the outer medullary cytoreticulum.

The walls of this type of cysts contained as predominating elements numerous epithelioid cells, most of which were spherical in shape (Fig. 47). The nuclei of these epithelial cells were usually excentric in position and had a few marginal fine chromatin granules with a sharply defined prominent nucleolus and an intensely TPA-stained nuclear membrane. Their cytoplasm was granular and generally TPA-negative. Some epithelial cells in the cyst walls possessed irregularly shaped nuclei and some avidly TPA-positive fibrillar elements in their cytoplasm (Fig. 47). A small number of lymphocytes were sometimes found between these epithelial cells. The lumen of these cystic structures was filled with a homogeneous colloid-like material, which reacted positively with PAS.

The second and most characteristic type of cystic spaces were lined by a unilayer of cuboidal or columnar ciliated cells, whose cilia and basal bodies stained avidly with TPA (Figs. 50 and 51). These cysts were sometimes bordered in part by ciliated epithelial cells and in part by non-ciliated ones (Fig. 50). In some instances, a cyst was lined exclusively by non-ciliated cuboidal cells, which were characterized by a TPA-positive terminal web which extended across the apical cytoplasm immediately below the cell membrane to insert into TPA-stained terminal bars circumscribing the cell apices and a lateral web running down the lateral cell walls (Fig. 46). The content of the lumen of these cystic structures was very variable. The lumen was usually filled with a PAS-positive colloid-like substance, portions of which contained small and large vacuoles,

giving it a net-like appearance, while other portions were granular in texture. Not infrequently, the lumen was occupied by a large number of normal and degenerated lymphocytes, which were suspended in a colloid-like substance (Figs. 50 and 51). These cysts were often accompanied by duct-like spaces lined by a simple cuboidal epithelium (Fig. 46).

The cysts of the second type were found to branch and to extend from the inner medulla of one lobule to that of neighboring ones by passing through the axial pedicle or stem of thymic tissue linking together the parenchyma of adjacent lobules. Furthermore, these cysts were traced in the outer medulla and the cortex, in company with lobular blood and lymphatic vessels, ensheathed in a perivascular connective tissue space, i.e., a prolongation of the inner medulla toward the periphery of the thymic lobule. Therefore, they also occurred in interlobular septa (Fig. 51).

Thus, typical thymic cyst and duct-like spaces were limited to the territory of the inner medulla and never occurred in the parenchyma of either the cortex or outer medulla. The "intracellular" and "intercellular cysts" observed in the cellular reticulum of the outer medulla were structures arisen from degenerating reticular-epithelial cells of the hypertrophic type.

Mitoses of Reticular-epithelial Cells:

Some reticular-epithelial cells, which showed a cytoplasm well outlined by TPA-stained tonofibrils, were found in mitosis (Fig. 53). After injection of ^{3}H-thymidine, the radioautographs revealed that

about 2% of the tonofibril-containing reticular-epithelial cells were heavily labeled (Figs. 54 and 55). However, the great majority of labeled reticular cells were recorded in the outer medulla.

SUMMARY OF OBSERVATIONS ON THE THYMUS OF ADULT RATS

The present work revealed that there were two distinct compartments in the thymic lobule: an epithelial compartment and a connective tissue compartment (see Fig. 62). In the epithelial compartment which was found to comprise the cortex and the outer medullary zone (for which the name "outer medulla" was proposed), the reticular-epithelial cells with cytoplasmic tonofibrils formed a continuous lining along the following structures:

1) the capsule;
2) the interlobular septa;
3) the intralobular septula accompanying blood capillaries;
4) the inner medulla and its extensions in the form of perivascular connective tissue spaces surrounding:
   (a) post-capillary venules in the outer medulla,
   (b) lobular blood and lymphatic vessels as well as occasional thymic cysts and duct-like spaces in the outer medulla and cortex.

In the parenchyma, these reticular-epithelial cells were found to constitute a supporting network, loose in the cortex and denser in the outer medulla, enmeshing lymphocytic cells.

The connective tissue compartment was demonstrated to consist of two main components, i.e., the inner medulla and its prolongations toward the periphery of the thymic lobule which were designated "perivascular connective tissue spaces". This compartment was characterized by the absence of reticular-epithelial cells, but contained numerous argyrophilic fibers which served as a supporting framework. It was also found to be heavily infiltrated with lymphocytes.

APPENDIX: OBSERVATIONS ON EMBRYONIC AND NEONATAL THYMUS

The epithelial nature of the reticular-epithelial cells, as indicated by their desmosome-cell web component, has frequently been taken to be a reflection of the fact that the thymic anlage arises from the endoderm (or ectoderm?) forming the lining of the third pharyngeal pouch (Beard, 1902; Hammar, 1905; Maximow, 1909; Norris, 1938; and others). In view of this, it was of interest to determine, with the help of the TPA technique, the early time of appearance of the cells containing TPA-positive cell web material. The following chapter is a brief description of the observations made on the distribution of reticular-epithelial cells with demonstrable tonofibrils in the embryonic and early postnatal thymus.

13-day-old Embryos:

At the thirteenth day of embryonic development, the thymic anlage was still associated with the parathyroid, forming bilateral common thymus-parathyroid epithelial complexes, each being non-vascularized and surrounded by a delicate continuous basement membrane, which stained with silver and PAS. The thymus and parathyroid portions of the epithelial complex on each side were easily distinguished in PAS-toluidine blue preparations (Fig. 63). The epithelial cells of the parathyroid portion were filled with PAS-positive granules which were digested by the alpha-amylase of saliva and were therefore glycogen granules, while the cells of the thymus were

essentially devoid of glycogen.

In TPA preparations, reticular-epithelial cells with demonstrable tonofibrils were not recognizable in the thymus at this stage of embryonic development. The thymic cells were essentially "undifferentiated" conical epithelial cells with a pale-staining, round or oval nucleus containing scant amount of chromatin and a sharply defined spherical or oval nucleolus. These cells were arranged in acinar configuration about the remnant of the primitive pharyngeal duct (Fig. 64). The residual lumen of the latter, however, was lined by columnar epithelial cells which had the same nuclear characteristics as the other thymic cells, but which contained a delicate TPA-stained terminal bar-terminal web complex (Fig. 64). The thymic anlages of the two sides were not always at the same stage of development. When traced through serial sections, the thymus of one side sometimes contained a few lymphoblasts, which possessed nuclei with a typically large, irregular, central nucleolus, while that of the other side was devoid of such cells.

14-15-day-old Embryos:

By the fourteenth to the fifteenth day of embryonic development, the thymic anlages of the two sides had approximated each other in the midline, and lobulation of the thymic surface had occurred (Figs. 65 and 66). Several small, endothelial lined vascular channels had begun to extend from the capsule into the thymus (Fig. 66). The penetrating vessels accompanied by a few mesenchymal cells were

always separated from the thymic parenchyma by a delicate uninterrupted membrane, which stained with silver and PAS. A few delicate reticular fibers could be observed in the parenchyma.

There was a marked increase in PAS-reactivity owing to an over-all glycogen increase in the thymus. However, no reticular-epithelial cells with visible TPA-positive tonofibrils could be distinguished, While numerous lymphoblasts were present, the thymus had not yet assumed a lymphoid character.

16-19-day-old Embryos:

By 16-17 days of gestation, in PAS-toluidine blue preparations, a small, pale-staining region constituting an early medulla was observed, under low power, in the more central portion of the thymus in close association with the vascular network (Fig. 69). It consisted exclusively of reticular-epithelial cells, some of which contained PAS-positive material in their cytoplasm. As shown in Figure 69, it is noteworthy that the first medulla developed in close association with an interlobular septum.

In TPA preparations, the reticular-epithelial cells of the early medulla were provided with a large amount of strongly TPA-positive tonofibrils (Figs. 67 and 68). Associated with the latter were avidly TPA-stained intracytoplasmic granules of varying sizes and shapes, which exhibited the same morphological characteristics and staining properties as keratohyalin-like granules observed in adult thymuses (Fig. 68). In fact, this medullary region was already the site of Hassall's body formation. However, reticular-epithelial

cells with demonstrable tonofibrils were lacking in the remainder of the thymic parenchyma, in which large lymphocytes were evident and medium and small lymphocytes were present in relatively large numbers.

As growth continued through the 19th day of embryonic development, the morphology of the thymus was essentially the same as that described for the 16-17-day-old embryos. The medulla was composed of tonofibril-containing reticular-epithelial cells and a few lymphoid elements.

20 Days Prenatal - 1 Day Postnatal Rats:

In the perinatal period, the medulla had developed tremendously, while the cortex showed a lesser degree of development. The medullary region contained, in addition to Hassall's corpuscles, an abundance of tonofibril-containing reticular-epithelial cells arranged in groups or forming a dense meshwork, in the small interstices of which large, medium and small lymphocytes accumulated. This region was richly vascularized. In the cortex, no reticular cells with demonstrable tonofibrils could be observed. Moreover, it was in this period, that mediastinal lymph nodes made their appearance on each side of the thymus.

2 Days Postnatal Rats:

By the second day of postnatal life, two zones, namely a thin and pale subcapsular zone and a broader and darker deep zone, could be easily distinguished in the thymic cortex in PAS-toluidine blue

preparations (Fig. 70). The subcapsular portion of the cortex was thin with a diffuse border toward the deep cortex. It was dominated by reticular cells and immature lymphoid cells with a high mitotic activity, the cell density nevertheless being greater than that in the medulla. Contrasting with this pale-staining, thin, subcapsular zone, the deep cortex was broad and dark, owing to the abundance of small lymphocytes, and showed a sharp border against the medulla.

In TPA preparations, under low power, the medulla and deep cortex were more darkly stained than the subcapsular, mitosis-rich zone, because they were dominated by anastomosing reticular-epithelial cells containing delicate TPA-positive tonofibrils. Reticular cells with visible tonofibrils could not be identified in the light subcapsular cortex.

3-5 Days Postnatal Rats:

In 4-day-old rats, the subcapsular cortex had disappeared, the histological picture being relatively more uniform throughout the cortex. By the fifth day of postnatal life, the cortex had increased in size, and reticular-epithelial cells rich in TPA-stained tonofibrils were visible throughout medulla and cortex but were not apparent along the capsule, interlobular septa and intralobular septula.

6-7 Days Postnatal Rats:

In 6-day-old rats, the thymic cortex was broader than the medulla, and reticular-epithelial cells with demonstrable tonofibrils

were still not recognizable where the thymic parenchyma bordered on connective tissue. However, by the seventh day of postnatal life, the morphology of the thymus, in regard to the distribution of tonofibril-containing reticular-epithelial cells, was essentially the same as that described for the thymus of adult rats.

Summary of Observations on the Embryonic and Neonatal Thymus:

In summary, then, the appearance of reticular-epithelial cells with demonstrable tonofibrils, some of which showing signs of degeneration, coincided with early medulla formation by the sixteenth day of embryonic development. In fact, at this stage of development, the medulla was already the site of Hassall's body formation. As growth continued through the first day after birth, tonofibril-containing reticular-epithelial cells were confined to the territory of the now well-developed medullary region, in which they accumulated into Hassall's corpuscles and were anastomosed by their cytoplasmic extensions to constitute a dense meshwork. It was only by the second day of postnatal life that reticular-epithelial cells with delicate TPA-stained tonofibrils were seen in the deep cortex of the thymus, which was easily distinguishable from the thin, mitosis-rich, less differentiated subcapsular area. With time, these cells became gradually more apparent in the peripheral portion of the cortex and along the connective tissue capsule, septa and septula, until each thymic lobule, by the seventh day of postnatal life, showed a histological picture similar to that obtained in the thymus of adult rats.

Fig. 12 Cross section of rat thymus. The connective tissue line which crosses the thymus in the center (arrow) separates right and left lobes. Similar, though shorter lines across the parenchyma are the interlobular septa which subdivide each lobe into lobules. Some septa, as can be seen, in the lobe on the right, do not completely severe the lobule, so that the parenchyma of one lobule is continuous with that of neighboring ones. The paler areas are the medulla which are surrounded by dark cortex. Elastic arteries may be seen below and mediastinal lymph nodes at the left side.

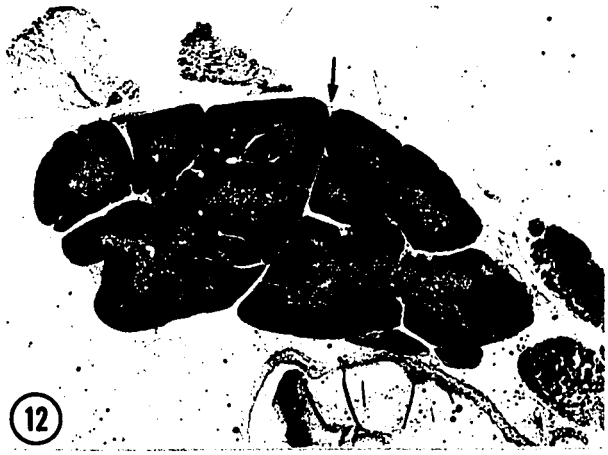
Carnoy fixation and silver (X10).

Fig. 13 Thymus. Portion of a lobule comprising a dark cortex and a paler medulla.

Carnoy fixation and silver

Fig. 14 Thymus. Portion of a lobule showing (going from left to right) cortex (C), outer medulla (OM) and inner medulla (IM). Two arrows point to a thin darkly stained under the unstained capsule. Note widely scattered, intensely stained elements in cortex (C). Such elements are numerous in outer medulla (OM) and absent in inner medulla (IM). A stained line separates outer medulla (OM) from inner medulla (IM).

Carnoy fixation and TPA (x225).



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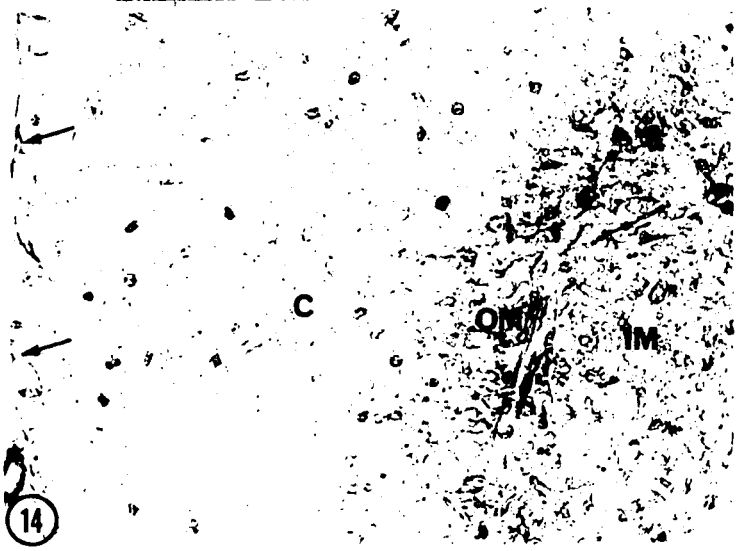

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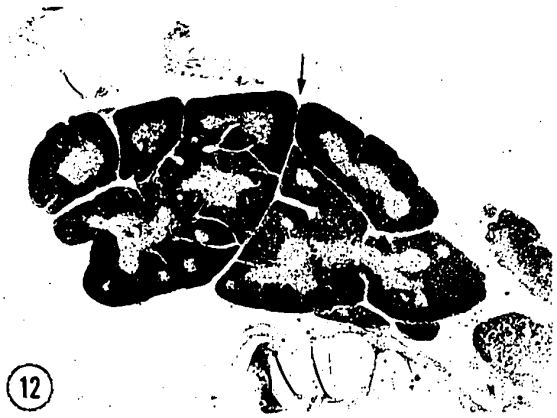

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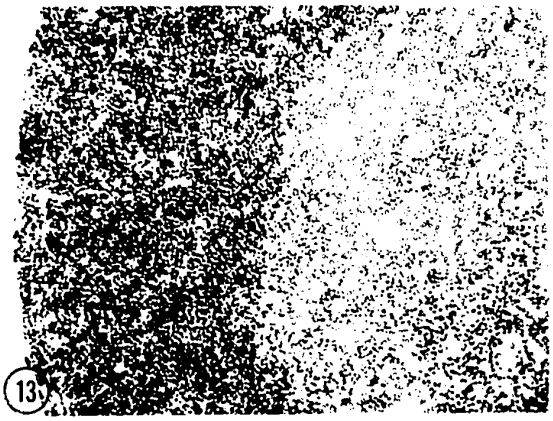

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Fig. 15 Thymus. An interlobular septum (IS) containing a blood vessel (BV) is completely delimited from the cortex (C) by a continuous layer of flattened reticular-epithelial cells. The arrow points to the nucleus of a flattened reticular-epithelial cell. Note the presence of intensely stained fibrillar material in the endothelial cells of the blood vessel (BV)

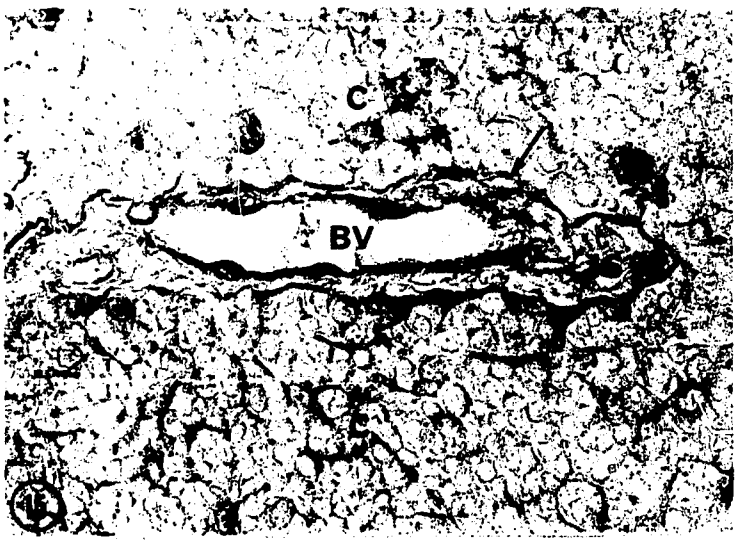
Carnoy fixation and TPA (x640).

Fig. 16 Thymus. An interlobular septum (IS) or large perivascular space infiltrated with pale-staining lymphocytes and containing a vein (V) and an artery (A) is completely demarcated from the cortex (C) on each side by a continuous layer of flattened reticular-epithelial cells. The 3 arrows point to the nuclei of the cells.

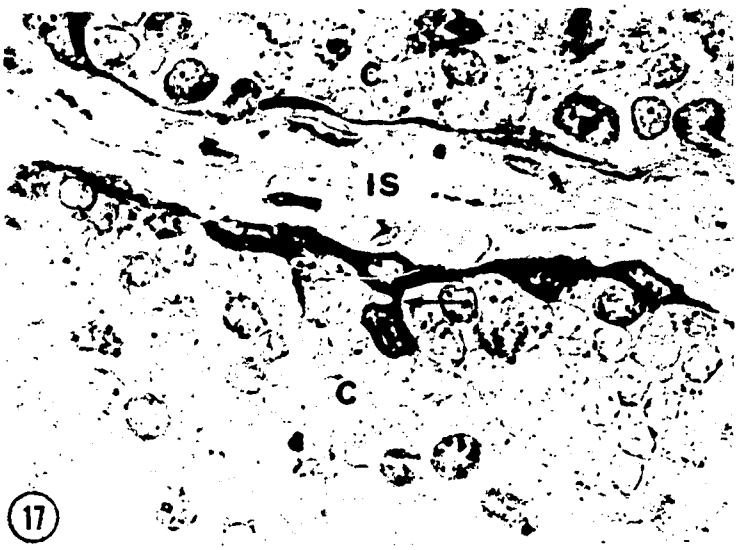
Carnoy fixation and TPA (x550).

Fig. 17 Thymus. An interlobular septum (IS) with TPA-negative collagenous fibers and two well-stained fibrocyte nuclei is separated from the cortex (C) on each side by a continuous layer of flattened reticular-epithelial cells with elongated or low triangular nuclei. The arrows points to a connection between a flattened and a stellate reticular-epithelial cell. Note that the nucleolus of the stellate cell is very prominent.

Carnoy fixation and TPA (x1160).


C
BV


C
IS
C
V
A


C
IS
C


C
BV
IS
C
15
C
IS
C
V
A
16
C
IS
C
17

Fig. 18 Thymus Cortex. Two capillaries (CP) surrounded by a continuous layer of intensely stained, flattened reticular-epithelial cells are visible. The arrow points to two stellate reticular-epithelial cells whose nuclei are present in pair.

Carnoy fixation and TPA (x1320).

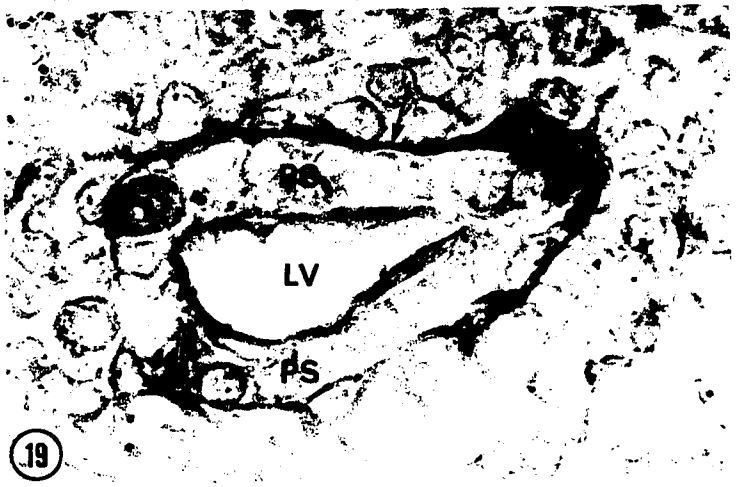
Fig. 19 Thymus Cortex. A lobular vein (LV) is surrounded by a perivascular space (PS) which is completely demarcated from the surrounding cortex by a continuous layer of flattened reticular-epithelial cells containing deeply stained tonofibrils (arrow).

Carnoy fixation and TPA (x1320).

Fig. 20 Thymus Cortex. Widely scattered among the pale-staining lymphocytes are well-stained stellate reticular-epithelial cells containing 1 to 3 nucleoli. The nuclei and the long, slender cytoplasmic processes of these cells are also visible. The processes are interconnected to form a loose network enmeshing lymphocytes. The arrow points to a pair of nuclei of two adjacent stellate cells.

Carnoy fixation and TPA (x650).

18



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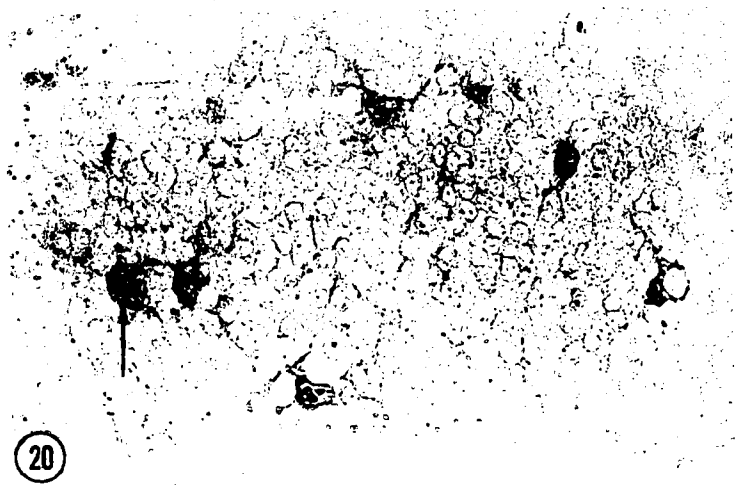

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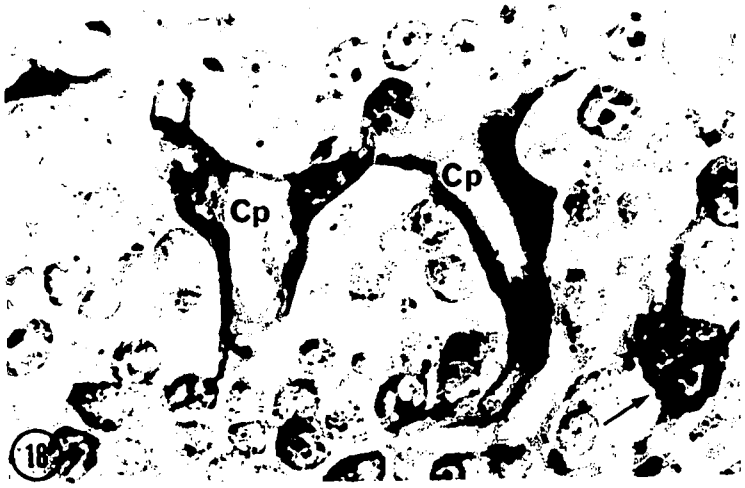

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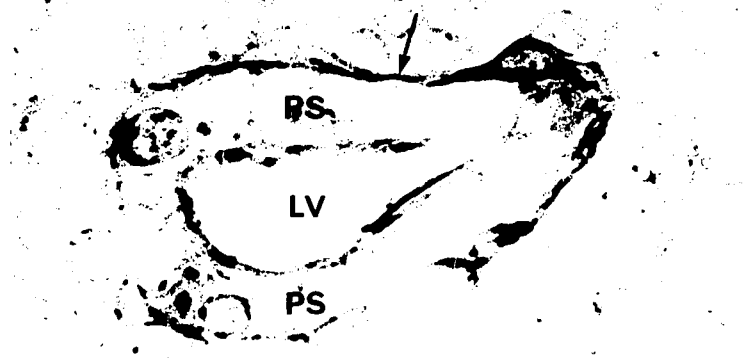

19

20

Fig. 21 Thymus Cortex. Above the artery (A) with intensely stained smooth muscle cells is a "cortical pocket" which is completely delimited from the surrounding cortex abobe and the TPA-negative collagenous bundles of the interlobular septum by continuous layer of flattened reticular epithelial cells (arrow).

Carnoy fixation and TPA (x550).

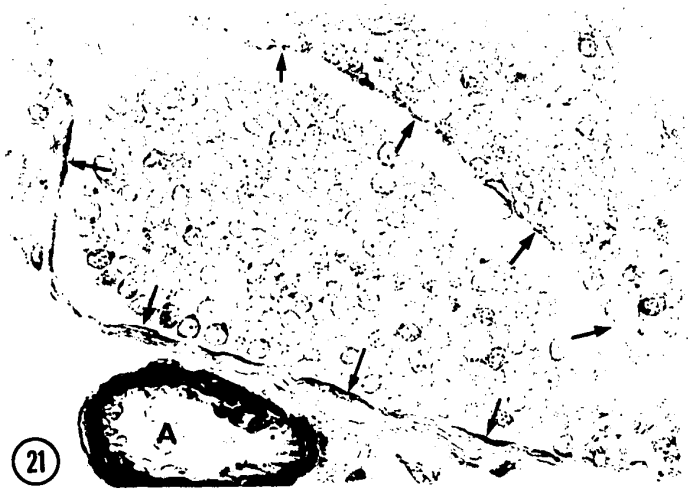

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Fig. 22 Thymus Medulla. From left to right, the end of an interlobular septum (IS) delineated by a well-stained line and infiltrated with lymphocytes, a cortex (C) with widely scattered, darkly stained elements, a dark outer medulla (OM) with numerous dark elements, a lighter inner medulla (IM), a dark outer medulla (OM), and a cortex (C) may be seen in succession. The inner medulla (IM) is surrounded by the outer medulla (OM).

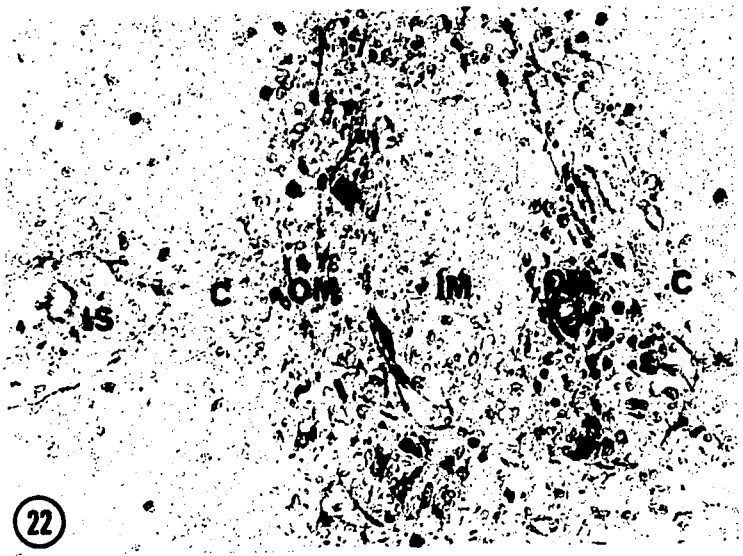
Carnoy fixation and TPA (x130).

Fig. 23 Thymus Medulla. The dark outer medulla (OM) may be seen to completely surround the lighter inner medulla (IM).

Carnoy fixation and TPA (x225).

Fig. 24 Thymus Medulla. In the outer medulla (OM), the intensely stained reticular-epithelial cells are interconnected to form a dense network holding lightly stained lymphocytes in its meshes. The lighter inner medulla (IM) is completely devoid of reticular-epithelial cells, contains large numbers of lymphocytes and is richly vascularized. The arrow points to a blood vessel.

Carnoy fixation and TPA (x550).



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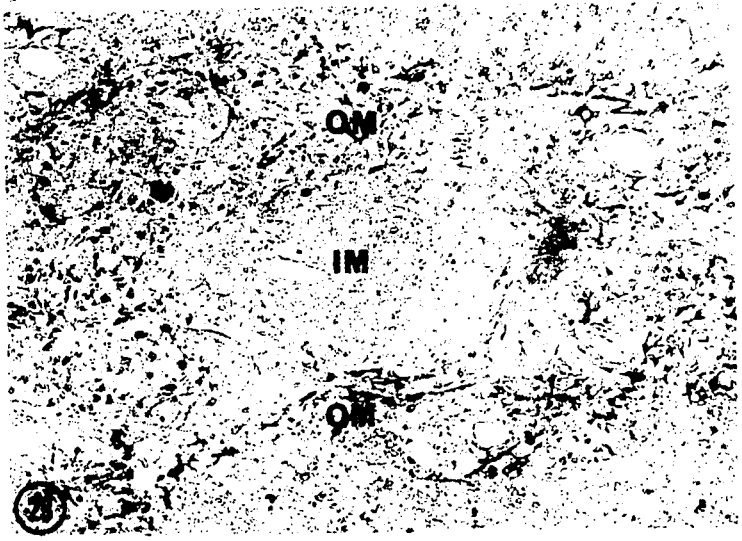

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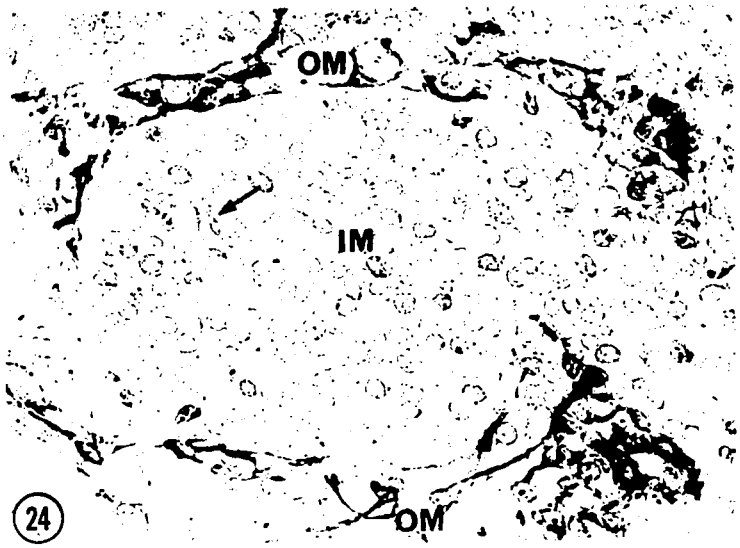

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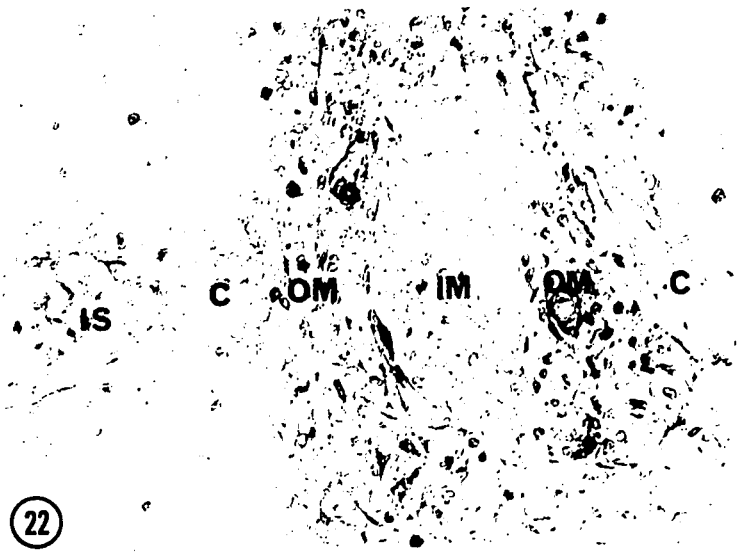

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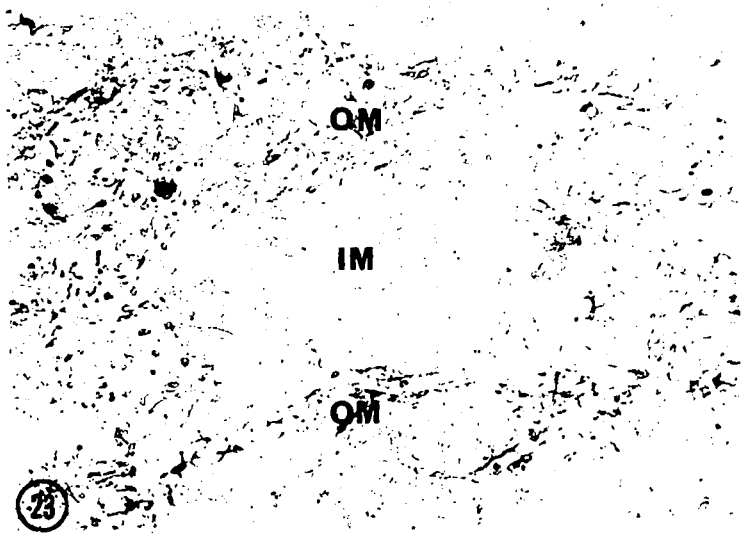

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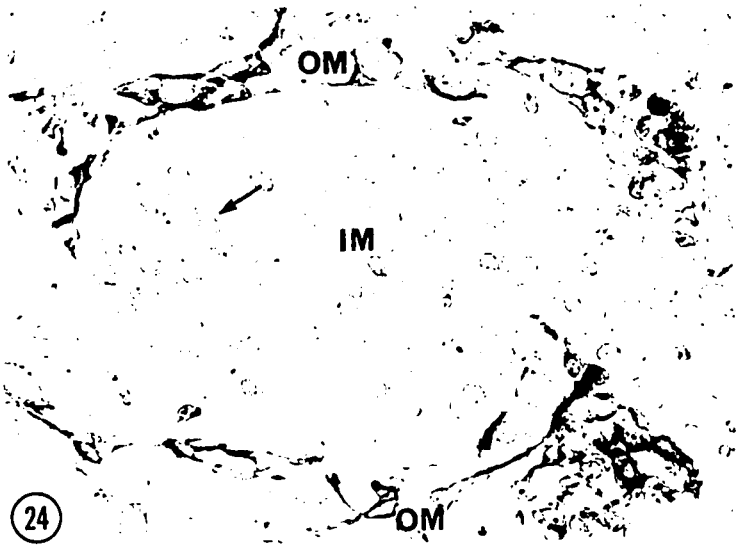


24

Fig. 25 Thymus Outer Medulla. Two post-capillary venules (v) are completely surrounded by perivascular spaces (PS). The latter are clearly demarcated by a layer of darkly stained, flattened reticular-epithelial cells showing connections (arrow) with the irregularly stellate reticular-epithelial cells of this zone.

