AGE CHANGES
IN THE PIG LYMPH NODE

THESIS FOR THE DEGREE OF PH. D.
MICHIGAN STATE UNIVERSITY

FREDERICK LUDWIG BOUWMAN, JR.

1959

THESIS

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This is to certify that the

thesis entitled

AGE CHANGES IN THE PIG LYMPH NODE

presented by

FREDERICK LUDWIG BOUWMAN JR.

has been accepted towards fulfillment
of the requirements for

Ph.D. degree in Zoology

Richard A. Fennell
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Date August 21, 1959

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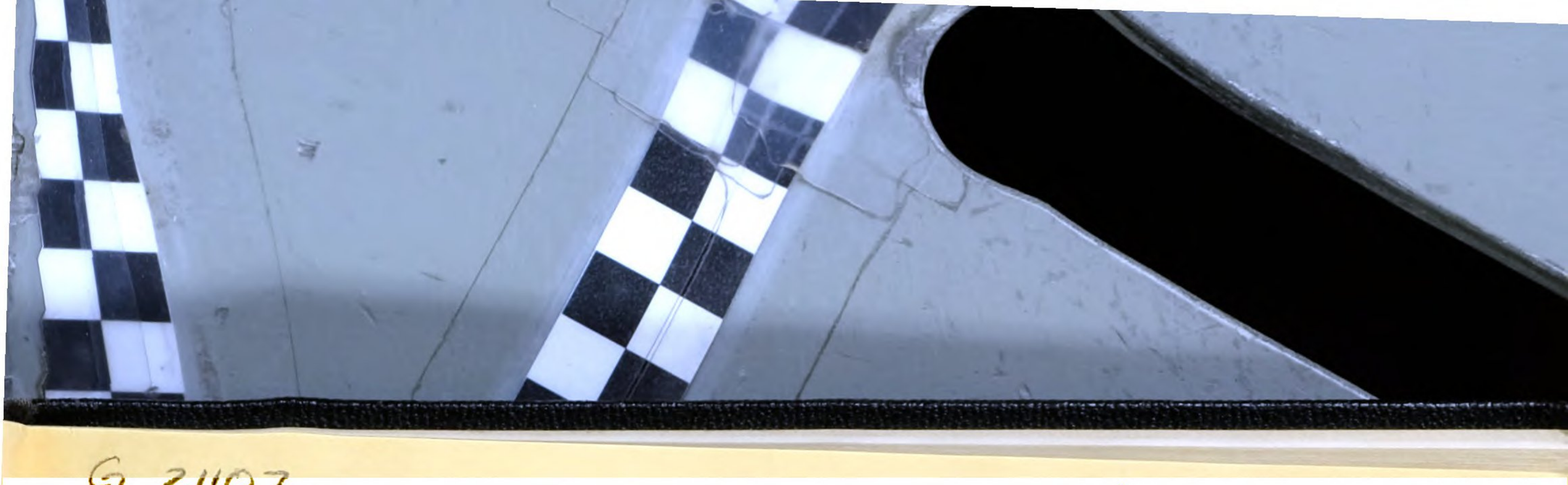
A THESIS

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DOCTOR OF PHILOSOPHY

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A total of thirty-eight lymph nodes, one or more from ten different body regions, were used in this study. Nodes were obtained from various ages of pigs (late fetal life to three years of age). Tissues were fixed in Lavdowsky's formal-acetic acid alcohol fixative and stained with either Harris' hematoxylin, Weigert Van Giesen connective tissue stain or Bielchowsky's reticular stain.

Late fetal nodes and those from young animals (1 to 6 days of age) exhibited a medullary region with an abundance of sinuses and scattered reticular cells. In nodes from older animals (3 weeks of age or older) lymph sinuses were occluded in part with reticular cells and lymphocytes. Thus the medullary sinuses, which characterized nodes of man and the dog, were not identified. The medulla usually surrounded the cortical tissue. However, in popliteal, superficial and deep inguinal nodes, the cortex was frequently at the periphery. In many of the older nodes examined, some of the nodules were surrounded by either collagenous fibers, reticular fibers or both.

With increased age the stroma became heavier and at the age of seven months elastic fibers were present as thin layers which in the capsule were not always near the inner edge. Elastic fibers were present in the parenchyma, par-

ticularly in older nodes.

Most of the changes noted above occurred most rapidly after birth, but continued until the age of sexual maturity at six or seven months.

Mesenteric, pharyngeal, mandibular and lumbar nodes were characterized by extensive fusion. The two bronchial nodes that were examined were small and showed no evidence of fusion. Popliteal, parotid, superficial cervical and deep and superficial inguinal nodes were conglomerates of many fused and partially fused nodes. Grossly these nodes had a lobed appearance. In some nodes the cortex was adjacent to the subcapsular sinus, and was divided into ampullae or alveoli as in the typical lymph node.

Afferent lymph vessels extended through the trabeculae to open, deep in the node, into sinuses adjacent to cortical tissue. Then lymph flowed to the medulla and drained into efferent vessels from several locations on the surface of the node.

ACKNOWLEDGEMENTS

I wish to gratefully acknowledge the assistance and many kindnesses of: Dr. R. A. Fennell, Professor of Zoology; Dr. M. Lois Calhoun, Professor and Head, Department of Anatomy and Mrs. Bernadette Henderson, Secretary, Department of Zoology, all of Michigan State University and my usually patient wife, Gloria Jane.

CURRICULUM VITAE

Frederick L. Bouwman, Jr., born in Grand Rapids, Michigan on January 16, 1916. Attended public schools and graduated from Ottawa Hills High School, Grand Rapids, Michigan. Received an A. B. Degree from Calvin College in 1940. Served in the Medical Department of the Army of the United States, in the Mediterranean Theatre of Operations, from 1942 to 1946. Awarded the degree of M. S. in Zoology by Michigan State University in 1947.

At present, Professor and Chairman of the Department of Biological Sciences at the Detroit Institute of Technology, Detroit, Michigan.

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I. INTRODUCTION

There is no adequate description of the pig lymph node in the English language and no thorough investigation of the structure of this organ has been made for about twenty years. The pig node differs so much in structure from the typical mammalian node that descriptions of the typical node are useless in attempts to interpret this organ. Further, there are no descriptions of the node in very young animals. Thus it was considered worth while to examine the organology, histology, and cytology of the node in pigs ranging in age from late fetal to seven months postnatal. In addition one node from a three year old pig was examined.

Dr. R. A. Runnells stated that the direction of the flow of lymph through the node is important to the pathologist. He suggested that a confirmation of Trautmann's (1926) and Goldkuhl's (1927) description of the lymph flow would be useful.

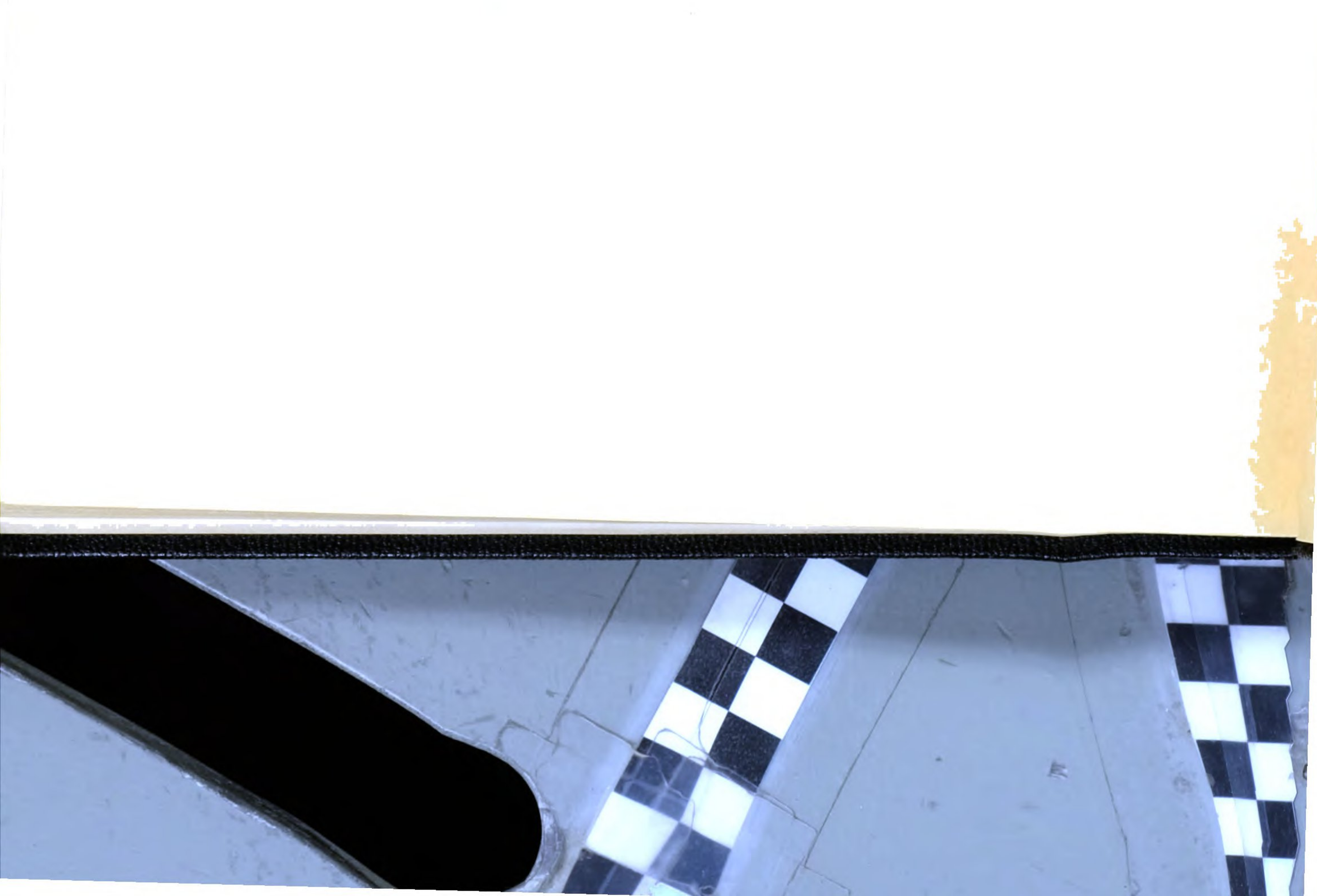


TABLE A

Age of pigs and regions from which nodes were taken

	Fetal 15 cm.	Fetal 21 cm.	1 da.	2 da.	6 da.	3 wks.	6 wks.	7 wks.	3 mo.	4 mo.	5 mo.	7 mo.	3 yrs.	Total
Mesenteric	1	1		1	1	1				2	2	1	1	11
Popliteal				1	1	2	1		1	1		2		9
Parotid		1	1	1						1				4
Pharyngeal										2				2
Sup. Cervical										1		1		2
Mandibular										1				1
Bronchial						1					1			2
Lumbar											1			1
Deep Inguinal	1						1			1				3
Sup. Inguinal						1			1	1				3

II. MATERIALS & METHODS

Lymph nodes were removed from nineteen pigs obtained from the following sources: i.e. the autopsy laboratory of the College of Veterinary Medicine (M.S.U.), the Department of Animal Husbandry (M.S.U.), a local meat packing company, and the General Biological Supply Company. Ten of the pigs were males, four were females and four were castrates. They ranged in age from late fetal to three years postnatal. Since many were crossbreeds, no attempt was made to classify them according to breed.

One or more lymph nodes were obtained from mesenteric, popliteal, parotid, pharyngeal, superficial cervical, mandibular, bronchial, lumbar, superficial inguinal and deep inguinal regions. A total of thirty-eight nodes were obtained. For further details see Table A.

Examination of Table A disclosed that nodes with a wide range of ages were obtained from two regions in order that age changes could be studied. These were the popliteal as an example of a superficial node and the mesenteric as an example of a deep node. The other nodes were obtained to learn if differences exist between nodes from different regions.

Animals from packing plants had very recently been slaughtered. Those from the autopsy laboratory were electrocuted. Others were killed by injection of an overdose of Halital[1], a barbitol anesthetic, into the peritoneal

1. Jensen Salsbery Laboratories, Inc.

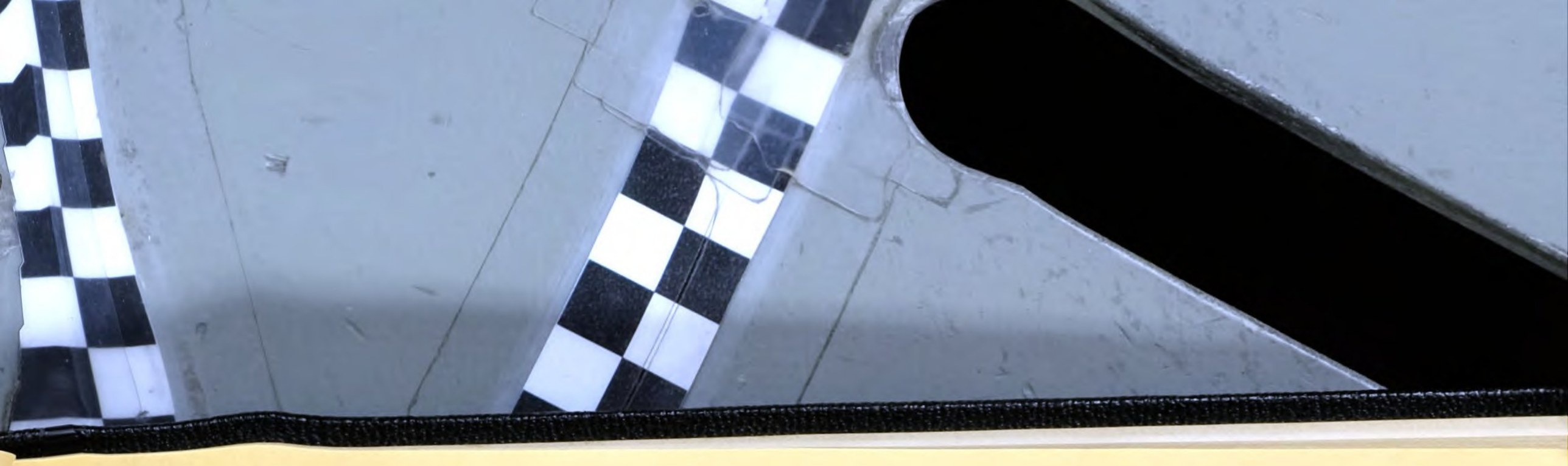
cavity. Two of the very young pigs, presumably killed by being rolled on by the sow, were found dead in the farrowing pens.

In most cases the desired nodes were removed from the animal and immediately placed in Lavdowsky's Formol-acetic acid-alcohol fixative, which consisted of commercial formalin (40 percent formaldehyde) 100 parts, 95 percent alcohol 500 parts, glacial acetic acid 20 parts, and distilled water 400 parts. The nodes were left in Lavdowsky's fixative for 24 hours and then stored in 70 percent alcohol. The smaller pigs were fixed and preserved entire in 10 percent formalin after the body cavities were opened.

In order to study the direction of the flow of lymph through the node, certain pigs were injected subcutaneously with trypan blue solution or India ink suspension before the animal was killed. The trypan blue was employed as a .05 percent solution in distilled water and the India ink as a 50 percent suspension in distilled water. The pigs were killed ten to thirty minutes after the injection. After ten minutes trypan blue was present in the node, but no appreciable quantities of India ink were found until 20 to 30 minutes had passed. One animal was kept alive after injection by removing the node surgically after intraperitoneal injection of Halitol. This procedure was followed under the direction of C. W. Titkemeyer, D.V.M. and was repeated until the proper time between injection and removal of the node was established and all of the desired nodes were obtained.

All nodes were dehydrated in a mixture of one-half 95 percent ethyl alcohol and one-half butyl alcohol followed by two changes of butyl alcohol. The nodes were left from one to three hours in each liquid. Following dehydration they were infiltrated in butyl mush, a 50 percent solution of paraffin in butyl alcohol, for ten to twelve hours, passed through three changes of paraffin remaining several hours in each change and then embedded in paraffin. Sections were cut at five, six or seven micra on a rotary microtome. In some cases about a dozen sections were cut and mounted on three or four slides. Other nodes were cut into serial sections. Usually sections were cut from two, three or four parts of one node so that various parts of one node could be compared.

Sections for routine examination were stained with Harris' hematoxylin and eosin. To study connective tissue, sections were stained by the Wiegert Van Gieson method. To show reticular fibers, the sections were stained by Bielchowsky's method as described by Bensley and Bensley (1938).



III. OBSERVATIONS

A. General Description of the Pig Lymph Node

The gross appearance of the pig lymph node differed from the typical node of other mammals only by the lack of a clearly defined hilus. The hilus was not found in the pig node because the entrance of blood vessels was not confined to one area of the node. They entered both with the afferent lymphatic vessels and where the efferent lymphatics took origin. Furthermore, arterioles and capillaries were seen which entered the parenchyma of the node through the capsule (fig. 13). Because efferent lymphatic vessels left the pig node at many locations the nodes did not possess a group of blood and lymph vessels which entered or left a specific area of the surface. Thus a hilus was not formed. On the other hand, microscopically visible hilus-like indentations were seen where afferent lymphatics entered the node. Goldkuhl (1927) referred to them as pseudohili. This terminology was undesirable since these indentations were, from a morphological point of view, essentially similar to the hili described by other authors.

Koelliker (1902) described the microscopic appearance of the mesenteric lymph node of the pig as follows. "Eigenartig sind die mesenterialen Lymphknoten des Schweines, wie v. Recklinghausen und Chievitz hervorheben und neuerdings wieder Ranvier bemerkt, dadurch, dass so zu sagen kein Markgewebe vorhanden ist und Follikel durch die ganzen Drusen verbreitet und dadurch die Lymphbahnen sehr eng sind." Prepared sections

of tissue from pig lymph nodes when examined under the microscope demonstrated that the region which corresponded to the cortex of other species may be located in the center of the node rather than adjacent to the capsule (figs. 4 and 7). The cortical tissue was well filled with lymphocytes and contained the lymph nodules. The other tissue of the node, which may be found at the periphery, did not resemble the medulla of the usual node in structure. In the literature it was usually termed the medulla, although Trautmann (1926) considered it to be a special tissue. Injection experiments to be described below showed that this tissue corresponded to the medulla of the typical node because lymph flowed into it from the cortical region and then into the efferent vessels (fig. 11). This was in agreement with the opinions of many authors who stated that this tissue should be termed the medulla. Goldkuhl (1927) stated that this region must be considered as medulla in order that the structure of the pig node could fit into the general structural scheme of the mammalian lymph node.

In the medulla lymphocytes existed as small clumps of cells distributed uniformly throughout this region (fig. 7). Small sinuses were numerous (figs. 10 and 14). The predominating cell was the reticular cell whose cytoplasm took a characteristic bright pink color with H. and E. stain (fig. 16). The color was evident because few lymphocytes were present to mask it. This region has been aptly described by the German histologists as the "helle" or "zellarme" region.

Silver precipitation stains showed that the network of reticular fibers found in nodes of other species was present in the pig lymph node. Collagenous fibers were common in the medulla and elastic fibers were present, particularly in nodes from older pigs. Large trabeculae occurred in the medulla only where the efferent vessels formed and during fusion of two nodes (fig. 11). This along with the presence of reticular cells suggested that the histological structure of the pig node was fundamentally like that of nodes from some other species, e.g., dog and man. However, as an organ the structure of the pig node was different i.e., the cortex was frequently surrounded by the medulla.

The capsule consisted chiefly of collagenous fibers with varying quantities of elastic fibers and smooth muscle fibers. It was thin when compared to the capsule of the nodes of other species (figs. 7, 10 and 12).

The subcapsular sinus was not regularly present. When it was present and adjacent to cortical tissue injection experiments demonstrated an abundance of dye particles in both lymph vessels and the subcapsular sinus. On the basis of the arrangement of valves within the lymph vessels, we interpret these observations as an indication of lymph flow from afferent vessels into the subcapsular sinus. Thus, from a functional point of view, the subcapsular sinus functions in essentially the same manner as the sinus of typical lymph nodes. Richter (1902) suggested that lymph could flow from the afferent vessels, through the subcapsular sinus into

efferent vessels thus bypassing the lymphoid tissue of the node. The lymphatic bypass around the lymphoid tissue was not seen in sections prepared from injected nodes. However, as these exist in some other species, i.e. the rat and dog, further investigation may disclose their presence in the pig. Trautmann (1926) maintained that efferent vessels leave the subcapsular sinus, but observations made during the course of this investigation did not confirm this point of view.

In the pig as in most other mammals the nodes possessed two systems of trabeculae. One of these arose at the hilus, or in the pig where the afferent vessels entered, and the other from many locations on the capsule. The latter system was usually poorly developed in the pig as few trabeculae of this type were seen. Branches of these two systems rarely joined. The system of trabeculae that arose where the afferent vessels entered the node carried these vessels, as well as blood vessels, into the interior of the node. In the older and larger nodes this system, at least near the surface, was very large and may have had a central cavity filled with loose fibrous connective tissue and fat (fig. 17). These trabeculae branched extensively throughout the cortex, but rarely entered the medulla. The trabeculae, especially the larger ones, were usually surrounded by a sinus which received lymph from the afferent vessels (figs. 9, 10, 15 and 21). Injection experiments as well as observations made on serial sections have demonstrated this connection. Thus, these sinuses should also be considered as corresponding to

the subcapsular sinus of the typical node as they received lymph directly from the afferent vessels. The other system of trabeculae when present arose from the capsule and usually penetrated cortical tissue (figs. 14 and 17).

The tissues of the trabeculae were the same as those of the capsule, i.e., collagenous fibers, elastic fibers and smooth muscle cells. Both collagenous and elastic fibers left the trabeculae singly and in groups which penetrated the parenchyma. The medullary cords and sinuses of the typical lymph node were not found in the pig node. Deposits of adipose tissue were characteristic of nodes from older pigs in a few cases.

Extensive fusion was characteristic of many pig lymph nodes. By this process many small adjacent nodes fused to form one large node. Observations made in this study indicate that fusion of the nodes began before birth. No evidence of continuing fusion was seen in the mesenteric node of a three year old pig. Trautmann (1926) described the process of fusion in detail.

B. THE MESENTERIC LYMPH NODES

The mesenteric lymph nodes of the pig were located in the mesentery of the small intestine. They were situated near the root of the mesentery and according to Sission and Grossman (1953) were adjacent to the anastomotic arches of the intestinal blood vessels. The nodes were arranged in a long chain that roughly paralleled the intestine. The afferent and efferent vessels of this group of nodes were the lacteals

of the small intestine. The efferent lymphatics carried lymph to the cisterna chyli.

1. The mesenteric lymph node in a 15 centimeter pig fetus. In the lymph node of the 15 cm fetus which would be approximately 30 days old, the capsule had not yet appeared, so the boundaries of the node were formed by sinuses at its periphery and by the surrounding connective tissue (fig. 1). This observation agreed with those of Sabin (1905) who described the capsule as first appearing in the 230 mm pig fetus. Trabeculae associated with the afferent lymph vessels and with blood vessels exhibited an abundance of fibroblasts and fine fibers were present (fig. 1). Trabeculae were extensions of the connective tissue adjacent to the part of the node where the afferent lymphatics and blood vessels entered the node. They penetrated deeply into the node, carrying the vessels with them and they frequently exhibited sinuses adjacent to their lateral surfaces. No trabeculae were seen entering the node from the surrounding connective tissue at any place in the periphery of the node except where afferent vessels also entered. Elastic fibers were not identified in trabeculae.

Both cortex and medulla could be identified in the node at this stage, but the latter comprised a very small part of the node. Within the cortex, nodules in early stages of development were seen (fig. 2). These were evident as areas where the dark stained nuclei of mature lymphocytes were closer to each other than those of the adjacent cortex.

Figure 1

Mesenteric node from a 15 cm fetus.

H. and E. stain 135 X

A. Trabeculum

B. Afferent vessel

Figure 2

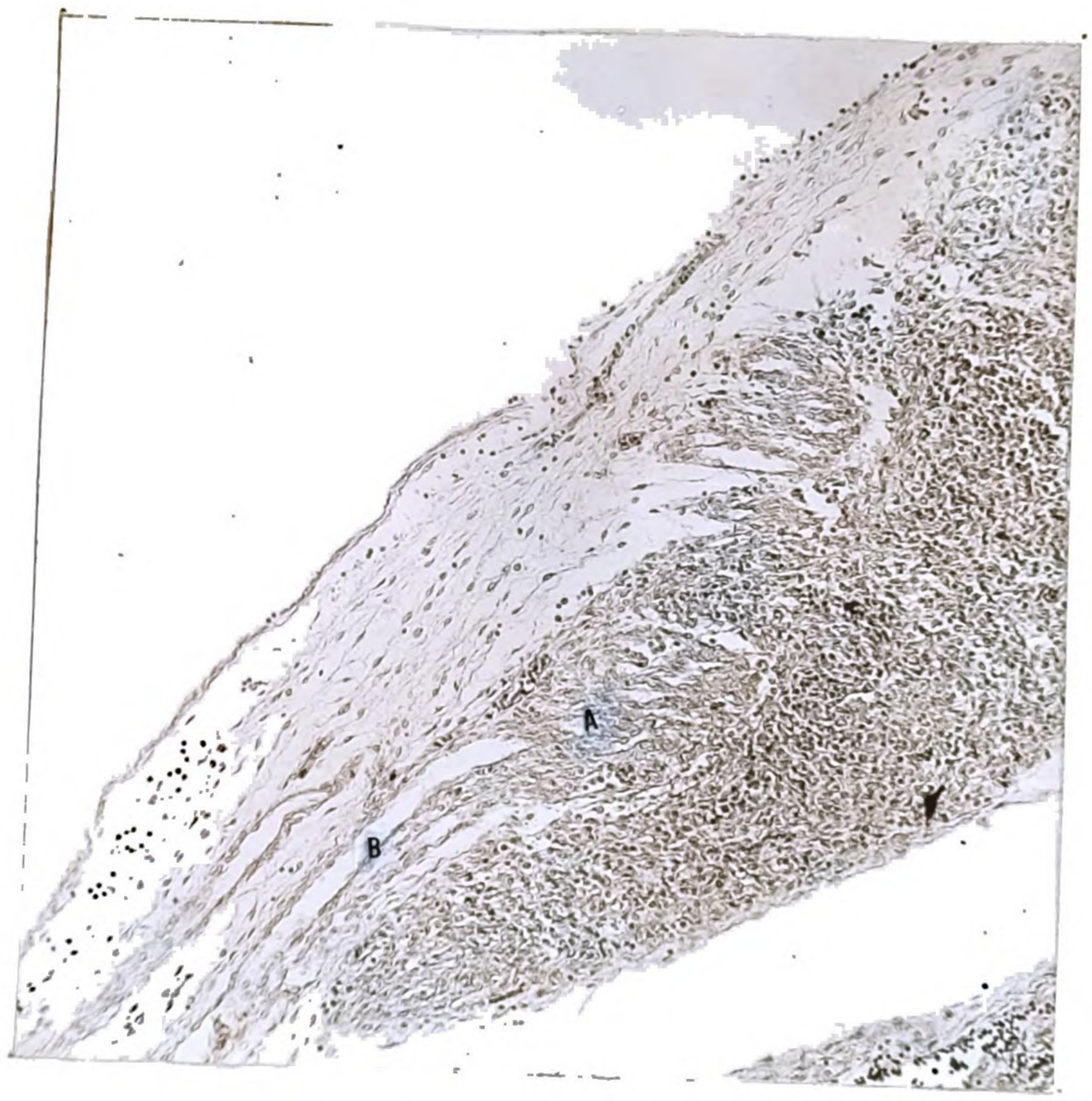
Same node as Fig. 1.

H. and E. stain 370 X

A. Nodule

B. Cortex

C. Connective tissue around node.


A
B

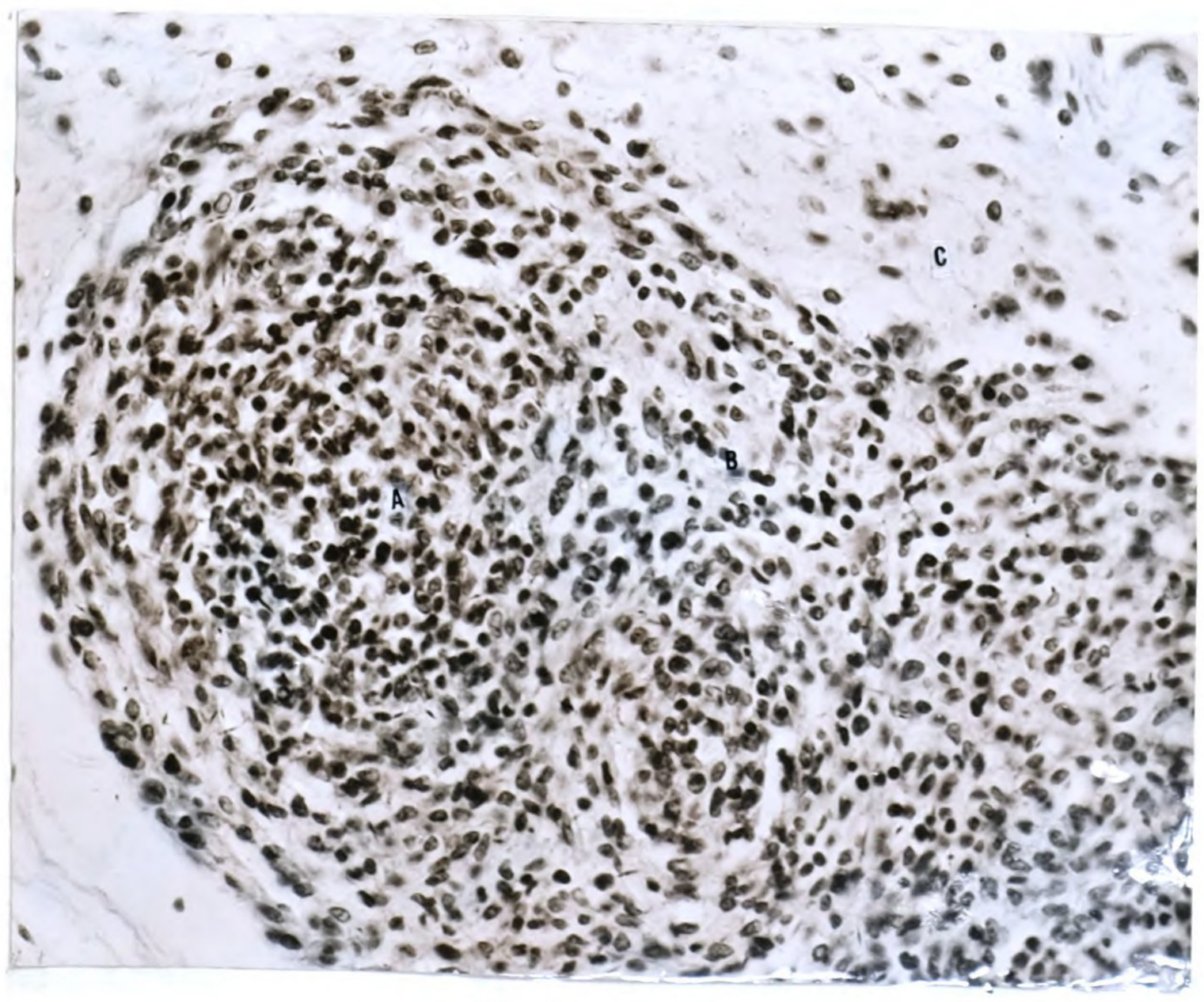

C
B
A

Figure 1

Mesenteric node from a 15 cm fetus.

