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Author(s)	HOSHI, Nobuhiko; HASHIMOTO, Yoshiharu; KITAGAWA, Hiroshi; KON, Yasuhiro; KUDO, Norio
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## BLOOD SUPPLY AND MICROVASCULATURE OF THE LYMPH NODES IN PIGS

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

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The blood supply and intranodal microvasculature in pig lymph nodes were studied by using a casting method and angiography.

Arterial supply: In mandibular, subiliac, superficial inguinal and superficial popliteal lymph nodes, each *nodular unit* received nodal arteries mainly at the *A-type hilus*, and to some degree at the *E-type hilus*. The jejunal lymph nodes were supplied by numerous nodal arteries from networks of jejunal arteries. Each nodal artery running into the jejunal node was divided into 3 to 5 small branches. One of them penetrated into each *nodular unit* at the *A-type hilus*. The others usually branched extensively over the nodal surface, and were often distributed and wrapped around a part or all of the node. They then penetrated into the *nodular units* at many peripheral convex surfaces and at the *A-* or *E-type hilus*.

Intranodal microcirculation of the jejunal lymph nodes: A nodal artery penetrated deeply into each *nodular unit* at the *A-type hilus*. Subsequently it was divided into many branches and ran into the trabecular trees. These smaller arteries became arterioles and capillaries in the cortex-like tissue (CT), and were distributed in the area of the CT and medulla-like tissue (MT). As the arteries entering via the *E-type hilus* branched into many smaller arteries within the MT, they extensively supplied the CT in the peripheral areas opposite the *E-type hilus*. The arteries entered from the peripheral convex surfaces supplied the CT, which occupied a conventional superficial position just underneath the capsule, and they formed capillary networks. Postcapillary venules (PCV) formed basket-like plexuses around germinal centers. These PCV converged into several large branches in the areas of the cortico-medullary junctions, and left the node through the *A-*, or *E-type hilus*.

Key words: arterial supply, lymph node, microvasculature, pig, scanning electron microscopy.

## INTRODUCTION

In conventional lymph nodes, circulating blood plays a role in immune responses as well as providing for metabolic needs.<sup>9,10)</sup> The traditional view of mammalian lymph nodes, except for those of pigs, is that blood enters the lymph nodes through one or more arteries at the hilus, a single indentation in the medulla.<sup>3)</sup> This view has been supported by observations in dogs,<sup>14)</sup> guinea-pigs,<sup>6)</sup> rabbits<sup>15)</sup> and rats.<sup>2)</sup>

Although histological studies have revealed that pig lymph nodes have a peculiar structure in comparison with those of other mammalian species,<sup>17,18)</sup> little information is available on the arterial supply or the microvasculature within the nodes.

Previous studies established that the vascular pattern in lymph nodes closely depended on the histological organization of the nodal parenchyma.<sup>24)</sup> Therefore, clarification of the vascular architecture in pig lymph nodes is also important to elucidate the peculiar histological architecture in this node.

The microvasculature has been studied by various methods in various species. Formerly, numerous histological investigations were carried out by using the injection of various dyes into the blood circulatory system; however, there were few studies on the tridimensional structure of vascular architecture.

Recently, using a casting method combined with scanning electron microscopy<sup>22)</sup> as well as the application of angiography<sup>14,15,24)</sup> and a Latex cast,<sup>10,11,25)</sup> morphological analyses of the minute blood circulations in lymph nodes were performed.

The aim of this study is to clarify the blood supply and intranodal microvasculature in pig lymph nodes by using the casting method and angiography.

## MATERIALS AND METHODS

*Animals*: A total of 40 Large White × Landrace pigs were used in this study. Eleven 4- to 6-week-old pigs were injected with Neoprene Latex and 29 6-month-old pigs obtained from a local slaughterhouse were used for angiography and injection replica scanning electron microscopy. Surgical procedures were performed under anesthesia with sodium pentobarbital (Somnopenyl, Pitman-Moore, Inc., Washington, USA).

*Angiography*: After an irrigation with physiological saline to flush out the blood through a pliable plastic cannula (internal diameter 2.0 mm) tied into the proximal end of the jejunal artery, injection of a barium sulphate suspension as a contrast medium (Ryubari-sol C or S, Maruishi Pharmaceutical Co., Ltd., Osaka, Japan) was performed by means of a 10 ml syringe with manual pressure. To prevent leakage after the injection, the proximal end of the artery was ligated after removal of the cannula. The jejunal lymph nodes were thereafter fixed in 4% buffered formaldehyde for 2 days before radiography. Some nodes were then cut into 2- to 10-mm-thick slices. The nodes and slices were radiographed on a soft X-ray photographic apparatus (Softex C-SM, Softex Co., Ltd., Tokyo, Japan).