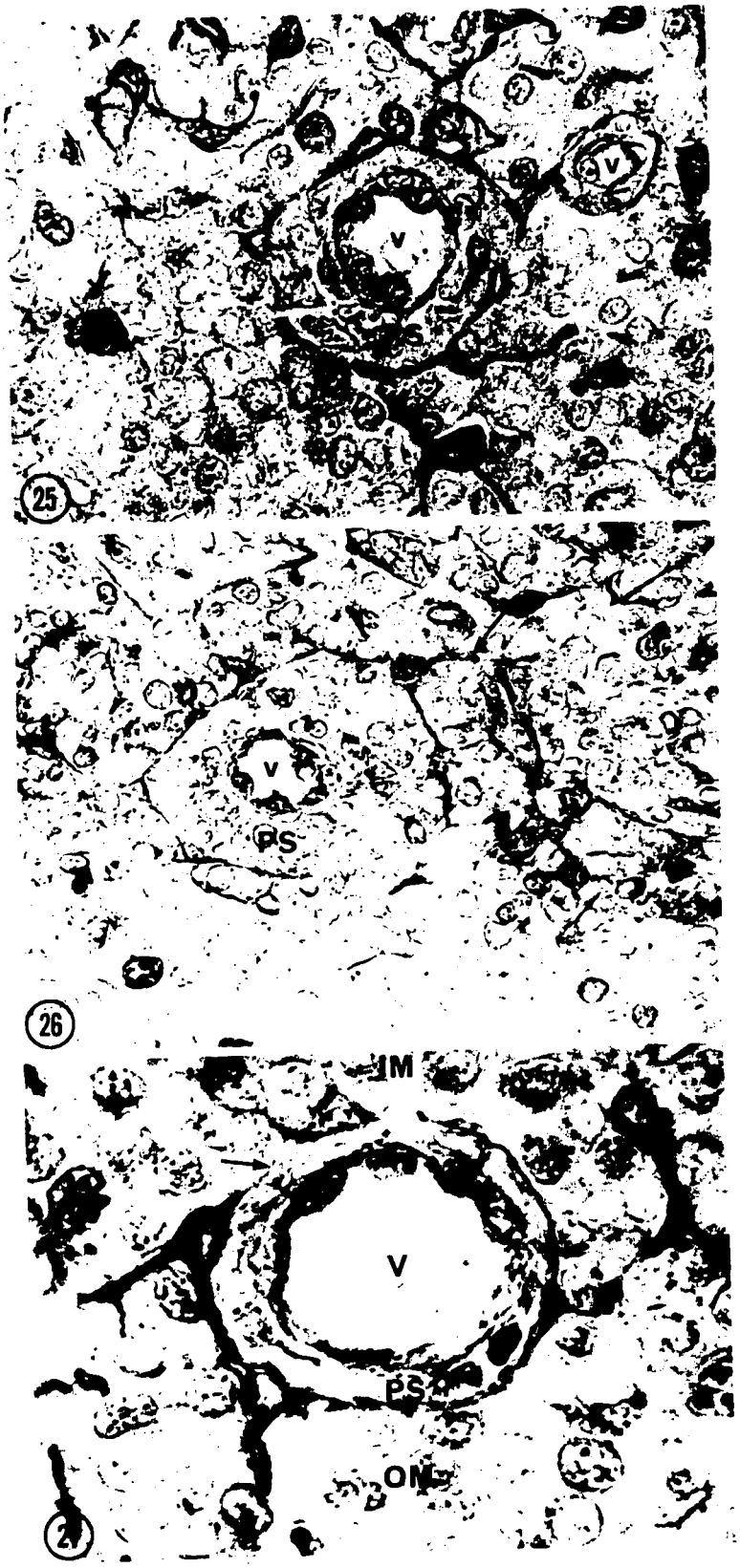
Carnoy fixation and TPA (x880).

Fig. 26 Thymus Outer Medulla. A post-capillary venule (v) is surrounded by a perivascular space (PS). The latter is delineated by a layer of intensely stained, flattened reticular-epithelial cells. This layer, however, is missing below, toward the pale-staining zone which corresponds to the inner medulla. The arrow points to a connection established between a flattened epithelial cell of the layer and the cellular reticulum of this zone. Note the dense network formed by the reticular-epithelial cells.

Carnoy fixation and TPA (x640).

Fig. 27 Thymus Outer Medulla. A venule (V) can be seen at the limit between outer medulla (OM) and inner medulla (IM). It is surrounded by a perivascular space (PS) which merges above with the inner medulla (IM). Note that the layer of reticular-epithelial cells delimiting the space is missing (arrow) on the inner medulla (IM) side where the PS becomes continuous with it.

Carnoy fixation and TPA (x1200).


V
V
25
V
PS
26
IM
V
PS
OM
27

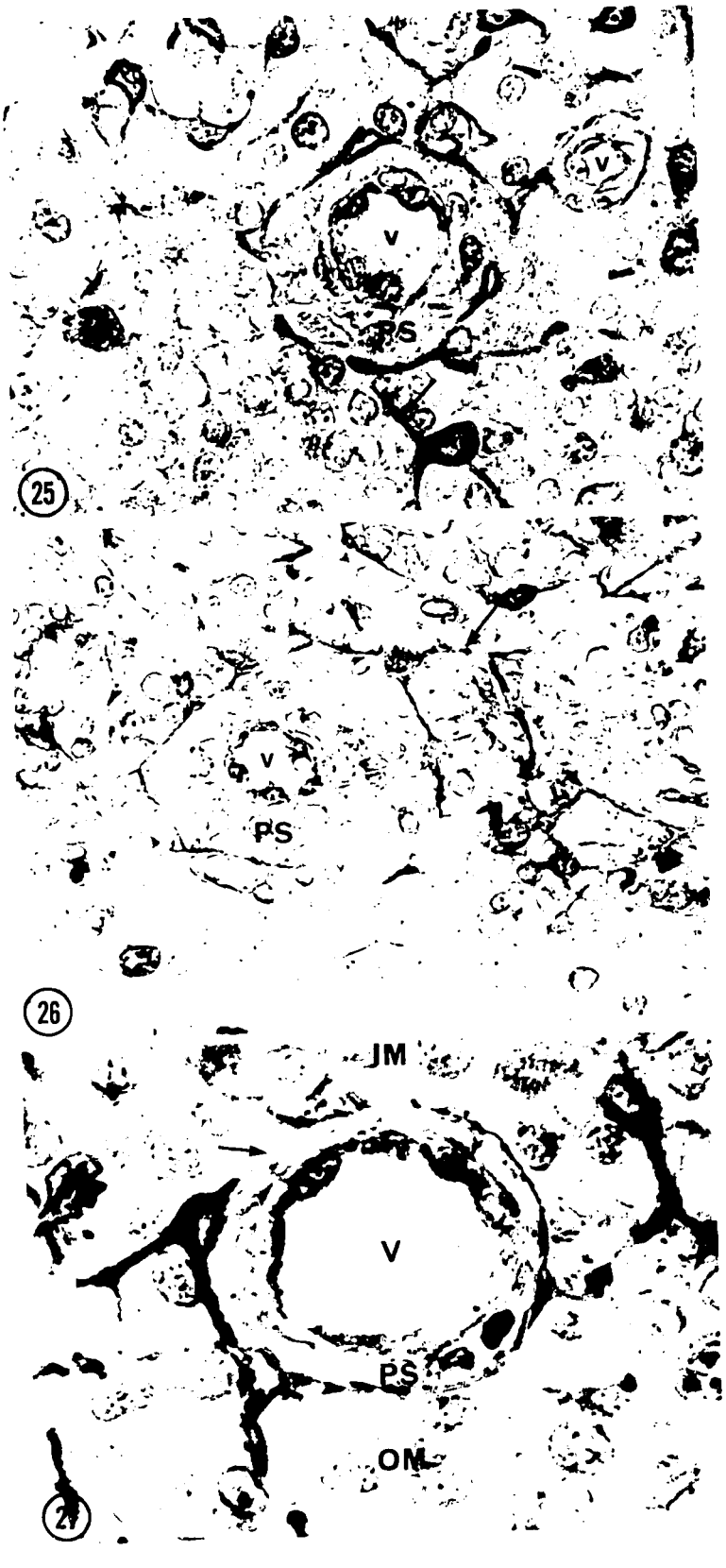
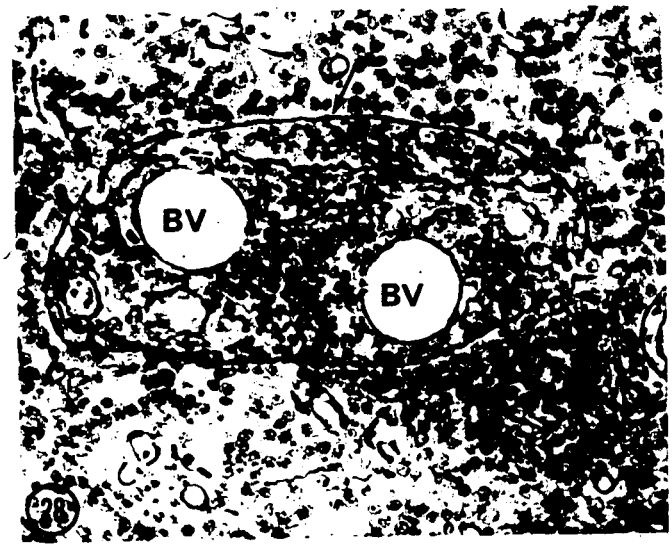

v
v
PS
25
v
PS
26
JM
V
PS
OM
27

Fig. 28 Thymus Outer Medulla. A perivascular space (PS) rich in argyrophilic fibers surrounds two blood vessels (BV). The arrow points to the continuous membrane or reticulum fiber demarcating the space from the surrounding tissue.

Carnoy fixation and PA-silver (x400).


BV
BV
28

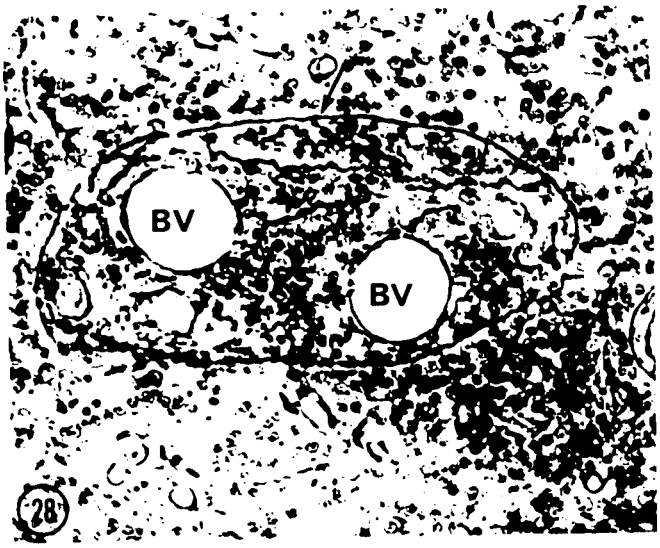
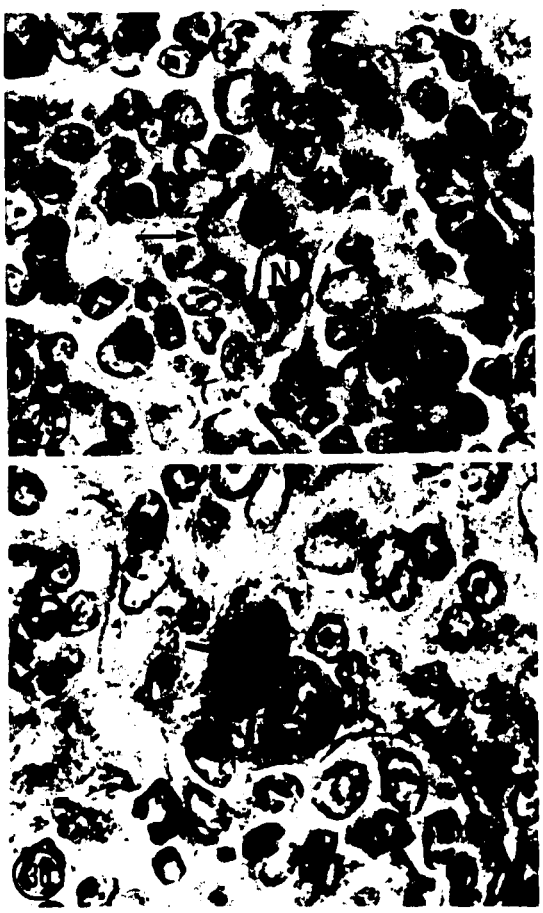

BV
BV
28

Fig. 29 Thymus Outer Medulla. The pale-staining nucleus (N) of a degenerating reticular-epithelial cell is clearly visible. In the cytoplasm of this cell, a single, large, PAS-positive inclusion (vertical arrow) is present. The horizontal arrow points to a TPA-stained tonofibril under the cytoplasmic membrane of this cell.

Carnoy fixation and TPA-PAS (x1120).

Fig. 30 Thymus Outer Medulla. An irregularly stellate reticular-epithelial cell (1) containing TPA-stained tonofibrils (arrow) is linked to a large, rounded, hypertrophic reticular-epithelial cell (2). The latter is characterized by a vacuolated nucleus with chromatin clumps and by a cytoplasm containing fine PAS-positive granules of uniform size and a thin rim of TPA-positive fibrils under the cytoplasmic membrane. To the left of this cell (2), a large mass of PAS-positive material (3) is visible and represents a degenerated reticular-epithelial cell which is still attached to the other cells of the outer medullary cellular reticulum.

Carnoy fixation and TPA-PAS (x1200).



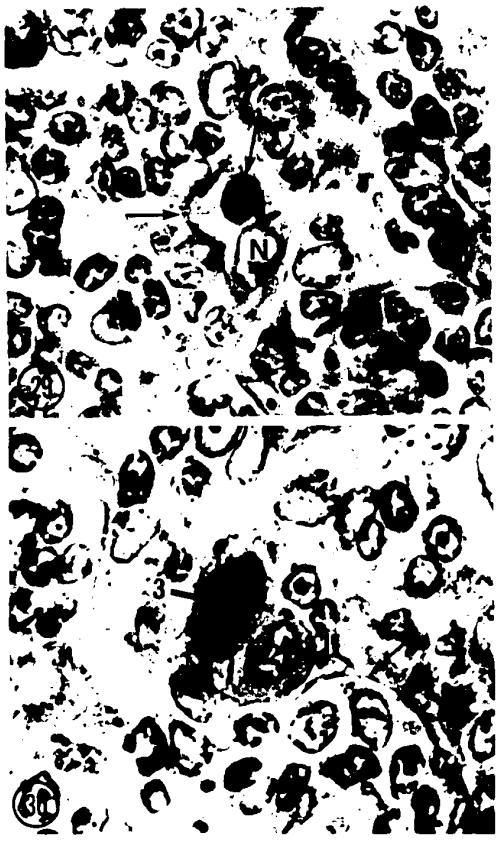

N
3

Fig. 31 Thymus Outer Medulla. Two small Hassall's corpuscles (H) are visible. The corpuscle on the upper left corner displays a pale-staining center surrounded by processes of reticular-epithelial cells rich in TPA-stained tonofibrils showing fragmentation (1) near the central core. In the other corpuscle the nuclei of the concentrically arranged epithelial cells rich in tonofibrils are visible. Note the connections (2 and 3) between the cells in the wall of the corpuscle and those of the outer medullary cellular reticulum. Note also an intensely stained, keratohyalin-like granule below and near the corpuscle.

Carnoy fixation and TPA (x880).

Fig. 32 Thymus Outer Medulla. A Hassall's corpuscle (H) is seen with a large central core containing degenerating cell nuclei and surrounded by concentric layers of tonofibril-containing reticular-epithelial cells. A capillary (Cp) surrounded by a layer of flattened epithelial cells is also visible. The arrow points to a darkly stained, stellate reticular-epithelial cell.

Carnoy fixation and TPA (x570).

Fig. 33 Thymus Outer Medulla. A large Hassall's corpuscle (H) is visible. Its central core contains a crescent-shaped nucleus of a degenerating epithelial cell above which are some degenerated lymphocyte nuclei. The core is surrounded by concentric layers of TPA-stained epithelial cells. The most peripheral cells seem to contain more TPA-stained material than the more central ones and send out processes (arrow) toward similar cells of the cellular reticulum.

Carnoy fixation and TPA (x1200).

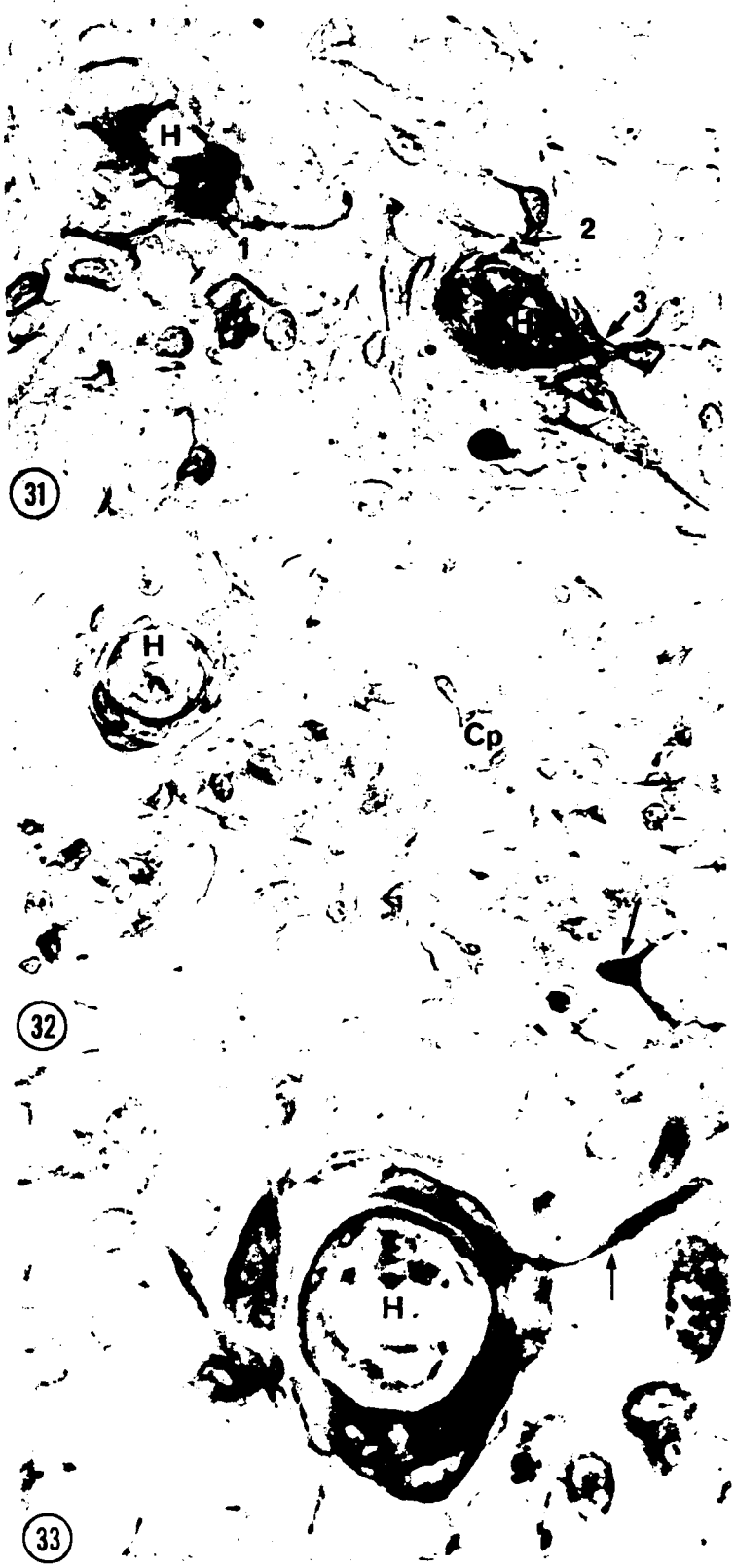
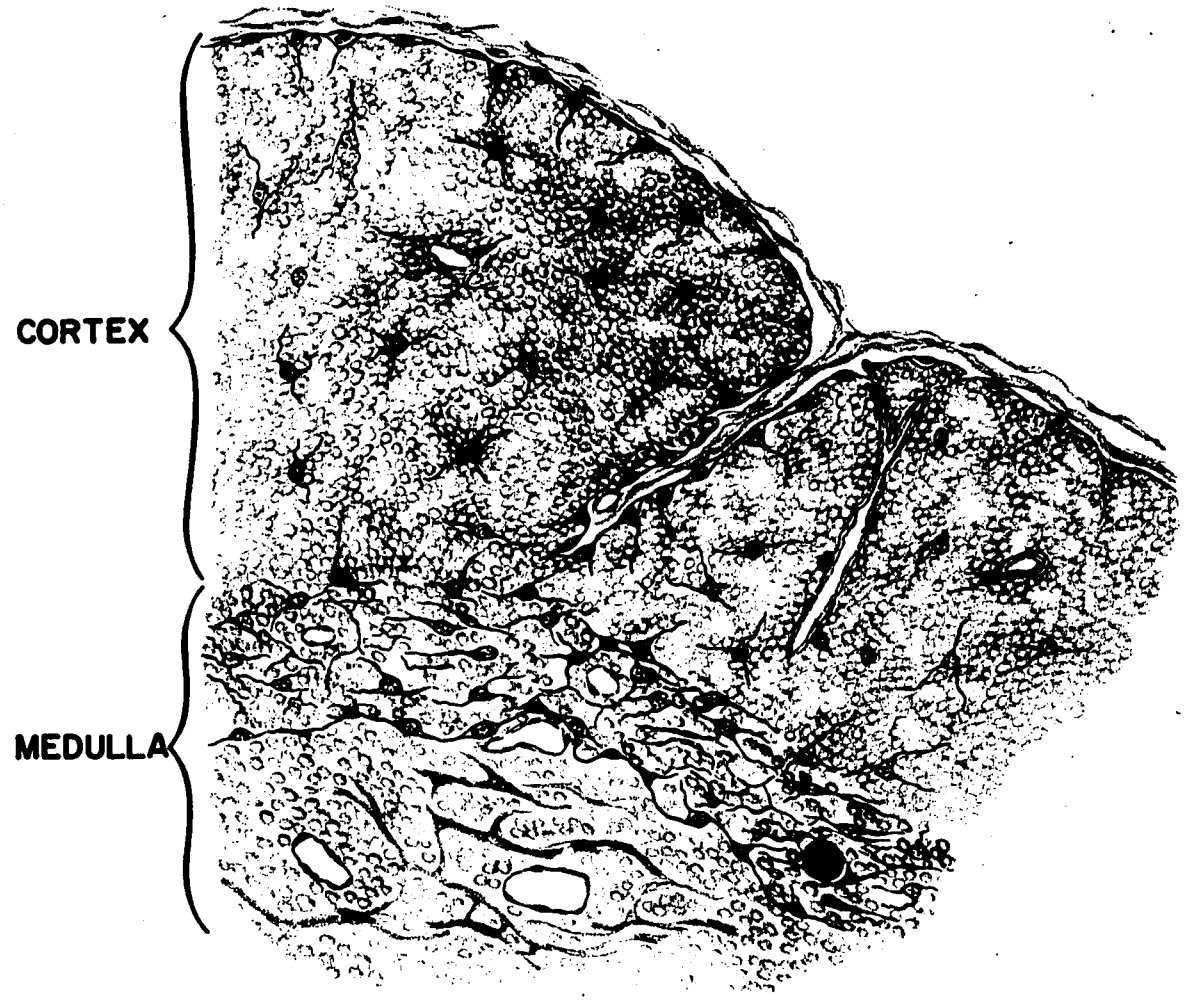

H
1
2
3
31
H
Cp
32
H
33

Fig. 34 Drawing showing the distribution of TPA-stained reticular-epithelial cells in the cortex and outer medulla of the thymic lobule (Description given in P. 85).


CORTEX
MEDULLA

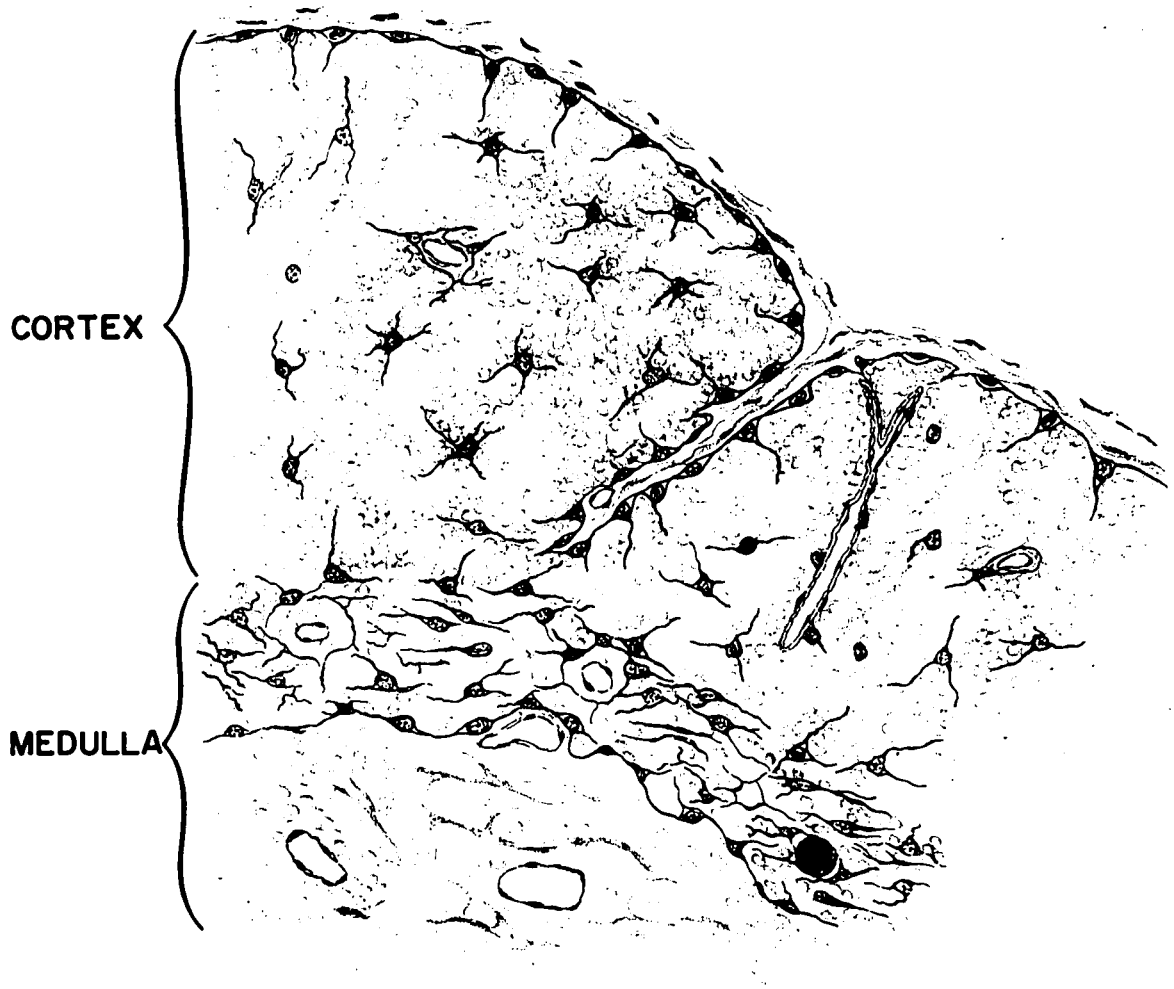
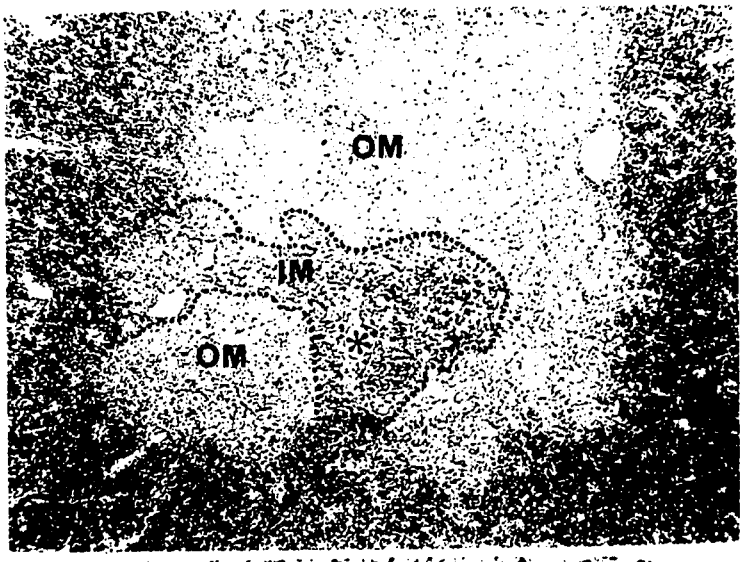

CORTEX
MEDULLA

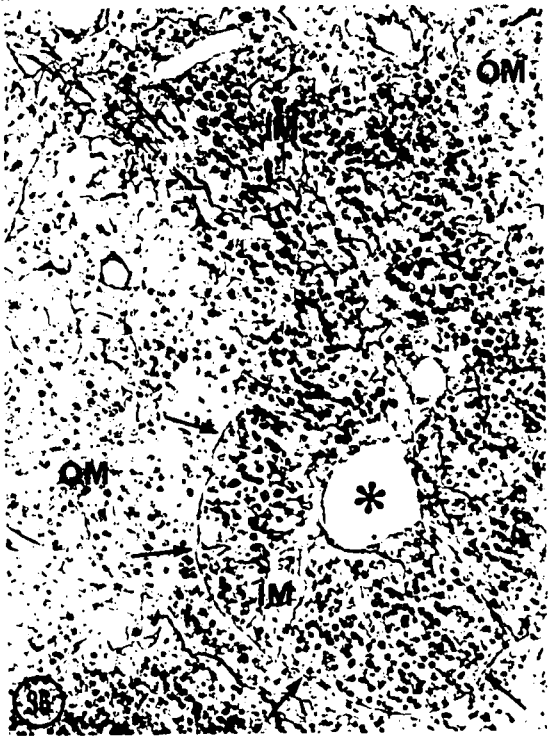
Fig. 35 Thymus. This shows a portion of a thymic lobule. The dotted line delineates the inner medulla (IM) from the surrounding outer medulla (OM) and darkly stained cortex. Note that the inner medulla (IM) extends through the outer medulla (OM) into the dark cortex. Note also that the inner medulla (IM) stains more intensely than the outer medulla (OM), owing to its greater concentration of lymphocytes. The asterisk is in the lumen of a blood vessel which is seen in Fig. 36 at a higher magnification.

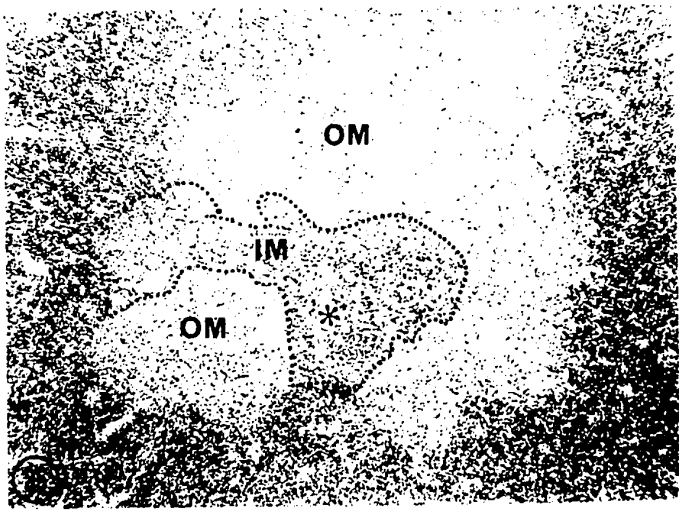
Carnoy fixation and PA-silver (x75).

Fig. 36 Thymus. This is a higher power view of a portion of the medullary region seen in Fig. 35. It is evident that the inner medulla (IM) contains more lymphocytic cells and more argyrophilic fibers than the outer medulla (OM). Note that no continuous argyrophilic membrane separates outer from inner medulla. However, the portion of the inner medulla (IM) which extends into the outer medulla (OM) is clearly delineated by an uninterrupted argyrophilic membrane (arrows).

Carnoy fixation and PA-silver (x250).


OM
IM
OM


OM
IM
OM
IM
38


OM
IM
OM

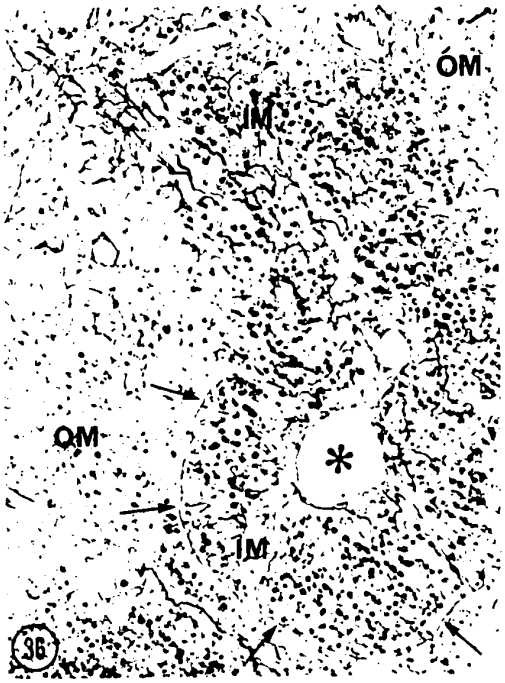
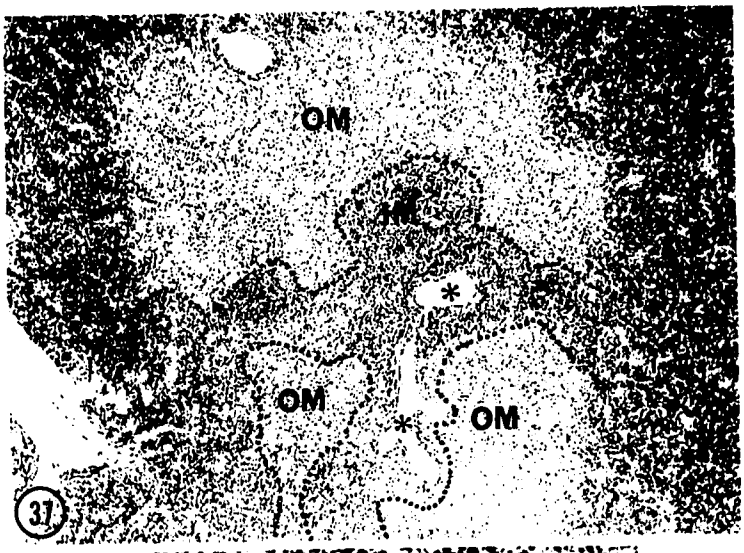

OM
IM
OM
IM
36

Fig. 37 Thymus. This shows a portion of a thymic lobule. The dotted line demarcates the inner medulla (IM) from the outer medulla (OM). Note that the inner medulla (IM) is almost as darkly stained as the cortex. Note also that the inner medulla (IM) crosses the outer medulla (OM) and penetrates the dark cortex. The asterisks are located in the lumina of blood vessels which are seen in Fig. 38 at a higher magnification.

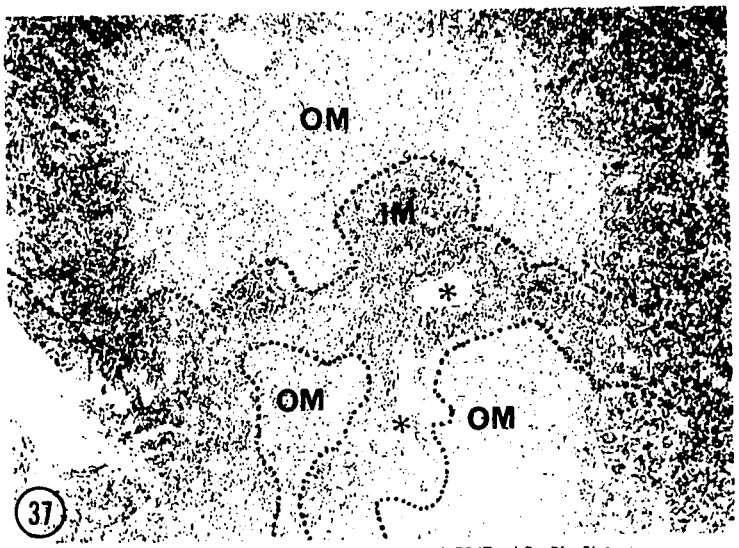
Carnoy fixation and PA-silver (x75).

Fig. 38 Thymus. This is a higher power view of the medullary region seen in Fig. 37. Again the inner medulla (IM) can be seen to contain more lymphocytes and argyrophilic fibers than the neighboring outer medulla (OM). Note that an uninterrupted argyrophilic membrane or fiber (arrows) delimits the inner medulla (IM) where it crosses the outer medulla (OM). Such a membrane is absent elsewhere.

Carnoy fixation and PA-silver (x250).