H. and E. stain 135 X

A. Trabeculum

B. Afferent vessel

A

B

Figure 2

Same node as Fig. 1.

H. and E. stain 370 X

A. Nodule

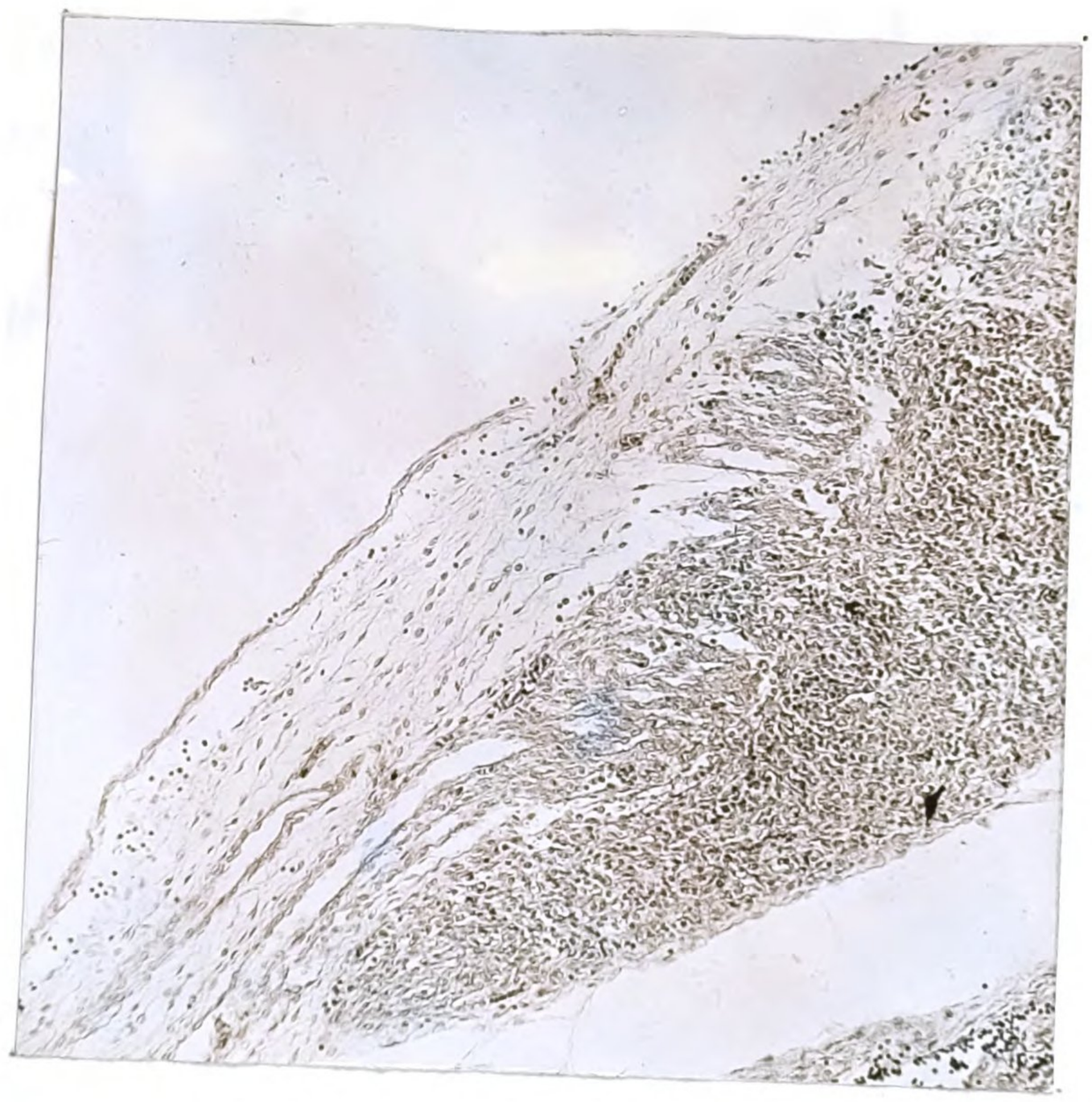
B. Cortex

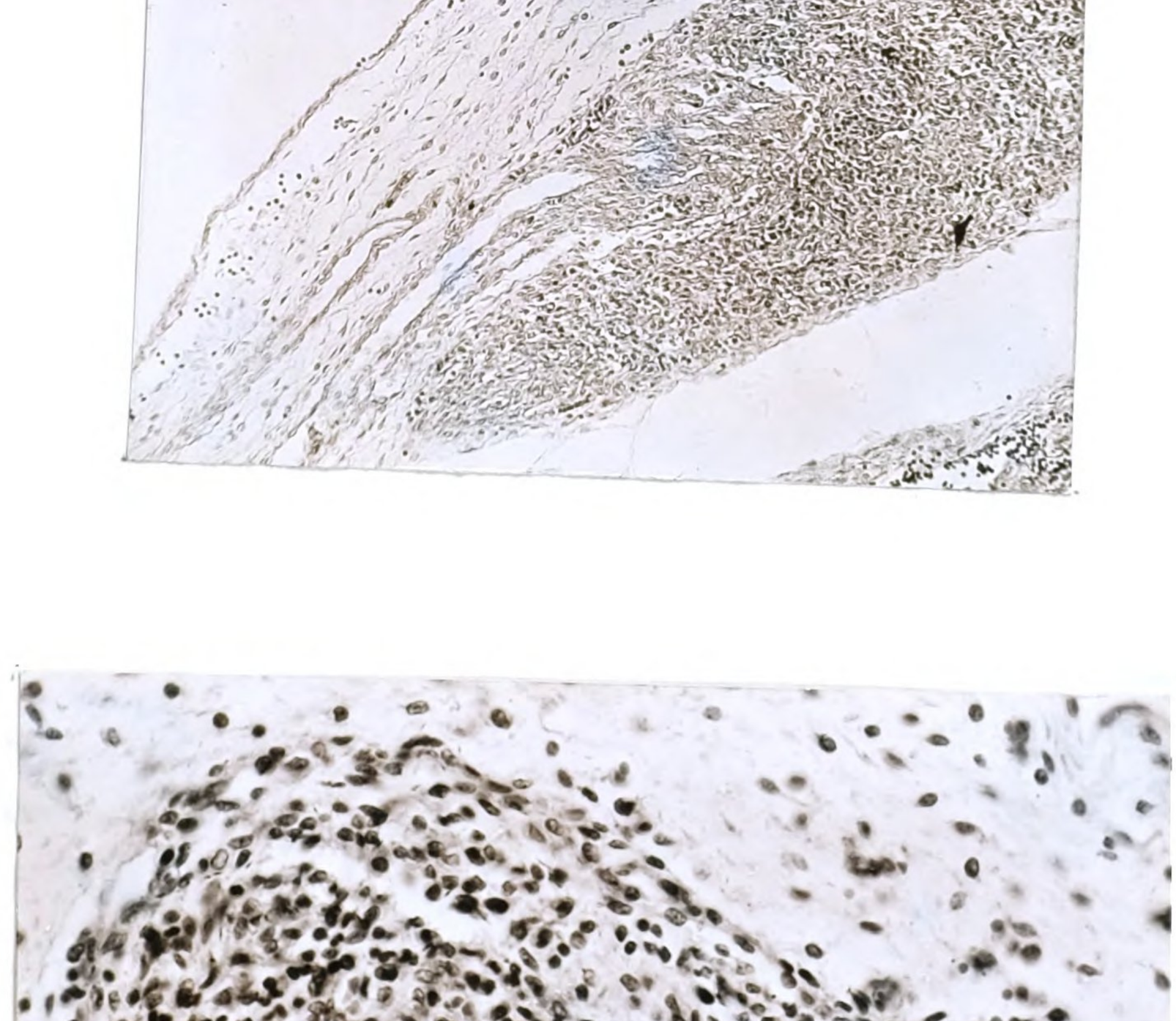
C. Connective tissue around node.

B

A







Occasionally, the blood capillary loop, described by Sabin (1905) around which the nodules develop, was seen. The heavy reticular and collagenous fibers which surrounded the nodule in the pig were not yet developed. The diameter of the nodules ranged in size from 25 to 125 micra. Sabin (1905) described the nodules of cervical and inguinal nodes from a fetal pig of this size as consisting of a few lymphocytes around a blood capillary. She also found, in these nodes, lymphatic cords composed of an arteriole surrounded by lymphocytes. The cords were bounded by sinuses. Lymphatic cords were seen as dense concentrations of lymphocytes, without clearly definable sinuses. Observations on one specimen of mesenteric node suggested that it was more advanced at this stage than the cervical node as described by Sabin (1905) and an inguinal node observed during this study. This was attributed to the greater development of the nodules and the absence of the sinuses around the lymphatic cords in the mesenteric node. Lack of definition in the latter was probably due to occlusion of their lumina by primitive reticular cells. This was suggested by observations made on a mesenteric node from a two day old pig and a popliteal node from a six day old animal (fig. 16). Obliteration of the sinuses occurred to some extent in lymph nodes of other species, but particularly in the pig nodes.

In the rest of the cortex a few small sinuses and blood capillaries were present. The lymphocytes were not as heavily concentrated as they were in nodes from older animals.

Two kinds of cells predominated in the cortex, namely, reticular cells and lymphocytes. Cowdry (1924) referred to the former as cells of the embryonic reticular syncytium. From these cells the reticulo-endothelial cells or macrophages were formed as well as the cells of the lymphocytic series. Reticular cells were stellate with a pale cytoplasm investing large nuclei with a sparse amount of chromatin (fig. 3). They surrounded the reticular fibers, but it is still open to question as to whether reticular fibers owe their origin to these cells. The cells were partially obscured by the great numbers of lymphocytes found in the cortex. At this stage large and medium sized lymphocytes predominated. Occasionally a lymphocyte undergoing mitosis occurred. Other cells frequently found in the cortex were myelocytes, metamyelocytes, and mature cells of both the neutrophilic and eosinophilic series. Macrophages were seen occasionally, as were monocytes, plasma cells, and megakaryocytes (fig. 3).

The medulla was distinguished by the presence of few lymphocytes. Consequently, the primitive reticular cells were prominent. Sinuses of various sizes were present, the average diameter of the smallest was about ten micra while the largest were approximately 45 micra in diameter. The large sinuses which anastomosed to form the efferent lymph vessels were evident in some sections (fig. 11). Occasional blood capillaries were found rather uniformly distributed in the medulla. In addition to the primitive reticular cells

Figure 3

Mesenteric node from a 15 cm fetus

H. and E. stain 1100 X

A. Recticular cell nucleus

B. Megakaryocyte

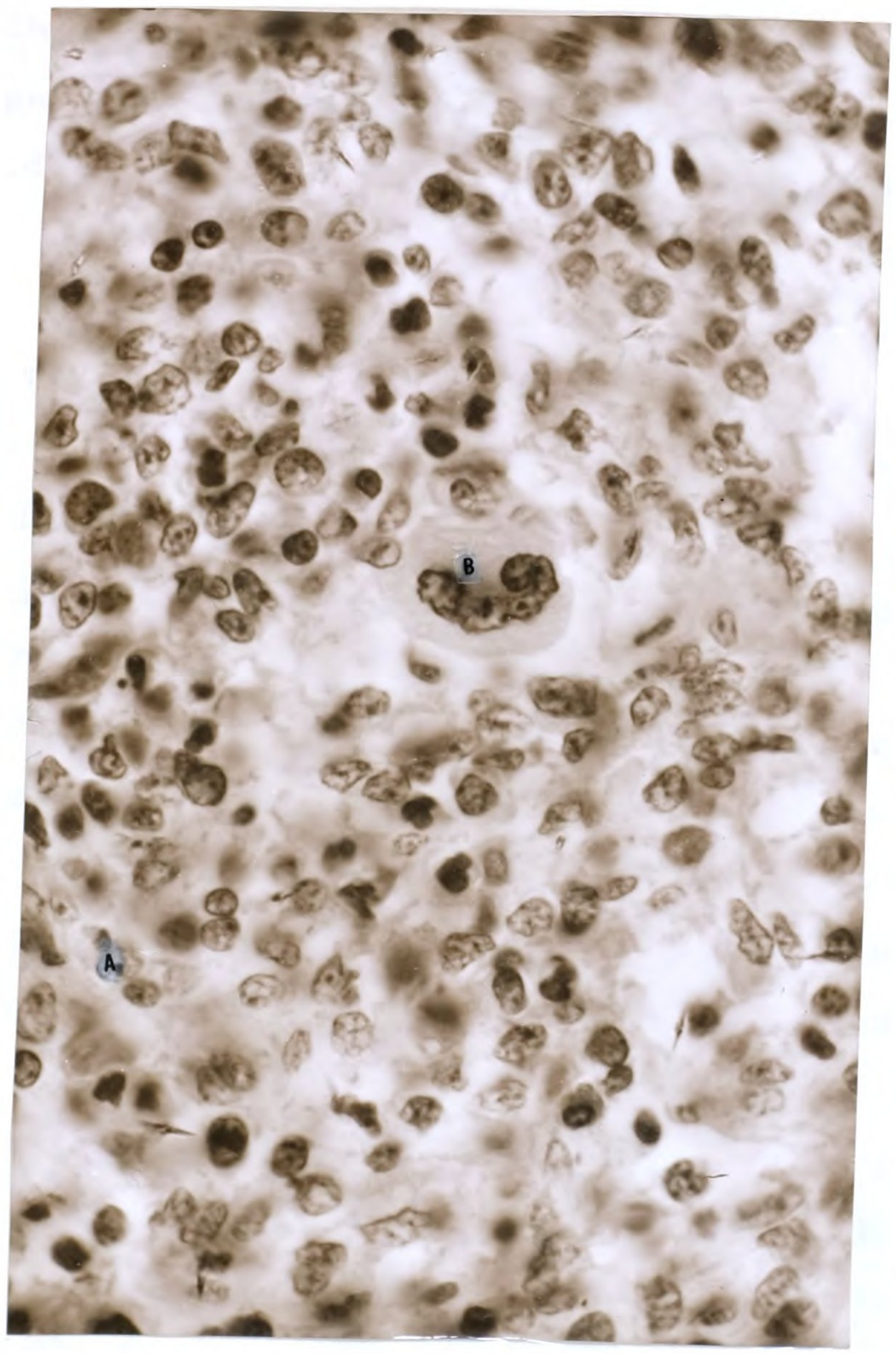

B
A

Figure 3

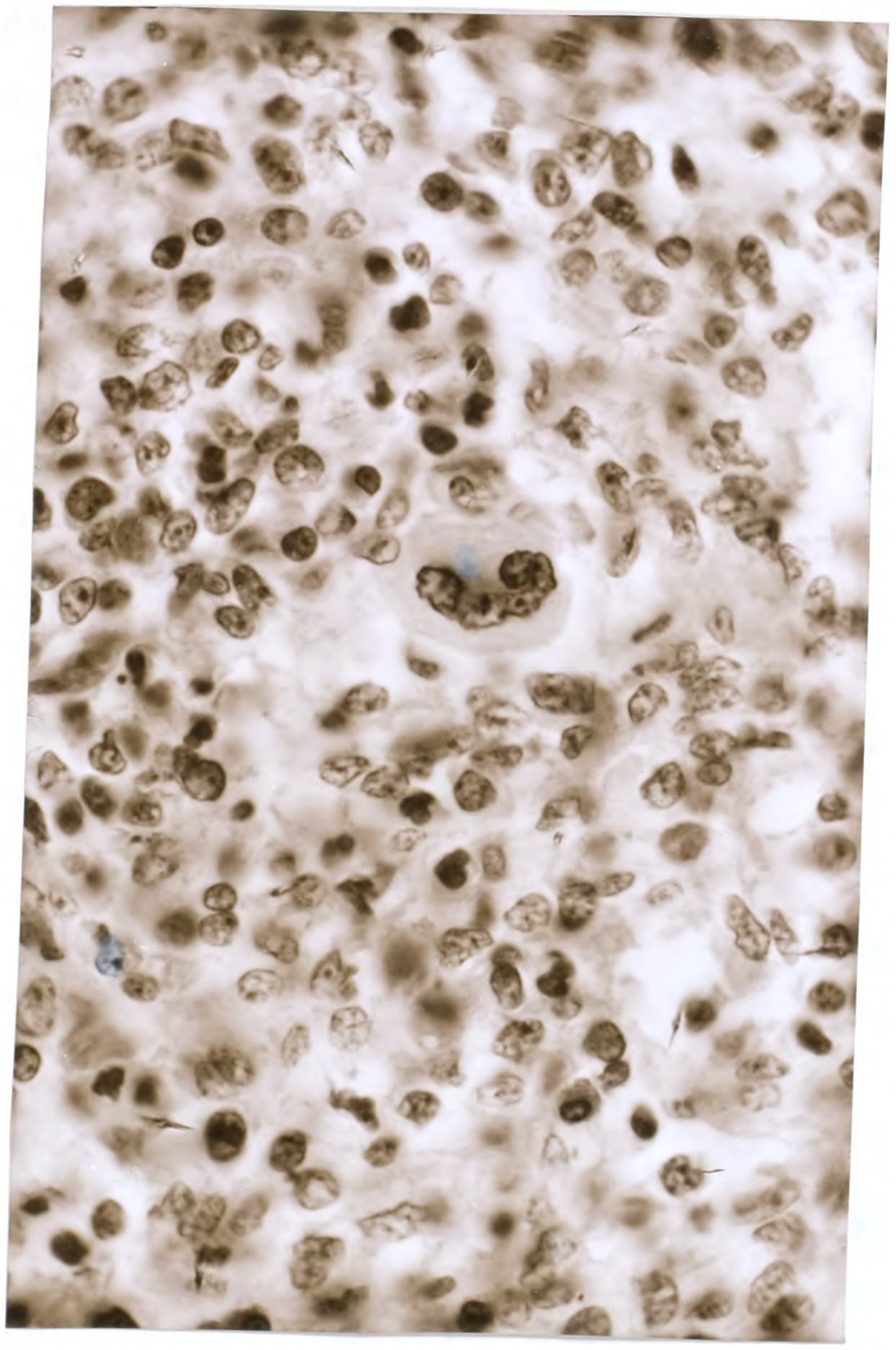
Mesenteric node from a 15 cm fetus

H. and E. stain 1100 X

A. Recticular cell nucleus

B. Megakaryocyte

A



and lymphocytes, some cells of the neutrophilic and eosinophilic series were found.

Sabin (1905) stated that embryos of 22 cm in length and also larger embryos had many minute nodes adjacent to the larger nodes. These minute nodes may have consisted of as little as a tuft of blood capillaries and a few lymphocytes. Clumps of lymphocytes and plasma cells were found adjacent to blood vessels in the connective tissue of the mesentery in the 15 cm pig fetus, and it was suggested that these were also developing nodes.

2. The mesenteric lymph node of the 21 centimeter pig fetus. The mesenteric lymph node of the 21 cm pig fetus resembled the definitive node in its general structure. Medulla and cortex could be recognized and the trabecular system with its efferent lymph vessels was well established (fig. 4). The plexus of lymphatics that Sabin (1905) described as surrounding the entire node of a 23 cm fetus was not yet evident.

A thin but definite capsule had formed around parts of many of the nodes examined and it was virtually complete around one node. The capsule was composed of fine collagenous fibers with few to many fibroblasts. These observations were in general agreement with those of Sabin (1905) who stated that in nodes from 23 cm fetal pigs the capsule was complete except for those locations where a plexus of lymph vessels was present on the border of the node.

Only that system of trabeculae which carried the affer-

Figure 4

Mesenteric node from a 22 cm fetus

H. and E, stain 90 X

A. Trabeculae with afferent vessels

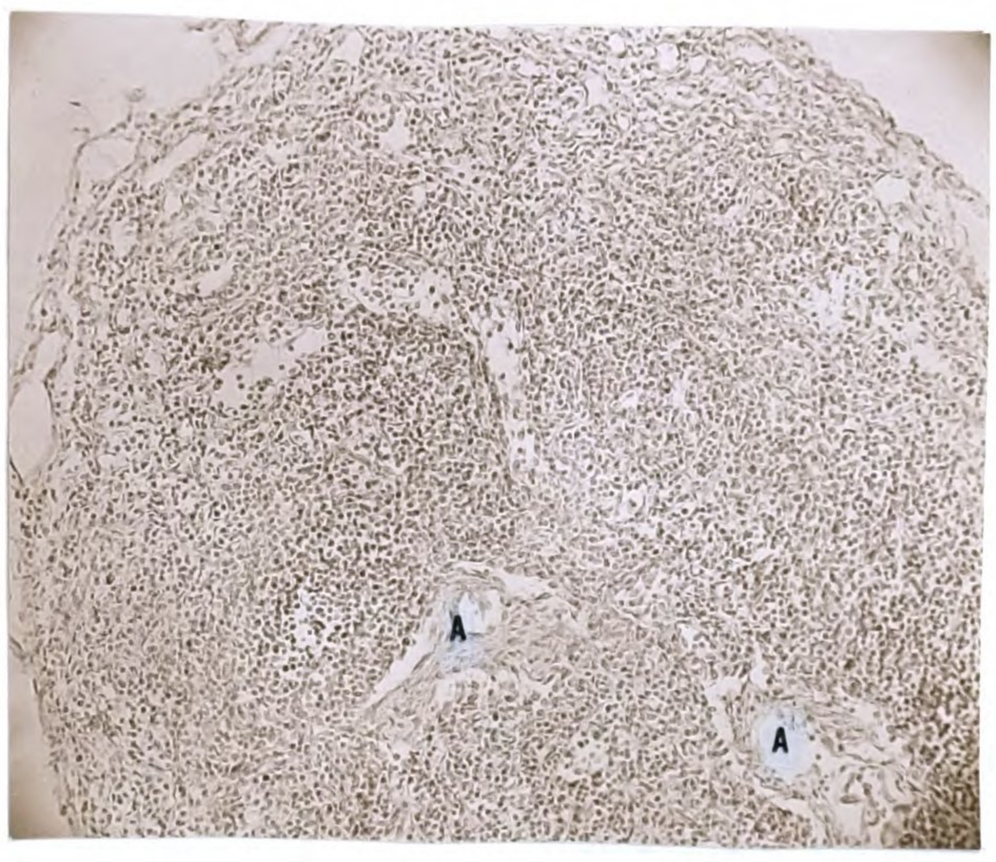

A
A

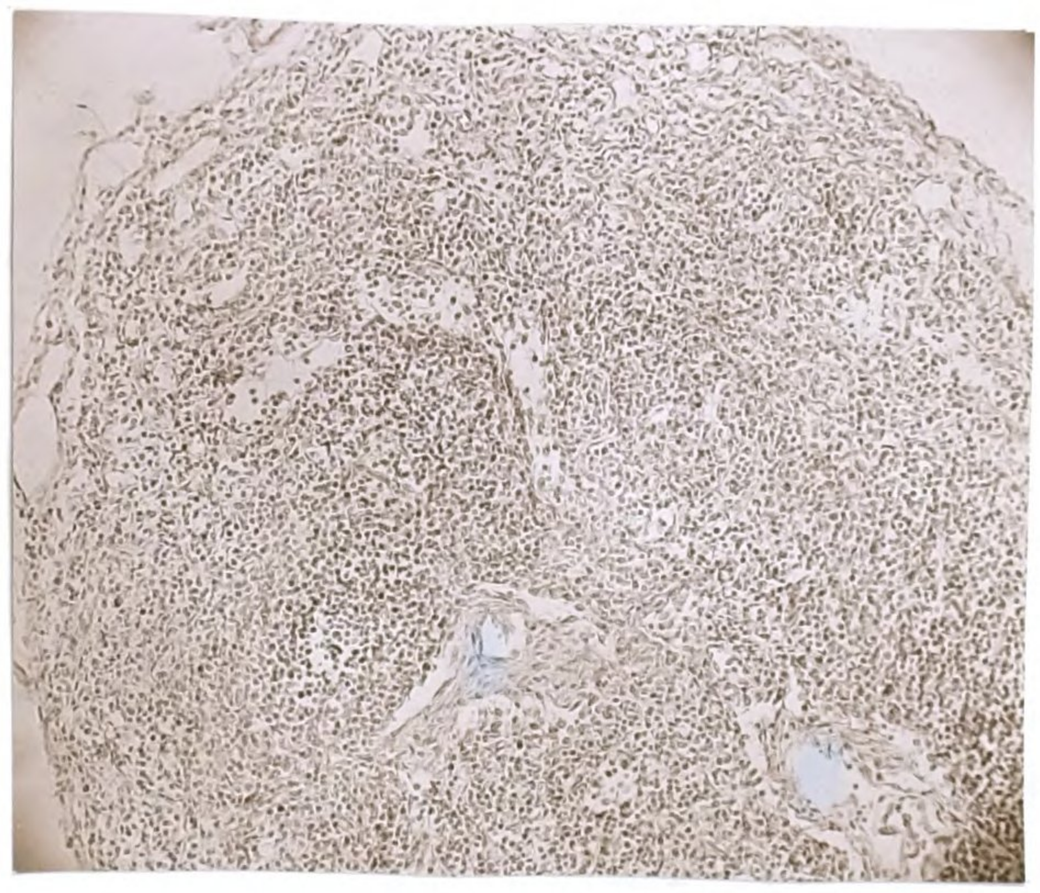
Figure 4

Mesenteric node from a 22 cm fetus

H. and E, stain 90 X

A. Trabeculae with afferent vessels





ent lymphatics was seen (fig. 4). It showed a considerable advance in development over the previously described node in which the trabeculae consisted largely of closely packed fibroblasts. In the node from the 21 cm fetus the trabeculae were formed of fine connective tissue fibers. Isolated lymphocytes were common within the trabeculae. Both blood and lymph vessels were contained within the large trabeculae. Valves to control the direction of the flow of lymph were seen in some afferent vessels. Fine fibers of connective tissue branched from the trabeculae in groups to enter the parenchyma and often a sinus surrounded part or all of the trabeculum. The sinuses were also traversed in a few places by reticular cells.

The cortex of the node at this stage showed very little change from the cortex of the mesenteric node in the 15 cm fetus. However, the lymph nodules were more numerous and their borders were more clearly defined. Chievitz (1881) described connective tissue fibers surrounding the nodule in mesenteric nodes from fetal pigs of sixteen to eighteen centimeters. Connective tissue fibers investing lymph nodules were not observed in nodes from fetal animals during the course of this study.

With the exception of eosinophils, leucocytes of the granulocytic series were not often seen. Eosinophils were frequently a common constituent of the cortex of pig lymph nodes. Blood capillaries were numerous and macrophages and monocytes were fairly abundant in both cortex and medulla.

It was apparent that the greatest difference between the mesenteric lymph nodes of the 21 cm pig fetus and the 15 cm pig fetus lay in the development of the stroma rather than the parenchyma.

3. The mesenteric lymph node of a two-day-old pig. Under the microscope the general appearance of the node which was examined resembled nodes in the fetal condition more than it resembled nodes from older animals.

Within the node the sinuses around the trabeculae were wide, and frequently a plexus of small lymphatics was found near the capsule (fig. 5). The presence of this plexus was possibly the reason Ranvier (1895) described this tissue as cavernous.

The mesenteric node at this age was completely surrounded by a very thin capsule of fine collagenous fibers. Blood vessels passed through the capsule adjacent to both afferent and efferent lymphatics. The connective tissue fibers of the trabeculae were still very fine and many young fibroblast nuclei were present. Occasional smooth muscle cells and elastic fibers were also present. Figure six illustrates this node.

In the cortex some of the nodules now showed a small secondary nodule. Many nodules were larger than those observed in the fetus. The largest nodule measured in the two day old node had a diameter of about 300 micra. In some areas of the cortex the lymphocytes were packed as closely as they were in nodes from older animals, but in others the

Figure 5

Mesenteric node from a 2 day old pig.

