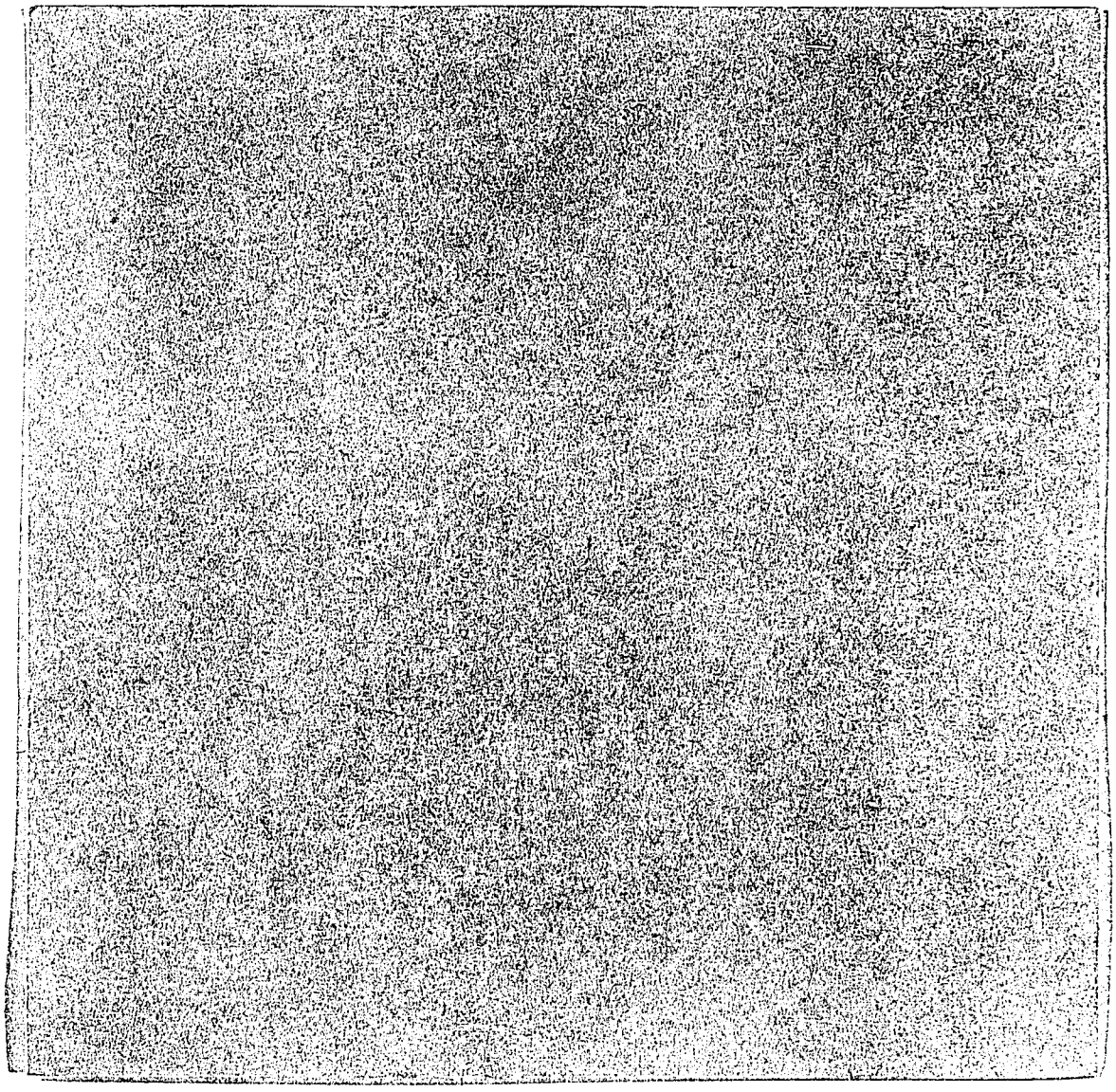
1967

A Three-dimensional Study Of The Normal Lymph Node Of The Young Adult Rat

Yoke Min Sin



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A THREE-DIMENSIONAL STUDY OF THE NORMAL LYMPH NODE OF THE YOUNG ADULT RAT

by

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Submitted in partial fulfillment
of the requirements for the degree of
Doctor of Philosophy

Faculty of Graduate Studies
The University of Western Ontario
London, Canada

July 1967

ACKNOWLEDGEMENTS

The author wishes to express his gratitude and sincere thanks to Dr. G. Sainte-Marie. His direction and suggestions were a most valuable help in carrying out this study.

The technical assistance of Mrs. J. Denisovs, and that of Mr. C. Chan of the University of Western Ontario are acknowledged. The artistic assistance of Miss E. Lambert and photographic assistance of Mrs. M. Miklosi and M. Cantin of the Université de Montréal are also indebted. To the typist, Miss G. Langevin, I am grateful.

Thanks are due to Dr. J.A. McCarter of the Cancer Research Laboratory of the University of Western Ontario for providing material facilities, as well as to Dr. P. Bois of the Département d'Anatomie de l'Université de Montréal where the thesis was completed.

My affectionate sentiments go to my father, mother and wife for their unfailing encouragement to undertake this thesis.

This study was supported by the National Cancer Institute and the Medical Research Council of Canada, to which the author expresses his thankfulness.

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LIST OF ABBREVIATIONS FOR FIGURES

A : Arteriole

AL : Afferent lymphatic vessel

C : Capsule

Cy : Capillary

DA : Dark area of the nodule

EA : Cortical extrafollicular area

EL : Efferent lymphatic vessel

F : Follicle

H : Hilus

L : Lobule

LA : Light area of the nodule

LF : Lymph flow

MR : Macrophagic reticular cell

MC : Medullary cord

MS : Medullary sinus

MV : Medullary venule

N : Nodule

PCV : Postcapillary venule

PF : Pseudo-follicle

S : Sinus

SS : Subcapsular sinus

T : Trabecula

ABSTRACT

This thesis constitutes an attempt to clarify the architecture of the node, by carrying out a three-dimensional analysis of the serial sections of a chosen node from a five-month-old rat, and by comparing the resulting observations with those from many other nodes and animals.

The chosen node was fixed in Bouin-Hollande, sectioned at 6.5 microns, and silver impregnated to show the reticular fibers. Each of 132 consecutive sections, cut longitudinally through the largest dimension of the chosen node and containing the hilus, was photographed in its entirety, area by area. The prints of the various areas of each section, at a magnification of 180X, were juxtaposed in the proper topographical order to constitute a photomontage of the section. The sections were then counterstained by the technique of Dominici; each was scanned to identify the structures on its photomontage and each type of structure was outlined in a specific manner. The marked photomontages were traced identically on sheets of clear glass and piled one above the other to enable three-dimensional analysis.

The node was semi-oval in shape, with a hilus located toward the center of its flattened face. It was formed of a thin layer of cortex, underlying the surface, and of medulla in the remaining space.

The capsule of the node was constituted mainly of collagenous fibers, the framework mostly of reticular fibers. Capsular collagenous fibers coalesced to yield coarse reticular fibers which were perpendicular

to the capsule and crossed the cortex. Upon reaching the medulla, these fibers fused with those outlining the medullary lymphatic sinuses. In between these sinuses, i.e., in the medullary cords, finer fibers formed a dense meshwork holding plasmocytes. In the cortex, fibers were few in the nodules (secondary nodules) and their follicles (primary nodules), being concentrated in the extrafollicular area, i.e. in the area outside these structures. The fibers adjacent to these structures encircled them, forming basket-like structures hanging from the capsule.

In silver-impregnated sections, it was possible to clearly outline the nodules and each associated follicle located in the cortex. Nodules were ovoid and each was formed of a light and a dark area. They were oriented in such a way that their largest diameter was perpendicular to the overlying capsule and their light area was nearest to it. With most nodules, the associated follicle entirely surrounded their light area and the outer portion of their dark area.

An artery entered the node at the hilus, where it branched into many arterioles that travelled along the medullary cords. Each medullary arteriole, upon reaching the cortex, branched into several smaller arterioles that penetrated it. One to three such arterioles reached the dark area of each nodule, where they transformed into capillaries. Many capillaries ran through the nodule, paralleling its long axis while branching in its dark and light areas. These capillaries also crossed the associated follicle, and upon leaving it, they transformed into peculiar postcapillary venules that had hypertrophied endothelial cells. Other arterioles yielded capillaries irrigating this area and abutting into the postcapillary venules. At the junction between the cortex and

medulla, the postcapillary venules joined to form the less numerous medullary venules, which, in turn, joined near the hilus to give rise to the vein leaving the node.

Examination of nodes from other localizations and from other rats yielded observations resembling those from the present chosen node. This indicated that the conclusions drawn on the architecture of the latter node can be applied to the architecture of the rat node in general.

INTRODUCTION

FOREWORD

The known functions of the lymph node are the prevention of dissemination of foreign substances into the organism, and the protection of the organism against those of these substances which are antigenic by producing agents that will neutralize them: the antibodies. After an antigen penetrates the organism, it commonly enters the lymphatic capillaries present at the site of penetration, via which it reaches the local draining node. There, a lymph filtering system retains the antigen to prevent it spreading any further in the organism. Moreover, some cells of the node that become aware of the antigenic penetration give rise to plasmocytes that produce a specific antibody against the antigen. This sequence of events constitutes the immune process, several cellular aspects of which remain to be elucidated. Such is the nature of the cells giving rise to the plasmocytes and the manner in which these cells become aware of an antigenic presence. The cellular reactions induced in a node by an antigen appear to be paralleled, at the histological level, by noticeable changes in the various structures of the node. The nature and significance of these changes are not known precisely, partly because our present knowledge concerning the histology of the node is incomplete. Since the cellular phenomena of the immune process take place within the various structures of the node and modify them, it would seem that a more adequate knowledge of these structures could enable a better understanding of the function of this organ. This thesis, therefore, deals with a three-

dimensional study of a well-developed node from a five-month-old rat, designed to obtain a better knowledge of the architecture of this organ.

REVIEW OF THE LITERATURE

On the one hand, much of the literature on the lymph node deals with pathological as well as experimental nodes, and it is known that the appearance of the node can vary with different conditions (Gillman, Gillman and Gilbert '52). This often renders it difficult to decide, a priori, whether an observation reported in the literature is relevant to the histology of the normal node of the rat and, consequently, whether it is appropriate to mention it in the present review. On the other hand, the present current concepts on the over-all histology of the node result from separate observations on the individual node structures, made in different species; so that this review cannot be restricted to the literature on the rat. Therefore, in view of above difficulties and in the interest of simplicity, it was thought appropriate to emphasize in this review the present current concepts, on the over-all histology of the node and on the composition of its various structures, which are found grouped together in textbooks of histology and in books devoted to the lymphoid organs. To facilitate comprehension, we have reproduced here the diagrams used in these books to illustrate these present current concepts (figs. 1-4). In the discussion, we will mention additional observations found in the literature and judged, a posteriori, to be relevant to the histology of the normal node of the rat.

A. Histology of the node. Nodes are known to vary greatly in size (Dunn '54), from being just detectable with the naked eye to measuring many millimeters in diameter. Nodes may be variously-shaped, but are commonly round, ovoid, or bean-shaped. For the sake of convenience, we will report what is known of this organ as it appears in a longitudinal section passing through the hilus of a bean-shaped node, the hilus of the node being located in the concavity of the bean. The node is enveloped in a capsule of dense collagenous fibers, which is often thicker at the hilus (Bloom and Fawcett '62, Ham '65, Weiss '66). Trabeculae, representing inner extensions of the capsule, are described as originating from the capsule, as well as from the hilus, and penetrating to variably depths in the node. Trabeculae, therefore, partition the organ extensively (figs. 1-3). Near the capsule, they divide the interior of the node particularly the cortex, into rounded areas sometimes called alveoli (Bloom and Fawcett '62). While it seems to be generally accepted that the node is partitioned extensively by numerous trabeculae, Yoffey and Courtice ('56) pointed out that the diagrams (figs. 1-3) used to represent the architecture of the human node are based on the dog. In the latter species trabeculae would be [illegible] abundant, but this would not be the case with the humans. In the humans, trabeculae would be few and the cortex would form a continuous layer (Heudorfer '21, Denz '47).

The parenchyma of the node can be divided grossly into two regions, like a unilobar kidney: an outer cortex and an inner medulla (Job '22, Bloom and Fawcett '62, Ham '65, Weiss '66). Both regions are delimited by a poorly defined cortico-medullary junction not known to correspond to any particular structure. The various components of the node

will be described separately.

1. Cortex. The cortex occupies a variably thick layer along the capsule and, at the concavity of the node, shows a gap filled by the node hilus. Often, however, the cortex does not come into contact with the hilus, but fades away at variable distances from it. In such cases, the hilus is surrounded by the medulla which, in this area, comes into contact with the capsule or rather the subcapsular lymphatic sinus, as will be seen later.

The cortex is constituted of a variably dense population of small lymphocytes. At its periphery are found rounded structures (figs. 1-4) often referred to as "germinal centers" (Flemming 1885, Bloom and Fawcett '62, Ham '65, Weiss '66) or "secondary nodules" (Maximow '27, Kindred '38, Ham '65, Leeson and Leeson '66), and sometimes as "reactive centers" (Hellman '30, Gillman, Gillman and Gilbert '52, Bloom and Fawcett '62, Leeson and Leeson '66). They are said to be fluctuating structures, i.e. they can disappear and new ones appear (Ham '53, Maximow and Bloom '57). They were termed "germinal centers" because they were considered by some authors as the sites of the node where lymphocyte formation occurs (Flemming 1885, Marshall and White '50). They were also termed "reactive centers" because they were considered by some other authors as sites of lymphocytic degeneration caused by the reaction of lymphocytes with toxic substances (Hellman '21, Heiberg '23). These structures, which may reach 1 mm in diameter (Maximow and Bloom '57), are commonly described as "pale areas" (Maximow and Bloom '57, Ham '65, Weiss '66), and their cellular constituents vary with time qualitatively as well as quantitatively (Kindred '55, Maximow and Bloom '57). Reticular

cells, small lymphocytes, and macrophages are always present in them but in variable amounts. As to large and medium lymphocytes, many of which are often in mitosis, their concentration varies to a greater extent than that of the above cells, since they may fill most of these structures or apparently be absent (Maximow and Bloom '57, Bloom and Fawcett '62). While the germinative or reactive centers (figs. 1-4) are commonly described and illustrated as homogeneous pale areas, a few authors have reported that each such structure is actually formed of two distinct areas: a dark and a light one (Rohlich '28, Taliaferro and Cannon '36, Kindred '38). The dark area, the most distant from the capsule, would contain the proliferating large and medium lymphocytes; the pale area, the nearest to the capsule, would be nearly devoid of these dark-staining cells.

The germinal or reactive centers are also termed "secondary nodules", because it is thought that each secondary nodule develops secondarily in the center of a structure often referred to as a "primary nodule" (Maximow '27, Yoffey and Courtice '56). The primary nodules are also referred to simply as "nodules" (Maximow and Bloom '57) and, occasionally, as "follicles" (Weiss '66). Primary nodules are described as rounded dense accumulations of small lymphocytes (figs. 1-3) that arise early in the lymphoid organs and in the loose connective tissue underlying the gastro-intestinal and the respiratory epithelia. The existence of the primary nodules as individual entities has been the subject of debate. Hellman ('30) pointed out that, during the development of the lymphatic tissues, only in the Peyer's patches do the so-called

primary nodules arise as isolated structures that later coalesce and in which secondary nodules develop. Therefore, Hellman maintained that only in this situation can one speak of primary nodules. In other locations, secondary nodules develop in diffuse masses of small lymphocyte accumulations (Hellman '30), so that the existence of individual primary nodules is doubtful. However, it is known that in the cortex of adult nodes, which is frequently thinly populated by small lymphocytes, there exist contrasting dense accumulations of small lymphocytes surrounding the secondary nodules to variable degree (Ehrich '29; Bloom '38, Kindred '55). The latter small lymphocyte accumulations are considered as primary nodules in adult nodes. No particular structures are known to constitute actual margins separating the secondary from the primary nodules and the latter from the remaining cortex. Nonetheless, Conway ('37) noticed that the limit of some secondary nodules can be sharply outlined by densely crowded small lymphocytes arranged in concentric layers.

2. Medulla. The medulla is formed of wide and anastomosing lymphatic sinuses, the "medullary sinuses", with so-called "medullary cords" between them (figs. 1-4), the sinuses and the cords converging towards the hilus. The medullary cords are said to consist of the same cytological constituents as the cortex, but rarely to contain nodules (Maximow and Bloom '57, Weiss '66); they are hence described as cords of dense lymphatic tissue. In diagrams, medullary cords are shown as extensions of the small lymphocyte population of the cortex penetrating between the medullary sinuses (figs. 1-3). It is known, however, that plasmocytes can be abundant in the medullary cords of some nodes (Ham '65,

Weiss '66), particularly in the nodes of the rat (Maximow '27, Dawson and Masur '30).

3. Lymphatic vascularization. Many "afferent lymphatic vessels" enter the node bringing a newly-formed lymph, while one or two "efferent lymphatic vessels" leave the node at the hilus carrying a filtered lymph (Furuta '47). The afferent lymphatic vessels pierce the capsule at regular intervals (Furuta '47) over the convex surface of the node to abut into the "subcapsular sinus", which is located between the capsule and the cortex (figs. 1-4). The subcapsular sinus extends along the trabeculae originating from the capsule, giving rise to the "subtrabecular sinuses". These latter sinuses open into the "medullary sinuses", which are seen between the cords of the medulla and are wider than the subcapsular and subtrabecular sinuses (fig. 4). The shape of the medullary sinuses is irregular and they anastomose one with the other (Denz '47, Bloom and Fawcett '62). Finally, the portions of medullary sinuses coming into contact with the hilus fuse together with each other and with the extremity of the subcapsular sinus adjacent to the hilus, thus giving rise to the efferent lymphatic vessels (figs. 1-4). The wallsof the various sinuses of the node are lined by reticulo-entothelial cells, as are the reticular fibers forming a network across the sinuses (Drinker, Wislocki and Field '33, Moe '63). These cells, and the leucocytes present in the lumen of the sinuses, remove the foreign substances that may be carried by the afferent lymph (Yoffey and Courtice '56). In this manner, the lymph is filtered during its successive passages from the afferent lymphatic vessels through the subcapsular, subtrabecular, and medullary sinuses.

The cortex being partitioned by trabeculae into numerous alveoli, each of which contains a nodule, the nodules are completely

separated from the capsule by the subcapsular sinus and from each other (figs. 1-3) by a trabecula and two subtrabecular sinuses (Bloom '38).

4. Blood vascularization. After an artery enters the node at the hilus, it branches in the medulla and transforms into progressively smaller vessels that irrigate the whole node (fig. 4). These arterial vessels, and the corresponding venous vessels returning towards the hilus, run through the medullary cords. Inside the cords, the arterioles give rise to a capillary network (Bloom and Fawcett '62). Little is to be found in the textbooks, regarding the vascularization of the cortex. In the diagram of Heudorfer ('21), which is still commonly used to illustrate the node vascularization, the vessels do not pass the cortico-medullary junction (fig. 4). Nonetheless, some textbooks indicate that a small arteriole enters each secondary nodule to supply it with capillaries (Bloom '38, Marshall '56), and also briefly mention the presence in the cortex of peculiar blood vessels with a cuboidal endothelium (Bloom and Fawcett '62).

While textbooks give little information on the vascularization of the cortex, a search of the literature revealed that Rohlich ('33) studied the vascular pattern of the nodules of the node. This author reported that an arteriole pierces the dark area of the nodule and runs along its longitudinal axis (fig. 5). When the arteriole reaches the light area of the nodule, it branches off at sharp angles into several capillaries that are present in the dark area (fig. 5). The capillaries run towards the surface of the nodule, where they give rise to "postcapillary venules". The latter are the peculiar blood venules of the cortex mentioned above (Bloom and Fawcett '62), which exhibit hypertrophied endothelial cells.

Their presence in the node cortex was observed long ago by Renaut (1881) and Schumacker (1899); but in spite of their particular appearance, little attention has been paid to the postcapillary venules. Nevertheless, in 1959, Smith and Hénon reconstructed segments of a group of postcapillary venules and found that they form an intricate pattern (fig. 6) before transforming into the medullary venules showing a regular appearance. These authors further pointed out that postcapillary venules are not seen in secondary nodules. Dabelow ('39) and Burwell ('62) reported that the postcapillary venules drain the capillary loops present under the subcapsular sinus, as well as the capillaries of the layer of the cortex adjacent to the medulla. According to the latter authors, the post-capillary venules usually commence a short distance below the subcapsular sinus and end at the cortico-medullary junction, towards which they run.

Besides noting the hypertrophy of the endothelial cells of the postcapillary venules, the early histologists (Renaut 1881, Thomé 1898, Schumacker 1899) observed that these endothelial cells were crossed by numerous migrating small lymphocytes. Until recently, it was thought that these small lymphocytes enter the venules (Schumacker 1899, Hummel '35, Pirro '54-55), a view now challenged by Gowans and Knight ('64), who proposed that these small lymphocytes leave the blood circulation. According to these authors ('64), the postcapillary venules constitute the main route by which the small lymphocytes can recirculate into the blood by passing from these venules into the node parenchyma and then into the node sinuses, returning to the blood via the efferent lymph.

5. Reticular network. A network of reticular fibers is reported to extend, like a continuous cobweb filling all the space of the node

(fig. 2), between the numerous trabeculae and between them and the capsule (Ham '65). Many reticulo-endothelial cells are attached to the fibers, which are frequently continuous with the collagenous fibers of the capsule and the trabeculae (Bloom and Fawcett '62). The network of reticular fibers is reported to be of variable density in the different locations of the node (Bloom and Fawcett '62, Ham '65). In the sinuses and the secondary nodules, the network is shown to be formed of coarse meshes (fig. 2). In the primary nodules and the medullary cords, the network is shown to be formed of fine meshes (fig. 2), thus holding together the free lymphocytes or plasmocytes present in these locations.

In reading the literature of previous investigators, we found observations on the reticular fiber framework of the node which seems to contradict the present current concept on this problem. Indeed, several investigators (Rössle and Yoshida '09, Orsós '26, Conway '37, and Denz '47) reported that in the cortex, reticular fibers are absent or rare in the nodules, being concentrated in the cortical portion outside these structures.

B. Terminology. In 1938, Bloom wrote: "There is much disagreement in the writings of the various authors as to the meaning of the terms follicle, nodule, primary and secondary nodule, germinal center and reaction center". On reading the literature, one comes across further terms to designate the primary or the secondary nodule, such as "marginal zone of small lymphocytes" to refer to the primary nodule (Bloom '38). The terms "nodule" (Bloom and Fawcett '62) and "follicle" (Verne '60, Weiss '66) are used, furthermore, to designate the over-all structure formed by the association of a primary and a secondary nodule.

The difficulty in terminology emphasized by Bloom still persists today, as it frequently happens that one is not sure to which structure an author is referring when using the above terms.

C. Orientation of present study. This study aimed to clarify the architecture of the normal node of the rat and to determine the topographical relationships existing between its various basic structures. It also aimed to elaborate a comprehensive terminology for these structures.

The node being a complex heterogeneous organ, we realized that only a three-dimensional study could yield satisfactory information on all aspects of its architecture. This type of study consuming much time and work, it appeared that not more than one node could be satisfactorily investigated in this manner during the present study. Therefore, we decided to carry out the present study on a chosen, well developed and normal node with an architecture likely typical of the normal rat node in general. This study is thus mainly a three-dimensional analysis of the architecture of a chosen cervical node from a five-month-old rat, which does not consider the morphological details of the cells constituting the structures of the node.

After the three-dimensional analysis of the chosen node, we compared the resulting observations with those from single, as well as serial sections of other nodes of various localizations and ages. Realizing that such comparisons could not yield always definite conclusions, the comparisons had for purpose to determine whether, in most likeliness, the determined architecture of the chosen node fitted with that of the rat node in general.

MATERIALS AND METHODS

Five-month-old Rats

A. Animals and tissues. Eight normal male Wistar rats were killed with chloroform and three cervical lymph nodes were removed for each rat, together with some adjacent tissues, without being touched directly. To prevent damage to the surface of the nodes, some fixative was poured over them prior to their removal. Tissues were placed in a slowly agitated fixative for two hours. The partially fixed nodes were then freed of excess adjacent tissues and returned to fresh fixative, using about 120 ml. solution per one cubic centimeter of tissue.

B. Histological methods.

1. Fixation. Fixation lasted three days in a slowly agitated variant of Bouin-Hollande composed as follow:

Distilled water	100 ml.
Neutral cupric acetate	2.5 gm.
Picric acid crystals	4 gm.
Neutral formaldehyde 38%	10 ml.
Trichloracetic acid crystals	0.75gm.
Acetic acid, glacial	0.75gm.

(The last two being added at the time of use).

After fixation, the nodes were washed in several baths of

70% ethanol until the alcohol remained colorless. They were then dehydrated in 100% ethanol, cleared in xylol and embedded in paraffin. The nodes were embedded in such a way as to yield longitudinal sections perpendicular to the hilus. Finally, the nodes were serially sectioned at about 6.5 microns.

2. Silver impregnation. The sections were deparaffinized and impregnated with silver for demonstration of reticular fibers, by the following variant of the technique of Bielschowsky:

Xylol (two baths)	4 min. each
Ethanol 100% (two baths)	3 min. each
Ethanol 95%	3 min.
Pyridine-ethanol 95% (1:1)	15 min.
Ethanol 95% (one slide at time)	2-3 sec.
Running tap-water	5 min.
Aqueous periodic acid 0.5%	20 min.
Running tap-water	5 min.
Distilled water (35-37°C)	5 min.
Ammoniacal silver solution (35-37°C)	2 hr.
Ammoniated water (one slide at a time)	2-3 sec.
Buffered formaldehyde (slides agitated)	5 min.
Running tap-water	5 min.
Gold chloride 0.1%	10 min.
Distilled water	rinse
Sodium thiosulfate 5%	2 min.
Running tap-water	5 min.
Ethanol 95%	3 min.

Ethanol 100% (two baths)	3 min. each
Xylol (two baths)	3 min. each
Mount with Permount	

The solutions were prepared as follows:

Ammoniacal silver solution:

Concentrated ammonium hydroxide (28-29%) was added, dropwise, to 10 ml. of a preparation of 10.2% silver nitrate until all the silver salt was in solution; 10 ml. of a solution of 3.1% sodium carbonate and 80 ml. of distilled water were then added. The mixture was stirred, filtered through paper, and placed in a 37°C water bath. Only a freshly prepared solution was used for staining.

Ammoniated water:

Distilled water	100 ml.
Concentrated ammonium hydroxide (28-29%)	one drop

Buffered formaldehyde:

Formaldehyde 5%	100 ml.
Sodium bicarbonate 1%	2 ml.

3. Counterstaining. After silver impregnation the sections were photographed as described below (see: Photomontages) and then unmounted by overnight immersion in xylol. They were counterstained by the technique of Domicici as follows, to permit further steps in their topographical analysis:

Xylol (two baths)	4 min. each
Ethanol 100% (two baths)	3 min. each
Ethanol 70% (two baths)	2 min. each

Ethanol 40%	2 min.
Ethanol 20%	2 min.
Distilled water (three baths)	2 min.eeach
Eosin Y-orange G	6 min.
Distilled water	10 sec.
Toluidine blue	10-15 sec.
Distilled water	10 sec.
Differentiation in ethanol 100%	2-20 sec.
Ethanol-xylol (1:1)	1 min.
Xylol (three baths)	3 min. each
Mount with Permount	

The staining solutions were made up as follows:

Eosin Y-orange G

Eosin Y	0.5 gm.
Orange G	0.5 gm.
Distilled water	100 ml.

Toluidine blue

Toluidine blue	0.5 gm.
Distilled water	100 ml.

In order to study the appearance of the structures of the node in sections stained by the technique of Dominici only, we had pre previously put aside a few sections, cut near the center of nodes, without impregnation with the ammoniacal silver solution. After deparaffinization, these latter sections were stained directly by the technique of Dominici as described above.

C. Choice of node. After silver impregnation, the sections of all nodes, most sections being silver-impregnated and a few stained by the technique of Dominici, were examined under the microscope to choose the most adequate node for three-dimensional study on the basis of the following criteria: a) the node should be sectioned longitudinally; b) the node should be represented by an adequate series of undamaged consecutive sections; c) the latter sections should pass through the cortex, a plasmocytic medulla, and the hilus of the node. A series of 132 consecutive sections of the chosen node, representing its largest dimension, was reconstituted three-dimensionally according to the various topographical steps described below. The serial sections of the other nodes served as controls of some observations made in the node of choice. The latter node will be referred to, from now on, as the node studied.

D. Topographical methods.

1. Photomontages. Using the silver-impregnated sections, overlapping negatives of the various areas of each of the 132 sections to be analyzed were taken with a 6.3X objective and 6X periplan ocular, and printed at an enlargement of about 180X. The prints of the various areas of each section were trimmed and placed in juxtaposition in the proper topographical order to constitute a photomontage representing each section (fig. 7). Each photomontage was about 45 inches wide, thus providing sufficient magnification to show the capillaries of the node.

2. Drawing on photomontages. After counterstaining by the technique of Dominici, each section was scanned under the microscope at a magnification of 600X. This permitted localization and identification

of the various structures of the node, as seen in the photomontages, which were of interest in this study. Each type of structure was outlined on the photomontages differently, by using Higgins inks, as follows:

(i) in the cortex:

a. the inner outline of the capsule, or of a trabecula, with a black line.

b. the outline of an artery, or of an arteriole, with a red line.

c. a cross-sectioned capillary was marked as a reddot, and a longitudinally-sectioned capillary with a single red line.

d. the outline of a postcapillary venule with a thick blue line.

e. the outline of a typical venule with a thin blue line.

f. the margin of the dark area of a nodule with a solid green line, the area itself being shaded with a few green lines.

g. the margin of the light area of a nodule with a broken green line.

h. the outline of a lymphatic sinus with a yellow line.

(ii) in the medulla:

a. the outline of an artery, or of an arteriole, with a red line as in the cortex.

b. the outline of a venule with a thin blue line as in the cortex.

c. the outline of a lymphatic sinus with a yellow line as in the cortex.

d. the surface of a plasmocytic medullary cord was covered by parallel violet lines.

It must be pointed out that only 3/4 to 4/5 of the entire surface of each of the 132 sections analyzed was marked in this way (fig. 7).

3. Drawing on sheets of glass. The structures under study, marked on the photomontage of each section, were then traced similarly, using colored Mirado 174 HB pencils, on a sheet of clear glass previously washed with ammonia. The thickness of the sheet of glass was closely proportional to the thickness and magnification of the section: for 6.5 micron-thick sections magnified 180 times on the photomontages, we used glass 1.63 mm-thick (6.5 x 180 = 1.17 mm). The sheets of glass representing the 132 sections of the node were laid one on top of the other in front of a light-box to allow three-dimensional viewing.

4. Three-dimensional reconstitutions. The three-dimensional appearances of the structures of the node were studied separately by examining them, in piles of up to 40 glass sheets at a time, while checking their outline in the corresponding tissue sections and photomontages whenever needed. Because of their complexity, some structures, such as the vascular pattern of the nodules, were not readily distinguishable in the pile of glass sheets. In these cases, individual analysis of several such structures was made by drawing them on separate small sheets of glass piled up as above, each drawing being checked by examination of the corresponding section under the microscope.

In addition, we made a volumetric cardboard model of the node under study (figs. 8-11). For this, we used photographs of every

eighteenth consecutive sections of the entire node, printed at a magnification of 40X. The outline of the section of the node, shown on each photograph, was reproduced identically on a sheet of cardboard proportional in thickness to the magnified thickness of the slice of node to be represented. The cardboard was then cut along this outline to provide a cut-out representing a thin slice of the node. The various cut-outs were glued together in the proper topographical order to form the shape of the node.

The same photographs of every eighteenth consecutive sections of the entire node, printed at a magnification of 40X, were also used to study the topography of the follicles or nodules with respect to the overlying capsule or trabecula. Each print was placed between a translucid paper and a light-box. For each print, we traced on the paper; the capsule, the trabeculae if any, the cortico-medullary junction, and the outline of the follicles or nodules. Each follicle or nodule was numbered, so that examination of the papers in consecutive order permitted us to determine how distant such a structure was from the capsule or from a trabecula.

Finally, we made a volumetric cardboard model of ten nodules and of their associated follicles in the cortex of the node. The latter models were made in the same manner as the cardboard model of the entire node, but at a magnification of 180X by using the above photomontages of the node sections.

Other Nodes and Animals

A. Animals. We will list below variously-aged, normal, male Wistar rats,

and nodes of various localizations, which we examined for comparing observations made in the node studied:

- one week-old rats: a cervical node in each of six rats.
- two-week-old rats: a cervical node in each of six rats.
- six-week-old rats: two cervical nodes in each of eight rats.
- ten-week-old rats: two cervical, mediastinal and mesenteric nodes in each of eight rats.
- five-month-old rats: two mediastinal, mesenteric and popliteal nodes in each of the eight rats which were used above for choosing the node studied.
- nine-month-old rats: two cervical, mediastinal and mesenteric nodes in each of eight rats.
- twopyear-old rats: two cervical, mediastinal and mesenteric nodes in each of eight rats

B. Processing of tissues. Rats were killed, nodes were removed and tissues were fixed in Bouin-Hollande fluid as previously described. All nodes from the one and two-week-old rats were sectioned serially at about 6.5 microns. Some of the other nodes were sectioned also serially, the remaining nodes were sectioned randomly through their largest dimension. Sections were stained either by the technique of Dominici or silver-impregnated as described previously.

RESULTS

Results will be presented in two sections. In the first section we will report solely the results from the three-dimensional analysis of the cervical node studied. In the second section, we will report the observations made in the other nodes and variously aged rats.

Node Studied

A. Constitution of the node.

1. External features. The volumetric cardboard model of the entire node, made at a magnification of 40X, showed the node to be roughly semi-oval in shape (figs. 8-11). The node thus had one convex surface and a more or less flattened one. Near the center of the flattened surface (figs. 9,10) was the hilus of the node, which appeared as a bifurcating invagination penetrating into the organ. For the sake of convenience, the flattened surface of the node was considered as the base and the convex surface as the top of the organ, so as to be able to speak of a top (fig. 8) and of a bottom (fig. 9) view of the node. Looking at the node from a top view, as illustrated in Figure 8, we distinguished further a left (fig. 10) and a right (fig. 11) view. The two portions of the node located at the extremities of its longest dimension were considered as the poles. Actually, the longest dimension of the node measured 6.4 mm.

The outline of the node was smooth and regular, except at the hilus and over an area of the convex surface more or less opposite to the hilus. There (figs. 8,11), the capsule curved in a circular manner and penetrated deeply into the node, almost completely separating

a rounded portion of the node measuring about 2 mm. in diameter from the remainder of the organ. This rounded portion was referred to as a "lobule", and its outline, as seen in longitudinal and in cross sections of the node, is schematized in Figures 12 and 13 respectively.

2. Internal features. The appearance of the sections of the node varied with the level at which the organ was sectioned. The main variations are seen in the eight sections shown in Figures 15 to 22, which were taken at the eight levels of sectioning indicated by the diagram in Figure 14. The sections passing through the periphery of the left side of the node (fig. 15) contained only cortex, throughout which were scattered nodules. Deeper into the node (fig. 16) but still quite a way from its center, one half of the sections were formed of a layer of cortex underlying the surface of the node except around the hilus, the other half being formed of medulla located in a semi-circular manner around the hilus. Opposite the hilus, such sections were cut nearly perpendicularly to the surface of the node, and the cortex exhibited nodules arranged in a single layer adjacent to the capsule. At both poles of the organ, such sections were cut through the cortex, closely parallel to the surface of the node, and nodules extended several layers deep beneath the capsule. The next sections, cut near and through the center of the organ (fig. 17), were mainly constituted of medulla, the cortex showing as a relatively thin layer underlying the capsule. In these sections, the over-all surface of the cortex was cut more or less perpendicularly to the capsule and contained a single layer of nodules adjacent to the capsule. Still no nodules were seen adjacent to the base of the hilus, which was totally surrounded by medulla. The sections

cut a little beyond the center of the node (fig. 18) resembled those passing through it except that in the portion of the node roughly opposite the hilus the cortex appeared much thicker and exhibited nodules variably distant from the overlying capsule. Examination of the consecutive serial sections revealed that this apparently thick portion of cortex was adjacent to a septal trabecula outlining the lobule located below. In the next series of sections, the hilus disappeared and the cortex appeared to underlie the over-all capsule (fig. 19). The portion of cortex adjacent to the lobule still showed thicker and exhibited more nodules than previous sections. The medulla now accounted for a smaller fraction of the node than was seen in the previous sections cut close to the center of the organ. In even deeper sections (fig. 20), passing through the lobule but very near the trabecula, the medulla became restricted to a narrow band totally surrounded by cortex. At this level, the sectioned septal trabecula appeared in single sections as two distinct and independant columnar trabeculae. Finally, in the last series of sections, the lobule showed as a rounded structure (fig. 21) separated from the remainder of the node (fig. 22). This last series of sections, cut through the cortex and closely parallel to the capsule, exhibited nodules scattered throughout, but no medulla (fig. 21,22) as did those in the first series (fig. 15).

Examination of the series of papers on which the nodules, the capsule, and the trabeculae of the node were marked at a magnification of 40X, revealed that the node contained no septal trabecula other than that outlining the aforementioned lobule. Besides this major trabecula, there were also fifteen short columnar trabeculae just slightly penetrating

into the cortex after arising from the capsule, mainly near the hilus. Examination revealed also that the cortex actually constituted a relatively thin layer underlying the over-all surface of the capsule. The thickness of the cortical layer was roughly constant under the whole convex surface of the node (figs.13,24), while it somehow progressively decreased, starting from both polar limits of this surface towards the hilus, where it had faded out. Hence, the apparently thick cortex seen in sections cut close to the center of the node (figs. 18,19) was in fact a thin cortical layer underlying the trabecula below, as can be seen in Figure 13. Correspondingly, it was found that all of 173 nodules of the node studied were disposed in a single thin layer, all being thus adjacent to the overlying capsule or trabecula.

B. Staining of sections.

1. Silver-impregnation.

a. Fibers. With this technique, the collagenous fibers, abundant in the hilus, capsule and the trabecula, showed variably as dark brown whereas the reticular fibers were black.

b. Nuclei. The silver salt also impregnated in black the nuclear chromatin of the cells located in restricted rounded structures of the node; these structures appeared as strikingly dark areas in the sections (figs. 23,25). These areas were observed to contain considerably fewer reticular fibers than the remainder of the node. Further, it was noted that the nuclei adjacent to the few fibers present in these areas had failed to stain (fig .27). In general, the coarser a fiber in these areas, the wider the band of unstained adjacent nuclei along it, ranging between 15 to 20 microns on both sides of a coarse fiber. Hence,

it was observed that the reticular fibers had prevented the silver impregnation of the nuclei adjacent to them, so that nuclei stained very little, if at all, in sites where these fibers were dense. The rounded areas of the node, in which most nuclei were silver impregnated, underlay the capsule in a manner resembling that of the nodules and were, in fact, nodules, as will be demonstrated later.

c. Macrophagic reticular cells and autofluorescent cells. The macrophagic reticular cells located in the nodules, and some present in medullary sinuses, stood out as very dark elements (figs. 27,33), as a result of the dense black impregnation of many variously sized cytoplasmic granules. When present in a section, the nuclei of these cells were unstained, appearing as a light zone among dark granules (fig. 27). It was observed furthermore that, in the nodules, the nuclei of the cells adjacent to these macrophagic reticular cells had failed to stain also. Thus, it was found that the impregnated granules of the macrophagic reticular cells had prevented the silver impregnation of their nuclei, as well as of those in adjacent cells, in the same way as did the reticular fibers.

Along the margin of some nodules, we observed other cells also exhibiting silver-impregnated granules (fig. 31). These cells were the peculiar "autofluorescent cells" described by Sainte-Marie (1965). They differed from the macrophagic reticular cells in the nodules in that most of their impregnated granules tended to show as brown rather than black as did most impregnated granules of the macrophagic reticular

cells. Moreover, the impregnated granules in the macrophagic reticular cells were usually relatively few and readily distinguishable one from another, while the cytoplasm in the autofluorescent cells was loaded with hardly distinguishable granules so that the colorless nuclei were not as frequently detectable as in the macrophagic reticular cells.

2. Technique of Dominici. In sections stained only by the technique of Dominici and examined at high magnification, the cytoplasm of the reticular cells was colorless, whereas that of the lymphocytes and plasmocytes stained blue. Reticular fibers exhibited a pink hue and were hardly detectable. In sections impregnated with silver salt and then counterstained by the technique of Dominici, the reticular and collagenous fibers as well as the impregnated granules in macrophagic reticular cells and autofluorescent cells retained the appearance they had presented after silver impregnation, while the other cells presented the same appearance as when stained only by the technique of Dominici.

C. Cortex and medulla. In sections passing near the center of the node, the cortex appeared as it actually was, i.e. forming a thin layer underlying the capsule. In such sections stained by the technique of Dominici (fig. 24), the cortical layer had a dark hue so as to be readily distinguished from the paler medulla, which filled the space between the cortex and the hilus. Grossly, the cortex was formed of a more or less homogeneous population of small lymphocytes and contained nodules. The medulla was constituted of wide lymphatic sinuses separated by plasmocytic cords. Comparison of two consecutive sections, one impregnated with silver salt (fig. 23) and the other stained by the technique of Dominici (fig. 24),

revealed that the dark areas seen in the former section correspond to the nodules seen in the latter. As to the cortical area outside the nodules and containing small lymphocytes, it contrasted with the medulla in the sections stained only by the technique of Dominici (fig. 24); but it grossly resembled the medulla in the sections impregnated with silver salt and examined at low magnification (fig. 23).

We will now describe successively the nodules of the cortex, the cortical area between these structures, the medulla, the lymphatic vascularization of the node, and the blood vascular pattern of the node. For the sake of simplicity, we will in general, first describe these structures as they appeared after three-dimensional reconstitutions. Later, their appearance will be reported as actually seen in single sections. Furthermore, we will describe with details only their most common appearance, while mentioning their main variations.

D. Terminology. When attempting to express the results of the present study, it was realized that this could not be done conveniently by using the current terminology for the node, which was felt to be confusing and inadequate. It was thought preferable to use, while completing and modifying it, the terminology for the node tentatively elaborated by Dr. G. Sainte-Marie ('66a), the supervisor of this thesis. The proposed terminology made as much use as possible of terms that are presently employed or were employed long ago by investigators. The basic suggestion of this proposed terminology is that the term "follicle" designates the structure currently termed "primary nodule" and that the term "nodule" designates the structure currently termed "secondary nodule

or germinal center". A series of adjectives were added to these two basic terms to describe their components, their variations, and the structures related to them. To avoid confusion resulting from the present terminology, from now on, the significance of the terms used will be as defined in this proposed terminology, except when stated otherwise. For the sake of convenience, the terms to be used are presented in a Glossary at the end of the thesis, examination of the accompanying Figures 124 to 130 will permit the reader to grasp readily the meaning of the terms.

E. Cortex. In silver-impregnated sections, the cortex was seen to be formed of two distinct parts on the basis of the reactivity of the nuclei with the silver salt. In one part, which appeared as rounded dark structures (figs. 23,25), the nuclei were silver-impregnated. These structures were the nodules and their associated follicles. In the other part, the nuclei were not silver-impregnated. This latter part filled the space outside the follicles and nodules (figs. 23,25) and will, therefore, be referred to as the cortical "extrafollicular area" (fig. 129).

1. Nodule.

a. Localization. All nodules and their follicles were localized within the outermost layer of the cortex, i.e. the "peripheral cortex", immediately adjacent to the overlying subcapsular or subtrabecular sinus that separates the cortex from the capsule or a trabecula respectively. These can be referred to as "peripheral nodules" or "peripheral follicles" as opposed to"deep nodules" or "deep follicles", reported by other authors (Conway '38, Gillman et al. '52) to be present in the deep layer of the cortex (fig. 126). The angle of sectioning, how-

ever, gave rise to sections that contained what appeared to be deep nodules (figs. 18,19). These were actually peripheral nodules adjacent to the overlying septal trabecula and its accompanying subtrabecular sinus present in further sections. Hence, it was found that each nodule and its associated follicle was adjacent to or in contact with, a subcapsular or a subtrabecular sinus.

b. Shape. Most nodules were ovoid (fig. 29) and oriented in such a way that their greatest diameter was more or less perpendicular to the overlying capsule or trabecula, their wider end being nearest to these structures (fig. 124). Some nodules exhibited similar forms but were variably wider than the latter nodules, so that occasionally their greatest diameter was parallel to the overlying capsule or trabecula. A few nodules were spherical, these nodules were found among the small nodules close to the hilus. In single section, the nodules appeared ovoid (fig. 31) to round (figs. 30,32,33), depending on their actual shape and on the angle of sectioning.

c. Histological light and dark areas. Each nodule was formed of a "light area" and of a "dark area" (figs. 29,124) that occupied, respectively, the half the nearest to and the furthest from the overlying capsule or trabecula. In single sections, both areas were seen simultaneously in nodules sectioned longitudinally (fig. 31) or tangentially (fig. 63), while only either the dark (fig. 32) or the light area (fig. 33) was seen in cross sectioned nodule. These areas of the nodule could be observed in sections stained by the technique of Dominici (fig. 30), just as well as in those impregnated with the silver

salt (fig. 31). In the former sections, the deeper hue of the dark area resulted from the fact that the large or medium lymphocytes, densely crowded in this area, exhibited a relatively abundant and very basophilic cytoplasm. The light area showed light because the small lymphocytes ands the macrophagic reticular cells populating it had little basophilic cytoplasm or none at all. In silver-impregnated sections, the light area appeared lighter than the dark one partly because the silver-impregnated nuclei were less crowded in the former than in the latter area and partly because the abundant macrophagic rèticular cells of the light area inhibited the silver-impregnation of their adjacent nuclei (figs. 31,33).

d. Margin and topographical zones. The shape common to most nodules permitted us to distinguish three portions in the margin of the nodule, i.e. the "nodular margin"; they were named on the basis of the particular orientation of the nodules (fig. 124). We will describe the actual make up of this margin, later. The first portion consisted of that outlining the wide end of the nodule and, being situated nearest to the overlying capsule or trabecula, will be termed the "outer nodular margin". The second portion consisted of that outlining the narrow end of the nodule and, being situated farthest from the overlying capsule or trabecula, will be referred to as the "inner nodular margin". The third portion, consisting of that located between both the above portions, will be referred to as the "lateral nodular margin".

Having distinguished three topographical portions in the nodular margin, it was thought that for future reference, it would be useful to similarly differentiate topographical zones in the nodule (fig. 125). Hence, we distinguished the:

- "outer zone of the nodule", i.e., the zone outlined by the outer nodular margin.
- "inner zone of the nodule", i.e. the zone outlined by the inner nodular margin.
- "lateral zone of the nodule", i.e. the zone along the lateral nodular margin.
- "central zone of the nodule", i.e. the zone surrounded by the lateral, the inner, and the outer zones of the nodule.

e. Types of nodules. Nearly all nodules were associated with a follicle; the association of both structures will be referred to as a "follicular nodule" (fig. 124). As the nodules were surrounded to variable extents by their follicles, they were distinguished into four types on the basis of (figs. 128):

- the "enveloped nodule", i.e. a nodule with a follicle outlining its entire margin.
- the "mantled nodule", i.e. a nodule with a follicle outlining its lateral and outer margins.
- the "capped nodule", i.e. a nodule with a follicle outlining its outer margin only.
- the "naked nodule", i.e. a nodule not outlined at all by a follicle.

Mantled nodules were by far the most numerous. The naked nodules were very few in number, being small and located near the hilus.

Some nodules were considerably larger than the others (fig. 39); they appeared to be formed by the fusion of two (fig. 34) to several (fig. 35) adjacent nodules and will be referred to as "complex nodules". In such nodules, it was usually possible to distinguish the light and

dark area of each of the fused single nodules, their orientation in respect to the overlying capsule or trabecula being the same as that seen in single nodules (figs. 42,43). The over-all shape of the complex nodules was variable, depending on the number and localization of the fused single nodules as well as on the extent of their fusion.

2.k Follicle.

a. Constitution and shape. In silver-impregnated sections, the follicle showed as a nearly homogeneous population of silver-impregnated small lymphocytes. The limit between each follicle and the adjacent cortical extrafollicular area was outlined clearly. In sections stained by the technique of Dominici, the latter limit was hardly detectable. The shape of the largest follicles outlining the over-all margin of their associated nodules resembled that seen in the diagram of Figure 124, a shape similar to that of the nodule.

b. Margin and topographical zones. As the orientation of the follicle and the shape of its margin, i.e. the "follicular margin", resembled those of the nodule, it was possible to distinguish zones and portions corresponding to those for the nodule. Hence, we similarly distinguished three portions in the follicular margin (fig. 124), the:

- "outer follicular margin", i.e. the portion of the margin parallel and adjacent to the overlying subcapsular or subtrabecular sinus.
- "inner follicular margin", i.e. the portion of the margin farthest from the overlying subcapsular or subtrabecular sinus.
- "lateral follicular margin", i.e. the portion situated

between both the above portions of the follicular margin.

We also similarly distinguished three topographical zones in the follicle (fig. 124), the:

- "outer zone of the follicle", i.e. the zone outlined by the outer follicular and nodular margins.
- "inner zone of the follicle", i.e. the zone outlined by the inner follicular and nodular margins.
- "lateral zone of the follicle", i.e. the zone outlined by the lateral follicular and nodular margins.

As reported above, the follicle consisted of an accumulation of nearly exclusively, small lymphocytes. In respect to the cell population, the outer zone differed from the two other zones of the follicle by the fact that it sometimes contained a few macrophagic reticular cells (fig. 25), which were absent in the latter zones.

c. Types of follicles. Having distinguished four types of nodules on the basis of the extent of their outlining by follicles, we similarly distinguished three types of follicles on the reverse basis of the extent of their outlining of the nodules. Hence, we distinguished (figs. 128):

- "the "enveloping follicle", i.e. a follicle outlining the entire margin of its associated nodule.
- the "mantling follicle", i.e. a follicle outlining the lateral and outer margins of its associated nodule.
- the "capping follicle", i.e. a follicle outlining only the outer margin of its associated nodule.

In single sections, only in longitudinally-cut follicular

nodules was it possible to realize the actual constitution of these structures,ii.e. the actual relationship between a nodule and its follicle. Indeed, mantled nodules cross-sectioned through their inner zone appeared as though formed solely of a dark area devoid of follicle (figs. 29A, 32), i.e. as naked nodules. The same nodules, cross-sectioned through their lateral or their outer zones, appeared as though formed solely of a dark or of a light area totally surrounded by a follicle (figs. 29A, 33), i.e. asenveloped nodules. In some tangential sections, these nodules appeared as if formed of a dark and light area totally surrounded by a follicle, i.e. again as enveloped nodules. Capped nodules appeared as though formed of a light or a dark area, or both, depending on the angle of sectioning, while appearing as naked nodules except when cut in a nearly perpendicular manner. Finally, in some tangential sections, occasional mantled and capped nodules with a thin inner zone exhibited what appeared to be a thick cap of small lymphocytes between the nodule and the overlying sinus and which, in fact, represented a tangentially-cut lateral zone of a follicle.