OM
OM
OM
37


OM
OM
38


OM
IM
OM
OM
37

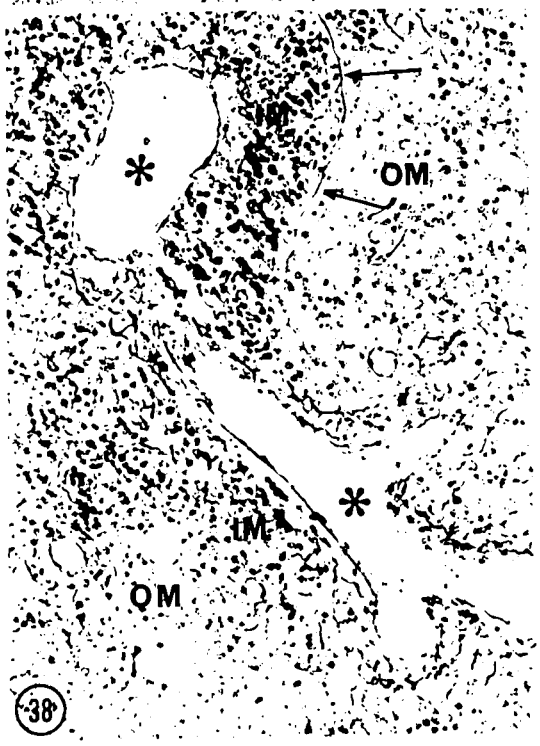
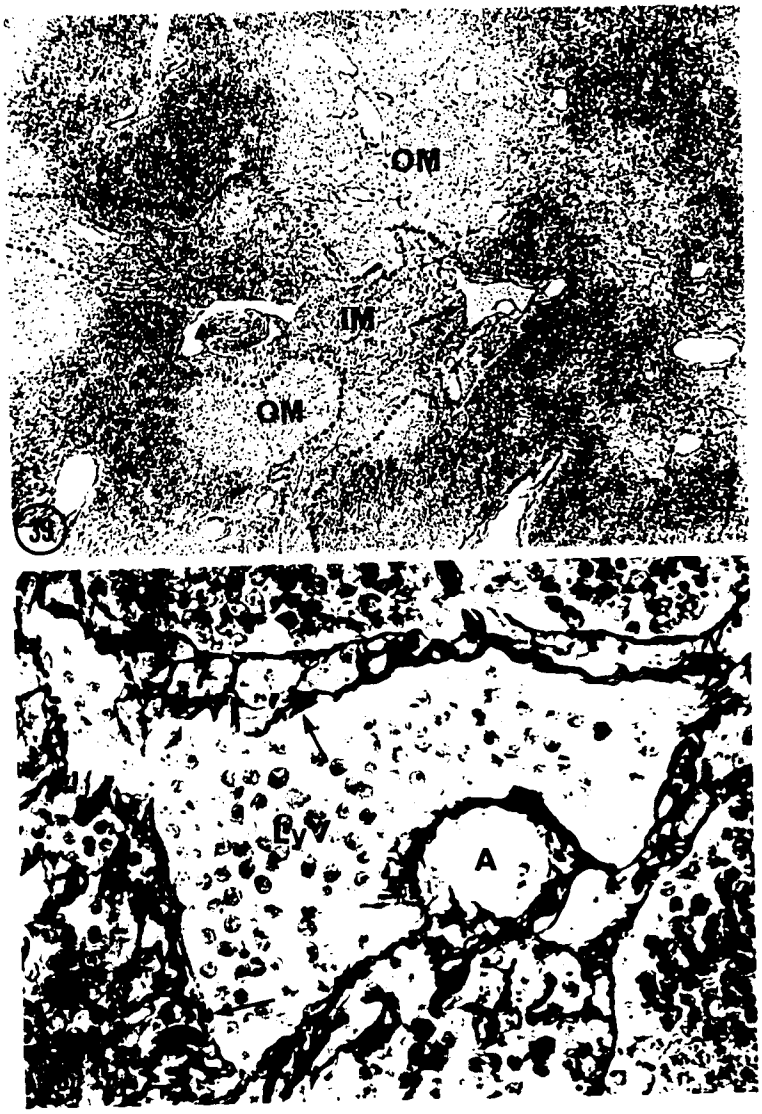

IM
OM
IM
OM
38

Fig. 39 Thymus. This shows a portion of a thymic lobule. The dotted line demarcates the inner medulla (IM) from the surrounding outer medulla (OM) and darkly stained cortex. The arrow in the inner medulla (IM) points to a typical lymphatic vessel. Note that the inner medulla (IM) of one lobule is connected to that of neighboring ones by an axial pedicle or stem of inner medullary tissue richly provided with argyrophilic fibers.

Carnoy fixation and PA-silver (x70).

Fig. 40 Thymus. This is a higher power view of the lymphatic vessel (LyV) seen in the inner medulla in Fig. 39. Such a vessel is endothelium lined (arrows) and usually accompanies an inner medullary arterial channel (A). The lumen of the lymphatic vessel in the inner medulla is filled with lymphocytes, and its wall is closely associated with a dense network of argyrophilic fibers.

Carnoy fixation and PA-silver (x570).


OM
IM
OM
39
LV
A

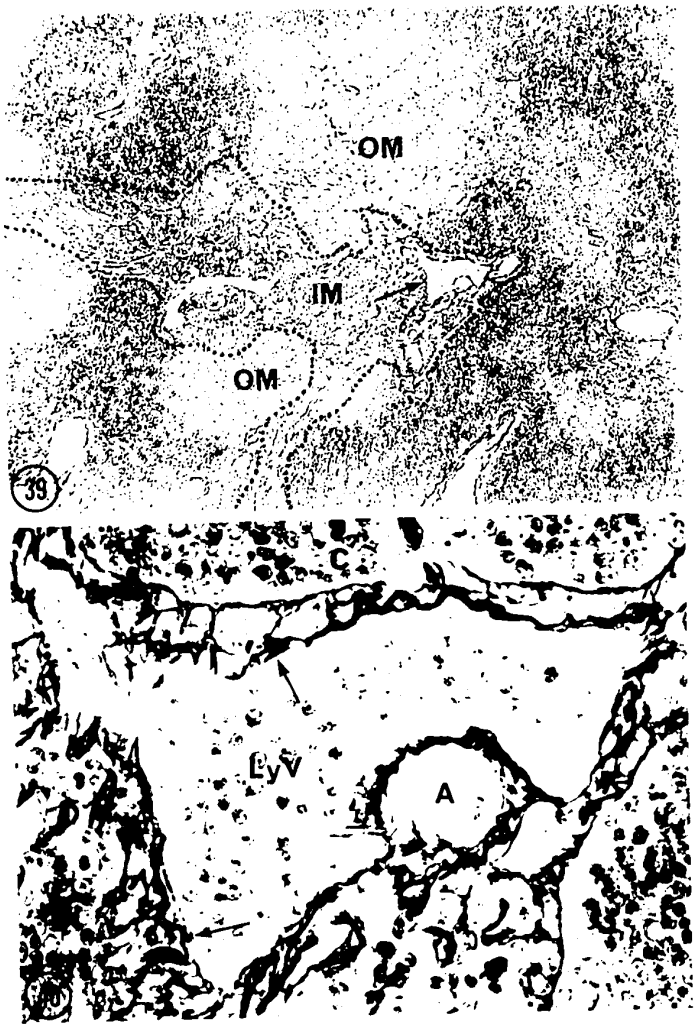

OM
IM
OM
39
C
LyV
A

Fig. 41 Thymus, A lobule is shown under low power. The pale-staining medulla is surrounded by the dark cortex. The asterisk is in the lumen of a longitudinally sectioned lobular vein which extends from the medullary region, through the dark cortex, into an interlobular septum.

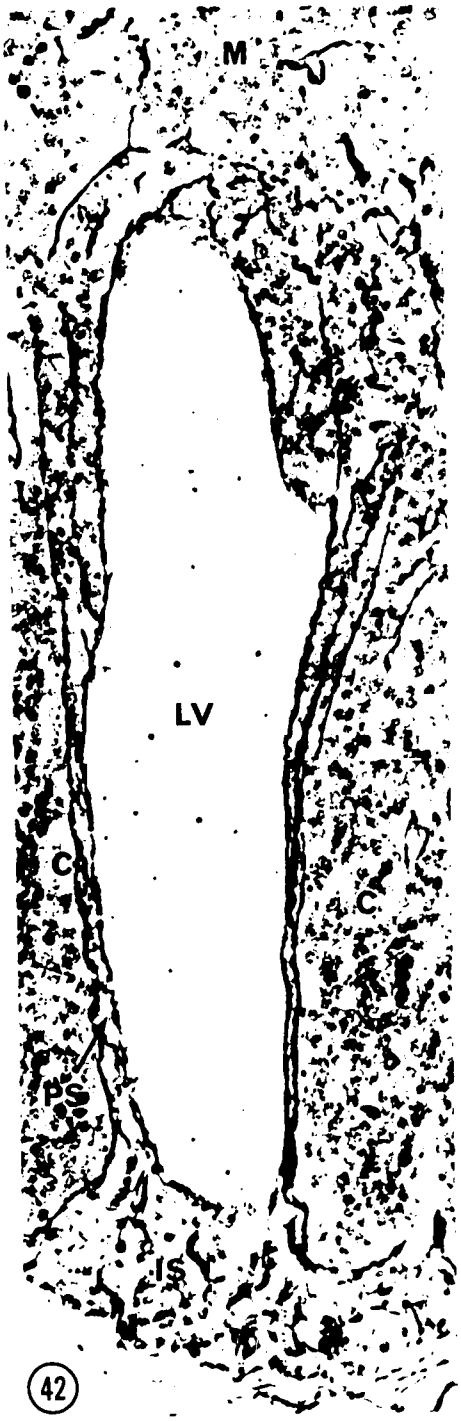
Carnoy fixation and PA-silver (x65).

41

41

Fig. 42 Thymus. This shows a higher power view of the same lobular vein seen with the asterisk in Fig. 41. The lobular vein (LV) is seen to extend from the medullary region (M) into an interlobular septum (IS)). A perivascular space (PS)), delimited from the cortical parenchyma by a continuous membrane, is visible on each side of the vessel. It merges with the medullary tissue (M) which is characterized by a network of argyrophilic fibers. The latter, however, are arranged in two or three concentric layers in the perivascular space (PS) toward the periphery of the lobule. Note the presence of lymphocytic cells in the interlobular septum (IS). When traced through serial sections, such extralobular or septal lymphocytes were found to fade out in the connective tissue.

Carnoy fixation and PA-silver (x420).



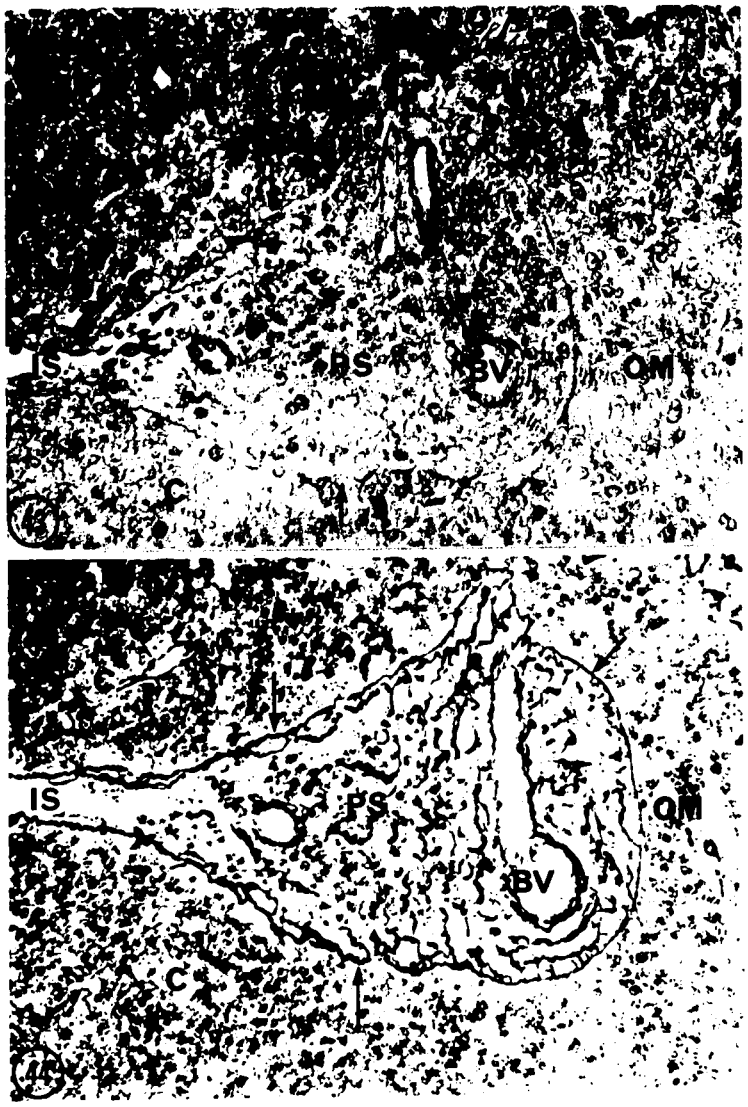
(42)

Fig. 43 Thymus. An obliquely cut perivascular space (PS) is visible around a blood vessel (BV). This space is continuous with an interlobular septum (IS) and is completely delimited from the outer medulla (OM) and the cortex (C) by an uninterrupted TPA-stained line (indicated by arrows) representing a continuous layer of flattened reticular-epithelial cells.

Carnoy fixation and TPA (x250).

Fig. 44 Thymus. This shows the same field as in Fig. 43, but impregnated with silver in an adjacent section. The perivascular space (PS) contains concentrically disposed lamellae of argyrophilic fibers surrounding a blood vessel (BV). This space is continuous with an interlobular septum (IS) and is completely demarcated from the outer medulla (OM) and the dark cortex (C) by an uninterrupted argyrophilic membrane (indicated by arrows) on which rests the epithelial layer seen in Fig. 43.

Carnoy fixation and PA-silver (x250).


IS
PS
BV
OM
C
IS
PS
BV
OM
C

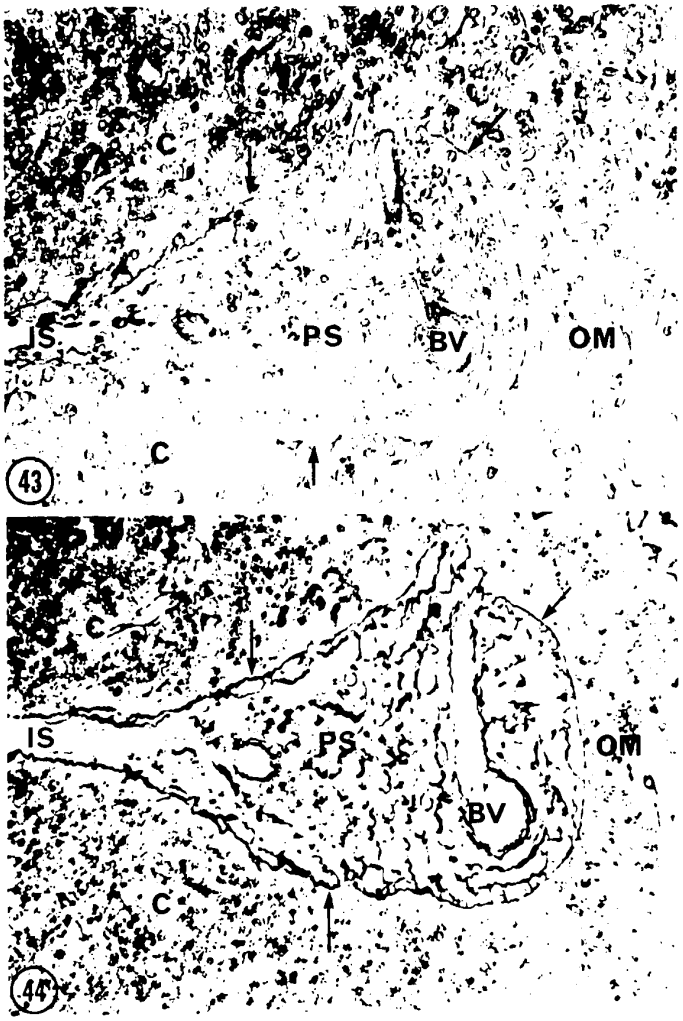

C
IS
PS
BV
OM
C
43
C
IS
PS
BV
OM
C
44

Fig. 45 Thymus Inner Medulla. This shows a large, solid mass of epithelial cells containing TPA-stained cytoplasmic fibrils in the inner medulla.

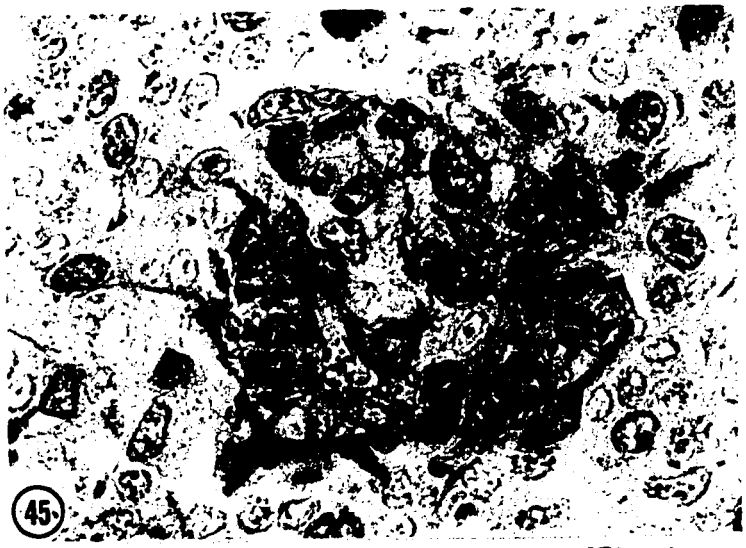
Carnoy fixation and TPA (x1200).

Fig. 46 Thymus Inner Medulla. When the solid epithelial mass seen in Fig. 45 was followed in serial sections, it was found to represent a tangentially sectioned duct-like space which is shown here in transverse section. The lumen (Lu) of the duct usually contains a TPA-negative, PAS-positive colloid-like substance in which are suspended a variable amount of degenerating lymphocytic cells. The duct is usually lined by cuboidal cells with a terminal bar-terminal web complex (terminal bars are indicated by arrows). The most peripheral cells in the wall of the duct are epithelial cells rich in TPA-stained fibrillar material.

Carnoy fication and TPA (x1200).

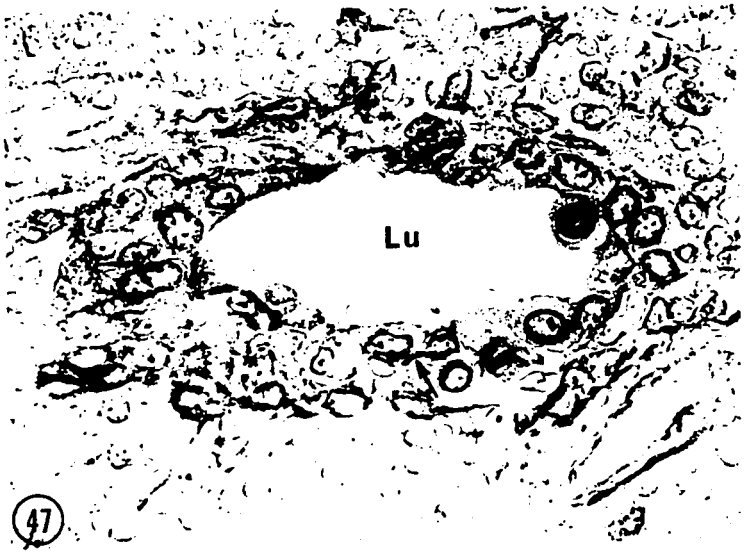
Fig. 47 Thymus Inner Medulla. This shows another type of cystic structures which are not infrequently encountered in the inner medulla. The lumen (Lu) usually contains a TPA-negative, colloid-like substance. The wall is formed by epithelioid rounded cells, some of which contain TPA-stained fibrils (indicated by arrows).

Carnoy fixation and TPA (x670).



45.

46



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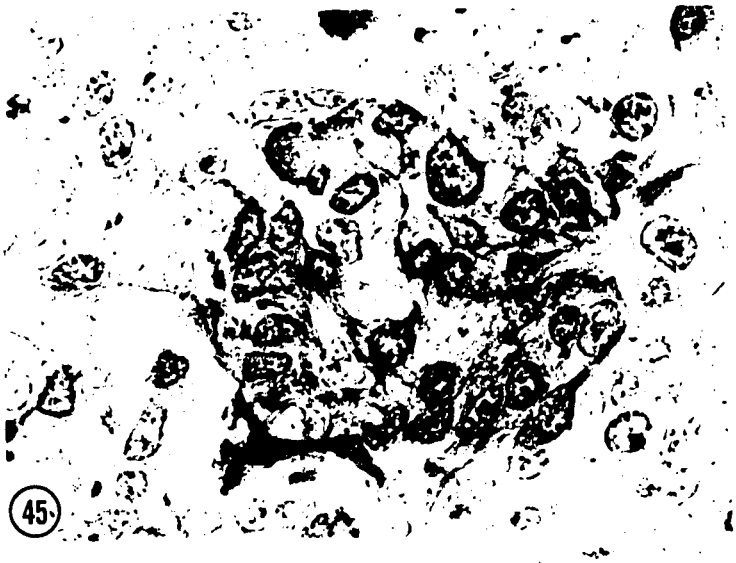

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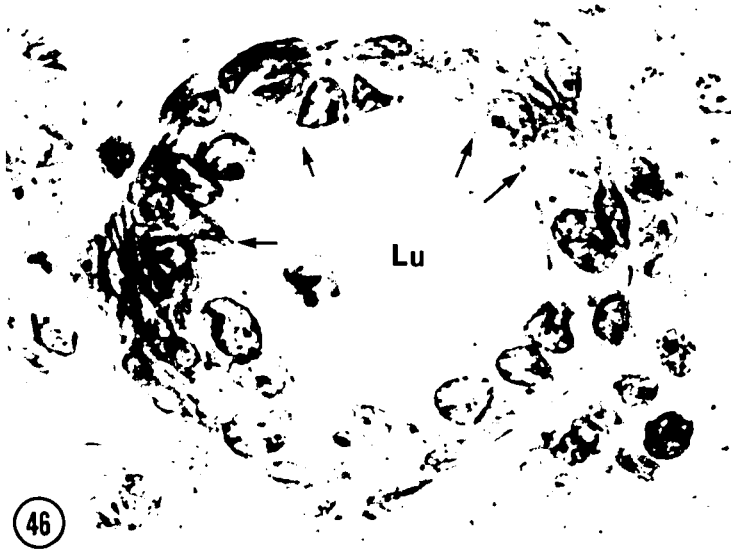

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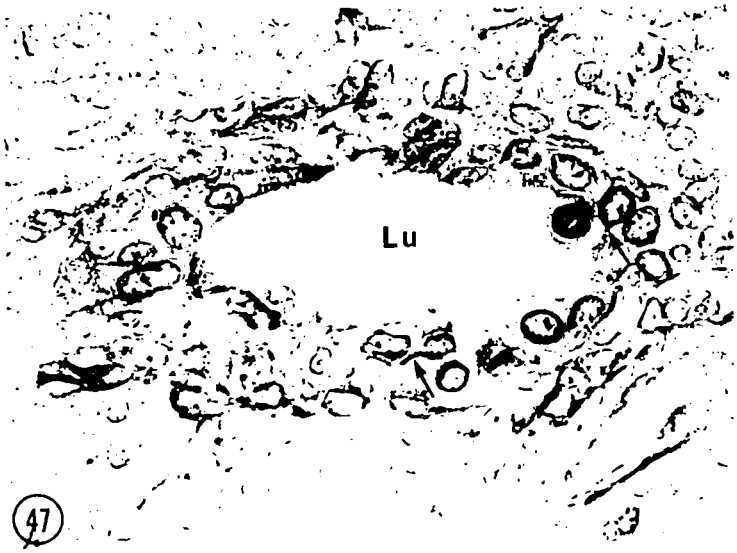


47

Fig. 48 Thymus Inner Medulla. This shows a portion of the inner medulla (IM) characterized by an abundance of argyrophilic fibers. The arrows point to a cyst in the inner medulla (IM).

Carnoy fixation and PA-silver (x120).

Fig. 49 Thymus Inner Medulla. The dotted line delineates the inner medulla (IM) in the central portion of a thymic lobule. The inner medulla (IM) is readily recognized by the absence of TPA-stained reticular-epithelial cells, which are seen as darkly stained elements in the neighboring outer medulla. The arrows point to the wall of the same cyst seen in Fig. 48, but in an adjacent TPA-stained section.

Carnoy fixation and TPA (x120).

Fig. 50 Thymus Inner Medulla. This shows a higher power view of the cyst seen in Fig. 49. Note that the cyst is lined partly by cuboidal cells, and partly by ciliated cells. The cilia with their basal bodies are intensely stained. The lumen (Lu) of the cyst contains normal and degenerating lymphocytic elements.

Carnoy fixation and TPA (x550).


IM


IM
49


IM
Lu
50

48

49

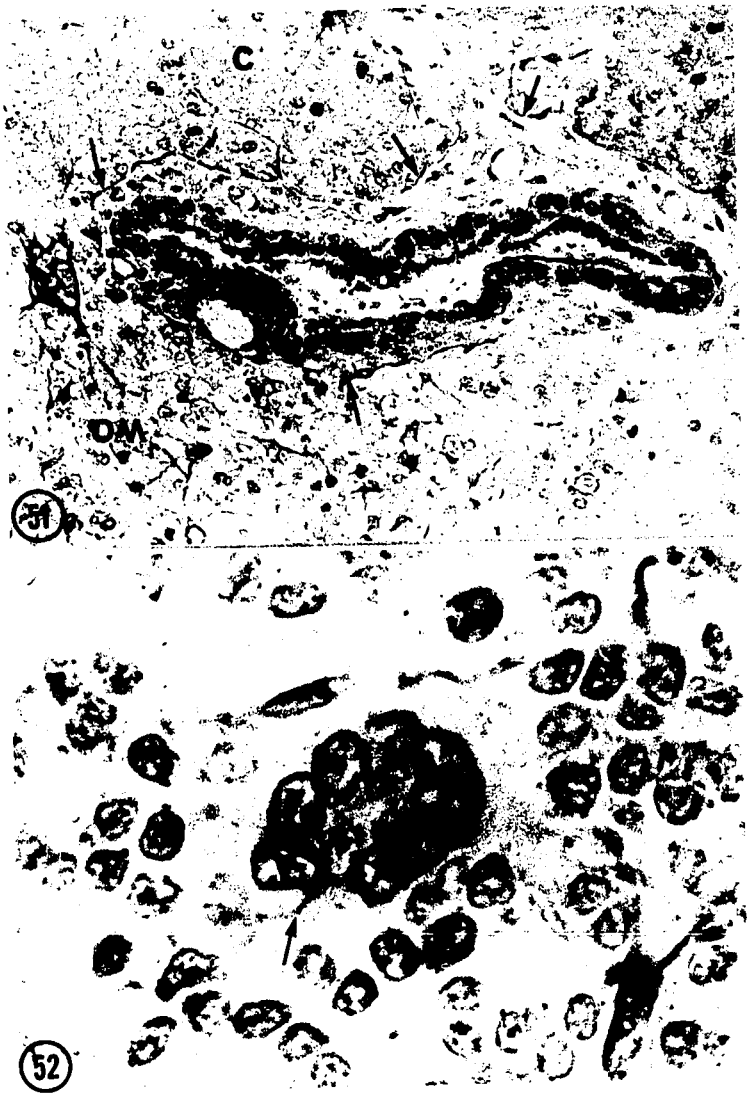
50

Fig. 51 Thymus. A cyst is visible in an interlobular septum with unstained collagenous bundles. The cyst is lined partially by intensely stained ciliated cells, and partially by cuboidal cells. Cellular elements are identifiable in its lumen. Note that the interlobular septum is completely demarcated from the surrounding cortex (C) and outer medulla (OM) by a continuous stained line (indicated by arrows) representing a layer of flattened reticular-epithelial cells. Note also that the epithelial cells of the outer medulla (OM) may accumulate into a small nest which can be seen close to the tip of the septum at the cortico-medullary junction.

Carnoy fixation and TPA (x275).

Fig. 52 Thymus Inner Medulla. A multinucleate giant cell with numerous darkly stained nuclei and an unstained voluminous cytoplasm (arrow) is visible in the inner medulla.

Carnoy fixation and TPA (x1360).


C
DM
51
52

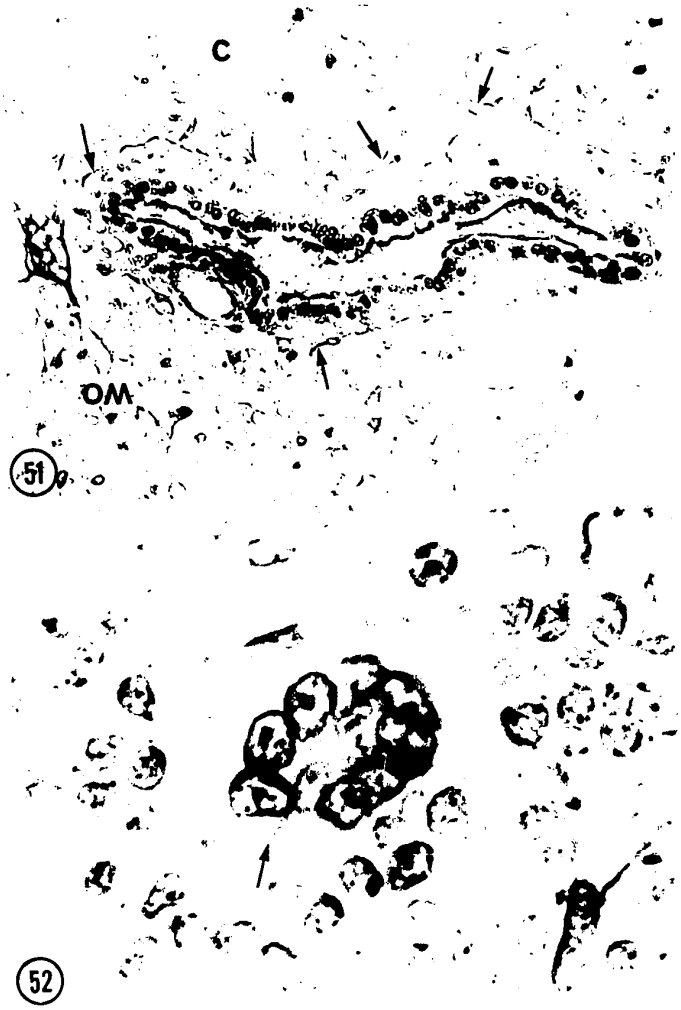

C
OM
51
52

Fig. 53 Thymus Cortex. An artery (A) with intensely stained smooth muscle cells in its wall is visible in an interlobular septum (IS). The latter is delineated from the cortical parenchyma by a well-stained epithelial boundary. Arrow 1 points to a flattened reticular-epithelial cell with a low triangular nucleus. Arrow 3 points to the more rounded nucleus of a stellate reticular-epithelial cell whose cytoplasmic processes are not visible in this photomicrograph. Note that the rounded nucleus (arrow 3) is in intimate contact with another reticular-epithelial in metaphase (arrow 2) with very little but still recognizable, TPA-stained material underneath the cytoplasmic membrane. Note also that the centrosomes and mitatic spindle are deeply stained. This is an example of a reticular-epithelial cell in mitosis.

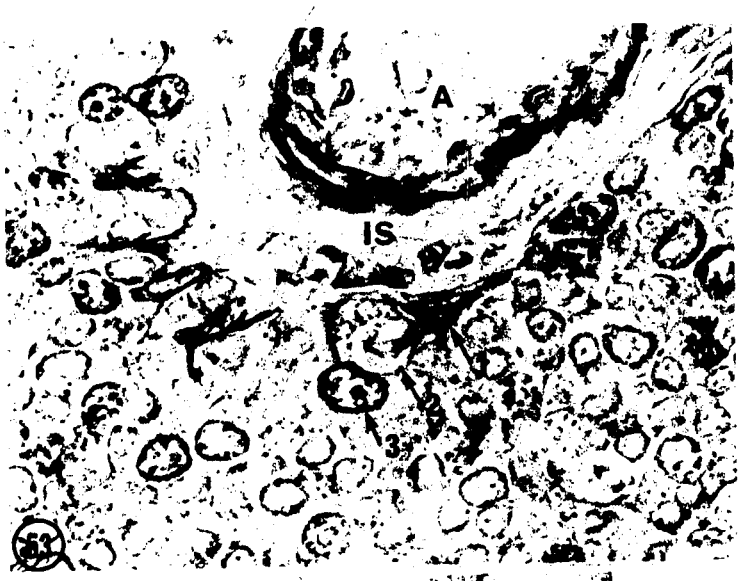
Carnoy fixation and TPA (x1200).

Fig. 54 Thymus Cortex (radioautograph). The arrow points to a labeled reticular-epithelial cell in the thymic cortex. Note the intensely stained tonofibril (indicated by arrow) in the cytoplasm of the labeled cell.

Carnoy fixation and TPA (x1200).

Fig. 55 Thymus Outer Medulla (radioautograph). The arrow points to a labeled reticular-epithelial cell of the irregularly stellate type. Note the intensely stained tonofibrils in the processes of this cell. Note also the dense network formed by the other epithelial cells.

Carnoy fixation and TPA (x1200).


A
IS
3
53


54


55

Fig. 56 Thymus. The arrow points to a terminal arteriole characterized by a narrow lumen and a single layer of intensely stained smooth muscle cells. The collagenous bundles of the interlobular septum (IS) are unstained and are separated from the cortex (C) by an epithelial layer (RE).

Carnoy fixation and TPA (x1200).

Fig. 57 Thymus. The arrow points to a precapillary sphincter characterized by a wide lumen, the absence of an elastica interna and the presence of a single layer of smooth muscle cells with well-stained nuclei. The interlobular septum (IS) is delimited from the cortex (C) by an epithelial boundary (RE).

Carnoy fixation and TPA (z1200).

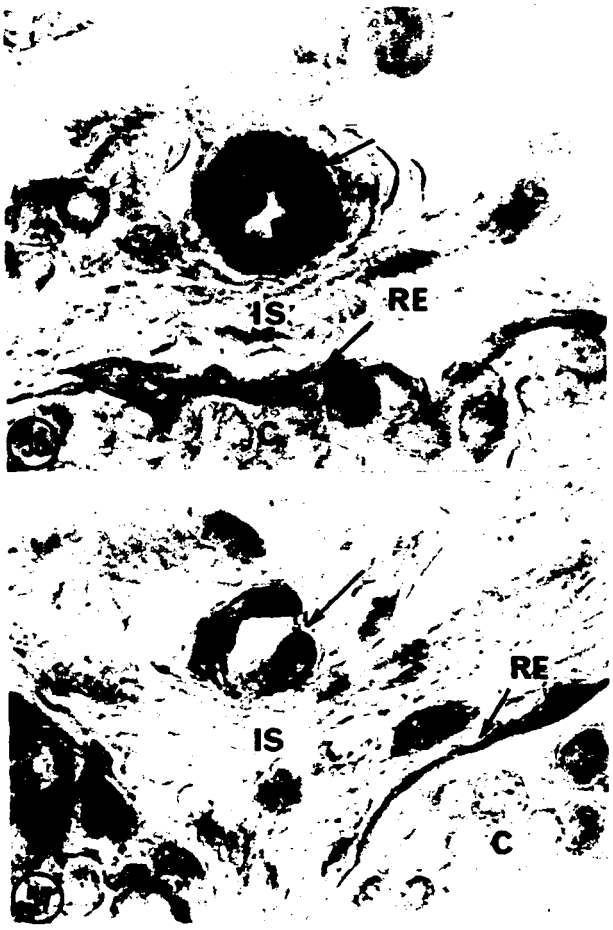

IS
RE
C
RE
IS
C

Fig. 58 Thymus. A radial capillary (indicated by arrows) can be seen to extend without branching from an interlobular septum (IS), through the cortex, into the outer medulla containing numerous darkly stained epithelial elements. Note that the capillary is delimited by an intensely stained line representing a layer of flattened epithelial cells.

Carnoy fixation and TPA (x235).

Fig. 59 Thymus Cortex. Arrows point to a many-branched capillary in the thymic cortex. Note the widely scattered, intensely stained, rounded nuclei of stellate reticular-epithelial cells.

Carnoy fixation and TPA (x570).

