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Author(s)	HOSHI, Nobuhiko; HASHIMOTO, Yoshiharu; KITAGAWA, Hiroshi; KON, Yasuhiro; KUDO, Norio
Citation	Japanese Journal of Veterinary Research, 36(1), 1-14
Issue Date	1988-01-30
DOI	10.14943/jjvr.36.1.1
Doc URL	<a href="http://hdl.handle.net/2115/3080">http://hdl.handle.net/2115/3080</a>
Type	bulletin (article)
File Information	KJ00002376917.pdf



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A SCANNING ELECTRON MICROSCOPIC STUDY  
ON THE ARCHITECTURE OF LYMPH VESSELS  
AND INTRANODAL LYMPH PATHWAYS  
OF LYMPH NODES IN PIGS

Nobuhiko HOSHI, Yoshiharu HASHIMOTO, Hiroshi KITAGAWA, Yasuhiro KON  
and Norio KUDO

(Accepted for publication November 18, 1987)

The architecture of lymphatic microcirculation of pig lymph nodes was studied by scanning electron microscopy using a corrosion cast method. There were two types of lymph vessels for afferent lymph supply as well as those for efferent lymph drainage. Most afferent lymph vessels entered the node at the A-type hilus and penetrated deeply into the node. Others divided from the larger afferents and distributed to the convex surfaces of the node. One type of efferent lymph vessel left from the E-type hilus by several efferent trunks and was formed by confluences of many smaller lymph vessels, and the other type arose from surfaces around the peri-hilar (A-type) region, encompassing the afferent lymph vessels with numerous initial efferent vessels. The afferent lymph vessels were followed by intra-trabecular lymph channels. The peri-trabecular lymph sinuses and the peri-hilar (A-type) sub-capsular lymph sinuses were connected subsequently with the efferent lymph vessels and there was a direct communication between the cortex- and the medulla-like tissue (CT and MT). A marked difference between the casting patterns of the CT and MT was recognized; that is, the CT was visualized as a dense coral-reef-like shape, whereas the MT exhibited a bead-like structure with numerous larger inter-spaces. These findings suggest that the intranodal lymph pathways not only share reversal in flow but are more minute and complex than those hitherto demonstrated.

Key words : lymph flow, lymph node, pig, scanning electron microscope.

INTRODUCTION

Attention has already been drawn to the morphological peculiarity of the pig lymph nodes, in which the course from the afferent to the efferent lymph vessels has resulted in an "inversion" of the cortex and the medulla.<sup>4,14)</sup> Early studies on the

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\* Department of Veterinary Anatomy, Faculty of Veterinary Medicine, Hokkaido University, Sapporo, 060, Japan

lymphatic sinus were only based on the intranodal injection of inert particles, such as India ink<sup>1,4,14)</sup> via the afferent lymph vessels; however, numerous problems still remain unelucidated.

Recently, as a result of re-examination of the structures in pig lymph nodes with light and transmission electron microscopes,<sup>8,9)</sup> it was revealed that the morphological peculiarity of the pig lymph nodes is not merely inversion, and that there are no medullary cords or sinuses in the MT.

There is little information on the lymph pathway system of lymph nodes in pigs, particularly the entry and exit patterns of lymph vessels and the three-dimensional architecture of intranodal microcirculation analyzed by scanning electron microscope.

The aim of the present study is to clarify the lymphatic microcirculation of the pig lymph nodes using a replica scanning electron microscope method.<sup>12)</sup>

#### MATERIALS AND METHODS

*Animals*: Six Large White×Landrace pigs, aged 4–6 weeks, were used in this study.

*Casting resin mixture*: Mercoc (Japan Vilene Co. Ltd., Tokyo), low-viscosity methacrylate resin was applied. In most preparations Mercoc was diluted with methyl methacrylate (20.0 g Mercoc : 5 g methylmethacrylate) before the addition of 0.5 g catalyst. This produced a resin with a lower viscosity and longer working time (20–30 min) than the Mercoc working time (5 min).