3. Variations in sizes of nodules and follicles. There existed noticeable variations in the size of the different nodules of the node. In fact, it was possible to distinguish three cortical areas in respect to the size of these structures, i.e. the area of cortex underlying the convex surface, the flat surface, and the septal trabecula outlining the lobule of the node (fig. 39). Along the convex surface, the size of the individual nodules was grossly similar, their longest dimension averaging 400 microns. Along the flat surface, the size of the nodules was on the average, the smallest seen in the node. The nearer they were to the

hilus, the smaller their size (fig. 18). This diminution in the size of the nodules paralleled the comparable diminution in the thickness of the cortex as it neared the hilus. The capped and particularly the naked nodules of the node were located in this portion of cortex. The nodules in the portion of cortex adjacent to the outline of the lobule were, on the average, largest of all (figs. 19,39). It must be pointed out that, although the complex nodules appeared to be the largest, each of the fused single nodules constituting them was actually no larger, on the average, than the other nodules in the area of the cortex in which it was localized (fig. 39). In general, the light and dark areas, each occupied about half of a nodule, irrespective of its size although, at times, one or the other area occupied two thirds of a nodule.

The over-all volume of the follicles varied, in general, somewhat proportionally to the size of their associated nodules; so that the area of cortex containing the larger nodules also exhibited the larger follicles (fig. 39) and for the area with the smaller nodules, the converse was seen. The larger follicles were of the enveloping and mantling types, whereas the smaller follicles were mainly of the capping types. In the enveloping follicles, the inner zones was very thin, except next to the lateral zone of the follicles. The thickness of the outer zone of the follicles varied quite independently of the over-all volume of a follicle and of its nodule. Often, this zone consisted of a thin layer of small lymphocytes separating the light area of a nodule from the overlying sinus. The lateral zone of the follicles was the main zone of the follicle, and its volume varied proportionally with the size of the associated nodules. In capping follicles, the follicle was most often reduced to a very thin layer of small lymphocytes in between the

associated nodules and the overlying sinus.

4. Outlining of nodules by autofluorescent cells. With most nodules, the portion of their margin not outlined by a follicle was associated with autofluorescent cells. These showed dark in silver-impregnated sections (fig. 44) and light-green in sections stained by the technique of Dominici (fig. 45). In some nodules, the autofluorescent cells were grouped in clusters (fig. 40), one to a few clusters being present along their inner margin. In other nodules, the autofluorescent cells formed a sheet outlining the portion of their margin not covered by a follicle (fig. 41). In other nodules, both clusters and a sheet of autofluorescent cells were seen. In general, the largest autofluorescent cells were located along the innermost portion of the nodular margin, their size progressively decreasing as they neared the outer margin of the nodules (figs. 41,46-49).

The nodules were associated with autofluorescent cells, irrespective of their localization in the node. However, in the thin cortex along the flat surface of the node, thennodules were associated with fewer and smaller autofluorescent cells on the average than nodules elsewhere.

In the reconstructed complex nodules, autofluorescent cells were found along the inner nodular margins of each of the fused single nodules (fig. 42) or what appeared to correspond to these margins (fig. 43).

5. Cortical extrafollicular area. The cortical "extrafollicular area" was the portion of cortex present outside the follicular nodules (fig. 129). As reported above, the limits between this area and the latter structures were clear-cut in the silver-impregnated sections but

not in those stained by the technique of Dominici. As to the limit between the cortical extrafollicular area and the continuous medullary cords it was vague in silver-impregnated sections and moderately detectable in the sections stained by the technique of Dominici. The latter limit, the cortico-medullary junction, was not constituted by a given component but rather by a change in cell population, the cortical extrafollicular area being populated mainly by small lymphocytes and the medullary cords predominantly by plasmocytes. It was constituted furthermore by changes in patterns of the reticular fiber networks, the lymphatic sinuses, and the blood vessels, as will be described later.

The thickness, or degree of development, of the extrafollicular area paralleled that of the whole cortex, except near the hilus. Extrafollicular area was absent around the few small follicular nodules located nearest to the hilus; the latter nodules were surrounded by medullary plasmocytic cords and sinuses.

6. Framework of reticular fibers. The capsule and the septal trabecula outlining the lobule of the node contained mainly collagenous fibers that showed variably dark-brown in the silver-impregnated sections. Along the capsule and this trabecula, the collagenous fibers coalesced to give rise to perpendicular "coarse" reticular fibers (figs. 50,55) whose diameter varied between 1.6 and 3.2 microns. The portions of these collagenous structures overlying the nodules and their follicles yielded considerably fewer fibers than that portion overlying the cortical extrafollicular area (figs. 50,61,62). In the latter area, the fibers crossed the thickness of the cortex, remaining nearly parallel to each other until they reached the cortico-medullary junction (figs. 50,61). In the

medulla, some of these coarse fibers fused with those outlining the medullary sinuses, while others bifurcated, became sinuous, and transformed into the irregular fibers of the medullary cords. In the cortical extrafollicular area "medium" fibers, varying in diameter between 0.8 and 1.6 microns, and "fine"fibers, reaching up 0.8 micron, extended between the coarse fibers and between each other, forming little polygonal chambers that often appeared as pentagons in single sections. In single sections, each such chamber contained about six small lymphocytes. Their outline was not continuous, so that the chambers frequently appeared to communicate one with the other (figs. 50C, 54).

The coarse fibers adjacent to the follicular nodules curved to encircle the inner zone of these structures with the medium and fine fibers extending between them, all those fibers presented the appearance of woven follicular baskets hanging from the overlying capsule or trabecula, each basket containing a follicular nodule. Hence, the follicular margin appeared to be formed of reticular fibers in the following manner. The outer follicular margin corresponded to the reticular fiber outlining the inner surface of the overlying subcapsular or subtrabecular sinus. The lateral follicular margin was constituted by the straight portions of the fibers forming the side of the baskets. The inner follicular margin was constituted by the curved portions of these fibers forming the bottom of the baskets (figs. 50,55). In general, the follicles themselves were devoid of a reticular network (figs. 50B,53); but in some, a network of fine fibers was seen mainly in their outer zone and in restricted areas of their lateral zone (figs. 55-58).

A few coarse fibers originating from the capsule or the

trabecula passed between the lateral zone of the enveloping and mantling follicles and their associated nodules (figs. 50,55). These fibers curved to encircle the inner zone of the nodules. Together,with the few finer fibers extending between them, they constituted thinly woven nodular baskets inside the follicular baskets. Hence, as with the follicular margin, the lateral nodular margin was constituted by the straight portions of the fibers forming the side of these latter baskets. The inner nodular margin was constituted by the curved portion of these fibers forming the bottom of thennodular baskets. The constitution of the outer nodular margin will be described later.

A few coarse reticular fibers originating from the capsule also penetrated into the nodules. A minority of them completely crossed the nodules and fused with the fibers constituting the inner nodular margin (figs. 50,60). These and the other fibers in the nodules constituted a very coarse network supporting the capillaries of the nodule (fig. 59). Except for this, the nodules appeared to be devoid of a reticular network.

In many nodules, when the inner and lateral zones of their associated follicles were devoid of small lymphocytes, the inner and lateral follicular margins were close to their inner and lateral margins, respectively. This often gave rise to what will be termed the "subnodular spaces", which consisted of one or two, more or less concentric spaces along the inner and portions of the lateral nodular margins not outlined by a follicle (figs. 63,67,68). The subnodular spaces were filled with the autofluorescent cells previously described (see: 4. Outlining of nodules by autofluorescent cells). In other nodules, mainly in those

located along the flat surface of the node, no subnodular spaces were detectable, the fibers of the follicular and nodular margins being in contact with each other where no follicles were present. Around these nodules, therefore, was seen a thick layer of densely woven reticular fibers (fig. 64), which, in cross sections, showed as a dense corona around the nodules (figs. 65,66).

It must be pointed out that, reticular fibers were cross-sectioned, appearing as dots or very short lines (fig. 7). It was only where fibers were cut longitudinally that the above structures were detectable readily.

F. Lymphatic vascularization.

1. Subcapsular and subtrabecular sinus. The subcapsular sinus and the subtrabecular sinus were two portions of a selfsame sinus overlying the entire surface of the cortex, the subcapsular sinus being the portion over the cortex along the capsule proper, whereas the subtrabecular sinus was the portion over the cortex along the trabecula. All along the convex surface of the node and along the septal trabecula of its lobule, the average thickness of this sinus was grossly constant. However, it was noticed that the follicular nodules often bulged into this sinus (figs. 31, 50), so that the thickness of the sinus over these structures was frequently only half of that over the cortical extrafollicular area, where it was about 25 microns. Along the flat surface of the node, this sinus became progressively thicker as it neared the hilus. There, its thickness appeared to be inversely proportional to that of the underlying cortex. Immediately around the hilus, there was no cortex and the sinus was at its maximum thickness, reaching up to 80 microns. There, in fact, where the sinus was in contact with the

medulla, the subcapsular sinus resembled a medullary sinus in thickness. It bore further resemblance to a medullary sinus in that, like the latter, its inner outline was quite sinuous, this outline being regular elsewhere, except of smooth curves over the follicular nodules.

2. Interfollicular sinuses. In some places, the inner outline of the subcapsular or subtrabecular sinus opened into similarly narrow and more or less straight sinuses that traversed the depth of the cortex (figs. 62,69). Because these sinuses passed through the cortical extrafollicular area between the follicles, they will be referred to as cortical "interfollicular sinuses" (fig. 129).

3. Medullary sinuses. Upon reaching the cortico-medullary junction, the interfollicular sinuses became wider, thus transforming into the "medullary sinuses" (figs. 69,84). The latter sinuses were very irregular in width, their outline was quite sinuous, and they anastomosed one with the other. On the whole, they ran in the direction of the node hilus, where they fused together to form the efferent lymphatic vessel leaving the node. It was also observed that, at the hilus, the subcapsular sinus opened into the adjacent medullary sinuses, forming the efferent lymphatic vessel (figs 69).

4. Framework of reticular fibers.

a. Subcapsular and subtrabecular sinus. Whereas the bulk of the fibers forming the capsule and the trabecula were brownish collagenous fibers, the inner outline of these structures, i.e. that in contact with the underlying sinus, appeared to be formed of a layer of black, silver-impregnated reticular fibers. This layer of medium reticular fibers constituted the outer margin of the subcapsular and subtrabecular sinus.

They coalesced in a funnel-like manner with some collagenous fibers of the capsule to give rise, as described previously, to the coarse perpendicular reticular fibers crossing the sinus and the cortex (figs. 50,55). The inner outline of the subcapsular sinus was formed of a layer of fine reticular fibers extending in between the above coarse fibers. The fine fibers of the latter outline appeared to form a continuous sheet with only a few narrow gaps in places (figs. 55,61,62). In restricted areas within the sinus, occasional medium and fine fibers formed a network extending between both outlines of the sinus (figs. 50, 62).

b. Interfollicular sinuses. These sinuses were outlined by a layer of reticular fibers that were variably and irregularly coarse, medium, or fine. The reticular outlining of these sinuses was rather regular and smooth, like that of the inner outlining of the subcapsular sinus (figs. 50,62). Here also, we observed what appeared to be a few gaps along the outline of the sinuses. Very few fibers extended across these sinuses. It must be pointed out that the interfollicular sinuses in the cortex along the flat surface of the node were wider than those elsewhere, thus resembling medullary sinuses.

c. Medullary sinuses. The reticular fiber outline of these sinuses resembled that of the interfollicular sinuses, except for the fact that it was not as smooth and regular and the gaps along it occured with greater frequency. Furthermore, variably thick fibers extended and branched across the medullary sinuses more often than in the subcapsular and interfollicular sinuses. In places, these fibers formed a coarse or fine network in the sinuses (figs. 50,65).

G. Medulla. The medulla was formed of the medullary sinuses and, in between them, "medullary cords" that were populated predominantly by plasmocytes at various levels of maturity. In these cords, the variably thick reticular fibers formed a network of polygonal chambers like those in the cortical extrafollicular area. Here, however, the chambers were on the average smaller than those in the latter area. In single sections, they frequently contained one, or two, small plasmocytes or a single large one. In silver-impregnated sections, this denser network and the frequently greater concentration of coarse fibers often imparted a darker hue to the medullary cords than to the cortical extrafollicular area.

Along the convex surface and the septal trabecula outlining the lobule of the node, the medulla was separated from the overlying nodules and the subcapsular or subtrabecular sinus by a relatively thick layer of cortical extrafollicular area. Along the flat surface of the node, this layer was progressively thinner as it neared the node hilus. Hence, the medulla became more and more in contact with the inner and lateral margin of the nodules and with the portions of the subcapsular sinus in between the nodules, the area immediately around the hilus being totally occupied by the medulla.

H. Blood vascularization.

1. Hilar artery. Upon reaching the node hilus, an artery branched to give rise to two arteries, one for each of the two portions of the bifurcating hilus (fig. 70). Within the connective tissue of these two portions of the hilus, each artery branched repeatedly, to yield many arterioles penetrating the medulla, the "medullary arterioles".

2. Medullary arterioles. These vessels ran radially, in the

direction of the various sites of the cortico-medullary junction, by-passing inside the medullary cords (fig. 69). Most medullary arterioles did not branch in these cords (figs. 69,70), but gave rise to very small lateral arterioles that yielded the capillaries of the cords, some of which ran parallel to and in contact with a medullary sinus. A minority of the medullary arterioles branched in the cords into a few smaller medullary arterioles that continued to run in the direction of the cortico-medullary junction (fig. 70). Upon reaching the cortex, each medullary arteriole branched into several fine arterioles that spread out in the cortex (fig. 70).

3. Arterial vessels of nodule and follicle. One to three fine arterioles formed at the level of the cortico-medullary junction ran towards each nodule (figs. 71,74) and branched into several very fine arterioles. The latter arterioles came in contact either with the inner and lateral nodular margin outlining the dark area of the nodule wherever this area was not covered by the associated follicle (figs. 71,73,80), or, with the follicular margin along the portion of a follicle covering the dark area of the associated nodule (figs. 73,79). Occasionally, one of these arterioles entered slightly into the dark area of a nodule (figs. 74,75) or ran along the lateral margin, between the nodule and the lateral zone of its follicle (fig. 73). At the inner and lateral follicular or nodular margins outlining the dark area of a nodule, the very fine arterioles transformed into capillaries that penetrated the follicle and, mainly, the nodule (figs. 73,79,80). Many capillaries ran through the nodule, often in a direction more or less parallel to its long axis, while branching off into its dark and light areas (figs. 74, 76,77) and, at times, anastomosing between them (figs. 73,79). Upon

reaching the outer nodular margin, these capillaries curved to form loops along this margin, so that there were no blood vessels in the outer zone of follicles (figs. 73,79). The loops were oriented towards the lateral margin of the nodule or of its follicle, where they ended (figs. 55,79,84). Capillaries, otherwise oriented, similarly ended at the lateral or inner margin of the nodule or of its follicle, at levels below that of the inner nodular margin (figs. 73,85,87). Upon leaving a nodule or its follicle, the capillaries transformed almost abruptly into postcapillary venules that will be described below.

All follicular nodules were irrigated solely by capillaries. In each of them, the arrangement of the capillaries followed the basic pattern described above, but with many minor variations. The few small naked nodules, located next to the hilus, made exception to this general observation. In the latter nodules, the very few capillaries ran along the nodular margin, not being found inside the nodules.

4. Arterial vessels of cortical extrafollicular area. Other fine arterioles, formed at the level of the cortico-medullary junction, ran into the cortical extrafollicular area in the direction of the overlying sinus (fig. 71), thus supplying the capillaries irrigating this area; they also abutted into the postcapillary venules present in the area. The capillaries running towards the overlying sinus did not come in contact with it, but curved near it, at distances ranging up to 15 microns, to form loops that resembled those in the follicular nodules. Slightly below the sinus, the ends of these capillary loops transformed almost abruptly into postcapillary venules.

5. Postcapillary venules of cortex. These were peculiar venules exhibiting variably hypertrophied endothelial cells and small lymphocytes in process of diapedesis (figs. 115-116A). Each postcapillary venule was surrrounded by a narrow space outlined by reticular fibers and referred to as a "perivascular channel". These channels contained flattened, small lymphocytes with ameboid forms. The postcapillary venules formed a complex network around the lateral and inner margin of nodules and follicles (figs. 69,72,78). Their diameter was very irregular and their outline was very sinuous (figs. 78,84). Wide and narrow anastomoses existed between the venules around a nodule, as well as between those around adjacent nodules (figs. 69,72,78).

On the basis of the degree of hypertrophy of their endothelial cells, the appearance of the postcapillary venules differed with the various areas of the node. Furthermore, some segments of these venules appeared to be "closed", as their narrow lumen contained no erythrocytes but a variable number of small lymphocytes (fig. 115). Such segments were located mostly in the outermost layer of the cortex underlying the convex surface of the node. Most segments were "open" and contained erythrocytes (fig. 116); they also showed numerous small lymphocytes in the proportion of one for every 50 erythrocytes to as many as one for every 5 erythrocytes.

Postcapillary venules were restricted to the cortical extrafollicular area of the node. The morphological features peculiar to the postcapillary venules were observed solely in the portion of cortical venules in contact with the cortical population of predominantly small lymphocytes (fig. 99). Hence, the portion of a venule wall in close contact with a nodule exhibited regular flat endothelial cells (figs. 88-

91). Moreover, in venules which were parallel to the cortico-medullary junction, the endothelium was hypertrophied on the cortical side of the venule only, the side in contact with the plasmocytes of the medulla having regular flat endothelial cells (figs. 99,100,101). Accordingly, the length or complexity of the network of postcapillary venules was proportional to the thickness and abundance of the cortical extra-follicular area in each site of the node. Along the flat surface of the node, the closer the postcapillary venules were to the hilus the less developed they were, being absent altogether immediately around the hilus (fig. 69).

Close to the cortico-medullary junction, there were capillary anastomoses between wide postcapillary venules (figs. 95, 96).

6. Complex nodules. Each of the fused single nodules forming the complex nodules had its own pattern of arterial vessels resembling that of the other single nodules. Similarly, the lateral and inner margin of each fused nodule was surrounded by a network of postcapillary venules. However, although each fused nodule had its own vascular network there existed anastomoses between the capillaries and the postcapillary venules of a nodule with those of adjacent nodules (fig. 92). The greater the extent of fusion between adjacent nodules, the more anastomosed were these vessels. It should be pointed out that, with complex nodules as with single nodules, all postcapillary venules were located outside the nodules and associated follicles, no venules passing through the nodular or follicular bridge in between fused nodules (figs. 34,35).

7. Medullary venules and hilar veins. The medullary venules continuous with the postcapillary venules followed the same course as the

medullary arterioles through the medullary cords, but in the opposite direction towards the hilus. These venules showed regular flat endothelial cells and no diapedesis of leucocytes. In such venules the small lymphocytes appeared to be in greater concentration than in the peripheral blood, but not in as great a concentration as in the blood of the postcapillary venules (figs. 101-104).

Close to and within each of the two branches of the hilus, the medullary venules fused to form two small veins which, in turn, fused to form the hilar vein leaving the node (fig. 105).

I. Pseudo-follicle

We reported that, in silver-impregnated sections, the small lymphocytes present in the cortical extrafollicular area failed to become silver-impregnated. Exceptionally, this was not true for an accumulation of small lymphocytes in a site of extrafollicular area adjacent to part of the septal trabecula outlining the node lobule. There, the extrafollicular small lymphocytes were silver-impregnated, like those of follicles (figs. 18,106,107). Because of this resemblance, this site will be referred to as the cortical "pseudo-follicular area". Examination of serial sections showed that the latter area occupied the cortical extrafollicular space in between and below the inner zones of the nodules, and extended much further below these nodules. The pseudo-follicular area seen in between nodules in Figure 106, was therefore part of an accumulation of small lymphocytes bulging below the over-all level of the cortico-medullary junction. The semi-oval mass of small lymphocytes, thus formed will be referred to as a "pseudo-follicle" (fig. 130). Like the cortical extrafollicular area, it contained postcapillary venules but

its concentration of reticular fibers was less than in the latter area. It also contained sinuses that bore more resemblance to medullary than to interfollicular sinuses.

Other Nodes and Animals

Except when otherwise mentioned, the observations reported in this chapter were from sections stained by the technique of Dominici. A. One-week-old nodes. The cervical nodes examined were very small, their longest dimension reaching up to 0.5 millimeter. In some of them, there were one or two septal trabeculae isolating one to three lobules in a node. Between the sinuses, the parenchyma of the nodes, which was devoid of nodules, showed as a more or less homogeneous population of predominantly small lymphocytes and reticular cells (fig. 117E). Hence, the nodes were not divided into a cortex and a medulla. It has to be pointed out that, in some cases, the presence of small lymphocytes in between the sinuses was observed throughout the nodes. In many cases, however, this presence of small lymphocytes was restricted from two to four rounded sites of the nodes. In silver-impregnated section, as in the node studied, capsular collagenous fibers coalesced to yield coarse reticular fibers that were perpendicular to the capsule and penetrated deep into the nodes (fig. 118). The latter fibers were few in number and distributed at more or less regular intervals beneath the capsule, so that there existed no concentrations of fibers like those seen in the cortical extrafollicular area of the node studied. Finally, the features peculiar to the postcapillary venules were not restricted to the outer, or

future cortical, portions of the venules. Venules localized in any sites of the nodes, populated by small lymphocytes, showed variably hypertrophied endothelial cells and small lymphocytes in the process of diapedesis. Those endothelial cells, which were hypertrophied, were usually not crowded but scattered along the vessels.

B. Two-week-old nodes. Some of the nodes looked like the one-week-old nodes described above. Other nodes appeared to be variably more differentiated, for they exhibited the following additional features. The separation of their parenchyma into a cortex and a medulla was in the process of realization or already realized (fig. 117A). The cortex was populated mostly by small lymphocytes, with a few islands of large lymphocytes that appeared to be nodules in process of development. Such structures will be referred to from now on as "developing nodules" or "developing follicular nodules". The latter structures showed as poorly outlined islands of large lymphocytes (fig. 117F), many of which were in mitosis. As to the medulla, the medullary cords contained a high proportion of plasmocytes (fig. 117F). In some nodes, we observed a few accumulations of small lymphocytes which resembled the pseudo-follicles (figs. 117A,117F). Finally, hypertrophied endothelial cells and diapedesis of small lymphocytes were seen mostly in the portions of the venules present in the cortex outside the developing nodules, i.e., in the future cortical extrafollicular area.

C. Six-week-to two-year-old nodes. Examination of nodes of different localizations (figs. 119-119B) and ages (figs. 117B-117D) revealed that, while the over-all appearance of the nodes varied, the basic features of their structures were constant and resembled those of the node studied.

Except for the variable degree of adipose transformation occurring in some nodes (fig. 120), the variations in the appearance of the nodes were seen in nodes of the same age as well as in nodes of different ages. Both well-developed and poorly-developed nodes were observed in ten-week-old as well as in two-year-old rats. Therefore, the main variations in the appearance of the nodes will be reported below without reference to the age of the animal.

1. General constitution. The size of a node varied from being hardly detectable with the naked eye to reaching up to 8 mm in its longest dimension. The shape of the nodes also varied considerably, often being round, oval, semi-oval, or flattened. The surface was smooth or it exhibited little bumps about 1 mm in diameter over variable extents of its area. The nodes differed, furthermore, in respect to the number of septal and columnar trabeculae. Some showed no septal trabeculae and, therefore, no lobule; other nodes exhibited up to three such trabeculae and, consequently, were divided into up to four variably isolated lobules. All nodes had at least a few short columnar trabeculae. It might be added that, as a general rule, the septal and columnar trabeculae were rarely found in the poorly-developed nodes. At times, we observed a trabecula, that penetrated half-way down into the peripheral cortex, between each of the follicular nodules in a restricted portion of a node. In such cases, the trabecula was very thin, and was either columnar or formed a narrow band partially separating two adjacent nodules. This latter finding was observed mostly near the hilus of some nodes as well as in a node area undergoing adipose transformation.

Independently of the above variations, all nodes exhibited two regions: a cortex and a medulla. The cortex in most nodes was arranged in the manner of the node studied, i.e., underlying the whole capsule

and the trabeculae; often, the cortex was becoming thinner and fading out as it approached the hilus. In such nodes, the cortical layer occasionally showed a gap (elsewhere than at the hilus) that was filled with medulla, thus coming into contact with the subcapsular sinus. The cortex around a cortical gap contained follicular nodules of average size, or small ones like those around the hilus in the node studied. In other nodes, the cortical layer underlay only a portion, down to about a half, of the capsule; the remainder of the outermost layer of the nodes contained medullary cords and sinuses. In all nodes, the medulla filled the space not occupied by the cortex, being located most commonly in a semi-circular manner around the hilus as in the node studied. Furthermore, in nodes with well-developed plasmocytic medullary cords, we frequently observed the extension of medullary cords, outside a node, in the form of variably thick plasmocytic sleeves along the hilar vein and the efferent lymphatic vessel, as well as along external veins running close to the capsule. In extreme cases, the node appeared to be nearly completely surrounded by a sleeve of plasmocytes. Occasionally, the plasmocytic population of the medullary cords of a node was seen to extend outside the node, elsewhere than through the hilus. This occurred when a blood vessel in a medullary cord pierced the subcapsular sinus and the capsule, at a site other than at the hilus. Then, a plasmocytic sleeve often accompanied such a vessel outside the node.

2. Sinuses. In all nodes, the basic pattern of the lymphatic sinuses was similar to that described for the node studied (figs. 117B, 117D,119-120), except for the following variations. Wherever a portion of the capsule or of a septal trabecula was underlaid not by the cortex

but by the medulla, the corresponding portion of the subcapsular or subtrabecular sinus was not thin like the remaining subcapsular sinus, but appeared wide and irregular like the medullary sinuses of the node concerned. Furthermore, wherever the cortex was thin, the corresponding portion of the subcapsular sinus and the corresponding interfollicular sinuses were often wider than those situated where the cortex was thick.

In general, the medullary sinuses of the mesenteric nodes (fig. 119A) appeared more dilated than those in nodes of other locations (figs. 119,119B). Irrespective of the site of the node, there were variations in the width of the sinuses, mainly of the medullary ones, in various nodes or various areas of a node. In extreme cases, seen mostly in the old rats, the diameter of a medullary sinus reached about 1,000 microns. Most frequently, the sinuses contained pale macrophages and small dark lymphocytes in a relatively sparse concentration, so that they were readily distinguishable, having an appearance lighter than the other constituents of the node. In some nodes, the sinuses contained a considerably greater concentration of mainly small lymphocytes, thus appearing dark like the other node constituents. In such cases, it was difficult to distinguish the medullary sinuses from the medullary cords unless the latter contained abundant and mostly plasmocytic cells.

3. Cortex. In all nodes, the cortex was formed of follicular nodules and of an extrafollicular area, as in the node studied (figs. 117B, 117D, 119-120). The great majority of the follicular nodules appeared to be located in the peripheral cortex (figs. 117B-117D, 119-120). Examination of the serial sections of a ten-week-old mediastinal node confirmed that all of its 59 follicular nodules were actually located in the peripheral cortex. Time did not permit us to check three-

dimensionally whether the few of these structures that in single sections seemed to be located in the deep cortex of some nodes were really deep nodules not coming into contact with the subcapsular sinus or subtrabecular sinus. In the nodes examined, we frequently observed that the size of the nodules decreased around the hilus, in the same manner as in the node studied (figs. 117C, 117D, 119, 119A).

In single sections of the nodes, the various appearances presented by nearly all the follicular nodules (figs. 121,121A) fitted with those to be expected from the different angles of sectioning of this structure as represented in Figure 29. It thus seemed that the basic constitution of nearly all follicular nodules corresponded to that illustrated in this Figure. Although it is not within the scope of the present topographical study, it is of interest to point out that we noticed great variations in the concentration of large and medium lymphocytes present in the dark area of nodules, in the degree of mitotic activity of the latter cells, and in the concentration of pycnotic nuclei present in the nodules. The latter cytological differences produced variations in the appearance of nodules, while the basic constitution seemed to be similar in nearly all of them. The relatively few nodules whose appearance differed from that described up to now were seen in the poorly developed nodes, particularly in those popliteal nodes undergoing adipose transformation. In such nodes, nodules with a dark and light area were few in the cortex. In some cortical sites where nodules were expected to be seen, there was a rounded accumulation of cells constituted of predominantly small lymphocytes with a few large lymphocytes and reticular cells. At times, such an accumulation was more densely populated than the area around it and was partially outlined by some

autofluorescent cells. Furthermore, examination of silver-impregnated consecutive sections revealed that, like the follicular nodules, such an accumulation contained few reticular fibers, while the area around it had abundant fibers, as does the cortical extrafollicular area. Intermediate forms between such an accumulation and nodules were also seen in these nodes, in which the features of the dark and light area of the nodule faded out progressively. All the latter observations were thought to represent various steps by which a nodule gradually loses its own cell population while keeping its other architectural features. From here on, the latter forms of nodules will be referred to as "resting nodules" or "resting follicular nodules".

The cortical extrafollicular area always resembled that in the node studied, except for the density of its population of small lymphocytes, which varied with the node as well as, in some cases, with the different sites in a node. In some nodes, the latter population was thick throughout the whole cortex, in others it was thin. There existed intermediate conditions in which the population was dense in the peripheral and thin in the deep cortex. In general, the density of this small lymphocyte population was greater in the peripheral cortex than in the deep one. The thickness of the whole cortex varied from node to node, and often with different sites in a node. The peripheral cortex being defined as that layer of cortex containing the follicular nodules, its over-all thickness was fairly constant in all nodes. It was therefore, the thickness of the deep cortex that showed considerable variation. Most often, this thickness was about a third of that of the peripheral cortex. At times, it was absent, the medulla then being in contact with the inner zone of the follicles or of the nodules. At

other times, it appeared to be as thick as the peripheral cortex. As will be seen below, the deep cortex was even thicker where a pseudo-follicle was present.

4. Medulla. In all nodes, the medulla was constituted of medullary sinuses and medullary cords as in the node studied. We previously described the variations related to medullary sinuses. The medullary cords varied from being wide and densely populated, predominantly by plasmocytes, to narrow and containing few plasmocytes and a more or less equal number of small lymphocytes. The medullary cords rarely appeared to be totally without plasmocytes. When the medullary cords were well developed, the sinuses were generally narrow and, conversely, medullary cords and sinuses were generally readily distinguishable one from the other. Distinction between the two structures was difficult when the cords were thin and contained small lymphocytes in a high proportion and when, simultaneously, the sinuses contained an abundance of small lymphocytes. It might be added that, as a general rule, the cervical and the mediastinal nodes appeared to be those of the nodes having the best developed plasmocytic medullary cords (figs. 117B-117D, 119). Plasmocytes were considerably fewer in the medullary cords of the mesenteric and popliteal nodes (figs. 119A,119B).

Finally, the limit between the cortex and the medulla was always vague and often irregular. However, the corticomedullary junction was best distinguishable when the medullary cords were highly plasmocytic, and it was the least distinguishable when plasmocytes were few in the cords.

5. Pseudo-follicles. Most of the nodes contained one to four

pseudo-follicles (figs. 117B,117C,119-120). Like the one found in the node studied here, these structures (fig. 108) appeared as semi-oval accumulations of small lymphocytes extending below the over-all level of the cortico-medullary junction of a node. Their constitution was similar to that of the cortical extrafollicular area. Hence, in sections stained by the technique of Dominici, pseudo-follicles were usually difficult to distinguish, as somewhat definite structures, from the remaining deep cortex. Consequently, wherever there was a pseudo-follicle, the latter layer of cortex appeared very thick. Pseudo-follicles were best distinguished from other surrounding constituents, in silver-impregnated sections. It should be pointed out that, usually, the lumen of the post-capillary venules present in the pseudo-follicles contained few or no erythrocytes at all. The segments of these venules, present in the pseudo-follicles, thus appeared to be closed (fig. 110).

Pseudo-follicles varied in size (figs. 119,120); in single sections passing through their center, they extended below two to six follicular nodules (fig. 108). They also varied in respect of the density of their small lymphocyte population, small lymphocytes being most often densely crowded within them.

6. Blood vascularization. The pattern of the blood vascularization of all nodes examined seemed to follow that observed in the node studied. Follicular nodules exhibited capillaries only, and postcapillary venules were restricted to the cortical extrafollicular area of nodes. The degree of development of the network of postcapillary venules was thus directly related to the amount of cortical extrafollicular area in a node, or in the various sites of a node. No postcapillary venules were found in the

outermost layer of a node whenever this layer was occupied by medullary instead of cortical structures. When the cortex was very thin, the post-capillary venules were correspondingly short, the peculiar features of these venules being restricted to small portions of venules beneath the subcapsular or subtrabecular sinus. Moreover, the denser the population of small lymphocytes in the extrafollicular area of a node, or of a site of a node, the more hypertrophied in general, was the endothelium of these vessels and the more abundant were the small lymphocytes in the process of diapedesis through them. In a node with an extrafollicular area thinly populated by small lymphocytes and with medullary cords thinly populated by plasmocytes, highly hypertrophied endothelial cells were seen only in portions of postcapillary venules next to the subcapsular sinus. In such a node, the remaining portions of the postcapillary venules resembled those of a two-week-old node, as they were just slightly hypertrophied. Moreover, occasional hypertrophied endothelial cells and diapedesis of small lymphocytes were also seen in the medullary portions of the node venules.

D. Reticular framework. Examination of the sections impregnated by the silver salt (figs 118A-118C) revealed that, basically, the framework of reticular fibers in all nodes was similar to that described for the node studied. What varied most in this framework was the concentration of fibers in the medullary cords (figs. 118B,118C).

E. Adipose nodes. Signs of adipose transformation were occasionally observed in nine-month-old nodes. In 24 month-old nodes, the process was more frequently observed and was more advanced. Many of these latter nodes, however, were almost untouched by the process (fig. 117D), such nodes

appearing similar to ten-week-old nodes. In nodes undergoing adipose transformation, the untouched portions were most often normal, i.e., showed the regular cortical or medullary structures of the node. At times, these portions exhibited involuted follicular nodules as described above. In nodes undergoing the early stage of adipose transformation, adipose cells were seen associated with the trabeculae and mostly with the hilus (figs. 119B,120). The process of adipose transformation appeared to progress by infiltrating the connective-tissue structures of nodes, mainly at the hilus. Often, the adipose cells formed cords trapping medullary sinuses or plasmocytic cords, while the cortex remained normal.

Furthermore, in some nodes, the portion of a node cortex encircling the hilus was seen to undergo adipose transformation.

DISCUSSION

The node studied here was chosen because it was thought to be well-developed and normal, and its architecture appeared to be representative of that of the rat node in general.

The node was judged to be well-developed because of its comparatively large size and mainly because, at the time it was chosen, it seemed to us to exhibit all the known histological features that can be encountered in nodes of adult rats, including well-developed plasmocytic medullary cords.

The cervical node studied was thought to be normal because it resembled the other cervical nodes that we examined. Moreover, it showed no features that could have been suspected of being pathological. Like most of the other cervical nodes, it contained abundant plasmocytes, which indicated that it was undergoing antigenic stimuli whether pathological or not. While the presence of plasmocytes in a node might reveal the occurrence of a pathological process in a region of the organism drained by the node, this presence constitutes a normal feature of the node, since plasmocyte formation represents the normal response of the organ to an antigenic stimulus (Leduc, Coons and Connolly'55).

After comparison of the observations from the cervical node studied with those made in other nodes of various localizations, we realized that the basic architecture of the node studied was representative of that of any node from rats more than six weeks of age. The differences observed in the various other nodes seemed to reflect variations in the intensity of their activities rather than basic

structural differences. For instance, whether plasmocytes were abundant in a node or not, the medulla was always constituted of medullary sinuses and cords, the quantity of plasmocytes in the latter cords most probably depending upon the intensity of the antigenic stimuli the node was subjected to and not upon structural differences. Since the architecture of the node studied appears to be representative of that of the rat node in general, in the present Discussion, we will deal firstly and mainly with the observations made in that node, as in all probability the conclusions are applicable to the rat node in general. We will discuss only those observations made in the various other nodes that differed significantly from the observations made in the node studied, so as to provide additional or different conclusions. At the end of this Discussion, we will consider briefly the problem of the architecture of the nodes in respect to the age of the animal.

A. Terminology. In carrying out this topographical study, we found it very difficult to report some observations, because of the inadequacy of the present terminology pertaining to the node, mainly that related to nodules. We also realized, as did Bloom ('38), that this terminology for nodules is confusing. Indeed, at present, the single term "nodule" refers to two different structures: i.e., "primary nodule" and "secondary nodule" are used synonymously to refer to the same structure, which is rarely in a purely germinative or reactive state. Moreover, even nowadays, there is still no evidence that the latter structure develops as a secondary structure within the so-called primary nodule, as was remarked by Hellman in 1930.

In view of the above difficulties encountered in the current

terminology of the node structures, we decided to use the tentative terminology previously proposed by Dr. G. Sainte-Marie ('66a), the supervisor of this thesis. Whenever necessary, the latter terminology was modified on the basis of the present observations. The modified terminology was thought to fit withethe observations made in all nodes examined in this study.k It will be seen from the present discussion that the rat node is basically similar to that of many species, including man, so that the presently used terminology, as such, most probably fits the nodes of these species.

In elaborating the present terminology, in collaboration with Dr. G. Sainte-Marie, we avoided adjectives for the nodules having a functional significance, such as germinative and reactive. This is because of the inadequacy of our knowledge regarding the function of these structures. Moreover, although it might have been desirable to use completely new terms, as much as possible, we used descriptive terms previously or presently used by investigators. Hence, "nodule" was proposed to refer to present "secondary nodule", and "follicle" for "primary nodule". The term follicle had previously been used commonly in this context (Prenant and Bouin '11, Jolly '23, Cowdry '38); and although it is still used by some authors (Marshall '56, Weiss '66); around 1940, the proposal was made to exchange it for nodule (Bloom '38). The term "center" was avoided here, for it does not lend itself to the making of adjectives such as "nodular" and "subnodular" as does the term nodule. As to the adjectives that we proposed to use for describing the various types of nodules, they have been employed also by previous investigators: "naked" by (Bloom ('38), "capped" by Ehrich ('29)

and Kindred ('55), and "mantled" by Kindred ('55). The proposed topographical terms were based on the fact that the capsule is "outside" the node, so that the portion of a structure nearest to it can be considered as its "outer" portion and the opposite portion its "inner" one. The qualifying "outer" had been used similarly by Bloom ('38). In the remainder of the discussion we will continue to use the terms of the presently proposed terminology, except when otherwise mentioned.

B. Capsule and trabeculae. In the schematic diagrams of the node presently used by various authors (figs.1-3), numerous septal trabeculae are shown to partition the organ extensively, each follicular nodule being surrounded completely by one. In the node studied, and in the other nodes examined, we did not observe any such phenomenon. The above current diagrams (figs. 1-3), therefore, do not represent the architecture of the node of the rat: they were based on the node of the dog as observed by Yoffey and Courtice ('56).

The columnar trabeculae, present in the node studied, were short and did not influence the architecture of the organ. As to the circular septal trabecula of the latter node, it simply separated the node into two portions, the cortex remaining as a single unpartitioned layer along the capsule and the circular trabecula. The additional observations made on trabeculae in the other nodes suggested that the latter phenomenon of the continuity of the cortical layer is a general rule whether there are one, two or three septal trabeculae separating a node into two, three or four portions or lobules, respectively. It was only in a minority of nodes that short band-like trabeculae were seen between each follicular nodule. This, however, occurred only in

restricted area of a node and, even then, the cortical layer remained continuous between the band-like trabeculae.

If the cortex formed a continuous layer, this layer did not necessarily underlie the whole surface of the capsule and the trabeculae of all nodes. In fact, observations made in the various nodes examined showed that, mostly around the hilus, variable portions of the surface of the latter connective tissue structure were underlaid by medulla instead of cortex, whether cortex had never existed along such portions or whether it had existed and eventually faded out to be replaced by medulla.

For the sake of comparison, it is of interest to point out that the human node resembles that of the rat rather than that of the dog. In fact, microphotographs of human nodes presented by various authors (Denz '47, Bloom and Fawcett '62, Ham '65) show no partition of the cortex into numerous small alveoli by septal trabeculae. As concluded by Heudorfer ('21) and Denz ('47), it thus appears that, in man also, the cortex forms a continuous layer.

C. Lymphatic vascularization.

1. Subcapsular and subtrabecular sinus. The subcapsular and the subtrabecular sinuses were two portions of a single sinus overlying the entire cortex. This terminological distinction is, therefore, not realistic, but was useful to permit topographical localization. Observations made in the node studied and in the other nodes revealed also that, when cortex was absent beneath a portion of the capsule or of a septal trabecula, the corresponding portion of the subcapsular sinus resembled a medullary sinus of the node rather than the remaining

subcapsular sinus. This indicated that, while the latter portion belonged topographically to the subcapsular sinus, most probably it behaved physiologically like a medullary sinus. This, and the fact that medulla was seen occasionally in the outer layer of nodes where cortex was usually present, indicated further that it is primarily the milieu of a given site of a node that determines the nature of the structures located in this site rather than the topographical localization. Of course, the organization of the nodes followed such a constant topographical order that the same type of milieu must normally exist in a comparable localization of all nodes and, therefore, this given localization usually exhibits the same histological structures. Consequently, it can be expected that, if a change in milieu occurs in a certain site of a node, the structures then present will be those normally existing in such a milieu rather than those usually present in this topographical site.

Observations revealed further that the subcapsular sinus was not homogeneous, as it presented two distinguishable zones, the first of which consisted of the separated rounded portions of the sinus over each nodule and its follicle, the second of the network-like zone over the cortical extra-follicular area. The variations between these zones suggest the existence of a difference in the dynamics of the lymph flow in the two zones. The sinus often being half as thin, or even thinner, over the nodules than over the cortical extrafollicular area, it is conceivable that the afferent lymph flows mostly or more rapidly over the latter zone. Moreover, the abundance of coarse reticular fibers in the sinus zone over the cortical extrafollicular area might conceivably

result in a trapping of the leucocytes in the afferent lymph an occurrence that could favor the diapedesis of these cells, particularly into the underlying extrafollicular area of the cortex rather than in the follicular nodules.

2. Interfollicular sinuses. In agreement with Heudorfer ('21) and Denz ('47), the cortex was found to be a continuous layer only pierced here and there by interfollicular sinuses, a term used also by Baillif ('64). Like the subcapsular sinus, these cortex-related sinuses were found to be narrow when the cortical layer was well developed and wide when the cortical layer was thin. This similarity is likely the result of a common cause, to be discussed further.

3. Medullary sinuses. The medullary sinuses were wider than the interfollicular ones, so that the lymph had, perforce, to flow more slowly across the medulla than over the cortex. Unlike the subcapsular and interfollicular sinuses, their width was very irregular and their outline was sinuous, a finding that might be partially explained in the following manner. Plasmocytic proliferation in the surrounding medullary cords is not usually a continuous process as it takes place only after an antigenic stimulus occurs in an area of areas of a node. This results in variations in the intensity of plasmocyte proliferation must yield a larger cell population locally which is likely to invaginate the adjacent sinuses, and hence, give rise to a sinuous outline.

The finding, in the present study, of small gaps in the reticular fiber outlining the various sinuses of the node fits with the observations made at the electron microscopic level of gaps in the wall of these sinuses (Moe '63).

D. Reticular fiber framework. Our pattern for the reticular fiber framework of the node studied, illustrated in Figure 50, fits with the observations of previous authors. Indeed, Rössle and Yoshida ('09), Orsós ('26), Conway ('37), and Denz ('47) reported the outlining of follicular nodules by a basket-like network of fibers. Moreover, Richter ('02), Rössle and Yoshida ('09), Orsós ('26), and Denz ('47) observed that the reticular fiber network of the node is denser in the medulla than in the cortex, the polygonal chambers outlined by the fibers being larger in the latter than in the former region.

Since trabeculae are rare in the rat lymph node, the reticular fibers obviously constitute the supporting framework of the organ. It is likely that in fulfilling such a function, the fibers are extensible and, at least the coarser ones, subjected to continuous squeezing. This could explain why the nodes can enlarge rapidly without disruption of their structures: relaxation of the squeezing force and elongation of the fibers under the stretching force caused by a rapid increase of fluid of cell population in an area of a node or in a whole node. As will be seen below, the orientation of the coarse fibers together with their probable squeezing force can be conceived as factors determining the shape of the node structures.

1. Cortical extrafollicular area. The numerous coarse fibers in this area most probably have for function to support the heavy network of postcapillary venules and arterioles in this area. The abundant squeezing fibers, attached to the capsule and to the heavy blood vessels, likely cause the invagination into the node of the portion of capsule over the extrafollicular area (fig. 50). This, in turn, likely accounts

for the bumpy or wavy appearance often exhibited by the surface of the nodes.

2. Follicular nodule. This structure contained only capillaries and a few fibers, most of the former running perpendicular to the capsule, i.e., in the same direction as the fibers to which they adhered. These observations indicated that the concentration of reticular fibers in the two cortical areas, the follicular and the extrafollicular, is proportional to the importance of the vascular bed of each area.

The follicular nodules take on the shape of the baskets of reticular fibers that surround them. Being anchored to the capsule, these baskets probably squeeze the follicular nodules against it. If this is the case, it is likely that the inner margin of these structures is the most compressed, and this would explain why the outline of this portion of the follicular margins is the most clear-cut. Such maximum compression at this site could also explain why follicles rarely extend beneath the innermost portion of the nodular margins. The squeezing of the follicles and nodules against the capsule can, furthermore, explain why the follicular nodules bulge into the overlying subcapsular sinus, thus reducing its thickness. Finally, the same force could explain the fact that the outer zones of the follicles is often thin, the small lymphocytesppreferably occupying the follicular zone between the lateral margins of the follicles and that of their nodules, where it is conceivable that the tension produced by the squeezing force is at a minimum.

While the squeezing force of the follicular and nodular

baskets of fibers participates in shaping the follicles and nodules respectively, there must simultaneously exist an expansion force likely responsible for the ovoid rather than round shape of the nodules. The latter force could arise from the proliferation of the lymphocytes in the nodules, the polygonal outline of the lymphocytes indicating that they are compressed. The cortical extrafollicular area containing a dense reticular network and many coarse venules, it likely opposes resistance to the lateral expansion of the nodules. The nodules could then extend more easily longitudinally, and this would serve to further explain the frequent thinness of the outer zone of the follicles and the bulging of the follicular nodules into the subcapsular sinus.

3. Medulla. The stretching force of the coarse fibers of the cortex that are oriented towards the capsule, likely fades out along the cortico-medullary junction where the perpendicular orientation of these coarse fibers to the capsule ends. In the medulla, where the fiber network extends across the medullary cords and few fibers cross the sinuses, the squeezing force of this network must tend to dilate the lumen of the sinuses. This could explain the fact that the lumen of the medullary sinuses is considerably wider than that of the subcapsular and interfollicular sinuses. In opposition to the squeezing force of the reticular network, the proliferation of plasmocytes in the medullary cords must give rise to an expansion force that dilates the cords and compresses the adjacent sinuses, as discussed above. The irregular intensity of plasmocytic proliferation along the cords can thus account for the sinuous shape of both the medullary cords and sinuses.

The same phenomenon can also explain the usual inverse variations in the average width of the medullary cords and sinuses: the more intense the plasmocytic proliferation, the wider the cords and, inversely, the narrower the sinuses. Hence, in general, the cervical nodes exhibited narrow sinuses and wide plasmocytic cords, whereas the mesenteric nodes showed the reverse situation. Finally, the variations in the plasmocyte population of the medullary cords could explain the quite variable concentrations of reticular fibers observed in the various areas of a node or in various nodes. It seems to us that an intense plasmocyte proliferation in a cord possible separates its fibers and, thus, dilutes them. If so, when the plasmocytic population of the same cord eventually diminishes, the reverse phenomenon occurs and, thus, the fibers once again become concentrated.

F. Follicular nodule.

1. Shape. The ovoid shape determined here for the nodule of the rat is similar to that found by Rohlich ('33) for the cat nodule and by Denz ('47) for the human nodule. The nodule being a non-homogeneous structure, it can be concluded in agreement with Rohlich ('33) and Kindred ('38) that only longitudinally-sectioned nodules reveal their over-all structure and that of their follicles: cross sections showing merely a part of it. As longitudinally and cross-sectioned nodules exhibit an ovoid and round shape respectively, these shapes can usually permit one to determine whether the nodule examined is revealed entirely or only partially, thus perhaps helping to avoid erroneous conclusions as to the actual structure of a cross-sectioned nodule.

2. Localization and orientation. In the cervical node studied, as well as in the mediastinal node examined three-dimensionally, all nodules were found to be located in the peripheral cortex adjacent to the subcapsular sinus including those which, in single sections of both nodes, appeared to be located in the deep layer of the cortex. In the other nodes examined, nearly all the nodules were also seen to be located in the peripheral cortex. Lack of time prevented us from determining the actual localization of the few remaining nodules of the latter nodes, but it is conceivable that they, too, were close to a non-distinguishable subtrabecular sinus. Hence, it seems reasonable to conclude that most probably all nodules are located in the peripheral cortex, i.e., are adjacent to the subcapsular sinus or its extension, the subtrabecular sinus. This is in agreement with the observations made, and the conclusion reached, by Ehrich ('29) for the humans, mice, rabbits, dogs and guinea-pigs, by Rothermell ('29) for calves, by Dawson and Masur ('30) for the rats, and by Denz ('47) for the humans. The report by Conway ('38), Ringertz and Adamson ('50) and by Gillman et al. ('52) of occasional nodules located in the deep cortex and even in the medulla (Conway '38) did not result from three-dimensional studies; so these authors could not have ascertained that these nodules were actually located as reported. Had three-dimensional reconstructions been carried out by the latter authors, they might have revealed that the nodules apparently located in the deep cortex were, in fact, peripheral nodules in contact with a portion of a subcapsular sinus not detectable in the single sections they examined. As to the occasional nodules reported to

be present in the medulla, they too could have been actually present in the peripheral cortex. Indeed, we have shown here (fig. 69) that, at times, the extrafollicular area of even the peripheral cortex is replaced by medullary structures. In such cases, nodules were seen to be almost completely surrounded by medulla when sectioned longitudinally, and completely surrounded by medulla when cross-sectioned. Unless a three-dimensional analysis is carried out, the latter observations could give rise to the conclusion that nodules can be located in the medulla, also.