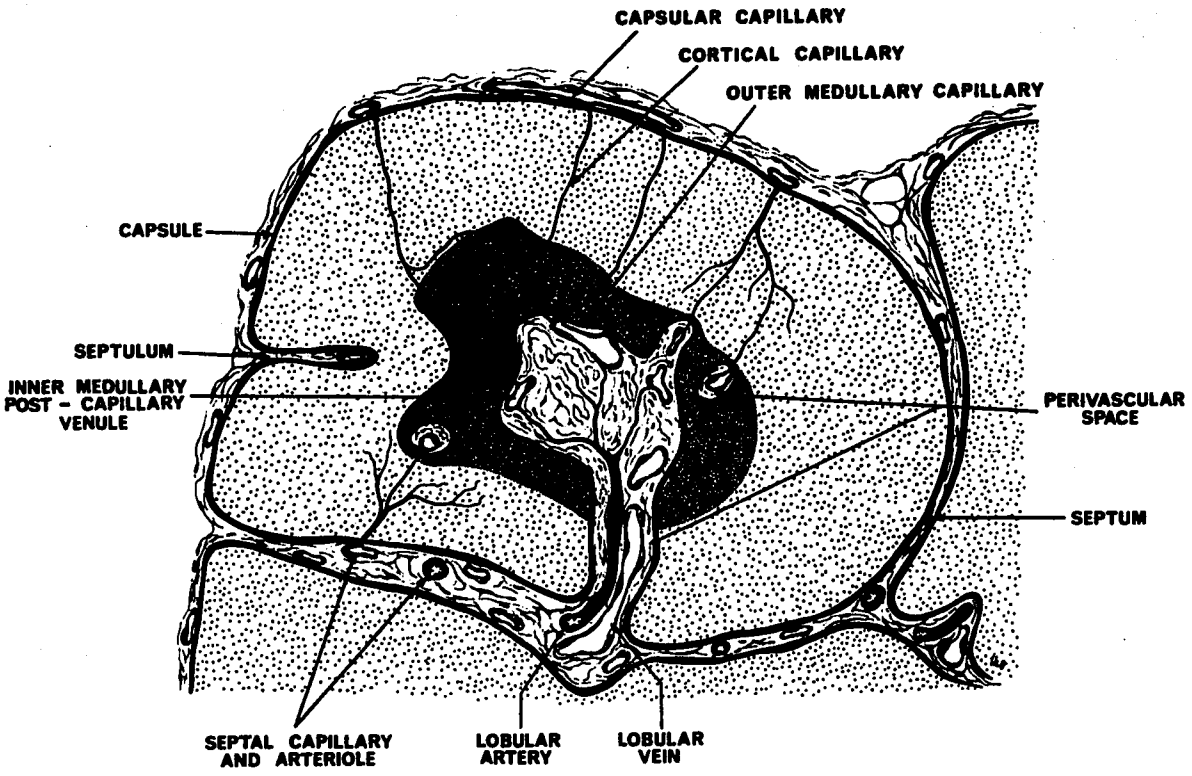
Fig. 60 Thymus Cortico-medullary Junction. A post-capillary venule (v) surrounded by a perivascular space (PS) and located at the boundary between cortex (C) and outer medulla (OM) is seen to receive a cortical capillary. The arrow indicates the point of confluence of the two vessels.

Carnoy fixation and TPA (x570).


IS
58
59
C
V
RS
OM
60


IS
58
59
C
V
RS
OM
61

Fig. 61 Schematic diagram of a thymic lobule showing the distribution of intralobular blood vessels (description given in pp. 69-71). Note that the lobule has a double arterial supply: one set of arterioles is distributed to the cortex and outer medulla, whereas another set supplies exclusively the inner medulla.


CAPSULAR CAPILLARY
CORTICAL CAPILLARY
OUTER MEDULLARY CAPILLARY
CAPSULE
SEPTULUM
INNER MEDULLARY POST - CAPILLARY VENULE
PERIVASCULAR SPACE
SEPTUM
SEPTAL CAPILLARY AND ARTERIOLE
LOBULAR ARTERY
LOBULAR VEIN

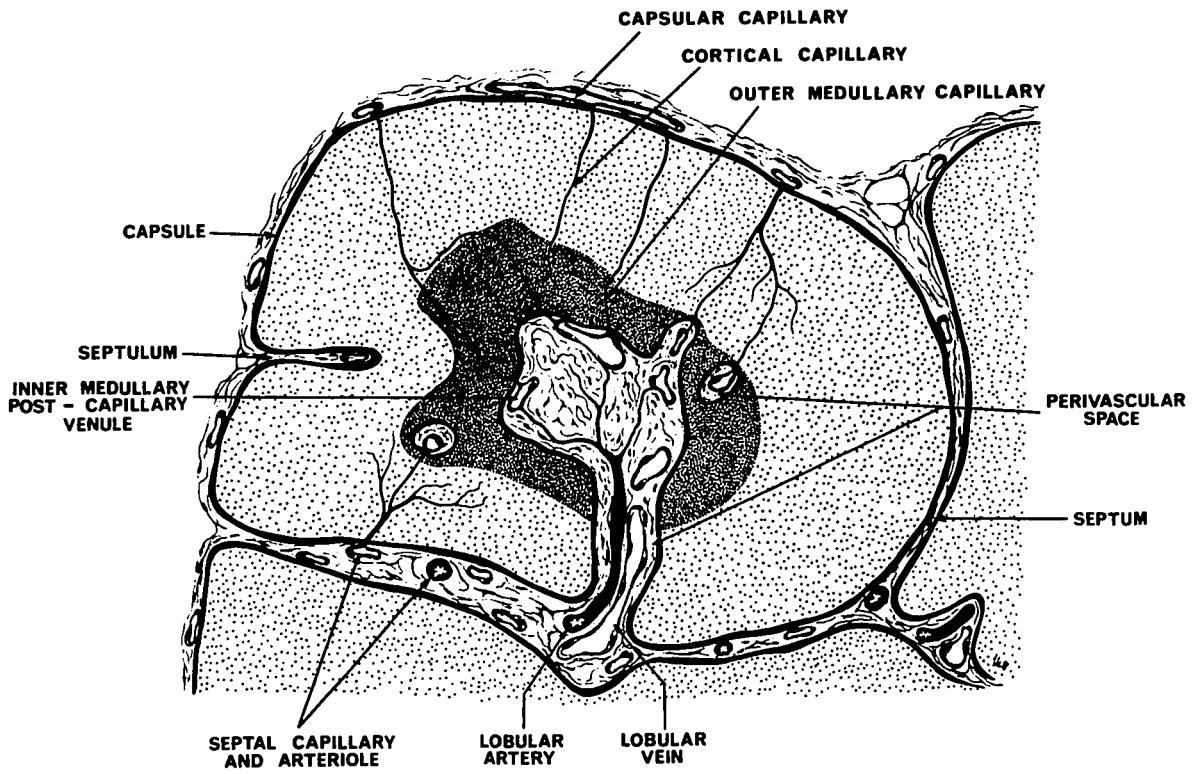

CAPSULAR CAPILLARY
CORTICAL CAPILLARY
OUTER MEDULLARY CAPILLARY
CAPSULE
SEPTULUM
INNER MEDULLARY POST - CAPILLARY VENULE
PERIVASCULAR SPACE
SEPTUM
SEPTAL CAPILLARY AND ARTERIOLE
LOBULAR ARTERY
LOBULAR VEIN

Fig. 62 Schematic diagram illustrating the two compartments of a thymic lobule (description given in pp. 85 and 86).

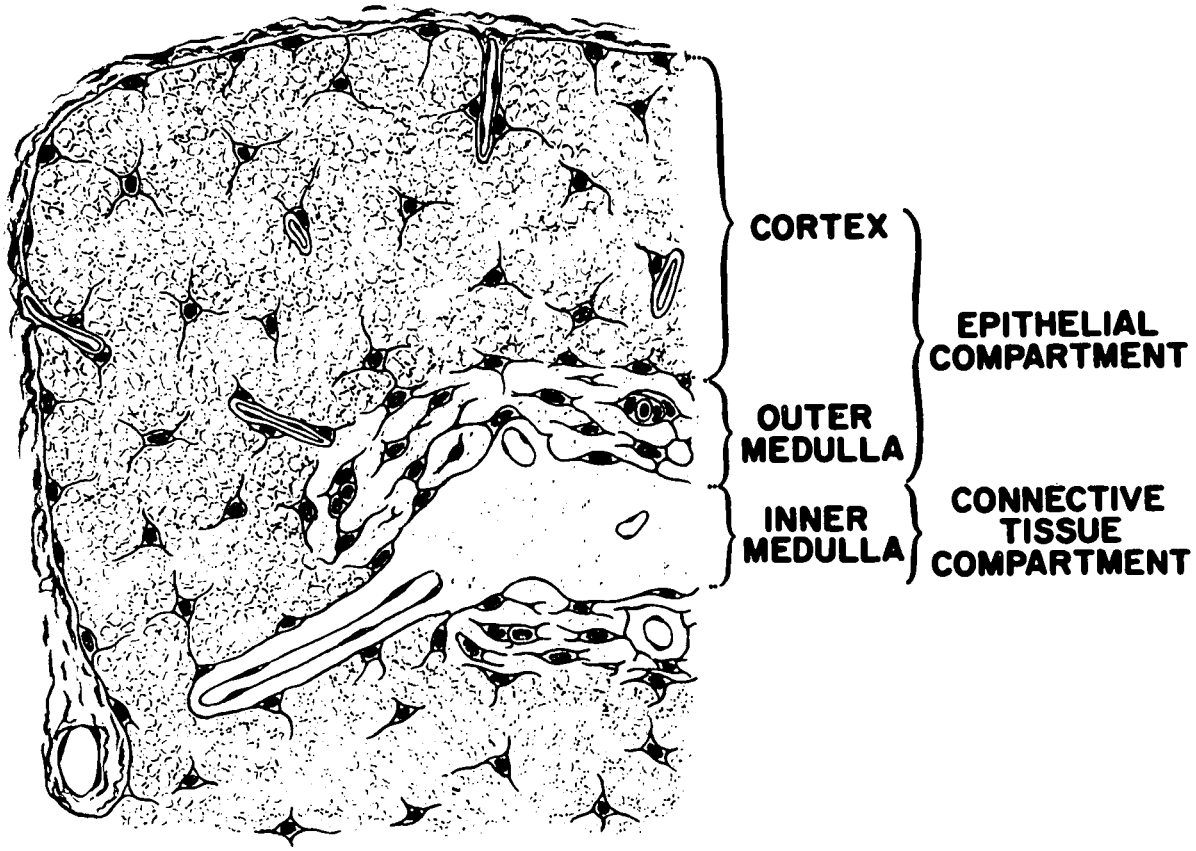
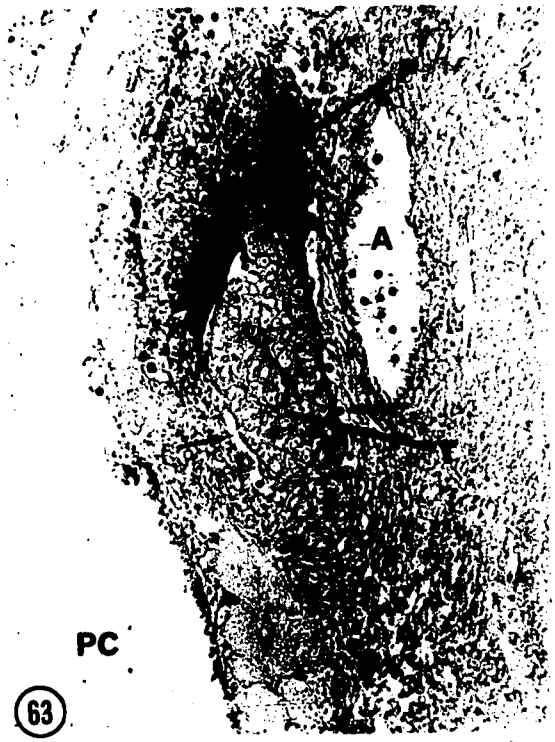

CORTEX
OUTER MEDULLA
INNER MEDULLA
EPITHELIAL COMPARTMENT
CONNECTIVE TISSUE COMPARTMENT

Fig. 63 Embryonic Thymus. This shows a sagittal section of the thymus-parathyroid complex (T and P) in the thirteen-day-old rat embryo in relationship to the aorta (A), vagus nerve (V) and the pericardial cavity (PC). In contrast to the thymus (T), the parathyroid (P) exhibits a strong PAS reaction due to its high glycogen content. At this stage of development the thymus (T) is outlined by a continuous basement membrane and is composed of only epithelial cells. Arrows indicate the presence of blood vessels containing nucleated cells outside the unvascularized thymic anlage.

Carnoy fixation and PAS-toluidine blue (x220).

Fig. 64 Embryonic Thymus. The residual lumen (Lu) of the third pharyngeal pouch is visible in a sagittally cut portion of the thymus surrounded by mesenchyme (Mes) in the thirteen-day-old rat embryo. The lumen (Lu) is lined by cylindrical epithelial cells displaying terminal bar-terminal web complexes (indicated by vertical arrows). The horizontal arrow points to an apical cytoplasmic bleb projecting into the residual lumen (Lu). Note that no lymphoblasts are present in the thymic parenchyma which is composed exclusively of undifferentiated stromal epithelial cells with acinar configuration (as can be seen in the middle right portion of the thymus).

Carnoy fixation and TPA (x1250).



63

64

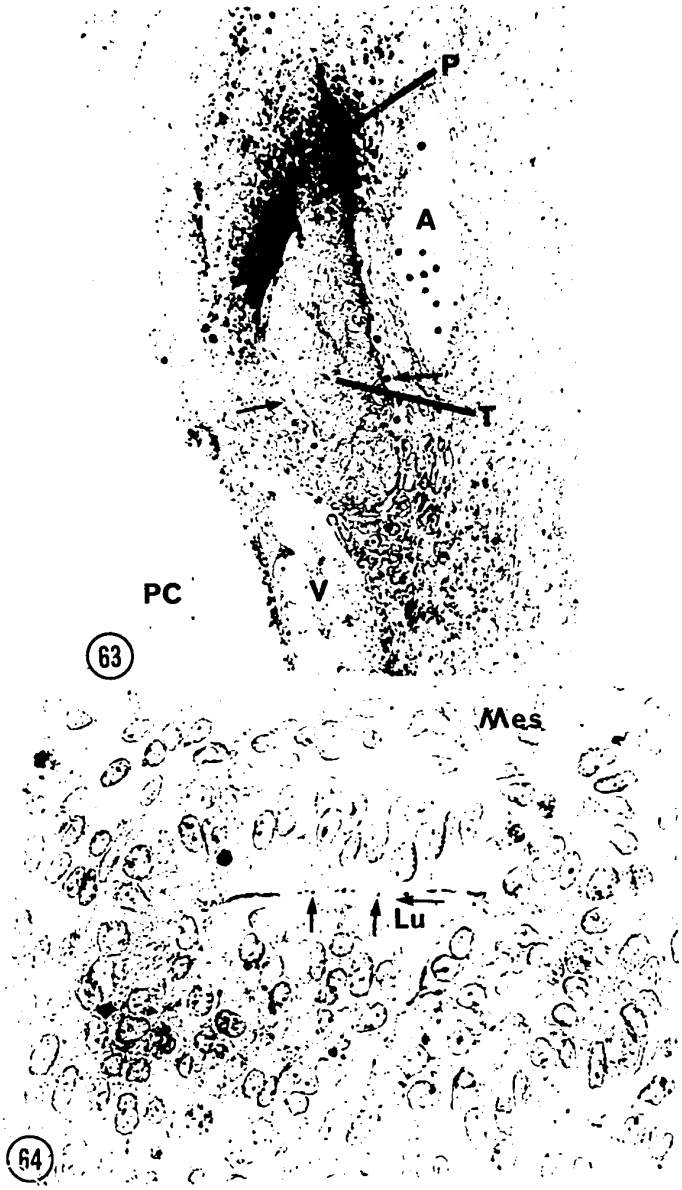
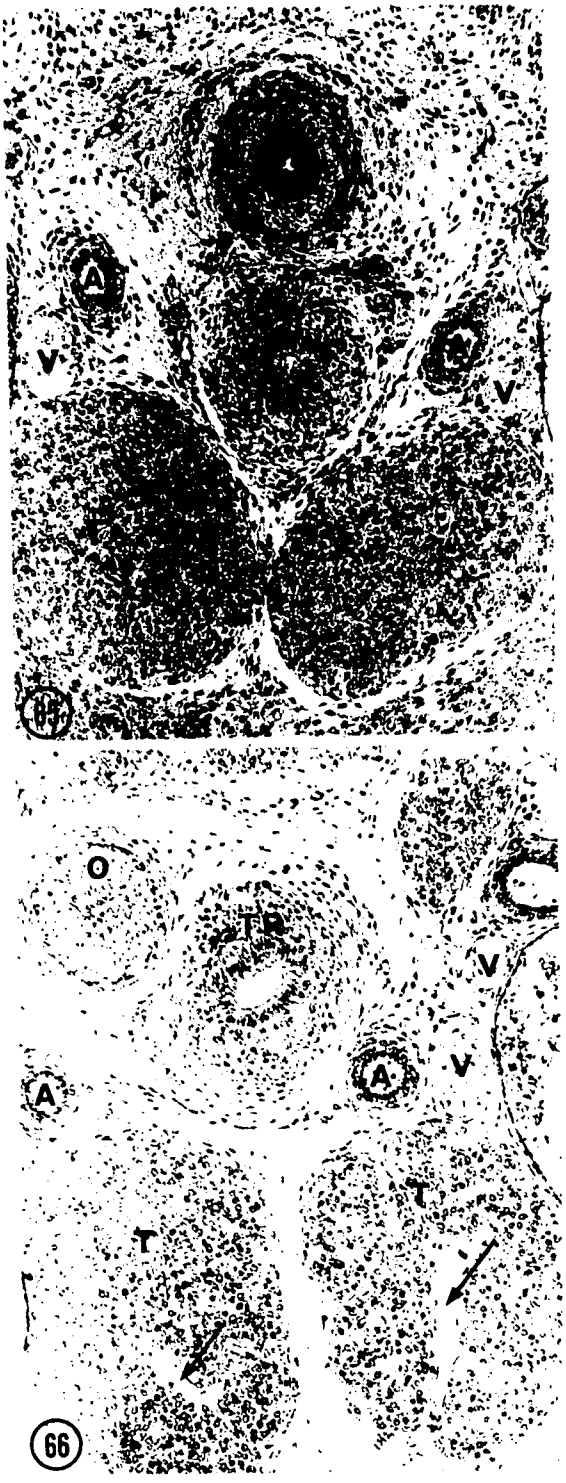

P
A
T
PC
V
63
Mes
Lu
64

Fig. 65 Embryonic Thymus. Cross section through the right and left thymus (T) in the fourteen-day-old rat embryo. The aorta (A), trachea (TR), esophagus (O) and vagus nerves (V) are also illustrated. No fibril-containing reticular cells can be identified in the thymus at this stage of development.

Carnoy fixation and TPA (x125).

Fig. 66 Embryonic Thymus. Cross section through the right and left thymus (T) in the fifteen-day-old rat embryo. The aorta (A), the vagus nerve (V), the trachea (TR) and esophagus (O) are visible. Arrows indicate two large vascular channels within the thymus. No TPA-stained reticular-epithelial cells are demonstrable.

Carnoy fixation and TPA (x125.)


A
V
V
O
TP
V
A
V
A
T
T
66

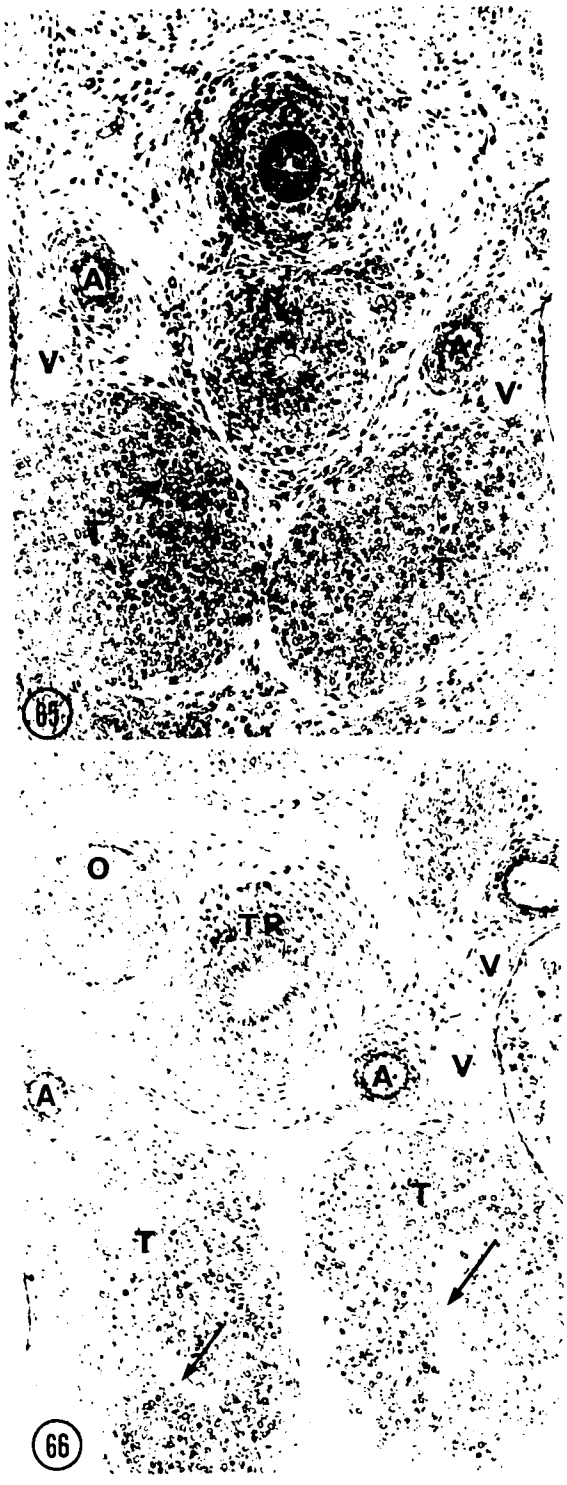

A
V
V
T
65
O
TR
V
A
V
A
T
T
66

Fig. 67 Embryonic Thymus. The thymus has assumed a lymphoid character and lobulation has begun in the thymus of the sixteen-day-old rat embryo. The arrow points to a group of intensely stained reticular-epithelial cells in the deeper portions of the thymus in close proximity to an unstained interlobular septum (IS).

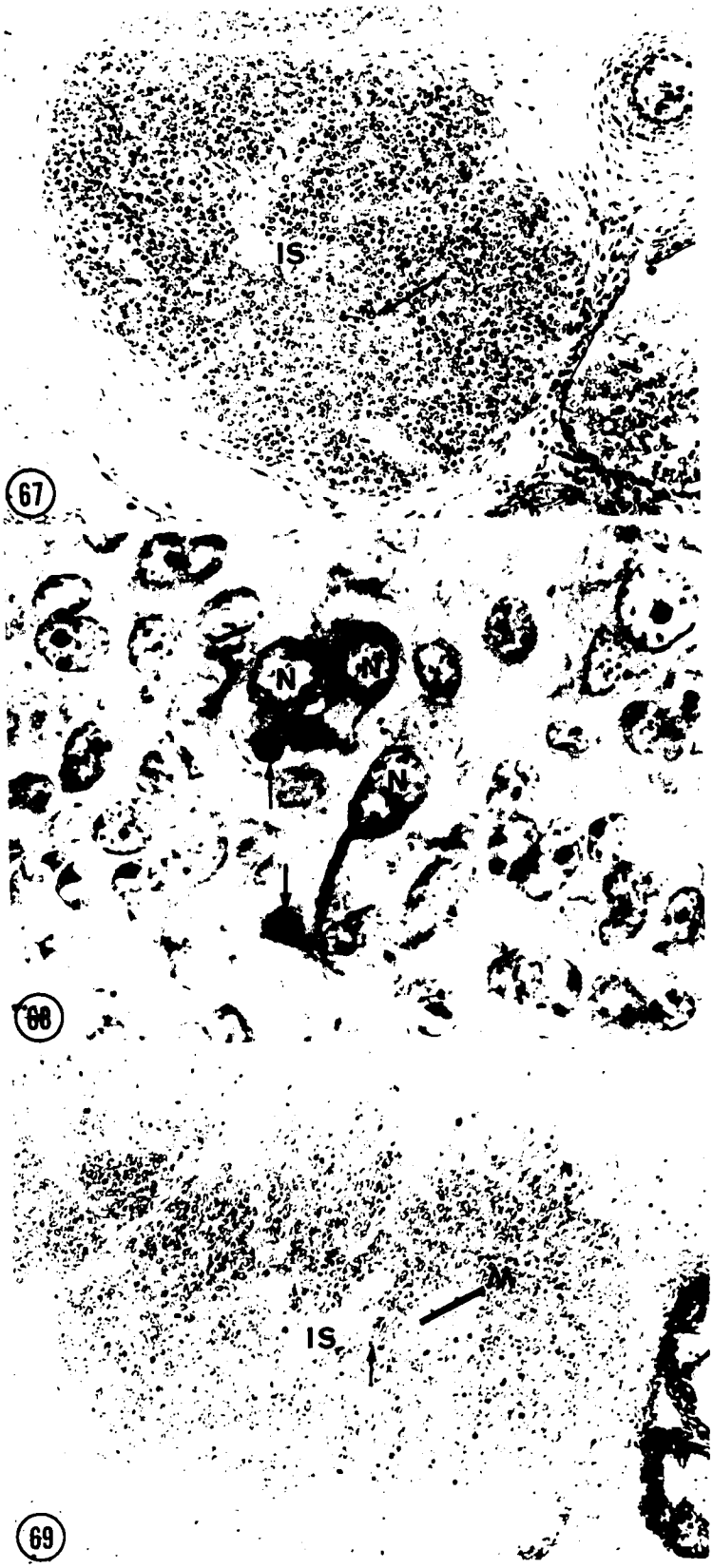
Carnoy fixation and TPA (x125).

Fig. 68 Embryonic Thymus. This shows at a higher magnification the group of deeply stained reticular-epithelial cells seen in Fig. 67. The nuclei (N) of the epithelial cells are lightly stained, are vacuolated and already show signs of degeneration. Their cytoplasmic processes contain avidly TPA-stained tonofibrils. Near or in their cytoplasm, large, rounded, intensely stained keratohyalin-like granules are visible (arrows).

Carnoy fixation and TPA (z1250).

Fig. 69 Embryonic Thymus. This shows the same thymus as in Fig. 67, but stained with toluidine blue in an adjacent section. This indicates that early medulla (M) formation in the thymus begins about the sixteenth day of development. The medulla is composed of an aggregation of reticular-epithelial cells and is already the site of Hassall's body formation. Note the intimate contact (arrow) that the first medulla establishes with a nearly interlobular septum (IS).

Carnoy fixation and toluidine blue stain (x125).


IS
67
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IS
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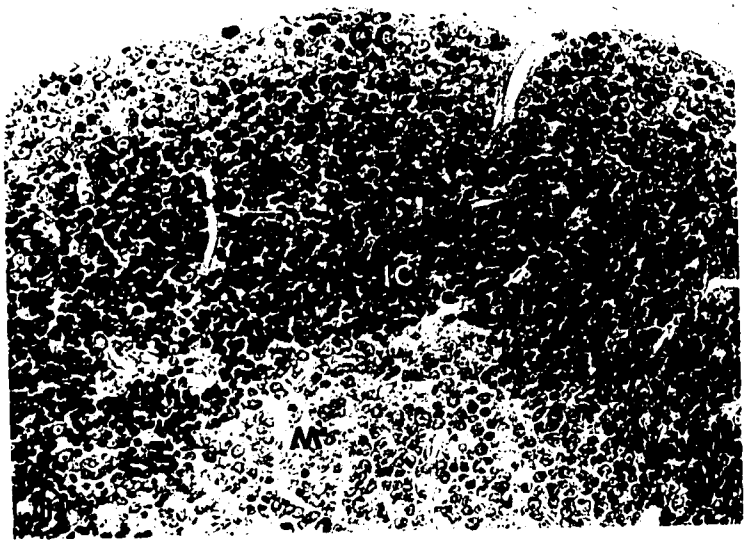
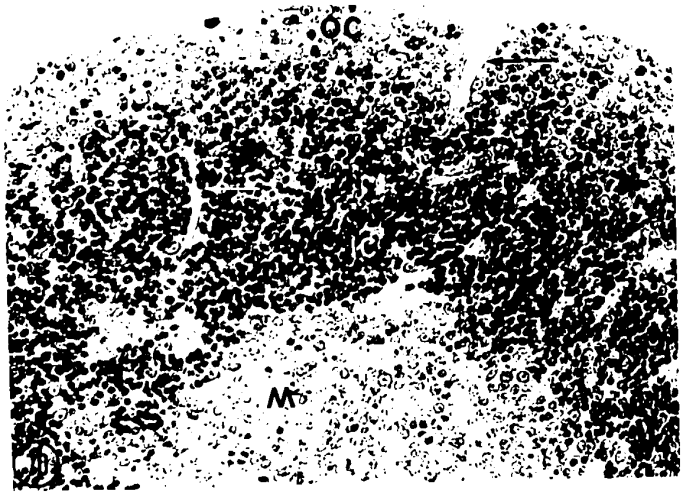
Fig. 70 Early Postnatal Thymus. A portion of a thymic lobule in a two-day-old rat. Three zones are readily distinguishable: a medulla (M), a darkly stained inner cortex (IC) and a lighter outer cortex (OC). Arrows indicate two intralobular septula accompanying blood channels.

Carnoy fixation and toluidine blue stain (x220).


IC
M


OC
M

DISTRIBUTION OF CELL WEB-CONTAINING RETICULAR CELLS IN THE ADULT RAT SPLEEN

The spleen of adult rats contained a large number of stellate reticular cells showing TPA-positive fibrils. In some of these cells the fibrils were coarse and clearly visible; such cells were preferentially located in certain areas of the organ. Other stellate reticular cells contained delicate TPA-staining fibrils often at the limit of visibility of the light microscope. They also appeared to congregate in certain areas of the organ. It is thus the purpose of the following description to analyze the distribution of these various types of TPA-stained reticular cells in the different regions of the spleen.

However, before proceeding to such a description from TPA-stained sections, it is essential to review, owing to the complexity of the spleen, the architecture of this lymphatic organ as seen in hematoxylin-esoin- and PA-silver-hematoxylin-stained preparations. Such a description which is different on several points from the classical textbook descriptions of the spleen is the result of a systematic analysis of serial sections.

General Description of the Adult Rat Spleen in H and E- and Silver-stained Sections:

A) Capsule and trabeculae.

The curved, finger-shaped rat spleen was enclosed by a thick,

firm fibro-elastic capsule containing some smooth muscle cells in its inner portion. In sections stained specifically for elastic fibers, the splenic capsule was found to be composed of a network of thin and thick elastic fibers, the latter occupying its deeper layers (Fig. 4). The flattened mesothelium of the peritoneum, which was often provided with a brush or striated border, closely adhered to its external surface (Fig. 4). At the level of the hilum, the capsule was thickened and deeply indented by the penetrating blood vessels (Fig. 71).

From the internal capsular surface, fibro-muscular trabeculae extended into the organ. Such trabeculae branched, tapered progressively and anastomosed to form a coarse supporting framework for the organ. Though they had a structure similar to that of the capsule, they were noticeably richer than the latter in elastic fibers and in smooth muscle cells which were arranged in small groups or in long cords; some of these cords constituted the wall of the large veins leaving the spleen (Figs. 1 and 5).

B) Regions and zones.

In hematoxylin-eosin-stained preparations three main zones could readily be distinguished in the spleen of adult rats: the white pulp, the red pulp, and the marginal zone.

The white pulp, particularly rich in lymphocytic cells (large, medium and small lymphocytes), accompanied the larger arteries of the organ and could be divided into two areas: the periarterial lymphatic sheaths and the lymphatic nodules (Fig. 71). The latter appeared as dilatations of the periarterial lymphatic sheath; therefore,

the demarcation between periarterial lymphatic sheath and lymphatic nodule was not clear cut. However, the distinction between these two areas remained useful and will continue to be used below.

The periarterial lymphatic sheaths, relatively thick and well structured with coarse argyrophilic fibers as the artery left the hilum of the spleen, became more delicate as the arteries reduced their caliber through several branchings (Figs. 71-74, 80). At the distal ends, just before the arterioles penetrated and terminated in the red pulp, each periarterial lymphatic sheath was reduced to a delicate, loose sleeve composed of few argyrophylic fibers and of one or two layers of lymphocytic cells (Figs. 71 and 74).

The lymphatic nodules, like those of the lymph node, were composed of a pale-staining germinal center surrounded by a dark corona or cortex (Figs. 71, 72, 84, 99). Some of them were well-developed, secondary lymphatic nodules with a germinal center dominated by rapidly-dividing lymphoid cells and by characteristic macrophages containing cytoplasmic phagocytosed nuclear debris, commonly referred to as "tingible body" macrophages. The nodules were separated from the surrounding marginal zone by a dense network of delicate argyrophilic fibers (Fig. 99).

The red pulp, particularly rich in large irregular splenic venous sinuses (or sinusoids) separated by cords or plates of loose vascular connective tissue (Billroth cords), was characterized by an abundance of red blood cells seen in both sinuses and cords and of macrophages laden with hemosiderin pigment granules within the cords (Figs. 72 and 73). Occasionally, the cords also contained

megakaryocytes which sometimes extended processes that reached the lumen of venous sinuses by passing through the gaps in the wall of these vessels (Fig. 104). The red pulp was richly provided with coarse argyrophilic fibers which formed a supporting framework for the splenic sinuses. This perisinal (or perisinusoidal) network was connected with the loose and more delicate meshwork of argyrophilic fibers forming the supporting framework of the cords of Billroth (Fig. 8).

All these argyrophilic fibers (including those of the white pulp) are now collectively referred to as "extracellular reticulum" (Weiss, 1966b). As pointed out in the Materials and Methods section, this reticulum is not exclusively composed of collagen and reticular fibers. It also consists of an amorphous substance with special histochemical properties.

The perisinal (or perisinusoidal) network with its characteristic "ring fibers" could be demonstrated upon staining with Gridley's periodic acid-silver technique (see Figure 8 in Materials and Methods section). However, this staining method was found to be not specific for reticular fibers, since it also stained the glomerular basement membranes of renal corpuscles which are known to be associated with neither reticular nor collagen fibers (see Fig. 10). In contrast, the ring fibers of the perisinal network failed to stain with Bielschowsky's silver reticulum method (see Fig. 9), which, unlike Gridley's technique, was specific for reticular fibers since it did not stain the renal glomerular basement membranes or any other basement membranes (see Fig. 11). In sections of spleen treated with

the periodic acid-sodium bisulfite-resorcin-fuchsin stain (which is believed to be specific for basement membranes), the perisinal network was stained selectively (see Fig. 7) and was thus considered to be a fenestrated basement membrane-like layer. Moreover, the above staining procedure revealed the presence of some delicate fibrillar elements within the coarse extracellular reticulum fibers of the periarterial lymphatic sheaths of the white pulp (see Fig. 6). Such delicate extracellular fibrils reacted positively to the Verhoeff's stain, thus indicating that the extracellular reticulum of the white pulp was composed of elastic fibrils in addition to collagen fibers suspended in an amorphous substance.

Separating the white pulp from the red pulp, there was in the rat a well-developed zone, known as the "marginal zone". This zone had distinct staining characteristics from both the white and the red pulp. Highly cellular but usually poor in small lymphocytes, it was then easily distinguishable from the heavily basophilic white pulp in hematoxylin-eosin-stained preparations (Figs. 72-74). Relatively poor in red blood cells, it was distinct from the eosinophilic red pulp. The marginal zone formed, in fact, a spongy cellular layer mainly composed of stellate reticular cells; in its portion adjacent to the red pulp, it showed an abundance of macrophages. Such a marginal zone surrounded the lymphatic nodules and periarterial lymphatic sheaths, but was missing around the thin layer of white pulp accompanying the terminal arterioles just before the latter penetrated the red pulp (Figs. 71 and 74).

C) Blood vascularization.

An understanding of the microscopic structure of each of the three main splenic regions was found to be dependent upon a knowledge of the distribution of blood vessels in it.

The splenic artery usually gave rise to three branches which entered the spleen on its concave dorsal side. The rat spleen thus usually had three hila. The latter, therefore, divided the organ into four approximately equal parts.

As shown in Figure 71, at the level of each hilum, a large arterial branch, known as the "central artery", as soon as it penetrated into the splenic parenchyma, was seen to radiate into the interior from the hilum region and to break up into central arterioles. Each central artery or arteriole was coaxially surrounded by a cylinder of lymphoid tissue, previously referred to as the "periarterial lymphatic sheath". The periarterial lymphatic sheaths persisted about the central artery and arterioles, attenuating progressively, until these vessels became terminal arterioles.

Within the periarterial lymphatic sheaths, the great majority of the radial branches of the central artery terminated as capillaries which ran toward the rim of the sheath to empty into the meshes of the marginal zone. Each lymphatic nodule received an arterial branch from the central artery, which entered the mid-portion of the germinal center and which, after reaching its central area, ramified into capillaries (Fig. 75). The latter traversed radially the germinal center and cortex of the lymphatic nodule to open into the meshes of the marginal zone. The central artery also provided other

branches to the cortex of the lymphatic nodules, which also terminated as capillaries opening into the marginal zone.