*Resin injection and corrosion casting*: Pigs were anesthetized with sodium pentobarbital (Somnopentyl) intravenously. They were injected subcutaneously with 10% patent blue violet (1 ml) into the dorsal site just proximal to the hoof and/or just distal to the tarsus of the pelvic limb, so that the lymphatics draining into these areas could be seen more clearly. Then an incision was made through the skin of the lateral areas of the hock joint, and superficial lymph vessels containing patent blue violet in the lymph were identified in parallel with the lateral saphenous vein. One of these was cannulated with a 27G venular indwelling needle (Venula V5: Top Co. Ltd., Tokyo, Japan). Subsequently, physiological saline solution (0.9% saline) was injected to flush out the lymph through this cannula. Immediately after this irrigation, the casting resin mixture was injected through the same cannula under manual pressure. Each pig was then killed by exsanguination, and after the polymerization of the injected resin was completed, the superficial popliteal, sacral and medial iliac lymph nodes were dissected. They were immersed in 20% KOH solution at 60°C for several days to dissolve the tissue. The casts thus obtained were washed in warm running water and by an ultrasonic generator (28 KHz for 10 min).

*Scanning electron microscope (SEM) observation*: To observe the cut surface of the casts more definitely, some of them were frozen in water and cut with a razor blade prior to SEM analysis. They were then air dried, coated with gold (JFC-1100,

JEOL Ltd., Japan) and observed by SEM (JSM-T200, JEOL Ltd, Japan) at an acceleration voltage of 10 kv.

## RESULTS

The injected resin mixture was confirmed in the lumber trunks via the superficial popliteal, sacral and medial iliac lymph nodes. The number of afferent and efferent lymph vessels varied according to the lymph node. Efferent lymph vessels were more numerous than afferent lymph vessels. However, no marked differences were found in the fundamental lymph vascular conditions or entry and exit patterns of the lymph vessels due to location of the lymph node.

*Afferent lymph vessels*: Well-developed lymphatic valves were situated at intervals of 0.8–1.2 mm in the afferent lymph vessels (Figs. 1, 2). One or two afferent lymph vessels penetrated deeply into the nodes at the hilar depression which was previously designated the “A-type hilus”<sup>8)</sup> (Fig. 1). However, there existed a variation in which the afferent lymph vessels gave off multiple branches just before they reached the lymph node. Some branches entered the convex surfaces of the node, and then joined sub-capsular lymph sinuses (SCLS). These SCLS were located between the capsule and the CT, which occupied a conventional superficial position underneath the convex surfaces (Fig. 2). Many small holes were found at almost regular intervals of 0.1–0.3 mm on the cast surface of the node because of the invagination of the capsular connective tissues, where direct communication between the SCLS and peri-trabecular lymph sinuses (PTLS) was observed (Fig. 2). The depression of the A-type hilus, which varied in size and depth, was observed commonly on the medial or on the lateral surface of the lymph node. The popliteal lymph nodes were usually furnished with one or two A-type hiluses. In the sacral and medial iliac lymph nodes, however, up to 4 or more of the A-type hiluses were situated according to the number of segmental “nodular units”.<sup>8)</sup>

*Efferent lymph vessels*: Efferent lymph vessels were more numerous and thicker than afferent lymph vessels. Two paths for leaving the efferent lymph vessels from the nodes were noted: one was a route from the shallow depression of the “E-type hilus”,<sup>8)</sup> and the other was that from the convex surfaces at the peri-hilar (A-type hilus) regions (Figs. 1, 3). In the former type, several small efferent vessels left the node at the E-type hilus, and formed several larger efferent ducts by confluences (Fig. 3). In the latter type, more numerous minute vessels (initial efferent lymph vessels) arose from the surfaces around the A-type hilus, and the vessels then converged to become larger main efferent lymph vessels (Fig. 1). The depressions of the E-type hilus were usually large but shallow, and situated on the dorsal or on the lateral surface of the node. These hiluses varied in number depending on the number of the nodular units in the pig lymph nodes.

*Intranodal lymph microcirculation*: The afferent lymph vessels, intratrabecular

lymph channels (ITLC), PTLs, peri-hilar (A-type hilus) SCLS and efferent lymph vessels were filled with resin mixtures. The cut surfaces of the casts displayed the lymphatic vasculature of the SCLS and PTLs as a thick limb, and the ITLC as a tubular or club shape (Fig. 4). The diameter of the cast of the afferent lymph vessels increased at the center of the nodular unit; that is, it could be called a central cisterna. Thereafter, the central cisterna was either connected to the PTLs or the peri-hilar (A-type hilus) SCLS successively (Fig. 4). In the peri-hilar region of the SCLS, however, the resin was injected intermittently so that casts were situated as an arrangement of dense-sparse-dense band structures (Figs. 1, 4).