The intimate relationship between the nodules and the node sinus that receives the afferent lymph is supported by the following exception. In the pig, the afferent lymphatic vessels open into the node towards the center of the organ, while the efferent lymphatic vessels leave it at its periphery. This situation is the reverse of that found in most mammals investigated. Correspondingly, in the pig, the nodules are located in the center of the node, in contact with the vessels carrying in the lymph (Chievitz 1881).

Besides determining the localization of the nodules, the subcapsular sinus further determines the topographical orientation of the components of the follicular nodule, i.e., the follicle, and the dark and light areas of the nodule. Indeed, in all nodules, these components were similarly oriented in respect to the overlying sinus, and orientation confirming that found by Rohlich ('28, '33) in the cat, and by Kindred ('38) in man, the dog and the rat. Since the particularity of the subcapsular sinus is that it is the site of entrance of antigens into the node, it is likely that this is the active phenomenon

that induces the localization of the follicular nodules and determines the orientation of their components. The nodules, therefore, probably carry out an immune function, a supposition that is supported by the fact that they are rare in germ-free animals (Glimstedt '36).

3. Constitution. The present study showed that, in rats more than six-weeks of age, nodules are not homogeneous structures but each one is formed of a dark and a light area, each area having a characteristic cell population. Furthermore, nearly every nodule was found to be associated with a follicle and these three components of the follicular nodule were shown to be localized, as regards one with the other, in a very definite topographical order. This supports the similar observations on the follicular nodule made by Rohlich ('33) for the cat, Taliaferro and Cannon ('36) for the monkey, Kindred ('38) for humans, the dog and the rat, more recently by Millikin ('66) for the humans, and by Sainte-Marie ('66a) for the rabbit and guinea-pig. The present finding that the nodule is vascularized by capillaries only, agrees with the view of Maximow ('27) and Rohlich ('33). However, our pattern for the vascularization of the nodule differs appreciably from that of Rohlich (fig. 5), as the arterioles in the present study were rarely found to penetrate into the nodules and they yielded a quite extensive network of capillaries as compared with that shown by Rohlich. Indeed, Rohlich's pattern (fig. 5) exhibits, in the dark area of the nodule, hardly any capillaries extending between the arterioles of the nodules and the postcapillary venules of the surrounding extrafollicular area, which is difficult to visualize. The present findings on blood

vascularization further indicated that a nodule and its follicle constitute a single structural unit, as they share a common capillary network transforming into a postcapillary venule network at the outer limit of this unit. Finally, we found that autofluorescent cells, variably outlining the nodular margin along the dark area of nodules, are also a component of the follicular nodule. Hence, the follicular nodule appeared as a single structural unit formed of five components related to each other in a definite topographical order: the follicle, the light and dark areas of the nodule, the outlining of autofluorescent cells and a common capillary network.

4. Development and evolution. Follicular nodules being absent in one-week-old nodes and in the process of formation in two-week-old nodes, it can be concluded that it is at some time between the second and sixth week of life that rat follicular nodules attain complete development as the complex structural units mentioned above. We did not investigated whether the light and dark areas of the nodule develop simultaneously or not during the latter period. Because of this structural complexity of the follicular nodule, and because the overwhelming majority of the follicular nodules examined, being more than six weeks of age, exhibited this complex constitution, it can be concluded that, once formed, follicular nodules are stable structures that, in most cases likely last as long as the animal itself. This fits the view of Ortega and Mellors ('57) that nodules are permanent structures. The present conclusion on the nearly permanent character of the follicular nodules does not imply that they do not undergo any variation. Indeed, we have reported great

variations in their size, in their population of large and medium lymphocytes, and in their content of mitoses as well as of pycnoses. All of which reveals the occurrence of large variations in the degree of a activity of the follicular nodules. Nevertheless, it remains that these physiological variations do not appear to alter the general architecture of the structures.

On the one hand, the presence, in some nodes from two-year-old rats, of follicular nodules similar to and apparently as abundant as those in nodes from two-month-old rats suggests that follicular nodules can be permanent structures. On the other hand, the presence of some follicular nodules appearing to undergo involution in nodes from rats of both ages suggests that follicular nodules are not necessarily permanent structures, some of them disappearing at various times during the life of the rat. The observations made here suggested, further, that such disappearance of follicular nodules can occur in two manners, the first of which was observed mostly in well-developed nodes not undergoing the process of adipose transformation, like the node studied. Here, the follicular nodules disappeared by becoming progressively smaller and smaller while usually maintaining their complex architecture, the surrounding extrafollicular area simultaneously becoming thinner and thinner. As this occurs in nodes with a proliferative population of medullary plasmocytes, the space thus emptied by the cortical structures is simultaneously filled by the medullary structures. In many nodes, this manner concerns the disappearance of follicular nodules from a restricted portion of their outermost layer elsewhere than around the hilus; in this portion, medulla was seen instead of the usual cortex.

The observations made in both the above portions of the outermost layer of nodes were taken to reveal the past and continuing disappearance of their cortical structures. Indeed, if the mere presence of medullary structures in a portion of the outermost layer of a node could mean that no cortex had ever existed there, it might also suggest the replacement of previous cortical by medullary structures, i.e. the disappearance of some follicular nodules. The latter explanation seems the more probable to us, because the presence of medullary components around the small follicular nodules that were nearest the aforementioned portions of the outermost layer of nodes can best be interpreted by the fading of the cortex around such portions and their replacement by medulla. Since follicular nodules disappear in this manner in restricted cortical portions of well-developed nodes, this phenomenon is not to be associated with the process of node involution, but probably arises from a progressive and permanent change in the nature of the lymph filtering through the subcapsular sinus, a change restricted to the portions of this sinus overlying the aforementioned portions of the outermost layer of the nodes. It is indeed conceivable that, for some undetermined reason, there could occur a progressive decrease in the amount of foreign or antigenic substances flowing through the latter portions of a subcapsular sinus. The greater would be this decrease in a site of the sinus, the thinner and smaller would be the underlying cortex and follicular nodules respectively. Hence, in many nodes, the closer they were to the hilus, the thinner and smaller became the extrafollicular area and the follicular nodules, respectively. This indicates that disappearance of the cortex had taken place, and was still in process, in a manner centrifugal from the hilus. This most probably results from a progressive decrease in the flow of afferent lymph in the overlying portion of the subcapsular sinus,

in a manner centripetal to the hilus. Indeed, the closer to the hilus, the shorter and less hypertrophied were the postcapillary venules; the degree of development of which depends on antigen drainage (Burwell '62, Sainte-Marie '66b). That the portion of a node cortex encircling the hilus is commonly an early site of cortical atrophy and disappearance is indicated also by the fact that it is frequently the first site in a node to undergo adipose transformation.

The second mode of disappearance was observed mostly in poorly developed nodes, whether they were undergoing adipose transformation or not. Here, the follicular nodules disappeared by progressively losing the characteristic cells of the dark and light area of their nodules while becoming populated mainly by small lymphocytes. Hence, follicular nodules faded out progressively, getting harder and harder to detect in the sections stained by the technique of Dominici. Since resting nodules were present in poorly developed and adipose transformation nodes, the disappearance of follicular nodules in this manner is likely to be associated with the process of node involution, which most probably arises from a progressive decrease in the amount of foreign or antigenic substances draining into the over-all or greater subcapsular sinus of a node. Indeed, a large decrease would be expected to result in the over-all atrophy of a node and in the simultaneous involution of its various structures. If atrophy persisted, the involuting or involuted structures of a node, cortical and medullary, would eventually be replaced by adipose tissue. The involuting cortical structures would not be replaced by medullary structures, because these structures would also be involuting, unlike in the first manner of disappearance of follicular

nodules, where there probably existed a tendency for expansion on the part of the medullary cords in nodes in which the plasmocytes of the well-developed medullary cords were proliferative. As the fading out of the characteristic cell population of some nodules likely results from a lack of antigenic stimulation, it would seem that such nodules could probably be reactivated if antigens were to reach themaagain. If, on the contrary, a node or a node area containing resting follicular nodules is not longer challenged by antigens, it is likely that the latter resting follicular nodules would then eventually disappear completely to be replaced by adipose tissue. Adipose transformation is the ultimate fate of the unstimulated node or area of a node. This is demonstrated by the fact that, in adult and old rats, the cervical nodes draining body sites of frequent antigenic challenges are well developed, whereasppopliteal nodes draining body sites of infrequent antigenic challenges are poorly developed and undergo an early adipose transformation.

It can be summarized from the above discussion that: first, the majority of follicular nodules are most probably permanent structures; second, in some active or well-developed nodes, a few follicular nodules located in a restricted cortical area disappear by becoming progressively smaller; third, in inactive or poorly-developed nodes, most follicular nodules enter a resting state by losing the characteristic cells of the light and dark area of their nodules; fourth, resting follicular nodules can likely become active again by redeveloping these cells; fifth, resting follicular nodules will eventually disappear by adipose transformation if they are not reactivated.

5. Current concepts. In the literature, we found opinions concerning the life history of nodules but not the life history of the follicular nodules as whole structural units. Discussion of the current concepts on this problem will, therefore, be limited to the life history of the nodules. Since a nodule and its follicle form a single unit, we feel that conclusions on the nodule are also likely to fit the follicle or the follicular nodule as a whole.

Presently, the nodules are commonly considered as temporary structures, which, after their formation, can fade out to disappear or to develop again at the same site (Maximow '32, Bloom and Fawcett '62), their number in a node showing marked fluctuations (Ringertz and Adamson '50, Bloom and Fawcett '62, Cottier, Odartchenko, Keiser, Hess and Stoner '64). It has been thought by some authors that new nodules can develop in adult nodes (Maximow '32, Conway '37, Ringertz and Adamson '50) and that as nodules form and fade out, they undergo a cycle of cellular changes involving a few phases (Flemming 1885, Downey and Weidenreich '12, Maximow '32, Conway '37), one cycle repeatedly following another (Maximow '32). During a cycle, a nodule would pass successively through active and resting phases, the resting nodule either disappearing or re-entering the active phase (Maximow '32). Maximow ('32) wrote that, as a rule, all nodules of a node, and perhaps of the whole body, would be in the same phase of such a cycle at a given time; but he made no mention of the duration of the cycle, and this time factor was rarely specified by the other authors cited above. However, the impression gained from reading the literature is that at least some phases of the cycle can occur quite rapidly. In fact, Conway ('37,38) reported that,

three hours after a first injection of bacteria, the mesenteric nodes of rabbits and guinea-pigs exhibited an increase in the number as well as in the size of their nodules. Cottier etaal ('64) reported that, starting from the second day, a secondary antigenic stimulation significantly increased the number of nodules in the challenged mouse nodes, but whether this represented the formation of new nodules or the activation of resting ones was not determined.

That the new nodules can develop rapidly in adult nodes and then undergo the cycle of cellular changes proposed by Maximow ('32) is taken to have been demonstrated by the observations made by Conway ('37, '38) in rabbit and guinea-pig nodes soon after an injection of bacteria. The latter author showed four microphotographs (reproduced in Yoffey and Courtice '56, Bloom and Fawcett '62), which she thought were demonstrating the formation of new nodules in the latter nodes and their evolution through four phases of activity. Having determined that the constitution of the rabbit nodule (figs. 122-122C) as well as the guinea-pig nodule resembled that of the rat (Sainte-Marie '66a), examination of these microphotographs revealed to us that, instead of representing four phase of evolution of nodules, they could be representing four cross sections of identical mantled or capped nodules sectioned at four distinct levels. The first of these four microphotographs (fig. 123) could represent a cross section passing through the innermost portion of the dark area of a nodule, since it presents a small dark nodule (dark center, in current terminology) not surrounded by a follicle, as is to be expected in such incidence of sectioning of a nodule. The

second microphotograph (fig. 123A) could represent a cross section passing at a higher level through the dark area of a nodule, since it shows a larger dark nodule with a very thin layer of follicular small lymphocytes around it, as is also to be expected in such cases. In both microphotographs, the nodules were populated by proliferative, large amd medium lymphocytes. This was interpreted as demonstrating the growth of a small into a largerddark nodule, and that nodules are active or proliferative during the growth phase of the cycle. The third microphotograph (fig. 123B) could represent a cross section passing at the level of the irregular junction between the dark and light area of a nodule, since it depicts a large greyish nodule containing a mixture of the cytological elements of both areas and surrounded by a thick corona of follicular lymphocytes. The fourth microphotograph (fig. 123C) could represent a cross section passing through the light area of a nodule, since it shows a large light nodule (light center, in current terminology) populated by reticular cells and small lymphocytes. The two latter microphotographs were interpreted as indicating the progressive passage of a nodule from the active or proliferative phase into the resting phase. As is to be expected on the basis of the present findings obtained on the appearance of cross-sectioned nodules, the shape of the four nodules shown by Conway was quite round. Moreover, the nodular margin was sharp in the first three microphotographs but not in the fourth one, which is also to be expected from cross sections passing through the dark and light areas of nodules, respectively. Hence, these four microphotographs fit perfectly with the images from cross-sectioned follicular

nodules. Since Conway did not verify the actual structure of the observed nodules by a three-dimensional analysis and since guinea-pig and rabbit nodules resemble the rat nodules, it seems to us most probable that the microphotographs in question actually represent four different cross sections of similar capped or mantled nodules.

As discussed above, the present observations support the view of the existence of variations in the degree of activity of the follicular nodules. They also support the view that a follicular nodule can enter a resting state, to eventually disappear or be reactivated. However, our findings can hardly fit with the view of Maximow ('32) that nodules undergo repeated cycles of cellular changes. We previously showed that, except for the resting ones, nodules in rats more than six weeks of age are not homogeneous structures, contrary to the proposal of Maximow and Conway. In all nodes, nodules were found to be populated simultaneously: in their dark area by the cellular elements of the first and second phases of Conway, and in their light area by the cellular elements of the fourth phase of Conway. Hence, in this study, we did not find any support for the view of Maximow that, at a given time, all the nodules of a node would be in a certain phase of his proposed cycle, thus having variable but homogeneous cell populations.

Can new follicular nodules be formed in nodes of rats more than six weeks of age, six weeks being roughly the postnatal period during which nodes and their follicular nodules develop? In nodes older than six weeks, the cortex is usually separated into follicular nodules and extrafollicular area, both cortical components having the characteristic features reported here. Hence, the formation of new nodules in these nodes would be expected to require considerable reorganization

in the local patterns of reticular fibers and blood vascularization. This, it seems to us, renders the rapid formation of new follicular nodules, as suggested by Conway ('38) to occur within three hours, quite inconceivable. It would seem more probable that, if an increase in the number of nodules is seen to occur within the first few days after an antigenic stimulus, it is the result of the reactivation of resting nodules rather than the formation of new ones. We found that, while resting follicular nodules could be detected quite readily in silver-impregnated sections, their existence was often hardly detectable in sections stained by the technique of Dominici. The reactivation of resting nodules would simply require the proliferation of large lymphocytes in the inner zone of the nodule, which could likely occur rapidly. This would make the resting nodules readily detectable, once again, as nodules, and give the impression of an increase in the number of nodules. If as is highly probable, follicular nodules cannot develop rapidly, it remains conceivable that new ones can form slowly under specific conditions. Indeed, it is obvious from previous and the present findings that there exists an intimate relationship between follicular nodules and the subcapsular sinus draining the afferent lymph. If new follicular nodules do develop they would be expected to develop in this context. In the node studied and in most other nodes, we found that follicular nodules are more or less regularly distributed along the peripheral cortex, and that there is little cortical space in between them. In fact, in most cases, there seemed to be hardly any space available in between the follicular nodules to accomodate new ones. New

follicular nodules could thus form, only if extracortical space were provided just beneath the subcapsular sinus. Antigens, which are known to induce hypertrophy of the node, simultaneously induce hypertrophy of the follicular nodules and an increase in the small lymphocyte population of the cortical extrafollicular area (Conway '38, Ringertz and Adamson '50). In this case, therefore, it seems that there is not much more free cortical space available for formation of new nodules, unless the increase in volume of a node is sufficiently large and lasts for a while. The time factor is also essential, because of the histological complexity of the new structures to be formed. For all these reasons, it seems to us doubtful that new follicular nodules develop in this manner in already large and well-developed nodes. Indeed, while a medium sized node can double its size after antigenic stimulation, nodes do not increase in volume over a certain point, at which they reach a maximum size. When a node is well-developed and large in size, it would, therefore, seem that the possibilities of its developing new follicular nodules in the above manner are limited. Such formation of new follicular nodules would be more conceivable in small and poorly-developed nodes. Even in these nodes, much of the additional space provided by their hypertrophy after antigenic stimulus could be expected to be filled by the enlargement of the cortical extrafollicular area and of its postcapillary venule network (Sainte-Marie '66b) of the follicular nodules themselves, and of the reactivated resting follicular nodules. Hence, even in these nodes, the formation of new nodules if it did occur, would likely be limited in a manner inversely proportional to the size of a node before hypertrophy. However, there exists another

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possibility for the development of new follicular nodules, which would consist in their development along trabeculae extending from the capsule and penetrating progressively deeper and deeper into a node. It was found in the present study that the subcapsular sinusnextends into the nodes along the trabeculae. As contact with a subcapsular sinus seems necessary for the development of follicular nodules, the conceivable extension of trabeculae could furnish the adequate environmental condition for the appearance of new follicular nodules. In the node studied, the follicular nodules associated with the circular septal trabecula outlining the lobule are larger, on the average, than those along the convex surface of the cortex. It was thought that this might result from a greater antigenic challenge in the area of the lobule than elsewhere in the node. Another possible explanation is that they are younger follicularnnodules, having evolved later in life than the others, their development accompanying the possible deeper and deeper penetration of the trabecula into the node. This cannot be confirmed or denied, because we cannot state whether the septal trabecula outlining the lobule already existed in the node at the time when follicular nodules developed after birth, since such trabeculae were found to be already present in one-week-old nodes.

F. Postcapillary venules. The postcapillary venules are the most characteristic element of the cortical extrafollicular area. In agreement with the observations made by Smith and Hénon ('59) and by Burwell ('62), we found no such venules in the nodules. We further demonstrated that they are not present in their associated follicles,

so that they are restricted to the cortical extrafollicular area. Clearly, there exists a close relationship between the three phenomena: P presence of small lymphocytes in the cortical extrafollicular area, diapedesis of small lymphocytes through the venules in this area, and hypertrophy of the endothelial cells in the latter venules. The nature of this relationship was proposed elsewhere (Sainte-Marie '66b, Sainte-Marie, Sin and Chan '67) as follows: diapedesis of small lymphocytes inducing the hypertrophy of the endothelial cells and small lymphocytes entering the venules can both occur only in the cortical extrafollicular area, where the small lymphocytes of the node are concentrated. This explanation can account for the fact that, in venules parallel to the cortico-medullary junction of nodes, the features of the postcapillary venules were observed solely on their side contacting the cortex (figs. 99,100).

We concluded in a previous study (Sainte-Marie et al. '67) that the small lymphocytes diapedesing through the postcapillary venules were entering the blood circulation . While it is conceivable that some of these small lymphocytes, i.e., those leaving the nodes, could be cells newly formed in nodes, the others could represent part of the lymphocytes coming to the nodes via the afferent lymph, which most often contains some of these cells (Yoffey and Drinker '39). The amount of small lymphocytes present in the subcapsular sinus of nodes, and probably coming from the afferent lymph, is variable. We pointed out previously that the great concentration of fibers across the zone of the subcapsular sinus located over the cortical extrafollicular area likely traps the afferent lymphocytes and thus favors their penetration into this area of the

cortex. Examination of serial sections of a node in which lymphocytes are abundant in this sinus, also showed an abundance of lymphocytes in its afferent lymphatic vessels (fig. 114). It appears that some of the small lymphocytes entering nodes via the afferent lymph eventually penetrate the postcapillary venules, while others leave by the efferent lymph, the intensity of both phenomena being subject to variations at any given time, dependingoon the areas of a node and on the node itself. The variations could account for the variable degree of hypertrophy shown by the endothelial cells of postcapillary venules in different areas of a node or from node to node, and for the variable concentration of small lymphocytes in the different sinuses of nodes.

G. Medulla and cortico-medullary junction. As we reported in the review of the literature, the medullary cords are said to consist of the same cytological constituents as the cortex, but to be devoid of nodules. Even if, topographically, the medullary cords can be considered as extensions of the cortical extrafollicular area running in between the medullary sinuses, morphologically they cannot be considered as such. The medullary cords differ from the cortical extrafollicular area in having a different type of venules, a different arrangement of reticular fibers and, usually, a different cell population. The medullary cords must thus be considered as a definite structure of the node to the same extent as are the cortical extrafollicular area and the follicular nodules. Of course, when poorly develpped, i.e., when containing a relatively small plasmocytic population, the medullary cords tend to look like the extrafollicular area. This does not prove the identity of the latter three node structures, but simply indicates that, when

unstimulated, the characteristic components of these three structures fade out progressively, the unstimulated node tending to return to the homogeneity it displayed during the first and second week of life.

The margin passing along the points where the morphological changes occur between the cortical extrafollicular area and the medullary cords constitutes the cortico-medullary junction. It is also at this junction that the narrow cortical interfollicular sinuses transform into the wide medullary sinuses. The junction is not readily detectable, partly because the morphological changes do not occur abruptly along the junction, for instance the cortical small lymphocytes and the medullary plasmocytes merge slightly along the junction, and partly because of its very irregular course. This irregularity suggests that the limit between the cortex and the medulla is not permanent but changeable. Hence, depending on local conditions, a cortical area of a node could become progressively replaced by medulla, or vice versa, replacement occurring along the changing junction and the cortico-medullary junction disappearing wherever the medulla comes to replace a portion of cortex.

H. Pseudo-follicle. The present description of the structure termed here pseudo-follicle fits in with that reported earlier by Ehrich ('29) and designated by him "pseudo-secondary nodule"; the existence of this structure is only rarely mentioned in the literature, (Yoffey and Courtice '56, Marshall '56). Ehrich ('29) named the structure thus, because its roundish shape and clear-cut outline reminded him of the nodule. He suggested, therefore, that like the nodule, the structure is lymphocytopoietic, and that it actually derives from a nodule. However, Ehrich ('29) added: "transitional stages from secondary nodules to

pseudo-secondary nodules are hard to find".

Clearly, the pseudo-follicle is composed of the same elements as the cortical extrafollicular area, except for a lesser concentration of reticular fibers, having nothing in common with the composition of the nodule. The views of Ehrich on the nodular origin and lymphocytopoietic activity of this structure, therefore, seems improbable. It appears to us that this structure can be explained simply as representing a local accumulation, after penetration into the cortical extrafollicular area, of small lymphocytes likely coming from the afferent lymph. The presence of abundant small lymphocytes in the afferent lymphatic vessel, and in the portion of subcapsular sinus related to the area of a node with a pseudo-follicle, indicated the arrival of abundant peripheral small lymphocytes in such areas of the node. Furthermore, the observation that the postcapillary venules inside the pseudo-follicles are mostly closed suggests that, because of the action of some undetermined local factor, these small lymphocytes penetrate very little into the blood circulation via the postcapillary venules but, instead, accumulate around them, thus forming a pseudo-follicle. The accumulation of small lymphocytes, in a half-circular manner, in the area of the deep cortex below and around the site of arrival of an afferent lymphatic vessel thus seems to provide the most fitting explanation for the observations made here on the pseudo-follicles.

Since the cortical extrafollicular area is normally already populated by small lymphocytes, it is likely that an accumulation of small lymphocytes leading to the formation of a pseudo-follicle can

occur only in the deep cortex and by invasion of the underlying medulla. An accumulation of small lymphocytes around venules of previous medullary cords would be expected to disperse and push away the reticular fibers, which would then become concentrated at the periphery of the pseudo-follicle, thus clearly outlining it as is the case. Such local accumulation of small lymphocytes and dispersion of reticular fibers would permit impregnation of these cells by the silver-salt, as with lymphocytes of the follicles. Hence, the larger the concentration of cells, the more dispersed the fibers and, as is to be expected, the darker the impregnation of the cells of the pseudo-follicles, which is the phenomenon observed here. If, as we think most likely, a pseudo-follicle develops partly by invasion of an underlining portion of the medulla, this would constitute a process opposite to that of the occasional replacement of a portion of cortex by medulla.

I. Age and lymph nodes.

1. Nodes less than six weeks of age. Except when a pathological process occurs in the fetus (Silverstein and Lukes '62), the parenchymal structures of nodes develop after birth. The present observations suggest that, starting from birth, the various structures of challenged node develop progressively to reach full development at about the age of six weeks. Most probably, nodes need to be antigenically challenged to develop their structures, as is shown by the near absence of nodules in germ-free animals. This must account for the present observation that some two-week-old nodes are more advanced in their development than others, likely as result of variations in the time and intensity of exposure to antigens.

Although this present work does not provide complete observations pertaining to all stages of the development of the node, the present observations do permit us to visualize this development as follows: in addition to its stroma, a node contains a network of sinuses at birth. The spaces between the sinuses and the stromal elements are filled with reticular cells. During the first week, the latter spaces are infiltrated by small lymphocytes, and small lymphocytes being present grossly throughout the node, diapedesis of these cells and hypertrophy of endothelial cells are seen at any portions of node venules. During the second or third week, the node becomes separated into a cortex and a medulla, with the development of a plasmocytic population in the portion of the latter spaces located around the hilus and the simultaneous development of nodules in the outermost layer of the cortex. Also simultaneously, the phenomena of diapedesis of small lymphocytes and of hypertrophy of the endothelial cells became restricted to the portion of venules present outside the developing medullary cords and follicular nodules, i.e., in the cortical extrafollicular area. This is because at least most small lymphocytes of the node do not originate locally (Miller '64) likely coming to the node via the afferent lymph (Sainte-Marie et al '67); these small lymphocytes no longer penetrate into the node sites now occupied by the developing nodules and the plasmocytic cords, so that their localization becomes restricted to the cortical extrafollicular area after they penetrate into a node via the afferent lymph. The small lymphocytes thus being restricted to the cortical extrafollicular area

diapedesis of small lymphocytes and, consequently, hypertrophied endothelial cells (Sainte-Marie et al '67) become concentrated along the portions of node venules that are present in the extrafollicular area and have become the postcapillary venules. The progressive development of these heavy venules probably provokes the formation of new supporting reticular fibers, which, hence, become more concentrated in the extrafollicular area of the cortex than in the developing follicular nodules. As the latter structures contain only capillaries, it is conceivable that they do not need numerous supporting reticular fibers. Reticular fibers simultaneously increase in number in the medullary cords likely to support the node arterioles and venules that go through them and not through the medullary sinuses. In this manner, the various structures of the node would be constituted. While developing follicular nodules are seen clearly in two-week-old nodes, only in about six-week-old nodes are well-developed follicular nodules detected. This indicates that node structures develop progressively, to commonly reach nearly full development during the sixth week after birth.

We reported the presence, in some nodes from two-weeks-old rats, of what appeared to be a definite pseudo-follicle. In some nodes, from one-week-old rats, there are accumulations of small lymphocytes resembling pseudo-follicles. It was not possible to determine here whether the latter accumulation represented early pseudo-follicles or a modality of the node development.

2. Nodes more than six week of age. The present study demonstrated that, in itself, ageing does not cause the atrophy and adipose trans-

formation of the node structures. This is indeed demonstrated by the fact that a two-year-old node can be similar to a two-month-old node. What thus seems to occur is that ageing is accompanied by local changes in some areas of the body, changes that yield to a more or less progressive decrease in the amount of antigens flowing into the draining nodes. Such a decrease would result, in turn, in the progressive atrophy of the draining nodes and, eventually, in their disappearance by adipose transformation. It can be concluded then, that the histological features of a node are determined, not by general bodily conditions, but by the local antigenic conditions in the region of the body drained by a node or by an area of a node.

GENERAL CONCLUSION

The present study confirmed the view of Rohlich ('30) and Kindred ('55) on the necessity of carrying out a three-dimensional analysis for determining the actual composition of some node structures, particularly the follicular nodules. It also demonstrated, for the first time, the necessity of carrying out a similar analysis in order to determine the actual over-all architecture of a node.

With respect to the histological technique, the present study demonstrated that a modified technique of silver impregnation enables restrictive staining of the cells of the follicular nodules. In particular, with the use of the modified technique we were able to distinguish, for the first time, the population of small lymphocytes of a follicle from that of the surrounding cortical extrafollicular area, which was, hitherto, not possible.

The observations in the present work showed that the node is formed of seven basic structures; a framework of reticular fibers, a network of sinuses, follicular nodules, a cortical extrafollicular area, pseudo-follicles, medullary cords, and a network of blood vessels.

With respect to the framework, the present study revealed that support of the structures of the node is provided by a framework of reticular fibers, not by abundant connective tissue trabeculae as is currently held to be the case. We proposed a pattern for this framework of reticular fibers, which is quite different from that presently illustrated in node diagrams. In this pattern, follicular nodules are held in baskets of fibers, in confirmation of occasional previous

report on this problem (Rössle and Yoshida '09, Denz '47).

As regards the sinuses, the present study confirmed the view of Denz ('47) on the existence of narrow interfollicular sinuses joining the subcapsular sinus and the medullary sinuses. The current concept of numerous subtrabecular sinuses joining both these types of sinuses was shown to be incorrect. Furthermore, we pointed out, for the first time, that the subcapsular sinus is divided into two zones on the basis of the concentration of reticular fibers crossing it.

With regard to the follicular nodules, the present study gave rise to the proposal of a pattern of their architecture, which differs considerably from the currently accepted pattern but which is in agreement with the observations of a few previous investigators (Rohlich '28, Taliaferro and Cannon '36, Kindred '38). Each follicular nodule was shown to be formed of five constant components: the follicle, the light and the dark areas of nodule, the autofluorescent cells, and a common capillary network, all of which were oriented in a definite order with respect to the overlying subcapsular sinus. We showed here, for the first time, that an outlining of autofluorescent cells is part of the follicular nodule. We further determined, in a more precise manner, the pattern of the capillary network of the follicular nodule. We also indicated, conclusively, for the first time, that a follicle and its nodule form a single structural unit. The present observations suggested that most follicular nodules are permanent structures that can undergo variations in the intensity of their activities and, also, that some follicular nodules can disappear in two possible ways, whereas others can come to rest, perhaps to be reactivated later on. Our proposal on

the life history of the follicular nodules introduces new views on this problem.

With respect to the cortical extrafollicular area, the present study emphasized the fact that this area constitutes a definite node structure with special morphological features, thus being distinct from both the follicular nodules and the medullary cords. To our knowledge, this concept has not been explicitly stated elsewhere; nor is it illustrated in current diagrams of the node. Our results further confirmed the statement of a few authors (Heudorfer '21, Denz '47) that the extrafollicular area, or the cortex, form a continuous layer although the presently held concept on this problem postulates the reverse situation.

As regards the pseudo-follicles, our present study confirmed the original report of Ehrich ('29) on the existence of such structures. Our observations, however, gave rise to quite a different conclusion from that of Ehrich concerning the nature of this structure, in as much as they indicated that, morphologically, pseudo-follicles are similar to the cortical extrafollicular area. Pseudo-follic-les likely represent restricted portions of the extrafollicular area in which the physiological conditions differ. The current concepts on the architecture of the node fail to mention the existence of this structure.

With respect to the medullary cords, the present study emphasized the fact that they constitute a definite structure distinguishable from the cortical extrafollicular area. This is partly a new concept, since medullary cords are commonly considered simply as being topographical extensions of the extrafollicular area.

With regard to blood vascularization, the present study

emphasized the fact that each of the three main regions of the node have a special vascular pattern: the follicular nodule, the cortical extra-follicular area, and the medullary cords. The present study also yielded more details as to the pattern of the capillary network of the follicular nodules. Many of the present observations on blood vessels simply confirm those of a few previous investigators (Rohlich '33, Smith and Hénon '59), but take on a new light if one considers the current concepts on this problem.

Hence, while some of the observations reported in this study represented original contributions to the architecture of the node, many constituted newly rediscovered facts that had been reported by a few previous investigators but remained buried in the literature; so that if one considers them in the light of the present context of the architecture of the node, most of the present observations appear to be new contributions. The originality of the present thesis lies in the simultaneous study of the various structures of the node to determine the topographical relationships existing between them. This permitted us to elaborate a diagram of the over-all structure of the node, which seems to us to be considerably more realistic than the presently available diagrams of this organ. While our diagram was constructed for the node of the rat, reports in the literature and a cursory examination of sections of nodes of other species suggested to us that, on the whole, it also fits the human, mouse, rabbit, and guinea-pig node. It is likely, however, that a detailed study of the node of these other species would reveal minor differences in the architecture.

Finally, the present study demonstrated that age per se is not a factor of node involution.

EXPLANATIONS OF FIGURES

Note.

Figures 1 to 6, and 123 to 123C are reproductions from various authors, the other figures are from us.

Except when otherwise mentioned, figures are from sections either stained by the technique of Dominici or impregnated with silver salt, which is referredtto in the explanations of figures simply as Dominici technique or silver-impregnation respectively.

Except when otherwise mentioned, figures are from the rat node here studied three-dimensionally.

In all explanations of figures, the terms used to refer to the various structures of the node are those defined in our proposed terminology (see Glossary).

In all figures showing a longitudinal section of a cortical structure, the figures are oriented such that the capsule or the subcapsular sinus are on the top of the figures.

In all cases, statements in the explanations of figures have been checked three-dimensionally by examination of adjacent sections whenever necessary.

Our diagrams are either "realistic" or "schematic". Realistic diagrams represent three-dimensional reconstructions of actual structures. Schematic diagrams represent schemes on the architecture of node structures. In the various diagrams, same structures are similarly represented.

LIST OF ABBREVIATIONS FOR FIGURES

A : Arteriole

AL : Afferent lymphatic vessel

C : Capsule

Cy : Capillary

DA : Dark area of the nodule

EA : Cortical extrafollicular area

EL : Efferent lymphatic vessel

F : Follicle

H : Hilus

L : Lobule

LA : Light area of the nodule

LF : Lymph flow

MR : Macrophagic reticular cell

MC : Medullary cord

MS : Medullary sinus

MV : Medullary venule

N : Nodule

PCV : Postcapillary venule

PF : Pseudo-follicle

S : Sinus

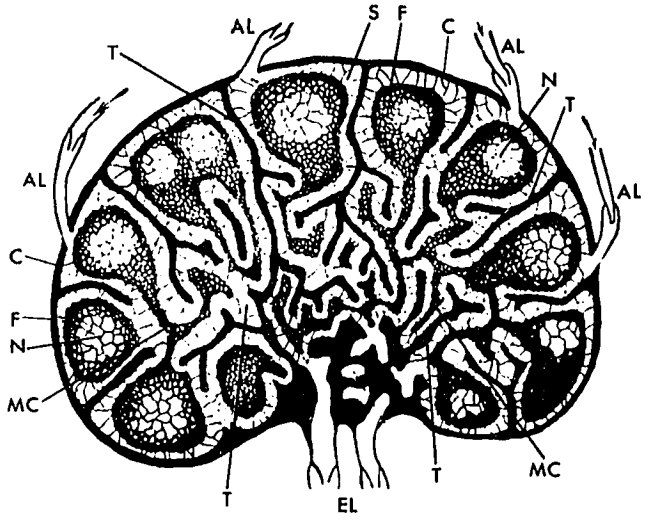
SS : Subcapsular sinus

T : Trabecula

Fig. 1. Diagram illustrating the architecture of the node, from the textbook of Bloom and Fawcett (1962).

Fig. 2. Diagram illustrating the framework of the node, from the textbook of Ham (1965). Capsule and trabeculae are formed of collagenous fibers, whereas the remainder of the framework is constituted of reticular fibers. The latter fibers are shown to be arranged either in a coarse mesh (sinuses and nodules) or in a fine mesh (follicles and medullary cords).

Fig. 3. Diagram illustrating the architecture of the node of the dog, from the book of Yoffey and Courtice (1956).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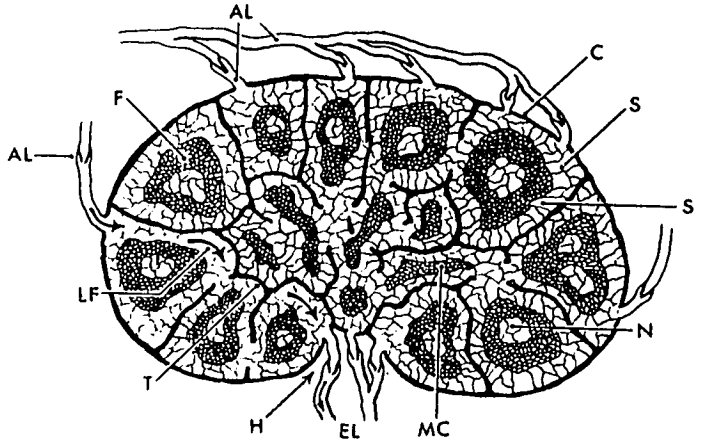
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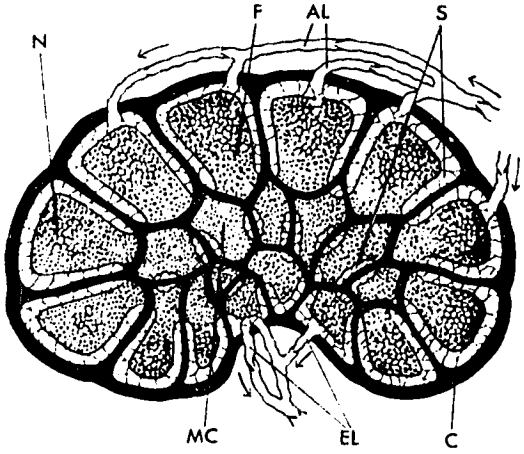
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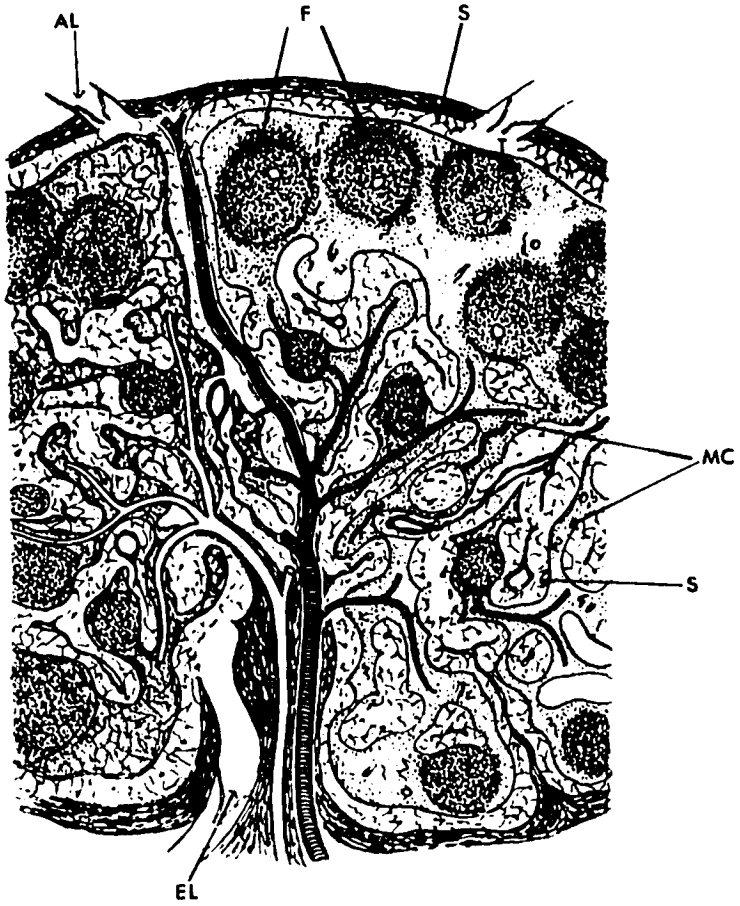
Fig. 4. Diagram of Heudorfer (1921), illustrating the blood vascularization of the node and still used today (Marshall, 1956). The arterial vessels aresshown as black with white stripes, the venous vessel as white.

Fig. 5. Diagram of Rohlich (1933), showing the blood vascular pattern of a nodule in the lymph node of the cat. The arteriole shown here is going through the dark area of the nodule.

Fig. 6. Reconstruction by Smith and Hénon (1959) of postcapillary venules below a non-outlined nodule (N) in a node of a mouse.



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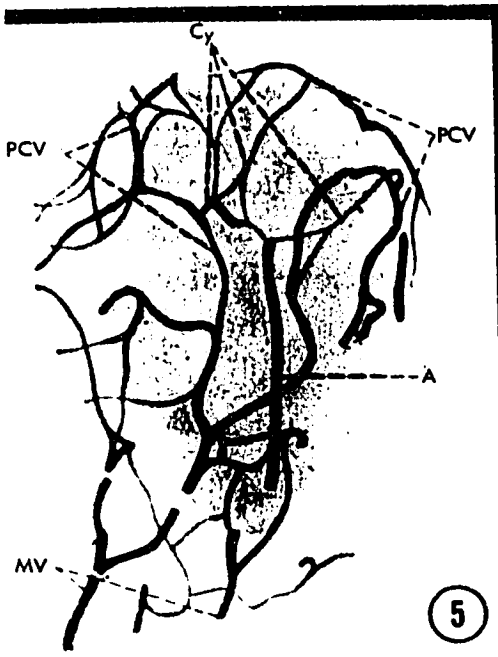

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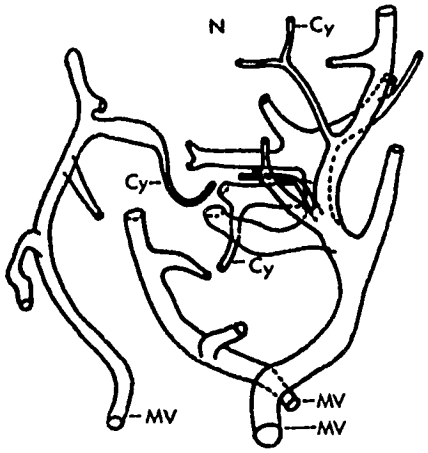

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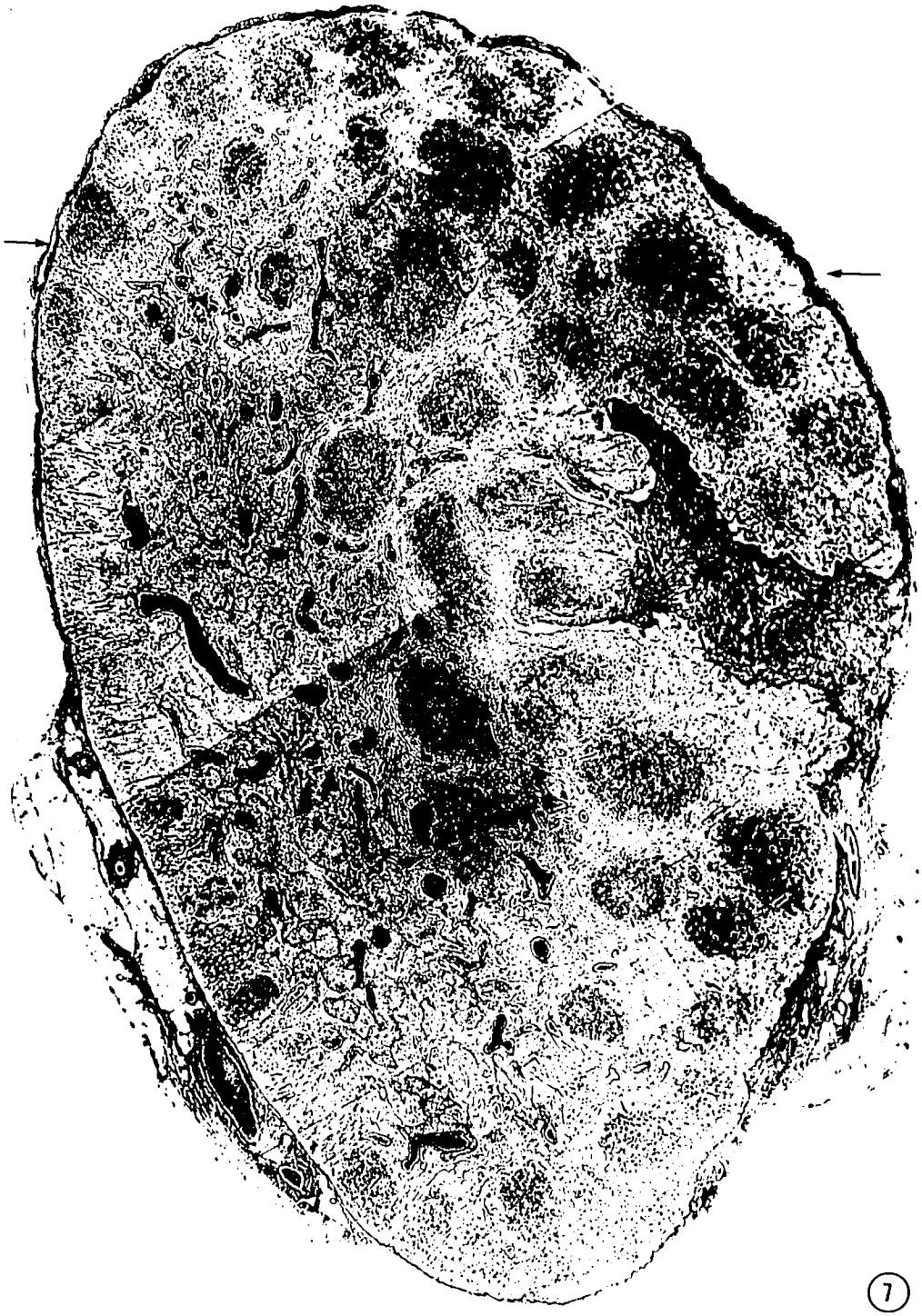
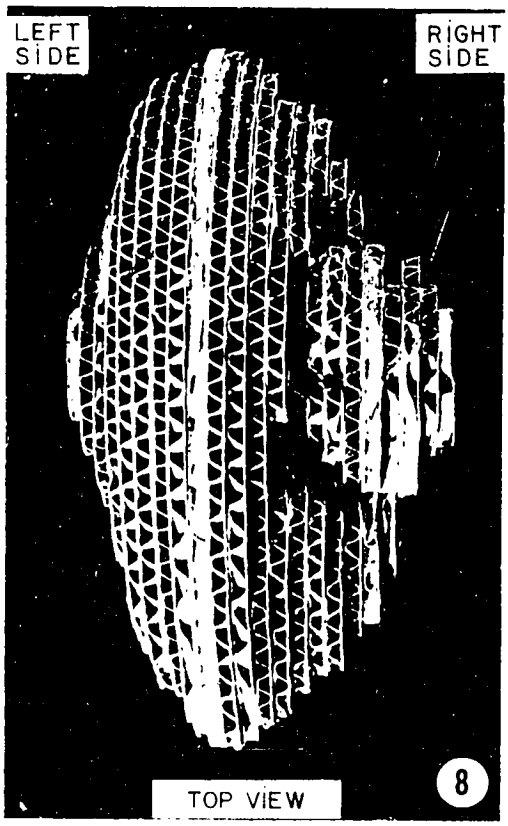
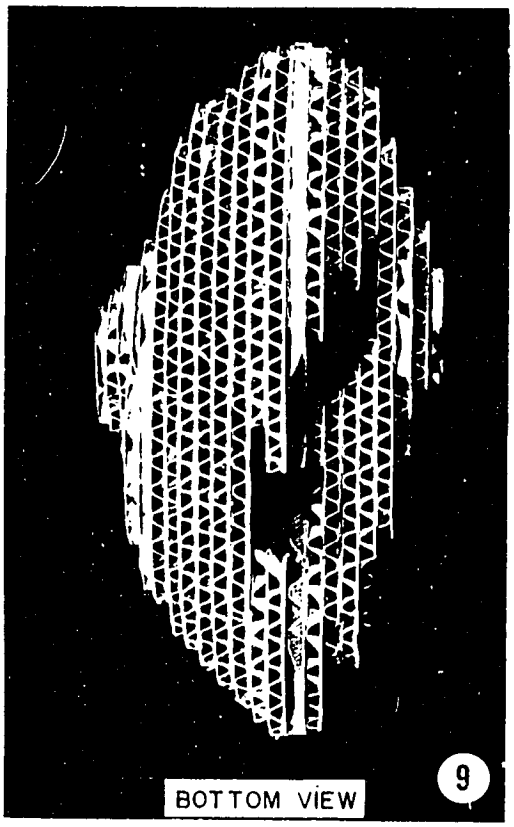
Fig. 7. Silver impregnation. Photomontage of a section. Below the arrows lies the portion of the serial sections reconstructed three-dimensionally in this study. The photomontage, whose actual measurement is 101 cm. in length, is a reconstitution of a longitudinal section cut through the trabecula outlining the lobule of the node shown in Figure 8. The dark, rounded areas (N) correspond to nodules. Note that beneath most of the left portion of the capsule, the reticular fibers hanging from the capsule are sectioned in a parallel manner so as to appear long and straight, whereas they are cross-sectioned in the remainder of the tissue.



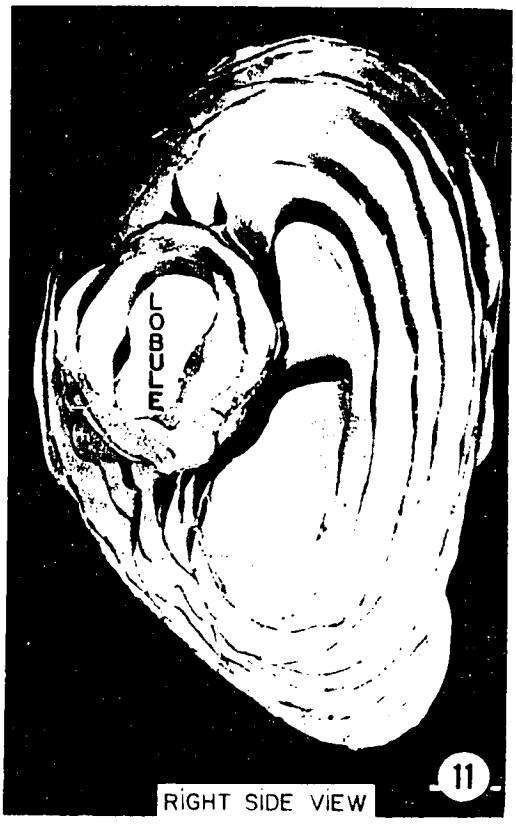
(7)

Figs. 8-11. The four longitudinal views of a volumetric cardboard model of the node studied. In Figure 8, the rounded structure at right is a lobule of the node shown laterally in Figure 11. In Figures 9 and 10, the shadowed area corresponds to the hilus.