In the red pulp, most of the terminal arterioles branched and ended in either the splenic cords or the splenic venous sinuses. The thin-walled sinuses then carried the blood from the terminal arterioles, the meshes of the marginal zone and those of the splenic cords into pulp veins, which in turn drained into trabecular veins. Thus, the whole red pulp was systematized about the venous channels and consisted virtually of two vascular structures, namely splenic sinuses separated from each other by thin plates of vascular tissue, referred to as "Billroth cords".

D) Lymphatic vessels.

The lymphatic vessels of the spleen were limited to a capsular plexus in the hilum regions with branches sometimes entering the larger trabeculae. They were all efferent channels and were not found as deep lymphatics in the splenic pulp (Fig. 83). They closely followed the course of the large arterial branches of the splenic artery outside the organ.

Observations on the White Pulp as Seen in TPA-stained Sections:

A) Periarterial lymphatic sheaths.

In TPA preparations, under low power, about four concentrically disposed, discontinuous, TPA-positive strands or plates, which seldom anastomosed and which were separated from each other by spaces containing cellular elements, were readily identifiable about each

central arterial channel (Figs. 76 and 81). The innermost TPA-stained plate closely invested the adventitia of the central vessel, while the outermost one, which was very prominent, delimited the periarterial sheath of the splenic white pulp from the contiguous marginal zone. Even at such a low magnification, the central artery was easily recognizable, owing to the strongly TPA-positive smooth muscle cells of its tunica media.

However, under higher power, each TPA-stained plate was found to represent a layer of fusiform reticular cells with elongate cytoplasmic extensions which were rendered clearly visible by the avidly TPA-positive cell web they contained in the form of fibrils. The latter showed a particular affinity not only for the cell membrane, but also for the nuclear membrane, so that the nuclei of these fixed reticular cells were more readily identifiable than those of other cell types in the region (Figs. 77 and 78).

At the limit between the periarterial lymphatic sheath and the marginal zone, cell web-containing reticular cells rich in coarse TPA-staining fibrils and nuclei facing inward, i.e., toward the central artery, formed a single or double conspicuous layer, which appeared to be continuous.

Similarly, elongated, TPA-stained reticular cells with elongate cytoplasmic projections formed a continuous layer, which delineated the adventitia of the central artery and its branches. As a rule, the nuclei of these cells tended to bulge slightly into the adventitial connective tissue (Figs. 78 and 79).

In the remainder of the periarterial lymphatic sheath, fusiform, almost squamous, TPA-stained reticular cells were organized into concentric layers separated from each other by spaces containing rows of mainly small lymphoid elements. These fixed-lining reticular cells rested upon the sides of the coarse extracellular reticulum fibers, which were TPA negative and which, in this portion of the white pulp, formed an open meshwork following a mainly circumferential pattern, so that each strand of this meshwork was sandwiched between two monolayers of fixed reticular cells (Figs. 77 and 78). Their nuclei always bulged slightly into the spaces they delimited (Figs. 76 and 77).

At the distal end of each periarterial lymphatic sheath where the marginal zone was missing, elongate TPA-stained reticular cells were seen to form one or two layers which ensheathed the terminal arteriole until the latter penetrated the red pulp.

In ordinary H and E preparations, the picture of the periarterial lymphatic sheaths was dominated by small and medium lymphocytes. These sheaths also contained a moderate number of plasma cells, free macrophages with phagocytosed material in their cytoplasm, and free reticular cells with large, irregular nuclei and an abundant, vacuolated acidophilic cytoplasm. In addition, there was another type of reticular cells which were characterized by large, ovoid or elongate nuclei with a pale-staining, homogeneous, finely granulated nucleoplasm without visible nucleoli, and by an indistinct cytoplasm. Such cells represented the TPA-stained reticular cells

described above.

In silver or PAS preparations, the picture was dominated by coarse strands of extracellular reticulum, forming an open meshwork with large interstices occupied by lymphoid elements and following a circumferential pathway about the central artery or arterioles (Figs. 80 and 82). About the rim of the periarterial sheath, the extracellular reticulum fibers, though they still had a circumferential disposition, where more delicate and constituted a denser meshwork with small interstices. As it was pointed out previously, some fine elastic fibrillar elements, which stained selectively with the Verhoeff's stain, were found in close association with the coarse argyrophilic reticulum fibers of this portion of the white pulp, hence the term "extracellular reticulum" applied to these mixed collagenous and elastic fibers.

With the exception of the innermost layer of TPA-stained reticular cells which covered the adventitia of arterial channels (Figs. 78 and 79) and which did not lay upon argyrophilic fibers, all the layers of reticular cells in the periarterial lymphatic sheaths encompassed the coarse extracellular reticulum fibers of the white pulp (Figs. 81 and 82). Furthermore, when traced through serial sections, the spaces delimited by layers of TPA-stained reticular cells resting on the coarse reticulum fibers of the periarterial lymphatic sheath were found in close association with the efferent lymphatic vessels in the hilum region (Fig. 83). It seemed that the endothelial lining of these lymphatics was fenestrated on the side.

facing the interior of the spleen, that is, the periarterial lymphatic sheath. This was suggested by the picture of a chicken wire or almost rectilinear net that the reticular fibers associated with that side of the lymphatic vessel presented (see Fig. 83).

B) Lymphatic nodules:

Like the periarterial lymphatic sheaths, the lymphatic nodules implanted on the central artery were clearly demarcated from the neighboring marginal zone by a conspicuous single or double layer of spindle-shaped reticular cells rich in coarse TPA-staining fibrils (Figs. 85-87). These cells were intimately associated with a dense meshwork of delicate extracellular reticulum fibers, which tended to have a circumferential disposition (Fig. 84). Similarly, all arterial channels in the nodules, excepting germinal center capillaries, were closely invested by a continuous layer of reticular cells.

Cell web-containing reticular cells also formed a delicate, uninterrupted layer at the limit between germinal center and cortex (see Figs. 88-92). This layer of reticular cells did not sit upon extracellular reticulum fibers (Fig. 84).

The cortex of lymphatic nodules was divisible into four areas: (1) an area capping the pole of the oblong germinal center which was directed toward the central artery, (2) an area capping the germinal center pole which was directed toward the marginal zone, (3) and (4) an area covering each side of the germinal center. In cortical areas (1) and (2), there were no blood vessels, no argyrophilic fibers and no reticular cells with TPA-staining fibrils. Thus cortical area (1)

was a space containing lymphoid cells and a few macrophages, which was delimited externally by the layer of TPA-stained reticular cells at the boundary of the germinal center and internally by the layer of reticular cells investing the adventitia of the central artery (see Figs. 88-90). In some planes of cut, as can be seen in Figure 89, cortical area (1) seemed to cover a large portion of the circumference of the central artery or arteriole. Cortical area (1) was found to be continuous with the spaces delimited by TPA-stained reticular cells in the periarterial lymphatic sheath (Figs. 90 and 91).

Cortical area (2), although similar to area (1) in most respects, did not contain macrophages and was bounded internally by the delicate layer of reticular cells delimiting the germinal center and externally by the conspicuous sheath of reticular cells demarcating the white pulp from the marginal zone.

In contradistinction, cortical areas (3) and (4) were supplied by branches of the central artery, which terminated as arterial capillaries in the meshes of the marginal zone. These areas were, in addition, traversed by the germinal center capillaries which also opened into the marginal zone. All the arterial channels in areas (3) and (4) were closely invested by a layer of TPA-staining reticular cells, which, at the limit between the lymphatic nodule and the marginal zone, became continuous with the conspicuous single or double layer of reticular cells there, thus contributing some cells to this layer. Around the arterioles supplying cortical areas (3) and (4), like in the periarterial sheaths reticular cells rich in coarse

TPA-staining fibrils made up concentric layers separated from each other by spaces containing rows of lymphocytes (Fig. 92). These spaces were continuous with those of the periarterial lymphatic sheath and communicated with cortical areas (1) and (2). The most peripheral periarteriolar layers of TPA-stained reticular cells in areas (3) and (4) often blended with the conspicuous sheath of reticular cells separating the lymphatic nodule from the marginal zone.

When well-developed germinal centers of secondary lymphatic nodules were traced through serial sections passing through their polar axis, they were found to be bipolar, oblong, polarized structures presenting a depression in their mid-portion and consisting of two merging hemispherical zones. One zone was pale-staining with TPA, while the other was more darkly stained. The light zone was directed toward the marginal zone from which it was separated by a cap of cortical small lymphocytes. The staining properties of this zone could be accounted for by the fact that it contained, in addition to large lymphocytes, macrophages and plasmoblasts, a moderate number of small lymphocytes whose nuclei were lightly stained with TPA. On the other hand, the dark zone, which was diametrically opposite to the light zone with its pole facing the central artery, was occupied by very few small lymphoid cells but a large number of macrophages and immature cells with deeply TPA-stained nuclei. The dark staining of this zone was also due to the fact that it tended to contain more numerous mitotic figures with intensely TPA-staining mitotic spindles and a larger number of mononuclear

phagocytes or "tingible body" macrophages with avidly TPA-positive nuclear fragments and other cellular debris in their cytoplasm.

It was at the level of the depression on the middle of the lateral aspects of the oblong germinal center that the branch of the central artery to the center entered the latter with its investing layer of TPA-stained reticular cells (Fig. 93). At the point of entrance of this arterial channel, the layer of reticular cells delimiting the germinal center was seen to invaginate and to be separated from that investing the arteriole to the center by a narrow space occupied by a row of mostly small lymphoid cells and a few free mononuclear phagocytes with an abundant, TPA-negative cytoplasm containing at times some avidly TPA-stained nuclear debris and phagocytosed material (Fig. 93).

This narrow periarteriolar space, when traced through serial sections, was not seen to traverse the whole thickness of the germinal center in its mid-portion (Figs. 94 and 95). On the contrary, the two layers delimiting such a space were found to be continuous in the central portion of the germinal center where the arteriole to the center broke up into radial capillaries. Such a space thus ended blindly along the proximal portions of the radial capillaries given off by the arteriole to the germinal center. Thus, unlike the other white pulp arterial capillaries, those within the substance of the germinal center were not invested by a continuous layer of TPA-staining reticular cells. However, as soon as these

capillaries passed out of the germinal center into the cortex, they regained their investment of reticular cells, which was continuous with the layer of cells demarcating the germinal center from the cortex. Thus, within the substance of the germinal center, there seemed to be no reticular cells with demonstrable fibrils in their cytoplasm.

Observations on the Marginal Zone as Seen in TPA-stained Sections:

The marginal zone was remarkably uniform in structure. In ideal planes of cut, 3 vascular areas could be distinguished in this zone: a marginal sinus area which was contiguous with the white pulp and to which succeeded a disorganized area, and lastly a more organized area.

As can be seen in Figure 98, the marginal sinus area was most prominent around the lymphatic nodules of the white pulp. The reticular cells in this area were stellate in shape and contained very little visible TPA-staining fibrils. They lined a series of anastomosing spaces lying against the periphery of the white pulp. Such marginal sinusoidal spaces were surrounded by very fine argyrophilic fibers which showed continuity with the coarser fibers at the periphery of the white pulp on the one hand and with those of the remainder of the marginal zone on the other (Fig. 99).

The great majority of the white pulp arterial capillaries, particularly those in the cortex of lymphatic nodules, opened into the marginal sinusoidal spaces at right or acute angles (see Fig. 92).

The latter were lined, along their inner surfaces which lay against the white pulp, by an endothelium which was continuous with that of the white pulp capillaries. However, their outer walls showed discontinuity and appeared to be formed by elongate reticular cells containing delicate TPA-positive fibrils in their apparently perforated or fenestrated cytoplasmic extensions (Fig. 92).

The disorganized area, which succeeded to the marginal sinus area, consisted of a meshwork of a fine argyrophilic fibers in association with reticular cells, apparently polymorphous and containing very little cell web fibrils. This meshwork supported an evenly-distributed population of cells, most of which were erythrocytes and medium-sized lymphocytes. No macrophages were seen in this area (Figs. 98 and 99).

The better organized, external area of the marginal zone was characterized by a meshwork of reticular cells, which contained more TPA-positive fibrillar material in their cytoplasmic processes and which were arranged in concentric lamellae separated from each other by vascular spaces rich in medium-sized lymphocytes and a few macrophages (Fig. 98). These reticular cells were in close association with coarser argyrophilic fibers which were continuous with those of the cords of the red pulp (Fig. 99).

In some rare instances, however, the marginal zone was particularly rich in lymphoid cells, and TPA-stained reticular cells became very difficult to identify (Fig. 97). The concentration of lymphocytes in the marginal zone might be such that it could not

easily be distinguished from the neighboring white pulp.

Red pulp sinuses did not usually penetrate the marginal zone (Fig. 97). However, arterioles of white pulp origin and capillaries of unclarified origin were sometimes seen passing through the zone.

Continuous with the marginal zone were the splenic cords of Billroth. Cordal reticular cells were abundant. They were branched, polymorphous cells with nuclei similar in structure to those of white pulp and sinal reticular cells. They were in close association with argyrophilic fibers, seemingly compartmentalizing the cord into communicating vascular spaces. A small number of them contained some TPA-stained fibrils in their cytoplasm (Figs. 100, 101, 104). At the limit of the cords, they clothed the sinal basement membrane. These cord-limiting cells sent long cytoplasmic projections that not only extended along the outer side of the sinal basement membrane but seemed to radiate into the cordal area as well. However, delicate TPA-staining fibrils were identifiable only in the cytoplasmic processes clothing the basement membrane (Figs. 101, 103, 104).

Distributed between the cords were the long, irregularly branching splenic venous sinuses. They often reached to and lay against the periphery of the marginal zone, conforming to its contour (see Fig. 97). They were lined by a single incomplete layer of spindle-shaped, nearly uniplanar reticular cells, often termed "reticulo-endothelial cells", though they showed little or no phagocytic activity. In longitudinally cut sinuses, these lining cells were seen to

run parallel to the long axis of the vessel with apertures or gaps between some of them and contained, in the portion of their cytoplasm adjacent to the fenestrated basement membrane, coarse TPA-positive fibrils, the so-called "basal plates", running parallel to the long axis of the cells (Fig. 102).

In cross sections of sinuses, therefore, the sinal cells were cut transversally and presented a cuboidal shape and intensely TPA-stained cross sections of basal plates, appearing as points or short bars bearing a slight indentation in their middle (Figs. 100-104). Similarly, in section, the fenestrated sinal basement membrane occurred either as long segments or as rows of short, rounded, discontinuous masses of PAS-reactive, or silver-impregnable, substance (see Figs. 7 and 8 in Materials and Methods section).

SUMMARY OF OBSERVATIONS ON THE ADULT RAT SPLEEN

In summary then, as shown in Figure 96, the splenic white pulp was clearly demarcated from the surrounding marginal zone, that transitional vascular tissue interposed between white and red pulp, by a conspicuous single or double layer of reticular cells rich in coarse TPA-staining fibrils.

In the white pulp itself, which consisted of periarterial lymphatic sheaths and lymphatic nodules, there was an abundance of reticular cells with coarse TPA-positive fibrils. They formed an uninterrupted investing layer around all arterial channels of the white

pulp, except around arterial capillaries supplying the germinal center of lymphatic nodules.

In the periarterial lymphatic sheaths, which were supported by a network of heavy argyrophilic fibers with large meshes having a mainly circumferential pattern, the TPA-stained reticular cells ran parallel to the central artery and arterioles and were arranged in concentric layers separated from each other by spaces containing rows of lymphocytic elements. These layers clothed the heavy argyrophilic fibers, so that the latter were sandwiched between the TPA-stained cytoplasmic projections of reticular cells.

In the lymphatic nodules implanted on the central artery, as represented in Figure 96, reticular cells with TPA-positive fibrils formed a delicate layer at the limit between germinal center and cortex. Like the investing layer of all arterial channels of the white pulp, excepting germinal center capillaries, this delimiting layer was not associated with argyrophilic fibers. By means of serial sections, it was found that such a TPA-stained layer was invaginated in the mid-portion of the germinal center at the point of entrance of the arteriole to the center and that it became continuous with the layer investing the arteriole where the latter broke up into radial capillaries in the central portion of the germinal center. Owing to this invagination, well-developed germinal centers appeared to be polarized, oblong structures, with one pole facing the central artery and another one directed toward the marginal zone. Moreover, the study of TPA-stained serial sections revealed that the

layer of reticular cells delimiting the germinal center formed an investment for the capillaries as they ran out of the germinal center to terminate in the meshes of the marginal zone. Thus, a germinal center was found to be a dilatation, accompanied perhaps by cellular infiltration, of the potential space between the walls of the radial capillaries given off by the arteriole to the center and the layer of TPA-stained reticular cells investing these capillaries.

Reticular cells with TPA-positive fibrils were also absent from the cortex of the lymphatic nodules, except around the arterioles supplying the cortical areas covering the lateral aspects of the germinal center, around which they made up concentric layers as in the periarterial lymphatic sheaths. The spaces delimited by these layers were continuous not only with the other cortical areas but with the periarterial sheaths as well.

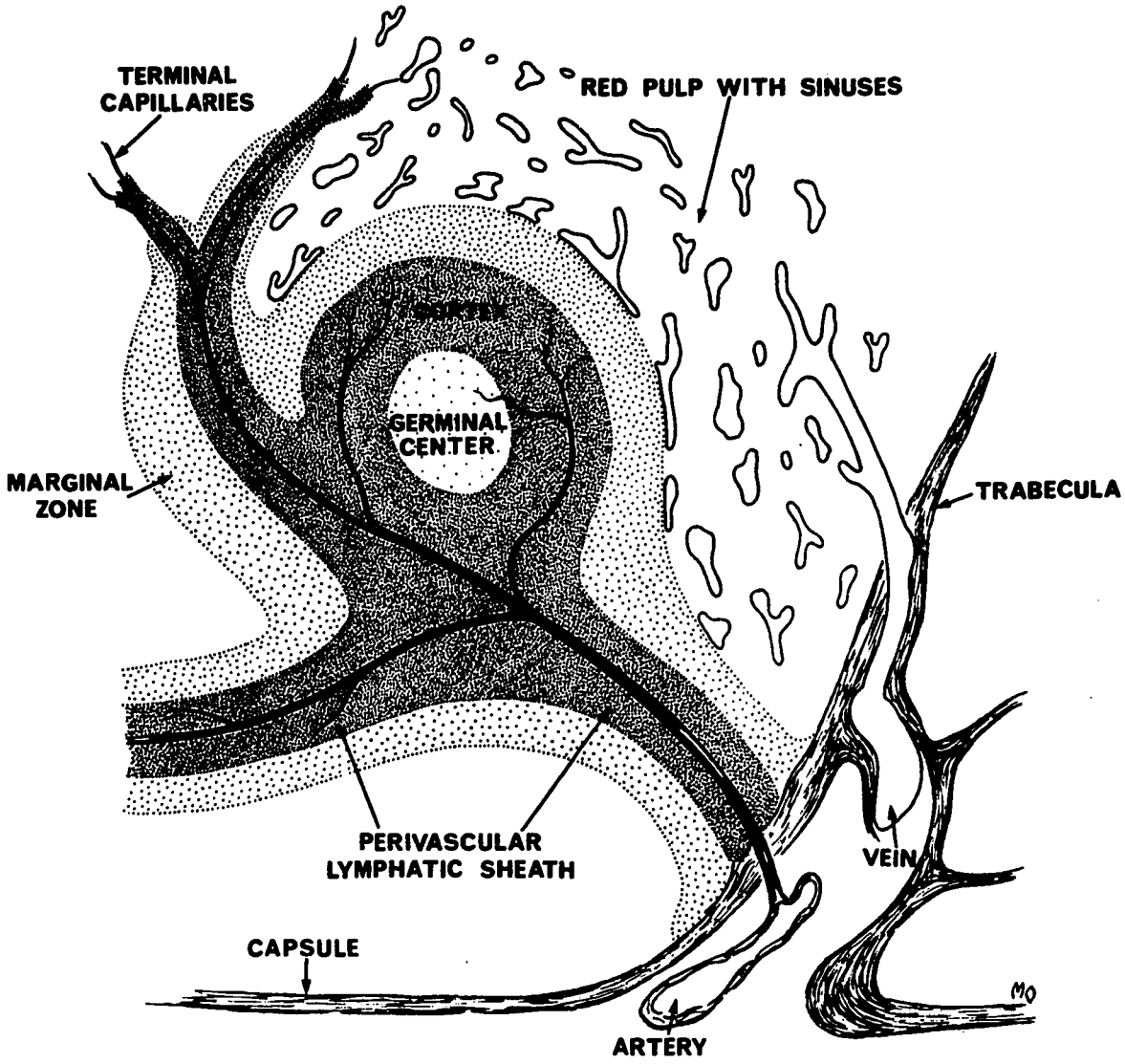
Thus the splenic white pulp seemed to be compartmentalized by layers of reticular cells with intracytoplasmic TPA-staining fibrils.

In the marginal zone, reticular cells containing discrete TPA-positive fibrils lay upon a meshwork of argyrophilic fibers. They were randomly distributed in the inner portion of the zone, while they were disposed in concentric layers separated from each other by blood spaces toward its periphery.

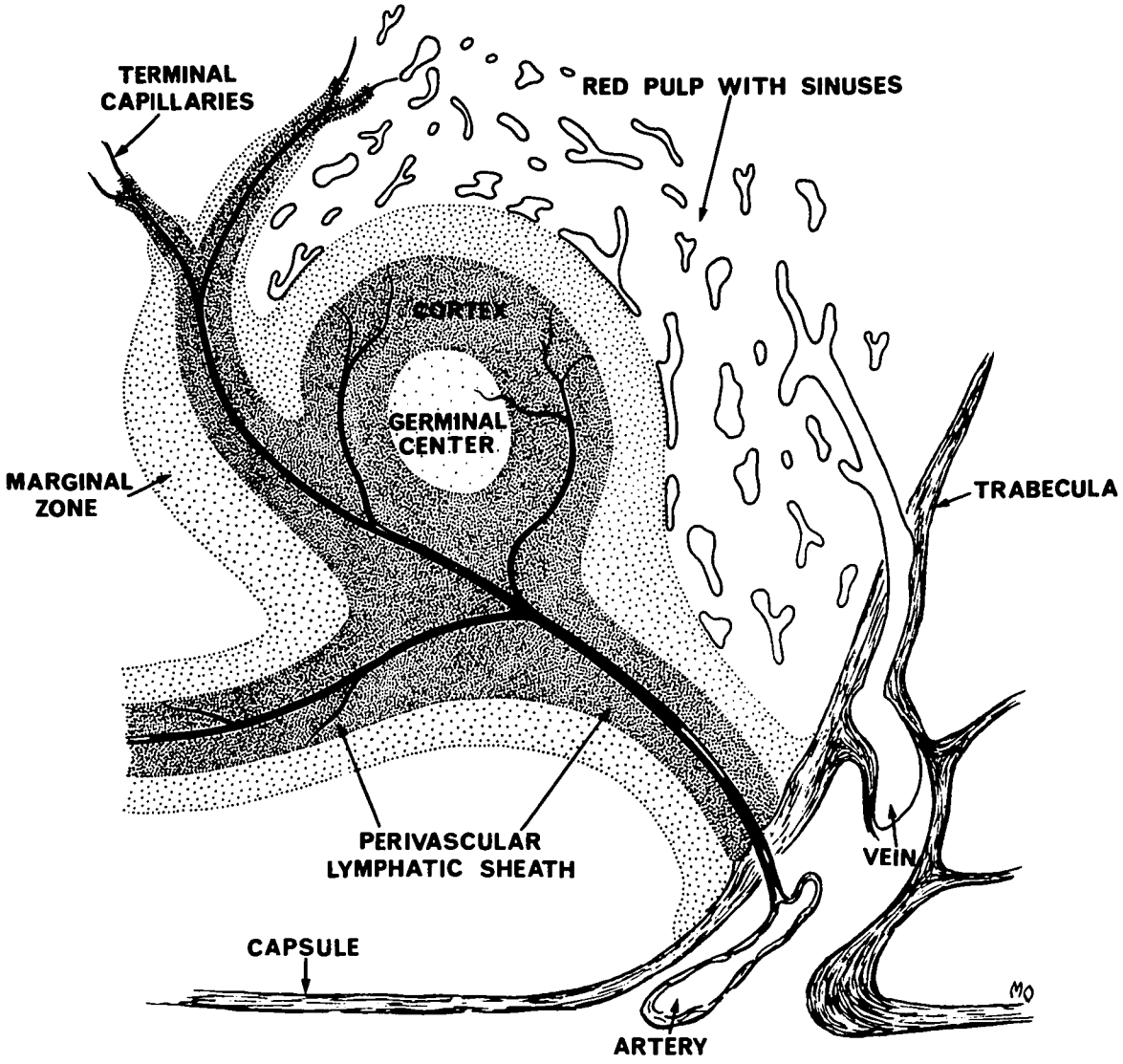
In the red pulp, some reticular cells with fibrils were found in the splenic cords between the sinuses. The wall of the sinuses contained three elements: a regularly fenestrated basement membrane sandwiched between an inner layer of sinal reticular cells, often

termed "reticulo-endothelial cells", and an outer layer of cord-limiting reticular cells. The long, spindle-shaped sinal reticular cells contained prominent TPA-positive fibrils forming "basal plates" in the portion of their cytoplasm adjacent to the basement membrane. Similarly, the cord-limiting reticular cells, characterized by long slender processes that not only radiated into the cord area but extended along the sinus wall as well, often showed TPA-staining fibrils in the cytoplasmic extensions that lay upon the sinal basement membrane. The latter, therefore, in section, was often seen to be enclosed by two incomplete TPA-stained lines.

Fig. 71 Schematic diagram illustrating a portion of the rat spleen at the level of the hilum (description given in pp. 93-99).


TERMINAL CAPILLARIES
RED PULP WITH SINUSES
GERMINAL CENTER
MARGINAL ZONE
TRABECULA
PERIVASCULAR LYMPHATIC SHEATH
VEIN
CAPSULE
ARTERY
MO

71


TERMINAL CAPILLARIES
RED PULP WITH SINUSES
CORTEX
GERMINAL CENTER
MARGINAL ZONE
TRABECULA
PERIVASCULAR LYMPHATIC SHEATH
VEIN
CAPSULE
ARTERY
MO

71

Fig. 72 Spleen. Typical hematoxylin-eosin preparation to show relation of the marginal zone (MZ) to the red pulp (RP) and the white pulp. Two lymphatic nodules, each consisting of a lightly stained germinal center (GC) surrounded by a dark cortex (C), are illustrated. Between the two lymphatic nodules is an obliquely cut central artery (arrow) with a periarterial lymphatic sheath (PLS) on each side. The white pulp thus comprises the lymphatic nodules and the periarterial lymphatic sheaths. The marginal zone (MZ) forms a halo around the white pulp.

Carnoy fixation and hematoxylin-eosin (x85).

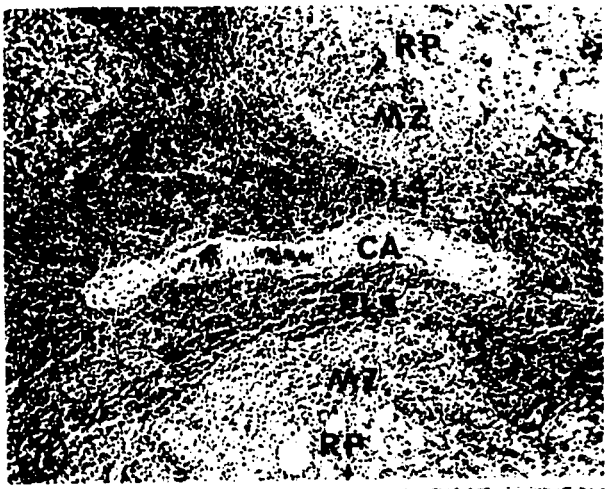
Fig. 73 Spleen. The marginal zone (MZ) separates each side of a portion of the white pulp from the red pulp (RP). This white pulp portion consists of a periarterial lymphatic sheath (PLS) around a central artery (CA).

Carnoy fixation and hematoxylin-eosin (x85).

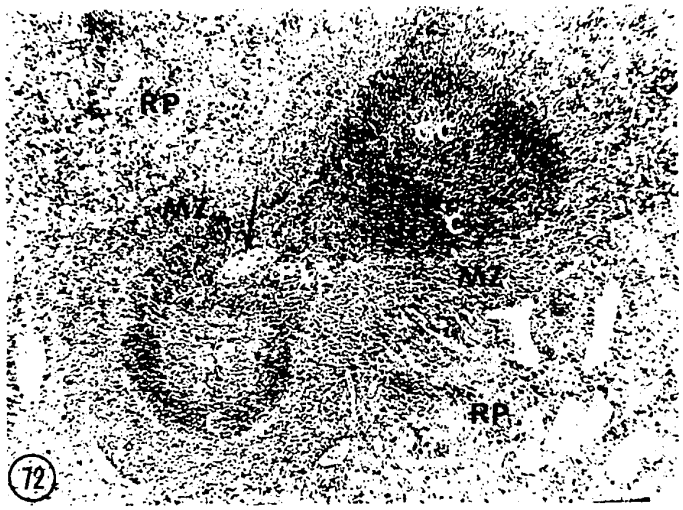
Fig. 74 Spleen. This shows the marginal zone (MZ) between the red pulp (RP) and a portion of the white pulp consisting of a periarterial lymphatic sheath (PLS) around a central artery (CA). Note that the terminal arteriole (TA) is surrounded by only a sleeve of lymphoid cells and is not separated from the red pulp (RP) by the marginal zone (MZ). The arrow indicates the point at which the marginal zone (MZ) stops.

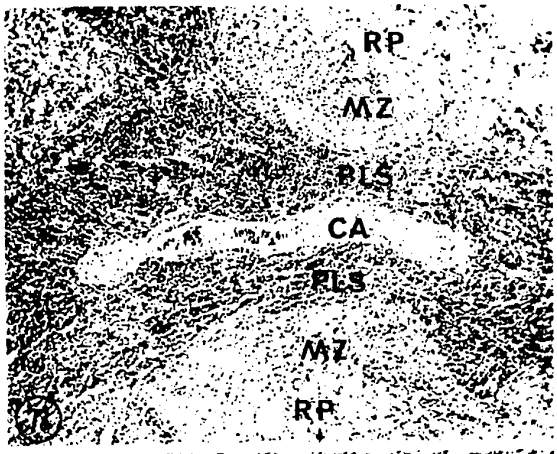
Carnoy fixation and hematoxylin-eosin (x85).


GC
C
PLS
GC
C


RP
MZ
CA
PLS
MZ
RP


RP
MZ
PLS
PLS
MZ
RP


RP
MZ
MZ
RP
72


RP
MZ
PLS
CA
PLS
MZ
RP
74

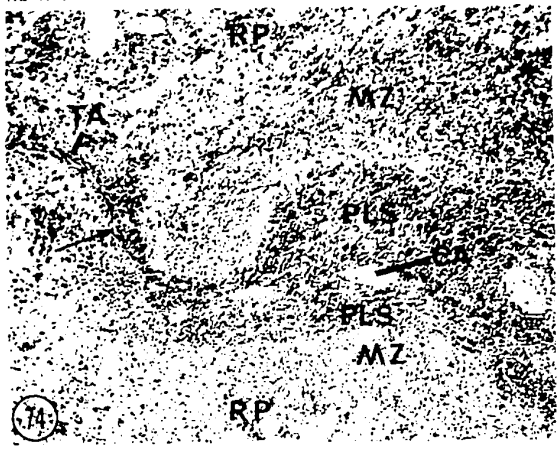

RP
MZ
TA
PLS
CA
PLS
MZ
RP
74

Fig. 75 Spleen. White Pulp. A central (eccentric) artery (CA) is visible between a periarterial lymphatic sheath (PLS) and a lymphatic nodule composed of a pale-staining germinal center (GC) surrounded by a cortex (C). This portion of the white pulp is covered on each side by the marginal zone (MZ). Note the arterial branch (AB) given off by the central artery (CA) to the germinal center (GC).

Carnoy fixation and PA-silver (x125).


MZ
GC
AB
MZ
75

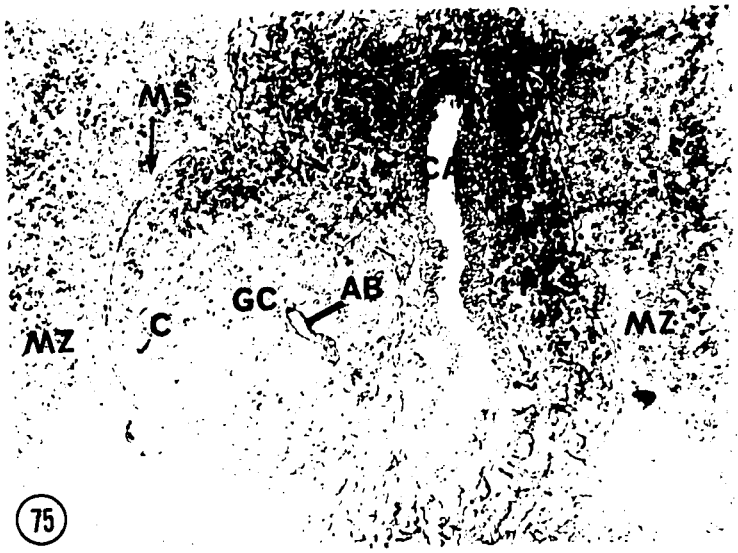
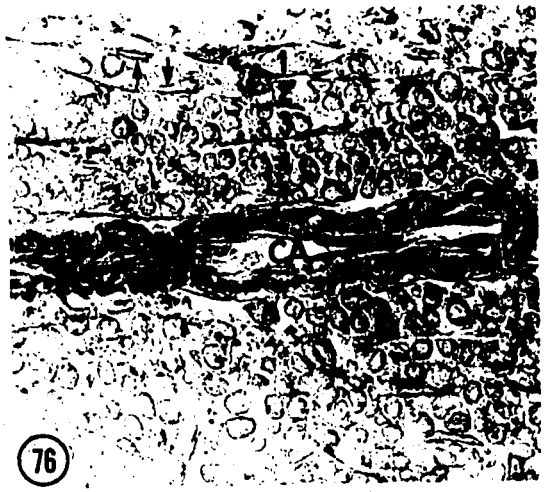

MS
GC
AB
MZ
C
MZ
75

Fig. 76 Spleen White Pulp. This shows a longitudinal section of a periarterial lymphatic sheath coaxially surrounding the central artery (CA) with its intensely stained smooth muscle cells. Above the artery, four spaces (1 to 4) are visible. Each space or compartment is delimited by two deeply stained lines representing layers of fusiform reticular cells with long cytoplasmic processes containing TPA-stained fibrils. The elongated, flattened nuclei of these reticular cells are indicated by arrows. Note that the innermost stained line or layer of reticular cells invests the adventitia of the central artery (CA).

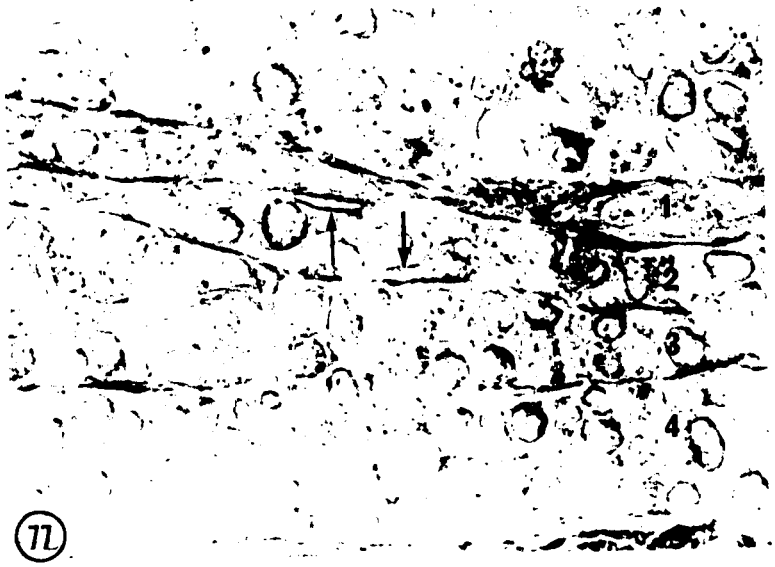
Carnoy fixation and TPA (x525).

Fig. 77 Spleen White Pulp. This is a higher power view of the periarterial lymphatic sheath seen in Fig. 76. The four spaces or compartments (1 to 4) are visible. Each compartment is occupied by rows of lymphocytes and is delimited on each side by a layer of elongated, almost squamous TPA-stained reticular cells whose nuclei are indicated by arrows. These cells can be seen to anastomose in space (1) and to form a continuous layer along the margin of the periarterial lymphatic sheath.