H. and E. stain 135 X

A. Plexus of lymphatics

B. Capsule

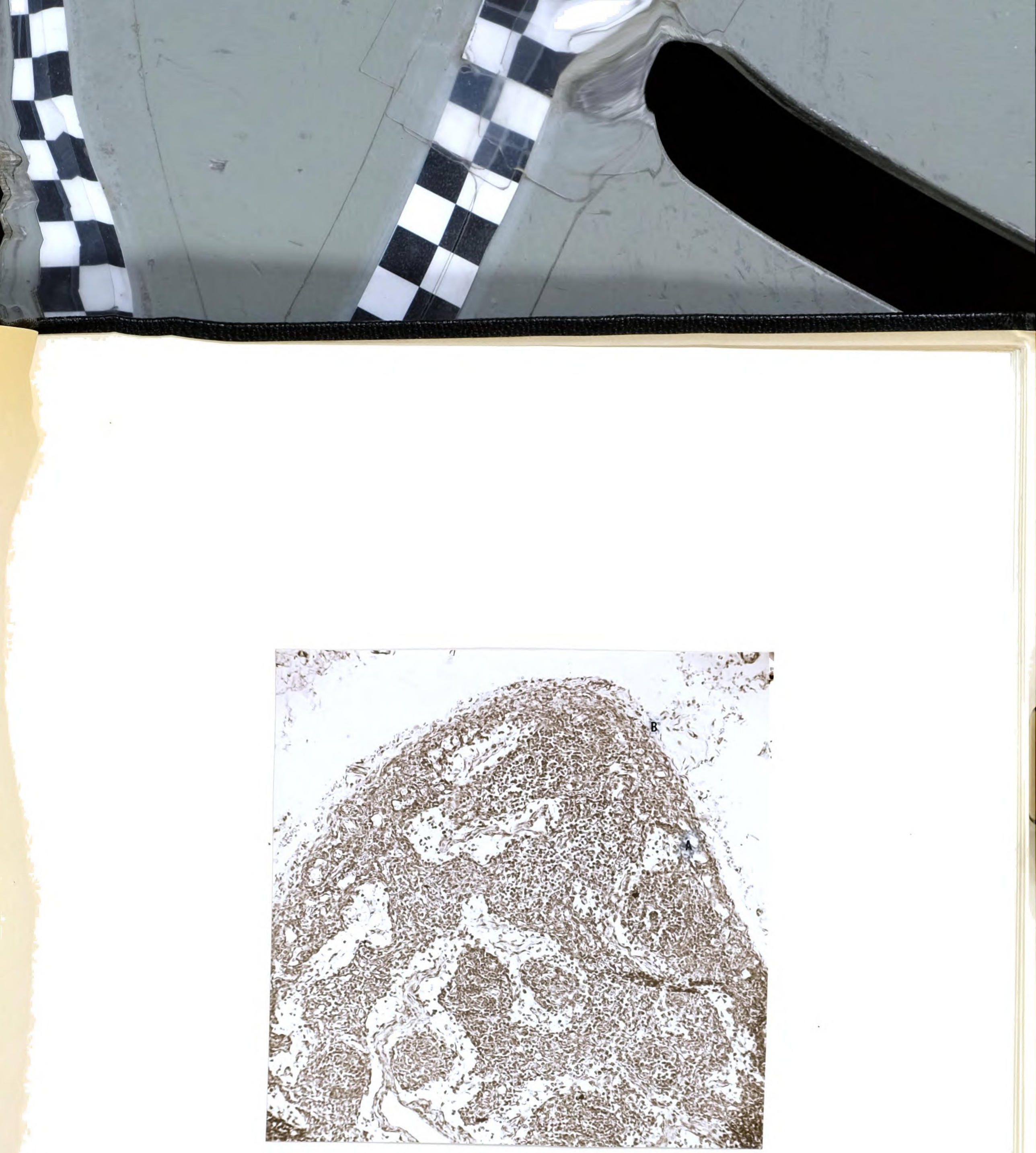

B
A



Figure 5

Mesenteric node from a 2 day old pig.

H. and E. stain 135 X

A. Plexus of lymphatics

B. Capsule

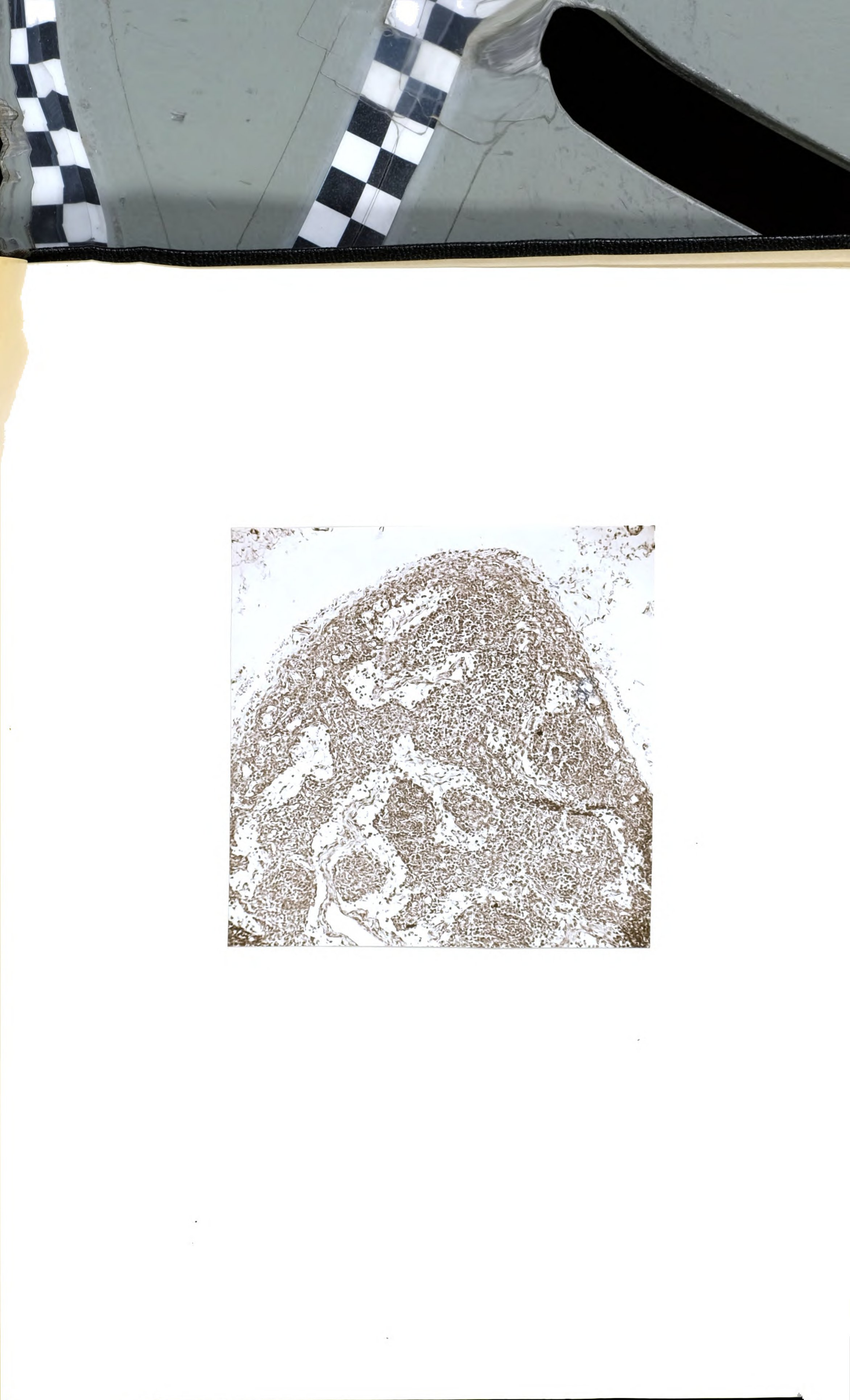


Figure 6

Mesenteric node from a 2 day old pig

H. and E. stain 180 X

A. Afferent vessel with valve

B. Cortex

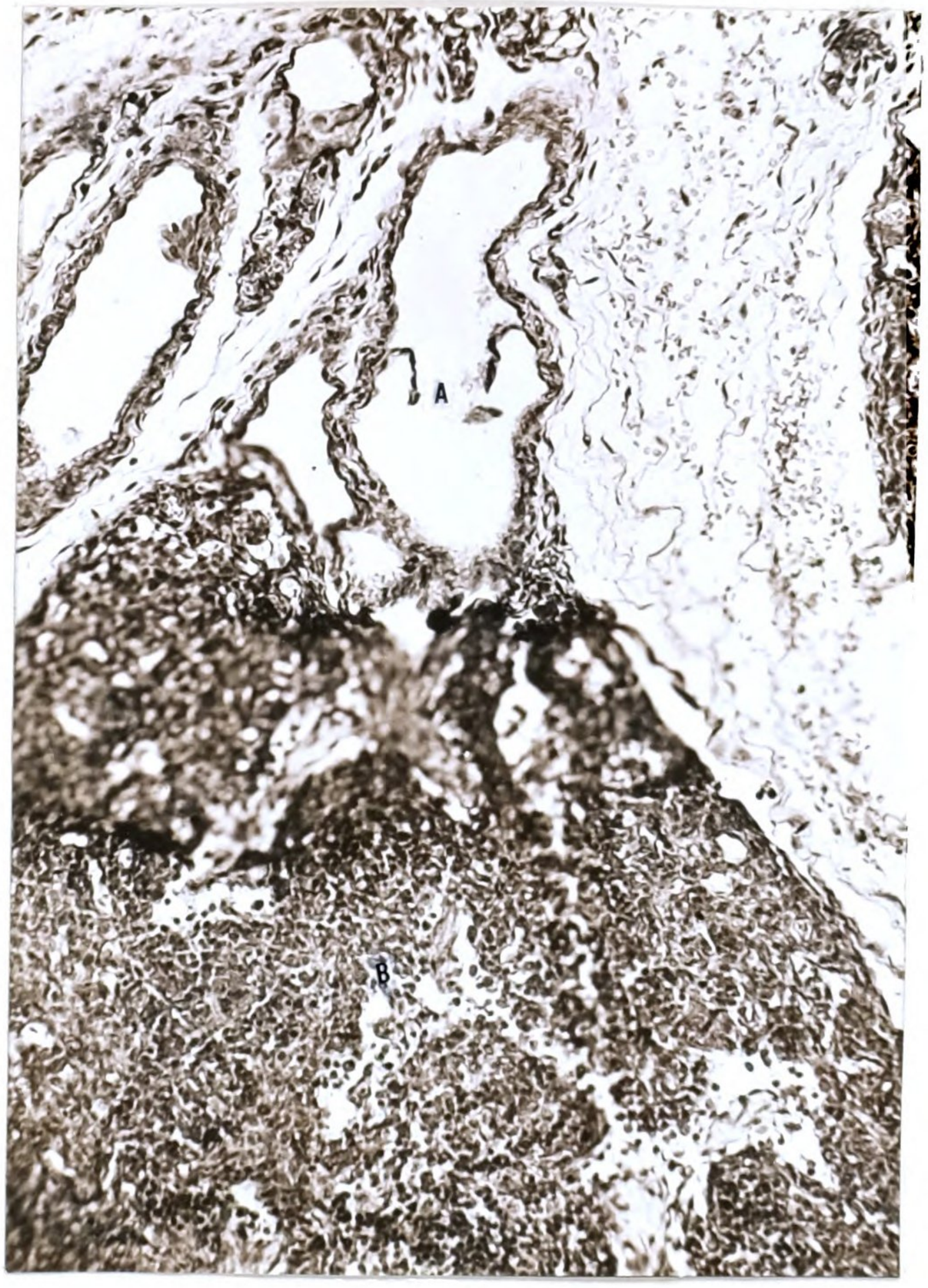

A
B

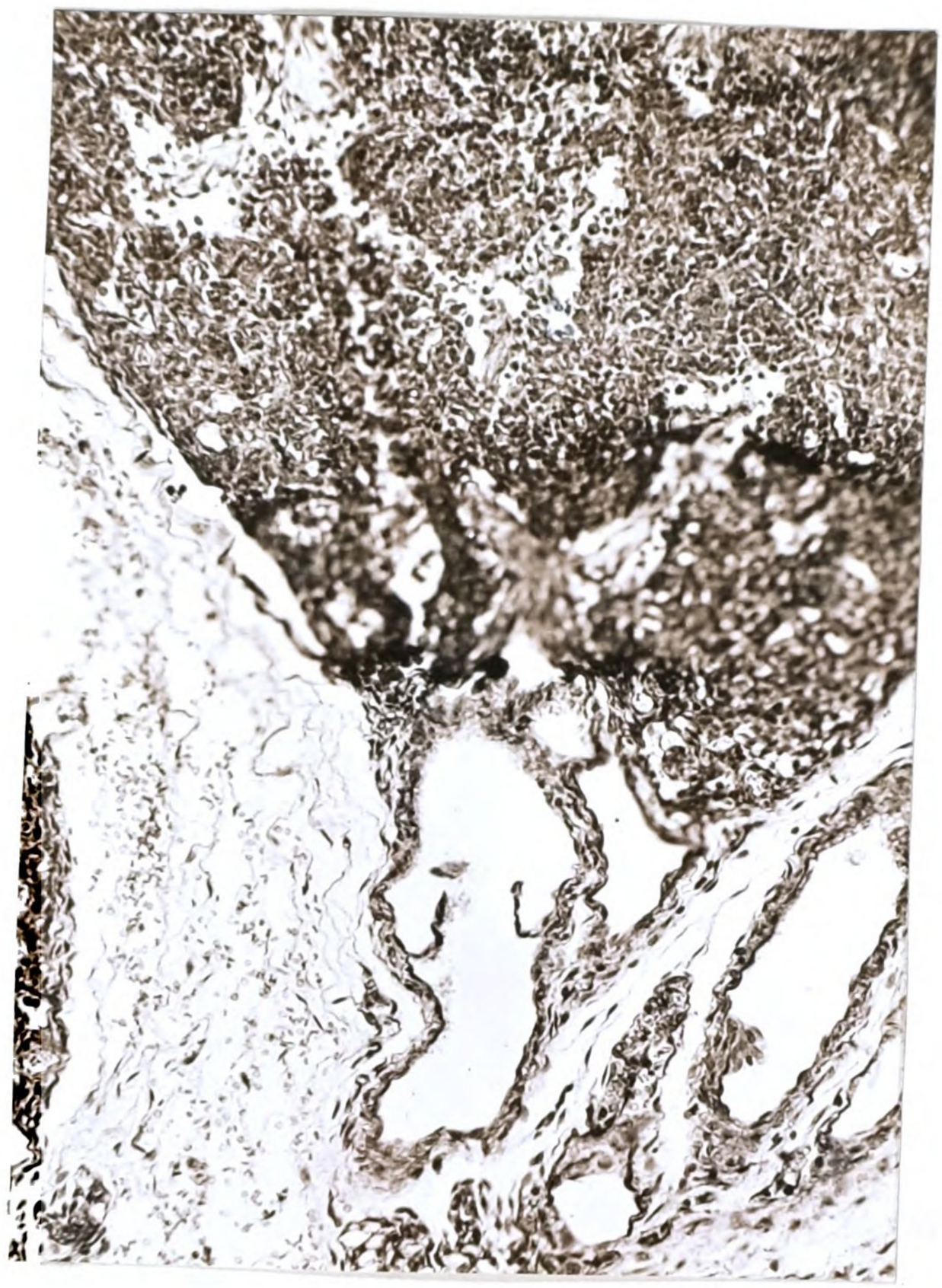
Figure 6

Mesenteric node from a 2 day old pig

H. and E. stain 180 X

A. Afferent vessel with valve

B. Cortex



density of these cells was no more than that found in fetal mesenteric nodes. Many eosinophilic metamyelocytes and eosinophils were seen as well as a few neutrophils, neutrophilic metamyelocytes, macrophages and monocytes. The eosinophilic cells were seen singly or in groups of up to four cells.

The medulla made up a very small portion of the periphery of the node. It showed little change from the medulla of the 21 cm fetal node. The plexus of lymphatics near the capsule when present penetrated the medulla as well as the cortex.

Examination of serial sections of one mesenteric node from a two day old pig disclosed the presence of two groups of afferent vessels entering it at two separate locations on the surface of the node. Each group consisted of two or three vessels. Further, this node had two systems of trabeculae which carried the vessels into the node. Thus it might be assumed that the node was formed by the fusion of two smaller nodes, and that each small node originally had only one set of afferent lymphatics and one system of trabeculae. This was also observed in a popliteal node from a two day old pig (fig. 15). Sabin (1905) stated that fusion of nodes occurred in the embryo after the capsule had formed and that the capsules of the small nodes became trabeculae in the fused node. Trautmann (1926) stated that the age at which fusion begins had not been reported, but that nodes in the beginning stages of fusion as well as completely fused nodes were found in the nine month old pig. He also stated

that the process of fusion continued until all traces of the capsules have disappeared and the parenchyma of the nodes had fused.

As stated before, the mesenteric nodes from a 21 cm fetus showed no indications of fusion. As a fetus of this size was approximately 100 days old and as the period of gestation of the pig was approximately 114 days it may be suggested that intrauterine fusion of mesenteric lymph nodes occurred late in pregnancy. Indications of the trabeculum that originally could have been a portion of the fused nodes were not found. Thus it may be stated that the description of fusion given by Sabin (1905) was incomplete because observations made in this study indicate that the fused capsules did form a trabeculum, but only in the earlier stages of fusion.

4. The mesenteric node of a six-day-old pig. The mesenteric node of a six-day-old pig which was examined showed some evidence of change when compared with the mesenteric node from the two day old animal. The capsule was about the same as that of the younger animal, but the connective tissue fibers of the trabeculae were less delicate in nodes from the older animals. A few lymphocytes were seen in the trabeculae. The sinuses adjacent to trabeculae were less prominent in nodes from older animals, and a subcapsular sinus was frequently present around at least part of each node. The sinuses near the periphery of the nodes from younger animals were not seen in sections made of nodes from a six

day old pig.

Little change had taken place in the cortex. Cells of the neutrophilic series were seen, and those of the eosinophilic series were very common. Monocytes and macrophages were also present. The medulla was more readily distinguished at this stage than in the stages previously described, because of a stronger development of the primitive reticular cell network, but it still formed only a small portion of the periphery of the node.

5. The mesenteric Lymph node of a three-week-old pig. A mesenteric node from a pig three weeks old showed evidence of continued fusion. It was provided with three groups of afferent vessels. Between these groups of vessels the parenchyma was crossed from side to side by narrow bands or regions characterized by many sinuses and small blood vessels (figs. 7 and 8). According to the description given by Trautmann (1926) these bands were indicative of the stage of fusion which follows resorption of the capsule.

The capsule showed a few short trabeculae which projected into the cortex. One of the trabeculae was seen to connect with a branch of the trabecular system which carried the afferent vessels. The larger trabeculae at this stage had a considerable quantity of smooth muscle and their collagenous fibers were heavier than those seen in the earlier stages.

Here and there blood vessels were seen passing through the capsule into the parenchyma, particularly where the cap-

Figure 7

Mesenteric node from a three weeks old pig

H. and E. stain 75 X

A. Medulla

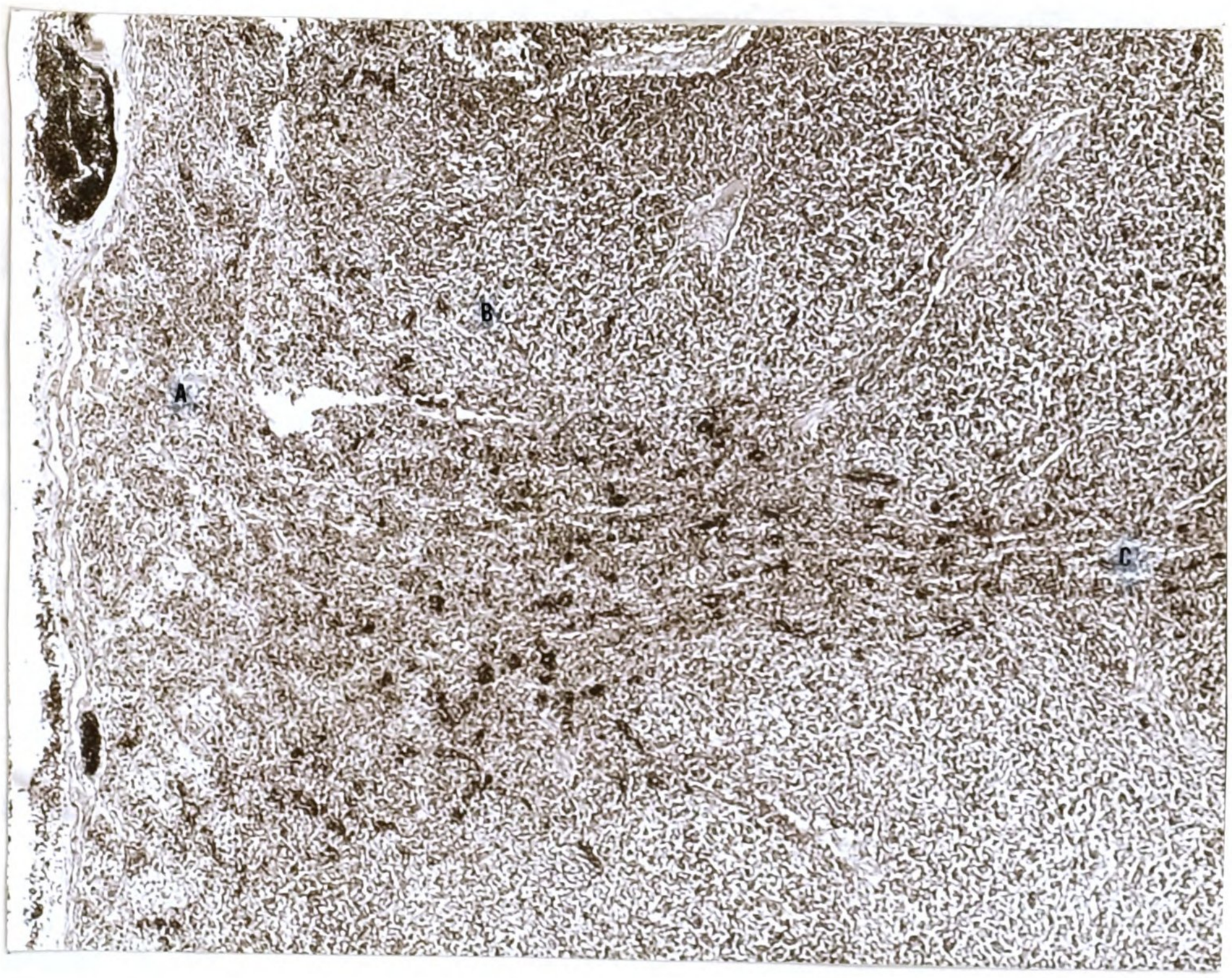
B. Cortex

C. Line of fusion with lymph sinuses and blood vessels.

Figure 8

Enlarged view of a portion of Fig. 7

225 X


B
A
C

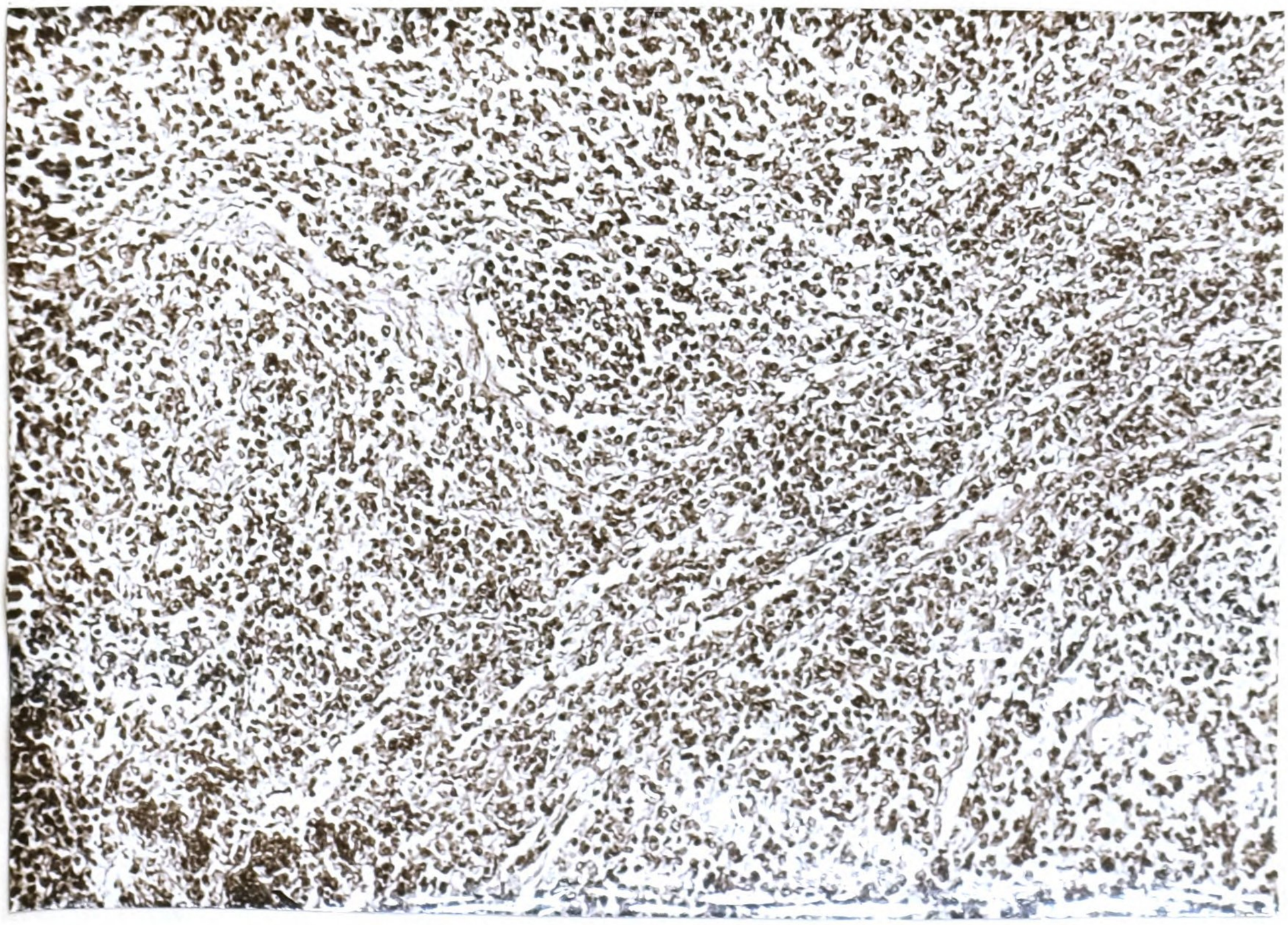


Figure 7

Mesenteric node from a three weeks old pig

H. and E. stain 75 X

A. Medulla

B. Cortex

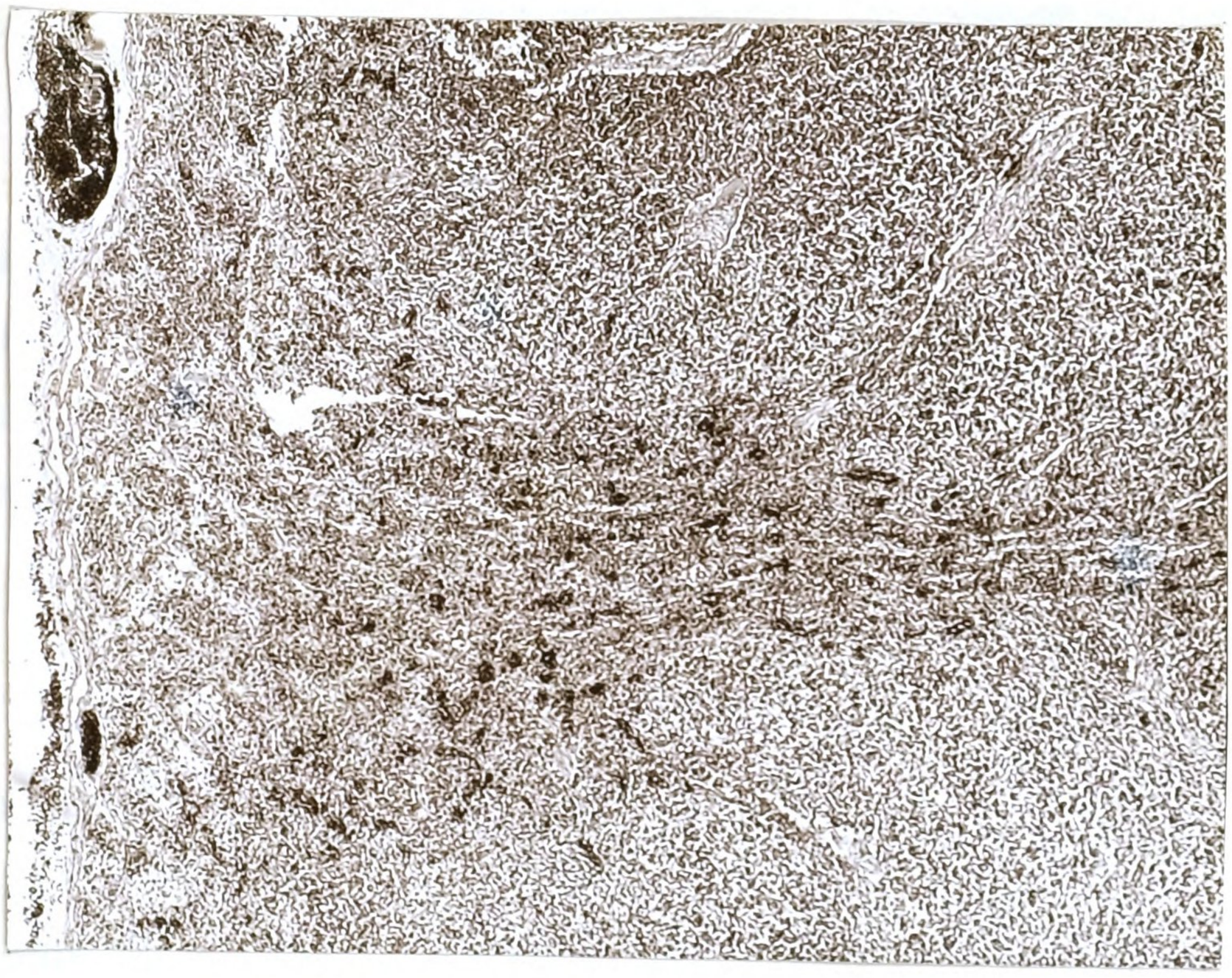
C. Line of fusion with lymph sinuses and blood vessels.