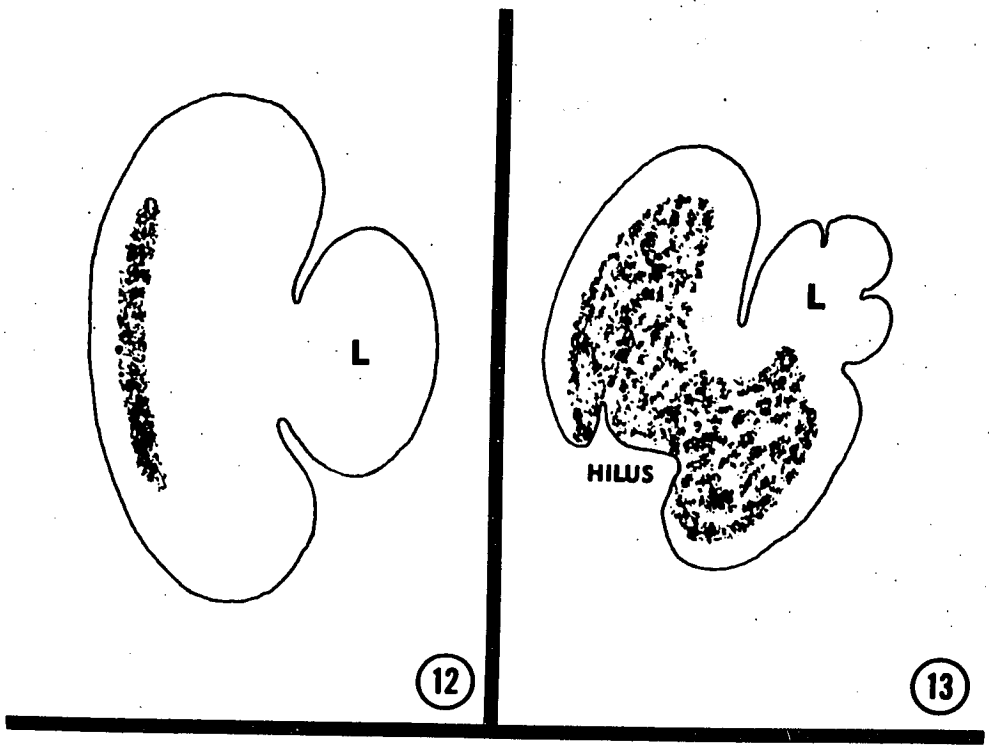

LEFT SIDE
RIGHT SIDE
TOP VIEW
8


BOTTOM VIEW
9


LEFT SIDE VIEW
10


LOBULE
RIGHT SIDE VIEW
11

Figs. 12,13. Realistic diagrams of a longitudinal and a cross-section, respectively, passing through the lobule of the node (L) seen in Figures 8 and 11. The solid lines outline the inner limit of the capsule and the circular septal trabecula outlining the lobule; the patchy dark areas represent the medulla; and the white areas represent the cortex. In both sections, the lobule is seen to be continuous with the bulk of the organ.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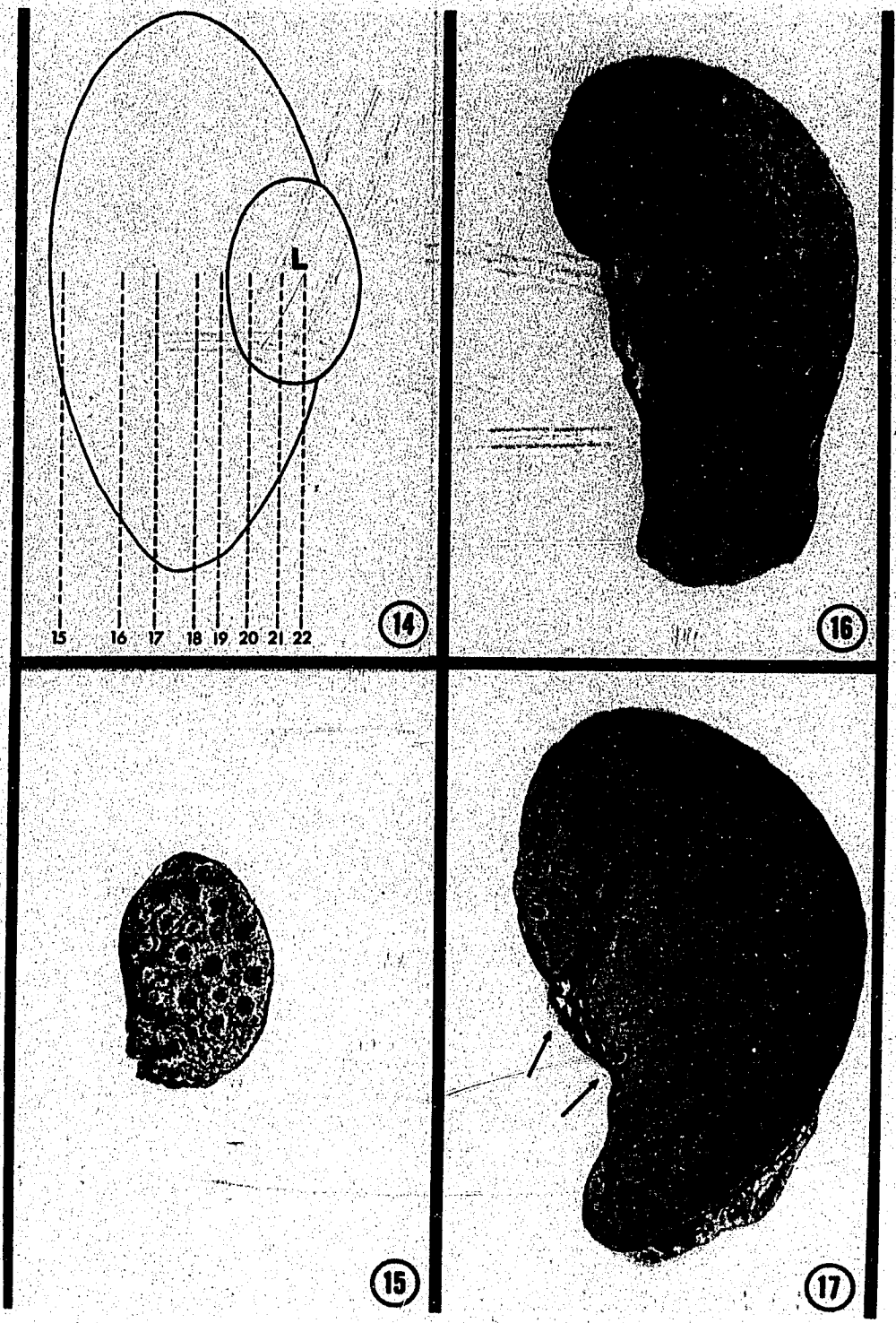
HILUS
13

Fig. 14. Outline of node and of its lobule (L) as viewed in Figure 8. The eight broken lines indicate the level of cutting of the eight longitudinal sections of the node shown in Figures 15-22, the numbers referring to the corresponding figure. A section passing through the septal trabecula of the lobule, intermediate between those in Figures 19 and 20, is seen in Figure 7.

Figs. 15-22. Silver impregnation. Longitudinal sections of the node as indicated in Figure 14. The dark rounded structures are nodules. The cortex appears as a variably thick layer containing these dark nodules, whereas the medulla is the remaining tissue devoid of nodules. In Figure 17, the arrows point to both branches of the bifurcating hilus. In Figures 20-22, L represents the center of the lobule. In Figures 18-20, note that the nodules adjacent to the septal trabecula outlining the lobule are larger and more darkly impregnated than those close to the hilus. Magnification about 15X.


L
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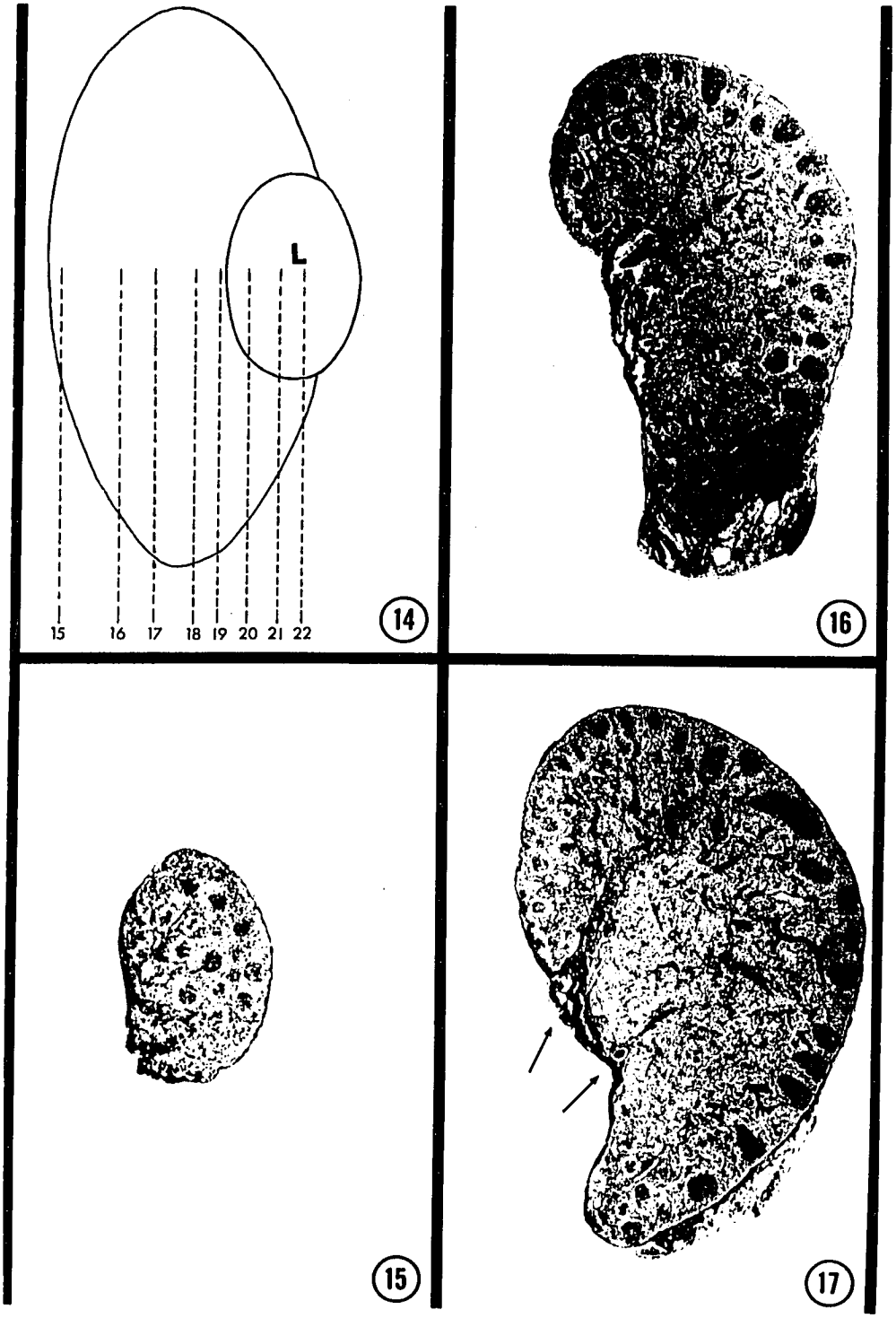
L
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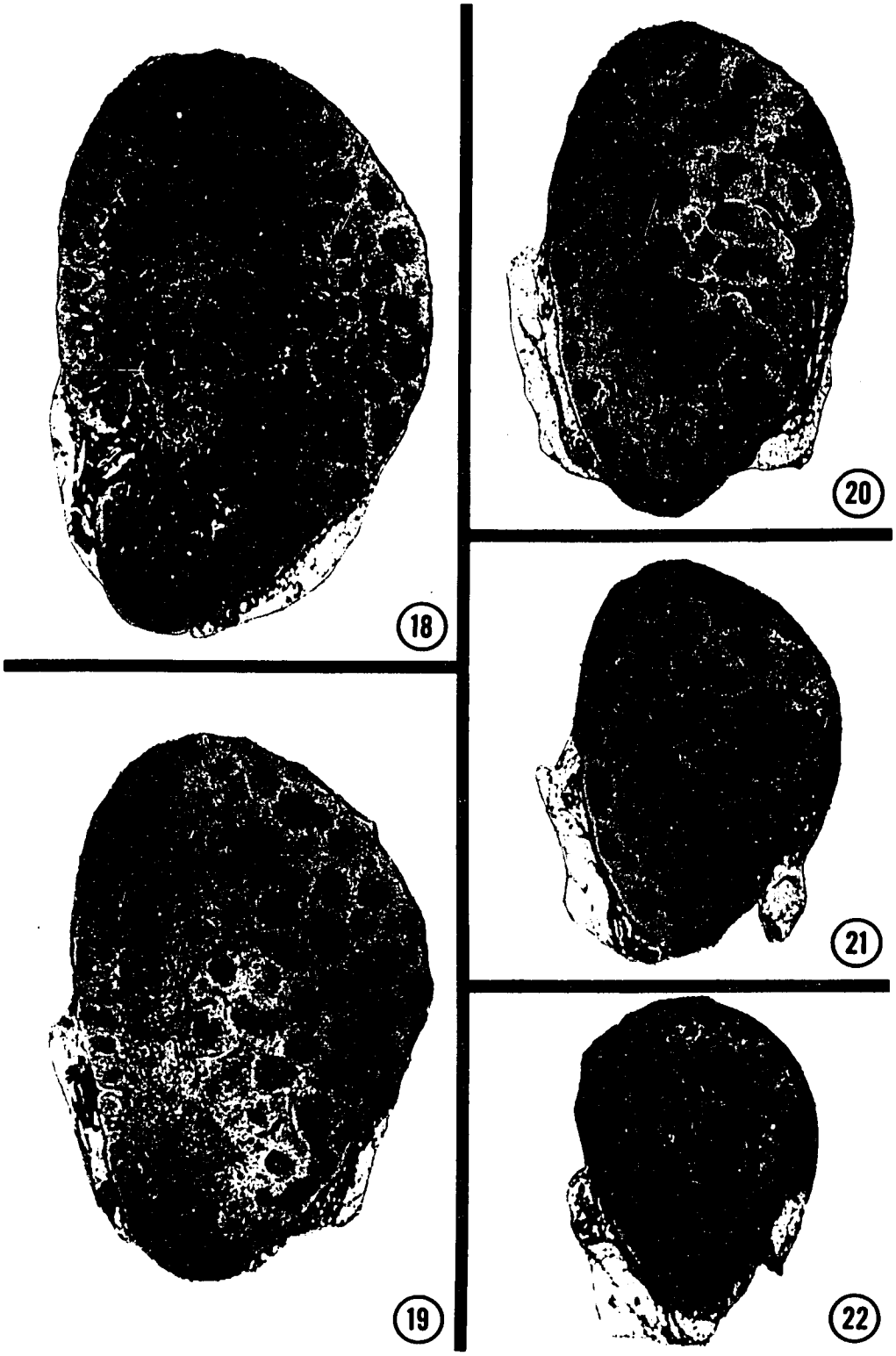
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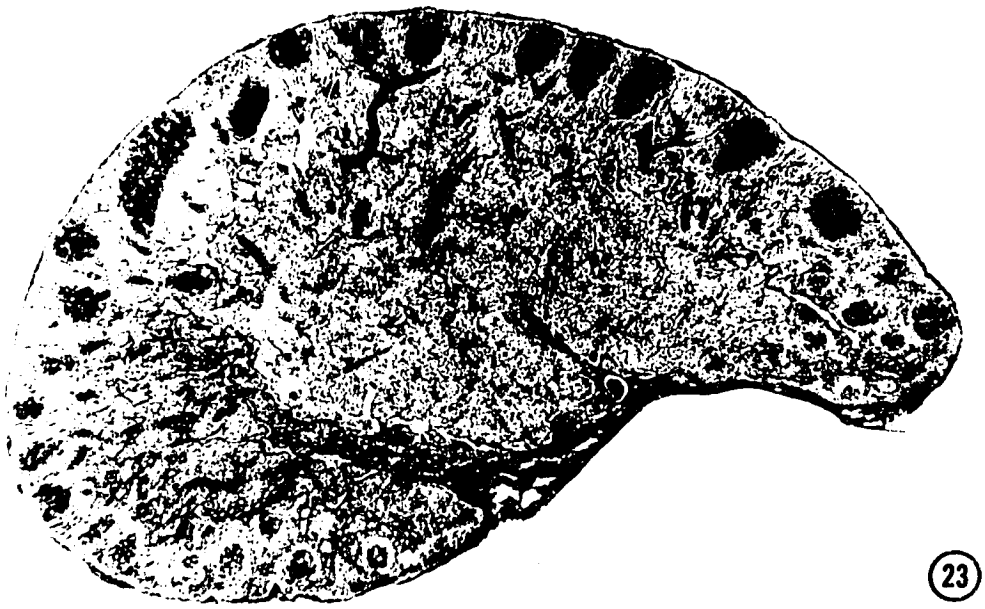
Figs. 23,24. Two consecutive sections passing near the center of the node: one silver-impregnated (fig. 23), the other stained by the technique of Dominici (fig. 24). In Figure 24, the cortex appears as a dark and relatively thin layer underlining the capsule. The remainder of the section is filled by the medulla, inside which bifurcates the hilus. In the cortex, numerous follicular nodules are adjacent to the capsule and stain slightly darker than the remaining cortical area, i.e., the cortical extrafollicular area. In Figure 23, the reticular fibers and the erythrocytes in the blood vessels show black impregnation with the silver salt, as do as the nuclei in the follicular nodules. The latter structures appear as dark areas contrasting with the remaining node parenchyma. At such low magnification, the cortical extrafollicular area resembles the medulla. Magnification about 20X.



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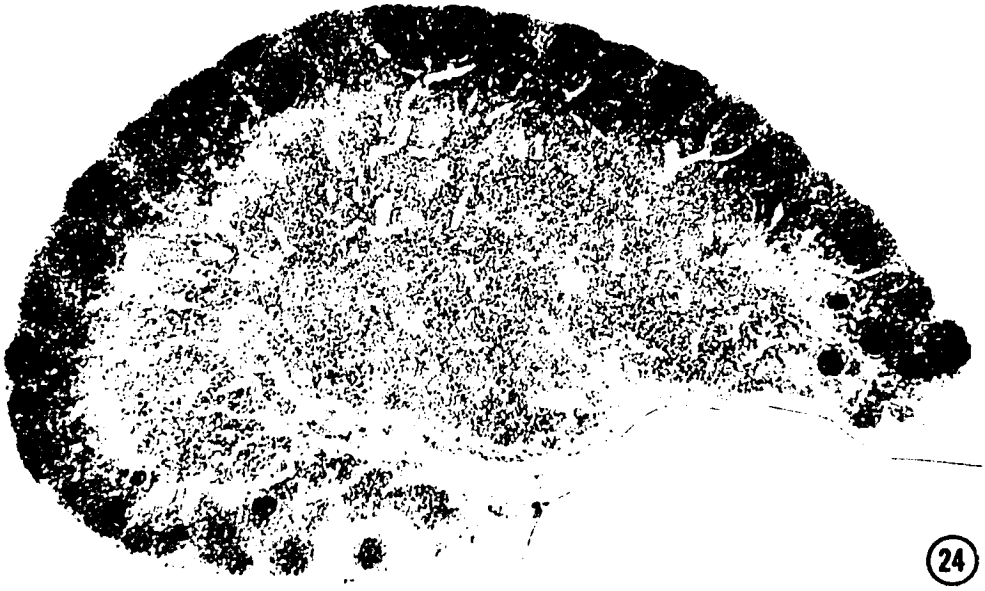

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Figs. 25,26. Same site in two consecutive sections: one silver-impregnated (fig. 25), the other stained by the technique of Dominici (fig. 26). In Figure 25, three nodules and their associated follicles are clearly outlined as dark impregnated areas separated from one another by a band of pale cortical extrafollicular area that contains many more reticular fibers than do the follicular nodules. In Figure 26, the three arrows point, from right to left, to the dark and light areas of a nodule and to the mantling follicle, respectively, of a longitudinally sectioned mantled nodule. Note that here, there is little contrast between the follicles and the cortical extrafollicular area, which appears poorly outlined. Magnification about 170X.

Figs. 27,28. Same site in two consecutive sections, stained as in Figures 25 and 26 respectively. Across the center of Figure 27 can be seen a band of cortical extrafollicular area, separating two follicular nodules. Above the PCV is a cross sectioned postcapillary venule that appears as a light area in Figure 28. In the nodules, the large black cells are macrophagic reticular cells. While the nuclei of cells present in the cortical extrafollicular area are not silver-impregnated, those in the follicular nodules are. However, even in the latter structures, the nuclei adjacent to the silver-impregnated reticular fibers

and macrophagic reticular cells are not impregnated. Note that the thicker the fiber, the wider the band of non-impregnated nuclei along it. The arrow points to a macrophagic reticular cell with a non-impregnated nucleus, and a light band around it as occurs with fibers. In the lower follicular nodule, the macrophagic reticular cells are disposed in a curved line in between the nodule (N) and its associated follicle (F). In Figure 28, all nuclei are stained. Magnification about 240X.


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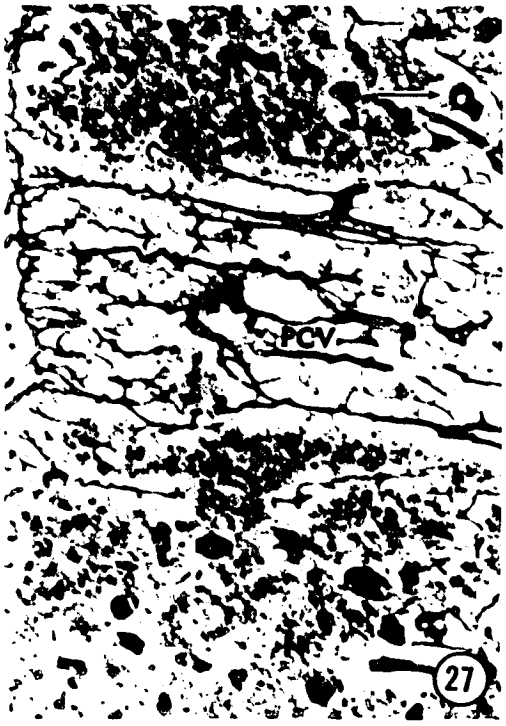
PCV
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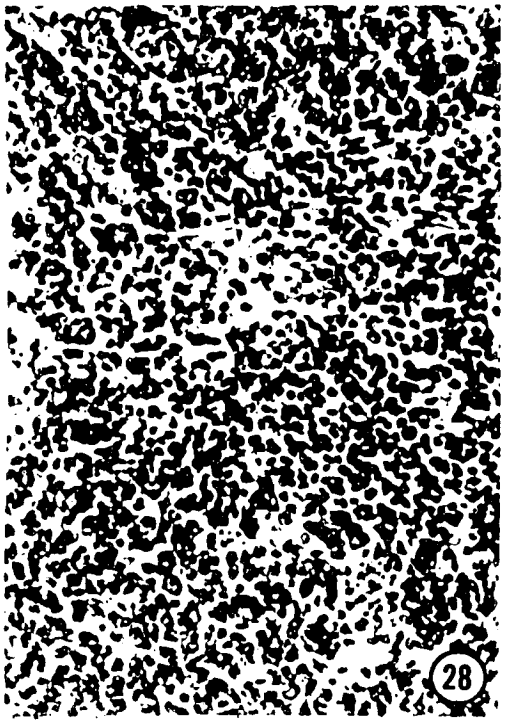
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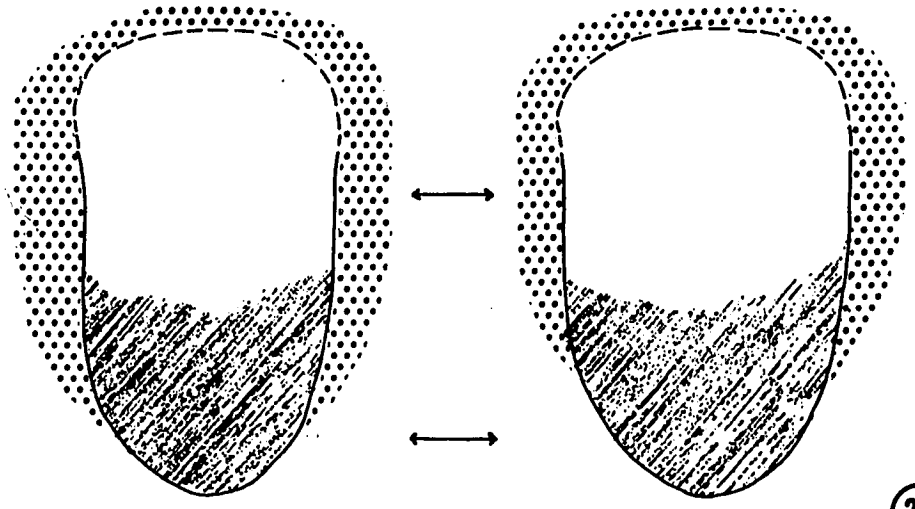
Figs. 29,29A. Schematic diagrams of a "mantled nodule". The dotted area corresponds to the follicle, the striated area to the "dark area" of the nodule, and the unmarked area to the "light area" of the nodule. In Figure 29, the diagrams represent longitudinal sections of the nodule cut at right angles to each other as indicated by A and B in the right-hand diagram of Figure 29A. This latter diagram is a cross section of the follicular nodule, passing through its light area as indicated by the upper arrow in Figure 29. The left-hand diagram in Figure 29A is a cross-section passing through the dark area of the follicular nodule, as indicated by the lower arrow in Figure 29. Here, the nodule does not appear surrounded by the follicle. Magnification about 180X.



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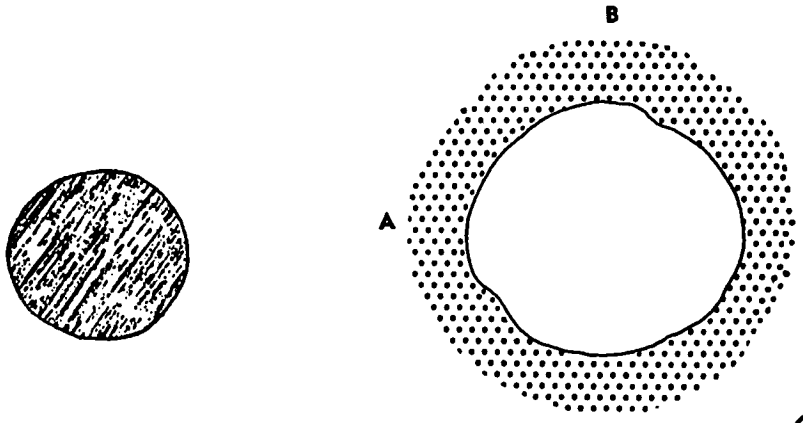


29A

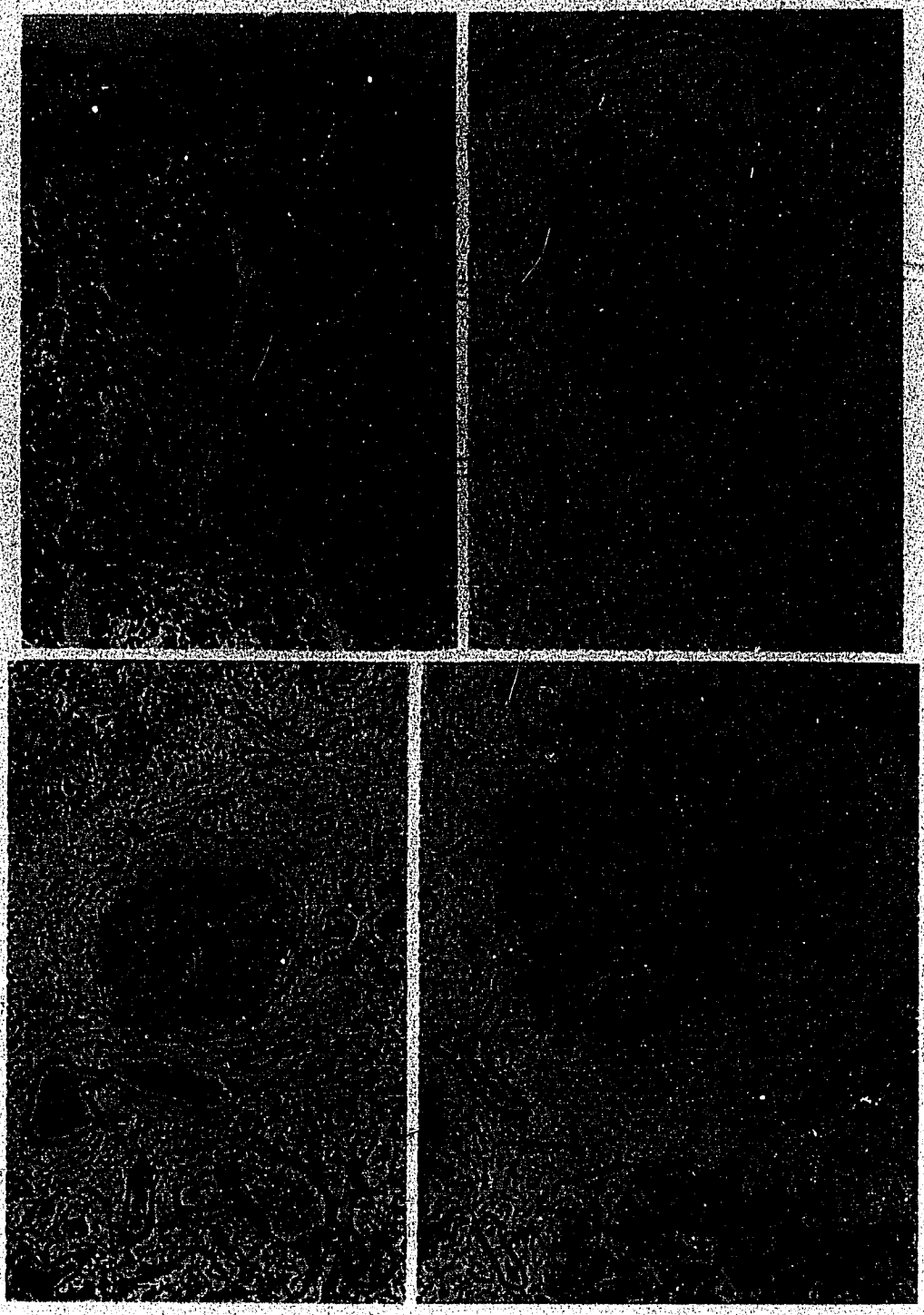
Fig. 30. Dominici technique. Section showing a tangentially cut mantled nodule. From bottom to top, the arrows point to the dark and light areas of the nodule, and to the associated follicle formed of small lymphocytes respectively. In the lower third of the figure are seen darkly stained plasmocytes of the medulla, which are separated from the nodule by a band of pale cortical extrafollicular area. Magnification about 110X.

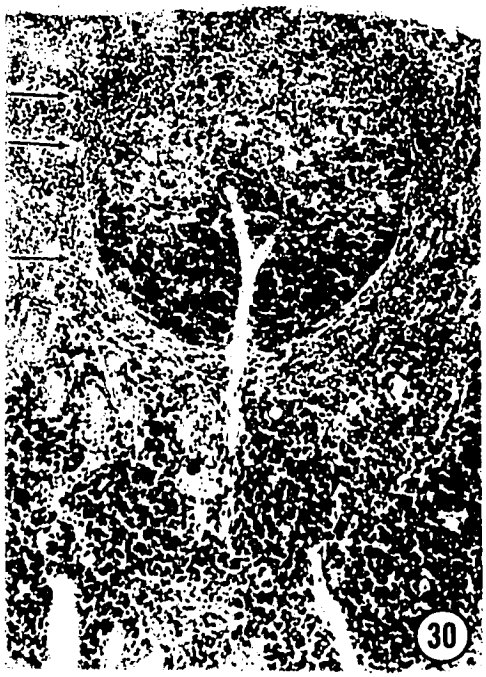
Fig. 31. Silver impregnation. Longitudinal section of a mantled nodule as illustrated in Figure 29. The three arrows indicate the same details as in Figure 30. In the dark area of the nodule, the dark cells along the inner nodular margin are autofluorescent cells grouped in clusters. In the light area of the nodule, note the numerous dark macrophagic reticular cells in which the colourless nuclei are more readily detectable than in the autofluorescent cells. The follicle appears as a narrow band along the lateral and outer margins of the nodules, which is devoid of macrophagic reticular cells and contains numerous similarly sized small nuclei. Magnification about 110X.

Fig. 32. Silver impregnation. Preparation showing a cross section through the dark area of a mantled nodule, passing at the level of the lower arrow in Figure 31 and as schematized in left diagram of Figure 29A. Several autofluorescent cells outline the section of the nodule, which resembles the dark

area in the nodule of Figure 31. Note the scarcity of macrophagic reticular cells in their area of the nodule. Magnification about 110X.

Fig. 33. Silver impregnation. Preparation showing a cross section through the light area of a mantled nodule, passing at the level of the middle arrow in Figure 31 and as schematized in the right diagram of Figure 29A. The section of the nodule exhibits many macrophagic reticular cells, like the light area of the nodule in Figure 31. The follicle appears as a more or less homogeneous band of small nuclei around the nodule and as nearly devoid of macrophagic reticular cells. Part of a similar section of a nodule is seen in the lower part of the figure. Magnification about 110X.

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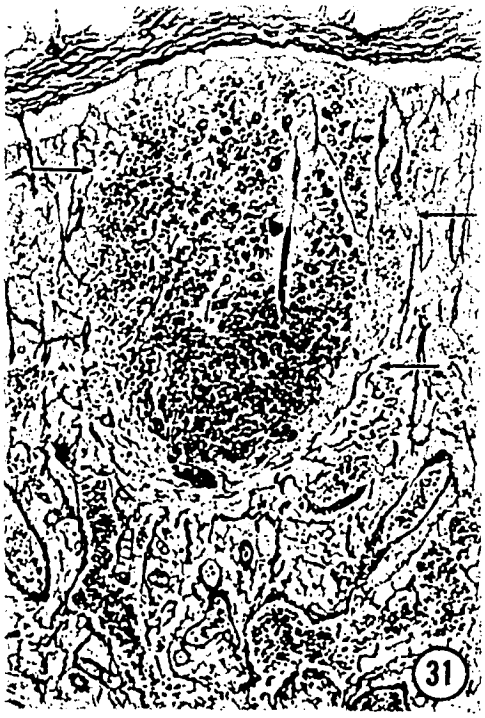
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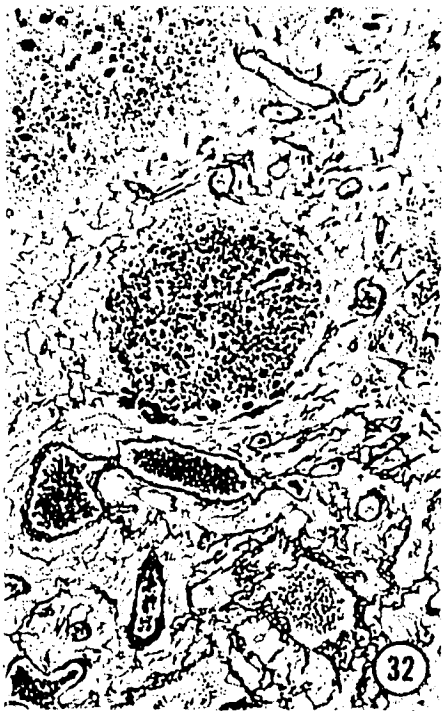
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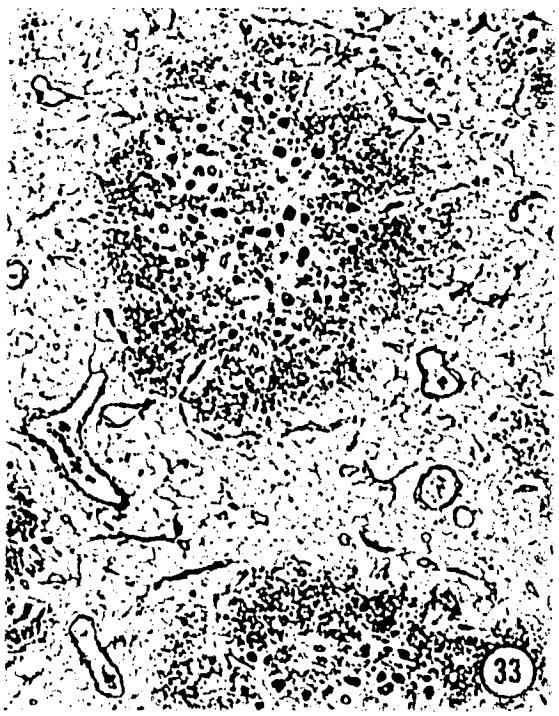
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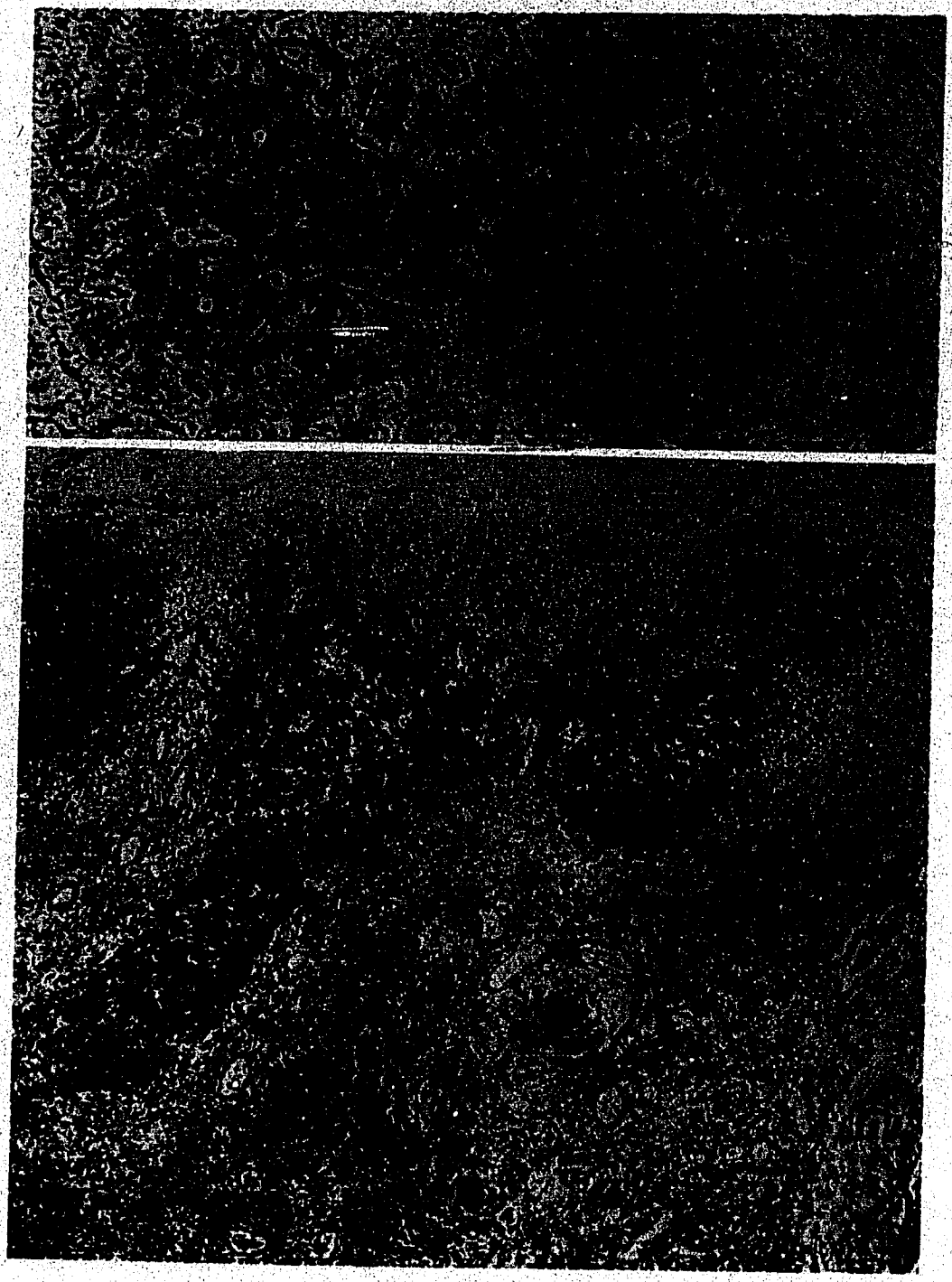
Fig. 34. Silver impregnation. Preparation showing a cross section through the light area of a complex nodule formed of two single nodules partly united in the center of the figure. Except for being hour-glass in shape, the nodule resembles that seen in Figure 33. (see diagram, Figure 42). The arrows indicate postcapillary venules, outside of but associated with the follicle. Magnification about 140X.

Fig. 35. Silver impregnated preparation counterstained by the technique of Dominici and exhibiting a tangential section through a complex nodule formed by the union of five single nodules (see diagram, Figure 43). The section passes mainly through the dark area of the united nodules. Magnification about 90X.

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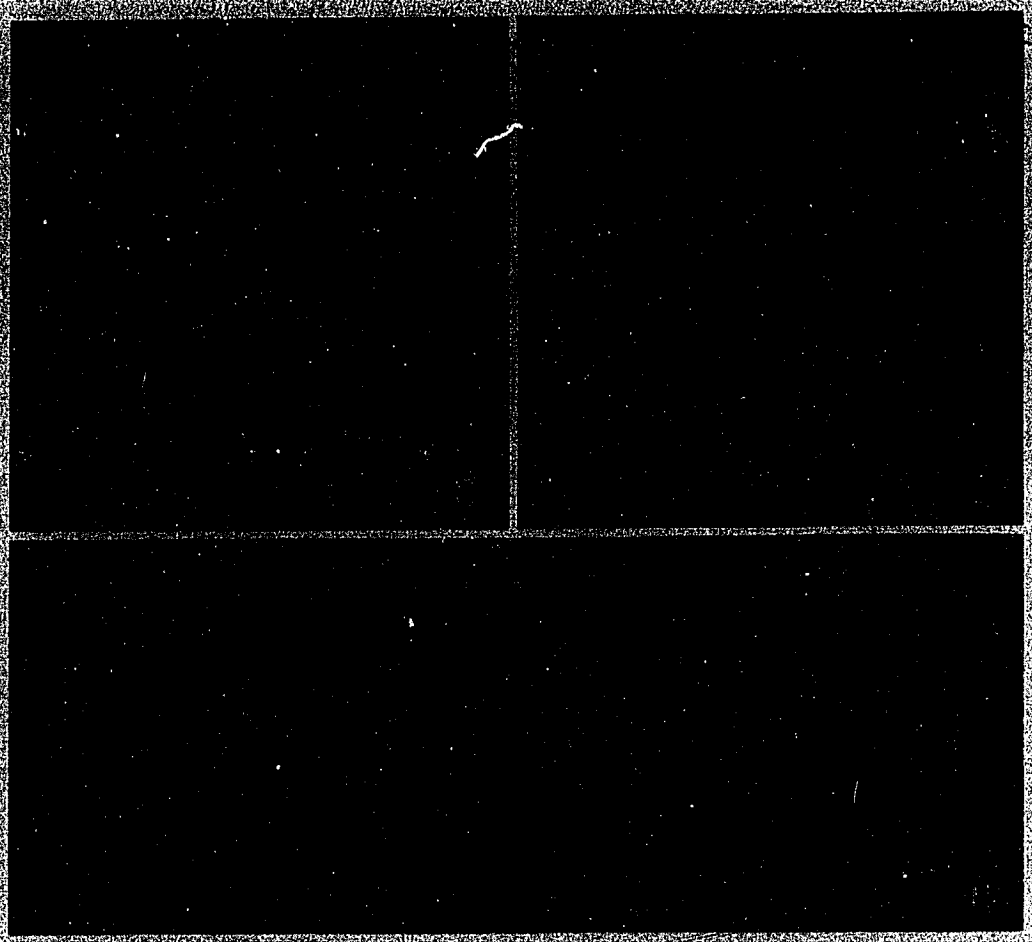
Figs. 36-38. Volumetric cardboard model, photographed from three angles of the complex nodule seen in Figure 35. The view in Figure 38 was taken in the direction indicated by the arrow in Figure 36. For composition of the nodule, see Figure 43.



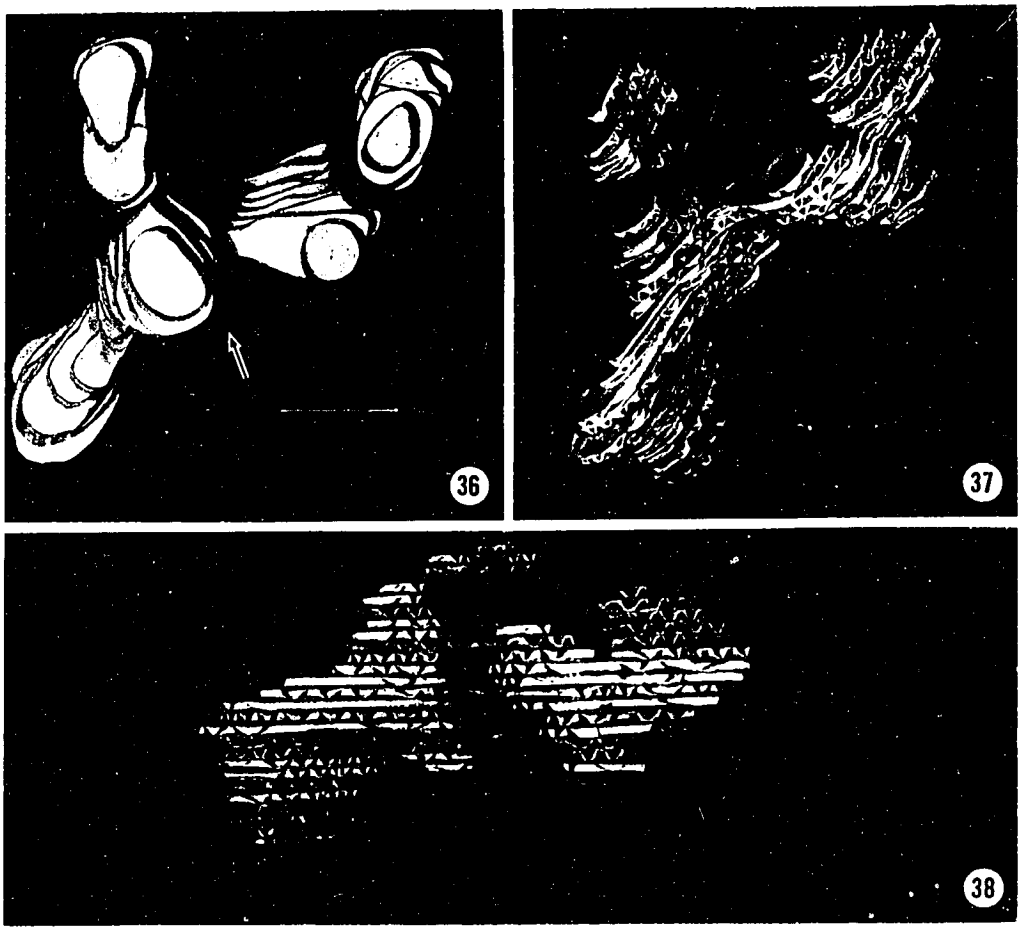

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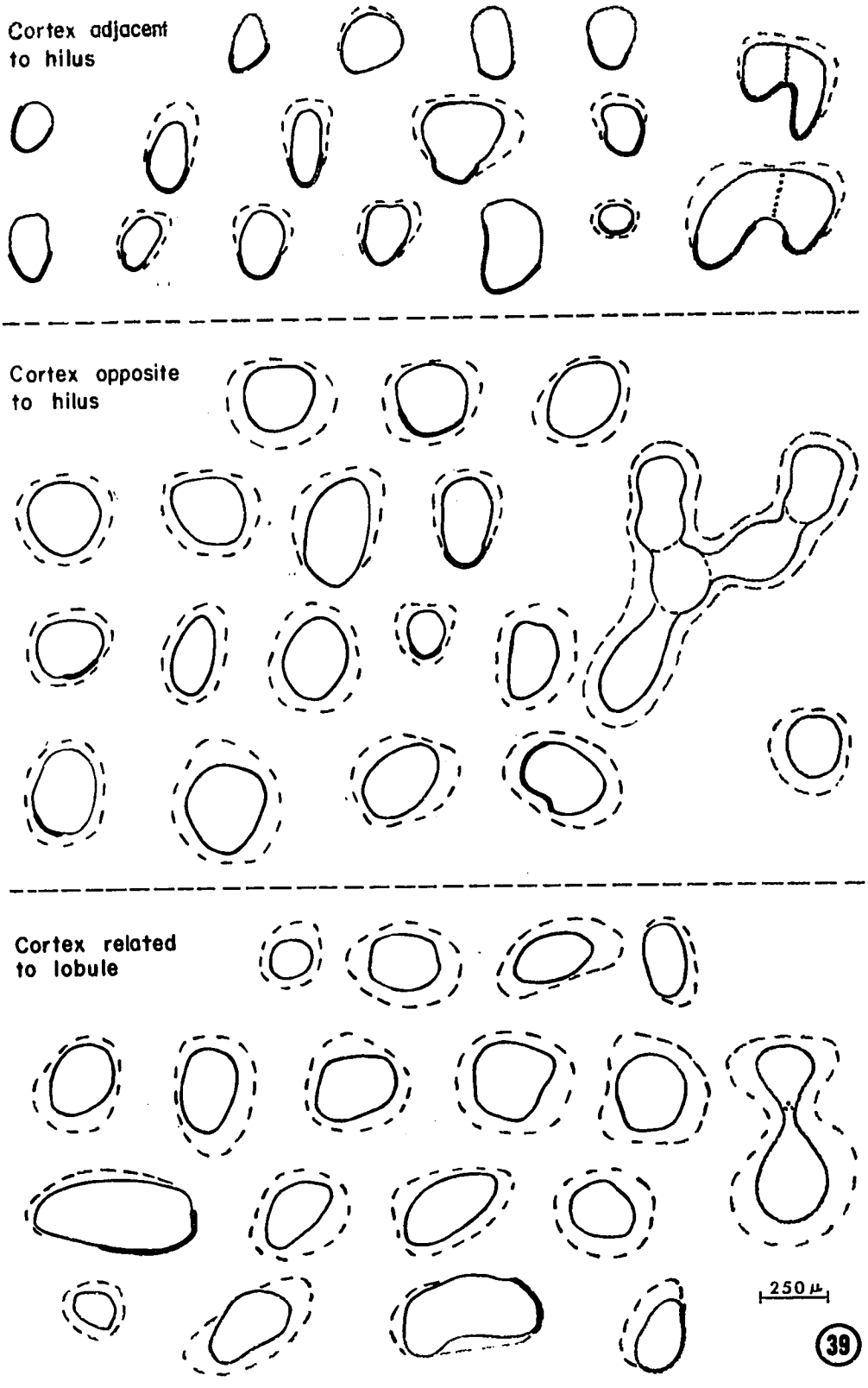
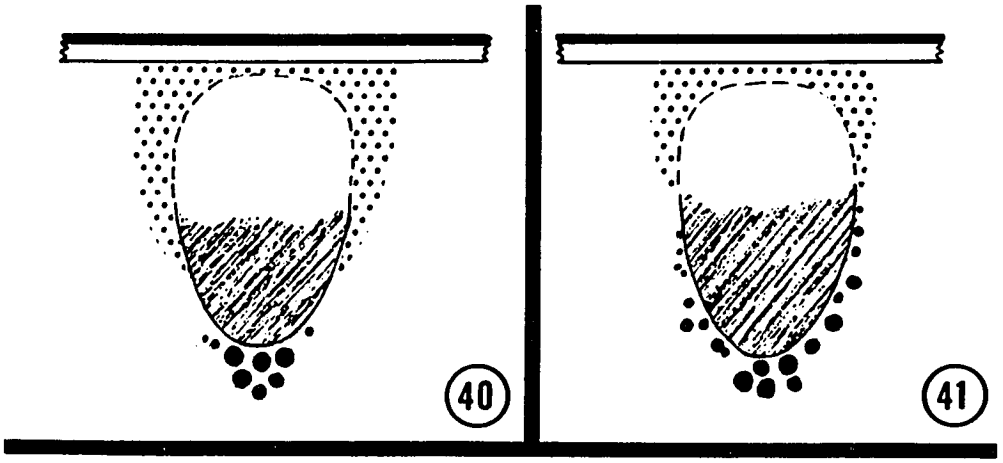
Fig. 39. Tracings of various follicular nodules selected from three different regions of the cortex. Solid lines indicate the margin of each nodule, the thicker part of which outlines the inner margin of the nodule. Broken lines indicate the margin of the follicle associated with each nodule. Two nodules were devoid of follicle (upper left corner) and four were complex nodules (right side); the dotted lines indicate the junction of the nodules in the latter structures. The sizes of the nodules are proportional, since each was traced at a magnification of about 40X in the section showing maximal size. Note that, in general, the smaller nodules are those adjacent to the hilus, whereas the larger are those related to the trabecula of the lobule; their associated follicles show the same tendency.