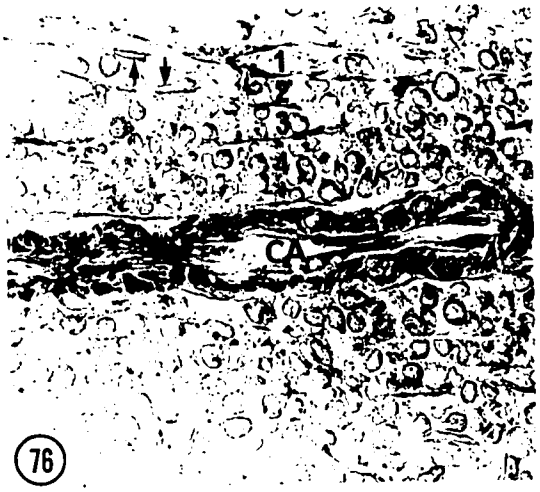
Carnoy fixation and TPA (x1100).



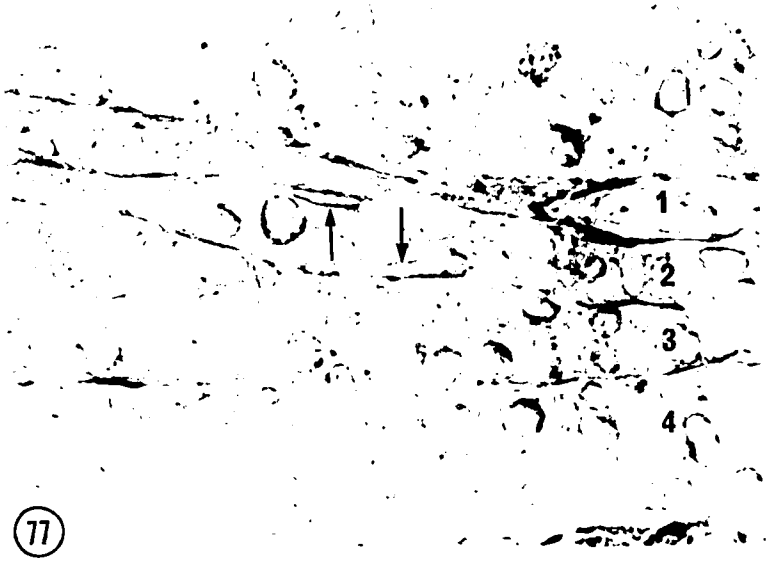
(76)



(77)



(76)



(77)

Fig. 78 Spleen White Pulp. A portion of a periarterial lymphatic sheath is visible above a central artery (CA) with intensely stained smooth muscle cells in its wall. Four spaces or compartments (1 to 4) are readily identifiable above the artery. Each space contains mainly lymphocytes and is delineated by fusiform TPA-stained reticular cells with long cytoplasmic extensions and elongate nuclei (N). Note again that the innermost layer of reticular cells closely invests the adventitia of the central artery (CA). The arrow points to a TPA-negative, coarse extracellular reticulum fiber sandwiched between two TPA-stained cytoplasmic processes of reticular cells.

Carnoy fixation and TPA (x1100).

Fig. 79 Spleen White Pulp. Arrows point to the layer of TPA-stained reticular cells investing the adventitia of the central artery (CA). The arrow below the artery points up to a reticular cell nucleus protruding into the unstained adventitial layer of the artery.

Carnoy fixation and TPA (x1260).

Fig. 80 Spleen White Pulp. The marginal zone (MZ) separates the red pulp (RP) from the periarterial lymphatic sheath (PLS) of the white pulp. In the sheath (PLS), the central artery (CA) is surrounded by concentric lamellae of coarse argyrophilic fibers.

Carnoy fixation and PA-silver (x250).


CA
CA
79
80


1
2
CA
CA
MP
CA
PLS

Fig. 81 Spleen White Pulp. Cross section of a periarterial lymphatic sheath. Arrows indicate the circumferential pattern followed by the elongate TPA-stained reticular cells around the central artery (CA).

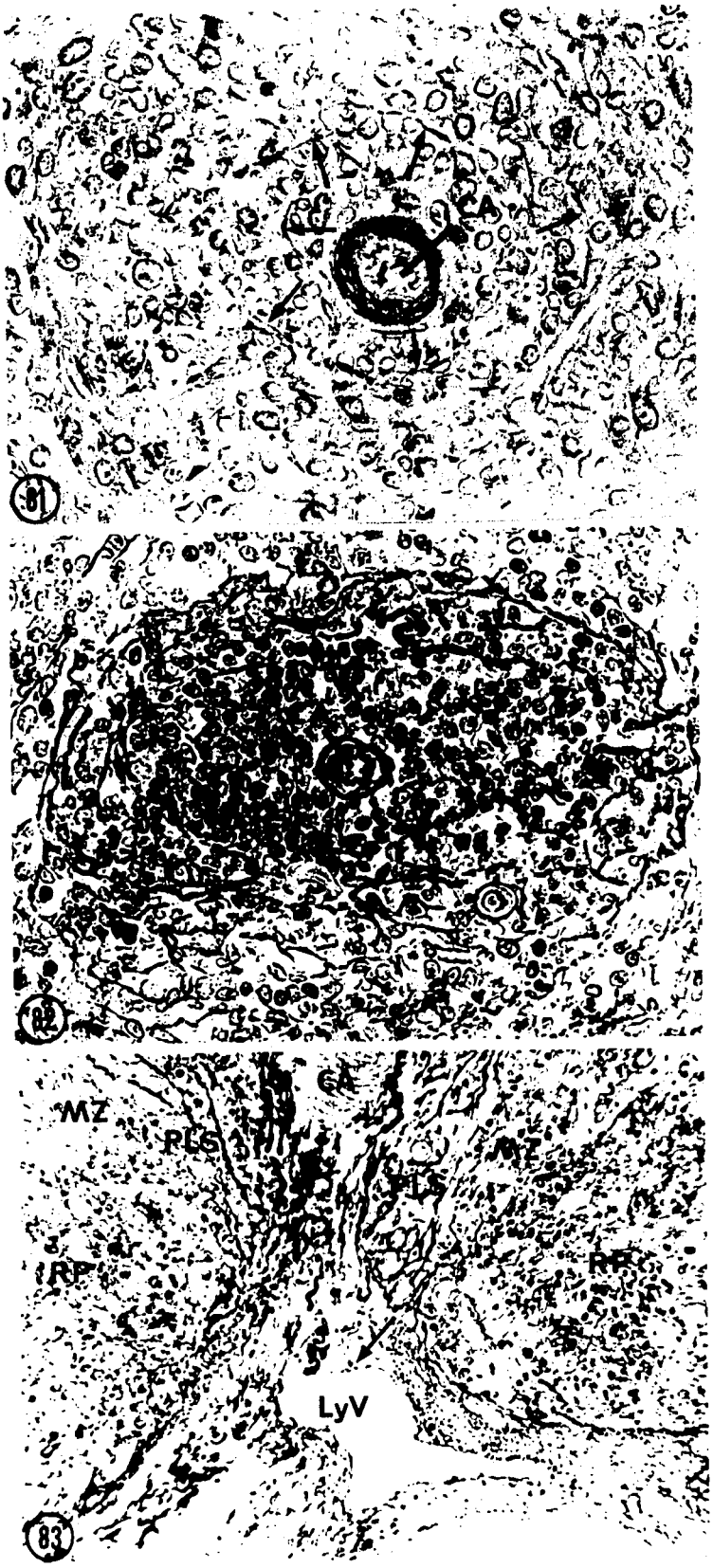
Carnoy fixation and TPA (x600).

Fig. 82 Spleen White Pulp. Cross section of a periarterial lymphatic sheath. Note the circumferential pathway which the coarse argyrophilic fibers commonly associated with the TPA-stained reticular cells follow around the central artery or arteriole (CA).

Carnoy and PA-silver (x465).

Fig. 83 Spleen White Pulp. Longitudinal section of the periarterial lymphatic sheath (PLS) and the central artery (CA) at the level of the hilum. The marginal zone (MZ) separates the sheath on each side from the red pulp (RP). In the hilum region, the spaces of the periarterial sheath are seen to come into close contact with an efferent lymphatic vessel (LyV). The portion of the wall of the lymphatic vessel (LyV) which faces the periarterial sheath appears to be discontinuous (arrow).

Carnoy fixation and PA-silver (x220).


81
82
MZ
PLS
RP
LyV
83

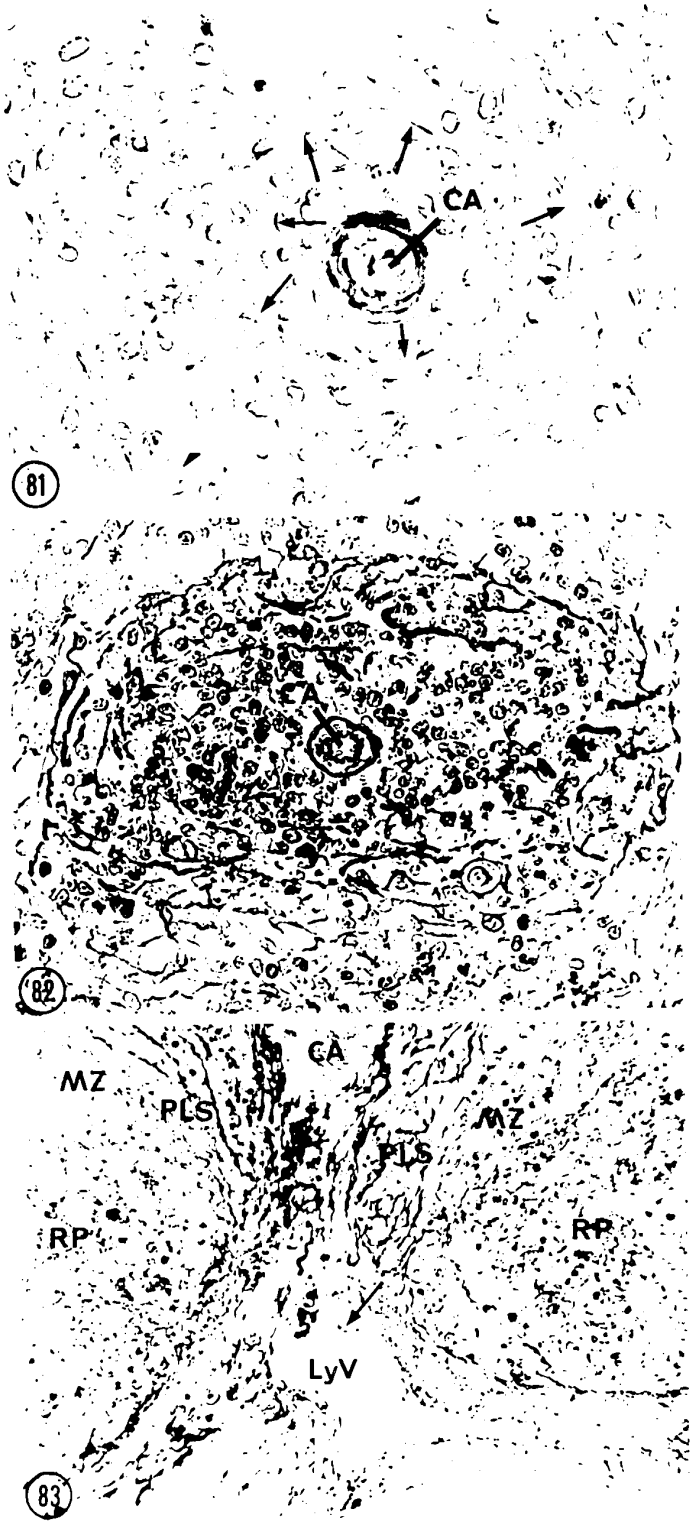
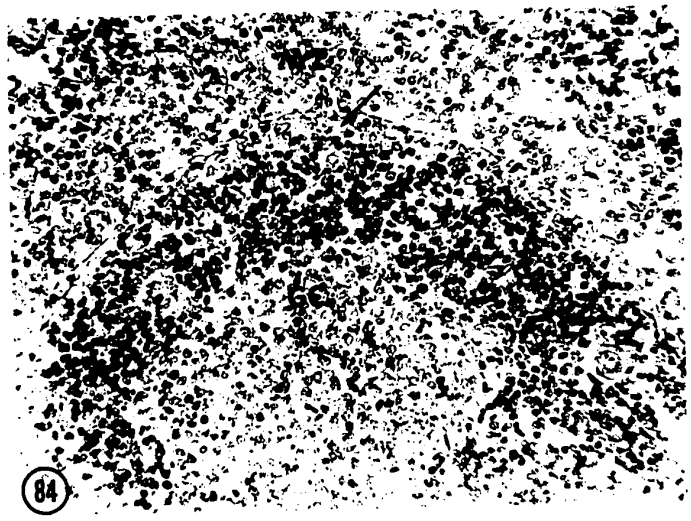

CA
81
CA
82
MZ
PLS
CA
PLS
MZ
RP
RP
LyV
83

Fig. 84 Spleen White Pulp. A portion of a lymphatic nodule with its pale-staining germinal center (GC) and dark cortex (C) is seen to be surrounded by the marginal zone (MZ). This portion of the nodule is not supported by argyrophilic fibers except along its margin (arrow).

Carnoy fixation and PA-silver (x225).


84

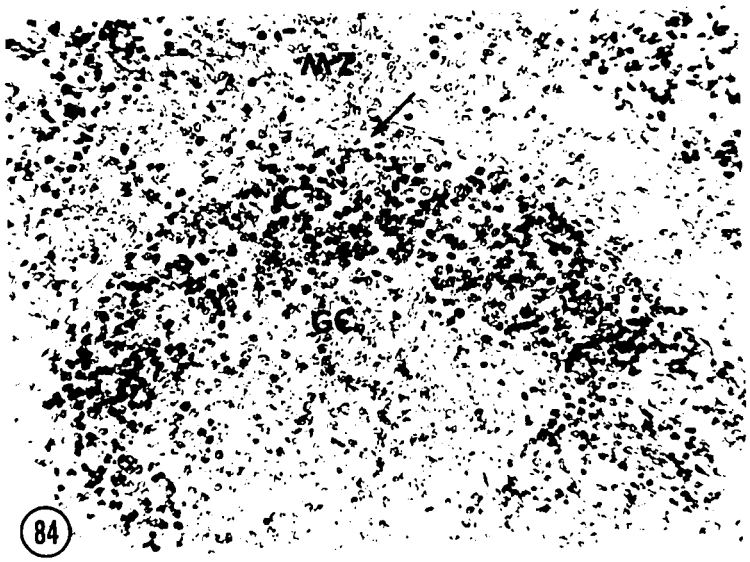

MZ
GC
84

Fig. 85 Spleen White Pulp. The arrow points to a single layer of elongate, TPA-stained reticular cells demarcating a lymphatic nodule (LN) from the marginal zone (MZ).

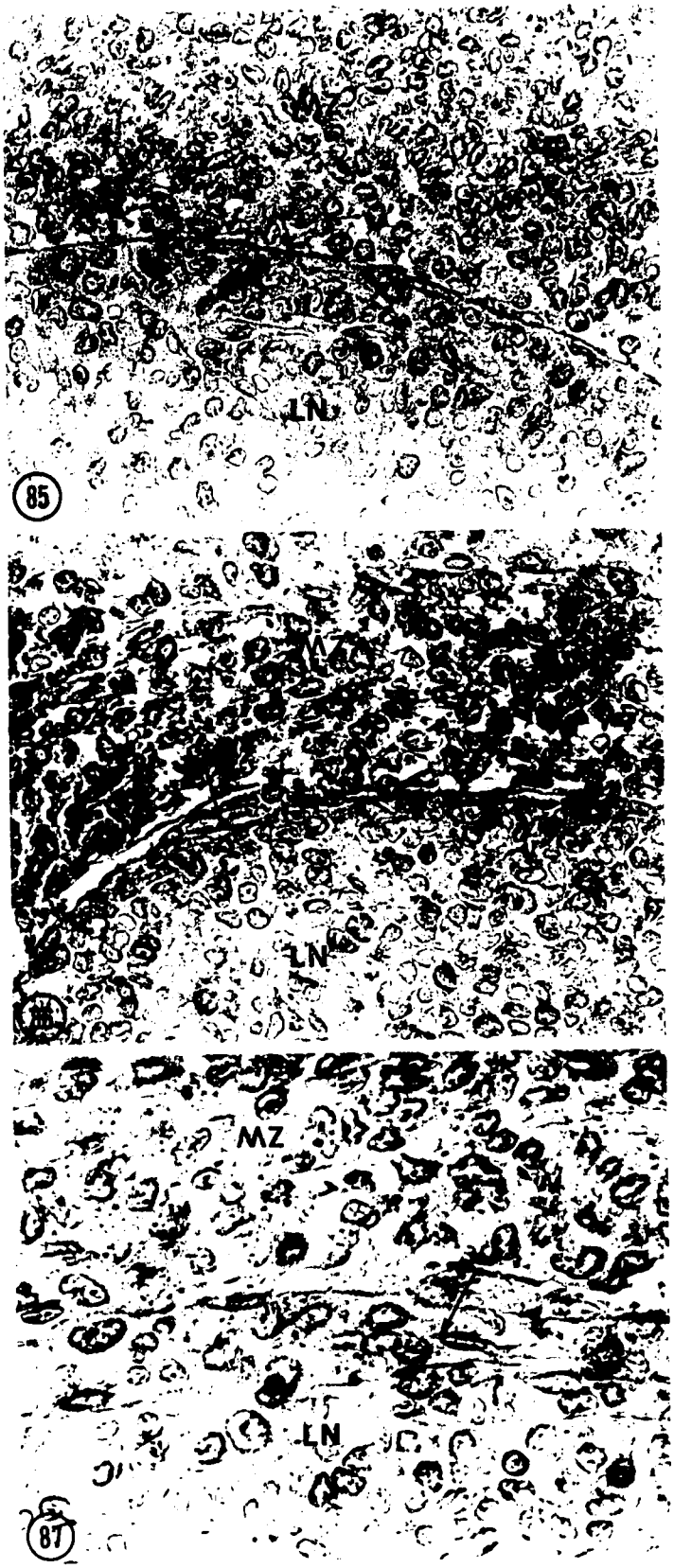
Carnoy fixation and TPA (x525).

Fig. 86 Spleen White Pulp. Arrows indicate a double layer of elongated, fibril-containing reticular cells forming a sort of barrier at the boundary between lymphatic nodule (LN) and marginal zone (MZ).

Carnoy fixation and TPA (x600).

Fig. 87 Spleen White Pulp. A conspicuous sheath (arrow) formed by two layers of TPA-stained reticular cells is visible along the margin of the lymphatic nodule (LN) and serves to demarcate it from the surrounding marginal zone (MZ). Note that some cells in the sheath tend to be rounded and to contain very little TPA-stained material.

Carnoy fixation and TPA (x850).


LN
85
MZ
LN
MZ
LN
87

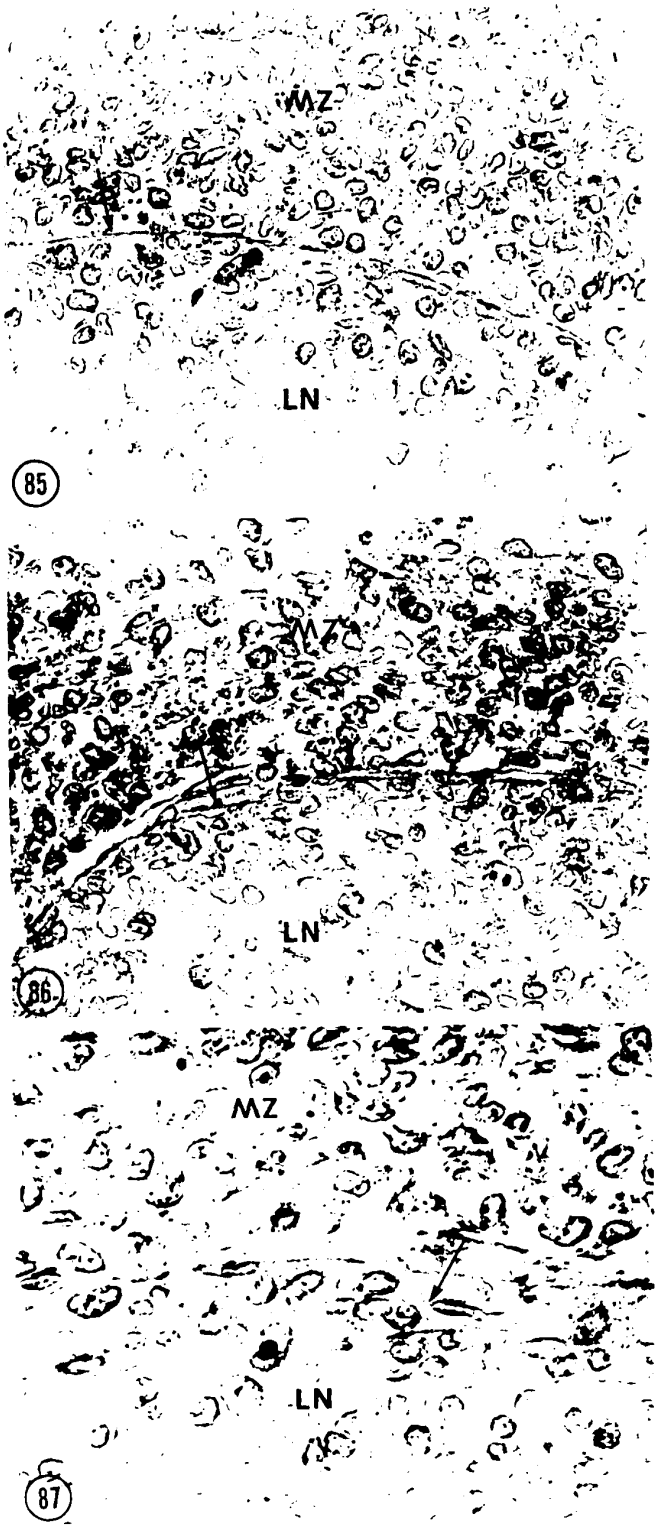

MZ
LN
85
MZ
LN
86
MZ
LN
87

Fig. 88 Spleen White Pulp. An obliquely sectioned central artery (CA) is visible between a periarterial lymphatic sheath (PLS) below and the proximal cortex (PC) or cortical area (1) in text of a lymphatic nodule above. The proximal cortex (PC) is devoid of TPA-stained reticular cells, but is delimited below from the central artery (CA) by the continuous layer of TPA-stained cells investing its adventitia (arrow) and above from the pole of the germinal center (GC) which is directed toward the central artery by another layer of TPA-stained cells (arrow). A macrophage (M) with a voluminous, TPA-negative, granular cytoplasm and a pyknotic nucleus (indicated by arrow within macrophage) is visible in the proximal cortex (PC).

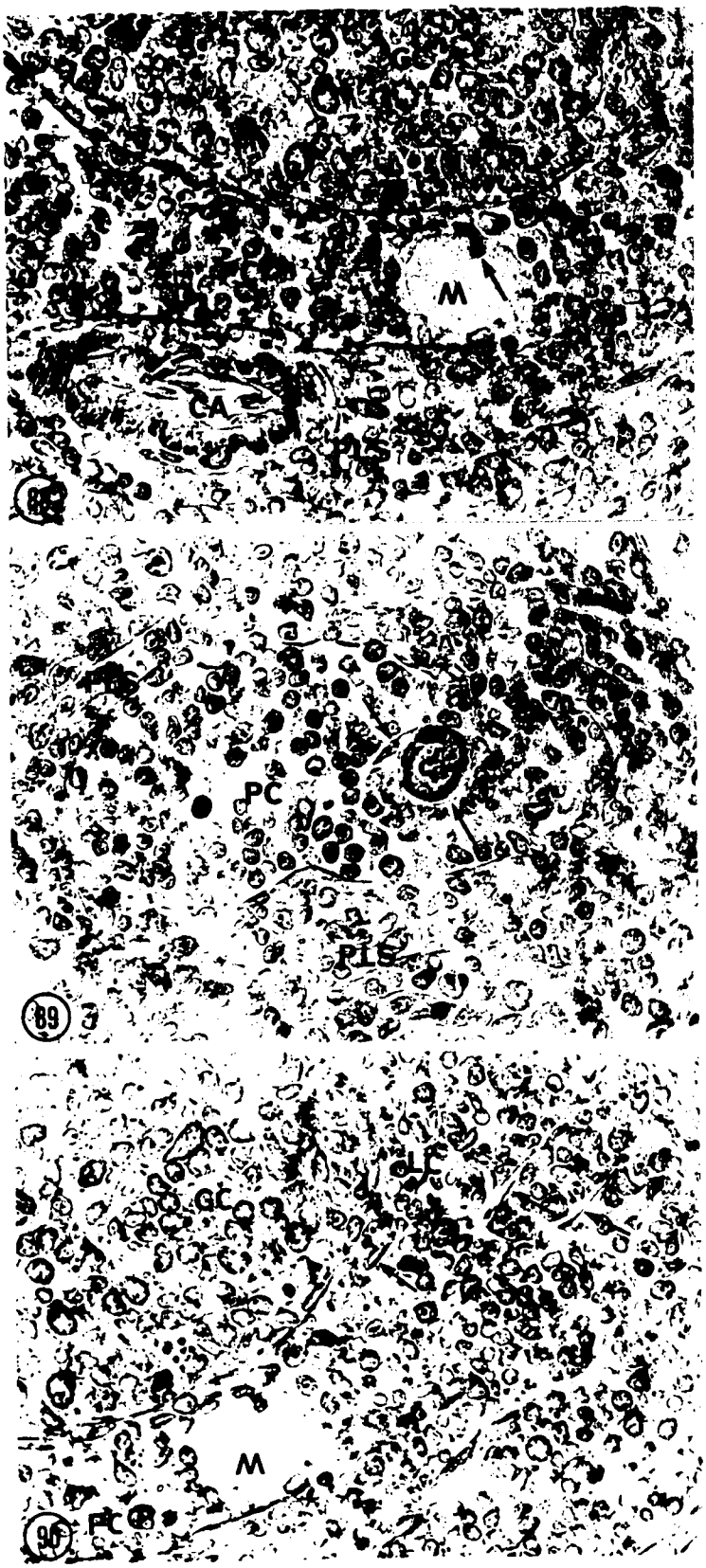
Carnoy fixation and TPA (x550).

Fig. 89 Spleen White Pulp. Toward the lateral cortex which corresponds to cortical area (3) or (4) in text, a branch of the central artery (ca) may appear to be almost completely surrounded by the proximal cortex (PC), but it is always separated from it by the layer of TPA-stained cells investing its adventitia (arrows).

Carnoy fixation and TPA (x525).

Fig. 90 Spleen White Pulp. The proximal cortex (PC) or cortical area (1) with a macrophage (M) is seen to be separated from the germinal center (GC) by a continuous layer of TPA-stained cells and to become continuous with the spaces of the periarteriolar lymphatic sheath in the lateral cortex (LC). Arrows point to TPA-stained cells lining spaces of the periarteriolar lymphatic sheath in the lateral cortex (LC).

Carnoy fixation and TPA (x550).


M
CA
PS
88
PC
PS
89
M
PC
90

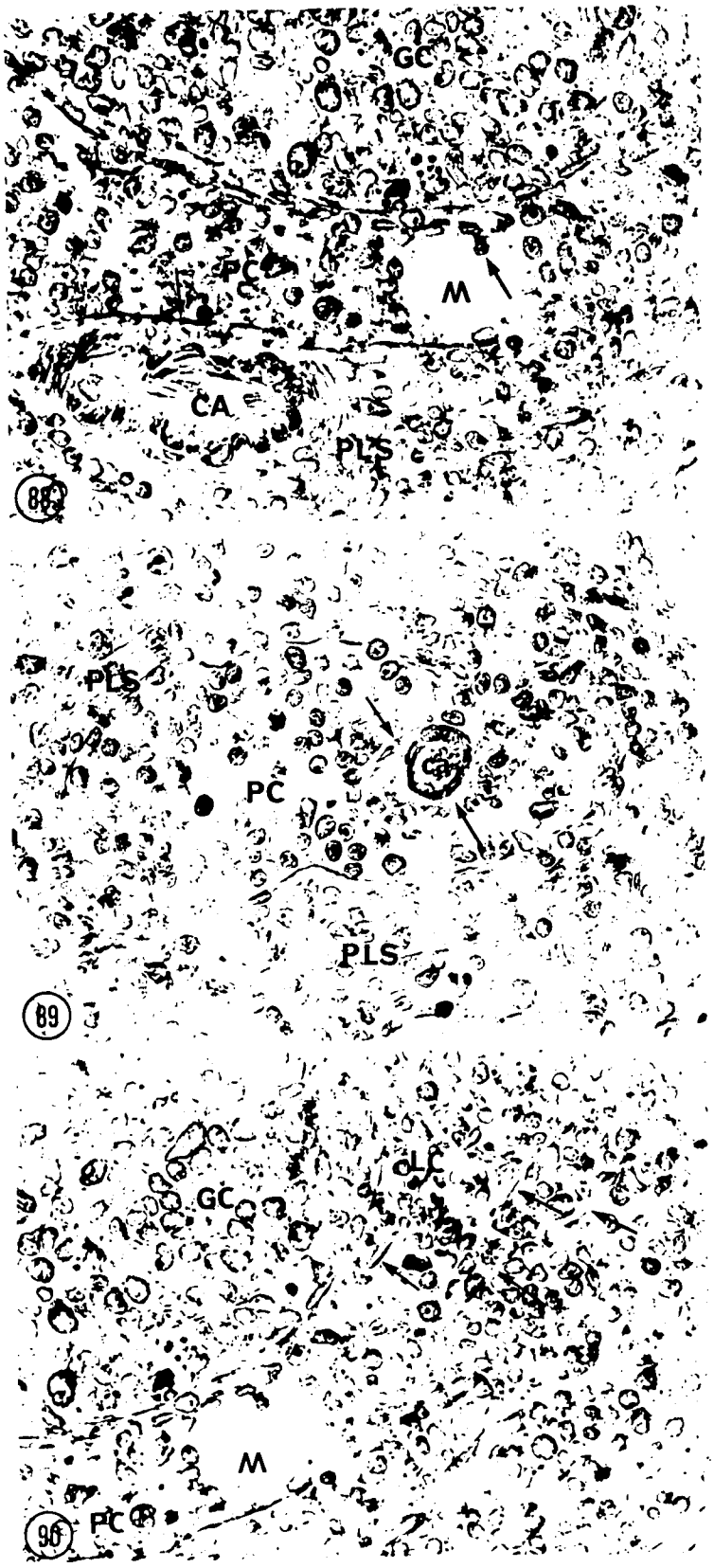
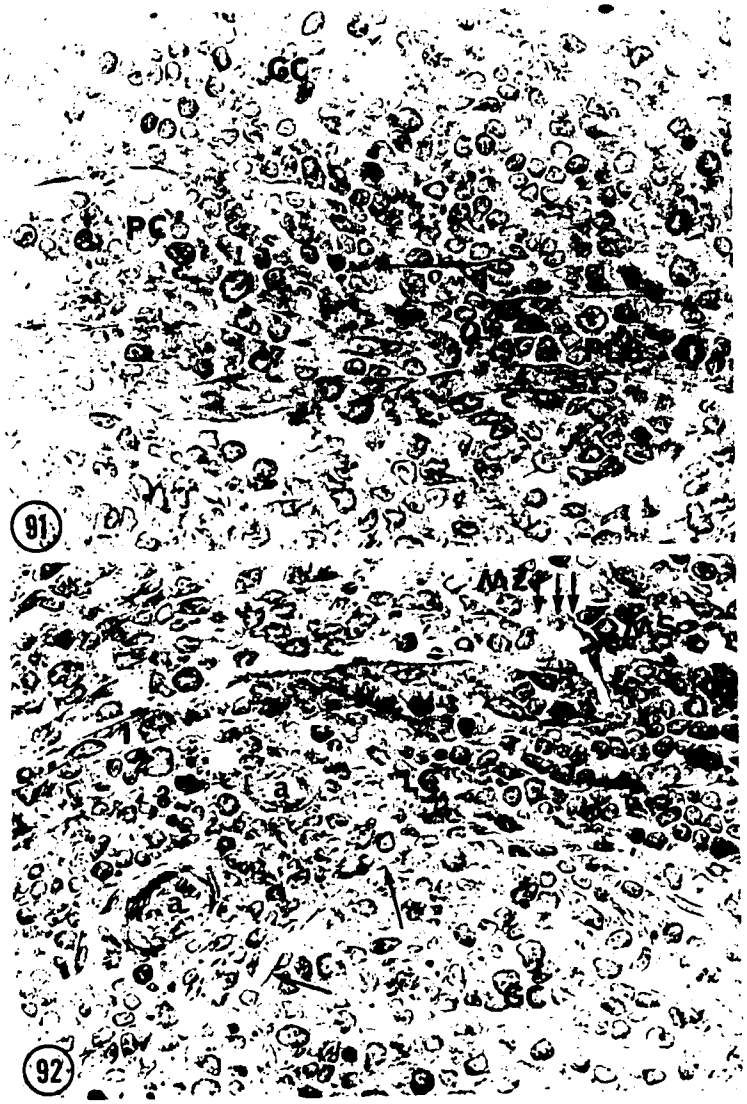

GC
M
CA
PLS
88
PLS
PC
PLS
89
GC
M
PC
96

Fig. 91 Spleen White Pulp. The proximal cortex (PC) or cortical area (1) of a lymphatic nodule is separated from the germinal center (GC) by a continuous layer of TPA-stained cells and shows continuity with the spaces (1,2,3, and 4) of the periarterial lymphatic sheath (PLS).

Carnoy fixation and TPA (x525).

Fig. 92 Spleen White Pulp. Two arterioles (a) given off by the central artery are visible in the lateral cortex (LC) which is demarcated from the germinal center (GC) by a layer of TPA-stained cells (arrows). Some of the spaces (1,2, and 3) of the periarteriolar lymphatic sheath in the lateral cortex (LC) are illustrated. At the limit between the lymphatic nodule and the marginal zone (MZ), a white pulp capillary is seen to open into a marginal sinus (MS). On the surface of the nodule, the sinus is lined by an endothelium continuous with that of the capillary. However, the wall of the sinus directed toward the remainder of the marginal zone (MZ) can be observed to be formed by a TPA-stained reticular cell (covered by a black bar) seemingly displaying discontinuity in one of its cytoplasmic processes (3 arrows).

Carnoy fixation and TPA (x525).


GC
PC
91
MZ
a
GC
92

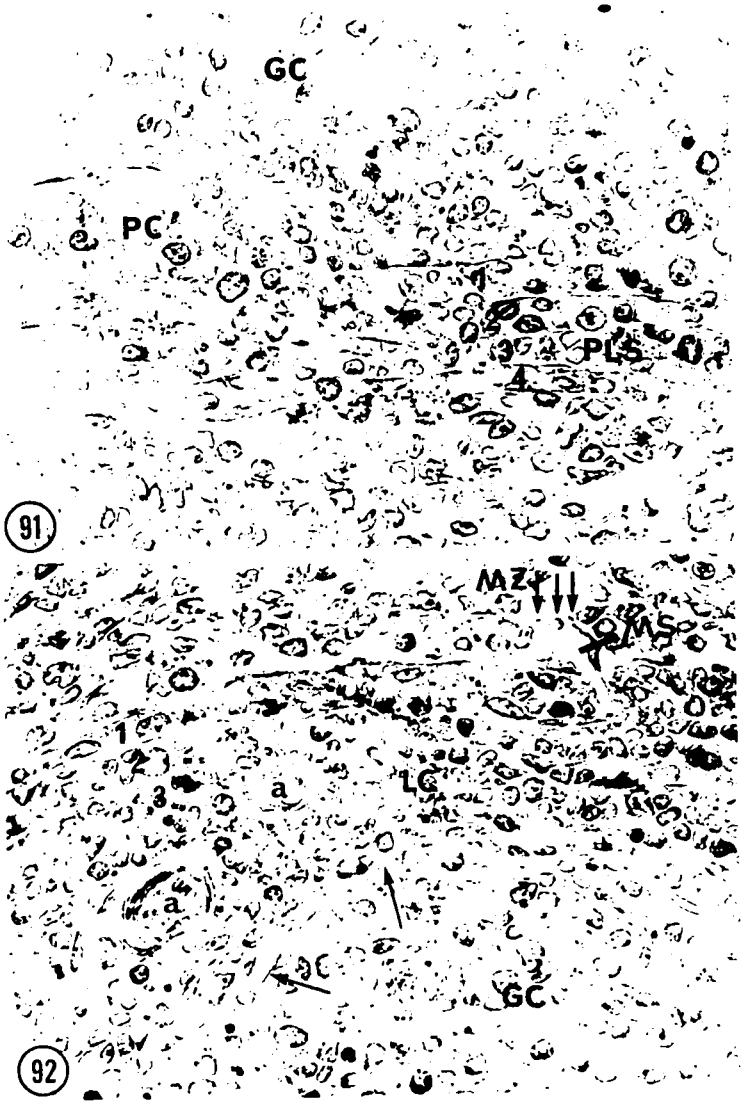

GC
PC
PLS
91
MZ
SWS
LC
a
GC
92

Fig. 93 Spleen White Pulp. A branch (a) is given off by the central artery (CA) whose wall contains intensely TPA-stained smooth muscle cells. The arterial branch (a) crosses the lateral cortex (LC) to reach the mid-portion of a germinal center (GC). The investing layer of reticular cells of the arterial branch to the germinal center delineates, together with the delimiting TPA-stained layer of the center, a space containing a row of lymphocytes (as indicated by arrows).