The CT and MT were also injected completely with the casting substance. In the CT, the pattern of lymphatic microvasculature by casting resin was visualized as a dense coral-reef-like shape, whereas the casts in the MT exhibited many dense bead-like structures containing larger interspaces (Figs. 5, 7). In the casts, the direct communications between the CT and MT were recognized clearly (Fig. 6). Areas corresponding to germinal centers were found sparsely but were not spaces. No differences in the pattern of lymphatic vasculature in the intra-nodular unit were confirmed among the lymph nodes in this study.

Present results on the lymphatic vasculatures of the afferent and efferent lymph vessels, and the intranodal lymph pathways in the pig lymph nodes are diagrammatically summarized in Figure 7.

#### DISCUSSION

It is widely believed that "reversal" is the genuine lymph flow pattern for lymph nodes of pigs in contrast to the other domestic animals; that is, the afferent lymph vessels enter the node at the hilar depression and emerge from many convex capsular areas.<sup>2)</sup> Functionally, it is considered that the flow of lymph in the pig lymph nodes is identical to that in other mammalian species, because the incoming lymph first flows through the lymphatic nodules.<sup>5)</sup> Although this general feature was evident in our morphological analysis, the flow pattern appears to be rather more complex.

Application of the injection replica scanning electron microscope method to the lymph pathway in the lymph node<sup>10)</sup> made it possible to replicate almost the entire lymphatic microvasculature in the pig nodes. In the present study, it was evident that the pattern of the afferent lymphatic supply is less complex and variable than that of the efferent lymphatic drainage. Moreover, there are two types of pathways in the afferent lymphatic supply: most of the afferent lymph vessels enter the nodes at the depressions of the A-type hilus and penetrate deeply into the nodes. The others enter the convex surfaces of the nodes, where the terminal afferent lymph vessels are divided from the larger afferents. These results were also reported by SPALDING & HEATH (1987) in the superficial inguinal lymph nodes in pig. This feature of vascularity in the lymph supply may be related to the unusual arrangement of the parenchyma

in the pig nodes, and account for the fact that some CT are located at the periphery of the node.

The results of the present study also show that there are two types of pathways in the efferent lymph drainage as well as those in the afferent supply : one leaves from the shallow depression of the E-type hilus by several large efferent lymphatic vessels which are formed by the confluence of many small lymph vessels, and many other minute lymph vessels arise from the surface area in the peri-hilar (A-type) region, where the afferent lymph vessels are encompassed by the efferent lymph vessels. The former type has an appearance common to the efferent drainage found in other mammalian species. However, it should be considered that the lymph nodes of pigs are composed of crowded nodular units, which are morphological units in the lymph nodes of pigs. In this cases, although it appears that there are lymph sinuses in the medulla-like tissue in histological preparations, they are efferent lymph vessels because many minute efferent lymph vessels are at the E-type hilus of the individual nodular units, and the sub-capsular lymph sinus follows these vessels.

Previous reports on the lymphatic valves of the lymph vessels in sheep,<sup>7)</sup> dogs<sup>10)</sup> and pigs<sup>13)</sup> studied using a casting technique were brief and did not refer to their numbers, locations and structures. In this paper, a number of well-developed lymphatic valves at intervals of 0.8–1.2 mm were revealed in the lymph vessels of the pig lymph nodes, and this result may signify the function of numerous lymphatic valves as an apparatus for the avoidance of lymph refluxing.

Although the MT in pig lymph node is considered to function like a connective tissue which impedes the passage of cellular elements and particle substances, and is a relatively impermeable area in the pig nodes, from our previous studies with colloidal carbon treatment<sup>8)</sup> and of ultrastructures of the MT and the lymph sinuses,<sup>9)</sup> and a study of the recirculation of lymphocytes in pigs,<sup>3,11)</sup> it became more clear that injected casting materials such as Mercocox in the present study or Microfil<sup>13)</sup> were permitted to pass through the MT very freely. SPALDING & HEATH (1987) concluded that these pathways were lymph sinuses in the diffuse tissue, contrary to our view that the lymph pathways are situated in the intercellular spaces of the MT, because no sinus endothelial cells are distinguished in the MT of pig lymph nodes even in an ultrastructural analysis.<sup>9)</sup>

It is known that there are no lymph sinuses within the parenchyma of the lymph nodes in pig and other mammalian species. Nevertheless, the CT was injected as well with the casting substance in this study as in the cortexes of humans,<sup>6)</sup> sheep<sup>7)</sup> and dogs.<sup>10)</sup> This suggests that the lymph node parenchyma in pigs and in other mammals are commonly saturated with lymph flow freely without lymph sinuses. However, there is a difference in the casting pattern of the parenchyma of the CT and MT in pig lymph nodes. This feature is considered to reflect the histological difference between the CT and MT of the lymph node in pigs.<sup>8,9)</sup>

## ACKNOWLEDGEMENTS

This work was supported in part by a Grant-in-Aid for Scientific Research (No. 61560330) from the Ministry of Education, Science and Culture, Japan.