Cortex adjacent to hilus
Cortex opposite to hilus
Cortex related to lobule
250 μ

Figs. 40,41. Realistic diagrams illustrating the distribution of the autofluorescent cells (large black dots) along the margin of nodules. In mantled nodules (fig. 40), the autofluorescent cells are restricted to the inner portion of the nodular margin, often forming clusters as shown here. In capped nodules (fig. 41), the autofluorescent cells extend along the lateral portion of the nodular margin. Note the decrease in size of these cells as their location becomes higher up along the nodular margin.

Figs. 42,43. Realistic diagrams of the localization of the autofluorescent cells associated with the two complex nodules in Figures 34 and 35 respectively. Autofluorescent cells were present along the inner margin of each single nodule united to form these complex nodules, in a manner similar to that in isolated single nodules (see figs. 40,41).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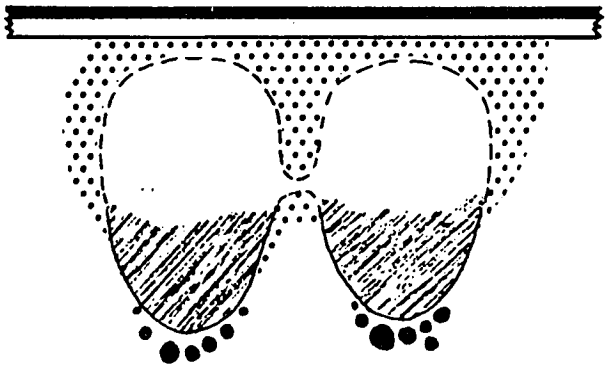
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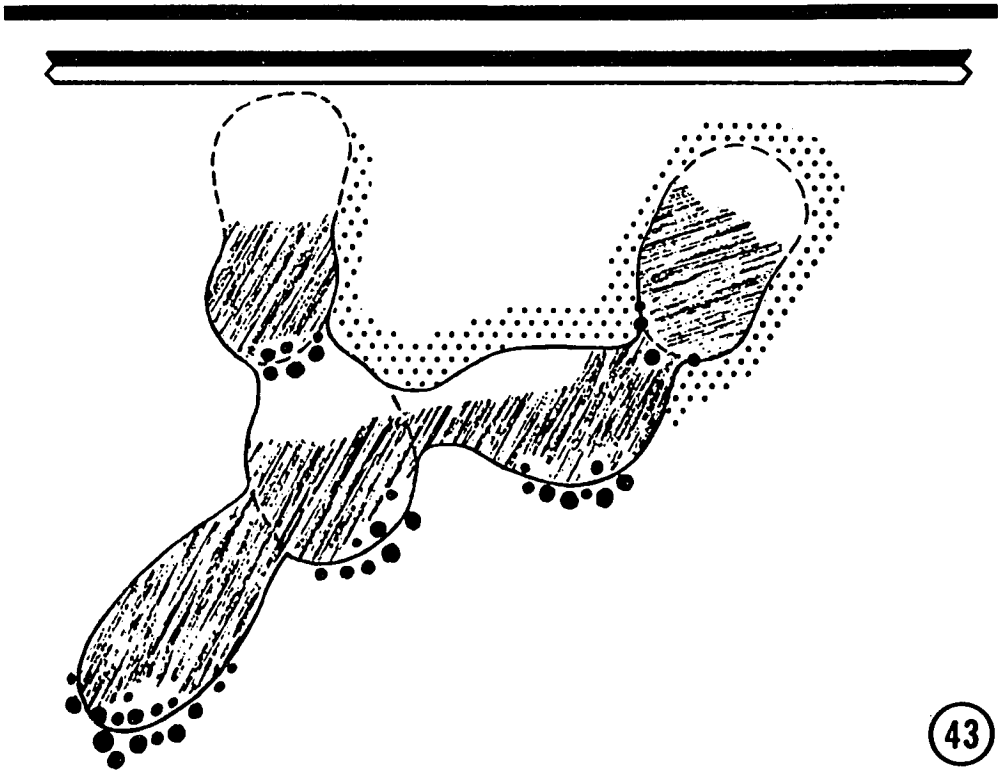
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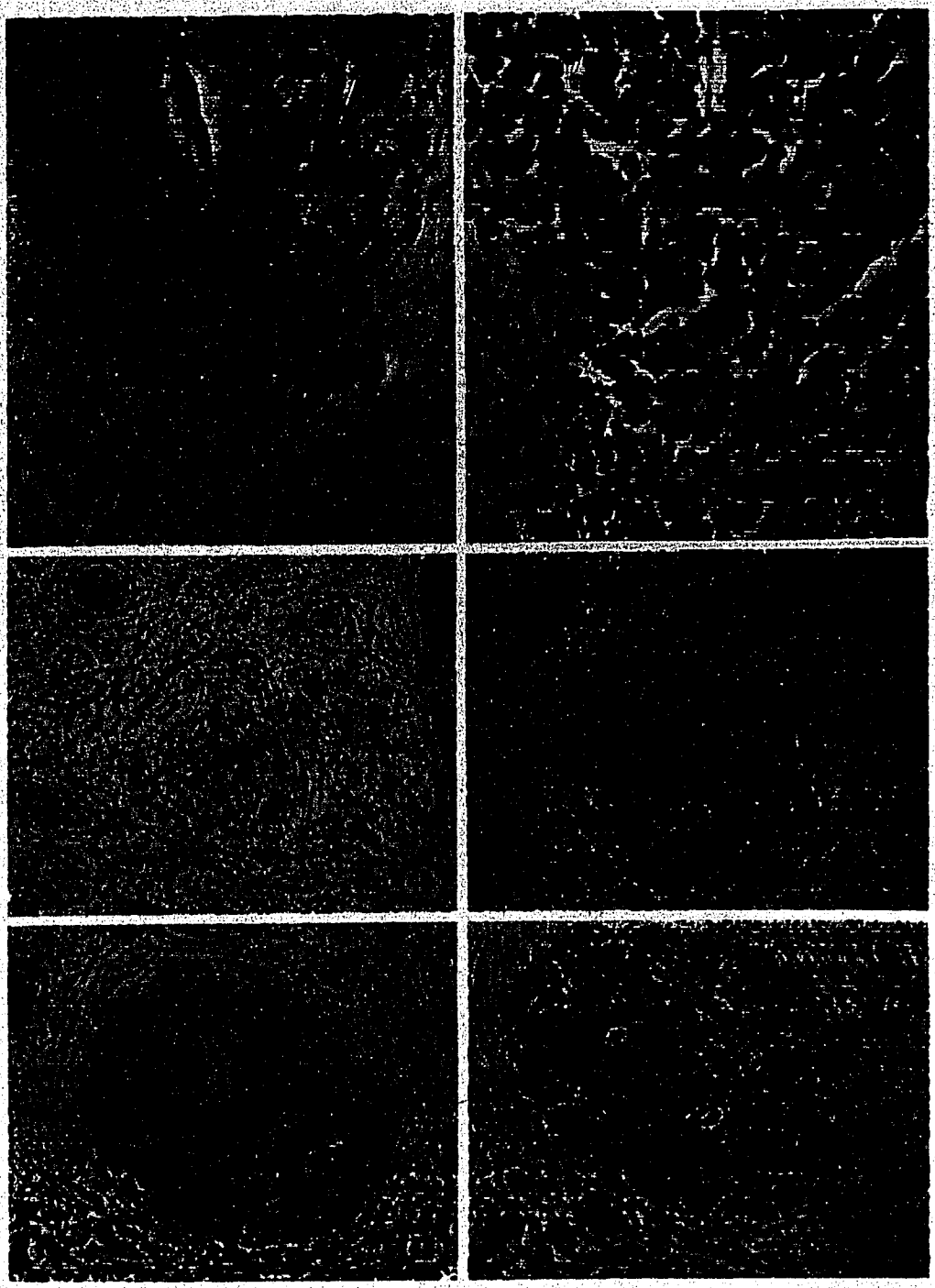
Figs. 44,45. Same site in two consecutive sections: one silver-impregnated, the other stained by the technique of Dominici. A cluster of autofluorescent cells is present in the subnodular space outlining the inner margin of a longitudinally-sectioned mantled nodule (compare with fig. 40). In Figure 44, the autofluorescent cells are darkly impregnated, whereas they are stained lightly (arrow) in Figure 45. Magnification about 460X.

Figs. 46-49. Silver impregnation. Preparations showing tangential sections of a same mantled nodule cut at four widely separate levels. In Figures 46 and 47, the sections pass through the dark area (DA) of the nodule, and the nodular margin is outlined by autofluorescent cells (arrows). In Figure 48, the section passes through the dark area and, on the upper part, through the follicle (F). The arrows point to small autofluorescent cells along the portion of the nodular margin not covered by the follicle. In Figure 49, the section passes in part through the dark and light (LA) areas of the nodule as well as through the follicle. Small autofluorescent cells are still present along the portion of the nodular margin not covered by the follicle. Below the MR is a macrophagic reticular cell of the light area. Magnification about 90X.



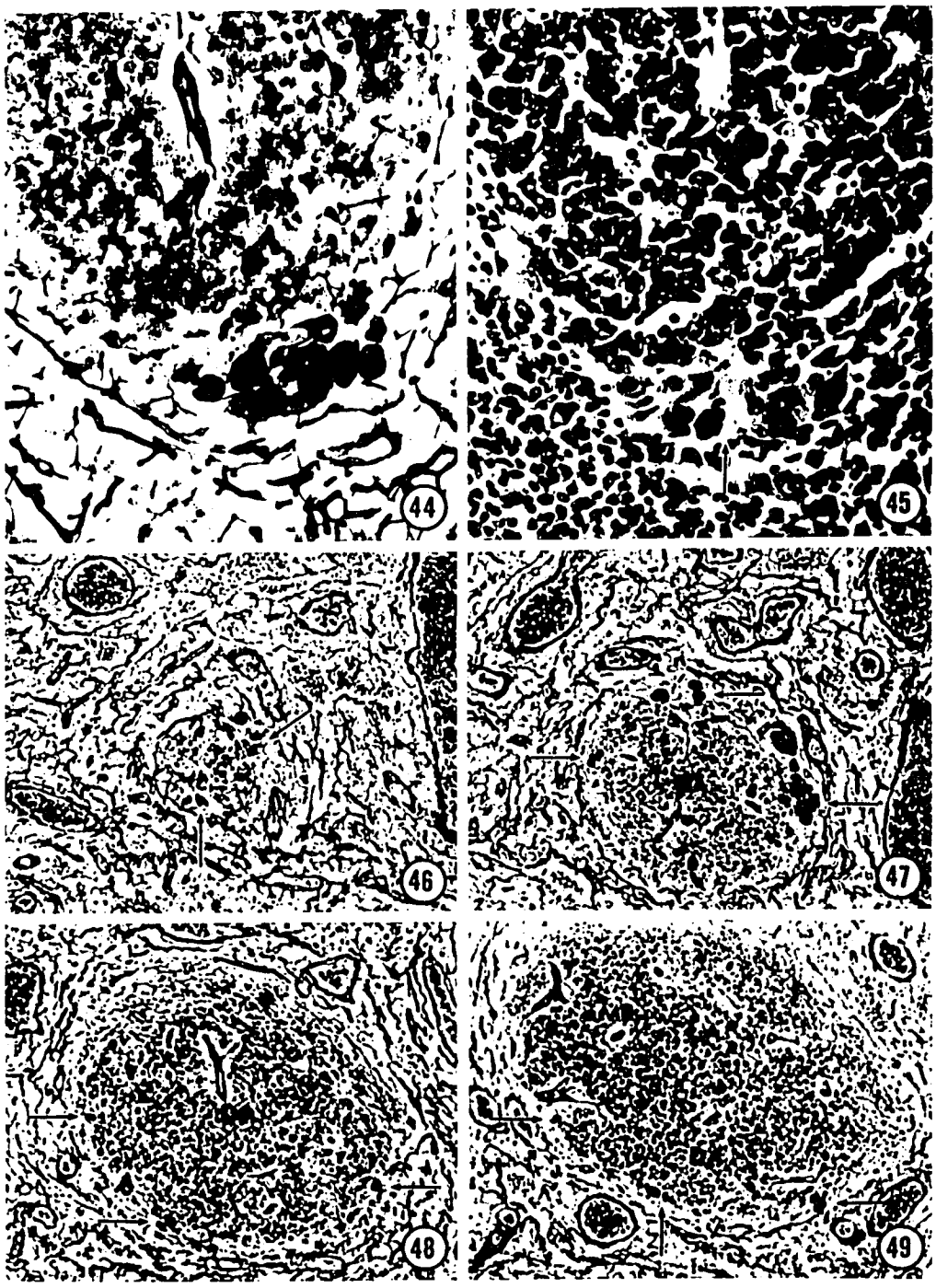

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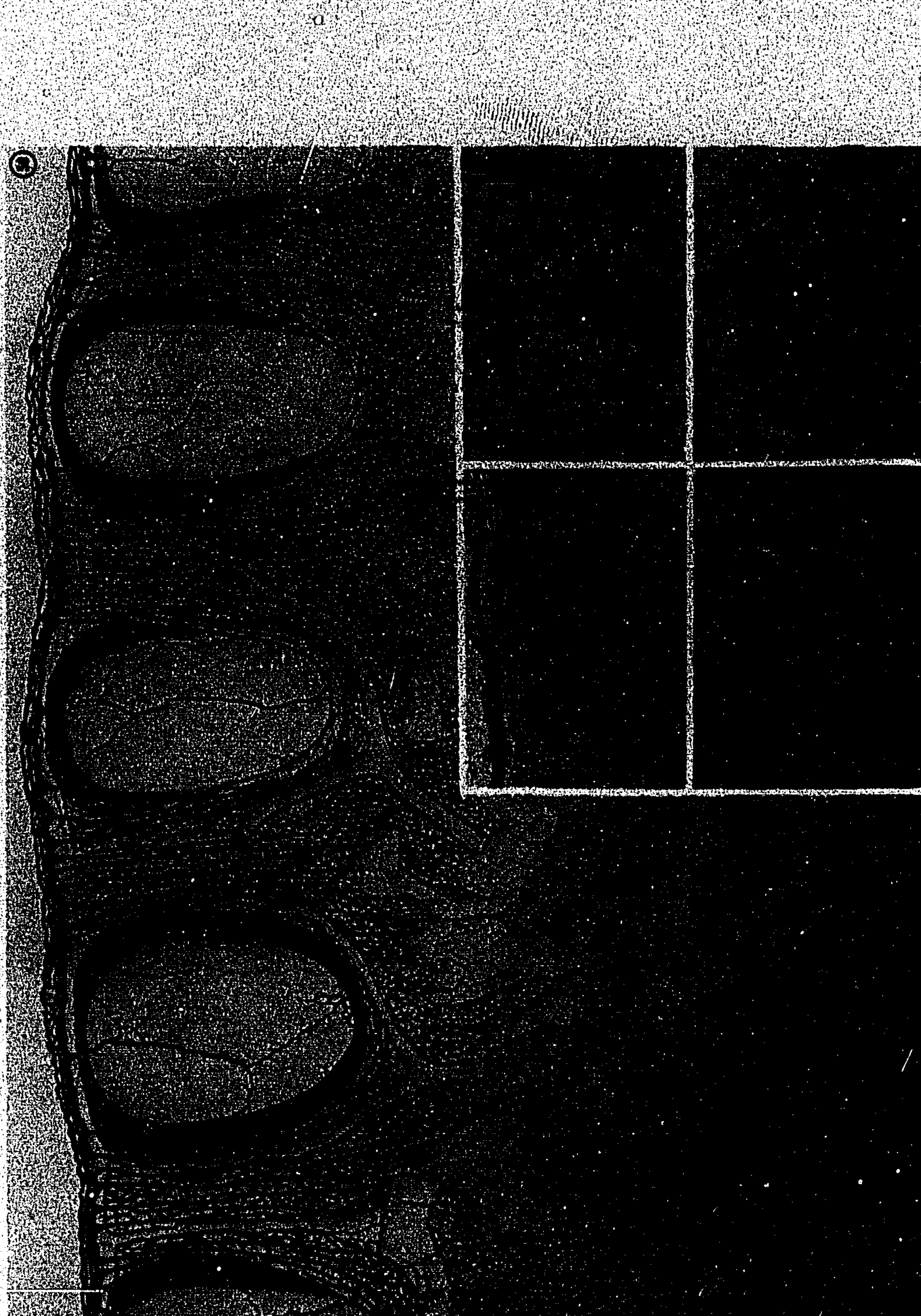
Fig. 50. Schematic diagram illustrating the main features in the framework of the node, based on reconstruction from silver-impregnated sections. The capsule (top of figure) contains mainly collagenous fibers; these impregnated variably dark brown and therefore appear paler than the remaining fibers, which are black-impregnated reticular fibers. The latter fiber underlies the inner surface of the capsule separated from the cortex below by the light subcapsular sinus. Collagenous fibers of the capsule appear to coalesce in a funnel-like fashion to give rise to the coarse reticular fibers that cross the subcapsular sinus and penetrate deep into the cortex. The coarse fibers adjacent to the follicular nodules encircle their inner margin, giving the appearance of basket hanging from the capsule and containing these structures. Microphotographs C and D represent sites in the cortical extrafollicular area and in the medullary cords, respectively. The dense concentration of fibers prevented the impregnation of the nuclei of cells in between the fibers, unlike in B. Note the variation in width of the fibers and the presence of parallel and nearly straight fibers in the cortical extrafollicular area. Microphotograph A shows the appearance, in single sections of longitudinally-cut follicular nodules, of these coarse fibers and of their baskets (magnification about 50X). The follicles (shadowed areas) and the nodules (pale areas) contain only a few fibers. Microphotograph B shows the scarcity of the fibers in the follicle, the cells of which are silver-impregnated. This microphotograph is at a magnification of 990X as are those in C and D taken outside the follicle. Most of the aforementioned coarse reticular fibers travel the thickness of the

cortex and, upon reaching the medulla, become the rêticular fibers underlying the medullary sinuses, which are the wide and variably shaped light areas in the lower 2/3 of the diagram and are crossed by a few fibers. Note that the reticular fiber outer linings of these sinuses do not appear to be continuous. In between the coarse fibers, medium and fine reticular fibers form a network that often shows denser in the medulla than in the cortex. Note that, along the inner nodular margin of the capped and mantled nodules, a few layers of more or less concentric fibers delimit a space under the nodule: the subnodular spaces. The postcapillary venules in the cortical extrafollicular area (light bands not crossed by fibers) are surrounded by fibers that form perivascular channels around them and make their outline irregular, whereas these structures are absent around the medullary venules.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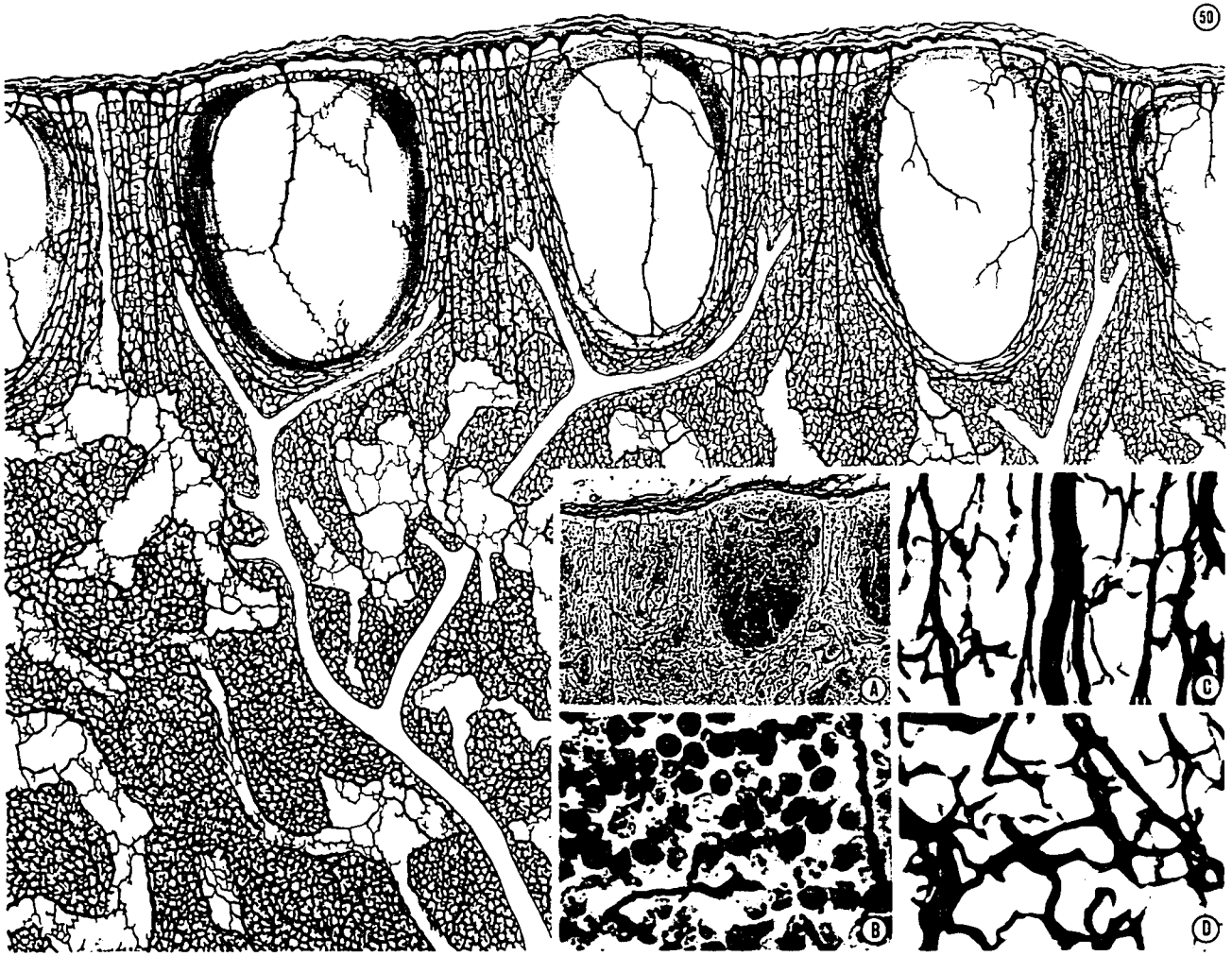

A
B
C
D

Figs. 51-54. Silver impregnation. Figure 51 shows a tangentially-cut mantled nodule surrounded by a pale cortical extrafollicular area at a magnification of about 160X. In Figures 52-54, the letters are located in exactly the same sites as in Figure 51. They indicate, at a higher magnification of about 1900X, a portion of the nodule (N), of the follicle (F) and of the cortical extrafollicular area (EA). Note the greater concentration of reticular fibers in the cortical extrafollicular area, in which the nuclei failed to become silver-impregnated. At the bottom of Figure 54 can be seen the follicle, so that nuclei are impregnated there.

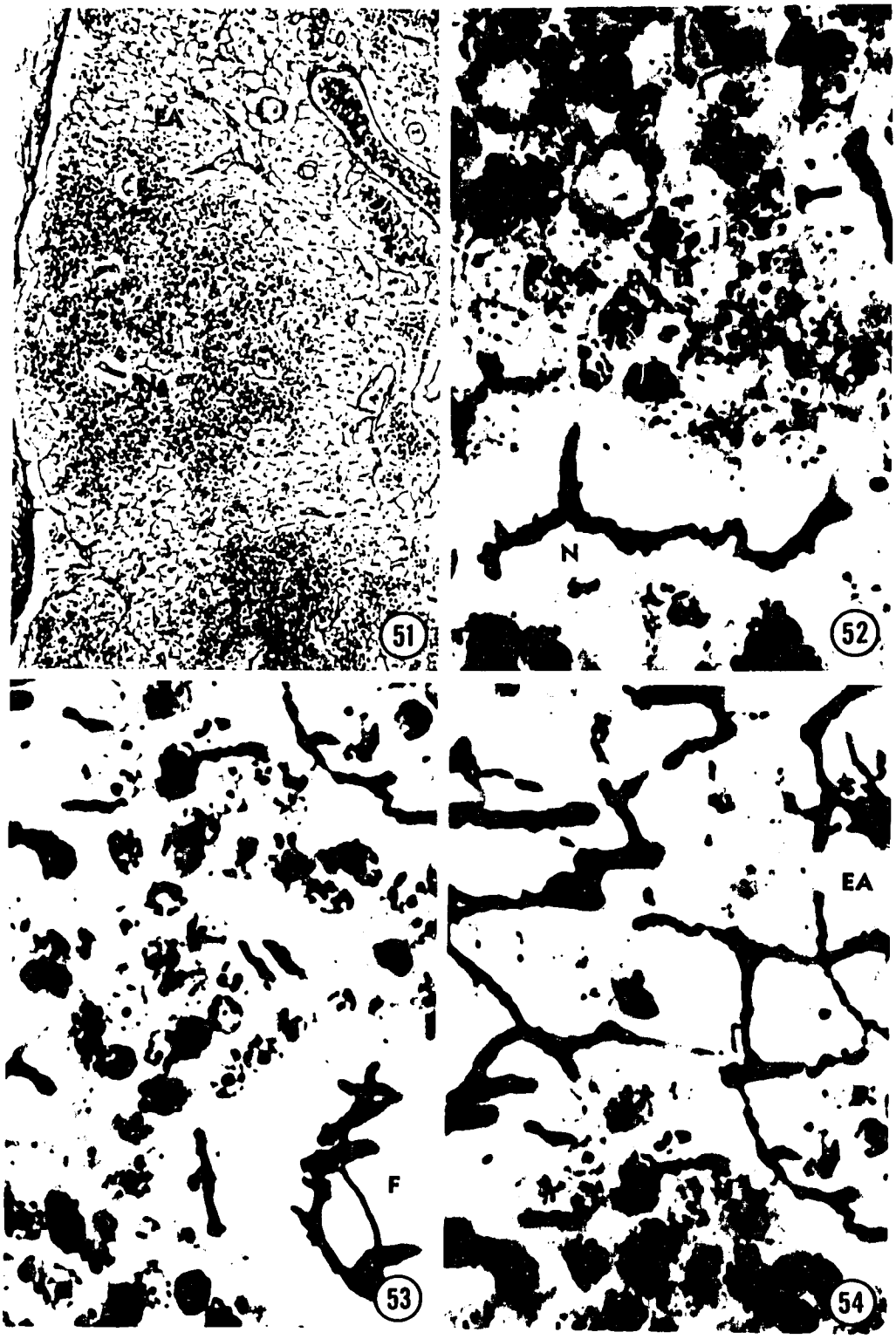
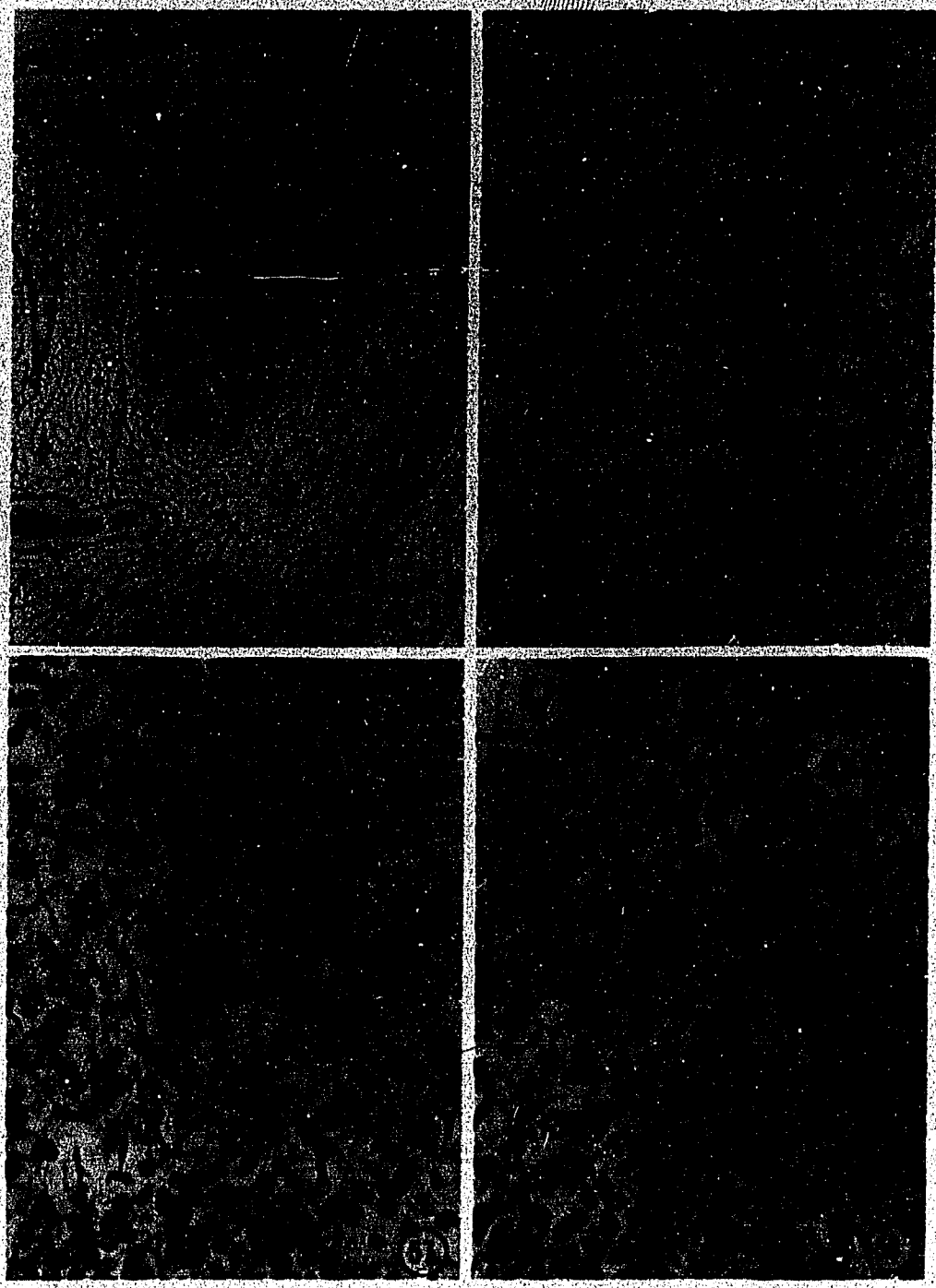

EA
N
51
N
52
F
53
EA
54

Fig. 55. Silver impregnation. Figure showing a longitudinally-sectioned nodule (N) with a mantling follicle (F). The arrows point to two fibers: one (left) constituting the lateral margin of the follicle, the other (right) located in between the follicle and the nodule. The portion of follicle in between these two fibers and below the left arrow exhibits a network of thin fibers, a concentration of which is unusual in follicles. By comparison, the portion of the same follicle in the upper right corner (F) contains few fibers, as is usual in follicles. Magnification about 160X.

Figs. 56-58. Silver-impregnation. Figure 56 shows a portion of two mantled nodules (N) cross-sectioned through their light area. EA indicates the cortical extrafollicular area in between the nodules. As in Figure 55, the follicles contain more reticular fibers than is usual in follicles. The two arrows point to a portion of each follicle, magnified at about 730X in Figures 57 and 58 respectively, to show the unusually dense reticular network. In the latter Figures, the arrows point to the same structures as in Figure 56.

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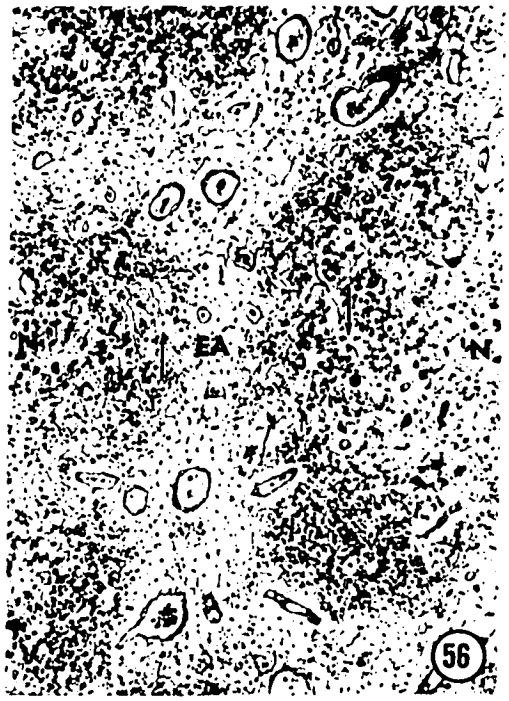
N
EA
N
56


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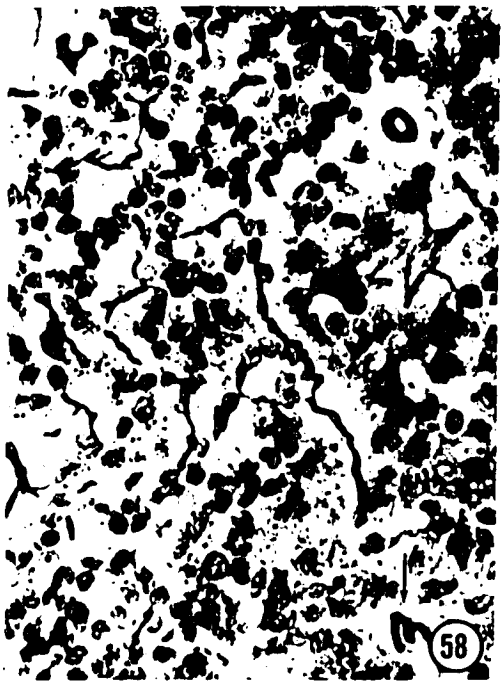
58

Fig. 59. Silver impregnation. A coarse reticular fiber, originating from the capsule, crosses the subcapsular sinus and the light area of the nodule. Towards the center of the nodule, the fiber is attached to a capillary appearing here as a widening of the fiber. The arrow points to a few layers of concentric reticular fibers encircling the inner margin of the nodule above it. Magnification about 170X.

Fig. 60. Silver impregnation. A coarse reticular fiber, originating from the capsule, crosses the entire length of a tangentially-sectioned nodule and, hence, reaches its inner margin (arrow). There, the fiber divides and anastomoses with those outlining the inner margin of the nodule, in the manner shown in Figure 59. Magnification about 170X.

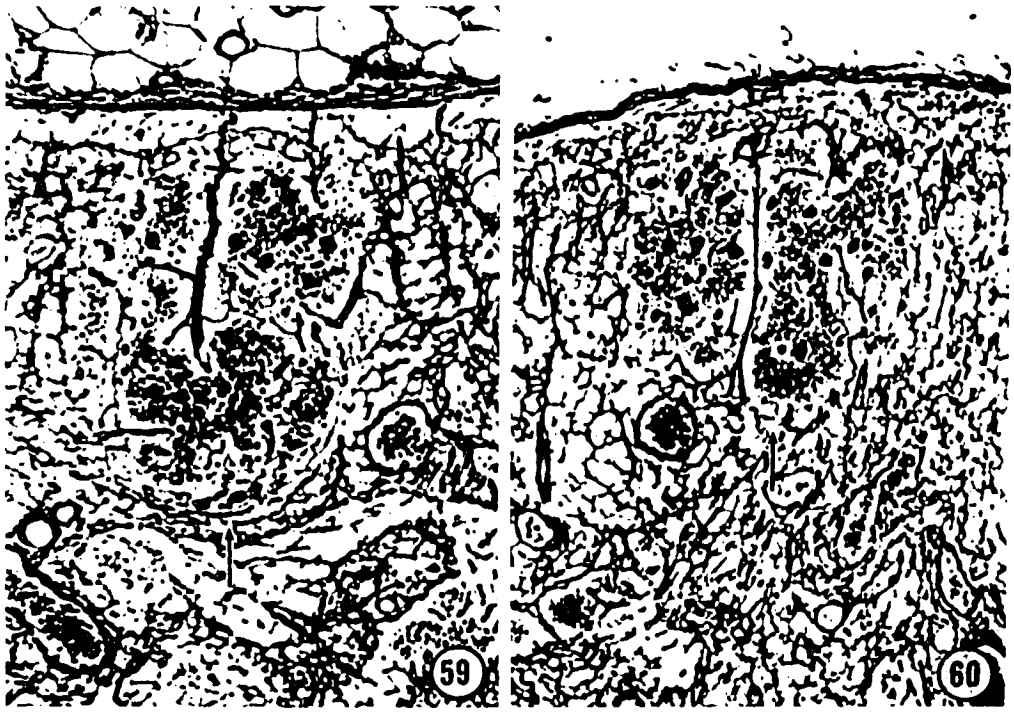
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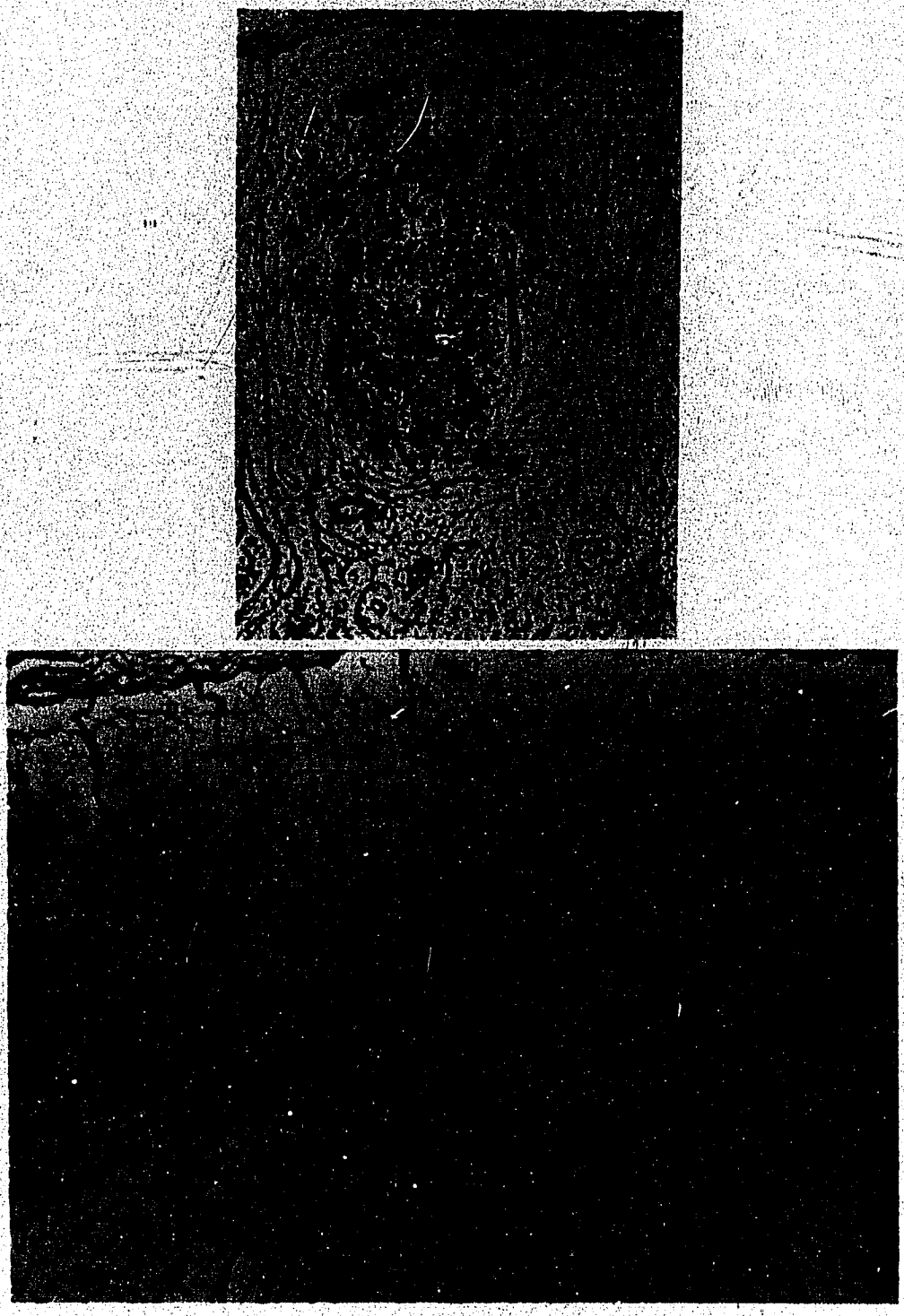
Fig. 61. Silver impregnation. Figure showing cortical extrafollicular area on both sides of a longitudinally-cut nodule, which exhibits a capillary at the margin between the nodule and associated mantling follicle. Two arrows point to coarse reticular fibers originating from the capsule and running down towards the medulla below the nodule. Magnification about 170X.

Fig. 62. Silver impregnation. Preparation showing portion of a nodule (N) on both sides of the figure. In the cortical extrafollicular area between nodules, the arrows point to an interfollicular sinus, which is narrow and not crossed by reticular fibers as in the subcapsular sinus (SS). The bottom of the figure corresponds to the cortico-medullary junction, where this narrow sinus transforms into a wide medullary sinus (MS). Magnification about 240X.

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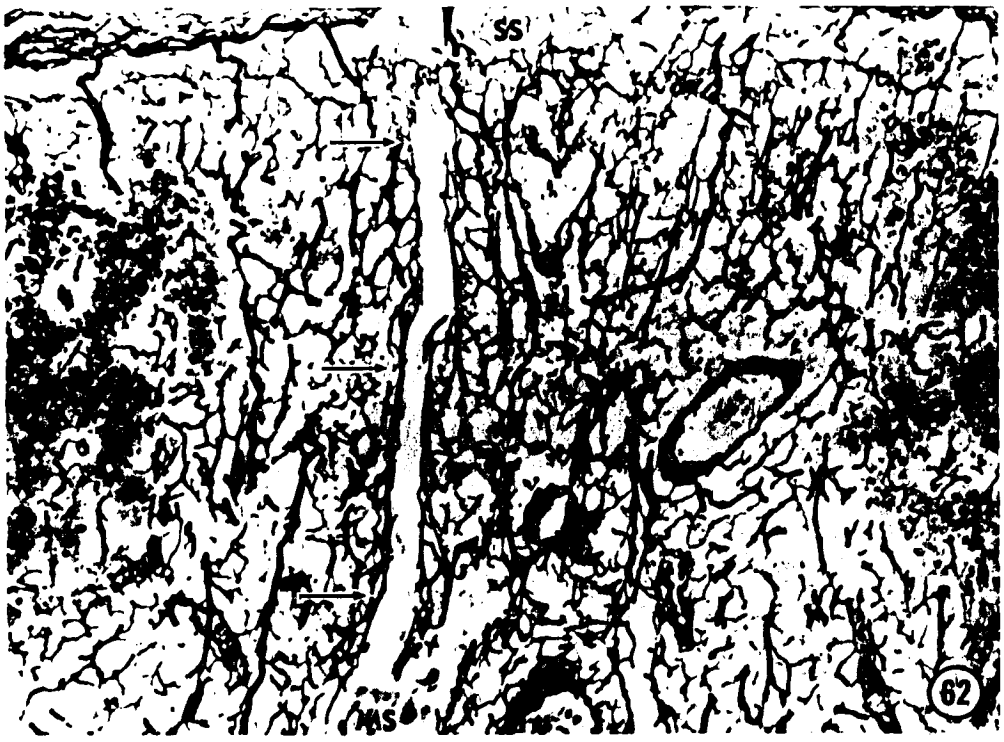
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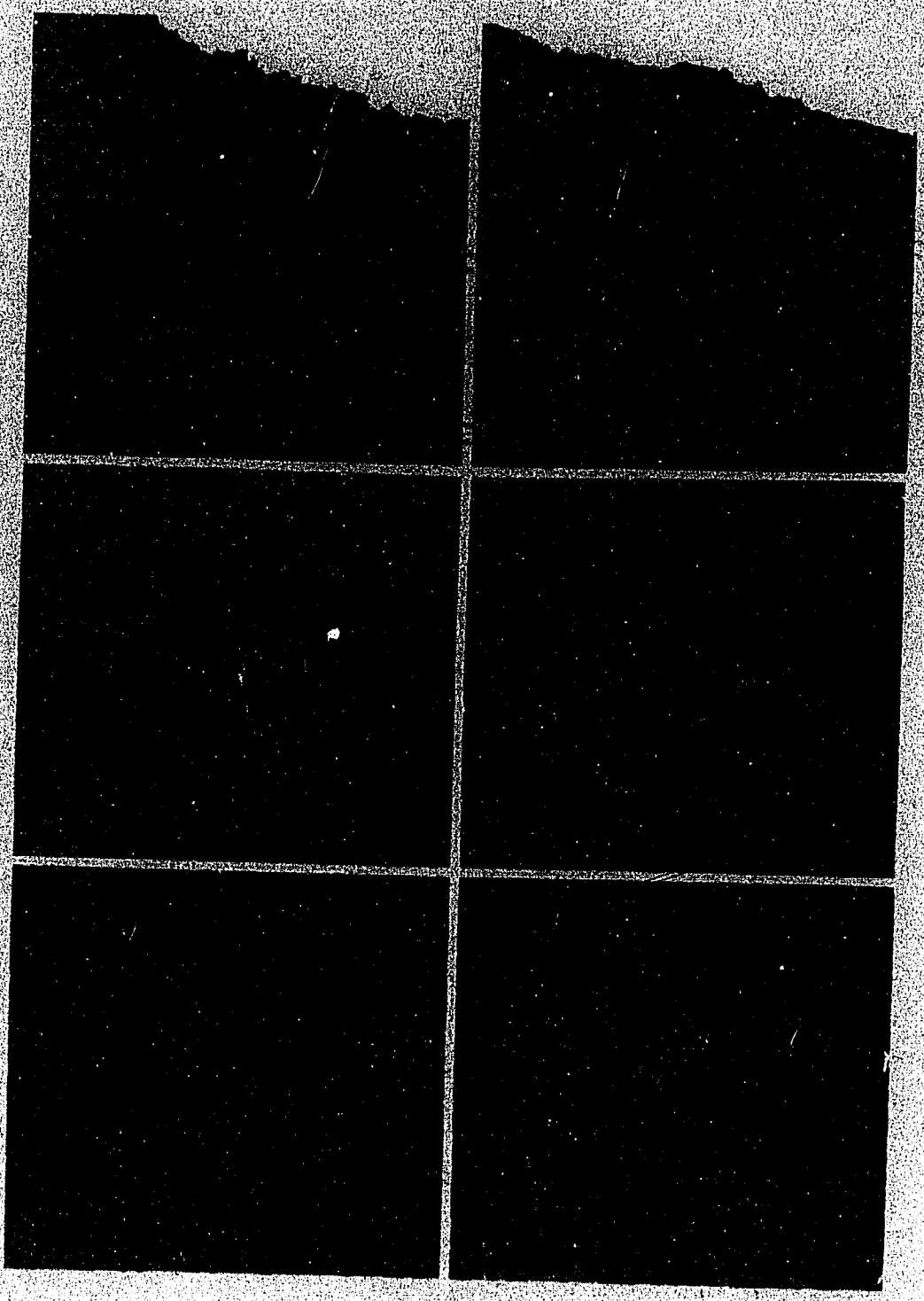
Figs. 63,64. Silver impregnation. Outlining of two nodules. In each figure, the lower arrow points to the reticular fibers arranged in semi-circular fashion around each nodule and its associated follicle, appearing like baskets hanging from the capsule. In Figure 63, the left arrow indicates a fiber curving towards the left to form an adjacent similar basket. Note the greater concentration of fibers along the inner margin of the tangentially-cut nodule in Figure 64, which was close to the hilus, whereas that in Figure 63 was in the cortex opposite the hilus. Magnification about 125X.

Figs. 65,66. Silver impregnation. Outlining of three mantled nodules cross-sectioned through their dark area uncovered by follicle. The right nodule in Figure 65 being heavily outlined with fibers, like that in Figure 64, its fiber outlining shows as a very thick crown. The nodule in Figure 66, and particularly the left one in Figure 65, shows a more regular type of fiber outlining. A subnodular space can be distinguished along the lower part of the latter nodule. Magnification about 75X.

Fig. 67. Silver impregnation. Figure showing the inner zone of a nodule and the reticular fibers constituting the inner margin of the nodule. Over the arrow are two concentric

layer of reticular fibers separated by a space: the subnodular space of this nodule. Magnification about 400X.

Fig. 68. Dominici technique. Same view as in Figure 67. The subnodular space outlining the inner zone of a nodule shows a light band in between the nodule and the population of small lymphocytes in the cortical extrafollicular area below the nodule. Towards the center of the figure, the subnodular space contains large and light-staining autofluorescent cells. Magnification about 500X.