Carnoy fixation and TPA (x550).

Fig. 94 Spleen White Pulp. Arrows point to the periarteriolar space seen in Fig. 93. The space delimited by TPA-positive reticular cells does not completely cut off the germinal center (GC).

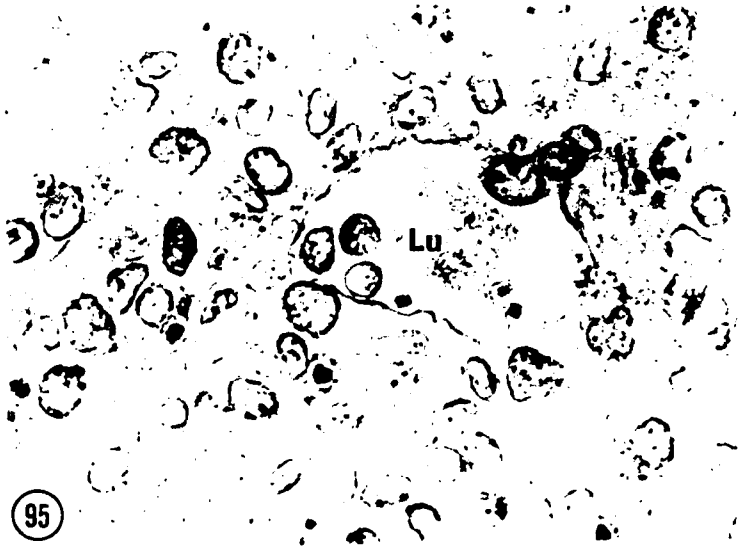
Carnoy fixation and TPA (x550).

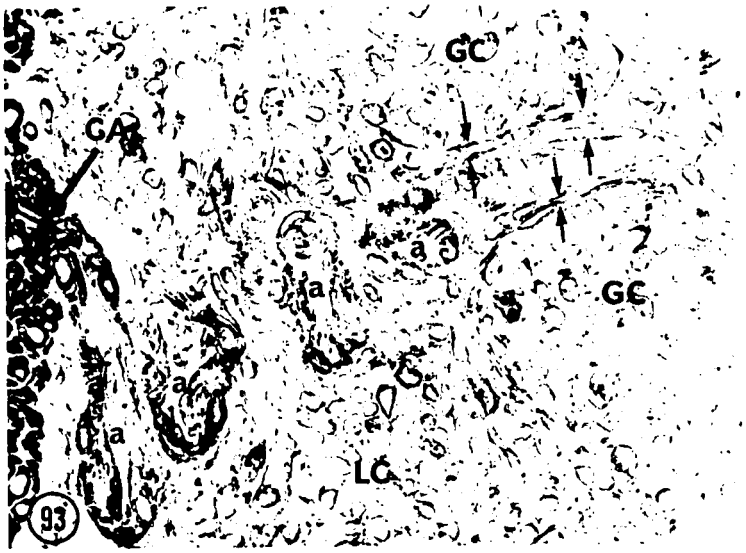
Fig. 95 Spleen White Pulp. Oblique section through the space seen in Figs. 93 and 94 in the central portion of a germinal center. The lumen (Lu) of this space can be seen to be delimited by a TPA-stained layer.

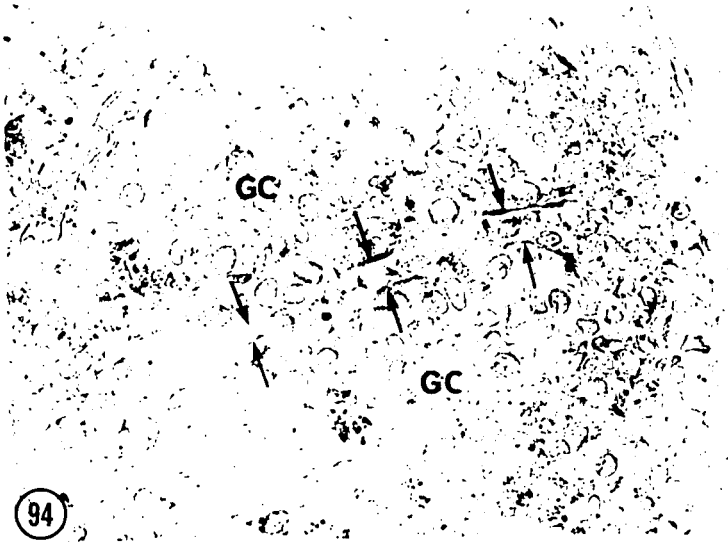
Carnoy fixation and TPA (x1100).


GC
GC
LC
93


GC
GC
94


Lu
95


GC
CA
a
a
a
GC
a
a
LC
93


GC
GC
94

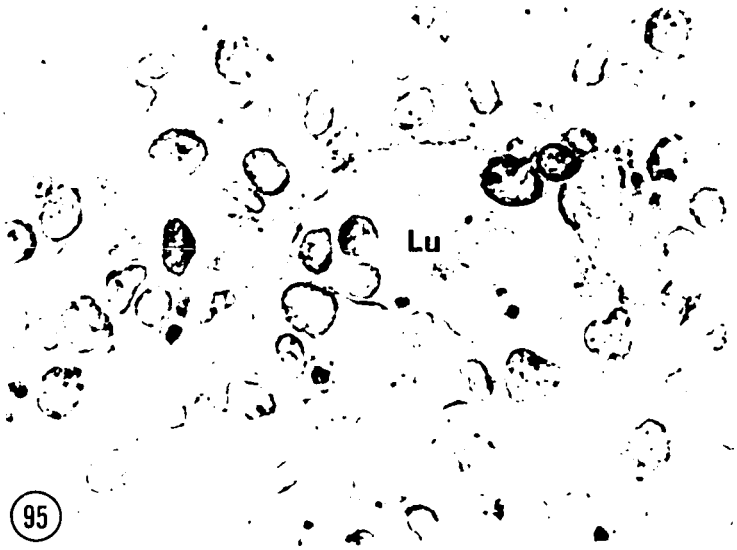

Lu
95

Fig. 96 Schematic diagram illustrating the compartments delimited by TPA-stained reticular cells (description given in pp. 110-112) in the white pulp.

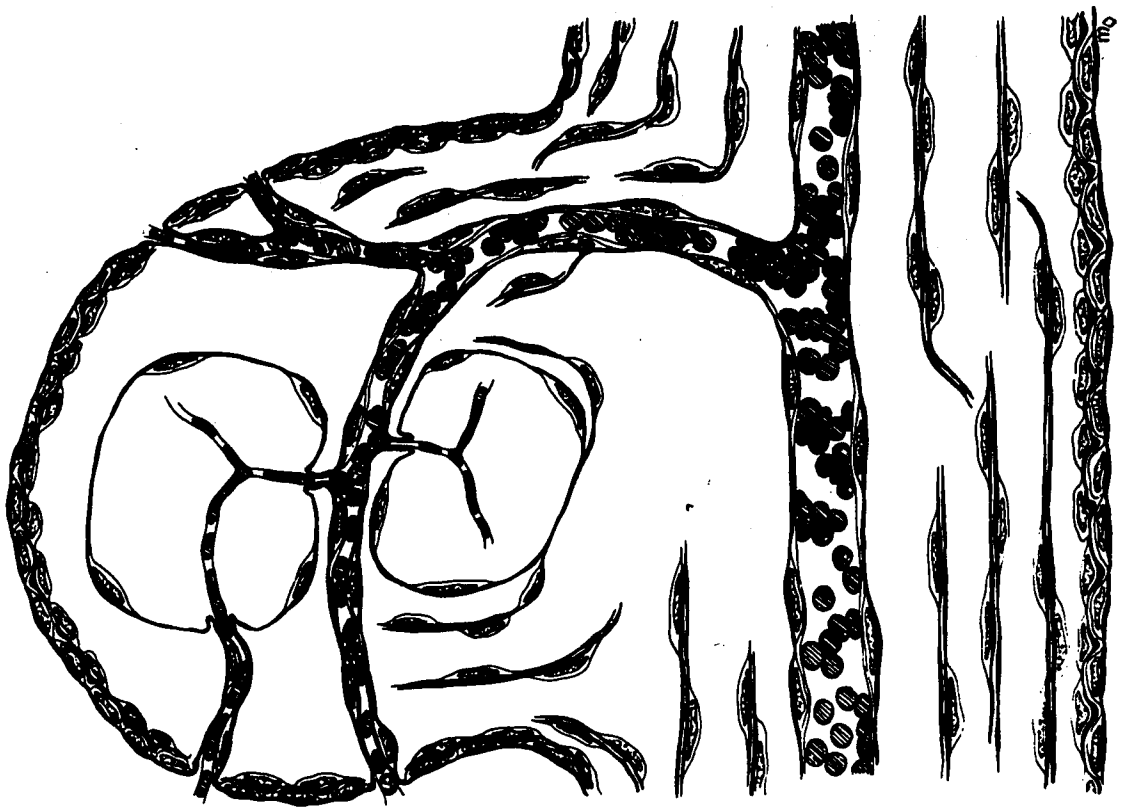


Fig. 97 Spleen Marginal Zone. In some instances, the marginal zone (MZ) may contain large numbers of lymphocytic cells. A marginal sinus (MS) is visible between the lymphatic nodule (LN) and the marginal zone (MZ). Venous sinuses of the red pulp were not found in the marginal zone. A venous sinus (S) conforming to the contour of the marginal zone is illustrated around the periphery of this zone.

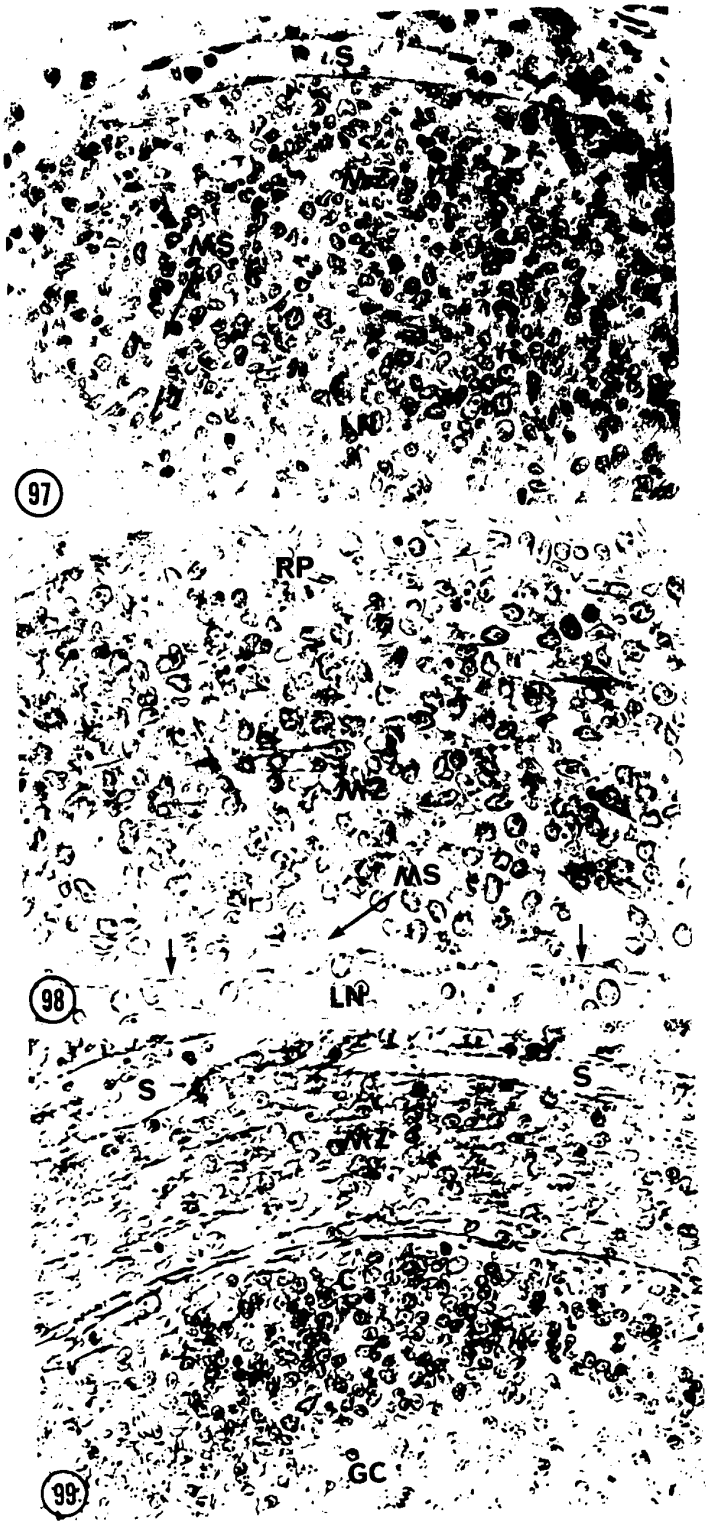
Carnoy fixation and TPA (x400).

Fig. 98 Spleen Marginal Zone. A marginal sinus (MS) is visible between the marginal zone (MZ) and a lymphatic nodule (LN). Arrows point to a single layer of TPA-stained reticular cells along the margin of the module. The marginal zone is usually divisible into three ill-defined vascular areas: 1) the marginal sinus area (MS) into which many white pulp capillaries open; 2) a disorganized area containing polymorphous reticular cells with a scanty amount of TPA-staining fibrils; and 3) an organized area toward the periphery of the zone in which concentrically disposed layers of elongate reticular cells with discrete cell web fibrils in their cytoplasmic processes (as can be observed in this photomicrograph) delimit communicating blood spaces. A portion of the red pulp (RP) is visible.

Carnoy fixation and TPA (x525).

Fig. 99 Spleen Marginal Zone. The supporting framework of the marginal zone (MZ) is formed by a network of delicate argyrophilic fibers which is continuous with the coarser network along the margin of the lymphatic nodule which is seen with its dark cortex (C) and light germinal center (GC). Two venous sinuses (S) are illustrated around the periphery of the marginal zone.

Carnoy fixation and TPA (x475).


S
MS
LN
97
RP
MS
LN
98
S
S
GC
99

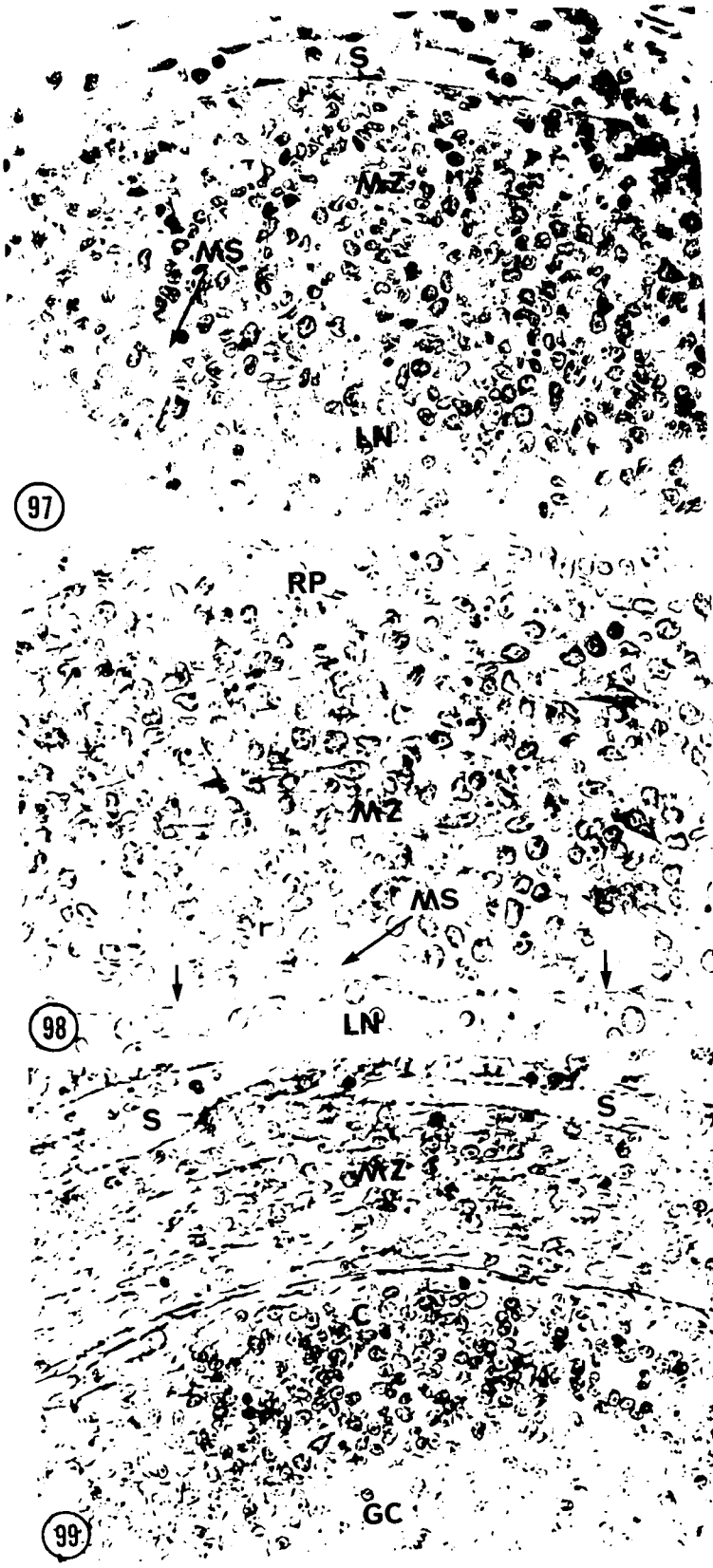

S
MZ
MS
LN
97
RP
MZ
MS
98
LN
S
S
MZ
C
GC
99

Fig. 100 Spleen Red Pulp. Cross section of a venous sinus (S). The reticulo-endothelial or sinal reticular cells appear cuboidal as they are sectioned transversely. Arrow inside sinus points to the cross section of a TPA-stained fiber, the so-called "basal plate". A TPA-positive fibril (F) is visible in the cytoplasm of a cordal reticular cell.

Carnoy fixation and TPA (x1100).

Fig. 101 Spleen Red Pulp. Cross section of a venous sinus (S). Arrow inside sinus points to a transversely cut "basal plate". The unstained sinal basement membrane (BM) is sandwiched between the dark cross sections of "basal plates" and a more lightly stained line in the cytoplasm of cord-limiting reticular cells. Some TPA-stained platelets (P) are visible.

Carnoy fixation and TPA (x1100).

Fig. 102 Spleen Red Pulp. Tangential section through a venous sinus. Arrows indicate longitudinally cut "basal plates" in the cytoplasm of the spindle-shaped sinal or reticulo-endothelial cells.

Carnoy fixation and TPA (x850).


S
P
BM
P
S
BM
101
S
102

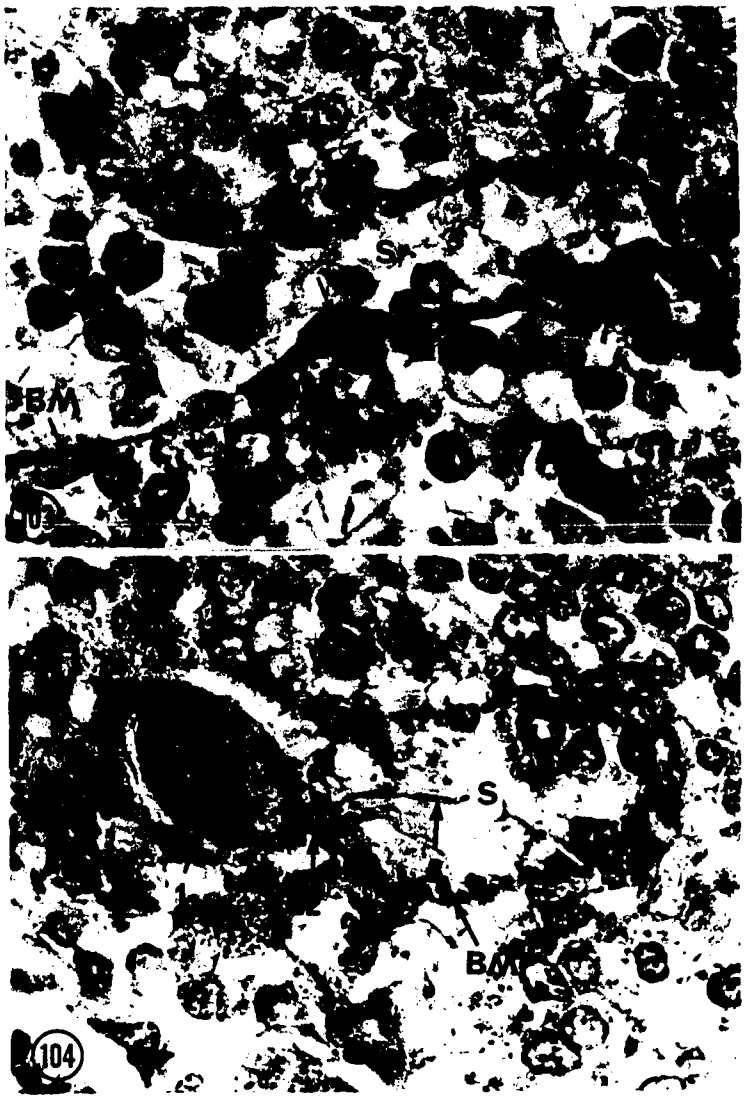

F
S
100
F
BM
P
S
BM
101
S
102

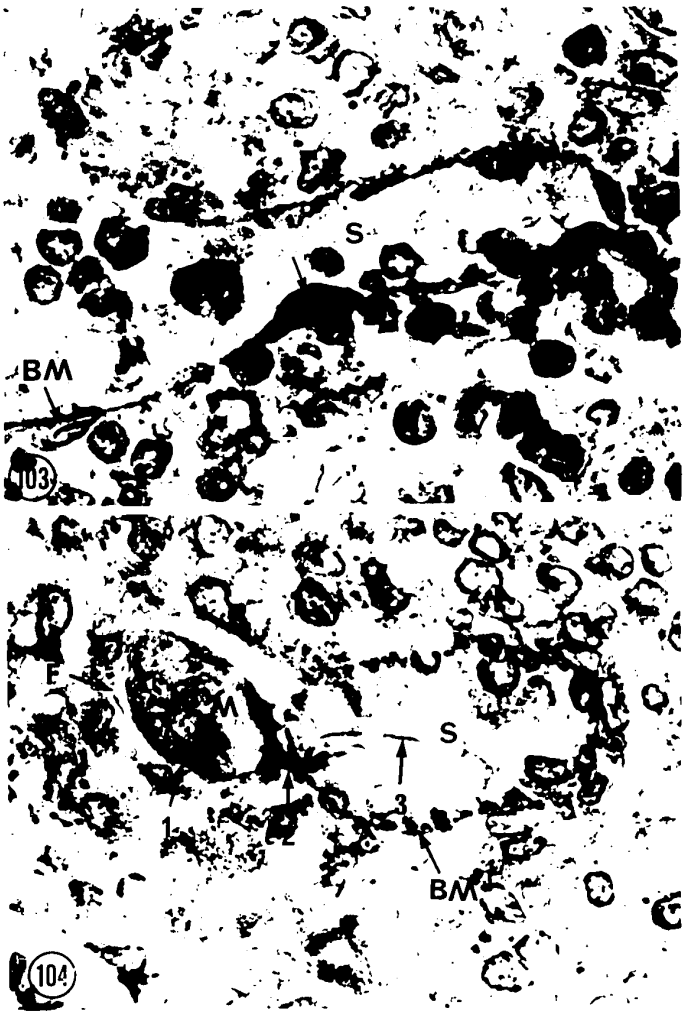
Fig. 103 Spleen Red Pulp. Longitudinal section of a venous sinus (S). Arrow points to a spindle-shaped sinal cell with longitudinally cut "basal plates" alongside the unstained sinal basement membrane (BM). The latter is sandwiched between two TPA-positive fibrils, one belonging to a sinal cell and the other to a cord-limiting reticular cell.

Carnoy fixation and TPA (x1100).

Fig. 104 Spleen Red Pulp. Cross section of a venous sinus (S). The unstained sinal basement membrane (BM) is sandwiched between two TPA-stained lines. A megakaryocyte (M) contains TPA-stained fibrils under the cytoplasmic membrane (1). This cell has extended a process rich TPA-positive material (2 and 3) through a gap in the sinus wall. A fibril (F) in a cordal reticular cell is visible.

Carnoy fixation and TPA (x1100).


S
BM
S
BM
104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DISCUSSION

THYMUS

From the evidence presented in this work, there are two distinct compartments in the thymic lobule: an epithelial compartment and a connective tissue compartment (Fig. 62). The epithelial compartment comprises the cortex and the outer medullary zone which is contiguous with the cortex and for which the term "outer medulla" was proposed. The connective tissue compartment is constituted exclusively by the inner medullary zone, referred to as "inner medulla", and its prolongations. The salient features of these two compartments, which have never been clearly described in the literature, will now be discussed.

Epithelial Compartment:

A) Nomenclature.

Using the electron microscope, Clark (1963) and Hoshino (1963) have observed "epithelial" and "macrophagic" or "mesenchymal" reticular cells in the mouse thymus. Subsequently, several investigators have presented no discrepancies in their studies of the ultrastructure of the thymus with the above-mentioned findings (Weiss, 1963; Cowan

and Sorenson, 1964; Izard, 1966a, 1966b; Blackburn and Miller, 1967).

As indicated in the Review of the Literature, it is usually very difficult to differentiate by light microscopy between thymic reticular cells of endodermal-epithelial origin and those of mesenchymal derivation, though Downey (1948), in studies on the rabbit thymus, has enumerated some morphological characteristics which, in well-fixed material, permit a distinction between these two cell types.

In this work, only one type of reticular cells could be safely identified. The term "reticular-epithelial cells" was used to designated such cells because 1) they were stellate in shape; 2) they did not appear to be phagocytic and displayed conspicuous epithelial characteristics, such as tonofibrils; 3) there was a basement membrane-like layer wherever they bordered on connective tissue; 4) they were interconnected by their cytoplasmic processes to form a continuous epithelium and were therefore judged to be "fixed" elements in contrast to mobile cells such as lymphocytes.

B) Cortex.

1) Cytology of reticular-epithelial cells

In the cortex, two main types of reticular-epithelial cells, namely flattened and stellate types, were recognized in TPA-stained preparations, though there were transitional cell forms between both types (Figs. 15-21). It appears very likely that the cells of the flattened variety correspond to the "subcapsular" cells observed in the rat thymus by Sainte-Marie and Leblond (1958a, 1964a).

In light microscopic studies on the rat thymus, Sainte-Marie

and Leblond (1958a, 1964a) have reported that the nucleoli and nuclear membrane of reticular cells stain a pale orange hue with the Dominici technique and thus display some acidophilia. Moreover, using the electron microscope, Clark (1963), Weiss (1963) and Kohnen and Weiss (1964) noticed that the tonofilaments of thymic reticular cells had a special affinity for membranous sites (nuclear and cytoplasmic membranes). It therefore seems likely that the consistently intense TPA staining of the nucleoli of reticular-epithelial cells is attributable to some acidophilic protein component of these structures, presumably their acidophilic core, as reported by Sainte-Marie and Leblond (1958a, 1964a). However, the TPA coloration of the nuclear membrane of these cells must be essentially due to an accumulation of tonofilaments on it.

Hoshino (1963) found under the electron microscope only occasional bundles of tonofilaments in the cytoplasm of stellate cortical reticular cells. This is confirmed by the present work. The slender cytoplasmic processes of these cells were often found to be less readily identifiable, i.e., to contain less tonofibrils, than those of the reticular-epithelial cells of the flattened type. This finding strongly argues against the view generally held by light microscopists that cortical reticular cells are not conspicuous, except for their nuclei, owing to the presence of closely packed lymphoid cells.

According to McNabb (1964) and Kallenbach et al. (1965), intercellular junctional complexes, i.e., terminal bars and desmosomes, are TPA negative, but their TPA staining is essentially due to an ag-

gregation of "cell web" filaments on the cytoplasmic membrane involved in these structures. It has also been noticed, in studies with the electron microscope, that the tonofilaments in the cytoplasm of thymic reticular cells do not always terminate in desmosomes (Clark, 1963; Hoshino, 1963; Kohnen and Weiss, 1964). Thus, two factors may explain the failure to observe desmosomes in TPA-stained sections of thymus: 1) a number of desmosomes are not associated with tonofibrils and are therefore TPA negative; 2) the desmosomes into which tonofibrils insert cannot be resolved by light microscopy as a result of an accumulation of tonofilaments on the cytoplasmic membrane which makes it difficult to differentiate between the portion of the membrane involved in these intercellular attachment devices and the rest of the membrane.

Lastly, "lipid-laden foamy cells" or "chromolipoid cells" have been described in the thymic cortex of the aging mouse (Loewenthal and Smith, 1952). Since their cytoplasm was filled with granules positive with the PA-Schiff technique, Metcalf and Ishidate (1961, 1962, 1963) referred to these cells as "PAS-positive reticulum cells". The latter were thought to be reticular-epithelial cells by Siegler (1964). However, in a recent study of the peculiar distribution of these cells in the lymphocytic tissue of the rat, Sainte-Marie (1965) found them to be extremely rare in the thymic cortex of animals of all ages. This author concluded that "PAS-positive reticulum cells" were not specific thymic cells for they also occurred in association with lymphatic nodules of the spleen, lymph nodes and Peyer's patches.

In the present work, the cortical reticular-epithelial cells of normal young and adult thymuses did not appear to contain PAS-positive granules in their cytoplasm. It therefore seems very likely that the "lipid-laden foamy cells" of Loewenthal and Smith (1952) and the "PAS-positive reticulum cells" of Metcalf and Ishidate (1961, 1962, 1963), which are known to be phagocytic, do not belong to the population of cortical reticular-epithelial cells. They may represent the "macrophagic" reticular cells of mesenchymal derivation which have been observed in very small numbers in the thymic cortex by Clark (1963), Hoshino (1963) and Izard (1966a).

2) Spatial distribution of reticular-epithelial cells

Around the periphery of each thymic lobule and around cortical blood vessels, a continuous boundary formed by reticular-epithelial cells with a basement membrane has been described as a "hematothymic barrier" by Clark (1963) and as a "thin sheet" by Hoshino (1963). It has been assumed that such a blood-thymus barrier inhibits the passage of antigens from the vascular system into the extravascular spaces of the thymus (Weiss, 1963; Miller et al., 1964). However, in 1964, Clark concluded from his data that there was no absolute barrier to the penetration of antigen into the thymus but only a decreased permeability of the thymic parenchyma to antigen as compared with other lymphoid organs. More recently, Kouvalainen and Gitlin (1967) confirmed and extended the data obtained by Clark (1964a).

Such a continuous boundary was observed in the present study and was found to be constituted by a continuous layer of reticular-

epithelial cells of the flattened category, which completely demarcated the cortical parenchyma of each thymic lobule from the surrounding connective tissue, i.e., the capsule, interlobular septa, intralobular septula accompanying cortical capillaries, and perivascular spaces enclosing lobular blood and lymphatic vessels (Figs. 34 and 62). This delimiting layer was always separated from the connective tissue on which it brodered by a continuous membrane, which stained with silver and PAS.

In some instances, the flattened reticular-epithelial cells were found to completely delineate "pockets" of lymphoid cells from the remainder of the cortical parenchyma (Fig. 21). These cortical pockets were filled with lymphocytes and consistently occurred at the periphery of thymic lobules, preferably right along the interlobular septa. Such lymphocytic compartments, whose functional significance is entirely unknown, have not been described in the literature surveyed.

In agreement with the electron microscopic findings of Clark (1963) and Hoshino (1963) in the mouse thymus, the present work revealed that the stellate reticular-epithelial cells were interconnected by their slender cytoplasmic extensions. Together with the cells of the flattened type, they were observed to form a loose network enmeshing lymphocytes throughout the cortical parenchyma (Figs. 34 and 62).

C) Outer medulla.

1) Cytology of reticular-epithelial cells

Under the electron microscope, Hoshino (1963) has recognized

two main types of epithelial cells in the medulla of the mouse thymus, namely "reticular" and "hypertrophic" types. In TPA-stained preparations, five types of reticular-epithelial cells could be easily identified by light microscopy in the outer medulla: "flattened", "irregularly stellate", "multibranched", "hypertrophic", and "degenerating". These five categories of cells are not, however, intended to be a rigid classification. In fact, all these outer medullary reticular-epithelial cells seem to belong to a single cell population.

The flattened reticular-epithelial cells were found to be morpholocically identical to those of the cortex. While Clark (1963) observed that the medullary epithelial cells were numerous and voluminous, Hoshino (1963) reported that most of these cells were stellate in form and often contained considerable amounts of tonofilaments in their cytoplasmic processes. The numerous reticular-epithelial cells of the irregularly stellate variety seen in the outer medulla of the rat thymus seem to correspond to the "voluminous medullary epithelial cells" of Clark (1963) and the "epithelial cells of the reticular type" rich in tonofilaments of Hoshino (1963).

Striated muscle cells, the so-called "myoid cells", have been reported in the fetal and perinatal thymus of some mammals (Hammar, 1905; Pappenheimer, 1910; Dustin and Bailez, 1914; Salkind, 1915; Wassjutotschkin, 1918), and even of man (Henry, 1966). In a recent paper, Raviola and Raviola (1967) confirmed with the electron microscope that striated muscle cells commonly occurred in the thymus of reptiles and birds. These authors described these cells as "round

or elongated myoid cells" resembling adult skeletal or cardiac muscle fibers and preferably encountered in the medulla of the thymic lobule. Interestingly enough, they observed that these cells were joined by desmosomes to reticular cells and to other myoid cells; they also noticed some degenerating myoid cells with pycnotic nuclei and an electron dense cytoplasm. They finally suggested that myoid cells might originate from reticular cells.

No myoid cells were recognized as such in the parenchyma of the rat thymus. The only type of reticular-epithelial cells that could conceivably be classified as myoid cells are those which were referred to as "multibranched reticular-epithelial cells" in the outer medulla. It may be recalled that these multibranched cells are provided with ramifying, dendrite-like cytoplasmic processes conspicuously richer in TPA-staining tonofibrils than the neighboring reticular cells with which they are anastomosed. The possibility that they may represent myoid cells, however, should be dismissed since their cytoplasmic filaments are not striated.

Furthermore, using the electron microscope, Clark (1963) described two types of cytoplasmic inclusions, which might be secretory, in some medullary reticular-epithelial cells of the mouse thymus. Hoshino (1963), on the other hand, observed that some large and round epithelial cells in the medulla of the mouse thymus had a few short cytoplasmic processes, sparse tonofilaments, and peculiar vesicles provided with microvilli or cilia in their wall; he described them as "hypertrophic" epithelial cells and stated that they often under-

went degeneration and were related to the formation of Hassall's corpuscles. More recently, Kohnen and Weiss (1964) noticed similar ciliated vesicles in the cytoplasm of some medullary reticular-epithelial cells in guinea pig and mouse thymuses and referred to them as "intracellular cysts".

In the present work, however, reticular-epithelial cells of the outer medulla were classified as "hypertrophic" when they displayed morphological characteristics, such a round shape, a thin rim of tonofibrils right under the cytoplasmic membrane and numerous PAS-positive cytoplasmic granules of uniform size. They were regarded as "degenerating" cells when their cytoplasm contained very little fibrillar elements and was occupied by either large PAS-positive granules of varying sixe or a single large inclusion presumably corresponding to an "intracellular cyst". They were finally considered to have degenerated when all cellular structures were obliterated by several globular, colloi-like masses and the whole cell was transformed into an amorphous mass or "extracellular cyst" reacting positively to the PAS technique. Such a classification does not differ considerably from that proposed by Hoshino (1963).

Lastly, since Metcalf (1958) has proposed that the thymus secretes a "lymphocytosis-stimulating factor", morphological and physiological evidence indicated that some thymic medullary epithelial cells may elaborate and secrete a hormone capable of stimulating lymphopoiesis within the thymus. As pointed out previously, electron microscopists have described several cytological features of thymic

medullary epithelial cells which may be indications of secretory activity (Hoshino, 1962, 1963; Clark, 1963; Weiss, 1963; Izard, 1965c). Moreover, Metcalf (1958) recorded a greater increase in the number of circulating lymphocytes in newborn mice and thymectomized adults when the animals were injected with extracts of thymic medulla than when they were treated with extracts of thymic cortex. Osoba and Miller (1964) stated that, within diffusing chambers, the thymus, though still capable of reversing the crippling effects of early thymectomy, consisted almost entirely of epithelial cells. More recently, Clark (1966) presented cytological and histochemical evidence indicating that some medullary epithelial cells of the mouse thymus were capable of elaborating and secreting a sulfated acid mucopolysaccharide. However, in a recent paper, Izard (1966) stated that he found no morphological evidence of granular secretion within medullary epithelial cells of the guinea pig thymus.