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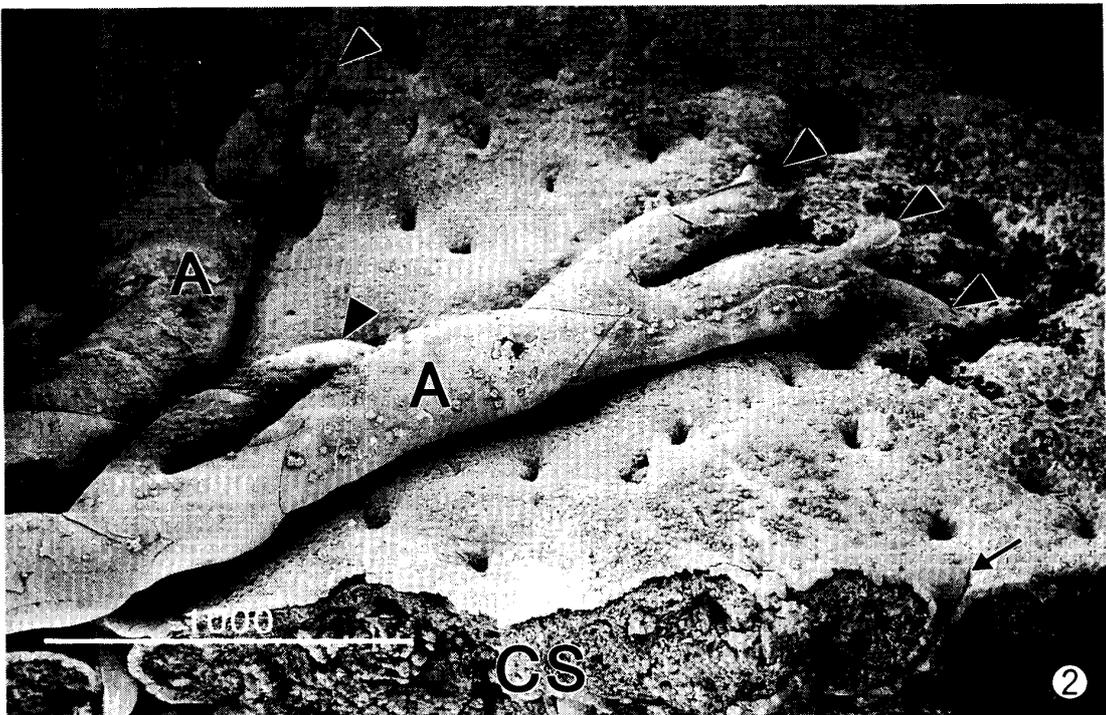
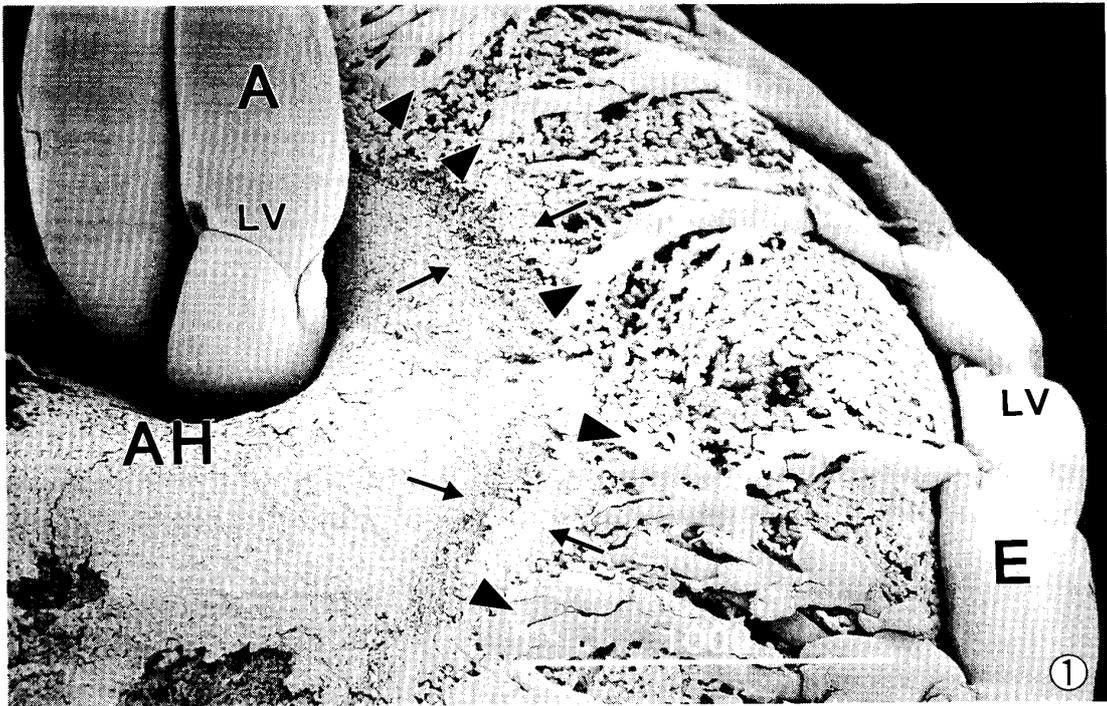
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## EXPLANATION OF FIGURES