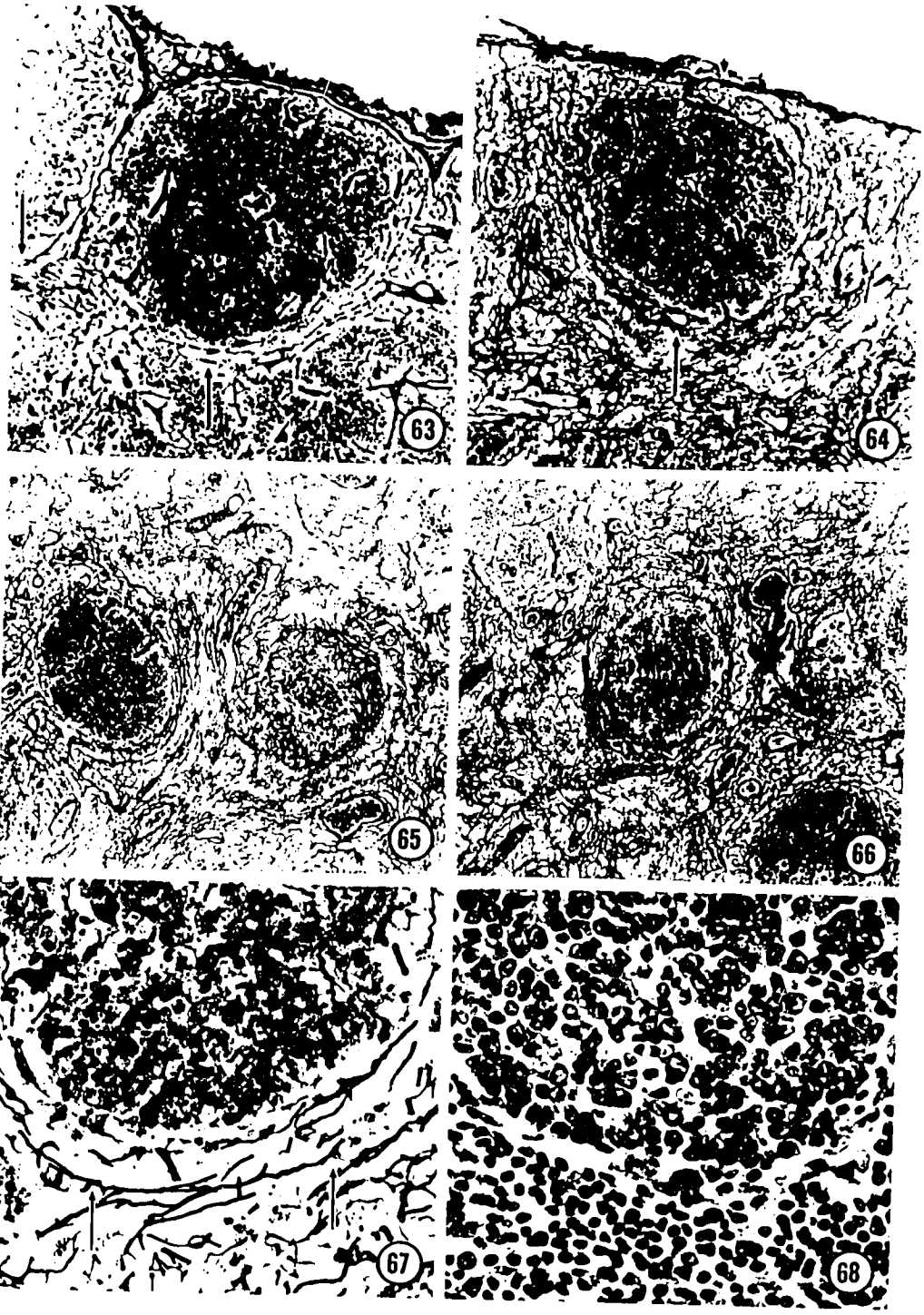

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Fig. 69. Schematic diagram showing the lymphatic vascularization of the node. Two afferent lymphatic vessels pierce the capsule to open into the subcapsular sinus (light area beneath capsule) located in between the capsule and the cortex below. In two sites, the sinus extends into the cortex forming long and narrow interfollicular sinuses. At the cortico-medullary junction, these latter sinuses transform in the wider and irregularly shaped medullary sinuses (white areas between patchy medullary cords). At the hilus (central part at bottom of figure) the medullary sinuses fuse together to form the efferent lymphatic vessel. Note that, close to hilus, the subcapsular sinus is wide and contributes to the formation of the efferent lymphatic vessel or opens into it. The diagram also illustrates the pathway of main blood vessels. The hilar artery as well as the medullary and cortical arterioles are black, whereas the hilar vein and the medullary venules are light and partly striped. The postcapillary venules in the cortical extrafollicular area are similar to the medullary venules, but shaded. Note that the medulla comes in contact with the nodules close to the hilus and that these follicular nodules are smaller than in the cortex opposite the hilus. Hence, close to hilus, the postcapillary venules are short and indistinct, or are absent altogether.

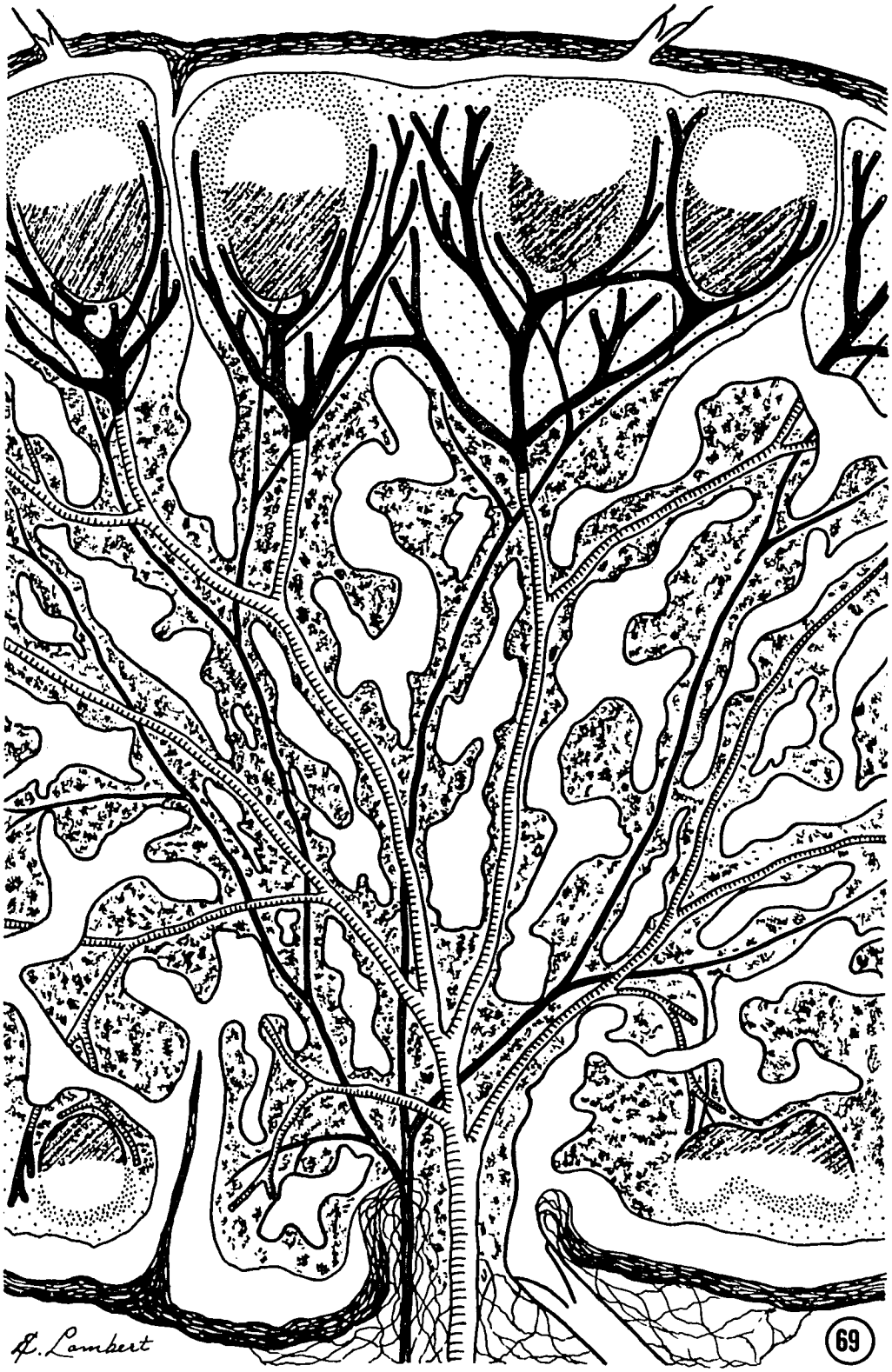

E. Lambert

Fig. 70. Realistic diagram of the arterial vascular pattern of the medulla to show the topography of the main arterial vessels, in a slice of the node including the hilus. The solid line represents the inner limit of the capsule and the broken line represents the cortico-medullary junction. The artery of the node divides at the hilus, giving rise to a major artery for each branch of the bifurcating hilus. Note that branching of arterioles occurs mainly within the hilus and along the cortico-medullary junction.

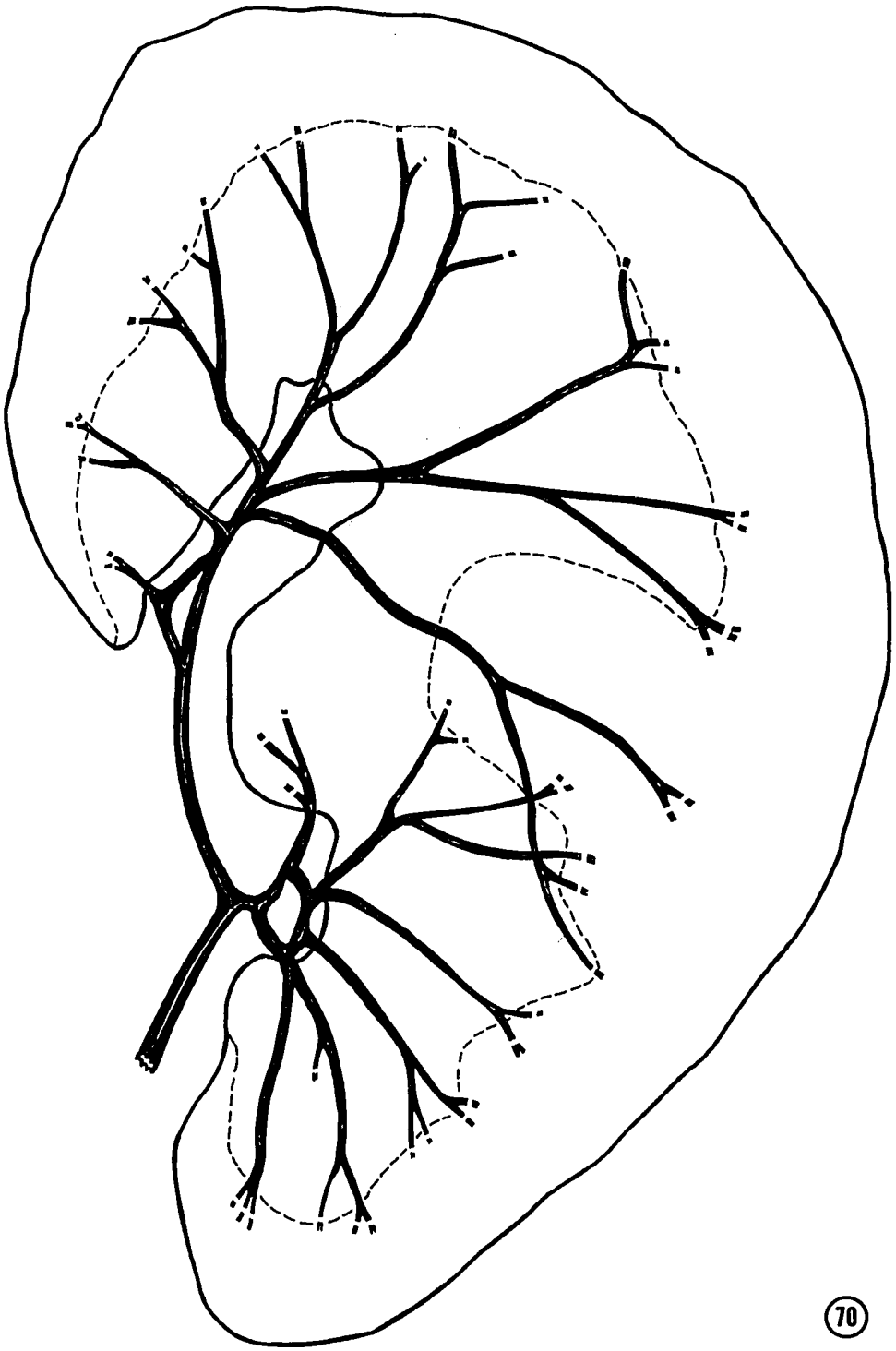
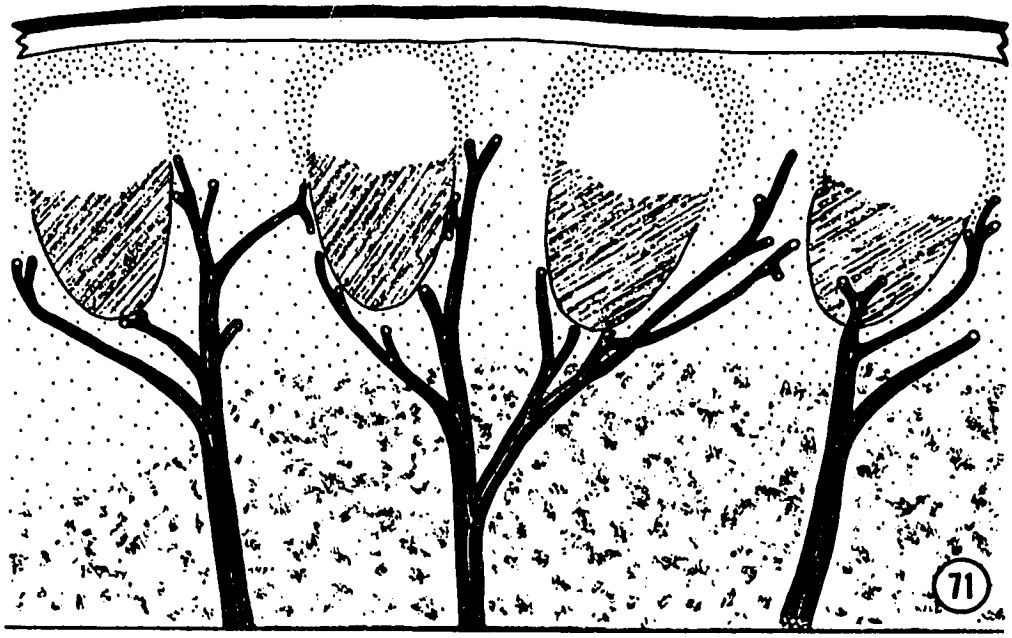


Fig. 71. Realistic diagram of the bed of fine arterioles irrigating a portion of cortex in a slice of the node similar to that seen in Figure 70. The areas of dense dots over the nodules represent the follicles, whereas the area showing spaced-out dots represents the cortical extrafollicular area. The blank circles at the ends of the arterioles indicate the origin of the continuous capillaries. Note that arterioles enter a nodule or a follicle by its inner or lateral margin and show no or very slight penetration inside these structures.

Fig. 72. Realistic diagram of the bed of postcapillary venules draining the same portion of cortex as that shown in Figure 71. The circles on the tip of the venules indicate the end of the drained capillaries. The indented opening at the beginning of a large postcapillary venule indicates continuation with another neighboring postcapillary venule. The postcapillary venules develope in the cortical extrafollicular area, the nodules and follicles containing capillaries only. The postcapillary venules that appear here as though located in the nodules or follicles are not actually inside these structures, but are adjacent to them and running in front of them (those passing behind are not represented). At the cortico-medullary junction, the postcapillary venules become transformed, in that they fuse together to give rise to the few medullary venules. Note the existence, close to the

junction, of anastamoses between postcapillary or medullary venules, the anastamosing segments often being narrower than the anastamosed venules.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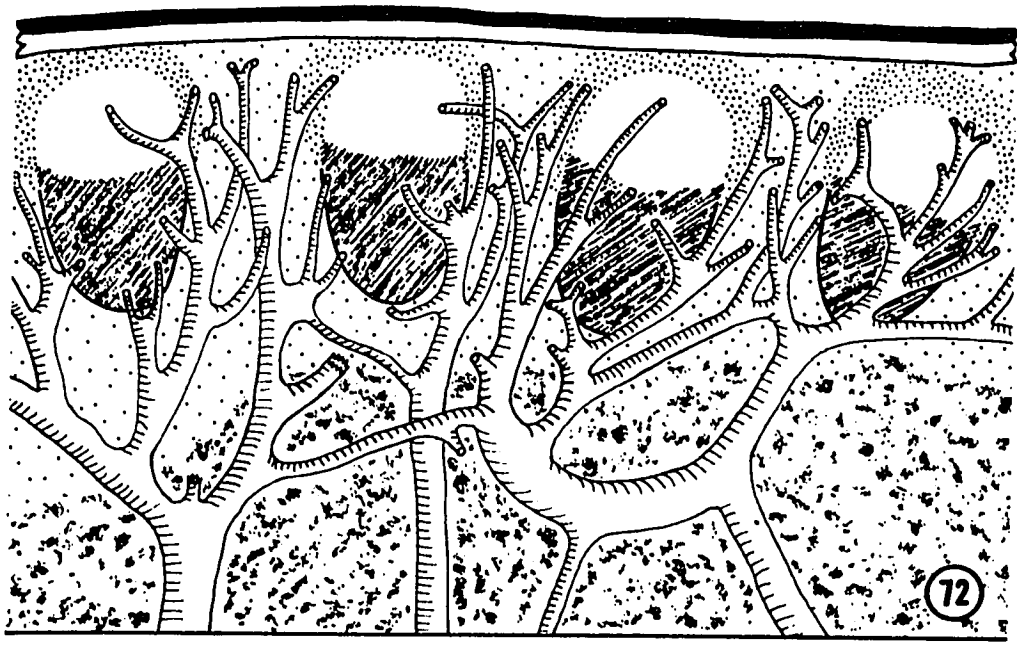
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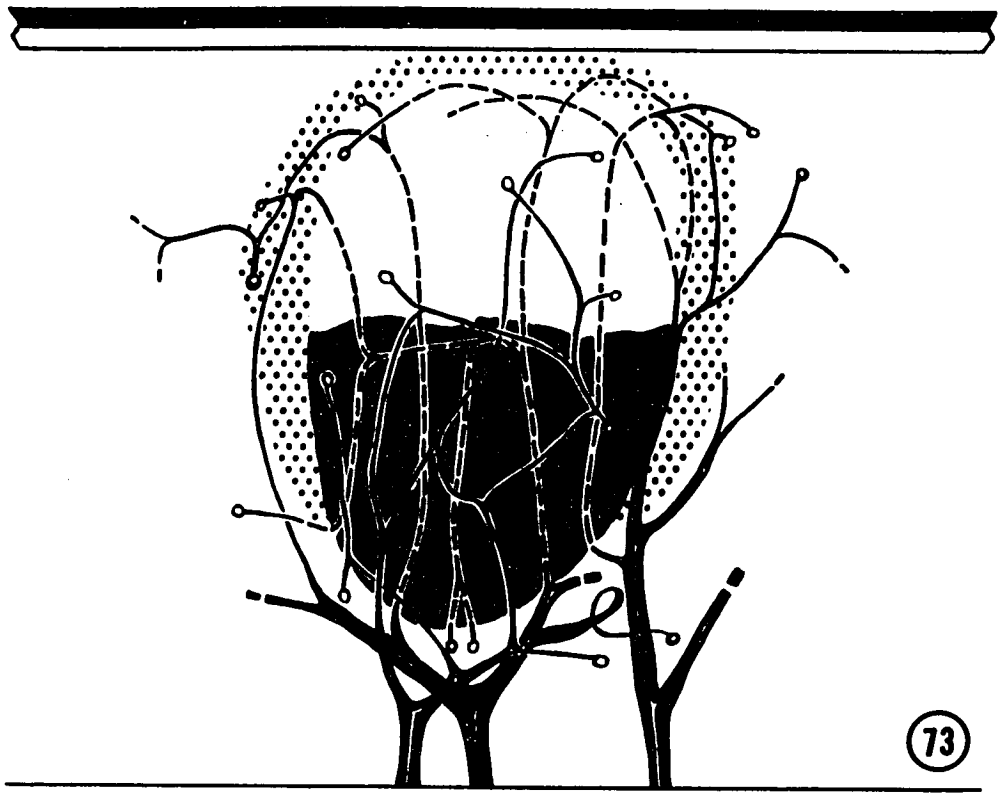
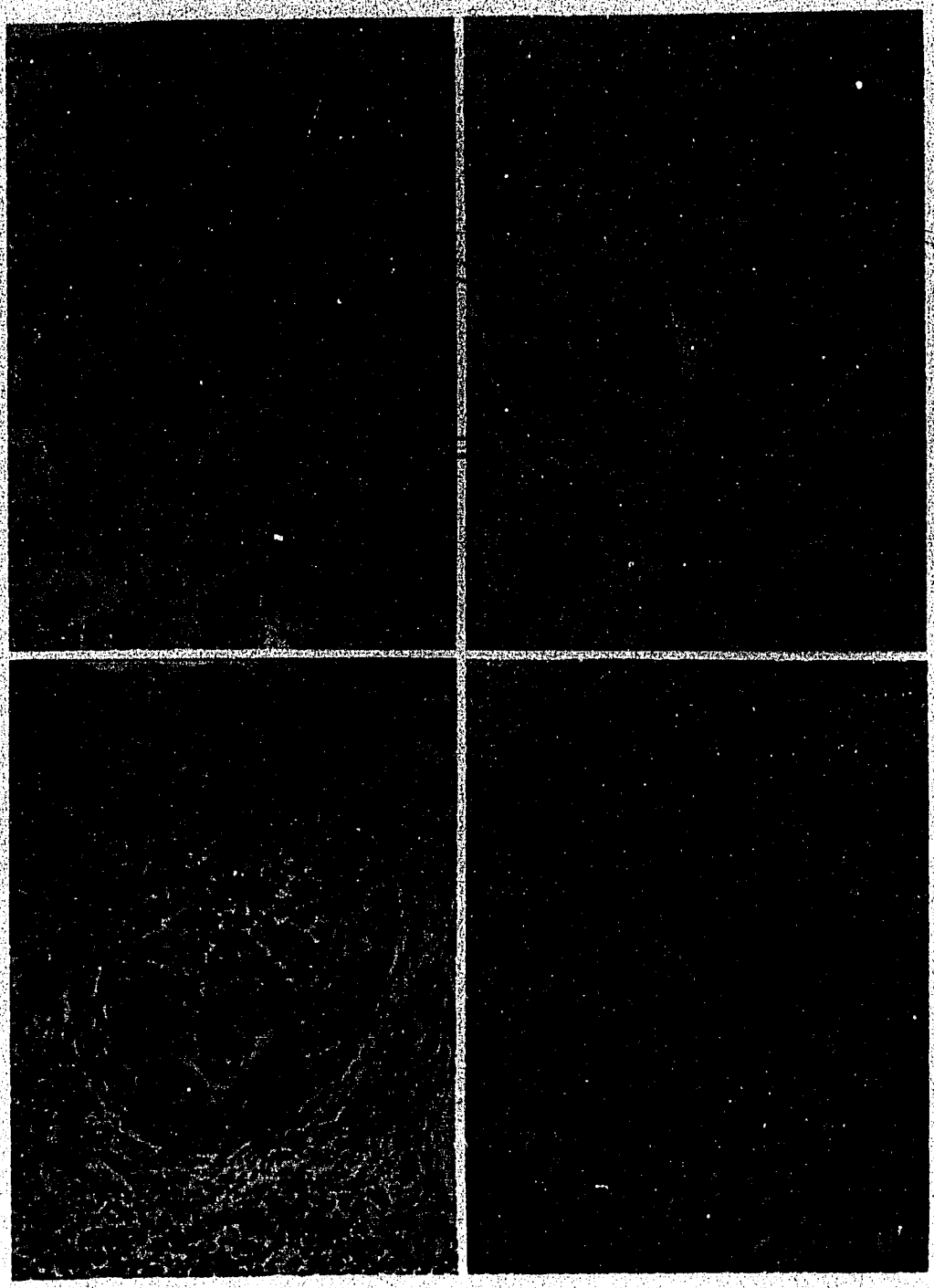
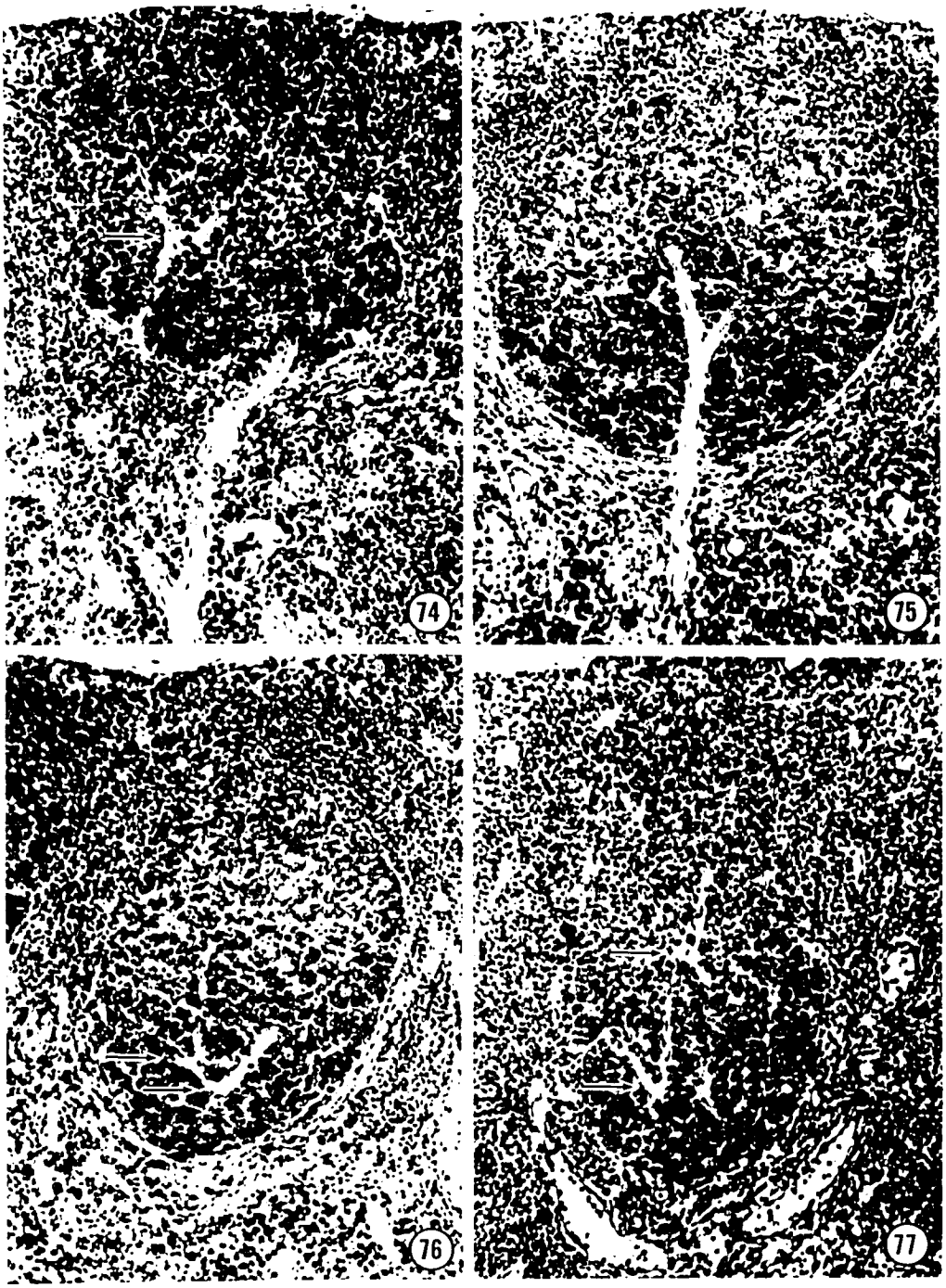
Fig. 73. Realistic diagram of most blood vessels of an entire mantled nodule, the segments of capillaries running towards, and leaving from the back of the nodule not being represented here. Three arterioles entering this structures give rise to capillaries along its inner and lateral margin. The capillaries passing through the nodule or its associated follicle are represented by broken lines, whereas those running along the margin of this follicular nodule are represented by solid lines. The circles at the end of capillaries indicate the site of their transformation into postcapillary venules. In the case of capillaries going through or along the follicular nodule, this transformation occurs along the margin of this structure. A capillary ending in a broken line indicates continuation of this capillary. Note that capillaries branch in the dark as well as in the light area of the nodule.



Figs. 74,75. Dominici technique. Tangentially-sectioned mantled nodules. In Figure 74, an arteriole branches at the cortico-medullary junction (bottom of figure), one of the branches running towards the dark area of the nodule. At the inner margin, it becomes a capillary entering that nodule. The arrow points to the branching of another capillary at the limit between the dark and light area of the nodule. In Figure 75, an arteriole similarly reaches the inner margin of a nodule, crossing its dark area where it branches into capillaries. Magnification about 170X.

Figs. 76,77. Dominici technique. Mantled nodules cut nearly longitudinally. Arrows in Figure 76 indicate a double branching of a capillary in the dark area of the nodule. Lower and upper arrows in Figure 77 indicate the branching of capillaries in the dark and light areas of a nodule, respectively. The wide blood vessel at the bottom of the latter figure is a postcapillary venule into which is opening a capillary leaving the left side of the associated follicle. Magnification about 170X.

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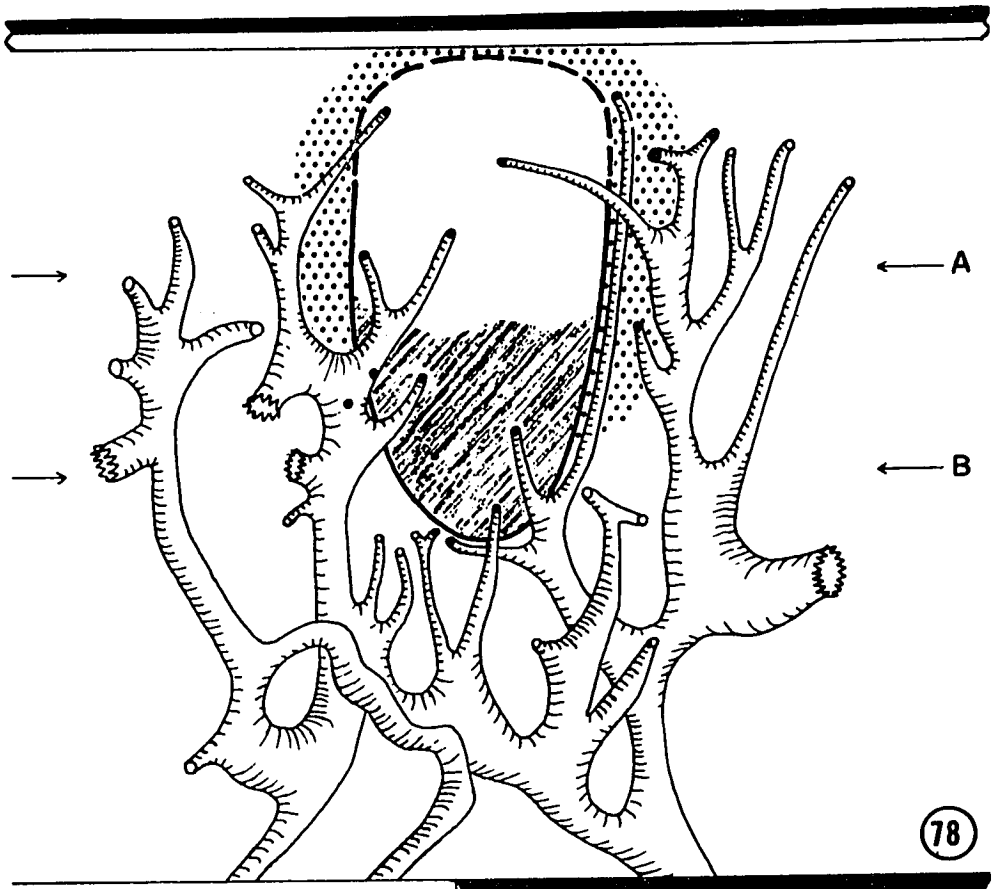
Figs. 78A,B, 79. Realistic diagrams of the blood vascular pattern of a mantled nodule. Figure 79 shows the capillary network of this nodule in the same manner as in Figure 73. In addition, the black dots ending most of the capillaries correspond to the black dots in Figure 78 and represent the point of transformation of the capillaries into the postcapillary venules of the latter figure. Figure 78 represents the front half of the network of postcapillary venules draining the capillaries of the nodule seen in Figure 79, as well as those in the surrounding cortical extrafollicular area. The blank circles at the beginning of some of the postcapillary venules indicate the site of transformation into these venules of capillaries of the cortical extrafollicular area. Arrows A and B indicate the level of reconstruction of the cross sections of the present follicular nodule, with its associated postcapillary venules shown in Figures A and B, accordingly (lower left corner of the plate). In A, note that the postcapillary venules (ovals and circles) are situated outside the follicle and, thus, do not come in contact with the nodule. In Figure B, the section passing through the portion of the nodule not covered by the follicle, the postcapillary venules come in contact with the nodule but not penetrate it.



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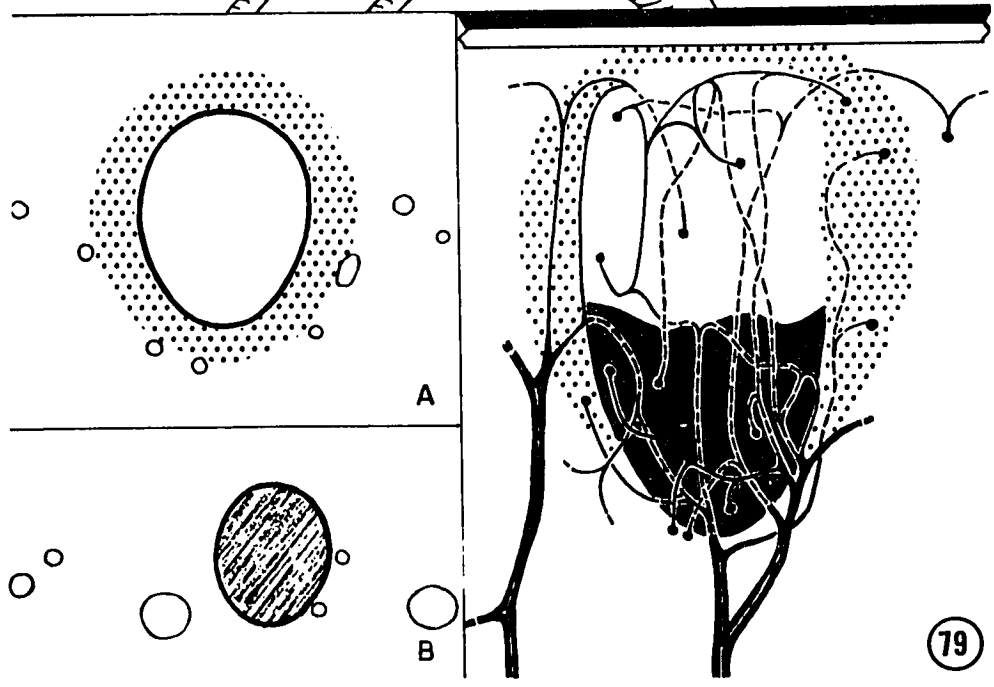

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Fig. 80. Silver impregnation. Preparation showing a cross section through the dark area of a mantled nodule, not covered by its follicle at this level of sectioning as in diagram B of Figure 78. The eight arrows point to cross sectioned postcapillary venules adjacent to but outside the nodule. In the upper part of the figure, a longitudinally-sectioned arteriole (A) is seen approaching the nodule inside which it gives rise to capillaries. Magnification about 130X.

Fig. 81. Dominici technique. This shows the same view as in Figure 80. Postcapillary venules are seen outside the nodule, one of which comes in close contact with its margin. Magnification about 160X.

Fig. 82. Silver impregnation. Preparation showing a cross section through the light area of mantled nodule, as in diagram A of Figure 78. The horizontal arrows point to capillaries, most of which are located along the nodular margin, i.e., between the nodule and its associated follicle. The vertical arrows indicate post-capillary venules located outside this follicle, many being adjacent to it. Magnification about 130X.

Fig. 83. Schematic diagram of a longitudinal section of a mantled nodule. This schematizes the location of the postcapillary venules (PCV) as being outside the follicles (F) and nodules, though they often come in contact with the inner margin of nodules, when not covered by the associated follicle, and usually with the lateral margin of the follicles.

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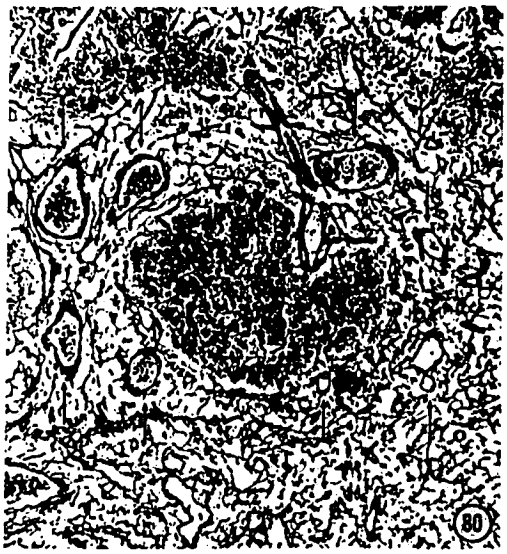
PCV



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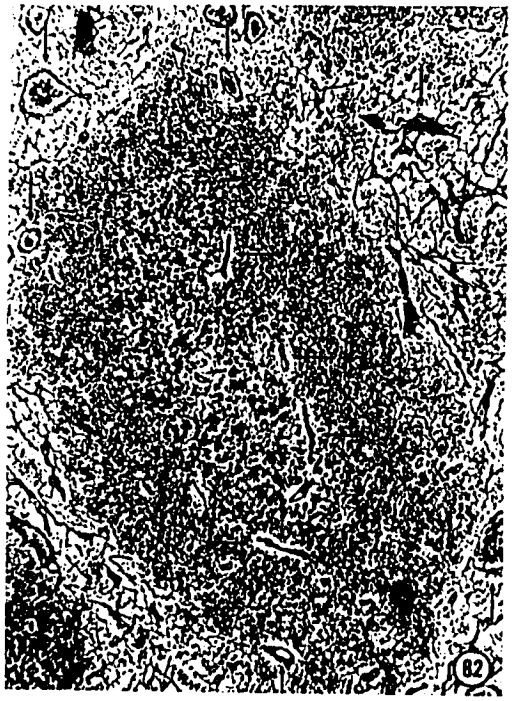

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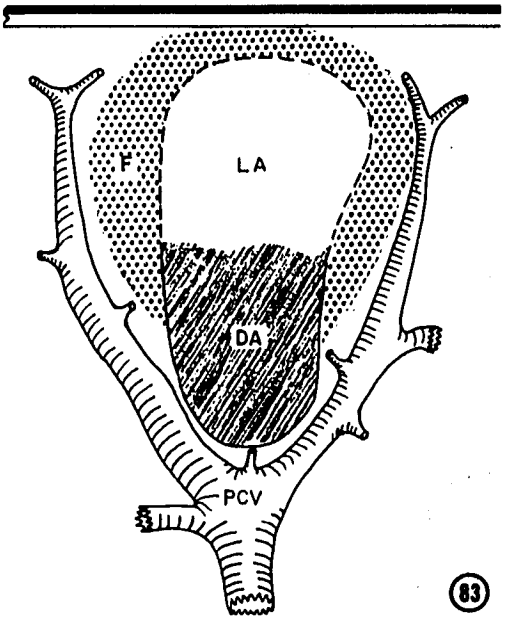

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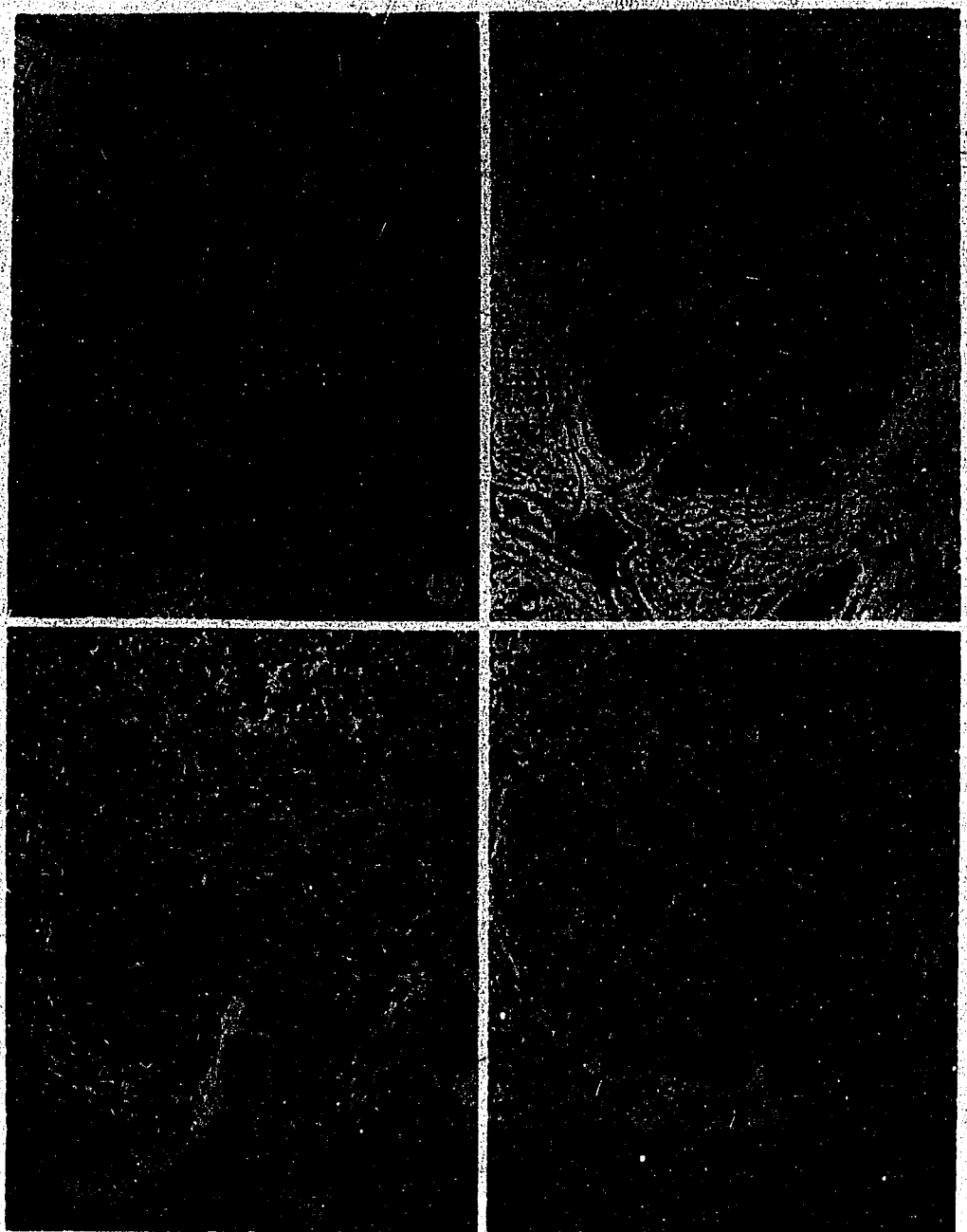
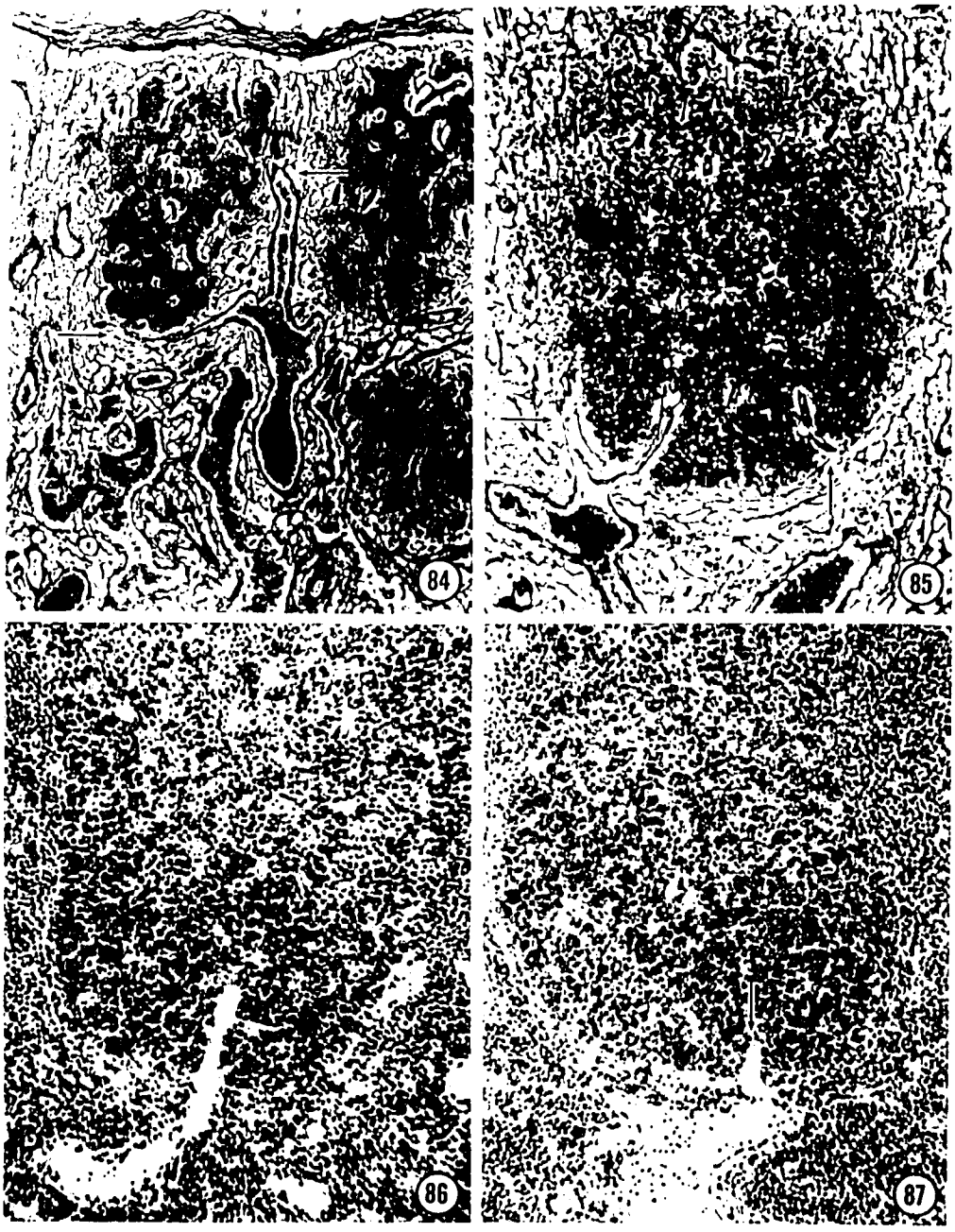
Fig. 84. Silver impregnation. Tangential section of a mantled nodule. The lower arrow points to a site of the inner margin of the nodule where a capillary leaving the inner zone of the nodule transforms into a postcapillary venule. The upper arrow points to a site of the lateral follicular margin where a capillary leaving the follicle transformed abruptly into a postcapillary venule. Magnification about 170X.

Fig. 85. Silver impregnation. Tangentially-sectioned mantled nodule. The right arrow indicates a capillary leaving the dark area of the nodule and which entered the postcapillary venule seen a little to the right of the tip of arrow. The left arrow points to one of two capillaries similarly leaving the nodule and joining the postcapillary below. Magnification about 170X.

Figs. 86,87. Dominici technique. Tangential sections of mantled nodules. In both figures, a capillary (arrow in Figure 87) leaves the dark area of the nodule to anastomose with a postcapillary venule outside the nodule, as occurs in Figure 85. Magnification about 170X.

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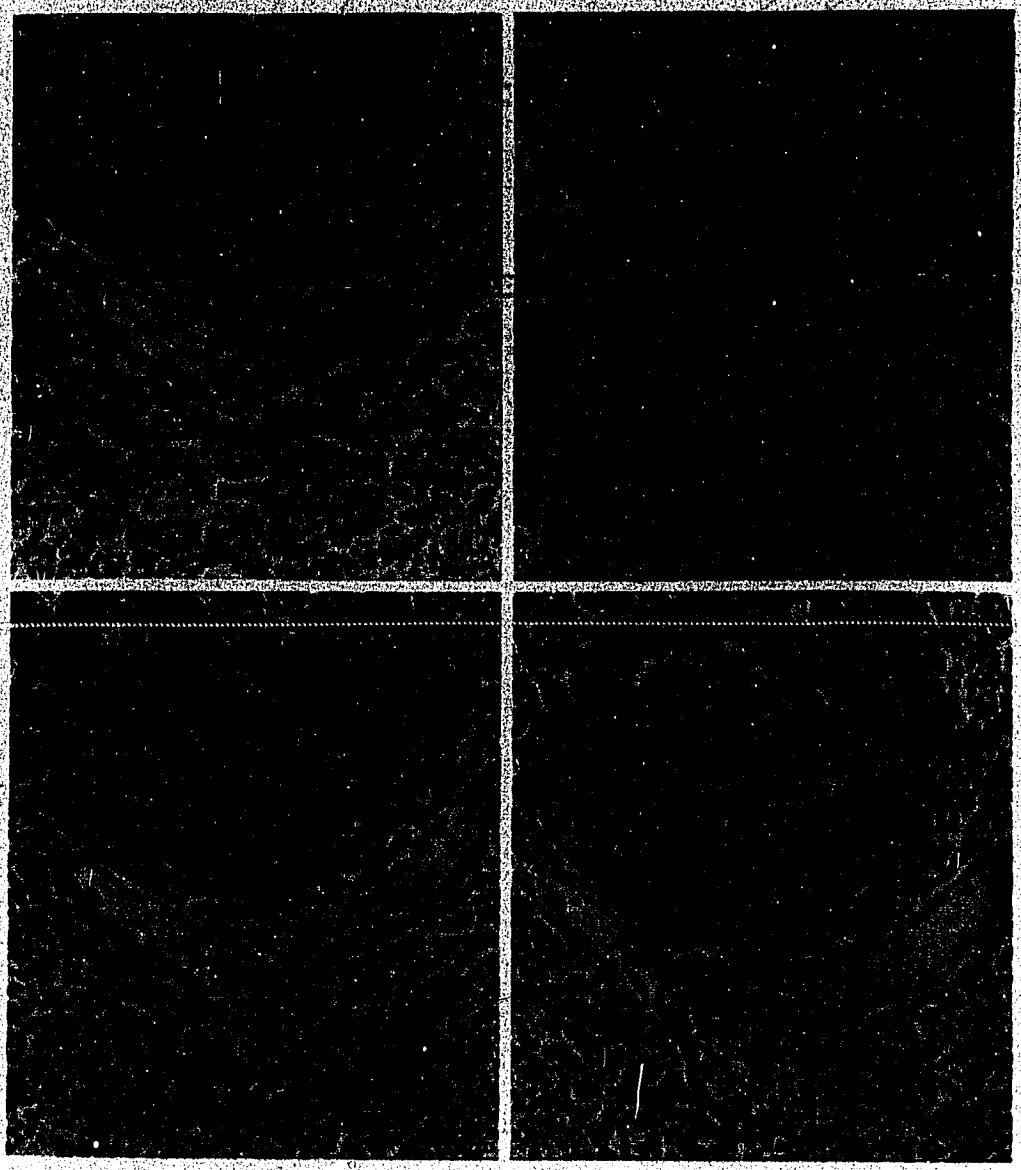
Figs. 88-91. Dominici technique. Four serial sections of a mantled nodule with a postcapillary venule adhering to its inner margin. The endothelial cells show no, or very little, hypertrophy on the upper side of the venule in close contact with the nodule; whereas they are quite hypertrophied on the opposite side in contact with the population of small lymphocytes of the cortical extrafollicular area. The arrow in Figure 91 points to a very hypertrophied endothelial cell bulging deeply into the lumen of the vessel. Magnification about 280X.