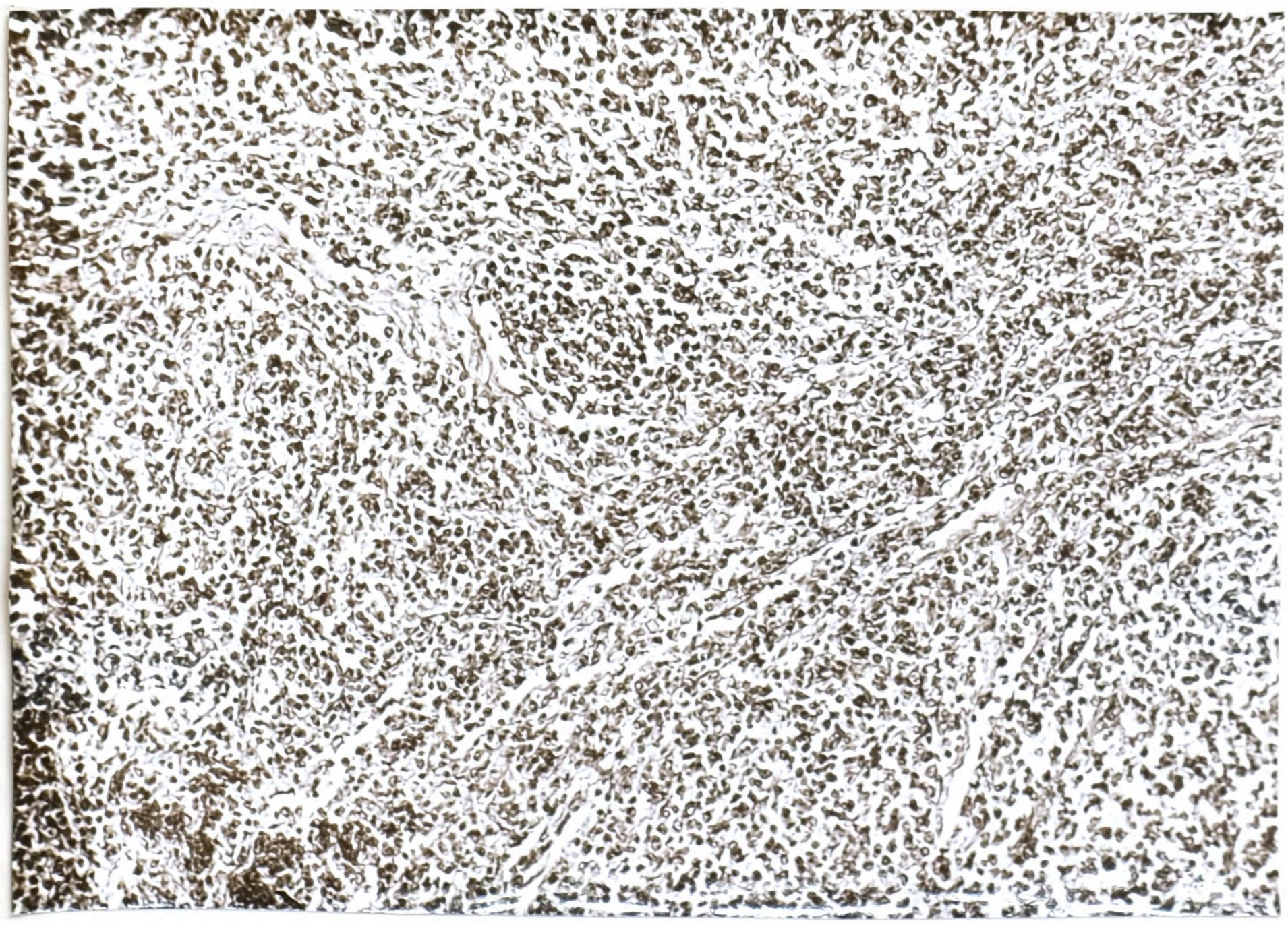
Figure 8

Enlarged view of a portion of Fig. 7

225 X







sule was adjacent to medullary tissue. This suggests that the major blood supply to the node entered through the capsule adjacent to the medulla rather than with the afferent lymphatics and through the trabecular system. This was in agreement with a statement made by Chievitz (1881), who reported that in the pig node the blood supply to the nodules entered the node at the place where the efferent vessels left the node.

Baum (1911) stated that the nodules in lymph nodes of the domestic animals were surrounded by concentric layers of reticular fibers. Richter (1902) reported that in the pig lymph node these fibers formed the boundary of the nodule. Goldkuhl (1927) and Hellman (1930) described these fibers in the pig node as having the appearance of a capsule which surrounded the nodule. In the present study sections of the mesenteric node from a three week old pig were subjected to silver precipitation stains to ascertain if the nodules were surrounded by a capsule-like concentration of reticular fibers. Examination of these preparations disclosed that this structure had not yet developed. Baum and Hille (1908) described the nodules of the mesenteric and other nodes of a three to four week old pig as being clearer and more numerous than those in younger animals, but without the appearance they had in older animals. Observations made during the present study were in agreement with their description.

The various cells of the leucocytic series present in mesenteric nodes of the previously described stages were al-

so seen in both the cortex and medulla of mesenteric nodes from a three weeks old animal. Cells of the eosinophilic series were the most numerous of the leucocytes seen.

6. The mesenteric lymph node of a four-month-old pig. The nodes from two pigs four months old which were examined as histological preparations showed evidence of continued fusion. However, the mesenteric node had not become a conglomerate of smaller nodes as had those from the popliteal and other regions (fig. 17). The capsule remained thin, but the trabecular system which carried the afferent vessels was well developed (fig. 9). Trabeculae which originated at the capsule were rarely found, but a few were seen in the medulla where fusion was occurring and others were seen where cortical tissue was in contact with the capsule. Thus sections from mesenteric nodes had the appearance of consisting largely of parenchyma with a small quantity of stroma.

Richter (1902) classified the lymph nodes of the pig according to the region from which the node was obtained and the development of the connective tissue septal system. He placed the mesenteric node, as well as some other nodes which received lymph from the viscera of the abdominal cavity, in the group which had the least stroma. Trautmann (1926) stated that the quantity of stroma observed in sections was determined by which plane of the node the section was taken from, and not the region from which the node was taken. He further stated that since hila were absent it was impossible to orient pig nodes in such a way that sections could be cut

Figure 9

Mesenteric node from a 4 month old pig

H. and E. stain 75 X

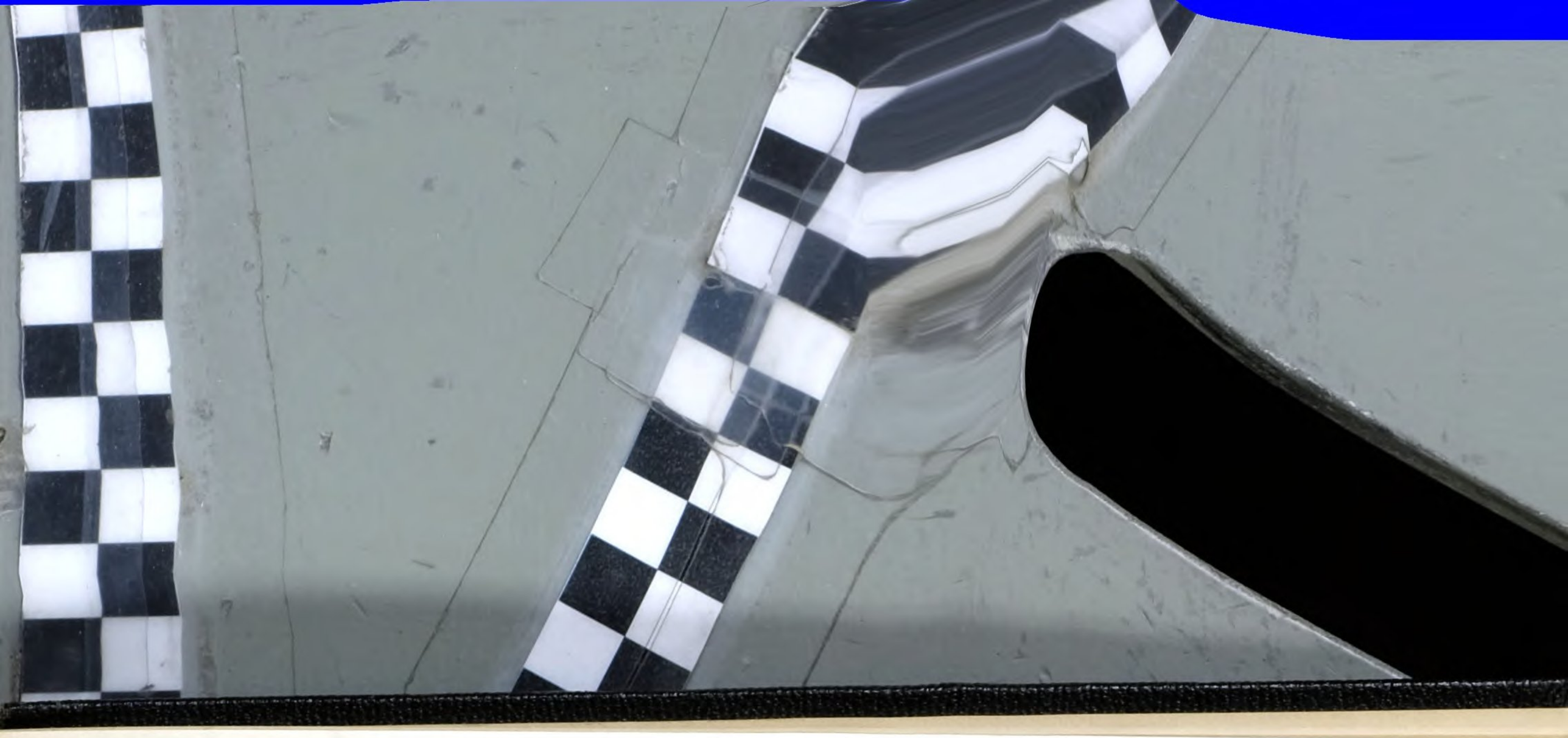
Area with heavy stroma

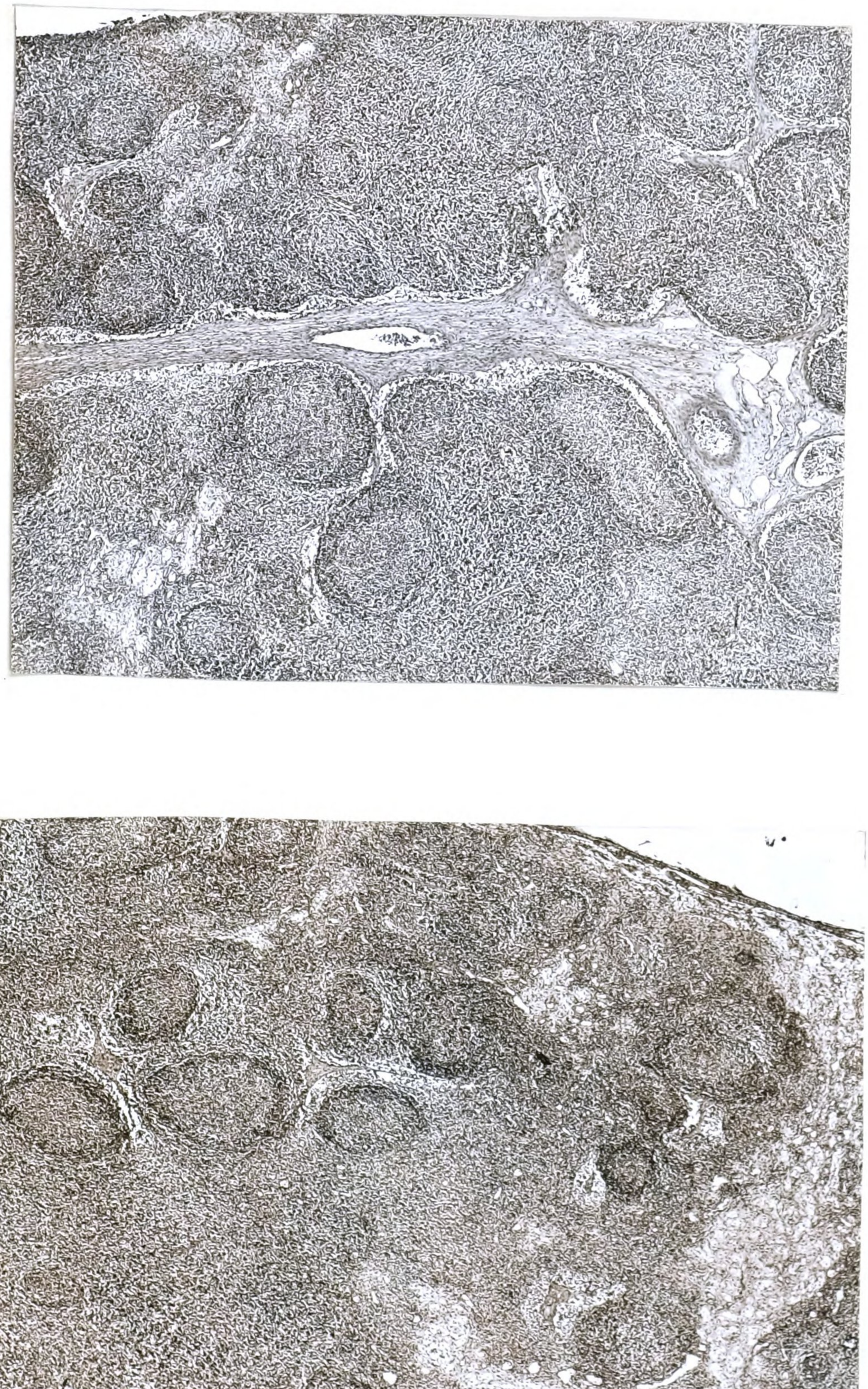
Figure 10

Same node as Fig. 9.

H. and E. stain 75 X

Area with light stroma





in the same plane from two or more nodes. The present study indicated that Trautmann (1926) was correct in both statements. Further, there was considerable variation in the quantity of stroma at different levels in the same node. When the section under examination was cut at a level where afferent vessels entered the node the heavy trabeculae which carried the vessels were evident, and the section showed a strong development of stroma (fig. 9). Examinations of sections above and below this level showed only the smaller branches of these trabeculae and consequently the stroma appeared poorly developed (fig. 10).

As stated above trabeculae that originated at the capsule were rarely seen in mesenteric nodes. However, examination of popliteal nodes disclosed the presence, in some regions, of many trabeculae originating at the capsule which in this node was frequently adjacent to cortical tissue. These regions appeared very much like the cortex of a typical mammalian node because the trabeculae divided the cortex into alveoli and frequently joined the trabecular system which carried afferent vessels. Further, the subcapsular sinus was confluent with sinuses adjacent to the trabeculae as it was in the typical node (fig. 17). These trabeculae gave the popliteal node the appearance of having a much stronger development of stroma than the mesenteric node. Thus it must be concluded that there was some justification for Richter's classification. Although examination of popliteal nodes also disclosed areas in the interior of the

node with few trabeculae the present study suggested that the mesenteric node had less stromal development than the popliteal node.

Bundles of elastic fibers were present in both the capsule and trabeculae of mesenteric nodes from a four months old pig, but their occurrence was sporadic (fig. 18). Both had many heavy collagenous fibers. In the capsule blood capillaries and pre-and post capillary vessels were occasionally seen. Probably some of these larger vessels were entering or leaving the parenchyma. Lymphocytic infiltration of the trabeculae, a characteristic of nodes from adult pigs, was common.

In the cortex most of the nodules had a thin dark staining border of closely packed lymphocytes, and a pale staining secondary nodule or germinal center. Mature lymphocytes were not nearly as numerous in the secondary nodule, thus the primitive reticular cells were more evident. Lymphoblastic cells with pale staining nuclei and a few cells in mitosis also were present in the secondary nodules. At this stage the largest nodules seen were approximately 400 micra in diameter.

The medulla formed a thin layer around most of the periphery, although cortical tissue was adjacent to the capsule at a few places (fig. 17). In some regions the medulla widened and extended well into the interior of the node, as was characteristic of nodes from adult pigs (fig. 17). Isolated areas of medulla surrounded by cortical tissue were

seen occasionally at this stage. This also was characteristic of nodes from adult pigs. Isolated areas of medulla have been described by numerous authors as transverse sections of medulla which project into the cortex from the periphery. Observations made during the present study support this view.

Cells of the leucocytic series seen in mesenteric nodes from four months old pigs were apparently largely confined to the medulla, and cells of the eosinophilic series were not numerous.

7. The mesenteric lymph node of the five-month-old pig. Two mesenteric nodes from one animal were studied, but no structural differences were noted from the nodes from four months old pigs, except in the development of the nodules. Nodules seen in mesenteric nodes of the five months old pig were less numerous and rarely showed secondary nodules although they were almost as clearly differentiated from the surrounding cortical tissue. Secondary nodules have been described by various authors as being centers of lymphocytopoietic activity, phagocytic centers or reaction centers to noxious substances with an accompaning increase of lymphocyte production. It was not the intent of this work to inquire into this subject, but merely to suggest that the development of nodules and secondary nodules was not closely related to the age of the animal. Figure 11, which illustrates the mode of formation of efferent lymphatics, was prepared from a mesenteric node from a five months old animal.

Figure 11

Mesenteric node from a five month old pig.

H. and E. stain 135 X

A. Origin of an efferent lymph vessel

B. Efferent lymph vessel

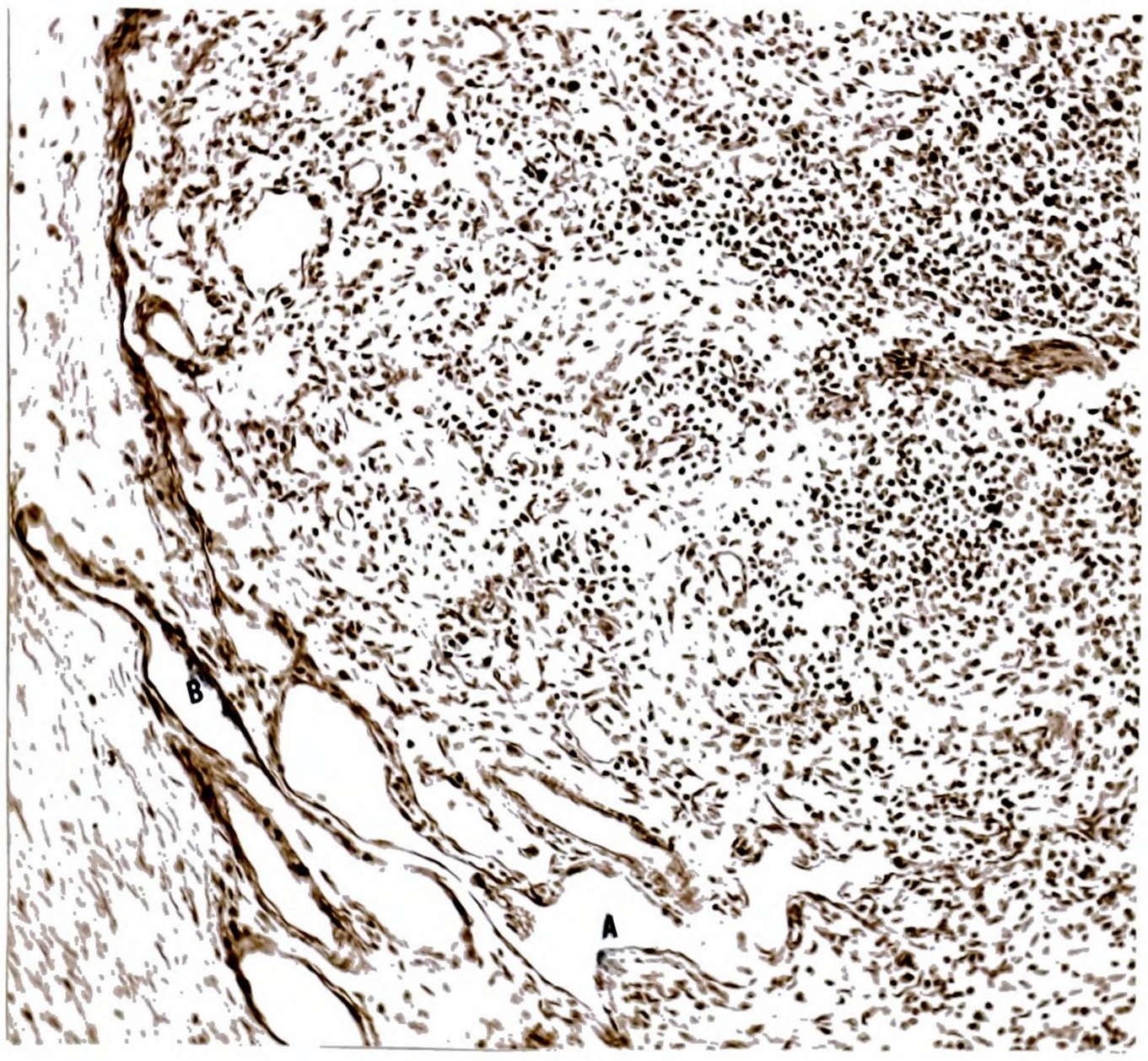

B
A

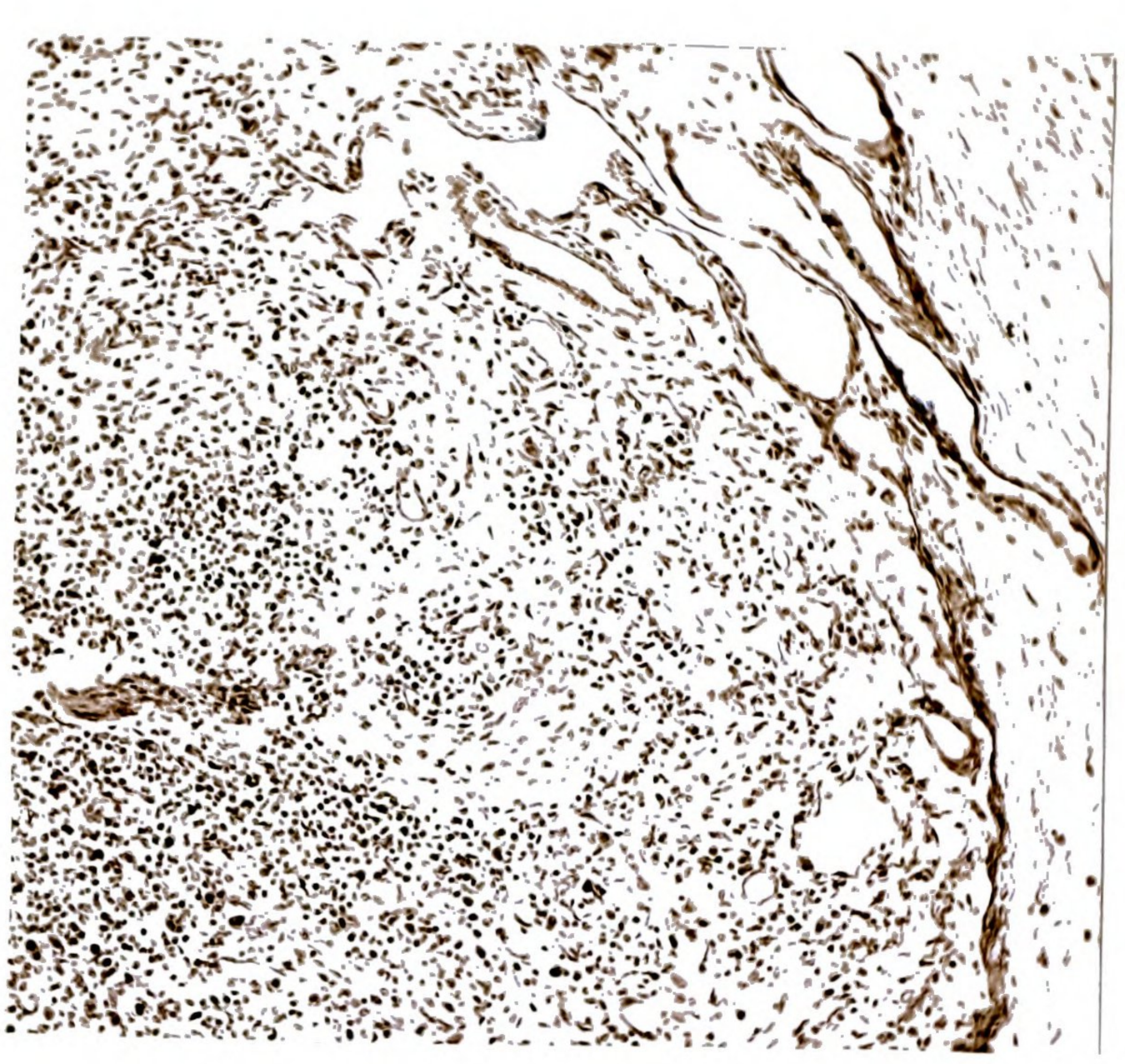
Figure 11

Mesenteric node from a five month old pig.

H. and E. stain 135 X

A. Origin of an efferent lymph vessel

B. Efferent lymph vessel



8. The mesenteric lymph node of a seven-month-old pig.

The mesenteric lymph node of a seven-month-old pig, when viewed as a histological preparation, showed little structural difference from mesenteric nodes of four and five months old animals. One distinct difference found between the nodes which were examined was in the development of the elastic tissue. In nodes from the older animal a thin layer of elastic fibers was observed in much of the capsule. In the remaining portion of the capsule elastic fibers were seen occasionally either as single fibers or in groups. Maximow and Bloom (1957) stated that the elastic tissue layer of lymph node capsules was located near the inner edge of the capsule. However, in the nodes examined in the present study the elastic tissue layer, when present, was found in the middle of the capsule as well as near the outer edge (figs. 12 and 18). In the trabeculae elastic fibers were exhibited more frequently than in nodes from younger animals, but much of the trabecular system was without them. Occasionally a suggestion of a small network of these fibers was seen in the parenchyma, but when present they were found to be either single fibers or a small net-like formation. Single fibers in the parenchyma probably were continuations of fibers from the trabeculae and were connected with the reticulum.

Goldkuhl (1927) reported the presence of small aggregations of lymphoid tissue within the substance of the trabeculae. Further, they were continuous with the rest of the

Figure 12

Mesenteric node from a 7 months old pig.

W. and V.G. stain 600 X

A. Adipose cells outside of capsule


A

Figure 12

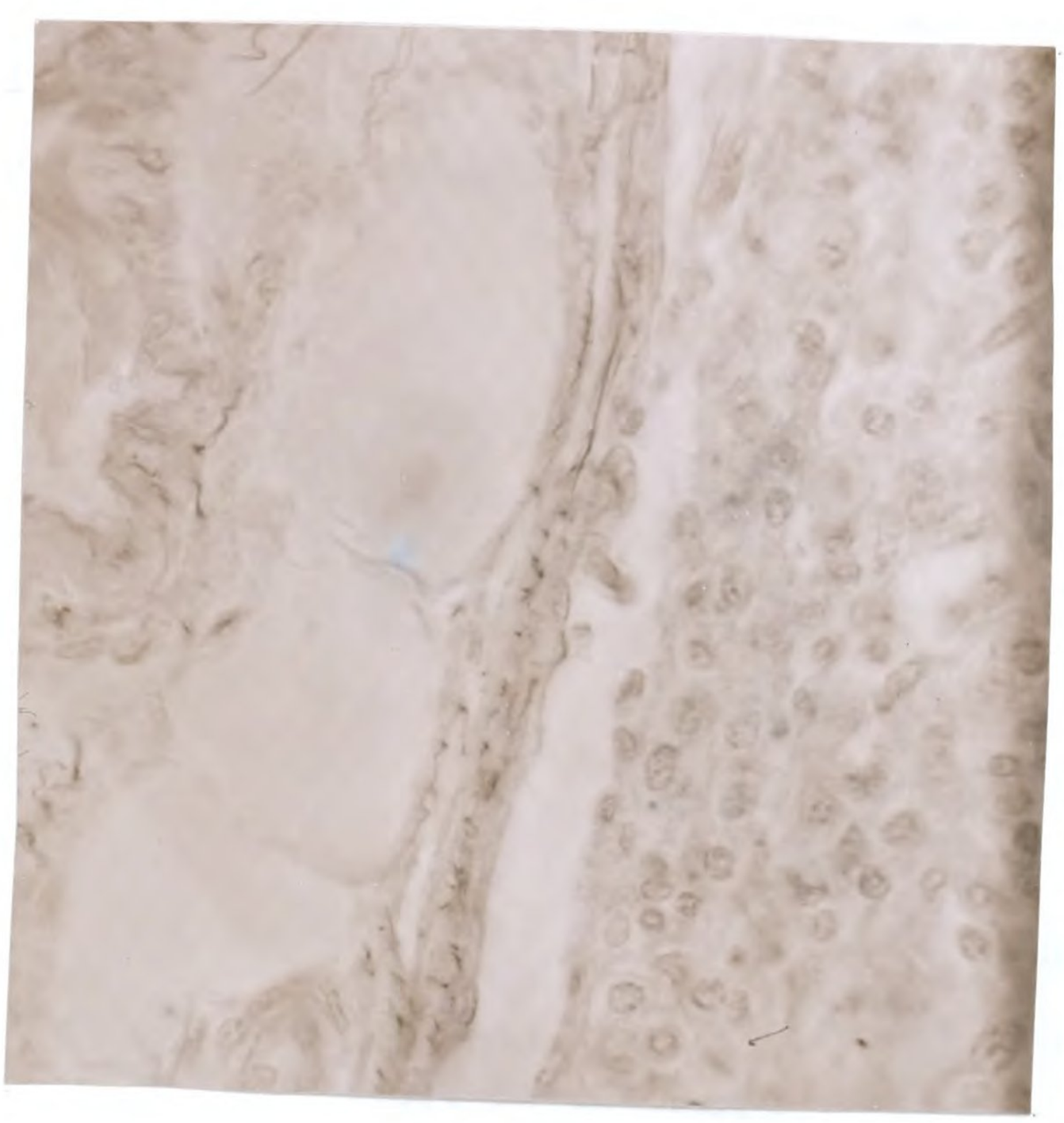
Mesenteric node from a 7 months old pig.

W. and V.G. stain 600 X

A. Adipose cells outside of capsule

A





lymphoid tissue. In the present study these structures were not seen in mesenteric nodes from pigs less than seven months old.

In the capsule capillaries and slightly larger blood vessels were frequently seen. Since the larger vessels were frequently adjacent to the inner and outer surface of the capsule, it is suggested that they conveyed blood to and from the parenchyma of the node as well as to the tissues of the capsule. Figure 13 shows a portion of medulla of this node and a small artery in the medulla.

Examination of the parenchyma showed that cells of both the neutrophilic and eosinophilic series were occasionally present. These cells as well as monocytes seemed to occur more frequently in the medulla than in the cortex. Some nodules were partly surrounded by a few short fibers.

Pigs reached sexual maturity when they became four to six months of age, but full maturity was not attained until eighteen to twenty-four months. Few important structural changes were detected in mesenteric nodes of pigs from four to seven months of age. This suggests that at sexual maturity mesenteric lymph nodes exhibited most of the characteristics they possessed in adult animals.