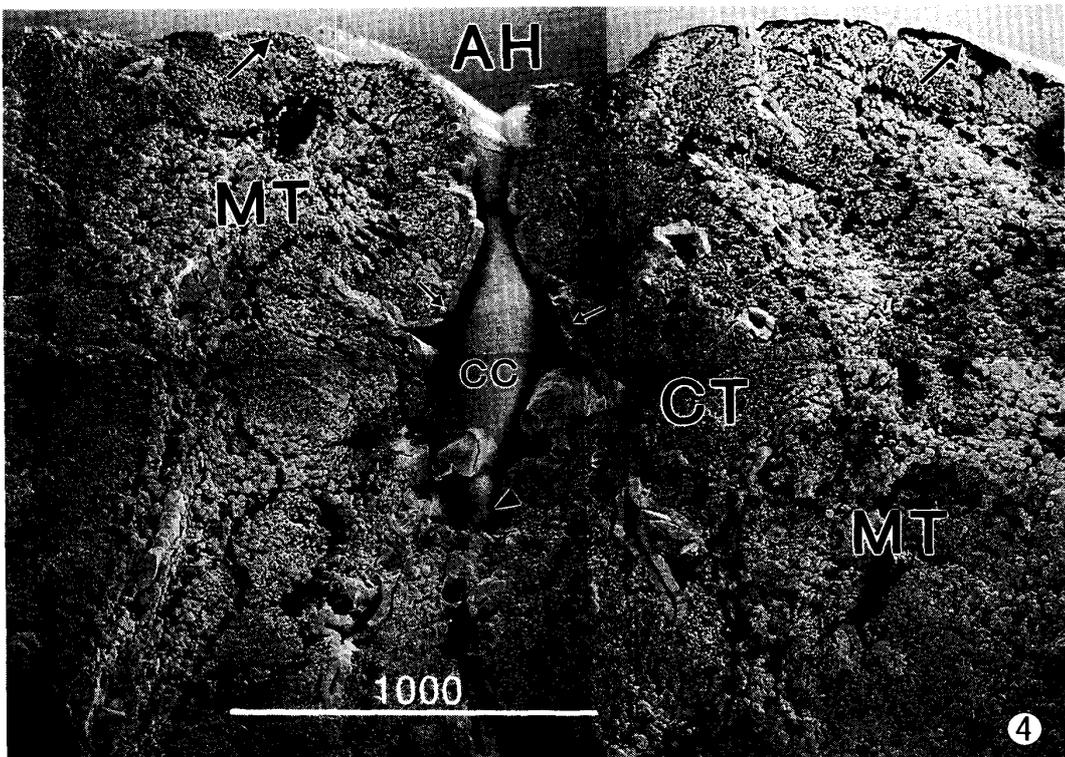
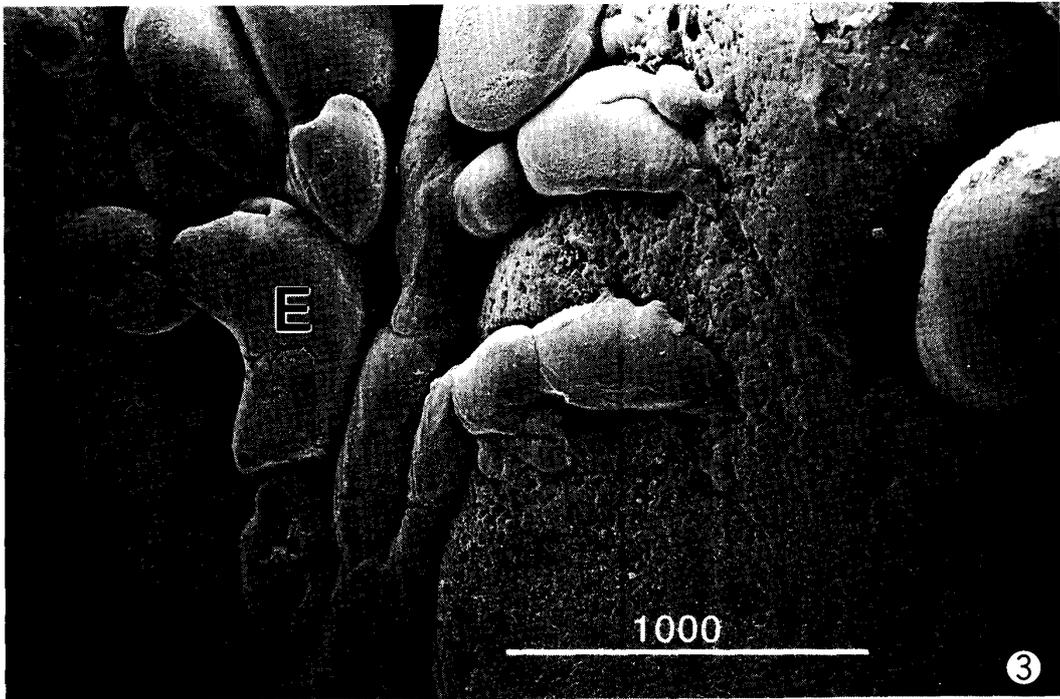
## PLATE I