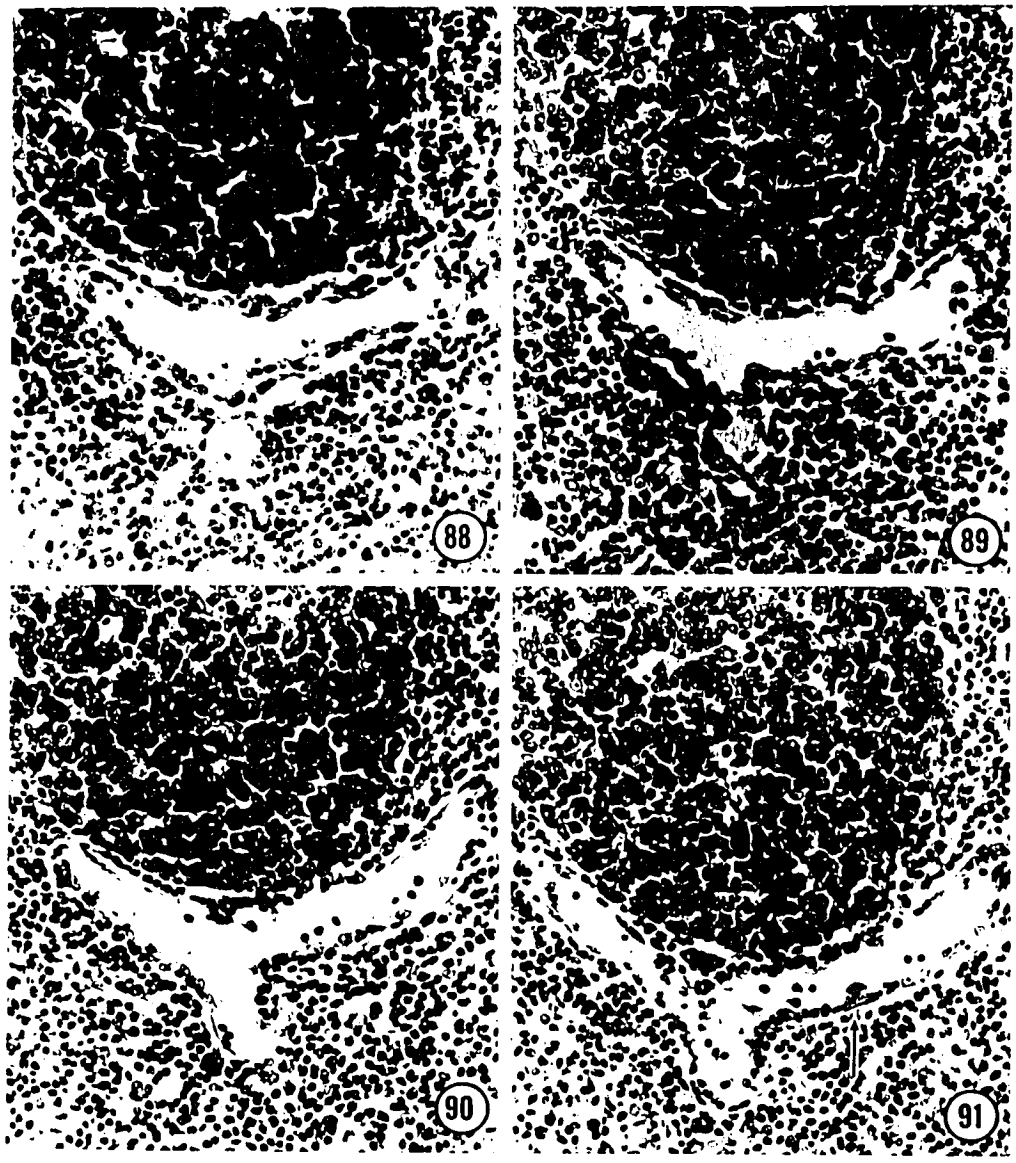

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Fig. 92. Realistic diagram like that of Figure 72, of the network of postcapillary venules around the complex nodule illustrated in Figures 35-38. No venules are present in the nodule or the associated follicle, all being located outside them as with the single nodules. The postcapillary venules draining each of the five single nodules united in this complex structure are quite anastomosed with one another.

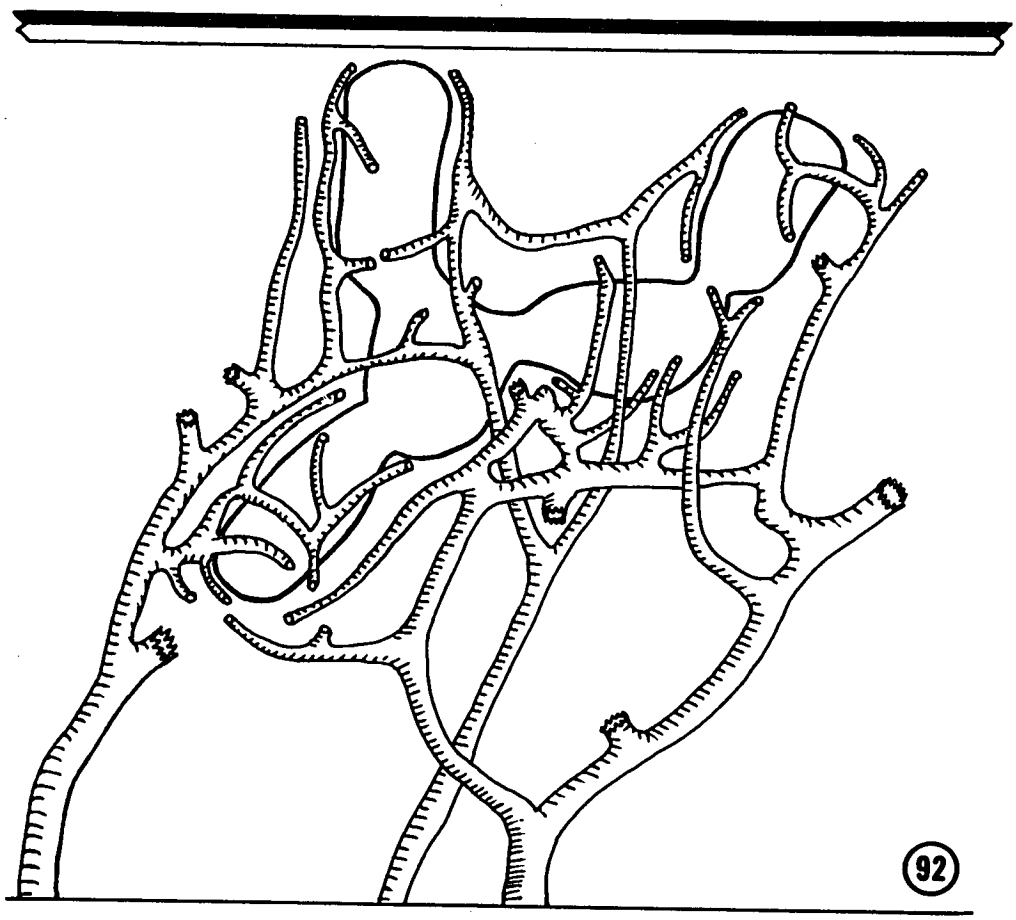
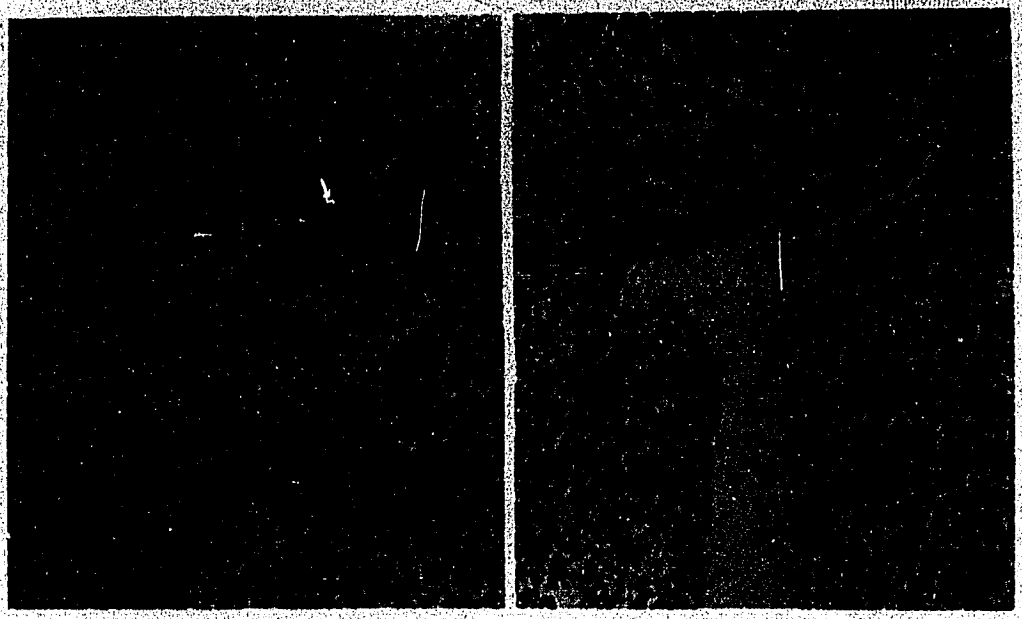


Fig. 93. Dominici technique. Figure showing a portion of the cortical extrafollicular area, which exhibits a venule originating from a capillary slightly below the capsule. An exceptional finding in the cortex, the segment of this venule has a regular flat endothelium seen above the arrow; the venule exhibiting the hypertrophied endothelial cells of a postcapillary venule is seen just below the arrow. Magnification about 180X.

Fig. 94. Dominici technique. Cortical extrafollicular area. The upper right corner of the figure shows a portion of the dark area of a nodule. The horizontal arrow shows a capillary leaving the nodule. The endothelium of this capillary becomes highly hypertrophied quite abruptly, the narrow postcapillary venule thus formed (vertical arrow) opening into a wider postcapillary venule. Magnification about 180X.



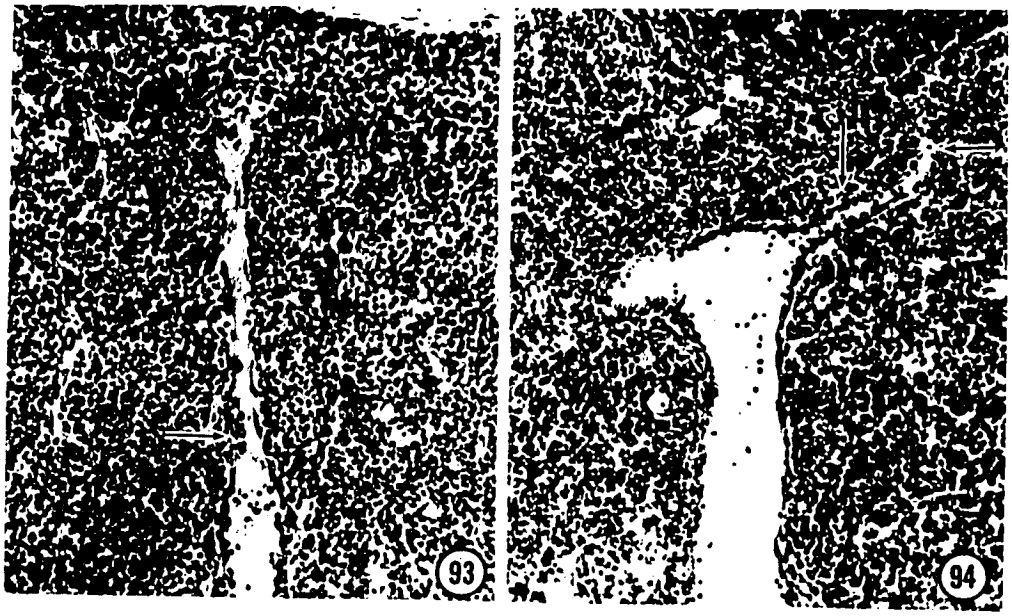

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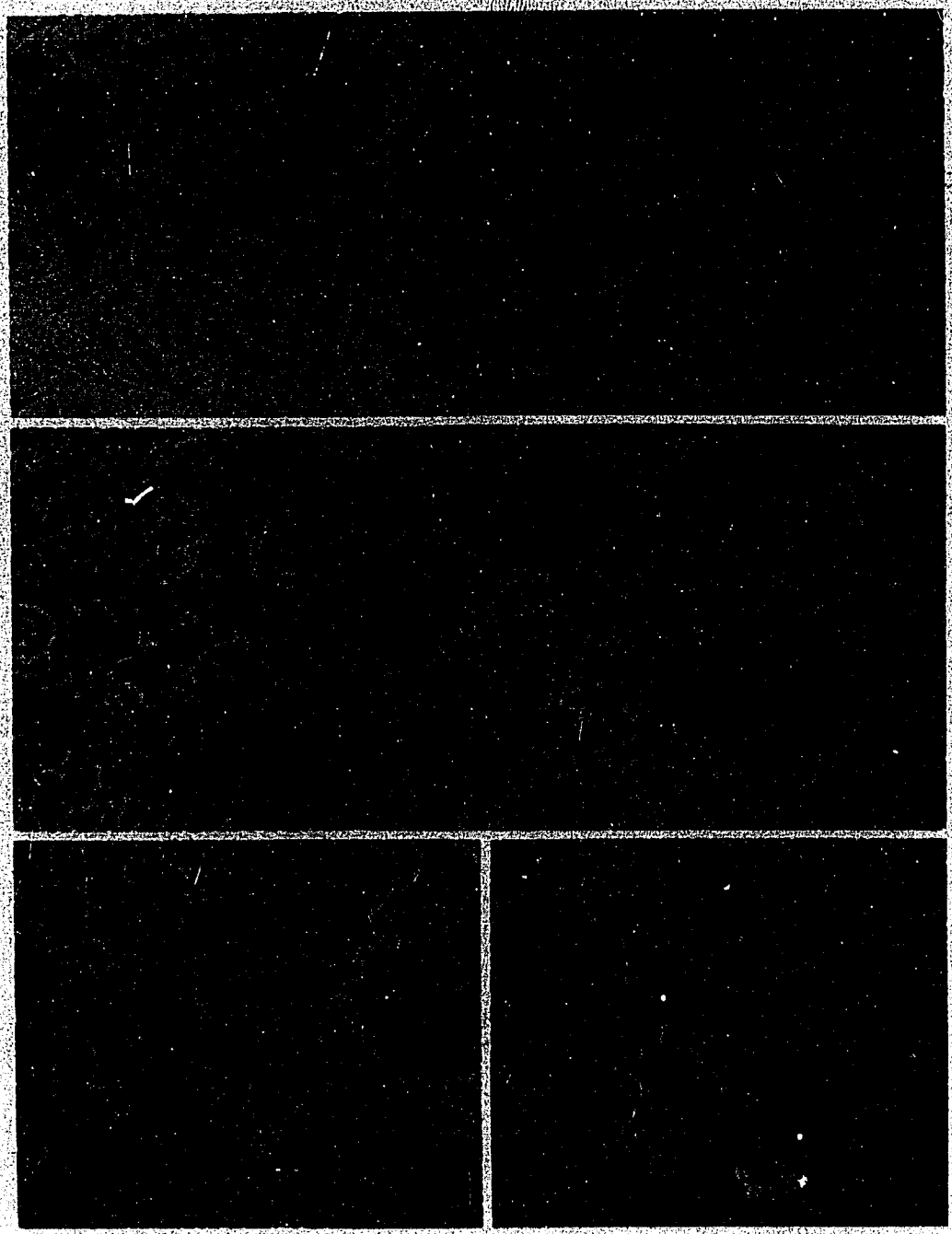
Fig. 95. Silver impregnation. Extrafollicular area close to the cortico-medullary junction. The arrows indicate three regular capillaries anastomosing between cross-sectioned, wide postcapillary venules. Magnification about 260X.

Fig. 96. Dominici technique. Same view as in Figure 95.

Fig. 97. Dominici technique. A venule (arrow) with a flat to low endothelium anastomosing between two wide postcapillary venules having a moderately hypertrophied endothelium. Magnification about 170X.

Fig. 98. Silver impregnation. Preparation showing a nodule (N) and a large postcapillary venule. The arrows point towards three narrow postcapillary venules opening into a larger one. Magnification about 170X.

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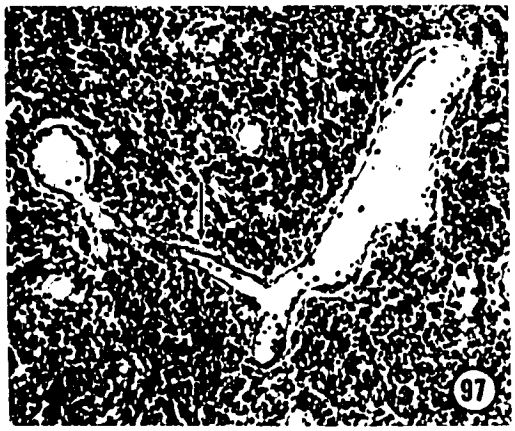
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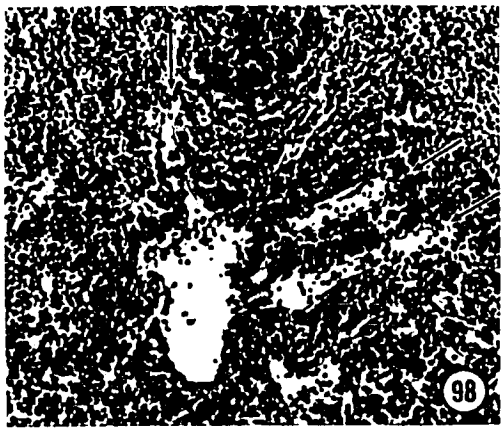
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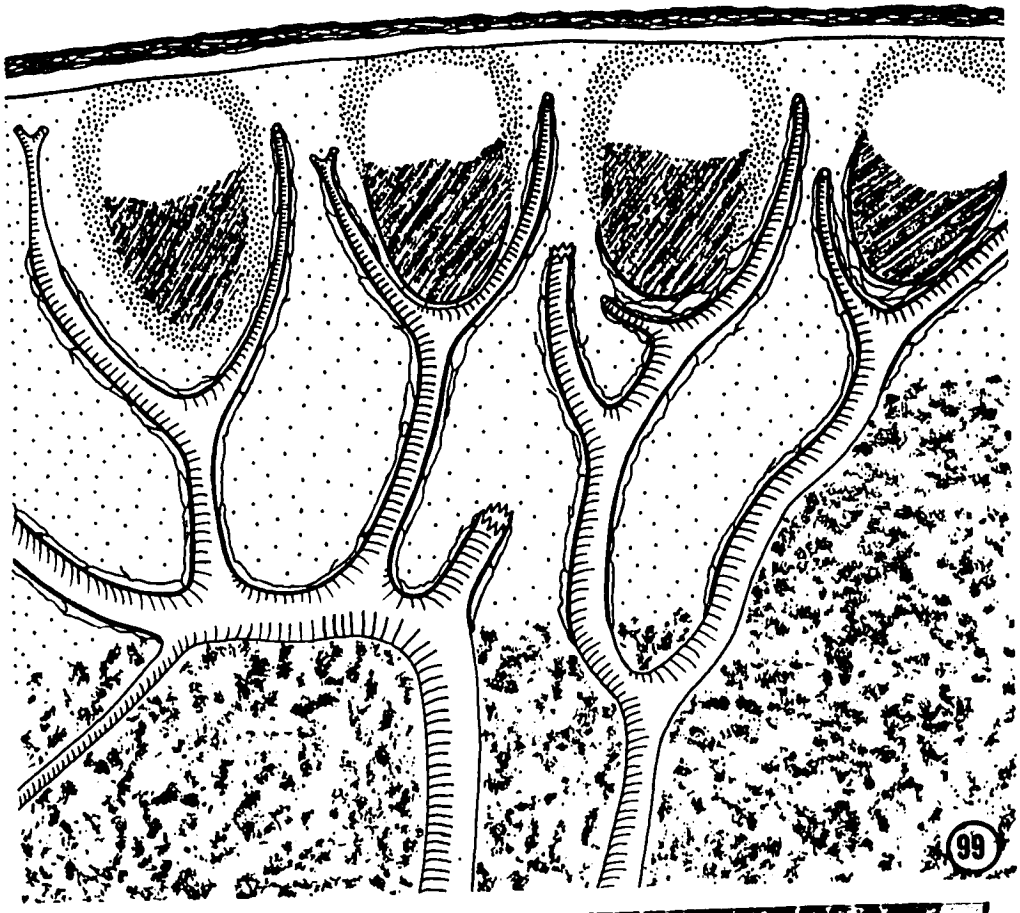
Fig. 99. Schematic diagram illustrating that only the portion of endothelium in contact with a cortical extrafollicular population of small lymphocytes has the hypertrophied endothelial cells and the perivascular channels characteristic of post-capillary venules. A hypertrophied endothelium is represented here by a thicker and darker outline of the venule wall, whereas a perivascular channel is represented by irregular lines along the thicker wall. Note that in the venules parallel to the cortico-medullary junction, the endothelium is hypertrophied on the cortical side of the venule only. Note also that the portion of venule in close contact with the inner nodular margin of the second left follicular nodule is not hypertrophied. Illustrating an exceptional case, the upper segment of the extreme left venule shows a regular endothelium.

Fig. 100. Dominici technique. A venule parallel to the cortico-medullary junction. The upper part of the figure is formed of an extrafollicular population of small lymphocytes, except over the two vertical arrows where there are plasmocytes in between the cross-sectioned and parallel-sectioned venules. Below the level of the two horizontal arrows, the figure shows medullary plasmocytes. Note that the portions of the venular wall in contact with cortical lymphocytes present hypertrophied endothelial cells, whereas other portions in contact with medullary plasmocytes have regular flat endothelial cells. Magnification about 190X.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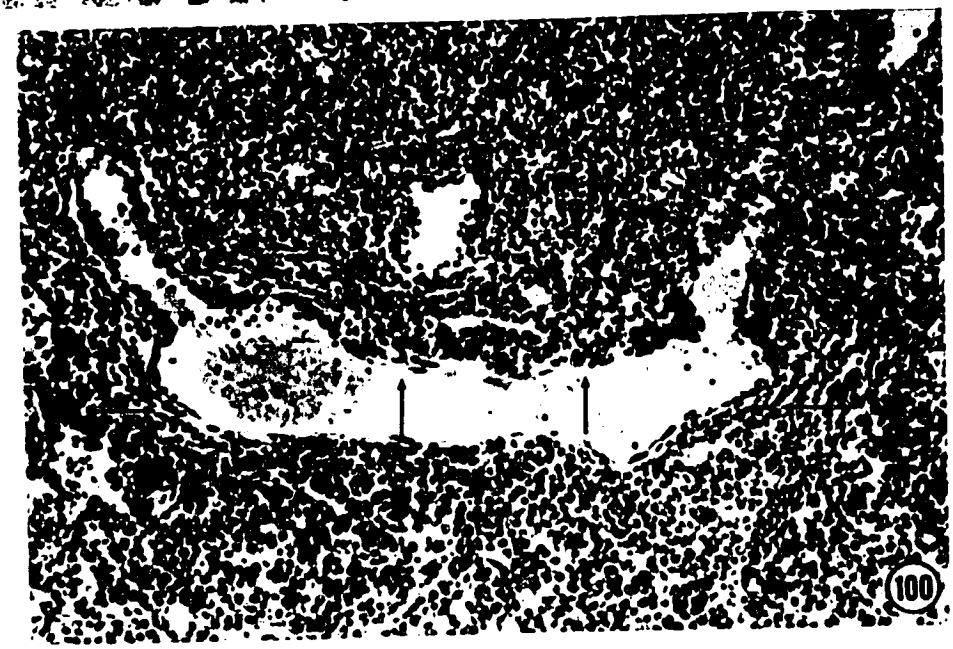
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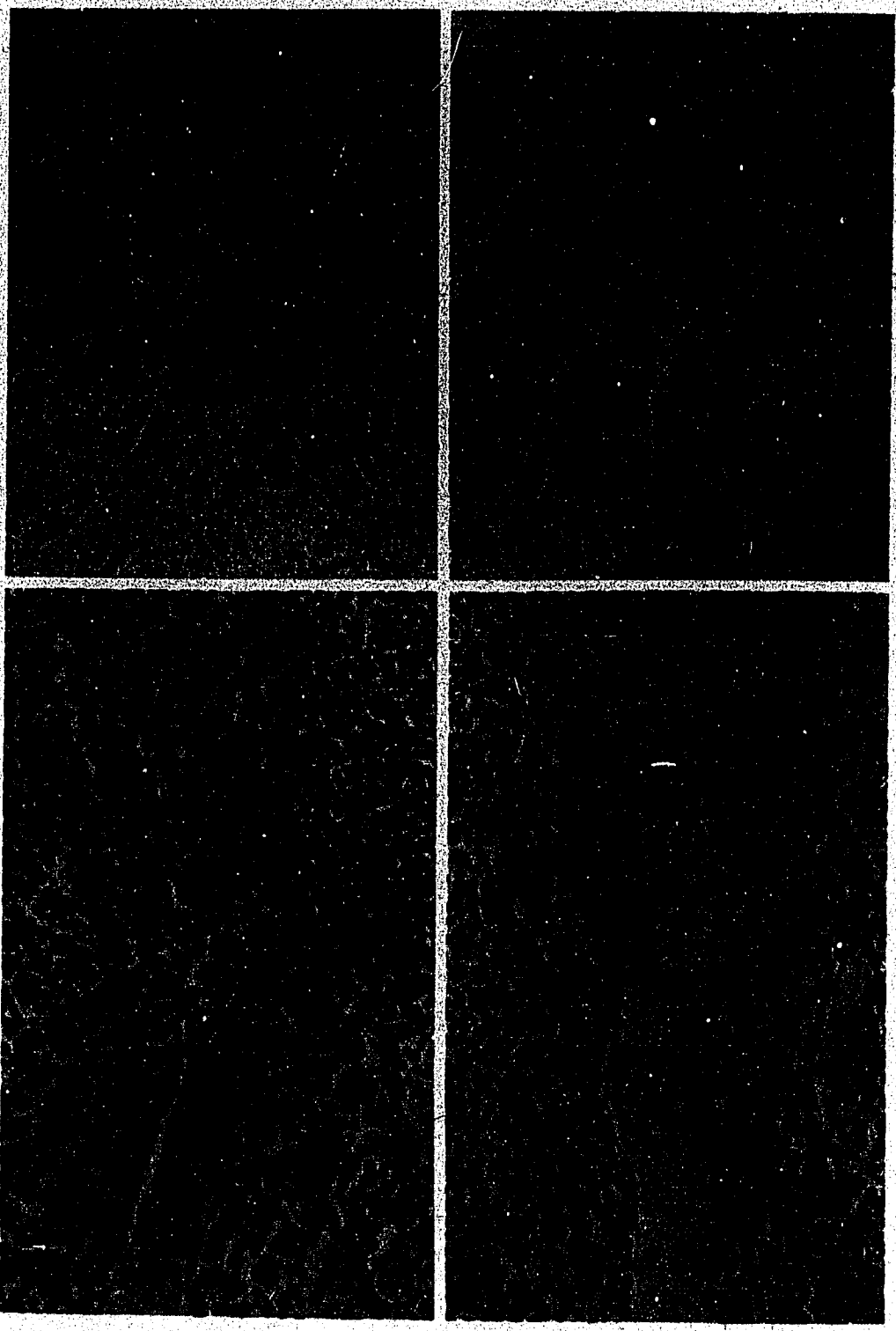
Figs. 101,102. Dominici technique. In both figures the arrows indicate the level of the vague cortico-medullary junction, the cortex being above the arrows the medulla below. The arrows also point to longitudinally-sectioned venules: postcapillary venules with hypertrophied endothelial cells above the arrows and medullary venules with flat endothelial cells below. Magnification about 110X.

Figs. 103,104. Dominici technique. Enlargement of parts of Figures 101 and 102, respectively, the arrows pointing to the same sites as in those Figures. In Figure 103, the change from a lymphocytic (above arrow) to a plasmocytic population is rather abrupt, as is the change from highly hypertrophied endothelial cells of the postcapillary venules to the flat ones in the medullary venules. In Figure 104, cortical lymphocytes and medullary plasmocytes are somehow mixed at the junction and the change in type of endothelial cells is irregular and progressive. Magnification about 320X.

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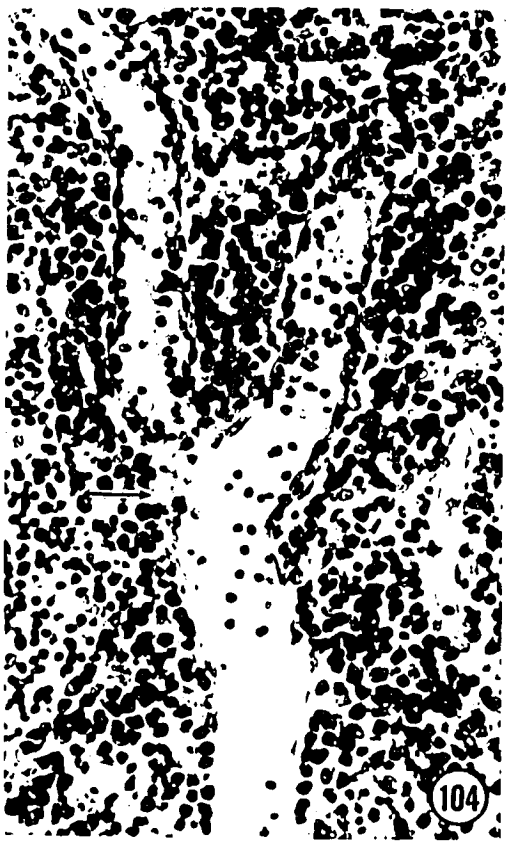
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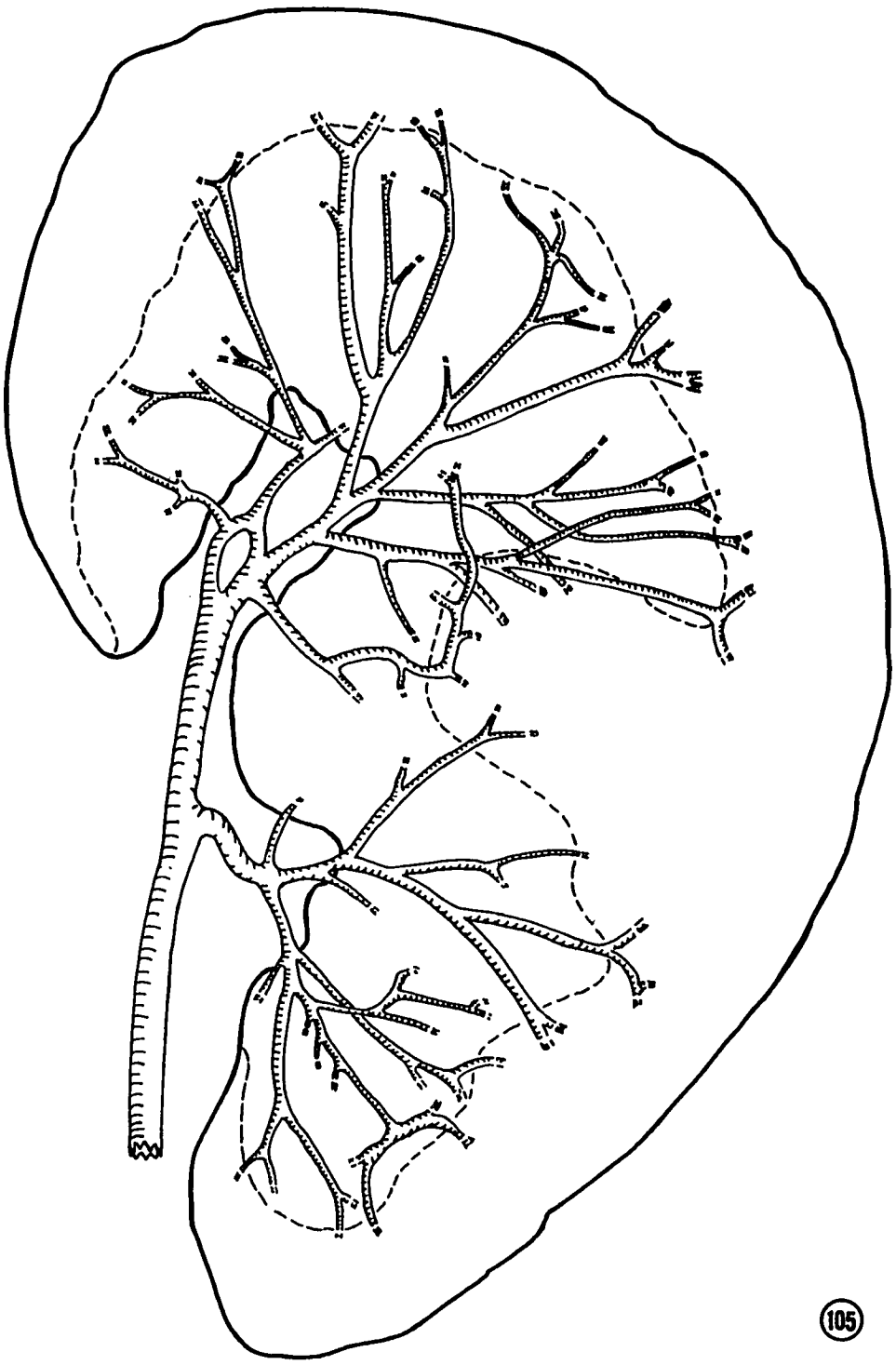
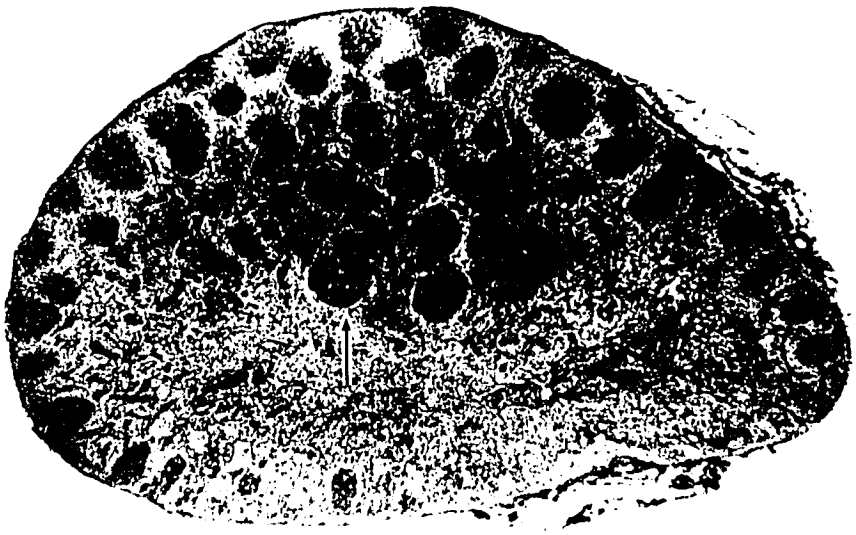
Fig. 105. Realistic diagram as in Figure 70, except that the medullary venules and the hilar vein of the node are represented. Branching of medullary venules is a little more complex than that of medullary arterioles.



Figs. 106,107. Silver impregnation. Figure 107 is an enlargement of the area pointed to by an arrow in Figure 106, and represents the cross-sectioned layer of cortex associated with the trabecula outlining the lobule of the node. In contrast to elsewhere in the node, the small lymphocytes present in the cortical extrafollicular area of this portion of cortex are silver impregnated. This cortical extrafollicular accumulation of impregnated small lymphocytes is referred to as a pseudo-follicular area. Magnification about 35X.



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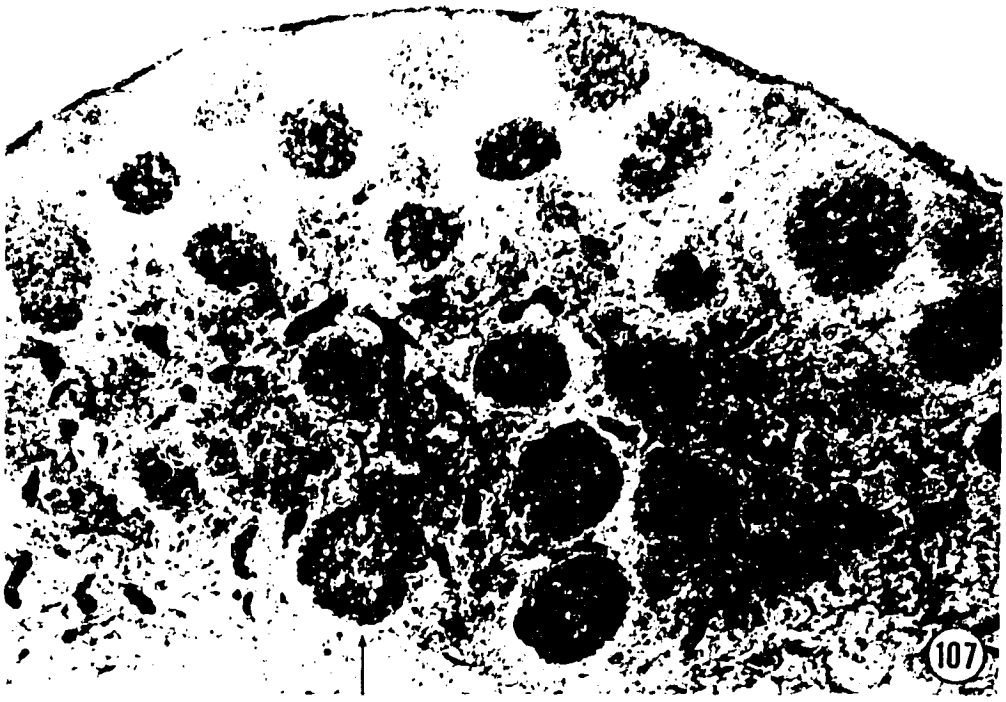

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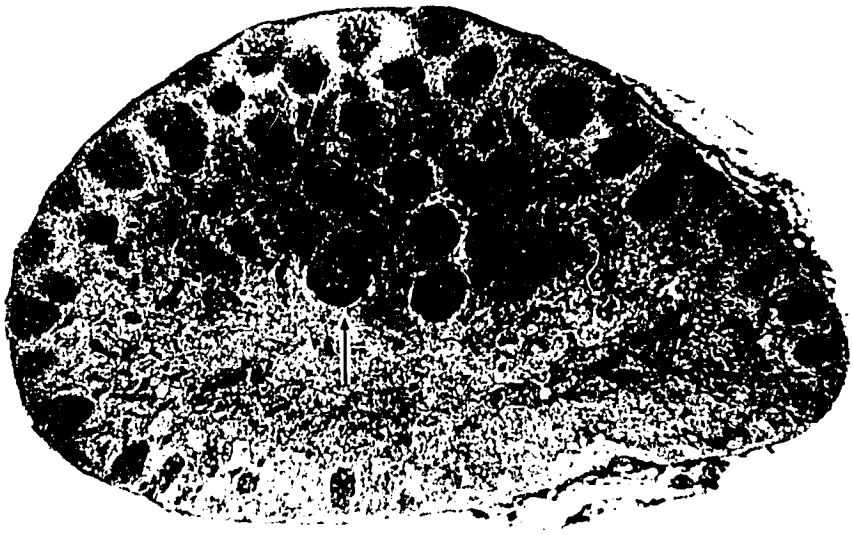

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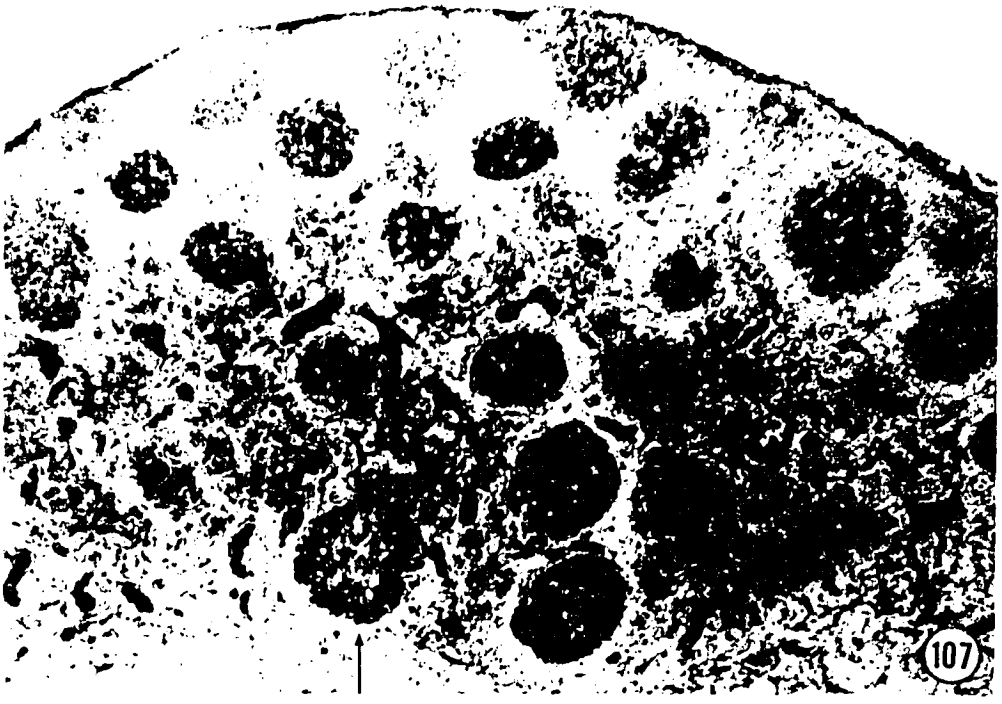

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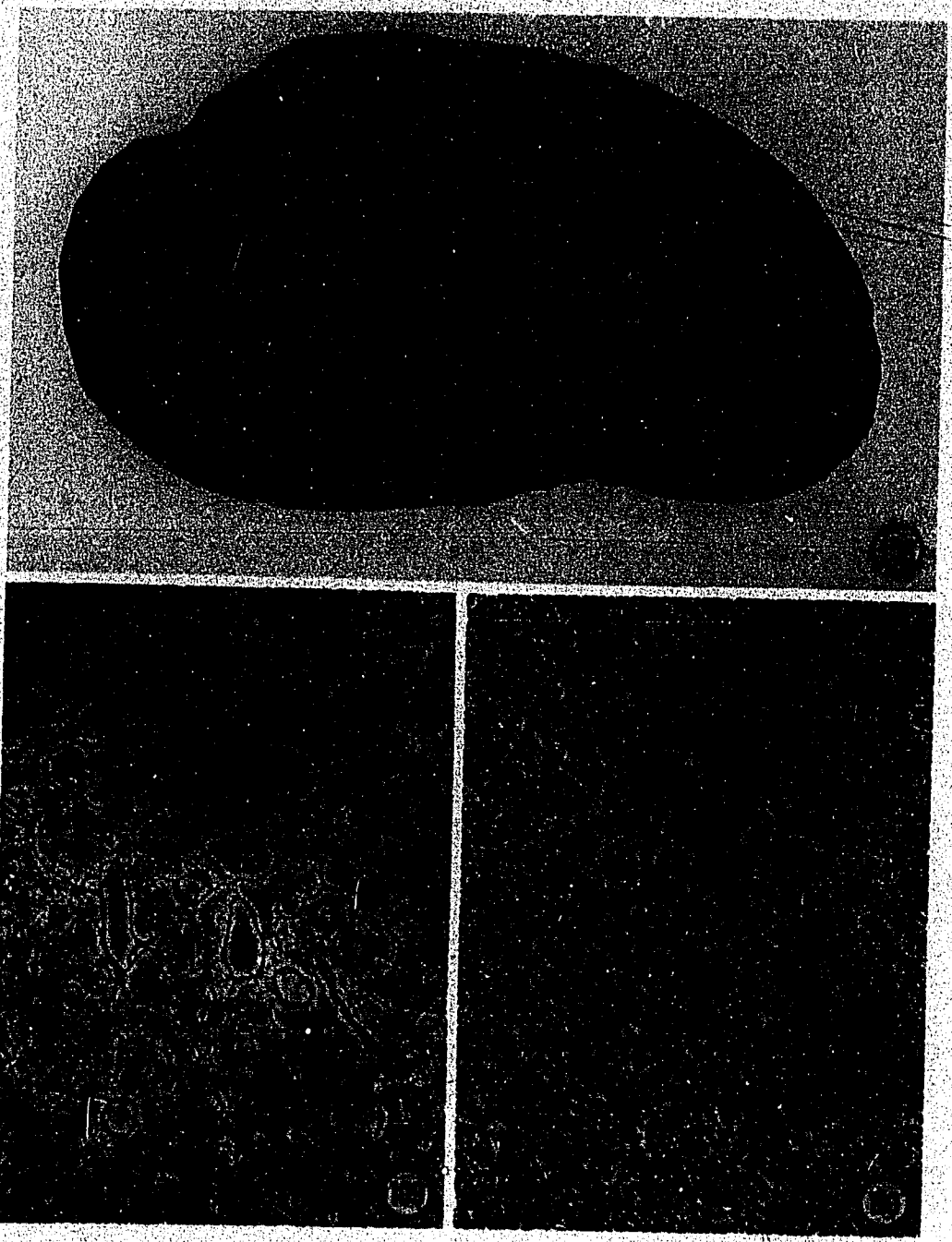
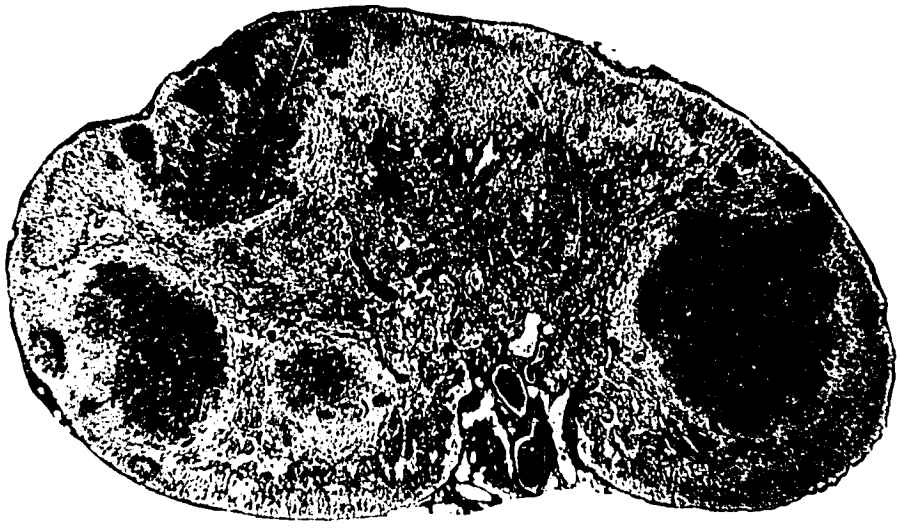
Fig. 108. Silver impregnation. Longitudinal section passing through the center of a cervical node from a 9-month-old normal rat used for this section but not depicted elsewhere in these studies. There are four pseudo-follicles (numbered 1 to 4) located in the deep layer of the cortex and in the peripheral layer of medulla adjacent to the cortex. These structures are constituted of a cortical extrafollicular accumulation of small lymphocytes, which are unusually silver impregnated like those of follicles. Note that the larger a pseudo-follicle, the darker its impregnation. Note also that the follicular nodules over these structures are darker than the others. Magnification about 25X.

Fig. 109. Silver impregnation. Enlargement of a portion of the pseudo-follicle number 3 of Figure 108. The S's are located in lymphatic sinuses filled with small lymphocytes, whereas the arrows point to postcapillary venules. Magnification about 150X.