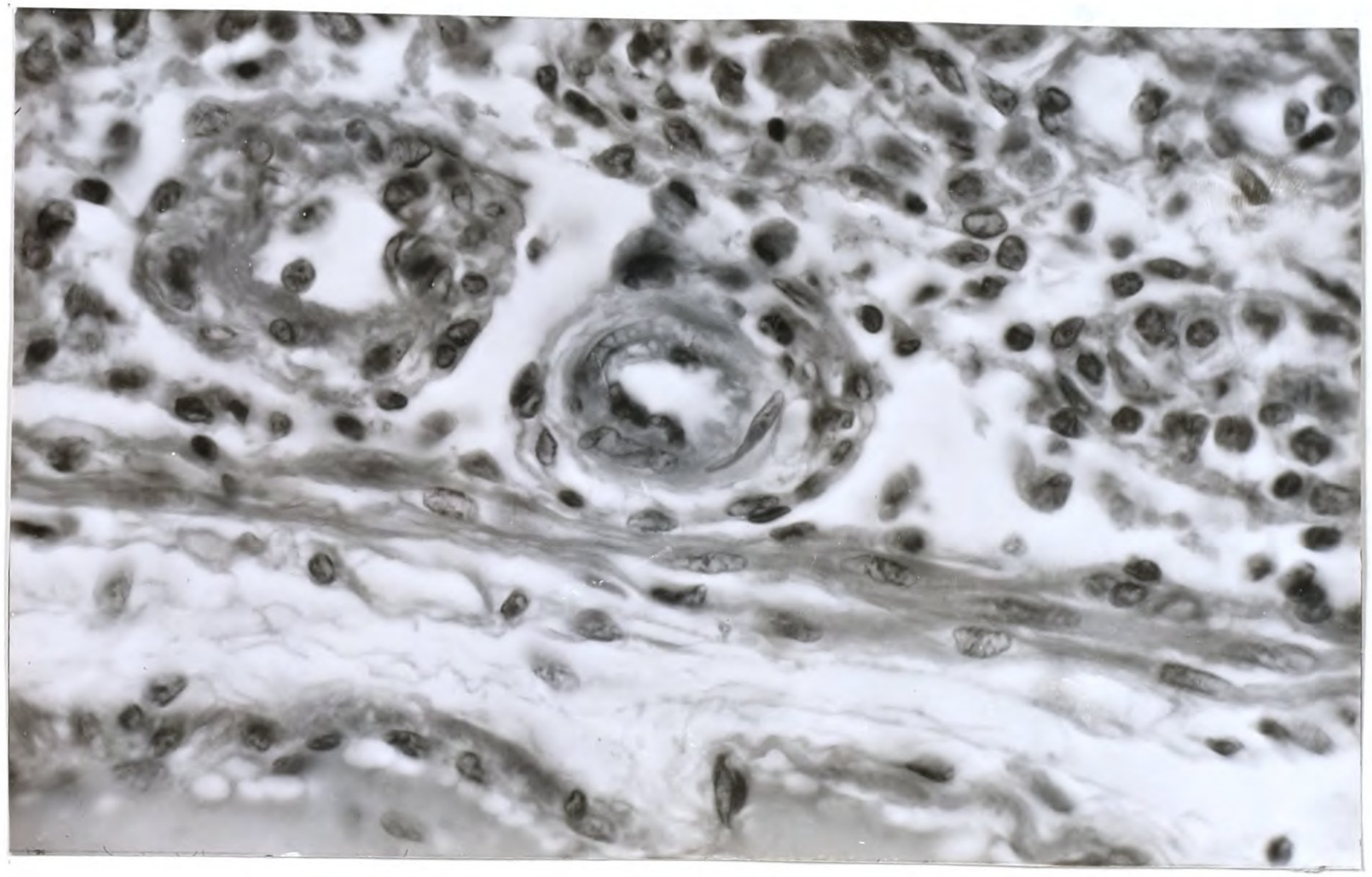
9. The mesenteric lymph node of the three-year-old pig. The capsule of the mesenteric lymph node of a three-year-old pig was reported by many authors to be thinner than the capsule of nodes from the other domestic animals. In the present study the thickness of the capsule was seen to vary

Figure 13

Same node as Fig. 12.

H. and E. stain 800 X





considerably, in part due to the presence of numerous blood vessels. As described above these frequently passed through the capsule into the parenchyma of the node. Examination of sections showed that the principal constituent of the capsule was heavy collagenous fibers. Some smooth muscle tissue was present, but its distribution was erratic. This was in agreement with the findings of various authors. Elastic fibers were seen to form either an irregular layer or a network in the capsule (figs. 12 and 13). They were not necessarily more numerous in the vicinity of blood vessels as reported by Engelmann (1907).

In the region of the medulla, where efferent vessels left the node the capsule sent short branches into the substance of the medulla. Most of these branches were continued in the formation of the walls of the efferent vessels within the node (fig. 11).

At the periphery of the node the capsule was continuous with the trabecular system that carried the afferent vessels. As stated above, trabeculae which branch from the capsule, except where afferent vessels entered, were rarely present in mesenteric lymph nodes of the pig. A subcapsular sinus was or was not present, but in either case reticular fibers which extend from the reticular network of the parenchyma formed the only important connection between the capsule and the parenchyma. However, some collagenous and elastic fibers branched from the capsule to enter the parenchyma.

The presence of lymphocytes that had infiltrated the

capsular tissue was not unusual, but no plasma cells were seen.

As stated above the mesenteric lymph nodes which were examined almost invariably exhibited only the trabecular system which carried the efferent vessels. This system branched extensively within the cortex. The termination of the finer branches was formed by many connective tissue fibers fanning out into the parenchyma. This system of trabeculae rarely entered the medulla although many of its branches extended to the border of the medulla, and it very rarely connected with the capsule. Its constituents were the same as those of the capsule. Smooth muscle tissue was not commonly found except in the larger branches. Occasional elastic as well as collagenous fibers branched from the trabeculae, crossed the sinus in contact with or surrounded by reticular cells and entered the parenchyma. A peritrabecular sinus was frequently present, but in some places it was completely occluded by lymphocytes. However, no sinus was present at the termination of the fine branches. The larger trabeculae, though thin, were frequently wide, hence the name given them by several German histologists was, "bindegewebeblatter." Arterioles, venules and capillaries were not seen as frequently in the trabeculae as they were in the capsule. As stated above, these vessels probably supplied blood to the tissues of the trabeculae and not the parenchyma.

Goldkuhl (1927) described many small trabeculae in the medulla, but these were very rarely seen. Trabeculae in the

medulla were usually present only where efferent vessels originated or where fusion was occurring (fig. 11).

Areas of cortex, which in some sections appeared to be completely surrounded by trabeculae were seen as described above. One of these areas included a well developed nodule. Many lymphocytes were seen among the tissues of the trabeculae.

Arteries were seen passing through the capsule into the cortex of the mesenteric node of a three-year-old pig. As suggested above this indicated that the blood supply to the parenchyma of mesenteric nodes was through the capsule. No evidence of continued fusion of nodes was seen in mesenteric nodes from a three-year-old pig.

The cortex of the mesenteric lymph node of the three-year-old pig was seen to constitute the greater part of the lymphoid tissue of the node. This observation was in agreement with various authors. Its periphery was occasionally adjacent to the capsule or a subcapsular sinus. However, it was always adjacent to the capsule or a subcapsular sinus where afferent vessels entered the node. This was observed by Goldkuhl (1927) as well as during this study. Isolated areas of medulla were frequently seen in the cortex, but as described above these were transverse sections of projections of medulla into the cortex. Likewise transverse sections of extensions of cortex into the medulla were seen. Here and there blood capillaries were present as well as slightly larger blood vessels which had one or two layers of smooth

muscle cells around the endothelial tube. Trabeculae were not numerous, but most of those seen were surrounded by a well defined sinus. The endothelial lining of these sinuses was incomplete on the side adjacent to the parenchyma. Rarely were trabeculae seen to join the capsule except where afferent vessels entered the node, consequently the cortex was not divided into ampullae or alveoli. The nodules were irregularly scattered in the cortex, some with and some without secondary nodules. Many nodules were adjacent to the peritrabecular sinuses. The connective tissue capsule which surrounded the nodule, as described above, was rarely present. The fatty degeneration described by many authors as occurring in the pig lymph node was not seen.

The medulla of the mesenteric node of the three-year-old pig showed no conspicuous change from that of younger animals except for the presence of large lymph sinuses which were completely lined by endothelial tissue. Trautmann (1926) described these as being caused by obstructive processes which apparently had occurred more centrally in the system of lymphatic vessels. He believed that under normal conditions the sinuses would be collapsed and thus not apparent. As in the cortex no fatty degeneration was seen.

There has been some controversy among investigators as to whether the, "bright zone" or "zellarme" region of the pig node was equivalent to the medulla of the typical node or whether it should be considered as a distinct tissue. Koelliker (1902) quoted von Recklinghausen, Chievitz and

Ranvier as stating that no medulla was present in the pig lymph node. Trautmann (1926) stated that the "bright zone" was not equivalent to the medulla. Goldkuhl (1927) and others stated that if the cortical tissue of the pig lymph node was comparable to the cortical tissue of other nodes, then the "bright zone" should be comparable to the medulla because the efferent vessels received lymph from it in both and because this interpretation enables the schema of the pig node to be fitted into that of the typical node. It was the opinion of the author of the present work that the "bright zone" was the equivalent of the medulla and that it was formed by the replacement, in the pig, of both medullary cords and sinuses with a uniform distribution of primitive reticular cells, scattering of lymphocytes and many small blood vessels. As it consisted of the same cellular elements in the pig and all other species that have been studied, it could not be considered a different tissue.

Trautmann's work (1926) and the present research have demonstrated by injection techniques (see below) that lymph permeated the medulla much more extensively than it did the cortical tissue. This enabled more of the lymph which passed through the pig node to make intimate contact with lymphocytes and primitive reticular cells. It was suggested that this provided greater efficiency in antibody formation by the lymphocytes.

C. THE POPLITEAL LYMPH NODE

The popliteal lymph node of the pig was located in the

subcutaneous connective tissue posterior to the knee or stifle joint. It was more superficial than the popliteal node of other species. Sisson and Grossman (1953) and Somers (1951) stated that this node was smaller in the pig than in the other domestic animals. According to Platsma (1928) and observations made during this study, the node was inconstant in its occurrence. Platsma (1928) found the popliteal node to be absent in one or both legs in three of six animals he examined. The popliteal node of the pig received lymph from the deep and superficial regions of the distal portions of the pelvic limb up to the region of the knee. Its efferent vessels emptied into the ischiatic lymph node and occasionally into the deep inguinal node.

Platsma (1928) stated that a node may be found in the subcutaneous fat three or four inches above the hock or tarsal joint. He named this the superficial popliteal lymph node. Sisson and Grossman (1953) merely stated that this node had been noted by another author. They did not name the node nor the author. An injection technique, described by Weinberg and Movius (1954), for vital staining of lymphatics was employed in the present study to determine the occurrence of this node and also the lymph channels through the popliteal node. Trypan blue (0.5% in physiological saline or distilled water) or India ink suspension (50% in distilled water) were injected subcutaneously or intramuscularly into the lateral and medial surfaces of the leg at various levels between the tarsus and one half of the dis-



tance to the knee. After a suitable time lapse the pigs were electrocuted. Dissection of these limbs disclosed that the popliteal node and many lymph vessels were stained. It was probable that the node described by Platsma (1928) as the superficial popliteal lymph node would also have been stained and readily found. It was not seen in any of the fourteen limbs examined, thus it may be concluded that this node was inconstant.

1. The popliteal lymph node in a two-day-old pig. The popliteal node of the two day old pig showed certain differences from the mesenteric node of the same age. In general the differences were those found to exist between mesenteric nodes and popliteal nodes rather than differences in the level of development. The differences between nodes from the two regions were noted by observations made on eleven mesenteric and nine popliteal nodes (table A). The capsule of the popliteal node was thicker and the connective tissue fibers were heavier than those of the mesenteric node, but the trabeculae, at this age, appeared the same in both nodes (figs. 5, 6, 14, and 15). Elastic fibers were rarely present in either node. The system of trabeculae which originated at the capsule was better developed in the popliteal node. Frequent small trabeculae extended from the capsule into cortical tissue suggesting the formation of ampullae (fig. 14). A distinct subcapsular sinus was present adjacent to the cortex.

Groups of afferent lymphatics were seen to enter the

Figure 14

Popliteal node from a 2 day old pig.

H. and E. stain 75 X

A. Medulla with many sinuses

Figure 15

Same node as Fig. 14.

H. and E. stain 90 X

A. Two systems of trabeculae with afferent vessels.


A

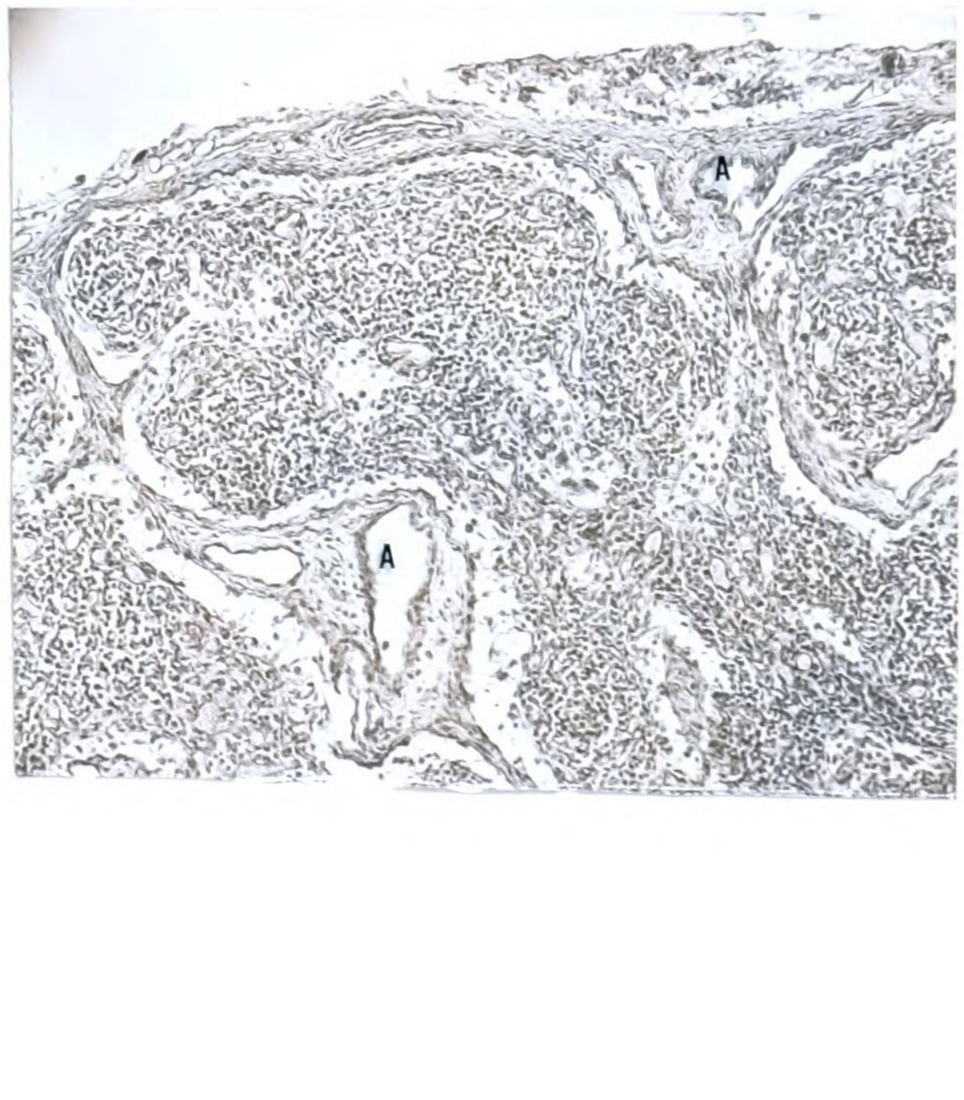

A
A

Figure 14

Popliteal node from a 2 day old pig.

H. and E. stain 75 X

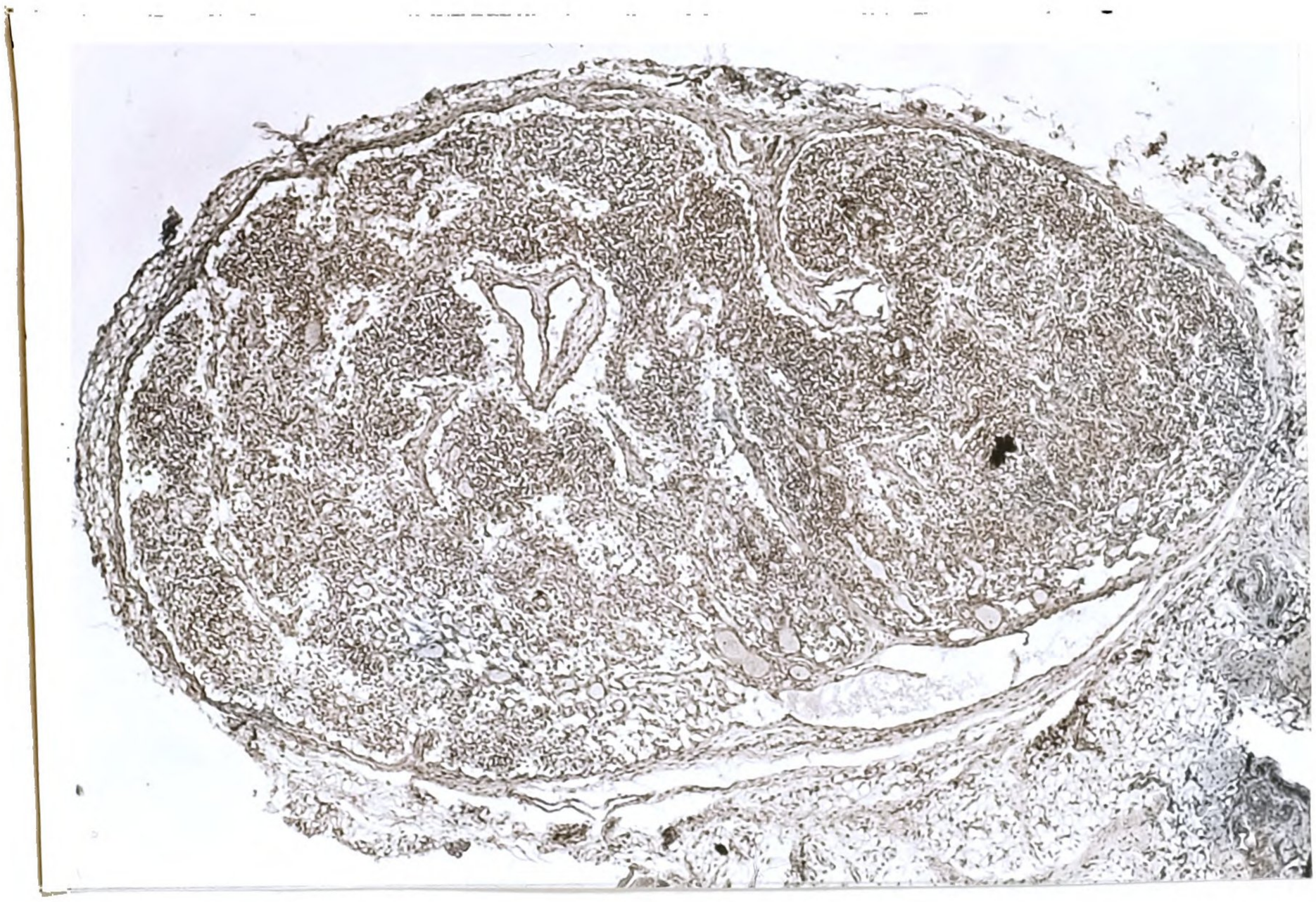
A. Medulla with many sinuses

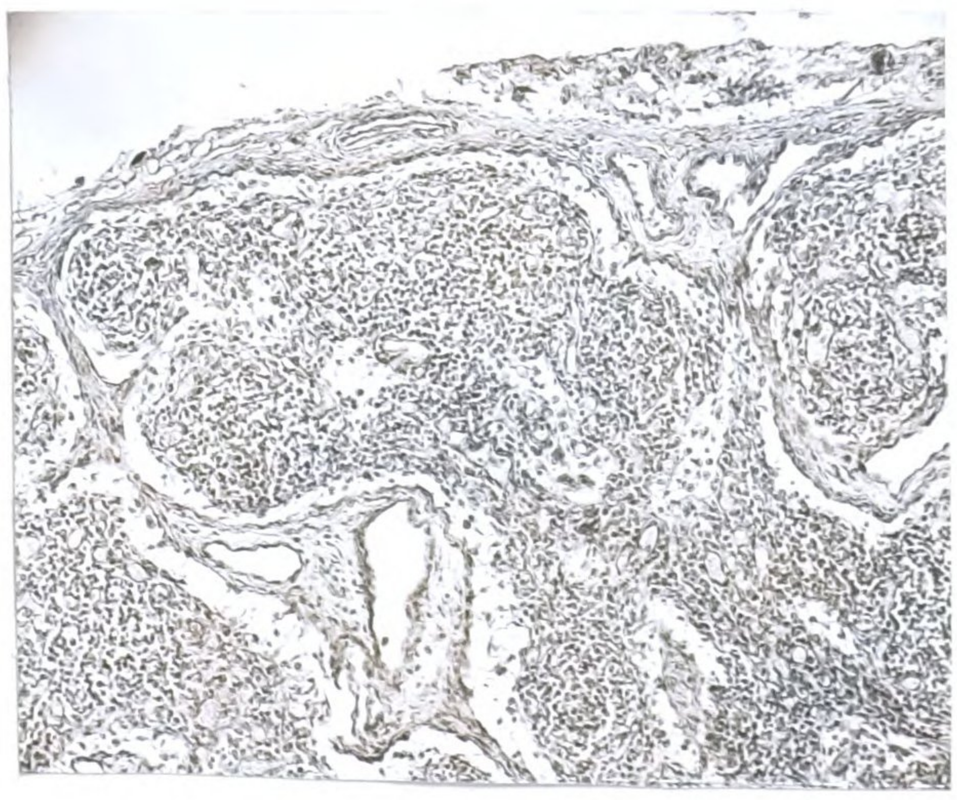
Figure 15

Same node as Fig. 14.

H. and E. stain 90 X

A. Two systems of trabeculae with afferent vessels.





node at several locations and each had its own system of trabeculae which penetrated deep into the node (fig. 15). If it was assumed that each node originally had only one group of afferent vessels, as is suggested by observations of mesenteric nodes from fetal pigs, then it must be concluded that this node was formed by the fusion of several smaller nodes. No evidences of partial fusion were seen so the process was completed before this node was removed from the pig.

The nodules resembled those of the mesenteric node, but they were smaller. The largest nodule seen in this node was approximately 200 micra in diameter. The medulla was characterized as much by the presence of many lymph sinuses as by the presence of primitive reticular cells not masked by lymphocytes. It did not closely resemble the medulla of adult nodes. The medulla was well supplied with blood sinuses and capillaries. There was no difference in the cellular constituents of this node and the mesenteric node of the same age.

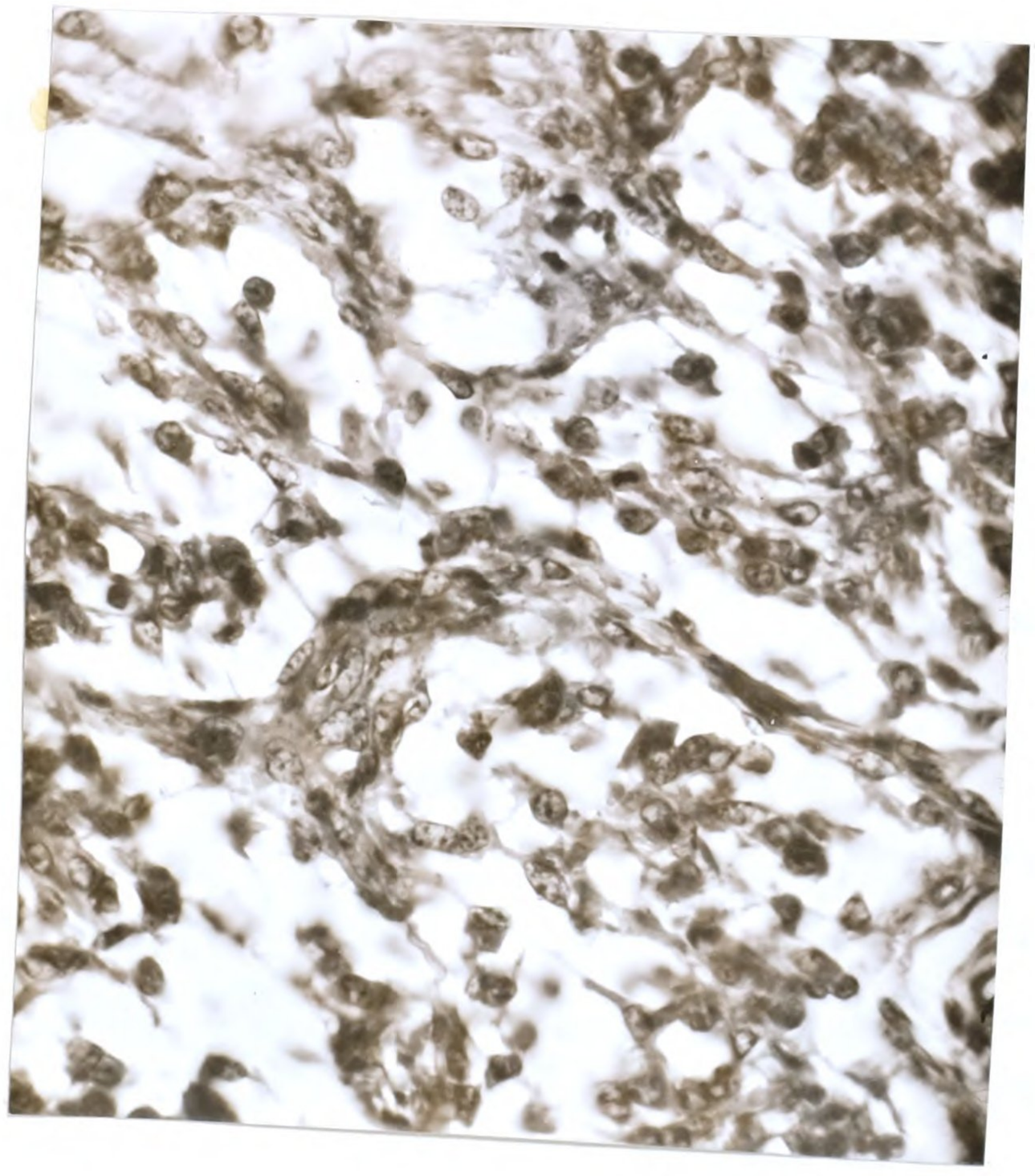
2. The popliteal lymph node in a six-day-old pig. The popliteal lymph node from a six-day-old pig had heavier fibers in the trabeculae than the same node in the two-day-old pig. In the cortex the nodules were larger and the medulla exhibited a greater development of the primitive reticular cells (fig. 16). The popliteal node was developed to about the same extent as the mesenteric node from a six-day-old pig.

Figure 16

Popliteal node from a 6 day old pig.

H. and E. stain 600 X

Medulla showing reticular cells and sinuses.



3. The popliteal lymph node from a three-week-old pig. The popliteal lymph node from a three to four-week-old pig showed little evidence of fusion. However, as serial sections were not made this is not conclusive. Sections cut from two nodes removed from the same animal were examined. Of these only one node appeared as though fusion was actively occurring, at the time the node was removed, and this fusion was seen only in a limited area. Trabeculae were much more numerous in both of the popliteal nodes examined than in the mesenteric node. However, there was a considerable variation between the popliteal nodes in this respect, because one was cut near the entrance of the afferent vessels, and the other was not. A very few small elastic fibers were seen in both stroma and parenchyma.

The cortex and medulla were more readily distinguished in the popliteal node than in the mesenteric node from an animal three weeks old. This was due to differences in the density of the lymphocytes. In the cortex of the popliteal node they were more closely packed together than in the cortex of the mesenteric node, thus the cortex of the mesenteric node had a younger or more fetal-like appearance. The nodules of the popliteal node were more easily distinguished from the surrounding cortex than were those of the mesenteric node because in the popliteal node they were frequently surrounded in part by a sinus (fig. 17). This structure was better developed in the superficial cervical node and a description of it was included in that section. Although

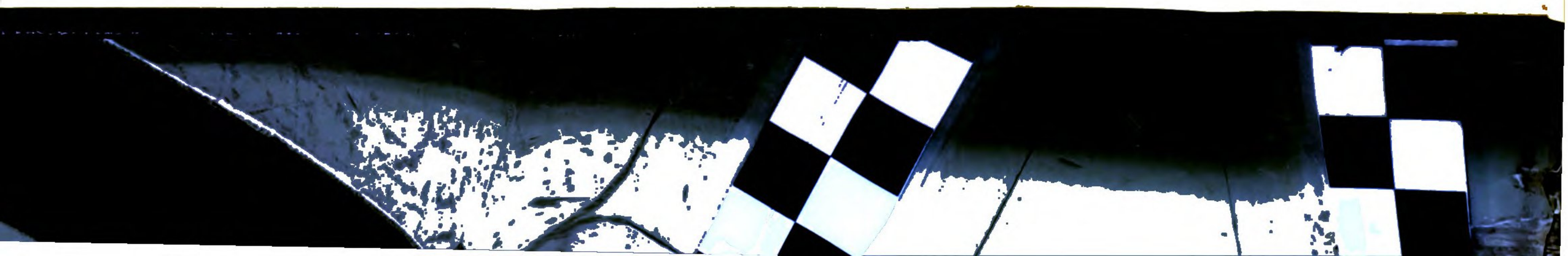


Figure 17

Popliteal node from a 3 week-old pig.

H. and E. stain 30 X

Illustrates the lobulated and conglomerate appearance and peripheral cortex typical of popliteal nodes.