From the present morphological study it appears that the only cells in the outer medulla of the rat thymus that could conceivably be candidates for such a secretory role are the reticular-epithelial of the hypertrophic type. Nevertheless, since these cells were found to be linked to adjacent degenerating and degenerated reticular cells (Fig. 30), it seems rather unlikely that they are involved in the secretion of a hormone. As they are usually found in close association with reticular-epithelial cells which are provided with the so-called "intracellular cysts" or which have undergone hyaline degeneration, it appears more likely that these hypertrophic cells represent reti-

cular cells in the initial stages of the degenerative process. It is suggested, after Hoshino (1963), that hypertrophic reticular-epithelial cells may degenerate and converge into Hassall's corpuscles. However, the PAS-positive granules that they accumulate or the cysts that may occur in their cytoplasm may be manifestations of synthesis and storage of a substance, perhaps the sulfated acid mucopolysaccharide which Clark (1966) found in some medullary epithelial cells. Such a sulfated acid mucopolysaccharide may be similar to those observed during keratinization of the epidermis (Wislocki et al., 1951) and may, according to Sylven (1950), provide sulfated redicals indispensable for the keratinization process. Such a process commonly takes place in Hassall's corpuscles, and the latter have been reported to contain sulfated acid mucopolysaccharides (Verne et al., 1956).

3) Hassall's corpuscles

There has been considerable controversy concerning the nature and origin of Hassall's corpuscles. Recent electron microscopic studies of these structures (Hoshino, 1963; Kohnen and Weiss, 1964; Izard, 1965c, 1966) seem to support the theory of Kingsbury (1928). According to this theory, Hassall's corpuscles are epithelial in nature and develop from hypertrophied reticular-epithelial cells.

From the evidence presented in this work, Hassall's corpuscles are restricted to and are not difficult to find in the outer medulla of the rat thymus. They constitute a diverse group of polymorphous bodies showing a great variability in size and structure. They may

vary from small solid masses of lamellated reticular-epithelial cells with massive quantities of tonofibrils (Fig. 31) to large structures displaying basically a central core filled with fluid or degenerating cells and surrounded by concentric lamellae of reticular-epithelial cells rich in tonofibrils (Figs. 31-33).

The central core of the corpuscles was found to vary in composition; it may consist of a single degenerating reticular-epithelial cell or may be composed of two or more such cells in addition to a few degenerate lymphoid elements. Unlike some investigators (Izard, 1965c; Kostowiecki, 1963, 1964; Jaroslow, 1967; and others), no other cell types or degenerating blood vessels were found to form the core of these bodies, findings which argue in favor of their exclusive development from hypertrophic or degenerating reticular-epithelial cells in the outer medulla of the rat thymus.

Furthermore, Kohnen and Weiss (1964) and Izard (1965c, 1966), in studies on the ultrastructure of Hassall's corpuscles, have reported that the cells making up the central core of these corpuscles, like those in the stratum corneum of the epidermis, have lost their nuclei and have become transformed into filamentous areas. Kohnen and Weiss (1964) added: "It is doubtful that all intracellular fibrils are inserted into desmosomes, and there is suggestive evidence that fibril deposition and membrane desintegration are associated processes." In the present work, no TPA-staining fibrils or desmosomes could be recognized in the central cells of well-developed corpuscles. It can reasonably be concluded that typical tonofilaments

are not present in these cells and that the filamentous material observed by electron microscopists in their cytoplasm, since it is TPA negative, does not belong to the "cell web" family and may represent the product of membrane desintegration. This finding also supports the view that degenerating reticular-epithelial cells in which no tonofibrils were observed form the center of Hassall's corpuscles.

The present study also demonstrated conclusively that the wall of a Hassall's corpuscles was composed of concentric lamellae of tonofibril-containing reticular-epithelial cells. As reported by Kohnen and Weiss (1964), the most peripheral cells of the wall was found to contain conspicuously more tonofibrils than neighboring reticular-epithelial cells in the outer medulla and was often seen to diverge from the main body to establish connections with these cells, i.e., with the outer medullary reticular framework. Unlike Izard (1965c, 1966), however, the tonofibrils were observed to be less conspicuous in the cytoplasm of the cells bordering the central core of corpuscles. In small, developing corpuscles, avidly TPA-stained granules of various sizes were seen in association with tonofibrils, particularly in the cytoplasm of reticular cells contiguous with the center of the corpuscle. It is noteworthy that these granules resemble the keratohyalin granules of epidermal cells which are also TPA positive (Kallenbach, 1963). Similar granules were reported in Hassall's corpuscles by Kohnen and Weiss (1964) and Izard (1965c) who also believed them to represent keratohyaline granules.

4) Spatial distribution of reticular-epithelial cells

The present work revealed, in accord with the electron microscopic findings of Clark (1963) and Hoshino (1963), that all the tonofibril-containing reticular-epithelial cells of the outer medulla were anastomosed by their cytoplasmic processes to form a continuous, dense network holding lymphocytes in its meshes. This network, at the cortico-medullary boundary, was continuous with the rather loose cellular reticulum of the cortex, thus indicating that the reticular-epithelial cells of both cortex and outer medulla belonged to a single cell population. Furthermore, as found by Sainte-Marie and Leblond (1964a), no membrane or network of fibers was observed at the junction between cortex and outer medulla.

However, at the limit between outer and inner medulla, i.e., between epithelial and connective tissue compartments, the reticular-epithelial cells of the flattened type were found to form a continuous layer. These cells also formed a continuous epithelial sheet around outer medullary capillaries and around the perivascular connective tissue spaces which enclosed the post-capillary venules that had extended from the inner medulla into the outer medulla to receive the blood carried by cortical capillaries.

Using the electron microscope, Clark (1963), Hoshino (1963) and Weiss (1963, stated that the epithelial sheet formed a continuous barrier around the periphery of each thymic lobule, around intrathymic blood vessels as well as around perivascular spaces. They also reported that this sheet was always separated from the surrounding connective tissue by a continuous basement membrane. This is in

agreement with the evidence presented in this work, except that the basement membrane associated with the flattened epithelial cells is discontinuous at the boundary between outer and inner medulla. The significance of such a discontinuous basement membrane will be discussed later.

In his electron microscopic investigation of the mouse thymus, Clark (1963) reported that medullary epithelial cells were often arranged in "clumps" or "solid cords". In the present study, small accumulations of epithelial cells were sometimes encountered in the outer medulla; however, they did not form compact, solid epithelial masses. Such masses, however, were found in the inner medulla.

D) Mitoses of reticular-epithelial cells.

Using the electron microscope, Clark (1963) stated that he observed no epithelial cells in mitosis in the thymic reticular cell network. However, in their light microscopic study of the rat thymus, Sainte-Marie and Leblond (1964a) found, in both the cortex and the medulla of the thymic lobule, dividing reticular cells which they related to the epithelial cells of Clark (1963).

In the present work, a number of cortical and outer medullary reticular-epithelial with tonofibrils were found in mitosis (see Fig. 53). A radioautographic experiment demonstrated that some thymic reticular-epithelial cells could synthesize DNA (see Figs. 54 and 55). However, more labeled reticular cells were observed in the outer medulla than in the cortex. This might be attributable to the fact that, unlike the reticular-epithelial cells of the cortex, those of

the medulla were numerous and contained large amounts of tonofibrils, which thus made them more readily identifiable in TPA-stained radioautographs.

E) Temporal distribution of reticular-epithelial cells.

From the evidence presented in this work, it was only with time and gradually that tonofibrils became visible in thymic reticular epithelial cells. It may be recalled that, between the thirteenth and the fifteenth day of embryonic development, no reticular-epithelial cells with demonstrable tonofibrils were observed.

In 13-day-old embryos, the thymic anlage was a solid epithelial mass completely delimited from the surrounding mesenchyme by a continuous basement membrane. At this stage of development, besides the columnar epithelial cells with a terminal bar-terminal web complex bordering traces of the primitive pharyngeal duct, the thymic anlage was populated by only conical epithelial cells arranged in acinar configuration (see Figs. 63 and 64). Such conical cells corresponded to the "undifferentiated epithelial" cells of Ackerman and Knouff (1965) and to the "primitive stromal epithelial" cells of Sanel (1967).

It was not until the sixteenth day of embryonic life that tonofibrils became visible in epithelial cells accumulated into a newly formed Hassall's body, which stained lightly in H and G preparations and which thus constituted the first or early medulla.

The present work revealed that, in the final quarter of embryogenesis of the thymus, it was not possible to demonstrate tonofibrils

in the cytoplasm of cortical reticular-epithelial cells. It was only by the second postnatal day that TPA-staining fibrillar material could be identified in the epithelial cells of only the deep portion of the thymic cortex. The latter was richly provided with small lymphocytes in contrast to the subcapsular cortex which was much less differentiated and was composed mainly of reticular cells and lymphoblasts.

Indeed, it was noticed that in 4-day-old rats the subcapsular cortex had disappeared, the whole cortex had increased in size, and was more uniformly populated by reticular cells with visible tonofibrils. However, it was only between the sixth and the seventh day of postnatal life that a layer of flattened reticular cells could be identified where the thymic parenchyma bordered on connective tissue.

Finally, it is noteworthy that the first medulla, as can be seen in Figures 67 and 69, was formed in close association with an interlobular septum. In a recent study of the first stages of development of the medulla and the cortex in the rabbit thymus, Kostowiecki (1967) confirmed this association and stated that the early medulla was a mixture of epithelial cells derived from the original epithelial anlage and mesenchymal cells provided by a nearly mesenchymal septum. This may explain the partitioning of the medullary region into an outer and inner medulla in the adult thymus.

Connective Tissue Compartment:

A) Topography.

From the evidence presented in this work, the connective tissue

compartment of each thymic lobule can be defined as a large, irregular, sharply defined connective tissue space which is divisible into two main components: 1) the "inner medulla", which occupies the deeper portions of the lobule; and 2) the "perivascular spaces", which are prolongations or extensions of the inner medulla enclosing blood and lymphatic vessels.

This compartment is completely demarcated from the epithelial one, which comprises the cortex and the outer medulla, by a continuous sheet of flattened reticular-epithelial cells resting on a basement membrane-like layer, which stains with silver and PA-Schiff. At the level of the perivascular spaces, the basement membrane was observed to be continuous. However, it was found to be discontinuous at the boundary between outer and inner medulla.

In his study of the histogenesis of dense lymphatic tissue of the intestine, Latta (1921) described the changes taking place in the basement membrane associated with the rapid increase of lymphocytes in the epithelium. He noticed under the light microscope that the basement membrane became indistinct as lymphocytes crowded into the epithelium. More recently, Shimizu and Andrew (1967) made a correlated light and electron microscopic investigation of lymphocyte-epithelial relations in the appendix of normal rabbits of various ages. These authors found with the electron microscope that the basement membrane at the level of the lymphatic nodules became discontinuous at two weeks and even disappeared in older animals. According to these workers, the basement membrane became discontinuous once lymphocyte migration into the epithelium was considerable.

They concluded that the discontinuity of the basement membrane was an indication of active lymphocyte migration from the lymphatic nodules into the epithelium.

Similarly, Toro and Olah (1967), using the electron microscope, observed that the migration of thymic lymphocytes across the wall of thymic capillaries was preceded by local dissolution of the collagenous fibers and disappearance of the basement membrane. In contrast to the above workers, Clark (1963) held that it was possible for lymphocytes to pass through the basement membrane. Moreover, Sainte-Marie and Leblond (1958b, 1964a, 1964b) observed figures of diapedezing small lymphocytes across the basement membrane-like layer forming the outer wall of perivascular spaces in the rat thymus.

From the above references, it appears that lymphocytes can pass through the basement membrane; however, when lymphocyte migration across the membrane is massive, the latter would become discontinuous and possibly disappear.

B) Content.

The connective tissue compartment of the thymic lobule was found to contain larger amounts of argyrophilic fibers than any of the two components of the epithelial compartment. The fibers were haphazardly distributed in the inner medulla, but formed, as previsouly observed by Smith and Ireland (1941) and Sainte-Marie and Leblond (1958b, 1964a, 1964b), concentric layers inside the perivascular spaces.

The concentration of lymphocytes in this area was slightly

lower than that in the cortex but higher than that in the outer medulla. It followed that, in silver preparations, the connective tissue compartment had a color intermediate between that of the cortex and that of the outer medulla (see Figs. 35-39).

This compartment was extremely well vascularized. In addition to post-capillary venules and lobular veins, it contained a number of arterial channels which ramified into numerous capillaries in the inner medulla area. Such capillaries were confined to the territory of the inner medulla and were never seen to penetrate the epithelial compartment. The arterial channels were often accompanied by typical, endothelium-lined lymphatic vessels.

Not infrequently, branching cysts and duct-like spaces were found in the inner medulla and in some large perivascular spaces which traversed the epithelial compartment to become continuous with interlobular septa.

C) Perivascular spaces.

The present work demonstrated conclusively that, in accord with Clark (1963) and Weiss (1963), perivascular spaces were not endothelium-lined as believed by Sainte-Marie and Leblond (1958b,, 1964a). They were found to be connective tissue spaces, continuous with the inner medulla, that enclosed blood vessels and that were delimited from the epithelial compartment by a continuous epithelial layer with an uninterrupted basement membrane. The belief of Sainte-Marie and Leblond (1964a) that these spaces may not completely surround a blood vessel obviously stems from the fact that the sections

often passed through the point where they become continuous with the inner medulla.

In section, perivascular spaces may be encountered in the outer medulla around a single post-capillary venule; however, in both the outer medulla and the cortex, they may be observed around a single lobular veins, around lobular blood and lymphatic vessels, or again around cystic and duct-like spaces. In all these locations, they were separated from the parenchyma of the epithelial compartment by a continuous epithelial sheet with a basement membrane.

D) Cystic and duct-like spaces.

According to Tesseraux (1959), typical cysts lined by cuboidal, columnar, or ciliated cells might be localized in the interlobular septa of the thymic lobe, but they most frequently occurred within the thymic lobule completely surrounded by thymic parenchyma. Schambacher (1903) and Shier (1963) proposed that Hassall's corpuscles might develop from the epithelial lining of tubular cysts.

The present work demonstrated conclusively that there were two main categories of thymic cysts: 1) the so-called "intracellular" and "intercellular" cysts, which were found exclusively in the outer medulla of the thymic lobule and which seemed to arise from degenerating medullary reticular-epithelial cells; and 2) frankly tubular or duct-like epithelial cysts which were lined by cuboidal, columnar, or ciliated cells and which were confined to the territory of the connective tissue compartment and of some interlobular septa of the thymic lobule.

The epithelial lining cells of the tubular cysts were found to be rich in cell web filaments. When such cysts were cut tangentially, they assumed the appearance of solid masses of epithelial cells richly, provided with cytoplasmic filaments (see Fig. 45). Such masses would correspond to the ones observed by Clark (1963) in the medulla of the mouse thymus.

Blood and Lymphatic Vascularization:

The present investigation clarified some points concerning the intralobular blood circulation in the rat thymus. First, it was confirmed that the pattern of arterial supply of the thymic lobule was centripetal. Secondly, it was clearly shown that the epithelial compartment of the thymic lobule, i.e., cortex and outer medulla, was characterized by the presence of only arterial capillaries which had three patterns of distribution. Thirdly, this work demonstrated conclusively that the thymic lobule had a double arterial supply: one set of septal arterial branches for the epithelial compartment, and another set for the connective tissue compartment.

It is generally believed that the thymus has only small efferent lymphatic channels which run mainly in the interlobular septa (Weiss, 1966a; Sainte-Marie and Leblond, 1964a; Smith, 1955). The present work revealed the presence of true endothelium-lined lymphatic vessels which originated in the inner medulla and accompanied the lobular veins and arteries to the lobar hilum.

SPLEEN

Like the reticular-epithelial cells of the thymus, most reticular cells in the spleen were found to contain TPA-staining fibrils, to form a continuous layer around most blood vessels and to delimit compartments within the splenic pulp. Unlike the thymic cells, however, their fibrils became visible late in postnatal life and these cells were not observed to form a continuous boundary around the periphery of the organ, i.e., under the capsule and along the trabeculae. The cytological characteristics and spatial distribution of these TPA-stained reticular cells in the splenic white pulp, marginal zone and red pulp will now be discussed.

White Pulp:

A) Cytology of TPA-stained reticular cells.

In the periarterial lymphatic sheaths of the white pulp, Galindo and Imaeda (1962), using the electron microscope, described two main types of reticular cells, both of which were elongate in shape in section: 1) "fixed reticular cells type A (FRCA)" rich in cytoplasmic free ribosomes which gave them a dark appearance, and 2) "fixed reticular cells type B (FRCB)" with fewer ribosomes. These workers found both cell types to be intimately associated with the extracellular reticulum and, on the basis of their structural pattern, suggested that FRCA might potentially transform into FRCB and/or macrophages.

Similarly, Weiss (1964) reported that most reticular cells in the periarterial lymphatic sheaths were fixed to the extracellular reticulum which they ensheathed. This author, however, noticed electron dense cytoplasmic material aligned in the portion of the cytoplasm of these cells that lay alongside the reticulum.

The present work revealed that all TPA-stained reticular cells in the periarterial lymphatic sheaths, in section, were fusiform and possessed elongate cytoplasmic extensions. With the exception of those associated with the adventitia of arterial channels, they were observed to encompass the coarse extracellular reticulum fibers of the white pulp stroma. In addition, delicate TPA-staining fibrils, most probably corresponding to the electron dense material seen by Weiss (1964), could be identified in the portion of their cytoplasm that was adjacent to the reticulum. It therefore seems most likely that such TPA-stained reticular cells represent the more differentiated "fixed reticular cells type B (FRCB)" of Galindo and Imaeda (1962) and the abundant "fixed reticular cells" with dense, aligned cytoplasmic material of Weiss (1964).

Moreover, Weiss (1964) noted that both endothelial cells and what he called "advential cells" of arterial capillaries contained dense basal material similar to that present in reticular cells. Such "adventitial cells" with dense cytoplasmic substance would correspond to the "unfixed", elongate TPA-stained reticular cells observed in this work in close proximity to the adventitia of arterial vessels.

It is noteworthy that the nuclei of TPA-stained reticular cells,

unlike those of thymic reticular-epithelial cells, were often not readily identifiable. This may be attributable to the fact that the relatively scant amount of TPA-staining material present in these cells preferably accumulates in their basal cytoplasm, i.e., the portion of the cytoplasm lying alongside the extracellular reticulum, and does not aggregate on the nuclear membrane as tonofilaments do in thymic reticular-epithelial cells.

It is equally noteworthy that, as a general rule, the nuclei of the TPA-stained reticular cells were observed to bulge away from the extracellular reticulum to which the cells were attached. One exception to that rule was the nuclei of the "unfixed" reticular cells that were associated with the walls of arterial vessels and that were not fixed to reticulum fibers; indeed, they were found to consistently protrude into the adventitial connective tissue. It follows that TPA-stained reticular cells in the periarterial lymphatic sheaths are oriented cells with a base resting on extracellular reticulum stainable with silver and PAS and containing TPA-staining fibrils analogous to the so-called "basal web" seen in the cytoplasm of kidney epithelial cells (Clermont and Pereira, 1966).

Little is known of the cytological characteristics of reticular cells in the uncommon secondary lymphatic nodules of the spleen. In a recent electron microscopic study of normal mouse spleens, however, Swartzendruber (1967) could distinguish two types of reticular cells in the germinal centers: 1) reticular cells with electron dense materials in their elongate cytoplasmic processes, and 2) "dark" reticular cells or "transitional" cells linked by desmosomes. In the pre-

sent work, the TPA-stained reticular cells in the lymphatic nodules were found to display the same cytological features as those in the periarterial lymphatic sheaths. They probably correspond to the "reticular cells with electron dense materials" of Swartzendruber (1967).

B) Spatial distribution of TPA-stained reticular cells.

The present study demonstrated conclusively that there was an abundance of TPA-stained reticular cells in the splenic white pulp. They were seen to form a conspicuous single or double layer richly provided with coarse TPA-staining fibrils which completely demarcated the white pulp from the surrounding marginal zone. Such a layer was found to be associated with a dense network of delicate argyrophilic fibers, which was called the "capsule of the follicle" by Krumbhaar (1948).

In light microscopic studies on the perifollicular region of the rat spleen, Snook (1964) found, at the margin of splenic lymphatic nodules, an unusual aggregation of reticulum-like cells which were blackened by Marshall's silver impregnation technique, hence the name "marginal metalophils" that he gave such cells. He stated that these marginal metalophils tended to be rounded with blunt processes in contrast to the highly branched metalophils or fixed reticular cells of the white pulp. Moreover, this author suggested that such cells represented potential phagocytes since they often reacted negatively to tests for iron. Recently, Pettersen et al. (1967), in their study of the primary and secondary immune responses in the

rat spleen, suggested that the marginal metalophils of Snook (1964) might initiate the antibody-forming process in the lymphatic nodules by coming into close contact with marginal zone lymphocytes.

It appears likely that the dense ring of marginal metalophils observed by Snook (1964) corresponds to the conspicuous sheath or barrier of TPA-stained reticular cells seen at the margin of the white pulp. Indeed, while most TPA-stained reticular cells composing the sheath were elongate, some were plump but still contained demonstrable fibrils in their cytoplasm; furthermore, an occasional cell with nuclear morphology similar to that of reticular cells and with phagocytosed material was sometimes recorded in the marginal sheath; however, such a macrophage did not display TPA-staining fibrils. It therefore seems plausible that the TPA-stained reticular cells making up the barrier at the margin of the white pulp are potential phagocytes which may be mobilized into active macrophages.

Snook (1946) described plexuses of deep lymphatic vessels in the periarterial sheaths of the splenic white pulp of some mammals but denied their existence in the rat. The present work confirmed Snook's finding that deep lymphatic did not occur in the splenic periarterial sheaths of the rat. Instead the periarterial sheaths were found to be compartmentalized by layers of TPA-stained reticular cells which encompassed the coarse fibers of the white pulp stroma and were concentrically disposed around the central artery. The compartments thus formed were seen to extend around the branches of the central artery which supplied the cortex of lymphatic nodules.

Similarly, at the level of the splenic hilum, these compartments were found to abut upon lymphatic vessels provided with gaps in the portion of their wall which is contiguous with the periarterial lymphatic sheath. This suggests that lymphocytes formed in the nodules may not pass into the red pulp in any large numbers and be supplied to the blood, for they may travel from the cortex through the intercommunicating compartments of the periarterial lymphatic sheath to be removed by the lymphatics in the hilum region.

An interesting finding was that the marginal zone was missing around the distal ends of the periarterial lymphatic sheaths, just before the arterioles penetrated and terminated in the red pulp. An even more interesting discovery was that, at their distal ends, the periarterial lymphatic sheaths were observed to be much attenuated and to be reduced to a delicate, loose sleeve composed of few argyrophilic fibers and of one or two layers of lymphocytic cells delimited by one or more continuous sheets of TPA-stained reticular cells. The terminal arterioles were found to retain their investing layer of fibril-containing reticular cells until the arterial vessels ended in the red pulp. At times, a double layer of TPA-stained reticular cells could be recognized about these channel. However, it was very difficult to follow these structures in serial sections as they were at the limit of visibility of the light microscope. More work thus needs to be done to clarify the structural peculiarities of such terminal arterioles and associated layers of reticular cells.

Nevertheless, since the single or double layer of TPA-stained

reticular cells investing arterial capillaries was seen, at the limit between lymphatic nodules and marginal zone, to diverge from the walls of these vessels to become continuous with the conspicuous sheath of reticular cells forming a barrier at the margin of the white pulp, it appears likely that the single or double layer of fibril-containing reticular cells ensheathing terminal arterioles at the distal ends of the periarterial lymphatic sheaths might do likewise and become an integrant part of the barrier decarmating the white pulp from the marginal zone. It is further suggested that, unlike the situation at the proximal ends of the periarterial lymphatic sheaths, few lymphocytic cells would follow the distal ends of these sheaths to reach the red pulp.

It therefore seems likely that the barrier of reticular cells found at the boundary of the white pulp, in addition to representing, as suggested by Snook (1964), a source of potential phagocytes which would, according to Pettersen et al. (1967), initiate the antibody-forming process by coming into close contact with marginal zone lymphocytes, would be effective to prevent the emigration of large numbers of white pulp lymphocytes into the marginal zone and eventually into the red pulp and the blood circulation. This belief appears to be substantiated by the finding of Pearce et al. (1918) that, in dogs, "the blood of the splenic vein does not differ greatly from that of the artery".

Furthermore, the TPA-stained reticular cells were observed to form a continuous uninterrupted investing layer around all arterial

channels in the white pulp, excepting around arterial capillaries supplying the germinal center of lymphatic nodules. Such a layer is morphologically "analogous" to the epithelial boundary formed by reticular-epithelial cells around intrathymic blood vessels. However, the cells making up the sheet investing white pulp arterial channels, unlike thymic reticular-epithelial cells, do not display epithelial characteristics, such as tonofibrils and desmosomes, do not have a basement membrane-like layer wherever they come into contact with connective tissue, and are known to be of mesodermal origin. Obviously, the presence of desmosomes does not imply per se that a cell is epithelial in nature, for endothelial and mesothelial cells, which are derived from the mesenchyme, may have such intercellular attachment devices. However, in the case of the thymus, this criterion has all its value owing to the presumably endodermal or perhaps ectodermal origin of this organ and to the epithelial character of its cellular reticulum.

The finding that germinal center arterial capillaries were the only vascular channels of the white pulp which were not closely invested by a continuous layer of TPA-stained reticular cells was significant, for it gave some insight into the peculiar structural organizations of large, well-developed secondary lymphatic nodules of the splenic white pulp. Indeed, from the evidence presented in this work, the germinal center of these transitory structures seems to have resulted from the cellular infiltration and subsequent dilatation of the potential space between the walls of the radial capil-

laries given off by the arteriole to the center and the layer of TPA-stained reticular cells investing these capillaries. This belief is substantiated by two findings: 1) capillaries within the germinal center are not invested by a layer of TPA-stained reticular cells; and 2) the delimiting cellular layer of the center not only is continuous with the investing layer of the arteriole to the center, but it also covers the capillaries that emerge from the center to run out toward the marginal zone. Thus, the layer of TPA-stained reticular cells investing white pulp arterial channels, since it was intimately associated with neither argyrophilic extracellular reticulum nor a basement membrane, would be a loose, "unfixed" membrane which could lend itself quite well to changes that might occur in the vicinity of these channels.

In a recent note, Millikin (1967) described well-developed germinal centers of the human spleen, lymph nodes and mucous membranes as bipolar spheroids consisting of two separate hemispheres. According to this author, in splenic germinal centers, one hemisphere was light and eosinopholic and was directed toward the marginal zone, while the other hemisphere was dark (owing to the presence of cells with scanty cytoplasm and darkly basophilic nuclei) and was diametrically opposite the light hemisphere. The present work confirmed that splenic germinal centers were bipolar, oblong structures, with one pole facing the marginal zone and another one directed toward the central artery. However, it was found that the two hemispheres are not separate, but that the germinal center was only inva-

ginated at the point of entrance of the arteriole to the center in its mid portion. Moreover, in contrast to Millikin (1967), the hemisphere facing the marginal zone was found to contain more small cellular elements (lymphocytes) than the other hemisphere, which were lightly stained with TPA and therefore made the hemisphere appear light.

The cortex of splenic secondary lymphatic nodules was thus found to be a corona around the germinal center from which it was separated by a continuous layer of TPA-stained reticular cells. It was observed to be demarcated from the marginal zone by the conspicuous sheath of reticular cells at the margin of the white pulp, and from the central artery by the layer of TPA-stained reticular cells which invested it.

From the evidence presented in this work, the cortex of the splenic secondary lymphatic nodules is divisible into four merging areas. The cortical area capping the pole of the oblong germinal center directed toward the marginal zone was found to bear a close resemblance to the area capping the other pole of the germinal center, as both areas were completely devoid of argyrophilic reticulum fibers and of blood vessels. These two cortical areas were observed to communicate through the spaces of the periarteriolar lymphatic sheaths which surrounded the arterioles supplying the cortical areas covering the lateral aspects of the bipolar germinal center. The TPA-stained reticular cells of such sheaths were found to show continuity with those forming the barrier at the margin of the white pulp. Moreover,

the spaces of the periarterial lymphatic sheaths were seen to merge imperceptibly both with those of the periarteriolar lymphatic sheaths forming the lateral cortical areas and with the cortical area capping the pole of the germinal center facing the central artery.

The above findings thus seem to indicate 1) that the splenic lymphatic nodules implanted on the central artery are developed within the periarterial lymphatic sheaths and represent dilatations of these sheaths, and 2) that all the reticular cells with TPA-staining fibrils of the white pulp belong to a single cell polulation.

Marginal Zone:

The present work confirmed the existence of a "marginal sinus" in the marginal zone, into which many pulp capillaries opened. In accord with the findings of Snook (1964), the marginal sinus was observed to be lined on the white pulp surface by an endothelium which was continuous with that of the white pulp capillaries, and laterally, i.e., toward the meshwork of the marginal zone, by a layer of cells showing discontinuity. However, this study revealed that the lateral wall of the marginal sinus was formed by fusiform reticular cells with elongate, slender cytoplasmic processes which, unlike the lining endothelial cells, contained TPA-staining cell web fibrils and appeared to be fenestrated (see Fig. 92). The fenestrae or pores in the cytoplasmic processes of these cells were separated from each other by TPA-positive dots and could thus be easily identified. Such apertures in the lateral wall of the marginal sinus

would thus allow the passage of blood into the meshes of the marginal zone.

If connections were seen between the endothelial cells of some white pulp capillaries and TPA-stained reticular cells of the marginal zone, no such connections could be ascertained between the latter and the reticulo-endothelial cells lining splenic venous sinuses at the periphery of this zone. Instead, in this area, fusiform reticular cells with delicate cytoplasmic fibrils were found to be disposed in concentric, discontinuous lamellae lying on similarly disposed, fine argyrophilic fibers and separated from each other by blood spaces. As venous sinuses were not found in the marginal zone, it seems likely that such concentric lamellae of TPA-stained reticular cells might be effective in preventing the invasion of this zone by venous sinuses. Moreover, as macrophages were found to be abundant only toward the periphery of the marginal zone, it is doubtful that this zone acts as a blood filter. Owing to the peculiar disposition of the TPA-stained reticular cells in concentric, discontinuous lamellae toward the periphery of the zone, the latter, as suggested by Lillie (cited by Krumbhaar, 1948), may instead act as a "pressure reducing mesh".

Red Pulp:

A) Cytology of TPA-stained reticular cells.

Electron microscopists have recently described three main types of reticular cells in the splenic red pulp of many mammals: "cordal",

"cord-limiting" and "sinal" reticular cells (Weiss, 1962b, 1963, 1966b; Galindo and Freeman, 1963; Roberts and Latta, 1964). From the evidence presented in this work, only a few cordal reticular cells contained TPA-staining fibrils. However, all cord-limiting and sinal reticular cells were found to accumulate cell web fibrils in the portion of their cytoplasm lying alongside the sinal basement membrane.

According to Weiss (1963, both cordal and sinal reticular cells were characterized by the presence of an electron dense substance which, in some instances, assumed a filamentous appearance in the part of their cytoplasm lying in close physical relation to the sinal basement membrane or cordal extracellular reticulum. As suggested by this author, such cytoplasmic filaments are related to the "cell web" described by Leblond et al. (1960). They also correspond to the fibrils observed in this work in the cytoplasm of all three types of reticular cells.

B) Spatial distribution of TPA-stained reticular cells.

As previously reported by many investigators (Weiss, 1962b, 1963, 1966b; Galindo and Freeman, 1963; Roberts and Latta, 1964), the basement membrane of the splenic venous sinuses was observed to be sandwiched between the TPA-staining cytoplasmic processes of the sinal reticular cells, also referred to as "reticulo-endothelial cells", and those of the cord-limiting reticular cells. It is felt that all three types of TPA-stained reticular cells of the splenic red pulp as well as those of the white pulp and marginal zone belong to a

single cell population.

It may be argued that, unlike other TPA-stained reticular cells, the sinal or reticulo-endothelial cells are not contiguous and contain a large amount of cell web fibrils in the form of "basal plates". However, Thomas (1967), using the electron microscope, has recently presented evidence that sinal cells are often contiguous and do not commonly alternate with gaps or apertures. Furthermore, Roberts and Latta (1964), in studies on the rabbit spleen, reported that the three types of red pulp reticular cells followed no definite pattern as to types of spaces they lined and that similar and dissimilar reticular cells might line a basement membrane on the same or opposite side. Lastly, it was observed that reticular cells sending out long cytoplasmic processes and subjected to mechanical stretch tended to accumulate larger quantities of cell web fibrils. For instance, cord-limiting reticular cells which sent out long cytoplasmic projections dying alongside the sinal basement membrane contained more demonstrable cytoplasmic fibrils than the other cordal reticular cells. It therefore appears likely that sinal cells, are spindle-shaped cells that can be subjected to mechanical stretch when venous sinuses are dilated, deposit massive quantities of TPA-staining filaments which, as suggested by Leblond et al. (1960), may serve to maintain their shape and integrity.

SUMMARY AND CONCLUSIONS

Staining sections of the thymus and spleen of the rat with the tannic acid-phosphomolybdic acid-amido black (TPA) technique made it possible to understand some structural characteristics of these two complex lymphatic organs. This was facilitated by the staining of fibrils, referred to as "cell web", in the cytoplasm of most reticular cells, which could thus be readily identified.

In the thymus, the reticular-epithelial cells were easily recognizable by the well-stained tonofibrils in their cytoplasm and were found to be distributed as follows.

In the cortex of each thymic lobule, the reticular-epithelial cells formed a continuous layer on the inner surface of the capsule and along the interlobular septa. They also closely invested all the small blood vessels. In the intervening space these cells formed a loose network enmeshing closely packed lymphoid cells.

In the medulla two zones were distinguished. In the outer zone, which was contiguous to the cortex, the reticular-epithelial cells, characterized by a voluminous cytoplasm rich in tonofibrils, were abundant and formed a dense network. In the inner zone no reticular-epithelial cells could be seen, but instead connective tissue cells and fibers made up the supporting framework. These two zones, referred to as "inner" and "outer" medulla, were clearly demarcated by flattened reticular-epithelial cells. The inner medulla was continuous with the perivascular connective tissue spaces seen around

the venules of the outer medulla and the larger blood vessels traversing the cortex.

Therefore, the reticular-epithelial cells constituted the supporting framework of the cortex and outer medulla, whereas the supporting tissue of the inner medulla (which was continuous with the perivascular spaces) was of a connective tissue nature. The thymic lobule was thus divisible into two compartments: an epithelial compartment comprising the cortex and outer medulla, and a connective tissue compartment composed of the inner medulla and its prolongations as perivascular spaces.

In the spleen, the white pulp was separated from the red pulp by the "marginal zone", a region in which reticular cells containing delicate TPA-positive fibrils were abundant. The white pulp in turn was demarcated from the marginal zone by a single or double layer of reticular cells rich in coarse TPA-staining fibrils.

In the white pulp itself, which was divisible into periarterial lymphatic sheaths and lymphatic nodules, there was an abundance of reticular cells with coarse TPA-positive fibrils. In the periarterial lymphatic sheaths, these cells ran parallel to the central artery and were arranged in concentric layers separated from each other by spaces containing lymphocytes. In the lymphatic nodules implanted on the central artery, they formed a delicate layer at the limit between germinal center and cortex. Reticular cells with TPA-positive fibrils were absent from the germinal center and cortex of the lymphatic nodules except around the arterioles supplying these zones,

around which they made up concentric layers as in the periarterial lymphatic sheaths.

In the marginal zone, reticular cells containing discrete cell web fibrils were randomly distributed in the deeper portions of this zone but were arranged in concentric lamellae separated from each other by blood spaces toward the periphery.

In the red pulp, some reticular cells with TPA-positive fibrils were found in the splenic cords of Billroth located between the venous sinuses. Finally, the long spindle-shaped reticulo-endothelial cells lining the sinuses contained a prominent TPA-positive cell web in the portion of their cytoplasm lying alongside the fenestrated sinal basement membrane.

Thus, the thymus and spleen of the rat appeared to be compartmentalized by reticular cells containing TPA-staining fibrils in their cytoplasm. Such a compartmentalization of these two intriguing lymphatic organs, which has never been clearly stated in the literature, therefore lay the morphological foundation for a study of their specific functions.

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