*Preparation of vascular casts and corrosion casting:* Two kinds of injectional materials for vascular casts were used. One was synthetic latex (Neoprene Latex 601-A, Kyoritsu-Kasei, Co., Ltd., Tokyo, Japan) which was used diluted with an equal amount of water for the investigation of arterial supply. Animals were anesthetized and exsanguinated through a carotid cannula. The cannula was then inserted into the abdominal aorta. Subsequently, physiological saline was injected to wash out the blood through the cannulae. After this irrigation, the Neoprene was injected through the same cannulae under moderate pressure. Each pig (carcass) was placed in a cold room for at least 48 hr to allow the casts to settle, then mandibular, subiliac, superficial inguinal, superficial popliteal and jejunal lymph nodes and their associated vessels were removed. They were dissected and the adipose and connective tissues were removed and photographed stereo microscopically.

Mercox, the other casting resin mixture is a low-viscosity methacrylate resin (Japan Vilene Co., Ltd., Tokyo, Japan). In most preparations Mercox was diluted with methyl methacrylate (20.0 g Mercox : 5.0 g methyl methacrylate) before the addition of 0.5 g of catalyst. The injection method followed the same procedure as was used for the angiography. The procedure and methods of corrosion casting are described in detail elsewhere.<sup>22)</sup>

*Scanning electron microscope (SEM) observation of microvascular casts:* The vascular corrosion casts of jejunal lymph nodes were dried in air and trimmed with a sharpened needle and forceps under a stereo-light microscope to expose the required parts. To observe the cut surface of the casts, some of the corrosion casts were frozen in distilled water and cut with a razor blade. The casts thus obtained were washed in distilled water and by an ultrasonic generator (28 KHz for 10 min). They were then air dried, coated with gold and observed by SEM (JSM-T200, JEOL, Ltd., Japan) at an acceleration voltage of 10 kv.

## RESULTS

An almost intact arterial system was obtained in both angiography and vascular cast specimens. Casts of Neoprene Latex revealed the patterns of arterial supply. These patterns and intranodal diverging patterns were clarified in radiographs after the injection of Ryubari-sol suspension.

These results on arterial supply in the pig lymph nodes are diagrammatically summarized in Fig. 1.

### 1. Arterial supply of the lymph nodes

Small variations in the arterial supply existed depending on the location of the lymph nodes; however, there was a marked difference between the superficially located lymph nodes (mandibular, superficial inguinal, subiliac, and superficial popliteal lymph nodes) and visceral lymph nodes (jejunal lymph nodes).

*Artery supplying the mandibular lymph nodes* (Fig. 1-A): The mandibular lymph

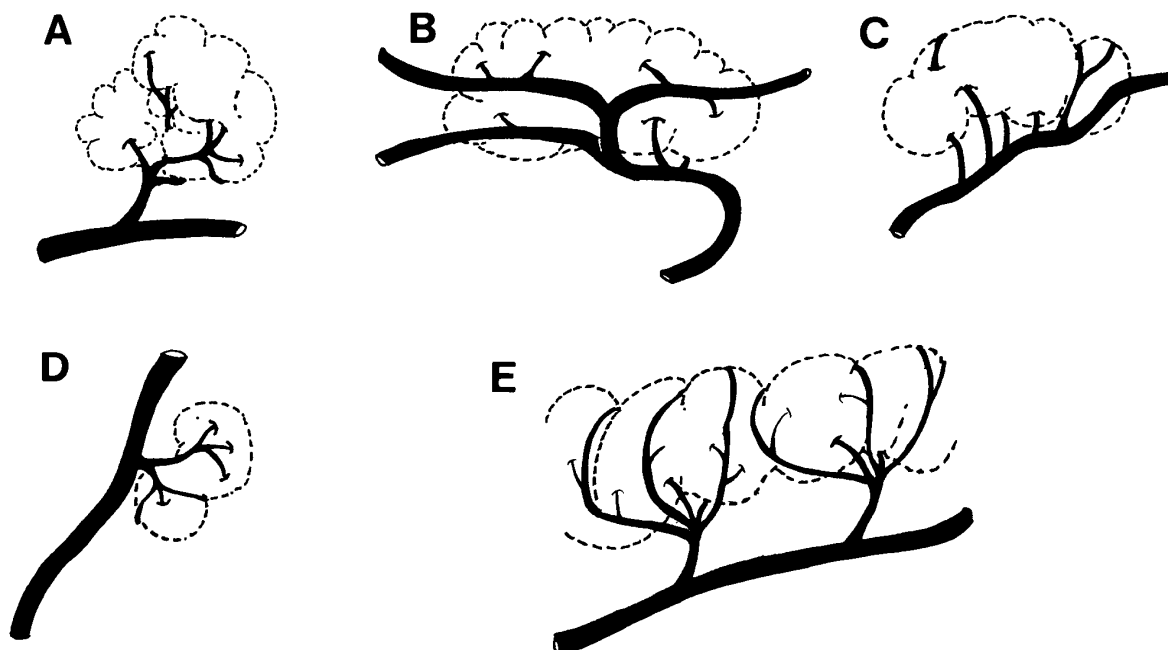


Fig. 1 A schematic drawing of the arterial supply in pig lymph nodes.