A. Cortex

B. Medulla

C. Carbon particles in afferent vessel following injection of India ink into leg below the level of the node.

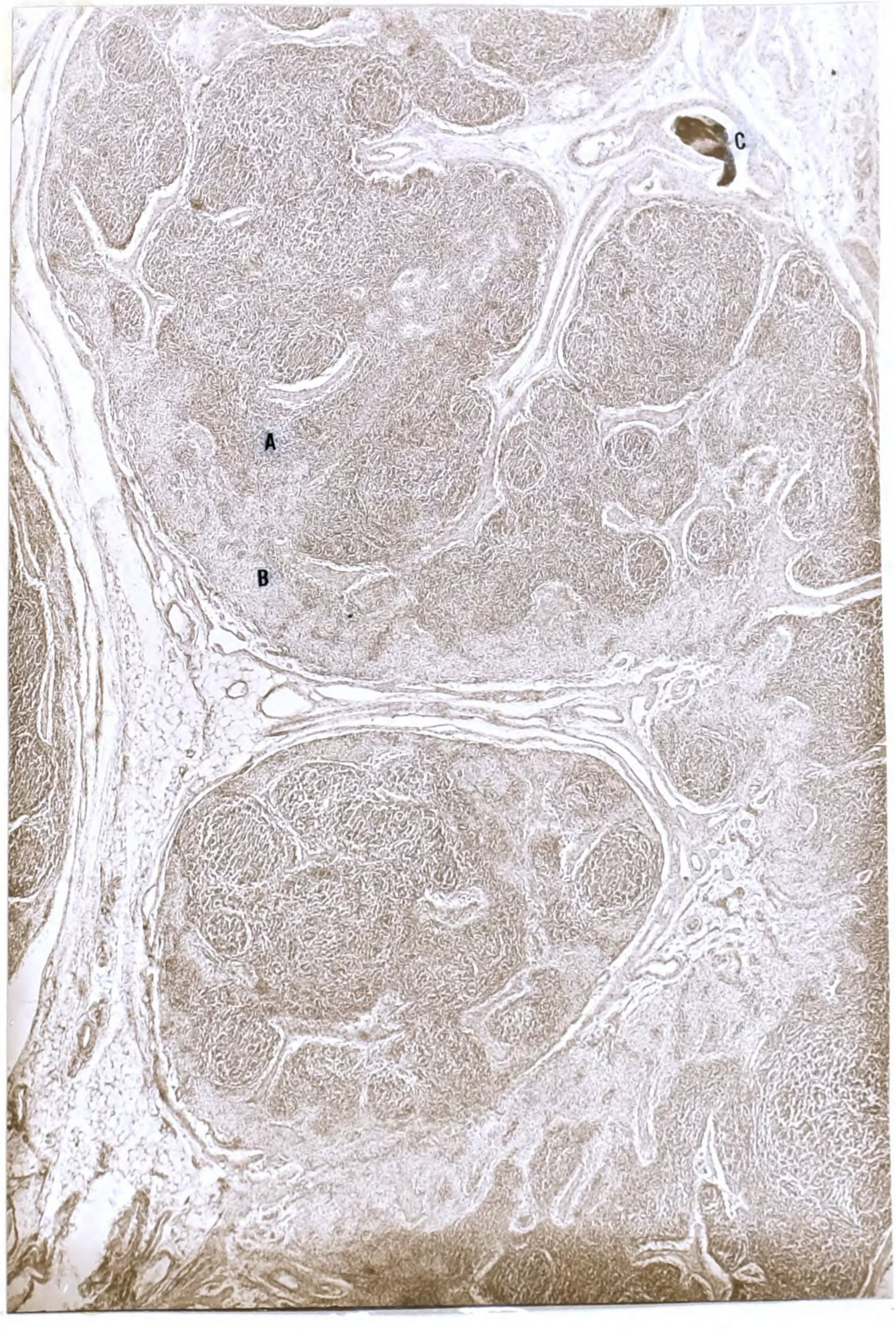

C
A
B

Figure 17

Popliteal node from a 3 week-old pig.

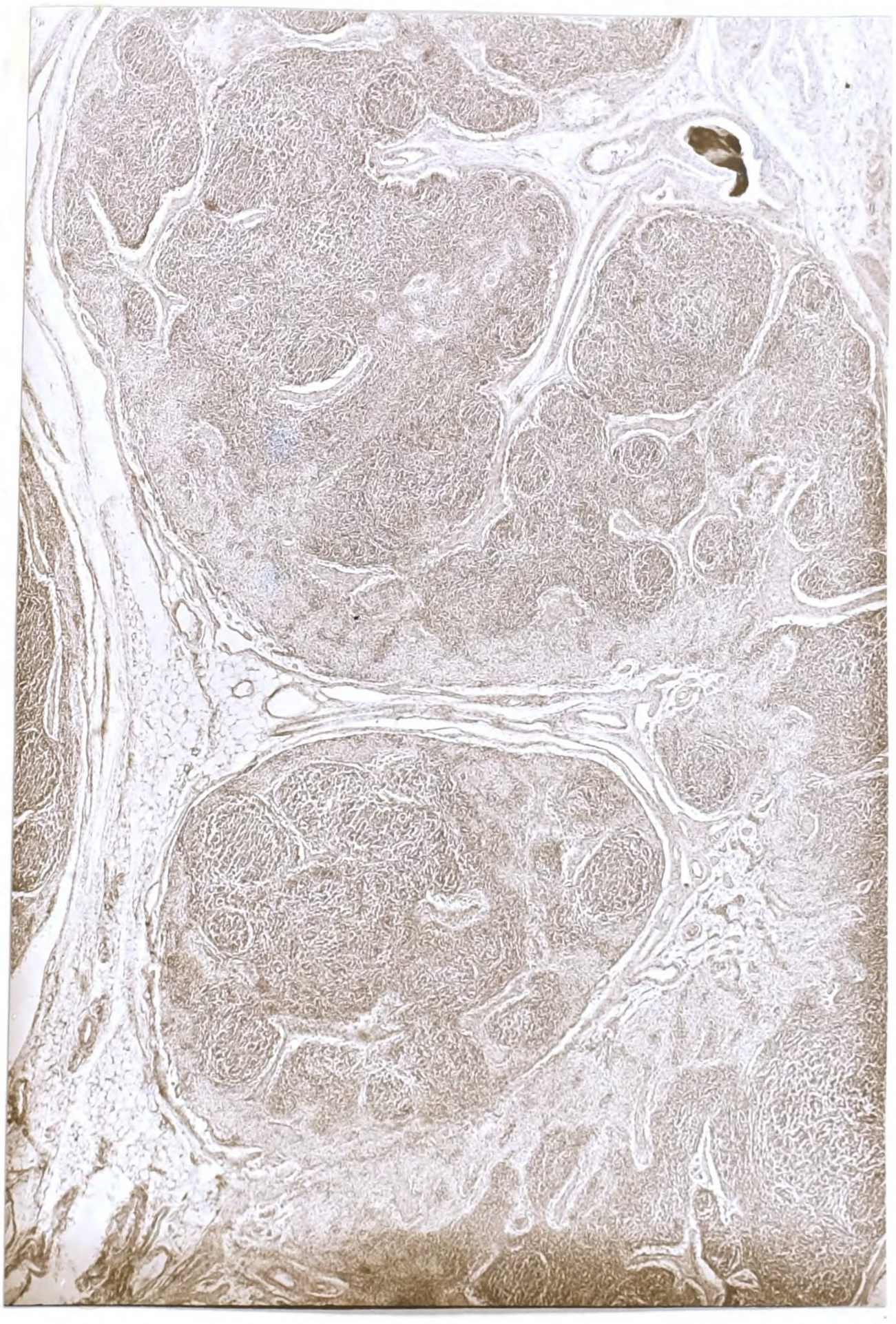
H. and E. stain 30 X

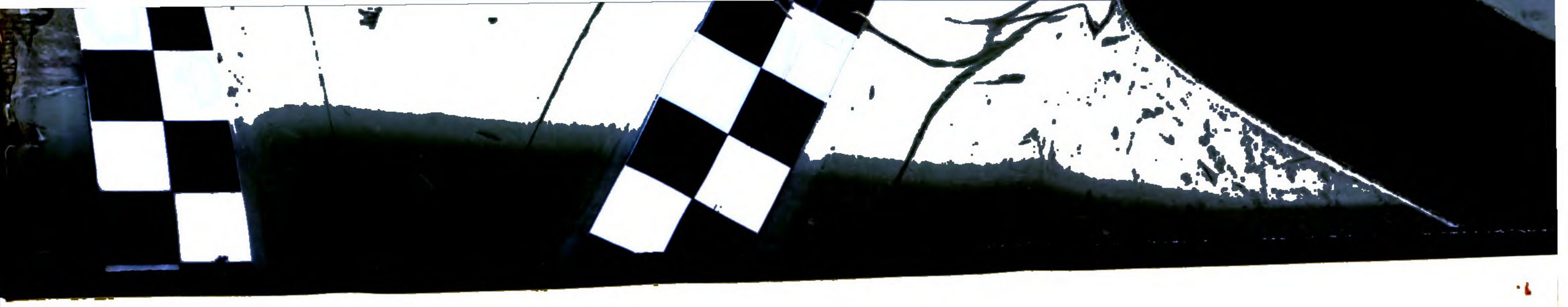
Illustrates the lobulated and conglomerate appearance and peripheral cortex typical of popliteal nodes.

A. Cortex

B. Medulla

C. Carbon particles in afferent vessel following injection of India ink into leg below the level of the node.



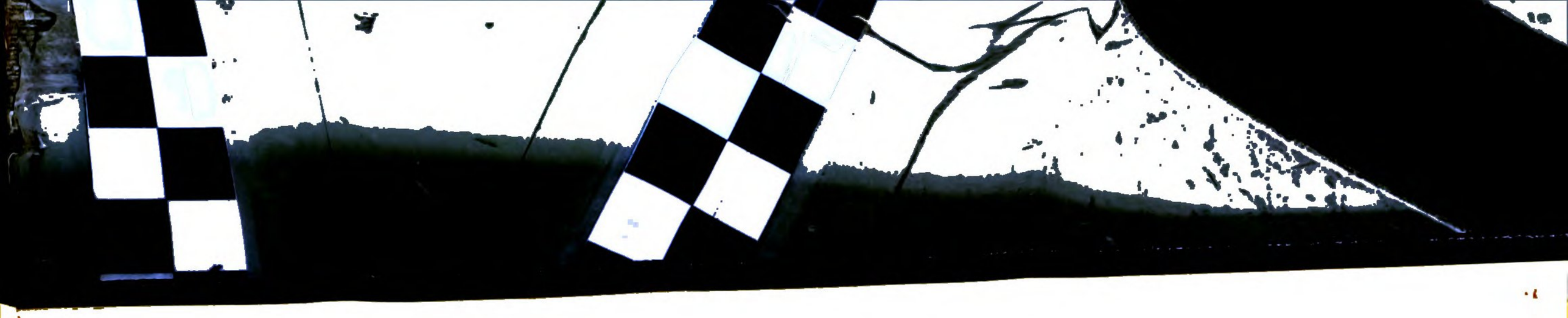


promyelocytes and the later stages of the granulocytic series of cells were frequently seen in this node, only one plasma cell was seen during a careful examination.

4. The popliteal lymph node from a six-week-old pig. The sections of popliteal nodes from a six-week-old animal that were examined did not exhibit any definite indications of fusion, however it was possible that fusion was occurring in other portions of the node. Most of the capsule was heavy, but it varied considerably in thickness. The trabeculae were well developed and those that carried the afferent lymph vessels often showed groups of smooth muscle cells. In many regions trabeculae that branched from the capsule divided the cortex into the ampullae that might be considered characteristic of popliteal nodes. However, where the ampullae were absent the popliteal node could not be distinguished from nodes from other regions (fig. 17). The occurrence of elastic fibers was sporadic, but where present they were usually large heavy fibers, frequently in bundles. They were present in both parenchyma and stroma.

In some sections examined most of the cortex was without nodules, but in others nodules were common. A few nodules exhibited indications of a capsule-like structure formed by a few short fibers.

5. The popliteal lymph node in the three-month-old pig. The histological preparations of the popliteal node from a three-month-old pig that were examined showed evidence of extensive fusion involving several nodes and various stages.



Both the gross and microscopic appearance of the node was that of a conglomerate of smaller nodes.

The capsule showed a great variation in thickness between two sides of the node as seen in the section. On one side it was what might be considered as normal thickness for a popliteal node. On the opposite side of the node where fusion was occurring the capsule was much thinner in some cases. However, there was no evidence to indicate that the occurrence of fusion and thickness of the capsule were related. The capsule was in contact with adipose tissue in most locations on each side. Because of its large size it was impossible to mount sections of the entire conglomerate that formed this node, but examination of the available sections showed that the thick capsule probably surrounded the entire conglomerate, or a portion of it, and that the thin capsule branched from it and surrounded the individual nodes. This structural feature was not noted during the gross examination of the node.

The trabeculae were not as heavy as some that had been seen in other popliteal nodes, but their arrangement, in relation to the distribution of cortex and medulla was important in interpreting the structure of other popliteal nodes and some nodes from other regions. In portion of this node the presence was noted of a region with a peripheral cortex somewhat divided into alveoli. This region surrounded the medulla which in turn surrounded trabeculae which carried efferent lymphatics. It was determined that this

type of structure was frequently present, but when sectioned in a different plane it was not readily recognized. It was also determined that this structure was radially symmetrical and that if it was sectioned in a transverse plane the trabeculae appeared similar to the afferent trabeculae, but were surrounded by medulla. The subcapsular and trabecular sinuses were usually well developed.

In addition to the type of structure described in the paragraph above, the popliteal nodes also were formed of the type of structure more typical of pig lymph nodes. This structure had a central cortex surrounded by medulla, but the thickness of the medulla varied tremendously and in places was absent so that the cortex was adjacent to the capsule. In this type the presence of a subcapsular sinus was not at all consistent although various authors have stated that it was present but occluded by compression or by free lymphocytes. In these nodes the trabecular sinuses carried lymph to the cortex, thus these sinuses were functionally equivalent to the subcapsular sinus of the typical mammalian node and thus a subcapsular sinus was not necessary for these nodes to function. Some of the popliteal nodes examined were composed of both types of structure and others consisted only of the type with the central cortex. As stated above in the description of the mesenteric node, the presence of the many trabeculae which surround the alveoli added to the impression that the popliteal node contained more stroma in relation to parenchyma than nodes

from some other regions.

A few of the nodules seen in this node showed some short fibers as evidence of the capsule-like structure at their periphery. Tremendous numbers of the eosinophilic series of cells were seen in both the cortex and medulla. In addition several neutrophilic myelocytes in mitosis were seen.

6. The popliteal lymph node in a four-month-old pig. Both the gross and microscopic examination of a popliteal lymph node from a four-month-old pig disclosed that it consisted of a conglomerate of smaller nodes. The microscopic examination showed that much fusion had occurred. There was a great variation in the size and number of trabeculae in different regions of the node. Where the stroma was lighter it was comparable to that of the mesenteric node. Occasionally small branches of the trabeculae which carried the afferent lymph vessels were seen to join the capsule. Both types of structure described in the preceeding section were present. The development of elastic fibers in the capsule was considerably greater than that observed in mesenteric nodes from pigs of the same age. In the capsule of the popliteal node a network of elastic fibers formed a thin but almost complete layer which varied in thickness and in its position in the capsule (fig. 18). This elastic tissue layer was occasionally seen to branch and form two layers which then anastomosed. In the trabeculae elastic fibers were not numerous and were scattered, or they rarely formed a heavy layer. Prominent groups of smooth muscle cells were

Figure 18

Popliteal node from a 4 months old pig.

W. and V.G. stain 800 X

Elastic fibers in the outer portion of the capsule.

Figure 19

Same node as Fig. 18

H. and E. stain 90 X

Clear lymph channel from sinus in cortex (A) to medulla (B).





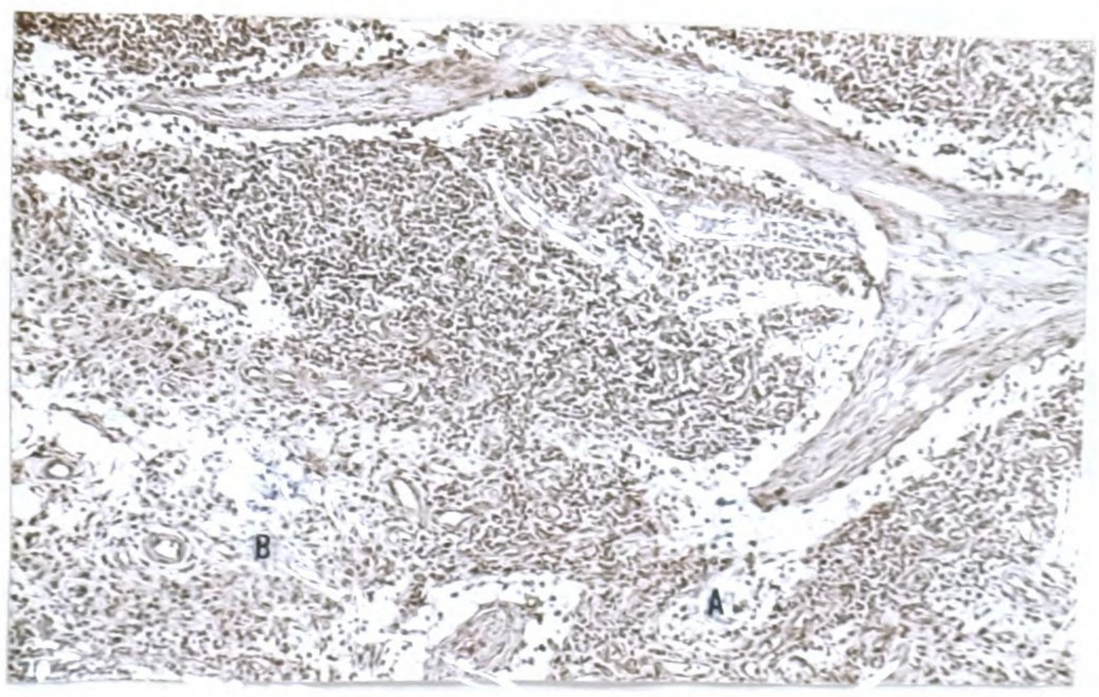

B
A

Figure 18

Popliteal node from a 4 months old pig.

W. and V.G. stain 800 X

Elastic fibers in the outer portion of the capsule.

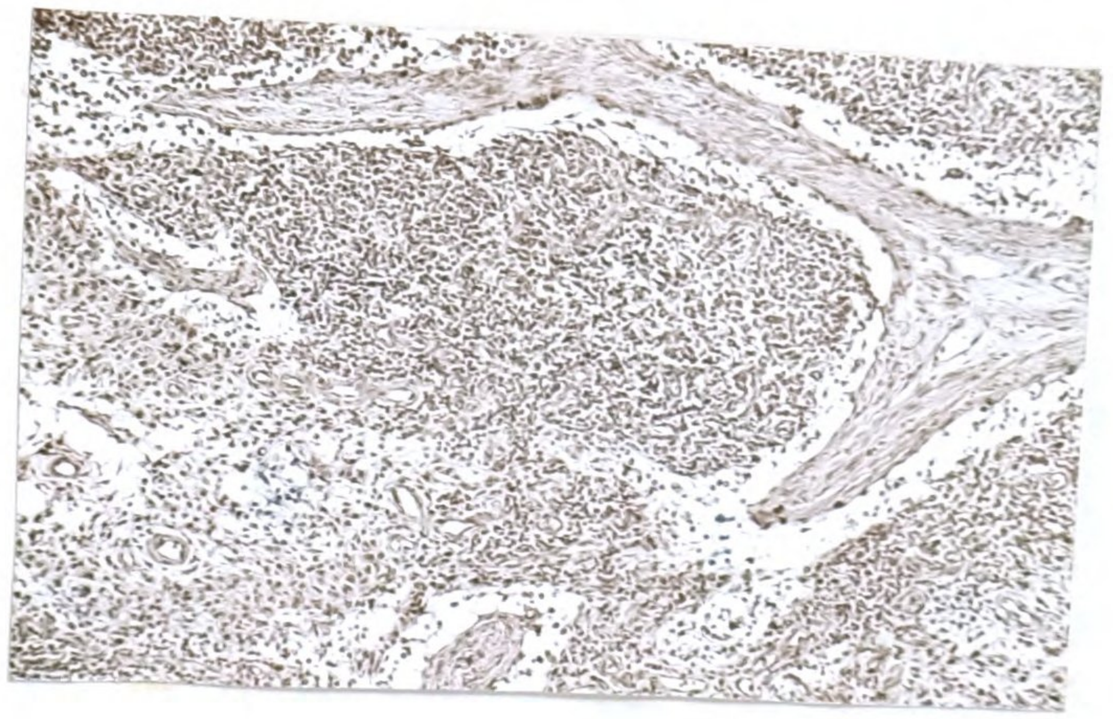
Figure 19

Same node as Fig. 18

H. and E. stain 90 X

Clear lymph channel from sinus in cortex (A) to medulla (B).





seen occasionally in the trabeculae.

The subcapsular sinus was well developed and present around most of the sections that were observed, and in some areas it was also adjacent to the medulla. A direct lymph channel from the afferent lymphatics directly to the medulla, but not to efferent vessels was observed in these sections (fig. 19). Groups of adipose cells were seen in a few places in the medullary tissue. The capsule-like structure around the nodules was seen very rarely and when present it was formed by a few short fibers. Except for the lymphoid cells, leucocytes were very rarely observed. No significant age changes between this node and the popliteal node from a three-month-old animal were noted.

7. The popliteal lymph node in a seven-month-old pig. The histological preparations of two seven-month-old pig lymph nodes that were examined were removed from three different portions of the node. No significant differences were noted between these sections and those of the node from a four-month-old pig that was examined. Both kinds of structures described above were observed in this node. The stroma was not heavy, but many trabeculae were seen which originated from the capsule. Usually these were seen only where the capsule was adjacent to the cortex. The subcapsular sinus was well developed and adjacent to both cortex and medulla except where efferent lymphatics were forming.

The capsule-like structure of the nodules was not completely present around any of the nodules that were examined,

and usually it was not observed at all. Many eosinophils were seen either in groups or singly, but some sections examined contained very few. A few cells in mitosis were seen. In other respects this node did not differ from the node described in the section immediately above.

D. THE PAROTID LYMPH NODE

The parotid lymph node consisted of a group of nodes lying anterior to and in part medial to the parotid salivary gland. Cranially they were related to the posterior aspect of the ramus of the mandible and the masseter muscle. Platsma (1928) stated that the afferent lymphatics of this node drain the surroundings, upper lip, rostrum, corners of the mouth, nose, front of the head and the anterior portion of the external ear. He further stated that the efferent vessels lead to the lateral retropharyngeal node.

Observations were made during this study on the parotid node of five animals. Grossly the node appeared to be a close conglomerate of small and large nodes. This was confirmed by histological studies. Examination of serial sections disclosed that the small nodes were frequently completely fused at small areas of contact in such a way that they could be considered as lobes of one larger node.

1. The parotid lymph node of the 21 centimeter fetal pig. The parotid node from a 21 cm fetus showed no important differences from the mesenteric node of the same age except for the following. The capsule of the parotid node was thicker and clumps of elastic fibers were occasionally

seen in some trabeculae. Evidence of an early stage of fusion was seen. A narrow band of medullary tissue penetrated by many small sinuses extended entirely across the node. According to Trautmann (1926) this was a stage of fusion. This observation confirmed the statement above (p. 17) that fusion occurred late in pregnancy. The node, at this age consisted of two closely adjacent nodes. This suggested that the formation of the conglomerate parotid node seen in older animals started forming before birth.

2. The parotid lymph node in a one-day-old pig. No differences were seen between parotid lymph nodes from a one-day-old pig and the late fetal pig described above.

3. The parotid lymph node in a two-day-old pig. The parotid node from a two-day-old pig was much like the mesenteric node from the same animal. This parotid node distinctly showed the conglomerate appearance of the parotid node of older pigs. Six lobes could be distinguished in one section. The parotid node also had a thicker capsule. The node of the two-day-old animal showed a concentration of lymphocytes comparable to that found in nodes from older pigs, and the stroma showed a much greater development than that found in the node of the one-day-old pig.

The changes observed between nodes in one and two-day-old animals were so great that it did not seem likely that they occurred in only one day. It seemed more plausable that such changes occurred during a period of several days. As these nodes were from two different individuals it could

be concluded that differences were due, at least in part, to differences in the rate of growth of the individual animals, rather than to the difference in age of the animals in days. However, the change from the appearance in the fetal state to that of the older pig had occurred within two days after birth in all nodes examined.

4. The parotid lymph node in a four-month-old pig. The parotid lymph node from a four-month-old pig in its gross appearance seemed to be a conglomerate. This observation was confirmed by microscopic examination. In proportion to parenchyma little stroma was seen even though very small trabeculae extended from the capsule into both cortex and medulla. The capsule was not heavy and a subcapsular sinus was present around most of the node adjacent to both cortex and medulla. Two stages of fusion were observed in the sections that were examined. The general structure of the node showed a large cortex much of which was surrounded by a narrow medulla. A few of the many nodules were surrounded by a well developed ring of collagenous fibers. Eosinophils were rarely seen.

E. THE PHARYNGEAL LYMPH NODES

Platsma (1928) described two lymph nodes in the region of the pharynx of the pig, but Somers (1951) and Sisson and Grossman (1953) described only one. In all cases these nodes were found on both the right and left sides. The node termed suprapharyngeal by Sisson and Grossman (1953) in the pig and by Somers (1951) was the same as the node that Platsma

(1928) named medial retropharyngeal. Platsma's description of the location of the node he called medial retropharyngeal was similar to the description of the parapharyngeal node in the horse described by Sisson and Grossman (1953).

According to Platsma (1928) the lateral retropharyngeal node received lymph from its surroundings, part of the ear, the posterior region of the head, skin, shoulder joint, tongue, tonsils and rostrum or snout as well as from the mandibular, parotid, and cranial cervical lymph nodes. Its efferent vessels carried lymph to the superficial cervical node. The medial retropharyngeal node drained the conchae, tongue, tonsils, larynx, pharynx and in some cases the superficial cervical lymph node. Its efferent lymphatics formed the tracheal lymph duct.

For this study one node from each of these pharyngeal regions was removed from one pig. No structural or histological differences were noted so these were treated as one node.

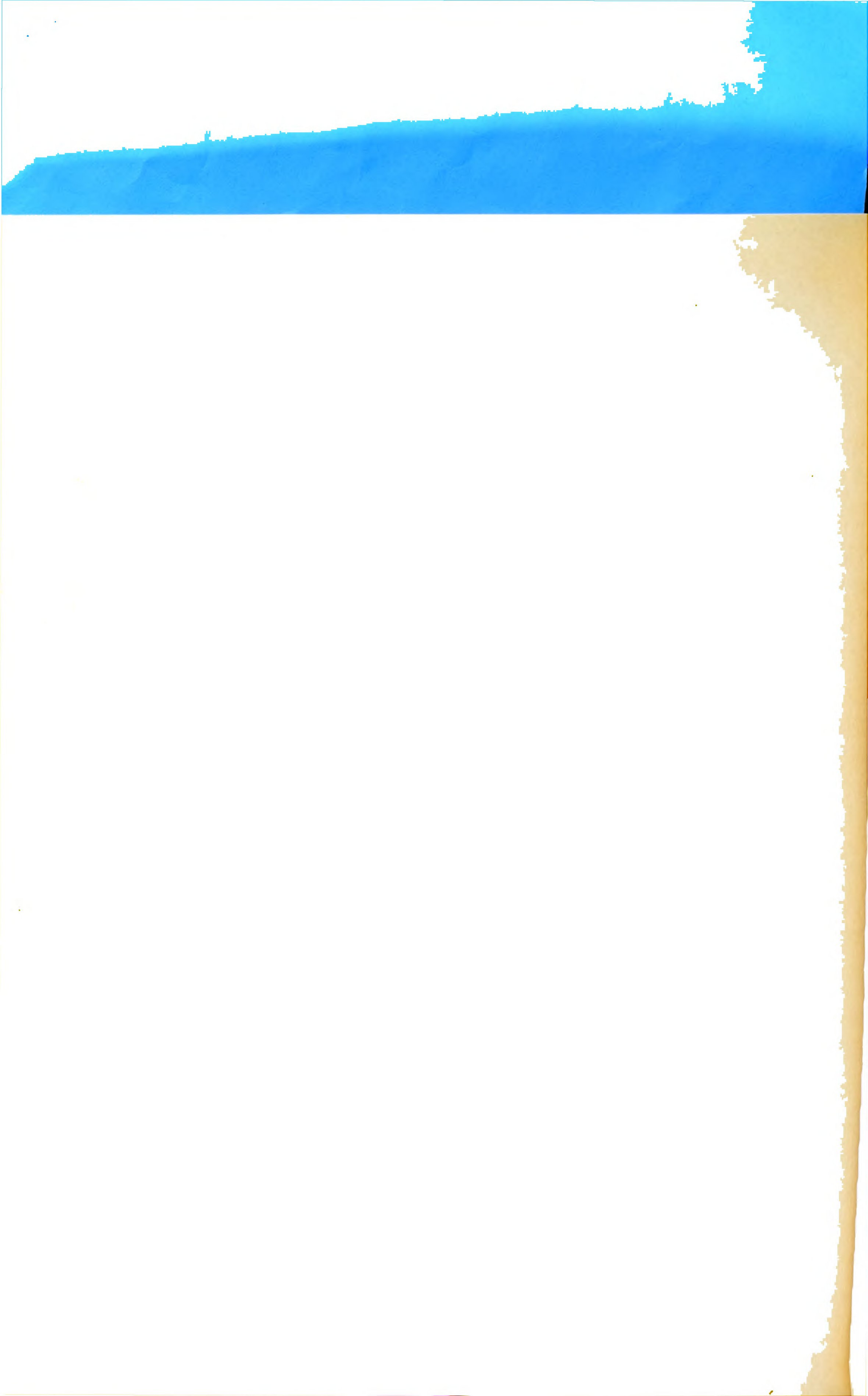
1. The pharyngeal lymph nodes of a four-month-old pig. The pharyngeal lymph nodes of a four-month-old pig, except for their lobulated appearance showed no important differences from the mesenteric node in a pig of the same age.

F. THE SUPERFICIAL CERVICAL LYMPH NODE

Sisson and Grossman (1953) used the term prescapular for this lymph node and gave the term superficial cervical as a synonym. They stated that it was located at the anterior border of the anterior deep pectoral muscle, and that

it drained lymph from the neck, chest, shoulder, and arm. Somers (1951) stated that the prescapular node was located in a deposit of adipose tissue slightly dorsal and medial to the shoulder joint and that several of these nodes formed a chain of partially or almost completely fused nodes. As a synonym for prescapular Somers used the term posterior superficial cervical, but did not clearly state that another superficial cervical node was present. The above named authors agreed in their descriptions of the areas drained by this node. Further they agreed that it emptied into the posterior cervical node. However, Somers stated that in some individuals it drained into the tracheal duct, and in some cases the efferent lymphatics of this node on the left side lead directly into the thoracic duct. Platsma (1928) described a superficial cervical node and a middle superficial cervical node. Neither of his descriptions agreed with Somers (1951) or Sisson and Grossman (1953). However Platsma (1928) reported that the middle superficial node received lymph only from the anterior limb and that the superficial cervical node drained the posterior region of the head, the neck and shoulder, and certain other lymph nodes.