- Fig. 1 A cast showing the surface architecture of lymph pathways at the A-type hilus (AH). Two afferent lymph vessels (A) enter the lymph node at the depression of the A-type hilus, and an efferent lymph vessel (E) emerges from the surface area. The efferent lymph vessels are encompassed by numerous minute initial lymph vessels (arrowheads). Lymphatic valves (LV) are located in the afferent and efferent lymphatic vessels. Note the intermittent structure which surrounds the afferent lymph vessels (between arrows). Sacral lymph node. Bar in  $\mu$
- Fig. 2 Casts of branches (arrowheads) from the afferent lymph vessels (A). They enter the lymph node at the convex surface. Note the numerous small holes penetrating the subcapsular lymph sinus, and the direct communication between the sub-capsular lymph sinus and the peritrabecular lymph sinus (arrow). CS : a cut surface of the cast. Superficial popliteal lymph node. Bar in  $\mu$



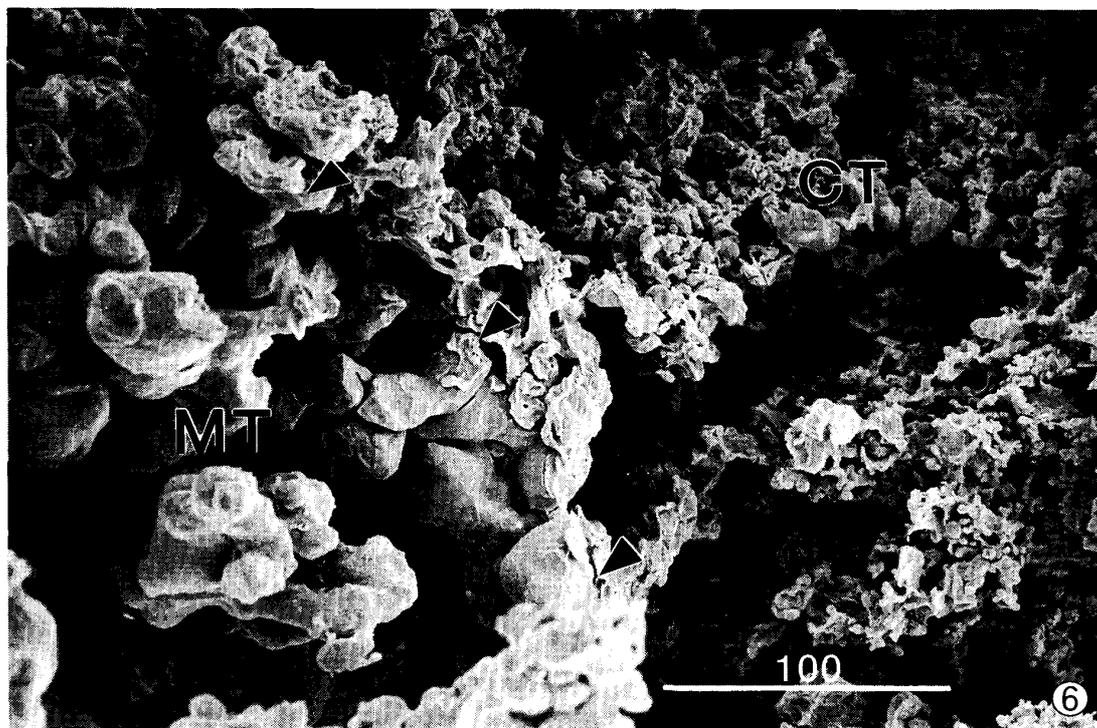
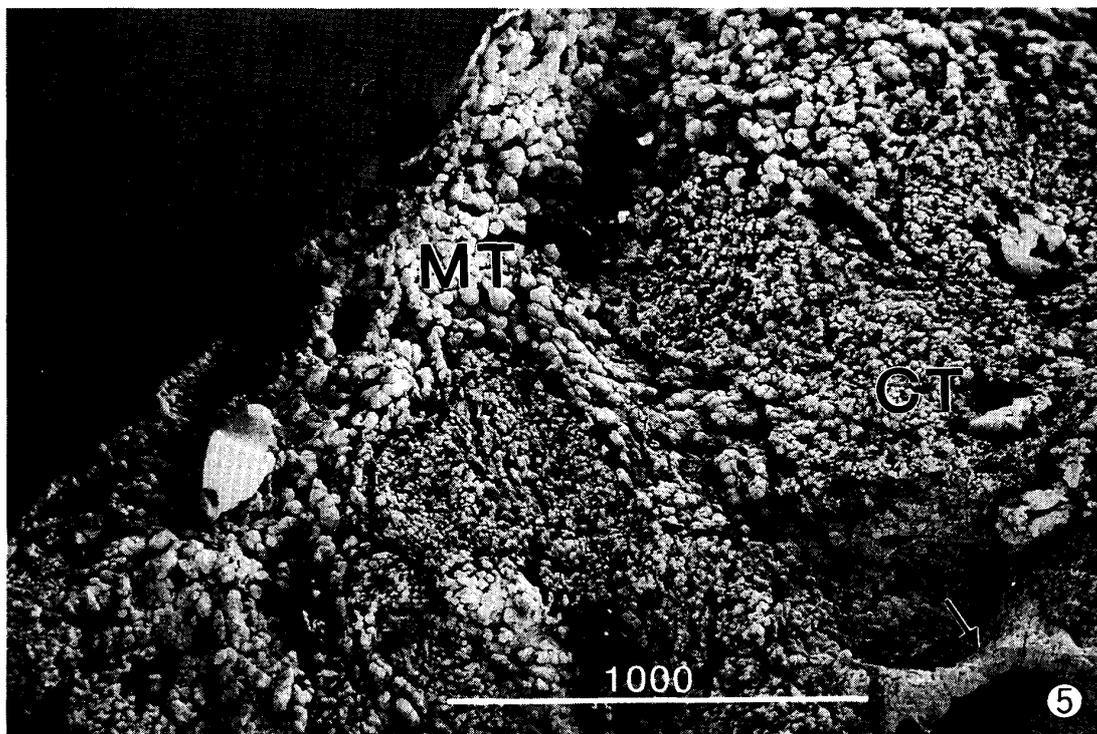
## PLATE II

- Fig. 3 A cast showing the efferent lymph vessels (E) at the shallow depression of an E-type hilus. Note the numerous small lymph vessels forming larger lymph vessels by confluences. Medial iliac lymph node. Bar in  $\mu$
- Fig. 4 A low-powered view of the intranodal lymphatic microvasculature of nodular units. Broken lines indicate the demarcation of the cortex-like tissue (CT) and the medulla-like tissue (MT). The central cisterna (CC) is followed by an intra-trabecular lymph channel (arrowhead). The peri-trabecular lymph sinus (small arrows) and sub-capsular lymph sinus (large arrows) are distinguished well. AH: A-type hilus. Medial iliac lymph node. Bar in  $\mu$



## PLATE III

- Fig. 5 A cut surface of the intranodal lymphatic microvasculature of a medial iliac lymph node. The broken line indicates the demarcation between the cortex- and the medulla-like tissue (CT and MT). The peri-trabecular lymph sinus is seen (arrow). Bar in  $\mu$
- Fig. 6 A higher magnification of a lymphatic cast of a boundary area between the cortex- and the medulla-like tissue (CT and MT). Note the corrosion cast communication between the CT and MT (arrowheads). Medial iliac lymph node. Bar in  $\mu$