A) mandibular lymph node, B) superficial inguinal lymph node, C) subiliac lymph node, D) superficial popliteal lymph node, E) jejunal lymph node

nodes were located at the caudoventral border of the mandible on the lateral side of the sternohyoideus muscle. Usually the lymph nodes were located ventral to the linguofacial vein, but often they extended to the dorsomedial side of the vein.

These mandibular lymph nodes generally consisted of 3 large segmental structures named the "nodular unit",<sup>17)</sup> which received lymph vessels independently.

The nodes were supplied by a single nodal artery which received blood from branches of the facial artery. As the nodal artery approached the mandibular lymph nodes, it was divided into 3 or more smaller arteries. They usually penetrated into each nodular unit at a hilar depression named the "A-type hilus", but some did so at the "E-type hilus".<sup>17)</sup>

*Arteries supplying the superficial inguinal lymph nodes* (Fig. 1-B): The superficial inguinal lymph nodes consisted of 10 or more nodular units, and appeared to have been formed by the fusion of small nodes. However, the blood supply to these nodes was not completely segmented.

The nodal arteries to these lymph nodes arose either directly from the external pudendal artery or from its three branches; the medial cranial, lateral cranial, and

caudal branches. These arteries penetrated directly into the lymphoid parenchyma through the trabeculae at the A-type hilus, and to some degree at the E-type hilus.

*Arteries supplying the subiliac lymph nodes* (Fig. 1-C): The subiliac lymph nodes were situated cranial to the tensor fasciae latae muscle embedded in subcutaneous adipose tissue along the ventral branches of the deep circumflex iliac vessels.

The nodal arteries supplying this node were divided into 3 to 5 branches directly from the deep circumflex iliac artery, and penetrated into each nodular unit at the A-type hilus, and to some degree at the E-type hilus.

*Arteries supplying the superficial popliteal lymph nodes* (Fig. 1-D): The superficial popliteal lymph nodes consisted of one or two nodular units located on the dorsocaudal surface of the gastrocnemius muscle, in a groove between the biceps femoris and the semitendinous muscles.

In these nodes the two nodal arteries typically branched off from the medial circumflex femoral artery. Each nodal artery penetrated deeply into the nodes at the A-type hilus. Some of its branches entered the nodes from the convex surfaces of the nodes, and then penetrated the area of the CT<sup>17)</sup> that occupied a superficial position.

*Arteries supplying the jejunal lymph nodes* (Fig. 1-E): The jejunal lymph nodes were composed of aggregations of nodes in the mesentery of the jejunum. These nodes appeared to fuse to varying extents and formed two convoluted chains, one on either side of the mesentery.

There were three patterns of arterial supply in the pig jejunal lymph nodes: via the A-type hilus, via the E-type hilus and via the peripheral convex surfaces of the node. These nodes were supplied by numerous nodal arteries which received blood from networks of jejunal arteries. As each of them approached the jejunal nodes, it gave off 3 to 5 smaller arteries. One of them penetrated into each nodular unit at the A-type hilus. The others usually branched off extensively over the node surface, and were often distributed and wrapped around a part or all of the node. Such arteries penetrated into the nodular unit on the peripheral convex surfaces or at the A- or E-type hilus.

## 2. Intranodal microcirculation of the jejunal lymph nodes

In the case of the arterial supply via the A-type hilus, a nodal artery penetrated deeply into each nodular unit. Subsequently it was divided into many branches and ran into the trabecular trees. The arterioles and capillaries from the smaller arteries then distributed in the area of the CT and MT<sup>17)</sup> (Figs. 2-5). The arterioles ran parallel to venules, and anastomosis between them was observed in the area of the CT-MT junctions (Figs. 4, 5).

In case of the arterial supply via the E-type hilus, where the nodal arteries went straight into the MT, they gave off many minute vessels within the MT, and extensively supplied the CT in the peripheral areas opposite the E-type hilus (Fig. 2).

In the case of the arterial supply via the peripheral convex surfaces, the arteries arose from the nodal arteries which were distributed and wrapped around the node in a cobweb-like manner (Figs. 2, 6). These arteries entered the CT and formed capillary networks (Figs. 2, 3).