1. <u>The superficial cervical lymph node from a four-month-old pig</u>. The superficial cervical lymph node from a four-month-old pig exhibited several stages of fusion. Five separate nodes partially fused into one conglomerate node were distinguished in the sections examined. The trabeculae were developed to about the same extent as those of the



mesenteric node from a four-month-old pig, but the capsule in the one node studied frequently was thicker in the superficial cervical node. Small trabeculae were seen occasionally projecting into the medulla from the capsule as well as those found where efferent lymphatics were forming. Elastic fibers were present in the stroma in approximately the same quantity and distribution as they were seen in the mesenteric node at this age. However, in one portion of the capsule of the superficial cervical node elastic fibers formed a very thin layer. This layer was in the middle of the capsule. This was contrary to the statements of authors who reported that the elastic layer of the capsule was closer to the inner edge (see above p. 24). In this respect it resembled the elastic tissue layer of the capsule of the mesenteric node from a seven-month-old pig. In those locations where fusion of nodes was in the earliest stage, i.e. where the two capsules had fused, no differences were seen in the quantity of distribution of elastic fibers from that seen in the capsule which surrounded the whole node.

A few plasma cells were seen in sinuses which were adjacent to the medulla. In other respects this node resembled the mesenteric node of the two animals of the same age that were examined.

This node was an excellent example of the difficulty or even futility of the attempts by various authors to classify pig lymph nodes according to the relative proportions of stroma and parenchyma, or the relative proportions of cortex



and medulla. Many variations of these proportions were observed in the sections examined.

2. The superficial cervical lymph node from a seven-month-old pig. The sections of a superficial cervical lymph node from a seven-month-old pig that were examined did not show the evidences of extensive fusion that were seen in the node from the younger animal. The capsule varied considerably in thickness, but it was thicker than the capsule of the mesenteric node from the same pig. In some trabeculae more smooth muscle cells were observed than were seen in the mesenteric node. As these observations were made on only one node from each region they may not be significant. The superficial cervical node at this age exhibited some small trabeculae which traversed the medulla and connected with branches of the trabecular system which carried the afferent lymphatics. However, their development was not as extensive as that observed in popliteal nodes. These trabeculae were not seen in mesenteric nodes from pigs older than three weeks.

The cortex and medulla of the superficial cervical node did not exhibit any particular differences from those of a mesenteric node from the same animal with the following exception. Most of the larger nodules, when viewed in histological sections, were seen to be partially or completely surrounded by a few heavy collagenous fibers which formed the capsule-like structure discussed above in the section on the mesenteric node of a three weeks old pig (fig. 20).



Figure 20

Superficial cervical node from a 7 months old pig.

H. and E. stain 185 X

Portion of a nodule and the adjacent cortex illustrating the collagenous fibers which partially surround the nodule.

It was suggested that this structure be termed the nodular capsule. This nodular capsule was more extensive in the superficial cervical node of a seven-month-old pig than that shown by mesenteric nodes from pigs of any age including the node from a pig of three years of age. Examination of this structure in the superficial cervical nodes suggested that the nodular capsule did not completely surround the nodule and that the lymphoid tissue of the nodule and the surrounding cortex were continuous in at least one area on the periphery of the nodule. Reticular and collagenous fibers were rarely seen within the nodule. This resulted in the circle of one or several fibers that formed the nodular capsule serving as an attachment for fibers in the surrounding cortex. This tended to make this capsule more conspicious in sections stained with precipitated silver than those stained by the usual histological stains (fig. 23). Frequently some fibers of the nodular capsule were also attached to adjacent trabeculae.

G. THE MANDIBULAR LYMPH NODE

Platsma (1928), Sisson and Grossman (1953), and Somers (1951) stated that the mandibular or submaxillary lymph node of the pig was located in the mandibular space adjacent to the anterior portion of the mandibular salivary gland. Sisson and Grossman (1953) stated that two mandibular nodes were often present on each side, one large and one small. The afferent lymphatics carried lymph from the surroundings and from most of the structures which formed the anterior

portion of the head. According to Platsma (1928) the efferent lymph vessels entered the cranial cervical, lateral retropharyngeal or superficial cervical node. However, Somers (1951) stated that they led to the atlantal node. In its gross appearance the mandibular node did not appear to be a conglomerate of smaller nodes. Only one node from this region was collected and examined.

1. The mandibular lymph node in the four-month-old pig. Examination of preparations of the mandibular lymph node from a four-month-old pig showed that it was an individual node and not a conglomerate of smaller nodes. This was also noted by Trautmann (1926). The stroma was not heavy nor present in proportionately large quantities. However, the sections examined were not taken from a location near the entrance of the afferent vessels and their heavy trabeculae. The capsule was variable in thickness, but it was never very thick. Trautmann (1926) stated that the capsule of non-fused nodes was stronger where blood vessels entered, but this was not observed. In the sections that were examined, the cortex was central. A subcapsular sinus was present around the node except where efferent lymph vessels were forming. As the cortex was completely surrounded by medulla this sinus was adjacent to medulla.

The nodular capsule was absent or poorly developed when present. A few plasma cells and only a few eosinophils were seen.

H. THE BRONCHIAL LYMPH NODE

Platsma (1926) stated that the bronchial lymph node was located at or near the bifurcation of the trachea. Sisson and Grossman (1953) agreed with Platsma's statement and further stated that an additional bronchial node was located at the apical bronchus of the right lung. Somers (1951) agreed with the statements of the authors named above. Furthermore, these authors agreed that this node drained lymph from the lungs, heart and esophagus, and that the efferent vessels carried lymph to the mediastinal nodes. Somers (1951) further stated that the bronchial node occurred in groups of two or four on each side.

1. The bronchial lymph node in a three-week-old pig.

The bronchial lymph node from a three-week-old pig did not show any evidences of fusion in the sections examined. Trautmann (1926) reported that little fusion occurred in the bronchial node. The capsule of the specimen examined was not particularly thin, but it was formed of delicate fibers. Only a few small trabeculae were seen and these were also formed of delicate fibers. No subcapsular sinus was observed. The node consisted of cortex with a thin rim of medulla along one edge.

A few nodules were present. They were distinct and all of those observed were surrounded in part by a fine nodular capsule. Some eosinophils were observed.

2. The bronchial lymph node in a five-month-old pig.

The bronchial lymph node from a five-month-old pig was small

when compared with the large conglomerate nodes from some other regions such as the popliteal and the parotid. It was also small when compared with a node that showed extensive evidences of fusion such as the mesenteric node. In the sections of this bronchial node that were examined no evidence of fusion was seen. The general structure of the node showed a central cortex with a thin rim of medullary tissue. The capsule varied in thickness from thin to fairly thick. Very few trabeculae were seen in the sections examined. The trabeculae were not thick and the collagenous fibers which were their principle constituents were fine. Elastic fibers were rarely seen in the stroma or the parenchyma. A well developed subcapsular sinus was present under most of the capsule.

A few nodules were seen in the cortex and all of these were in part surrounded by the few connective tissue fibers which formed the nodular capsule. A few cells of the eosinophilic series and a very few plasma cells were seen.

I. THE LUMBAR LYMPH NODE

The lumbar lymph nodes comprised a row of nodes adjacent to the aorta in the sublumbar region. These were a part of a chain which included the renal and internal iliac nodes. Platsma (1928) named the lumbar nodes the lumbar aortic nodes. He stated that the afferent vessels arose only from the skin of the lumbar region and possibly the dorsal parietal peritoneum, and that the efferent vessels led to the pelvic or lumbar lymphatic trunk. Somers (1951)

stated that the lumbar nodes received lymph from the dorsal lumbar region and the upper ventral abdominal wall. In addition he stated that they received lymph from most of the nodes located further posteriorly, thus most of the lymph from the posterior part of the body would pass through the lumbar nodes. He also stated that the efferent lymphatics led to the lumbar trunk and cysterna chyli. Sisson and Grossman (1953) reported that the lumbar nodes located near the hilus of the kidney could also be called renal nodes.

1. <u>The lumbar lymph node in a five-month-old pig</u>. The lumbar lymph node from a five-month-old pig showed little evidence of fusion in the histological sections from one specimen that was examined. However, it could not positively be stated that fusion had not occurred as the node was several times larger than the possibly non-fused bronchial node from an animal of the same age, and the medulla in one area extended deep into the node. This extension of the medulla would have been present during the later stages of fusion if fusion had occurred. However, it is not known if this is invariably due to fusion. The medulla in general was peripheral, except for the situation described above. Although much of the periphery was formed of cortical tissue rather than medulla, the type of structure described above in the section on the popliteal node was not seen. Thus the lumbar node which was studied more closely resembled the mesenteric than the popliteal node. The capsule and trabeculae were not heavy and in places the capsule was very thin.

A subcapsular sinus was seen to surround some portions of the node adjacent to both cortex and medulla.

In the cortex only a few small nodules that were indistinctly separated from the surrounding tissue were seen and no indications of nodular capsules were seen. No eosinophilic cells were seen, but one plasma cell and a cell of the basophilic series were seen.

J. THE DEEP INGUINAL LYMPH NODE

Platsma (1928) stated that the deep inguinal node of the pig was located in the pelvic cavity adjacent to the external iliac artery. According to Sisson and Grossman (1953) it was most ventral of several nodes situated along this artery. Somers (1951) did not describe this node in the pig. Platsma (1928) further stated that the afferent vessels of the deep inguinal node drained lymph from the deep portions of the rear limb, part of the parietal peritoneum, the skin of the lumbar region, the superficial inguinal node, and sometimes the popliteal and sacral hypogastric nodes. The efferent lymphatics went to the pelvic lymphatic trunk or occasionally to the medial iliac, or renal node.

1. The deep inguinal lymph node in the 21 centimeter pig fetus. The deep inguinal lymph node from one 21 cm fetal pig, when viewed as a histological preparation, did not appear to differ greatly in structure from a mesenteric node from the same fetus.

Very little stroma was seen in this node. The capsule was very thin and the few trabeculae that were seen were

small and delicate. No evidences of fusion were seen in the sections examined. A few lymph sinuses were seen at the periphery and more than a few in the parenchyma, but the network of peripheral sinuses as described by Sabin (1905) was not seen. A valve was observed in an efferent lymphatic close to the node. Although other authors had stated that valves were usually found close to the node in efferent vessels they were not seen in any of the nodes described above.

It was not possible to make a clear distinction between the cortex and medulla although it was possible in the mesenteric node at this age. As in the mesenteric node a few small nodules were seen. The cellular constituents of the parenchyma were like those seen in the mesenteric node.

2. The deep inguinal lymph node in a four-month-old pig. This node from a four-month-old pig was large and examination of histological sections disclosed evidence that it was formed by the fusion of several smaller nodes. The capsule varied in thickness from thin to heavy. The trabeculae were usually heavy and they were observed to be more numerous in the cortical tissue than they were in the same tissue in mesenteric and other nodes. The node resembled the popliteal in this respect. Elastic fibers were seen occasionally in both the parenchyma and stroma. A subcapsular sinus was present in places adjacent to both cortex and medulla. The general structure also resembled the popliteal node as both the central and peripheral cortex types were seen.

The presence of nodular capsules was rare. In slides of one portion of the node that were examined no nodules were seen. The cortical tissue consisted only of a thick tube-like structure around a trabeculum. These were scattered throughout the medulla which formed most of the node in the sections examined. This structure probably was a variation of the central cortex type. No eosinophils were seen.

K. THE SUPERFICIAL INGUINAL LYMPH NODE

The superficial or external inguinal lymph node was termed the supramammary node in the female because of its relation to the posterior mammary gland. No important discrepencies were found in the descriptions of this node given by Sisson and Grossman (1953), Somers (1951), and Platsma (1928). They occurred as subcutaneous groups at and adjacent to the external inguinal ring. The afferent lymphatics drained the external genitals and the skin of the surrounding area and rear limb. In addition Platsma (1928) reported that they drained a portion of the peritoneum and the deeper portions of the upper rear limb.

1. <u>The superficial inguinal lymph node in a three-week-old pig</u>. The superficial inguinal node from a three-week-old pig resembled the popliteal nodes from the pigs of the same age in three respects. Proportionally large quantities of stroma were seen, most nodules were adjacent to a trabeculum, and considerable fusion had occurred. On the other hand, the general structure was the central cortical type. Because of the extensive fusion that had occurred the general

structural type was obscured but it clearly was not the peripheral cortical type. A subcapsular sinus was present around most of the node in the sections that were examined. Elastic fibers were seen more frequently than in other nodes from pigs of this age. However, they were not often seen and examination of nodes from several animals might indicate that this situation was exceptional.

Large blood vessels were seen entering and leaving the medulla of the node near the locations of the efferent vessels. This was observed in other nodes also. Where present the nodular capsule was formed of one or two layers of heavy fibers. This capsule was more prominent than it was in most of the other nodes where it was seen. The usual cellular constituents including a moderate number of eosinophils were observed.

2. The superficial inguinal lymph node in the seven-week-old pig. The superficial inguinal node from a seven-week-old pig differed only in a few respects from this node in the three-week-old pig. Fusion was continuing with at least three nodes involved in this process. There was less stroma in proportion to parenchyma in this node than in the same node from the younger pig. However, the proportion of stroma was high and almost every nodule was adjacent to a trabeculum. The capsule was thin and the presence of the subcapsular sinus was not constant. The general structure was the central cortex type.

The nodular capsule was rarely seen in the sections

examined and when present it was thin. No eosinophils were seen.

3. The superficial inguinal lymph node in the three-month-old pig. The superficial inguinal node from a three-month-old animal showed a larger proportion of stroma to parenchyma than was seen in most of the nodes that were examined. Most of the nodules were adjacent to a trabeculum. The capsule showed a great variation in thickness and the subcapsular sinus was nearly complete. No evidence of fusion was seen. Different parts of the node in the same histological preparation showed both types of general structure.

A thin nodular capsule was present around some nodules. Many eosinophils, chiefly immature forms were seen in the parenchyma. Frequently these occurred in clumps suggesting that extramedullary myelopoiesis might have occurred within the node.

4. The superficial inguinal lymph node in the four-month-old pig. The superficial inguinal lymph node from a four-month-old pig differed only in some minor characteristics from that of the three-month-old pig. There was less stroma in proportion to parenchyma and only a few nodules were present, but those that were observed were adjacent to a trabeculum. A few trabeculae were seen to have branches which joined the capsule. The capsule varied in thickness. The subcapsular sinus, when present, was narrow. The elastic fibers observed in this node were more numerous and much

heavier than in any other nodes that were examined. However, many areas of the node were devoid of these fibers. These differences were probably due to differences between individual animals rather than to age changes. In all of the preparations that were examined the general structure was of the central cortex type.

No nodular capsules were seen around any of the nodules that were observed. Eosinophils were rarely seen.

L. LYMPH FLOW THROUGH THE NODE

Trautmann (1926) studied the direction of lymph flow through the pig node by injection of dye into the afferent lymphatics and ascertaining its position in microscopic preparations. Goldkuhl (1927) injected dye directly into the node and demonstrated that it left through efferent vessels which did not originate at a hilus. Valves prevented reverse flow of dye from the afferent vessels. Both methods could be criticized because the pressure used could conceivably have induced artifacts in the reticular tissues of the nodal sinuses. The methods described below were less likely to cause artifacts and were sufficient to delineate the pathways taken by the lymph.

Three techniques were used to study the circulation of lymph through the node. Two of these involved injection of materials into the pig and the third consisted of examination of sections to ascertain the position of the valves.

Subcautaneous and intramuscular injections of living pigs with an aqueous suspension of India ink or of 0.5

percent aqueous trypan blue were used for localization of phagocytic cells within lymph nodes. Regions of the nodes which exhibited the phagocytized materials served as means of identification of passages. Thus some of the regions of the node through which lymph had passed were demonstrated. Subcutaneous injections permitted the injected material to be carried to the node by normal lymph flow, thus artifacts were not likely to be produced. In another experiment 0.5 Percent aqueous trypan blue was injected into the submucosa Of the small intestine of a pig immediately subsequent to death by exsanguination. Injection of materials was discontinued when the lacteals in the mesentery exhibited an abundance of dye. As the pressure used to inject dye into the submucosa was not directed against the nodal tissues it was believed that no artifacts were induced. The node adjacent to the injected area, subsequent to fixation, dehydration and staining was used for study of lymph channels in nodes deep in the body.

As stated above the third technique used was, the examination of sections that exhibited valves. These valves had the same structure as those that occur in the veins. They were found in pairs on opposite sides of the vessel with one edge attached to the wall of the vessel and one edge free. The direction of lymph flow past the valves was from the attached end toward and beyond the free end. Thus by examination of the valve the direction of lymph flow could could be determined.

The injection of trypan blue into the gut wall demonstrated the pathway of lymph through mesenteric nodes. Although little phagocytosis of dye materials had occurred within the node there were sufficient accumulations of dye to make evident the channels through which it flowed. In all sections examined the adjacent tissue was well stained in areas where the dye had passed through. Trypan blue could be seen in the afferent lymphatics within the trabeculae (fig. 21). It could also be seen in the sinuses adjacent to the smaller trabeculae that lead to the medulla. Very little was seen in the cortical tissue. On the other hand, large areas of the medulla were uniformly stained indicating that lymph had diffused widely in the medulla. Evidence of dye was also seen in some efferent vessels.

In sections of popliteal nodes following subcutaneous injection of India ink suspensions phagocytosis had occurred in the cortex adjacent to the sinuses around the trabeculae. No carbon particles were seen elsewhere in the cortex or in the medulla, except in afferent vessels (figs. 17 and 22).

The evidence cited above shows that lymph was transported deep into the node through afferent vessels which were located in trabeculae, and which drained into sinuses adjacent to the trabeculae. These sinuses continued as far as the medulla although near the medulla they became very small. Lymph passed through the sinuses and upon reaching the medulla infiltrated large areas of medullary tissue. Undoubtedly lymph could also flow through cortical tissue and then

Figure 21

Mesenteric node from a 5 months old pig.

H. and E. stain 90 X

A. Trypan blue in afferent vessel

B. Trypan blue in adjacent sinus


A
B

Figure 21

Mesenteric node from a 5 months old pig.

H. and E. stain 90 X

A. Trypan blue in afferent vessel

B. Trypan blue in adjacent sinus



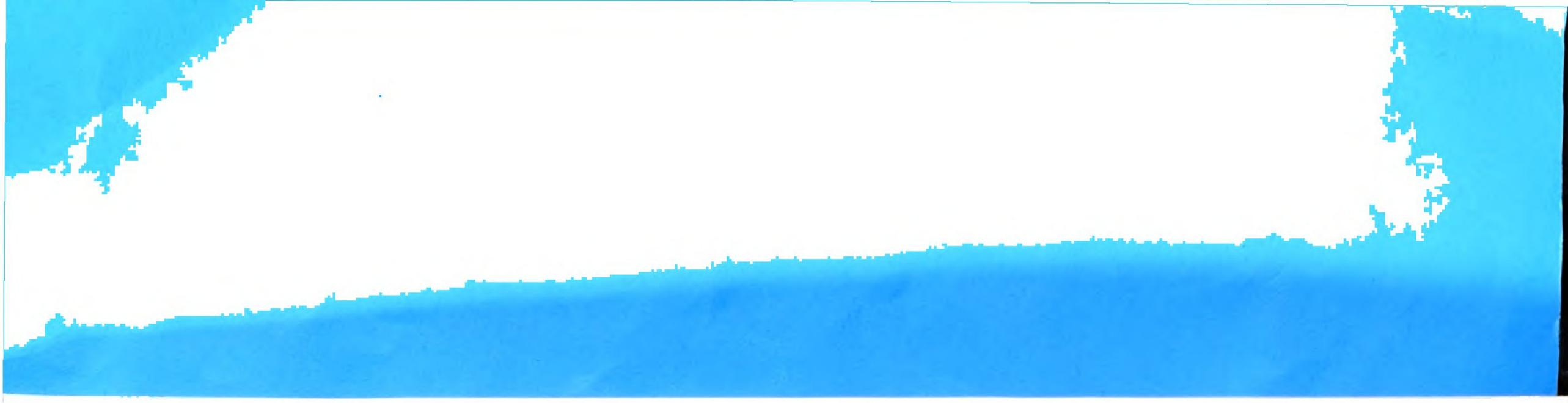


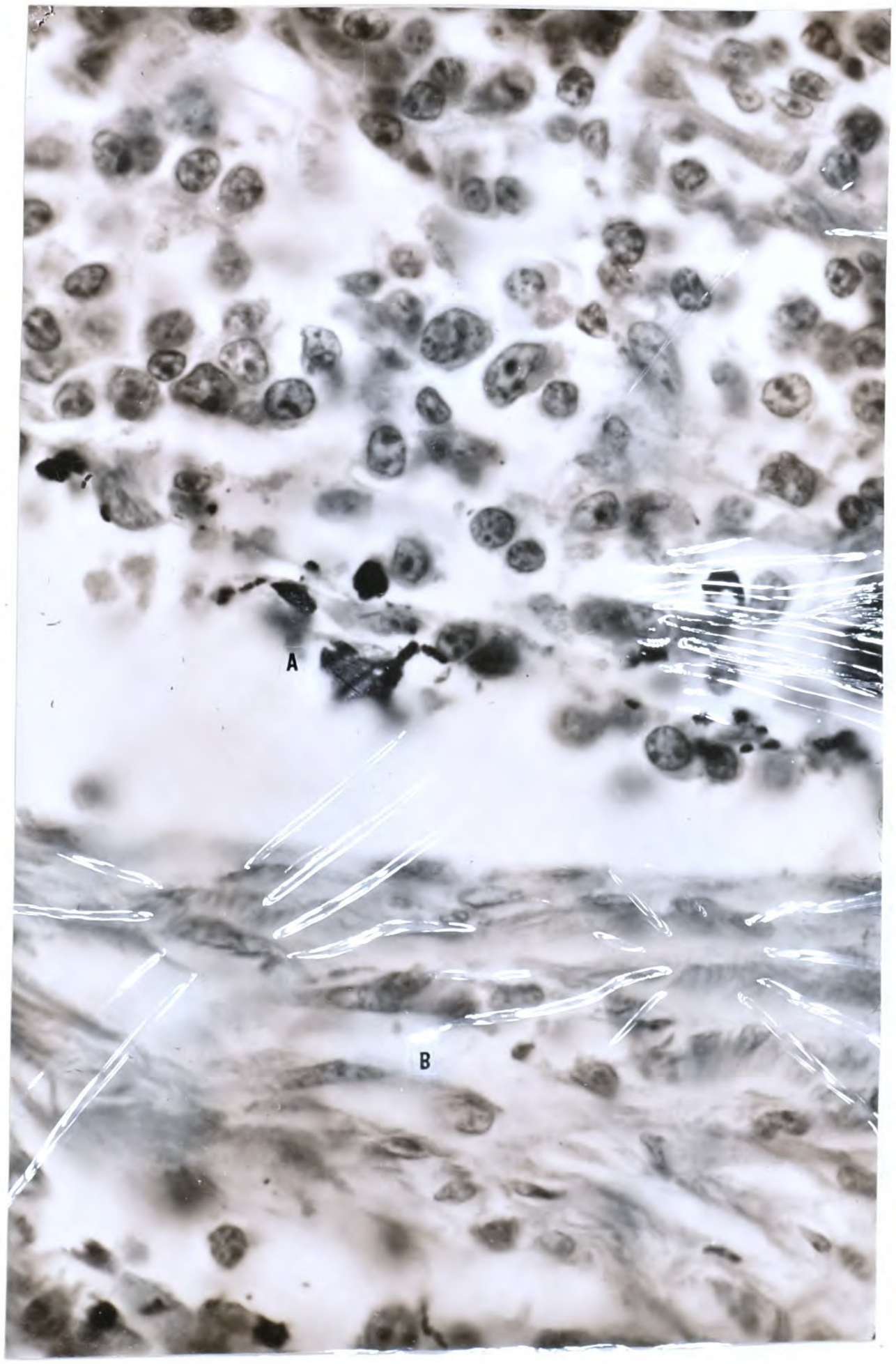
Figure 22

Popliteal node from a 3 week old pig.

H. and E. stain 1100 X

A. Reticular cells which have phagocytized carbon particles.

B. Trabeculum


A
B

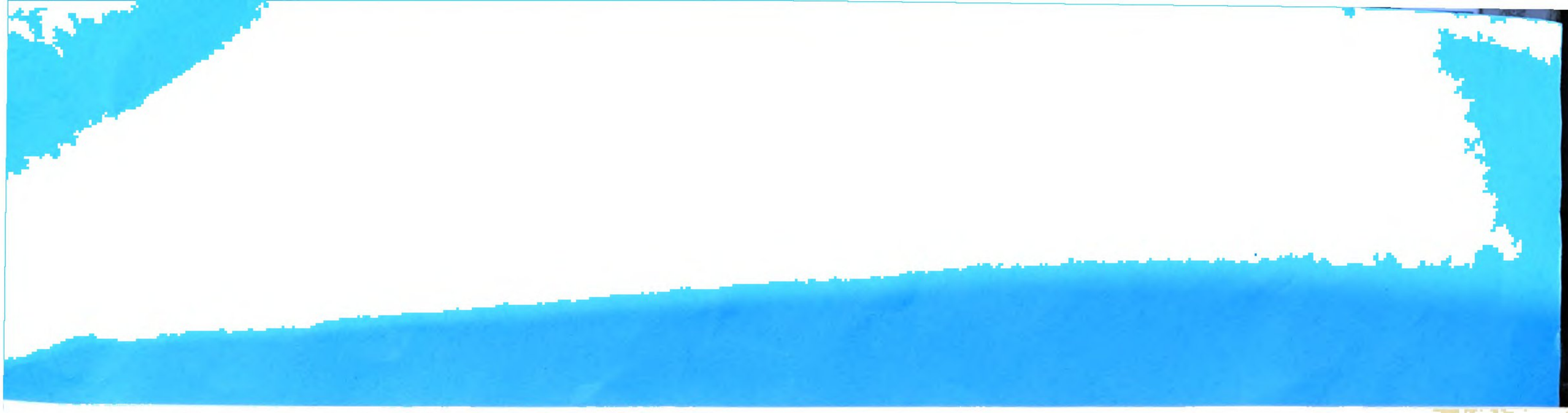


Figure 22

Popliteal node from a 3 week old pig.

H. and E. stain 1100 X

A. Reticular cells which have phagocytized carbon particles.

B. Trabeculum

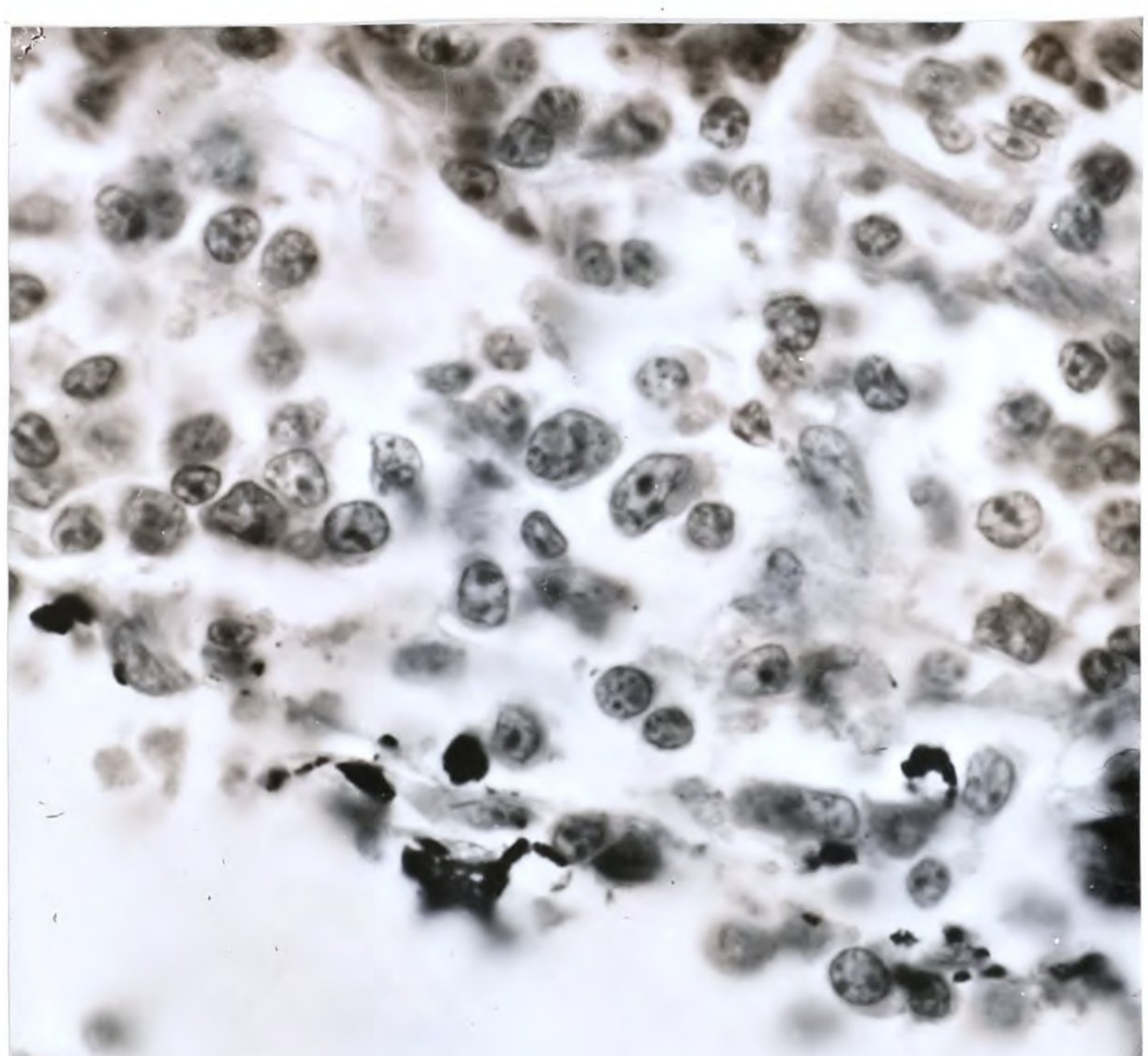


Figure 23

Parotid node from a four months old pig.

Bielchowsky's method. 300 X

A. Nodular capsule of reticular fibers.

Figure 24

Mesenteric node from a five months old pig.