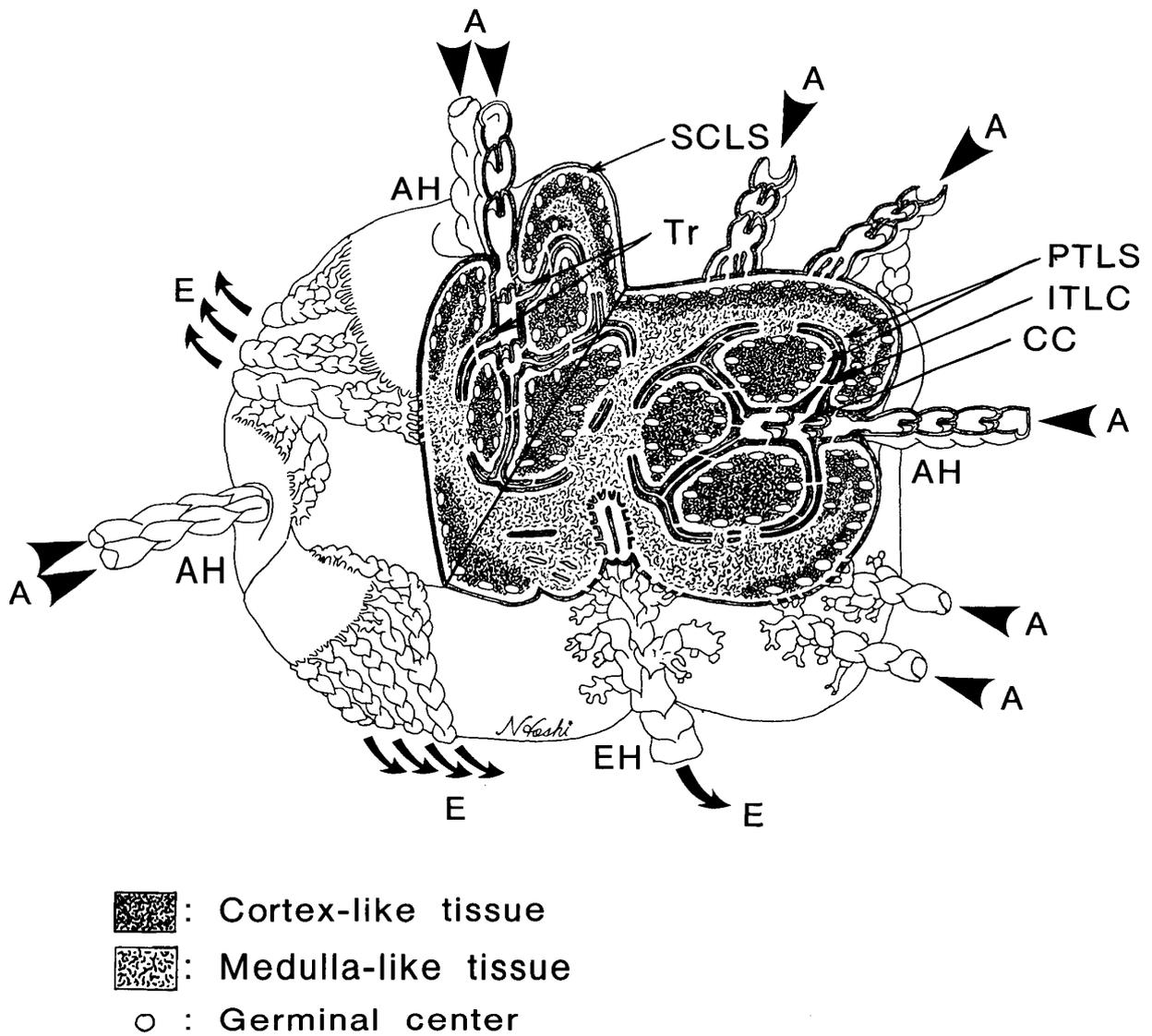


Fig. 7 A diagrammatic representation of the extra- and intranodal lymphatic pathways of the pig lymph nodes. A : afferent lymph vessel, AH : A-type hilus, CC : central cisterna, E : efferent lymph vessel, EH : E-type hilus, ITLC : intra-trabecular lymph channel, PTLS : peri-trabecular lymph sinus, SCLS : sub-capsular lymph sinus, Tr : trabecula.