Fig. 110. Dominici technique. Enlargement of a portion of the pseudo-follicle number 3 of Figure 108 as seen in a section adjacent to that in the latter figure. The arrows indicate cross-sectioned postcapillary venules that appear to be closed. Magnification about 150X,





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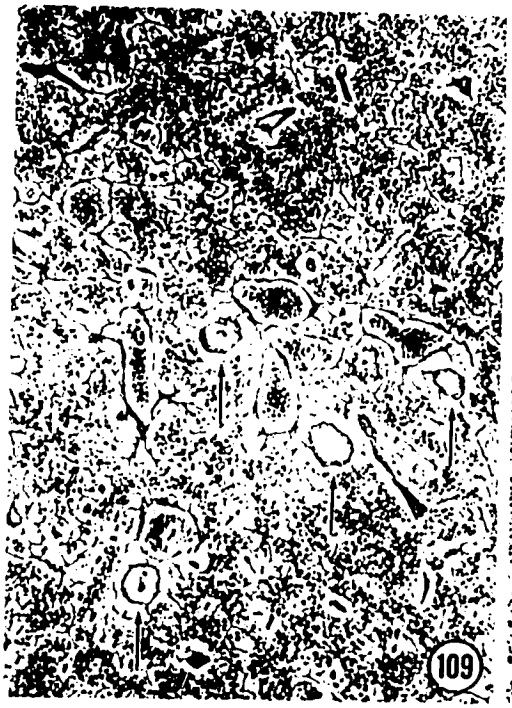

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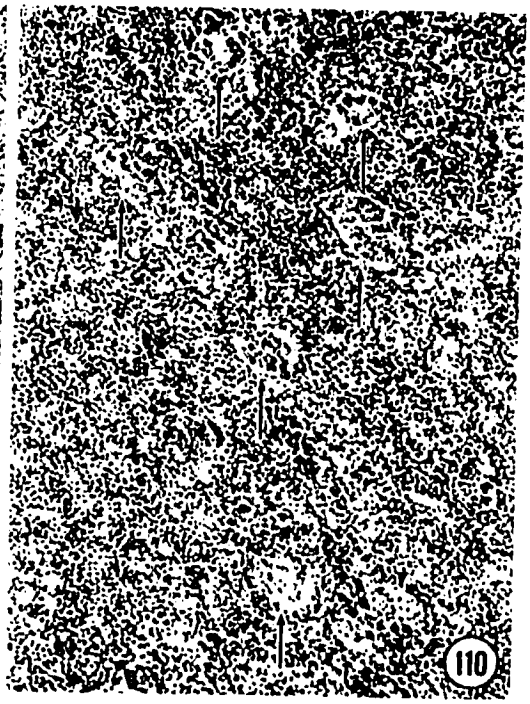


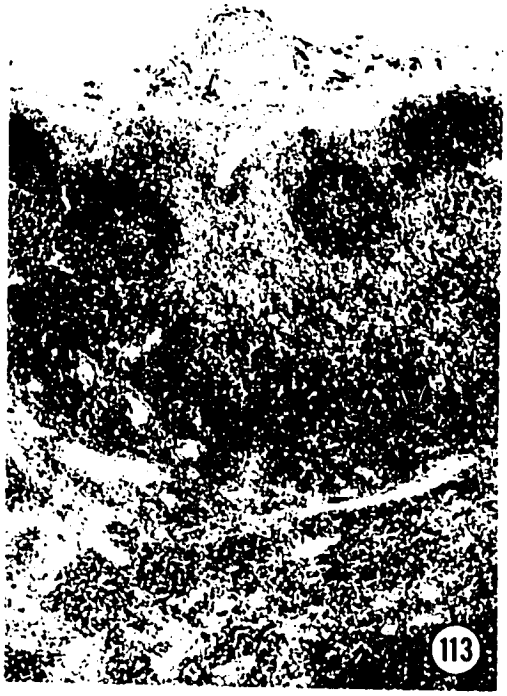
110

Figs. 111,112. Silver impregnation. Both figures show the same area of the node seen in Figure 108. In Figure 111, the large, darkly impregnated area (PF) is a pseudo-follicle. Above the pseudo-follicle is seen a triangular-shaped structure, which is an afferent lymphatic vessel. In Figure 112, taken in the eighth section consecutive to that in Figure 111, the vessel pierces the capsule to become continuous with the subcapsular sinus. Note the greater concentration of fibers in the medulla (below the pseudo-follicle) than in the cortical extra-follicular area (above pseudo-follicle). The pseudo-follicle contains still less fibers than the latter area. Magnification about 60X.

Figs. 113,114. Dominici technique. Same general area is that seen in Figures 111 and 112, taken from a section close to that of Figure 112. Figure 114 is an enlargement of 113. It can be realized that the afferent lymphatic vessel and the subcapsular sinus contain a high concentration of leucocytes, which are mainly small lymphocytes. Magnification about 65 and 195X.


PF
111


112


113

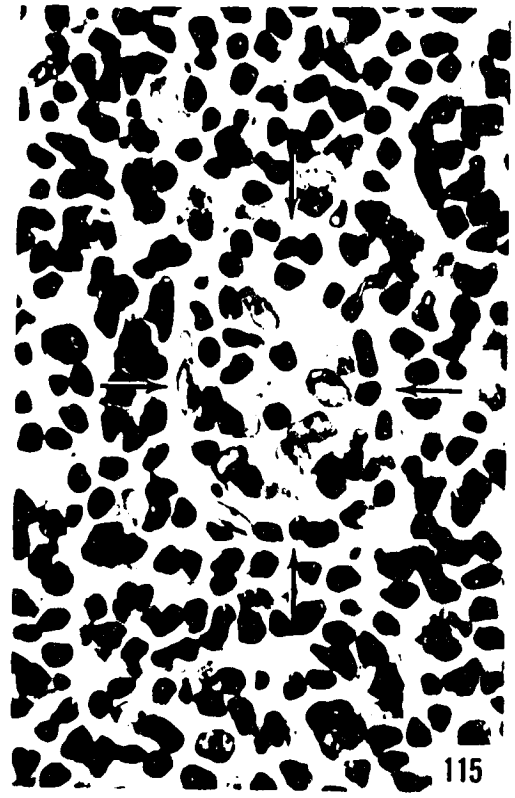

114

Fig. 115. Dominici technique. The four arrows outline the cross section of a closed segment of a postcapillary venule. No erythrocytes, but a few small lymphocytes are present in the lumen. A few small lymphocytes are in the process of diapedesis through the wall of the vessel. Magnification about 1000X.

Fig. 115A. Enlargement of part of Figure 115. At least three small lymphocytes in the process of diapedesis can be detected. Note the irregular shape of the large and pale nuclei of the hypertrophied endothelial cells. Magnification about 1950X.

Fig. 116. Dominici technique. Longitudinal section of an open segment of a postcapillary venule. The lumen contains erythrocytes with several small lymphocytes. The arrow points to a small lymphocyte in the process of diapedesis. A similar cell is present just above it. Magnification about 1000X.

Fig. 116A. Enlargement of part of Figure 116. To show better the two small lymphocytes in the process of diapedesis through the wall of the vessel. Magnification about 1950X.



115

116

115A

116A

Figs. 117B-117D. Dominici technique. Cervical nodes from normal rats of various ages. Magnification about 15X.

Fig. 117. Node from an one-week-old rat, the node is enlarged in Figure 117E (see below).

Fig. 117A. Node from a two-week-old rat. The node is separated into a cortex and a medulla. The cortex contains several developing follicular nodules (see the enlargement in Figure 117F) as well as a pseudo-follicle. In the medulla, the cords and sinuses resemble those in older nodes (figs. 117B-117D).

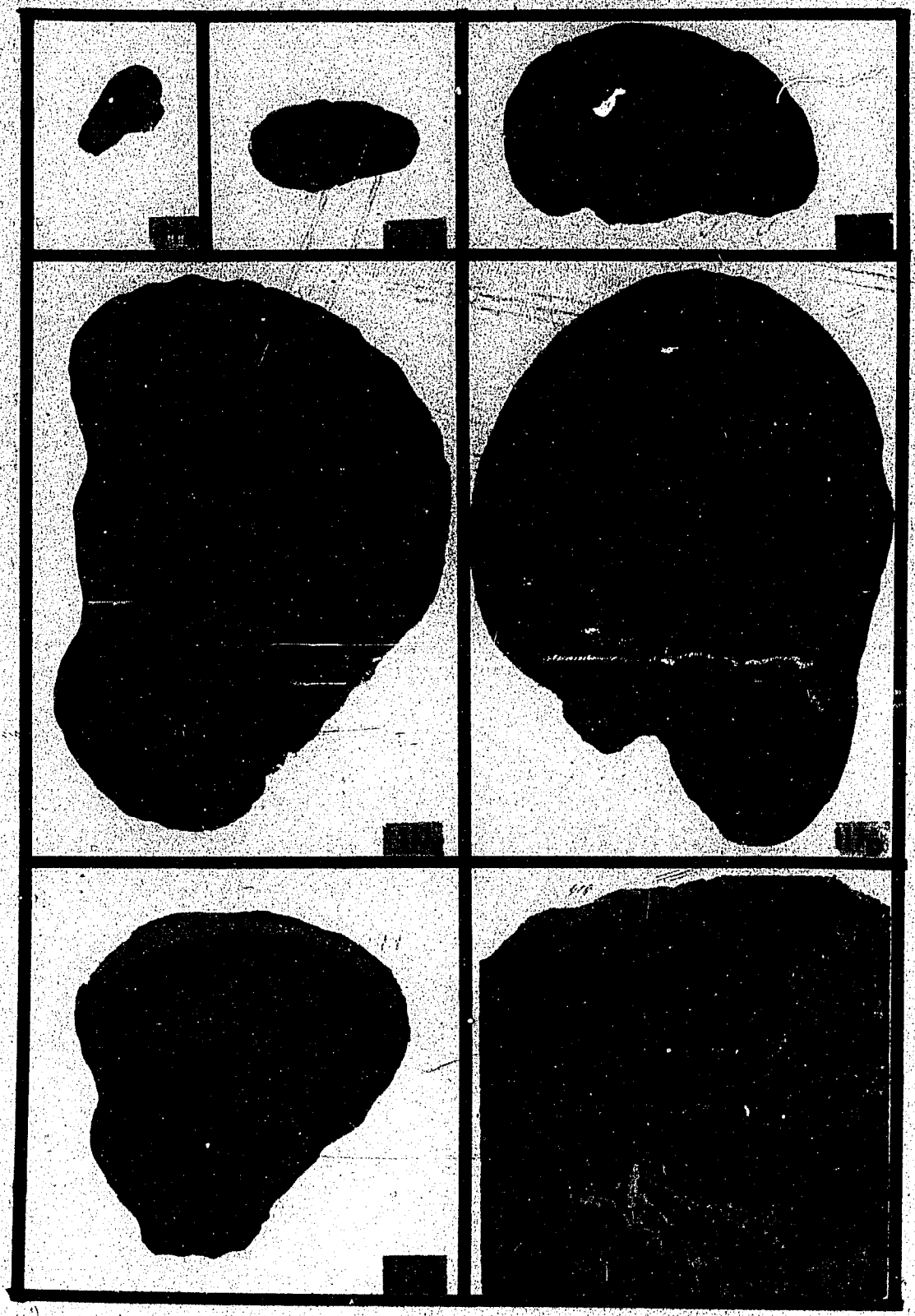
Fig. 117B. Node from a six-week-old rat. Except for its smaller size, this node is similar to the five-month-old nodesstudied here. Note the presence of three pseudo-follicles.

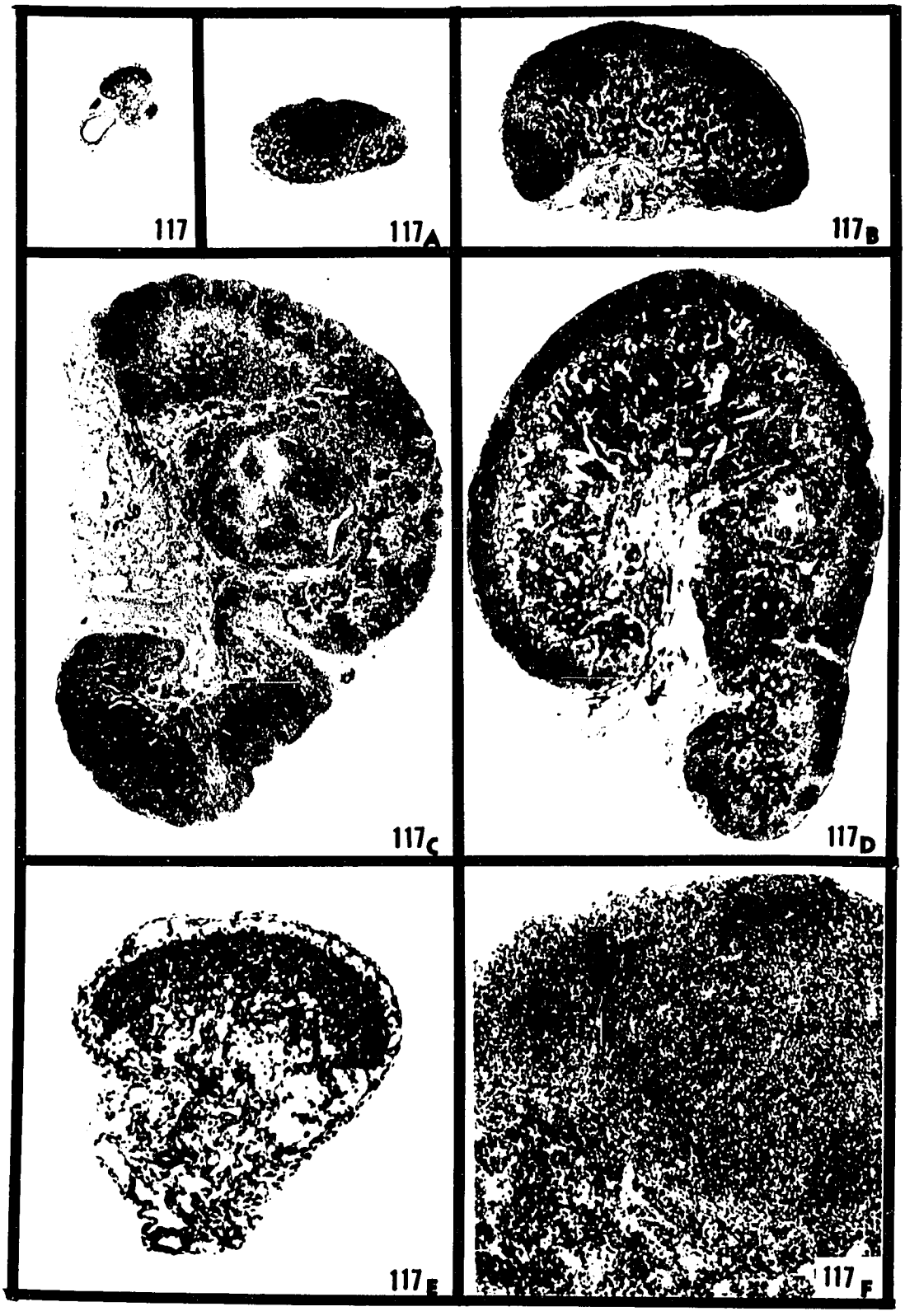
Figs. 117C. Node from a nine-month-old rat. Except for adipose transformation in the hilar area, the basic architecture of this organ is similar to that of the node studied here.

Fig. 117D. Node from a two-year-old rat. This node show a slight degree of adipose transformation at the hilus. Otherwise, it is similar to the five-month-old node studied here. Note the nearly regular distribution of follicular nodules along the outermost layer of the node. Note also that in many nodules, the light and dark area are readily detectable.

Fig. 117E. Enlargement of Figure 117. Note that the portion of the outermost layer of the node shows homogeneous appearance and which is populated mostly by small lymphocytes. Note also that the subcapsular sinus and other sinuses are wide. Magnification about 100X.

Fig. 117EE Enlargement of part of Figure 117A. The arrow points to a developing follicular nodule. Another one is present at left of the arrow, and a larger one is seen in the upper right corner. The lower left corner contains medullary plasmocytic cords and sinuses. The central right portion of the figure is occupied by part of a pseudo-follicle. Magnification about 100X.

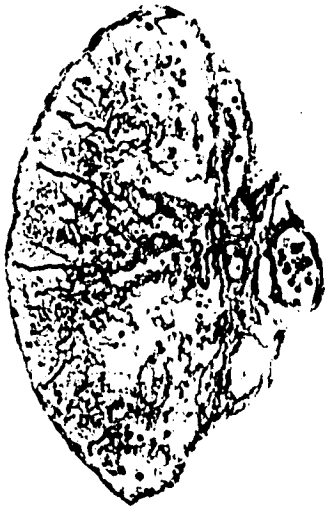



117
117A
117B
117C
117D
117E
117F

Figs. 118-118C. Silver-impregnation. Cervical nodes from normal rats of various ages to show resemblance in pattern of reticular fibers. Magnificationaabout 130X.

Fig. 118. Node from an one-week-old rat. Coarse and medium reticular fibers are distributed more or less regularly in the developing cortex (at left). They originate from the capsule and cross the cortex perpendicularly until they reach the developing medulla.

Figs. 118A-118C. Node from a six-week-old, a nine-month-old, and a two-year-old rat, respectively. The distribution of the reticular fibers in the cortex of these nodes is similar to that shown in Figure 25 for the node studied here: fibers are rare in the follicular nodules and numerous in the extrafollicular area. The only difference is that, with ageing, fibers are more concentrated in the medulla (right side of each figure).



118

118A

118B

118C

Fig. 119. Dominici technique. Mediastinal node from a normal five-month-old rat. The portion of the outermost layer of the node around the hilus (central left) is occupied by the medulla. Note the well-developed medullary plasmocytic cords and the presence of two-pseudo-follicles beneath the cortex at right. Magnification about 15X.

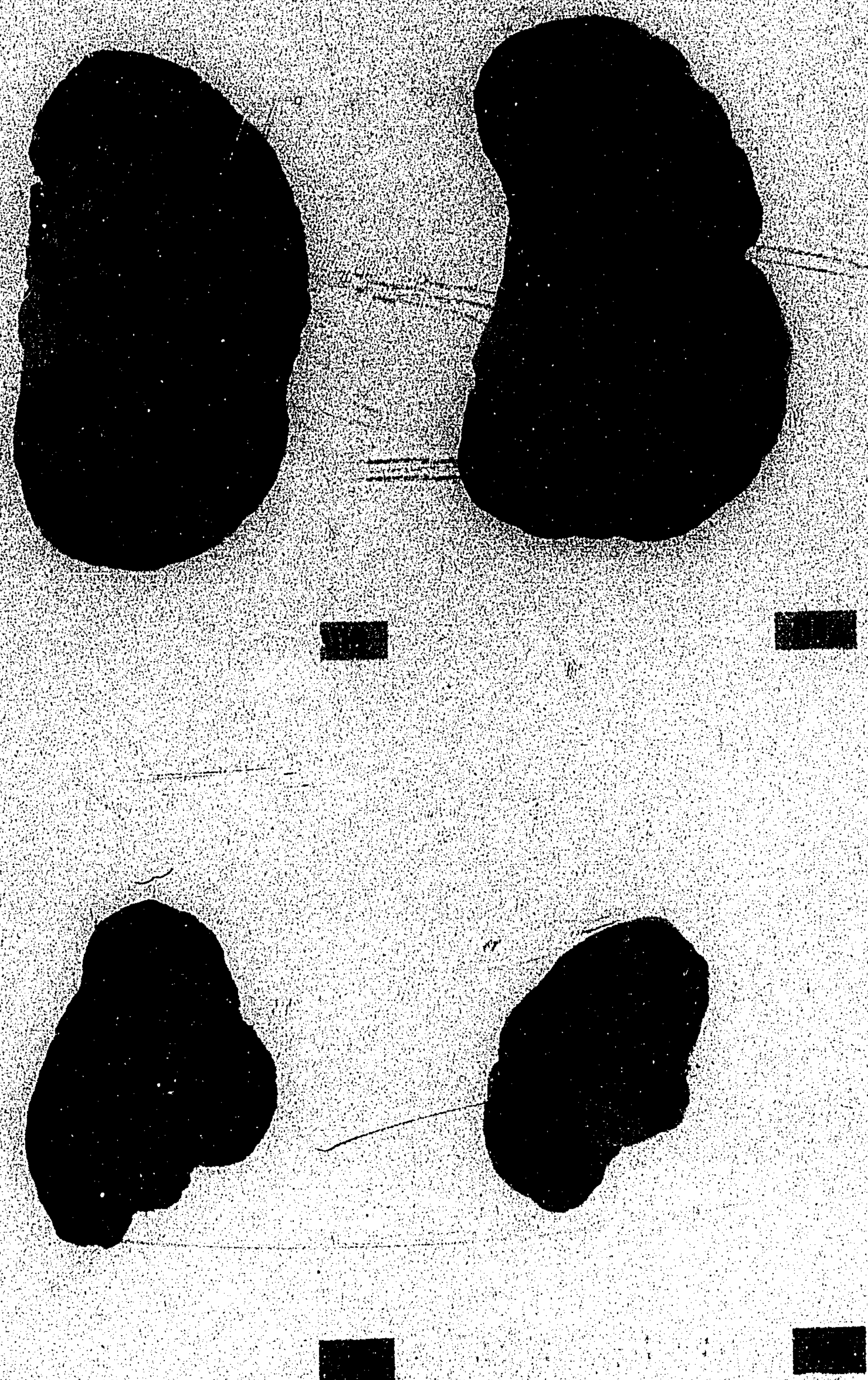
Fig. 119. Dominici technique. Mesenteric node from a normal five-month-old rat. In the medulla, the cords (small dark areas) are poorly developed while the sinuses (remaining light areas) are wide. In the lower right portion of the node, the presence of a few nodules appearing distant from the capsule resulted from the incidence of sectioning. Magnification about 15X.

Fig. 119B. Dominici technique. Popliteal node from a normal five-month-old rat. The node is actually smaller than those in Figures 119 and 119A and the hilar area has undergone adipose transformation. The remaining of the node is divided into a cortex and a medulla as usual. In the medulla (light area) the cords were poorly-developed and were not readily distinguishable from the sinuses. Note the presence of two pseudo-follicles. Magnification about 15X.

Fig. 120. Dominici technique. Mediastinal node from a two-year-old rat. Note the slight adipose transformation of the hilar area. This small and old node shows continuous cortical and medullary

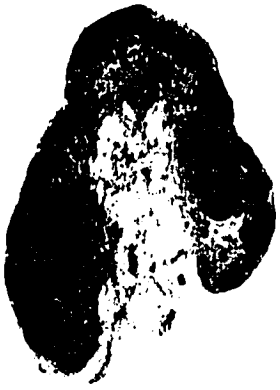
layers as usual. The darker band adjacent to the hilus represents a dense population of plasmocytes. Magnification about 15X.

layers as usual. The darker band adjacent to the hilus represents a dense population of plasmocytes. Magnification about 15X.



119

119A

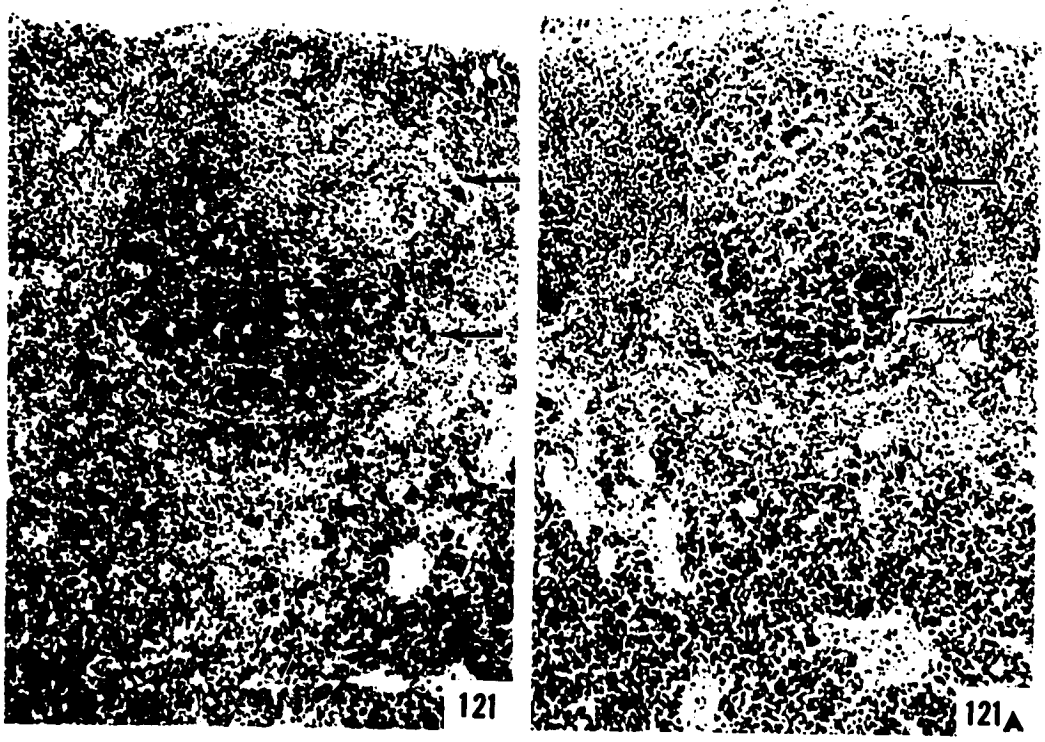


119B

120

Fig. 121. Dominici technique. Cervical node from a normal six-week-old rat. From bottom to top, the arrows point to the dark and light areas of the tangentially-cut mantled nodule respectively. Magnification about 130X.

Fig. 121A. Dominici technique. Cervical node fromaa normal two-year-old rat. The two arrows pointing to the tangentially-cut mantled nodule show the same details as in Figure 121. Maghification about 130X.


121
121A

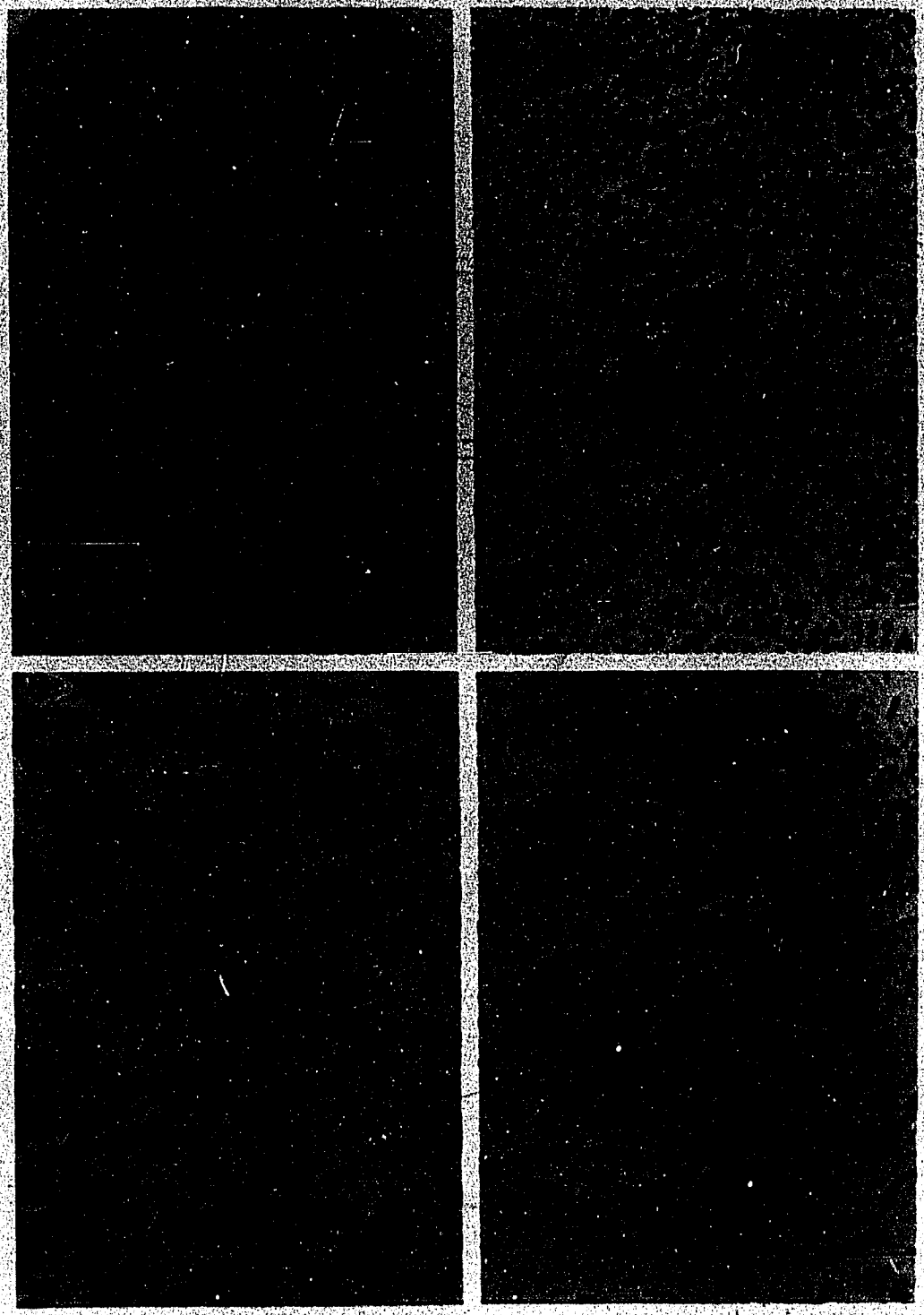
Figs. 122-122C. Microphotographs from a normal five-month-old rabbit obtained from Dr. Guy Sainte-Marie.

Fig. 122. Dominici technique. The upper and lower arrows indicate respectively to the light and dark areas of a capped nodule. Magnification about 120X.

Fig. 122A. Dominici technique. The arrow indicated a cluster of pale-stained autofluorescent cells adjacent to an inner nodular margin. Magnification about 200X.

Fig. 122B. Dominici technique. Enlargement of the cluster of autofluorescent cells seen in Figure 122A. The cluster has been turned at an angle of 90 degrees toward the left. The arrow is in same position as in Figure 122A. The outline of these large and pale stained autofluorescent cells is hardly detectable. Above each P a small plasmocyte is present. Magnification about 800X.

Fig. 122C. Silver-impregnation. The horizontal arrow points to a silver-impregnated macrophagic reticular cell present in the light area of a longitudinally-sectioned nodule. The central vertical arrow points to a few silver-impregnated autofluorescent cells adjacent to the inner nodular margin. Magnification about 300X.



122

122A

122B

122C

Figs. 123-123C. These are slightly reduced reproductions of microphotographs from Conway (1937). The text below is the explanations of Figures from the latter author.

Explanation of Figures 123-123C

Microphotographs showing different stages in the cycle of the lymphatic nodule in diffuse lymphatic tissue of cortex of mesenteric lymph node of guinea pig, 5 days after injections of B. monocytogenes. X450, reduced by one-fourth. Hematoxlyin-eosin-azure II.

Fig. 123. Small new nodule containing six mitotic lymphocytes. It is poorly demarcated from the surrounding diffuse lymphatic tissue and its margin are indicated by the arrow heads.

Fig. 123A. Slightly later stage in the development of a lymphatic nodule, which is actively lymphocytopoietic. It contains twenty-seven mitoses in medium-sized lymphocytes. This nodule lacks completely, a peripheral zone of small lymphocytes such as is seen in Fig. 123B. This nodule, histologically, is a typical "germinal center" of Flemming.

Fig. 123B. The central portion of this nodule is still actively lymphocytopoietic and contains fifteen mitoses in medium-sized lymphocytes. It is demarcated from the surrounding diffuse lymphatic by more or less concentrically arranged layers of small lymphocytes. This is the type of nodule

which is often regarded as typical, consistingoof an outer dark-staining zone and a lighter central area.

Fig. 123C. This nodule has a very pale-staining central portion consisting mainly of reticular cells, few macrophages and scattered smaller lymphocytes. The macrophages contain small amounts of cellular debris. The periphery of this nodule consists primarily of small lymphocytes which merge gradually into the surroundingddiffuse lymphatic tissue.

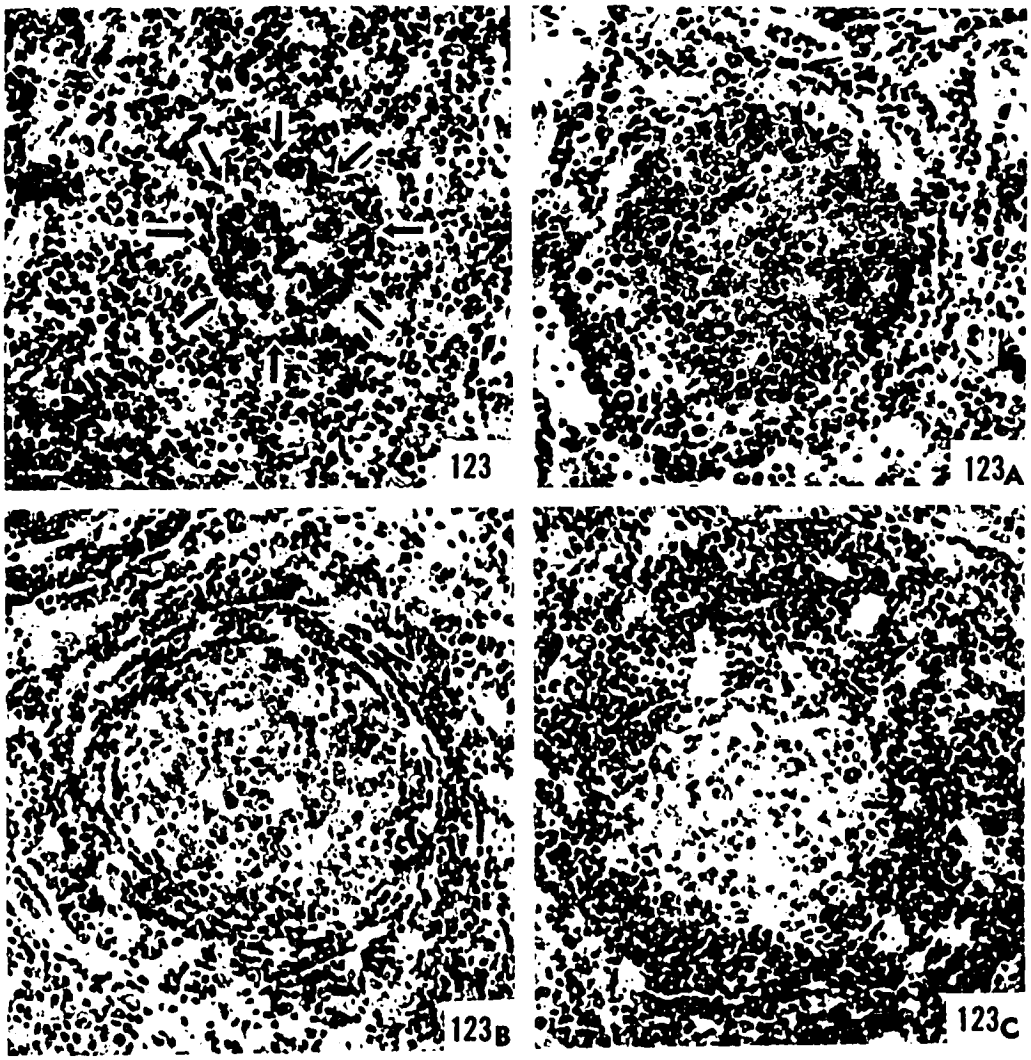
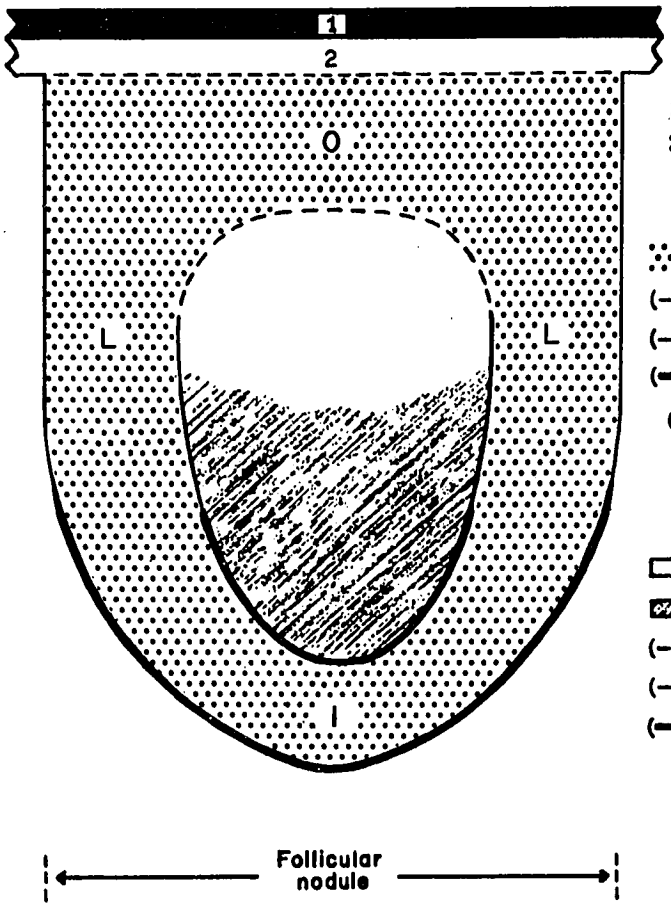

123
123A
123B
123C

Fig. 124. Schematic diagram illustrating the various components of a "follicular nodule" and their topographical orientation in respect to the adjacent subcapsular of subtrabecular sinus. The dotted surface represents the follicle, Inside the follicle, the nodule is actually composed of a light (upper) and a dark (lower) areas.

Fig. 125. Schematic diagram of a nodule to illustrate how it can be divided into four topographical zones.

Fig. 124. Schematic diagram illustrating the various components of a "follicular nodule" and their topographical orientation in respect to the adjacent subcapsular of subtrabecular sinus. The dotted surface represents the follicle, Inside the follicle, the nodule is actually composed of a light (upper) and a dark (lower) areas.

Fig. 125. Schematic diagram of a nodule to illustrate how it can be divided into four topographical zones.

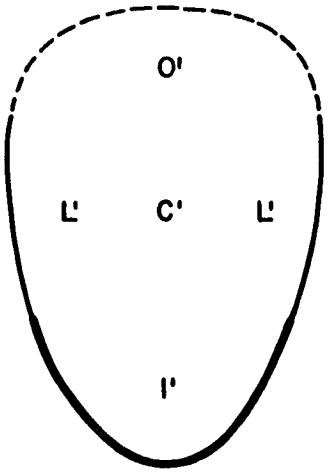


1 Capsule or Trabecula
2 Subcapsular sinus or Subtrabecular sinus

Follicle
(- -) Outer follicular margin
(——) Lateral " "
(▬▬) Inner " "
O Outer zone of follicle
L Lateral " " "
I Inner " " "

Light area of nodule
Dark " " "
(- -) Outer nodular margin
(——) Lateral " "
(▬▬) Inner " "

124



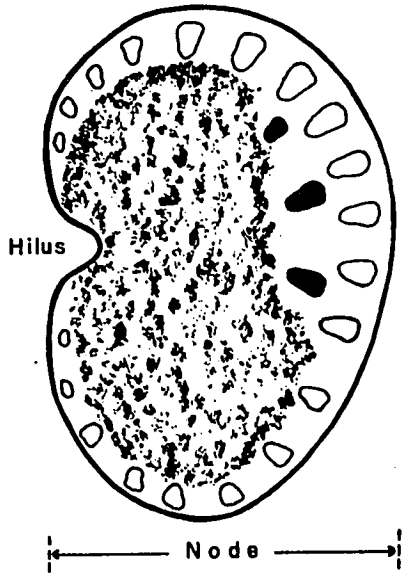
O' Outer zone of nodule
C' Central " " "
L' Lateral " " "
I' Inner " " "

125

Fig. 126. Schematic diagram of a longitudinal section of a node to illustrate the current concept of deep and peripheral nodules. The medulla is represented by the patchy area, and the cortex by the blank area. In the cortex, most follicular nodules (each nodule being associated with a follicle) are arranged into a single layer adjacent to the subcapsular sinus beneath the capsule. These are the peripheral nodules or follicles. A few follicular nodules appear to be located deeper into the cortex below the above layer of peripheral follicular nodules. These would be the deep nodules or follicles.

Fig. 127. Schematic diagram showing the more or less concentric subnodular spaces which may outline the inner and the lateral nodular margin when these are not covered by the follicle associated with a nodule.

Fig. 128. Schematic diagrams illustrating the classification of the "nodules" into four types, on the basis of the extent to which their margin is surrounded by the associated follicles. Correspondingly, the diagrams illustrate the classification of the follicles into three types, on the basis of the extent to which they surround the nodule to which they are associated.



Cortex

Medulla

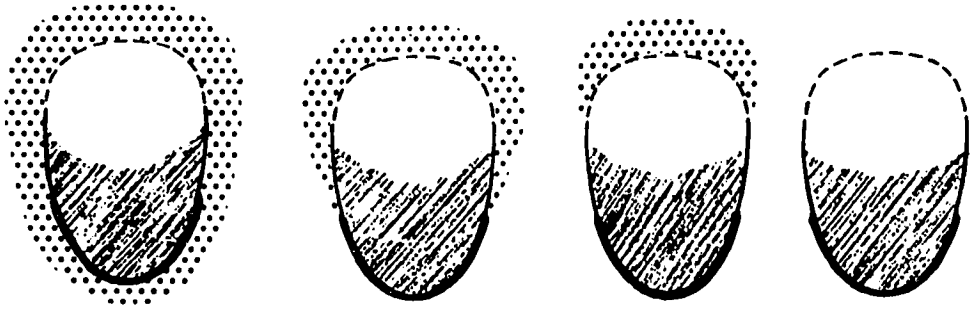
Peripheral follicles
or
Peripheral nodules

Deep follicles
or
Deep nodules

126

Subnodular
spaces

127



Enveloping follicle
or
Enveloped nodule

Mantling follicle
or
Mantled nodule

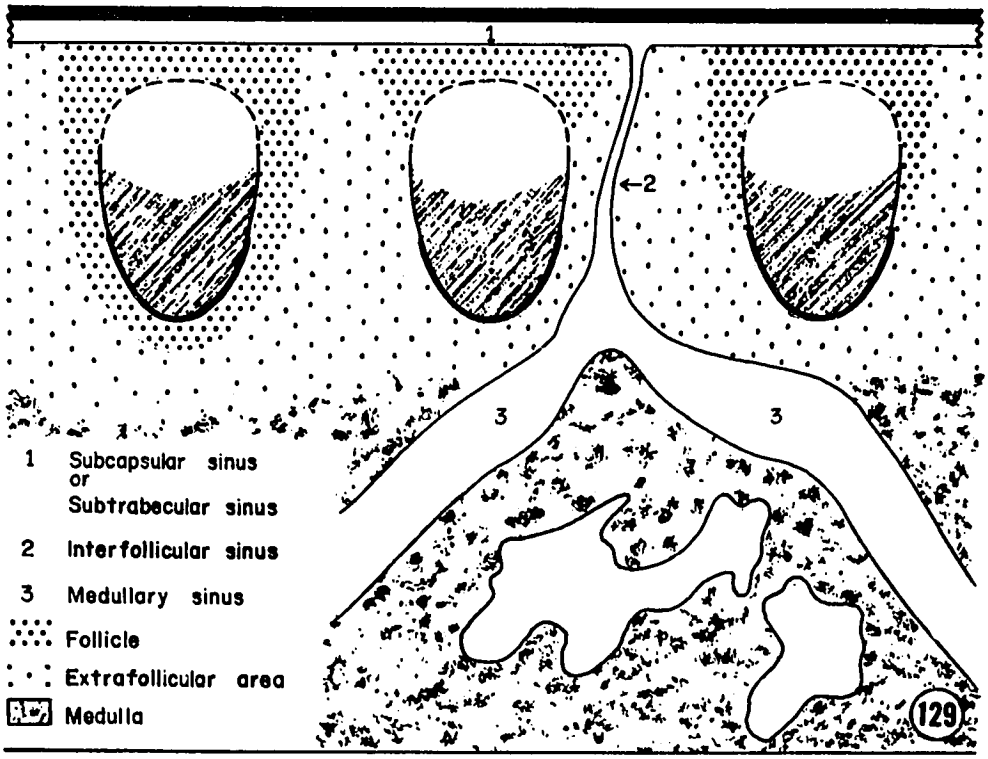
Capping follicle
or
Capped nodule

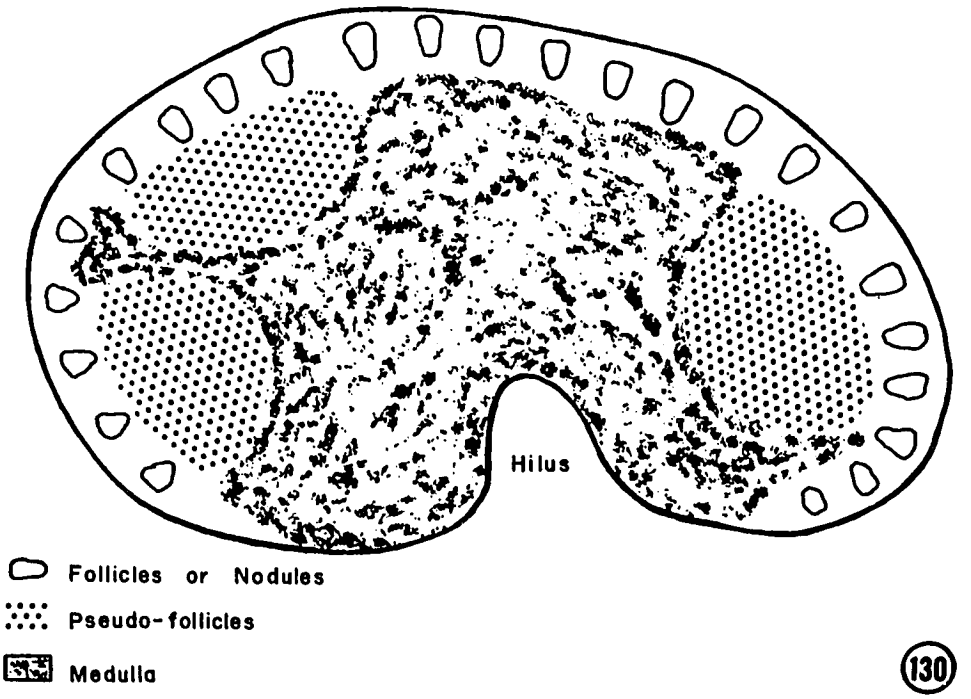
Naked nodule

128

Fig. 129. Schematic diagram to show some of various structures in the cortex of the node and, the variations in the extent of contact of the cortical extrafollicular area with the nodules depending on the type of their associated follicles.

Fig. 130. Schematic diagram to show the shape and localization of well developped pseudo-follicles.


1
←2
3
3
1 Subcapsular sinus or Subtrabecular sinus
2 Interfollicular sinus
3 Medullary sinus
Follicle
Extrafollicular area
Medulla
129


Hilus
Follicles or Nodules
Pseudo-follicles
Medulla
130

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GLOSSARY

Capping follicle: a follicle showing as a "cap of small lymphocytes", i.e., an accumulation of small lymphocytes over the outer nodular margin only (fig. 128)

Capped nodule: a nodule associated with a capping follicle (fig. 128)

Central zone of the nodule: the zone of the nodule surrounded by the lateral, inner and outer zones of the nodule (fig. 125)

Complex nodule: a variably-shaped association of several adjacent nodules.

Cortico-medullary junction: the virtual margin delimiting the cortical and the medullary structures.

Dark area of the nodule: the area of the nodule populated mainly by large, medium and small lymphocytes, and which often show many mitoses (fig. 124)

Deep cortex: the portion of the cortex located between the peripheral cortex and the medulla, it usually contains no follicles or nodules.

Deep follicle: a follicle present in the deep cortex (fig. 126)

Deep nodule: a nodule present in the deep cortex (fig. 126)

Developing follicular nodule: a structure which, in very young animals, is developing into a follicular nodule.

Developing nodule: a structure which, in very young animals, is developing into a nodule.

Enveloping follicle: a follicle showing as an "envelope of small lymphocytes", i.e. an accumulation of small lymphocytes over the entire nodular margin (fig. 128)

Enveloped nodule: a nodule associated with an enveloping follicle (fig. 128)

Extrafollicular area (cortical): the portion of the cortex outside the follicles and nodules present in the cortex (fig. 129)

Follicle (lymphocytic follicle): a structure today commonly referred to as a "primary nodule", consisting of an accumulation of small lymphocytes which outlines the nodular margin to variable degree (see: capping, mantling and enveloping follicle).

Follicular margin: the margin of a follicle.

Follicular nodule: a structure consisting of a nodule and of a follicle which are variably associated (see: naked, capped, mantled and enveloped nodules).

Inner follicular margin: the portion of the follicular margin that is the furthest from the overlying capsule or trabecula i.e. from the overlying subcapsular or sub-trabecular sinus (fig. 124)

Inner nodular margin: the portion of the nodular margin that is the furthest from the overlying capsule or trabecula, i.e. from the overlying subcapsular or sub-

trabecular sinus (fig. 124)

Inner zone of the follicle: the zone of the follicle over the inner follicular margin (fig. 124)

Inner zone of the nodule: the zone of the nodule over the inner nodular margin (fig. 125)

Interfollicular sinus (cortical): a lymphatic sinus originating from the subcapsular or a subtrabecular sinus and crossing the depth of the cortex, in between follicles and nodules, to abut into a medullary sinus (fig. 129)

Lateral follicular margin: the portion of the follicular margin located between the outer and inner portions (fig. 124)

Lateral nodular margin: the portion of the nodular margin located between the outer and inner portions (fig. 124)

Lateral zone of the follicle: the zone of the follicle along the lateral follicular margin (fig. 124)

Lateral zone of the nodule: the zone of the nodule populated mainly by small lymphocytes, reticular cells and macrophagic reticular cells (fig. 124)

Light area of the nodule: the area of the nodule populated mainly by small lymphocytes, reticular cells and macrophagic reticular cells (fig. 124)

Lobule: portion of a node variably separated from the remaining of the node by an outlining septal trabecula.

Mantling follicle: a follicle showing as a "mantle of small lymphocytes", i.e. an accumulation of small lymphocytes over the outer and lateral nodular margins (fig.128)

Mantled nodule: a nodule associated with mantling follicle (fig. 128)

Medullary cord: a region of the medulla occupying the space in between two medullary sinuses and which may be populated by predominantly plasmocytes.

Medullary sinus: a lymphatic sinus located in between medullary cords.

Medullary venule: the portion of a node venule present in the medulla in which has typical flat endothelial cells.

Naked nodule: a nodule with no follicle around it (fig. 128)

Nodular margin: the margin of a nodule (fig. 124)

Nodule (lymphatic nodule): a structure today commonly referred to as a "secondary nodule", a "germinal center" or as a "reactive center" (fig. 124)

Outer follicular margin: the portion of the follicular margin that is the nearest to the overlying capsule or trabecula, i.e. the overlying subcapsular or subtrabecular sinus (fig. 124)

Outer nodular margin: the portion of the nodular margin that is the nearest to the overlying capsule or trabecula, i.e. to the overlying subcapsular or subtrabecular sinus (fig. 124)

Outer zone of the follicle: the zone of the follicle beneath the outer follicular margin (fig. 124)

Outer zone of the nodule: the zone of the nodule beneath the outer nodular margin (fig. 125)

Peripheral cortex: the outer layer of the cortex underlying the subcapsular and the subtrabecular sinuses, and in which are usually present follicles and nodules (the peripheral follicles or nodules)

Peripheral follicle: a follicle located in the peripheral cortex (fig. 126)

Peripheral nodule: a nodule located in the peripheral cortex (fig. 126).

Perivascular channel: a space, or a few concentric spaces, outlined by reticular fibers and which surround each postcapillary venule.

Postcapillary venule: the portion of a node venule present in the extrafollicular area of the cortex and which has hypertrophied endothelial cells and is surrounded by a or a few perivascular channels.

Pseudo-follicle: an often rounded accumulation of small lymphocytes that may occur in the deep cortex and, which is usually much larger than a follicle and contains no nodule (fig. 130)

Pseudo-follicular area (cortical): an accumulation of small lymphocytes in the cortical extrafollicular area and which is part of a pseudo-follicle.

Resting follicular nodule: a follicular nodule which has lost its characteristic cells.

Resting nodule: a nodule which has lost its characteristic cells.

Subcapsular sinus: the lymphatic sinus located in between the capsule and the cortex (fig. 129)

Subnodular space: a space, or a few concentric spaces outlined by reticular fibers and which may be seen along the basal and lateral margins of a capped or mantled nodule (fig. 127)

Subtrabecular sinus: an extension of the subcapsular sinus located in between a trabecula and the cortex.