W. and V.G. stain 125 X

Trabeculum with an afferent vessel opening through a valve into the adjacent sinus. This lies within the cortex.


A



Figure 23

Parotid node from a four months old pig.

Bielchowsky's method. 300 X

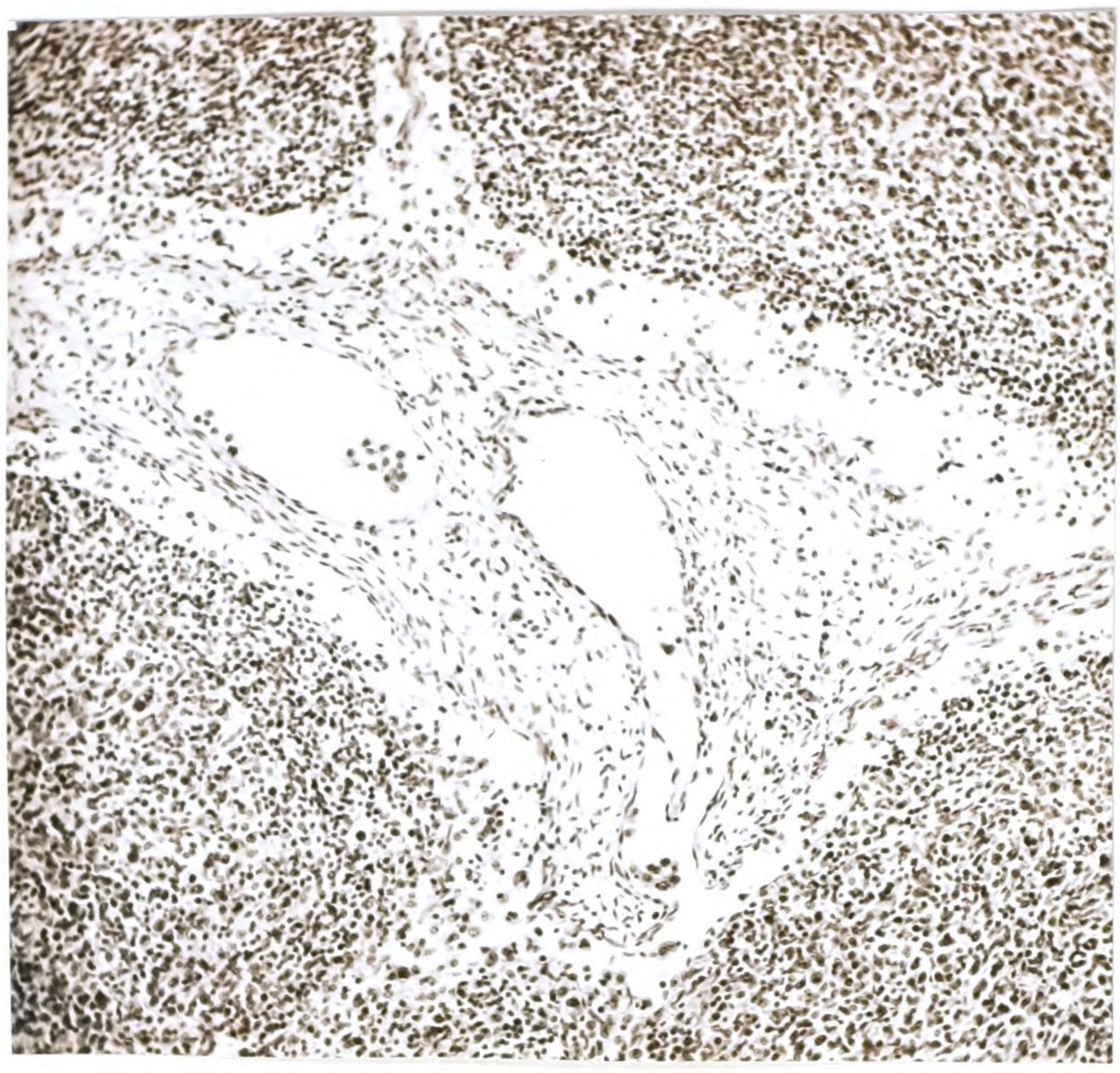
A. Nodular capsule of reticular fibers.

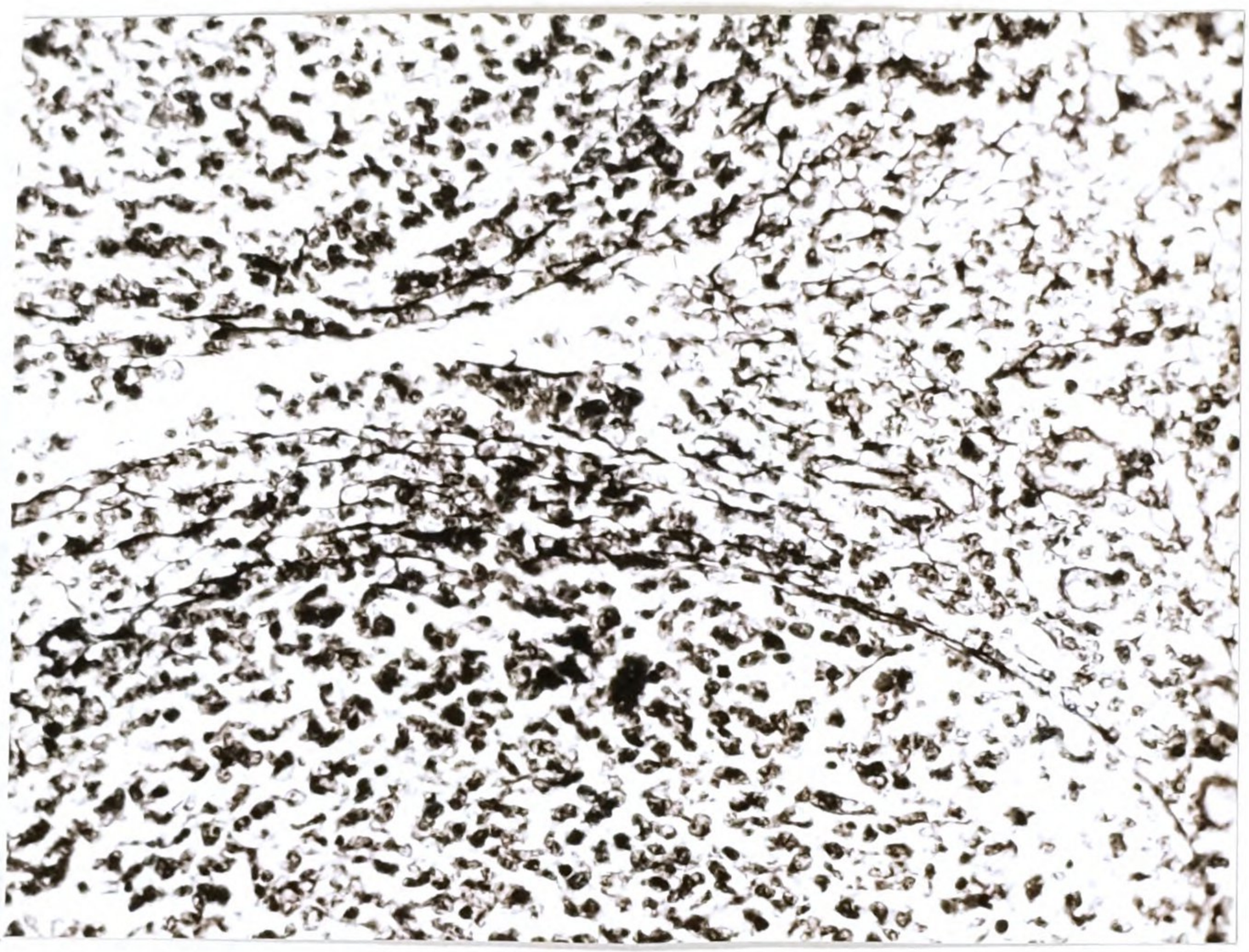
Figure 24

Mesenteric node from a five months old pig.

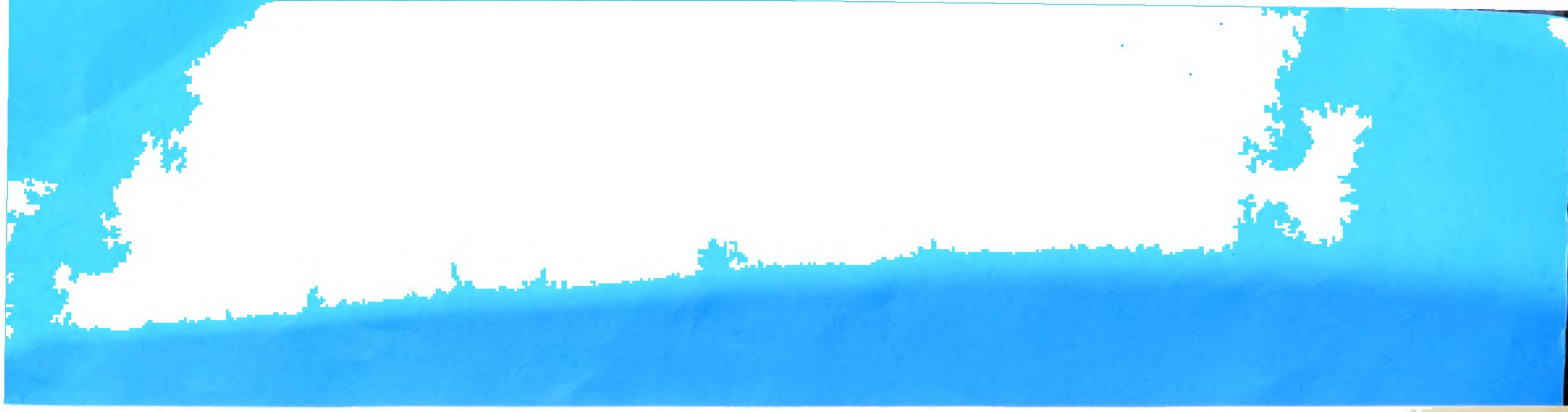
W. and V.G. stain 125 X

Trabeculum with an afferent vessel opening through a valve into the adjacent sinus. This lies within the cortex.





on to the medulla. From the medullary tissue lymph passed into sinuses located at certain areas on the periphery of the medulla. These sinuses joined to form the efferent vessels. This circulation was very much as Chievitz (1881), Trautmann (1926), Goldkuhl (1927) and others have described it. Fundamentally it was the same as the circulation of lymph through the typical mammalian node because in both cases lymph flowed first through sinuses adjacent to the cortical tissue and then into the medulla from which it left the node through efferent vessels.



IV. DISCUSSION

Age changes in pig lymph nodes were readily detected in nodes removed from pigs whose ages ranged from late fetal to three or four weeks post natal. The changes observed were as follows. The stroma became heavier and the individual collagenous fibers of the strome became thicker. There was little increase in the number or size of the elastic fibers. The network of lymph sinuses at the periphery of the node had disappeared within six days after birth. The lymph sinuses of the medulla decreased greatly in number, probably because they were being filled with primitive reticular cells. In the cortex the density of the lymphocytes had increased and the nodules had become larger and more clearly defined. These changes occurred most rapidly within a few days after birth, but continued for some time.

Differences between nodes from various regions were not often found to be present, although in a few cases structural differences were observed. Differences in gross appearances were noted, such as the tendency of the mesenteric nodes to form long rather cylindrical masses. Nodes from other regions, as the popliteal formed clumps, groups or conglomerates. In addition other nodes, as the bronchial were small and were not found in groups. These gross characteristics were reflected in the microscopic appearance. The mesenteric nodes showed much evidence of fusion. The popliteal nodes appeared lobed which suggested that they were only partially fused. The bronchial nodes showed no evidence

of fusion.

A structural characteristic found in many popliteal nodes and rarely in other nodes was noted. The pig lymph node, when examined in histological preparations, at times presented a clear picture of a central region of cortical tissue surrounded by a rim of medullary tissue. However, at other times a confused picture of various relationships between cortical and medullary tissue that were without system were seen. A study of the perinatal development of these nodes, examination of nodes from several regions, and examination of serial sections of some of the nodes disclosed several possible causes of this apparently confused picture. The peripheral medulla varied in thickness and was even absent in some parts of the nodes. Thus the cortex was adjacent to the capsule or subcapsular sinus. In other locations in the node, particularly where the efferent lymphatics formed, the medulla thickened and penetrated deeply into the cortical region. Further in some nodes from the popliteal and deep inguinal regions the structure of a portion of the node resembled the structure of the typical mammalian node. A third cause of this apparent confusion was the frequent presence of various stages of fusion.

Although attempts to relate specific structural features to the region from which the node had been removed were largely unsuccessful, in one case at least an indication of such a relationship existed. If the type of structure with the peripheral cortex, i.e. resembling the typical

mammalian node, formed part of the node there was a strong possibility that the node was popliteal or deep inguinal. However, if this type of structure was not seen it could not be assumed that the node was not popliteal or deep inguinal. Goldkuhl (1927) stated that he saw ampullae in inguinal and mesenteric nodes.

The fibrous capsule around the nodules described as characteristic of the pig lymph node by several authors was found to be variable in occurrence in nodes from various regions and even in individual nodes. Its greatest development in size and frequency was observed in the superficial cervical node. However, it was usually present around some nodules in most of the nodes that were examined irrespective of the region that they were removed from.

The occurrence or non-occurrence in the pig lymph node of the characteristics discussed below was not correlated in any way with the region from which the node was removed nor with the age of the animal from which it was removed.

Many authors have noted that eosinophils were a common constituent of the parenchyma of pig lymph nodes. It was noted during the examination of the nodes that there were great differences between them in the quantity of these cells. Either they were very common or they were rather rare. A relationship was found between the source of the pig from which the node was removed and the quantity of eosinophils found in the node. Pigs grown under farm conditions, where heavy infestation with intestinal parasites

may be expected, exhibited lymph nodes with the greatest number of eosinophils and other granulocytes. Under these conditions granulocytes may have entered the node either directly from the capillaries of the blood vascular system or from tissue spaces by way of the afferent lymphatics. It is well established that increased numbers of eosinophils in the peripheral blood are related in some manner to parasitic infestations of the digestive tract. Further research including counts of eosinophils in the blood as well as examination of lymph nodes might prove useful in establishing this relationship. The eosinophils and other blood cells were observed more frequently in the medulla than in the cortex, but possibly this was due to the fact that in the cortex they could have been obscured by the numerous lymphocytes.

Plasma cells were described as occurring in the lymph node of the rat by Hellman (1930) but they were very rarely seen in the pig nodes that were examined. Because several megakaryocytes were seen in a node from a fetal pig the possibility that other myeloid cells were formed there should have been considered.

Observations showed, as had been reported by several authors, that blood vessels entered the pig lymph node at various places. The largest vessels entered the medulla near the point of origin of the efferent lymphatics. These would probably be homologus with the blood vessels of the typical mammalian node. The artery which entered the me-

dulla usually branched into small vessels, usually capillaries, almost immediately after it entered the node. However, occasionally arterioles were seen in the medulla. The larger trabecular which carried the afferent lymphatics also carried blood vessels, but none were seen to leave the trabeculae and enter the parenchyma so it was assumed that these supplied only the trabeculae. Small vessels entered the capsule and in some cases penetrated it and entered the parenchyma. It was assumed that these supplied the capsule and the parenchyma.

The presence or absence of a subcapsular sinus, or whether it was present but collapsed or occluded has been discussed by several authors. This sinus was not constant in its occurrence, but the fact that it was often present indicated that it was not necessary to inject the node to demonstrate the sinus. The subcapsular sinus was seen to be present adjacent to both the cortex and the medulla although both Goldkuhl (1927), and Trautmann (1926) stated that it was usually present only adjacent to the cortex. As the subcapsular sinus was not greatly concerned with the circulation of lymph through the node, as it was in the typical form of this organ, the presence or absence of the sinus probably was not important. Where it was continuous and adjacent to both cortex and medulla it could have possibly served in conjunction with a peritrabecular sinus as a bypass for lymph around the parenchyma of the node. The sinuses which were adjacent to those trabeculae which car-

ried afferent lymphatics had a function equivalent to the function of the subcapsular sinus of the typical node. These sinuses were usually present, and when they apparently were not present observations suggested that they were completely filled with lymphocytes. The larger of these sinuses usually had an almost continuous endothelial lining which was usually lacking in the smaller sinuses. Although Goldkuhl (1927) stated that a sinus was present around the smallest trabeculae, even those trabeculae which extended into the medulla, these sinuses were not always seen.

At no time were myelinated nerve fibers seen. The fatty degeneration of pig lymph nodes that had been described by many authors was observed in only a few of the older nodes and consisted of only a few adipose cells. This degeneration probably corresponded to the fibrous degeneration that had been observed in nodes from other species.

V. SUMMARY

Lymph nodes from ten body regions, which varied in age from late fetal to three years, were examined using various standard histological methods.

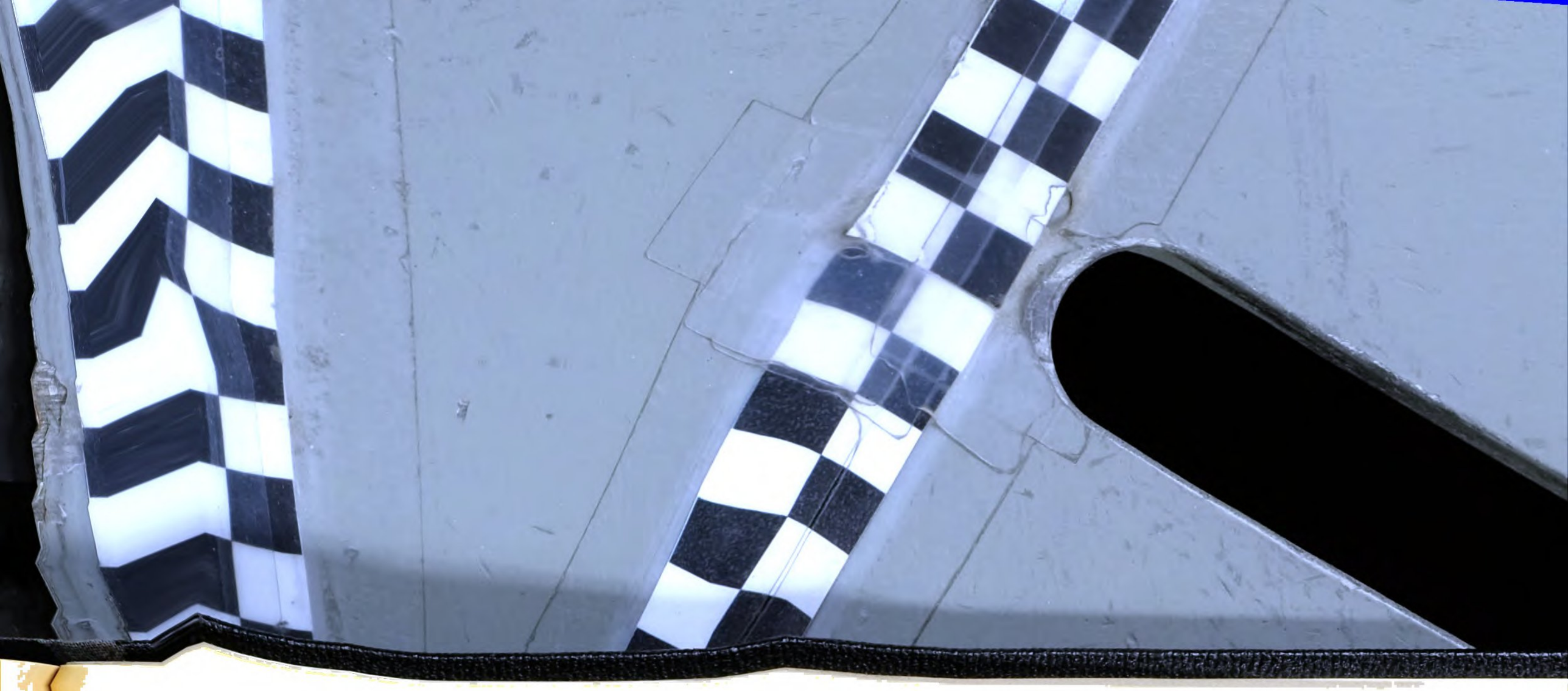
The medulla of nodes from animals up to six days of age, consisted of reticular cells, lymphocytes, and many sinuses. In nodes from older animals, only minute sinuses were exhibited, due to the increased numbers of reticular cells in the medulla. As a consequence, the medullary cords and sinuses characteristic of this region were not observed. In all the nodes, except popliteal and superficial and deep inguinal, the medulla usually surrounds the cortex.

As the age of the animal increased, the stroma of the node became heavier. Elastic fibers formed layers in the trabeculae and capsule and were present in the parenchyma at the age of seven months. Attempts to classify nodes according to varying proportions of the stroma and parenchyma were found to be unsuccessful.

Nodules in nodes from the older animals frequently were surrounded by collagenous fibers, reticular fibers or both.

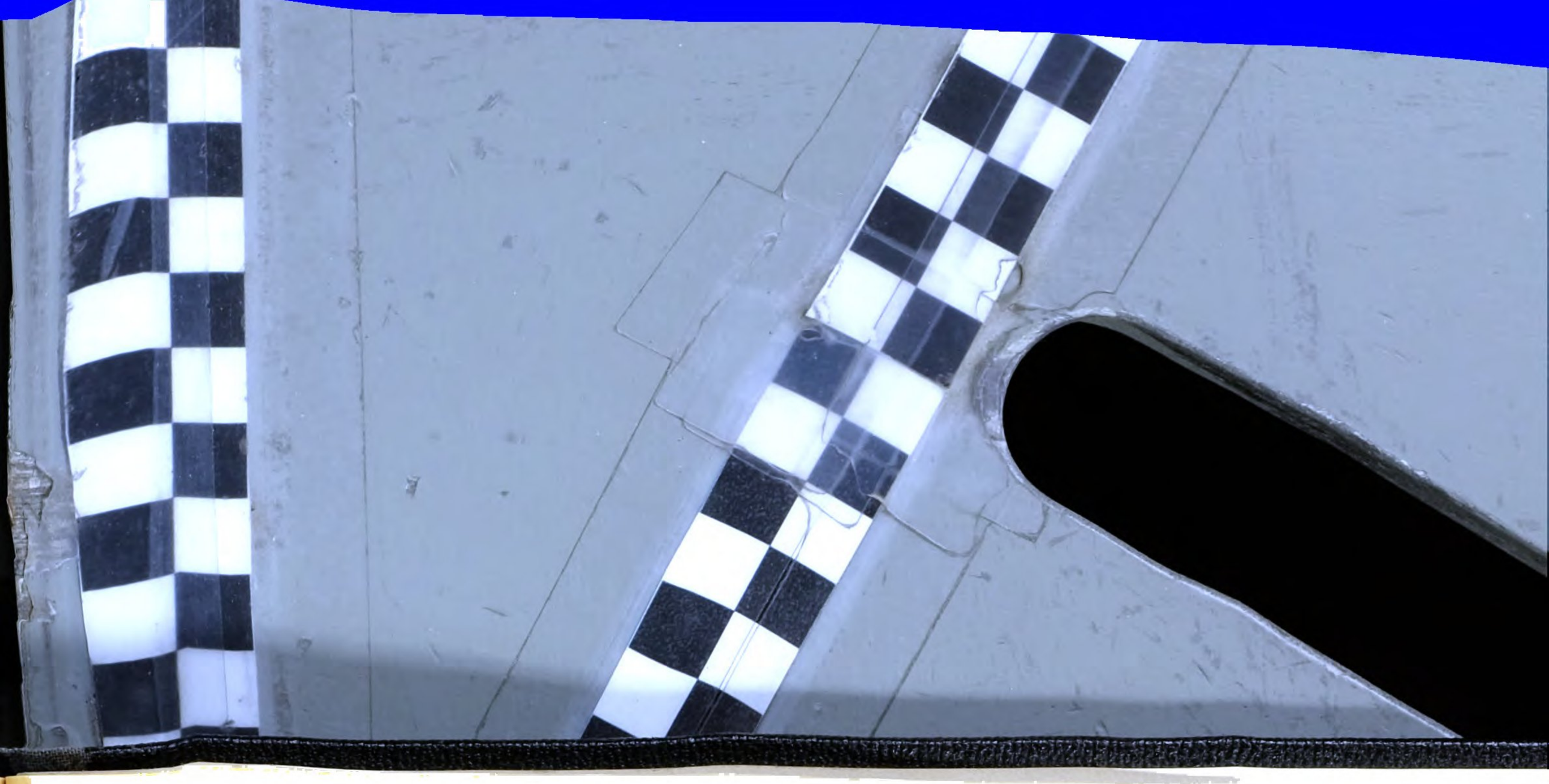
The most rapid changes occurred within days after parturition. However, they continued until attainment of sexual maturity, i.e., at six or seven months.

The nodes examined could be grouped into three general classes. First, large nodes, characterized by extensive fusion. These were the mesenteric, pharyngeal, mandibular and lumbar nodes. Second, bronchial nodes, which were small



and showed no evidence of fusion. Third, large nodes, which grossly were lobed and microscopically appeared as conglomerates of fused and partially fused nodes. These were the popliteal, parotid, superficial cervical, the deep and superficial inguinal nodes. In some cases, structures similar to the cortical sinuses or alveoli characteristic of human nodes were observed.

Lymph entered the cortex from afferent vessels, which followed trabeculae. From the cortex lymph entered the medulla and hence into efferent vessels.



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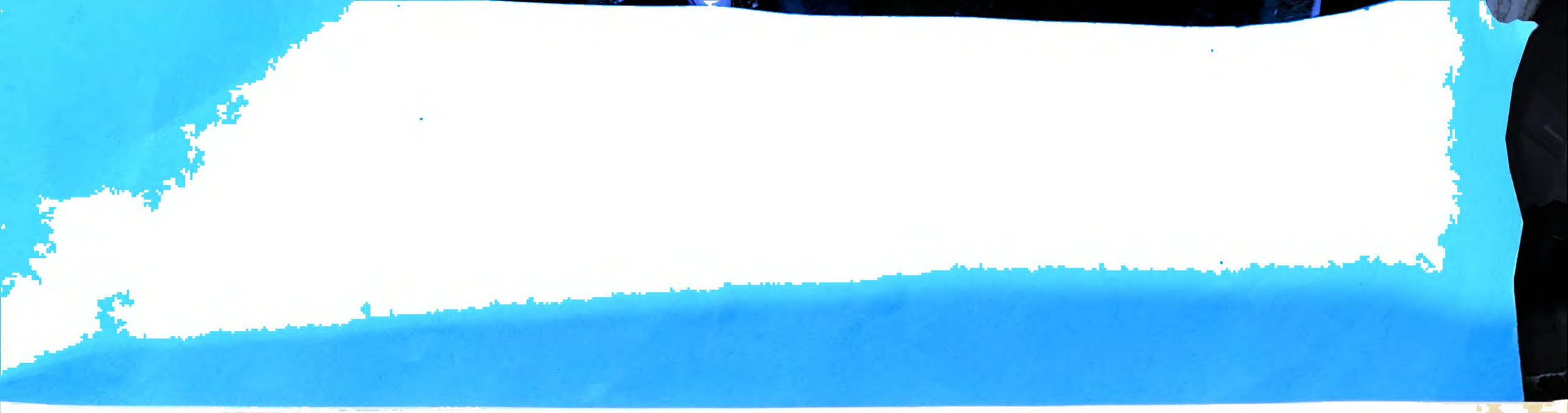
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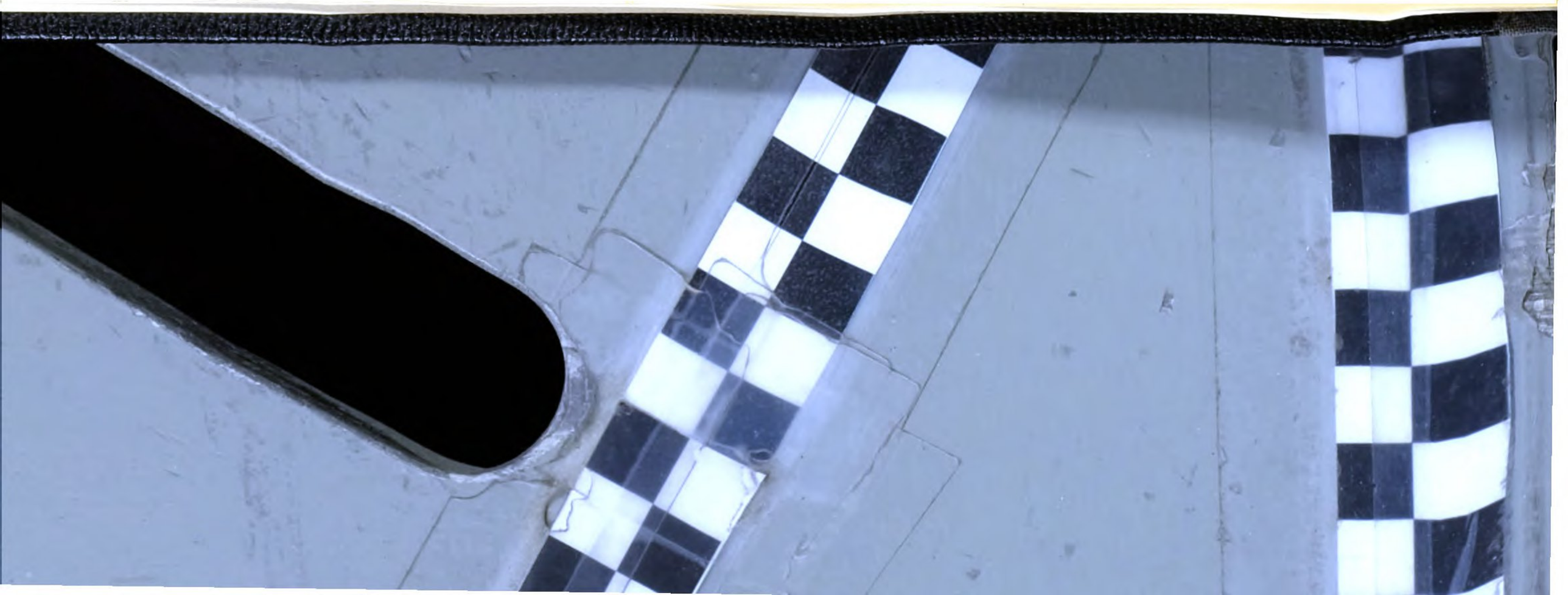
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