Postcapillary venules (PCV) from the capillaries were distributed in the area of the CT. Some of these PCV surrounded the germinal centers, and communicated with capillaries within them (Fig. 7). The PCV drained into larger venules in the CT-MT junctions, and left the node at the A-type hilus (Figs, 2, 3). The capillary networks in the MT merged consequently into veins, and left the nodes at the E-type hilus (Fig. 2).

#### DISCUSSION

Numerous investigations have been performed on the structure and function of lymph nodes. Recently, studies on microcirculation within the lymph nodes have been carried out actively because of their immunological importance, since the selection of small lymphocytes from the blood into the lymphatic tissues also occurs in the lymph nodes.<sup>9)</sup> As the relation between the immune response and blood circulation in the lymph nodes became clearer, morphological changes of PCV and an increase of lymphocyte traffic via the PCV within the nodes or of the blood flow into the node after antigenic stimulations were pointed out.<sup>1,10,12,13,15,16)</sup>

However, even in given species, man included, there is a marked difference in the pattern of arterial supply between superficial (inguinal and axillary) and deep (mesenteric) lymph nodes. This difference is a reflection of the microanatomical structural differences between superficial and deep lymph nodes.<sup>4,8,15,20,24)</sup>

In the present study, a marked difference was found between the vascular architecture of the superficial and the visceral (jejunal) lymph nodes. It was suggested that even in the lymph nodes of pigs, the pattern of blood circulation reflected their morphological differences.

As a results of this investigation the pattern of blood supply in pig lymph nodes is shown to be different from that in other mammalian lymph nodes; in other mammals, the nodal arteries enter the lymph nodes at the hilus and pass through the medulla to the cortex, whereas the nodal arteries in the pig lymph nodes first entered the CT, except for the nodal artery which entered via the E-type hilus. In addition, the jejunal nodes in pigs showed that there were three additional patterns of arterial supply: via the A-type hilus, via the E-type hilus, and via the peripheral convex surface.

BOUWMAN (1959) reported that the majority of vessels supplying the parenchyma of the pig lymph node branch from arteries within its capsule. However, he also concluded that blood vessels generally penetrate the lymph node where the lymphatics enter and leave it, and that the largest arteries enter where the efferent lymphatics

originate. This conclusion has some similarities with our present view.

On the other hand, SPALDING & HEATH (1986) reported that most of the arteries from surface arterial networks penetrate the capsule directly to supply the lymphoid parenchyma in the pig lymph node. Based on our earlier investigation,<sup>17)</sup> we do not support their observation that the pig lymph node lacks hilus.<sup>7,26)</sup> However, their opinions include the view that the arterial supply in pig lymph nodes is unique in pattern in comparison with that of other mammalian species.

The present results suggest that the pig lymph nodes appear to have a high density of blood vessels, especially PCV in the CT, and many anastomoses between arteries, as well as arteriovenous anastomoses. These anastomoses were observed in rats<sup>2)</sup> and rabbits.<sup>16)</sup> In these small animals, it has been shown that the anastomoses permit regional control of blood flow through the PCV and that flow through them increases during the immune response.<sup>2,16)</sup> It seems likely, therefore, that they play some role in the regulation of the rate of lymphocyte recirculation. It is not clear, however, whether this occurs in pigs, as differences in the pattern of lymphocyte migration within the lymph nodes occur between species.<sup>5,21,23)</sup>

From the point of view of the phylogeny of the lymphatic system from amphibia to man, SUGIMURA (1976) indicated that there is a fixed tendency toward differentiation, a development and increase of the lymph node according to the development of the lymph vessel system.

In addition, KAMPMEIER (1969) reported that in the taxonomic spectrum in mammalian species, the Suidae, from the point of view of the lymphatic system, is nearer the perissodactyls than to any family of artiodactyls. Considering, for example, the number of lymph nodes in the individual regional stations, the pig stands midway between the horse and the cow.

Considering the views mentioned above and those hitherto reported,<sup>17,18)</sup> as other mammalian lymph nodes continue to occur with the development of lymph vessels, they are differentiated independently to communicate with the lymph vessels. On the other hand, it is considered that in the pig lymph nodes immature lymphoid tissue fuses in a relatively early stage of genesis. Thereafter, as they communicate to the lymph and blood vessels, a numerical reduction of lymph nodes in pigs is brought about by the coalescence of contiguous nodes within separate regional groups, thus producing large and lobulated nodes. There are indications that adjacent lymph nodes can continue to fuse together after birth in the pig lymph node;<sup>7)</sup> however, this process is not clear before birth.

Further studies on ontogenical morphology are needed to elucidate the intranodal architecture in pig lymph nodes.

#### ACKNOWLEDGEMENTS

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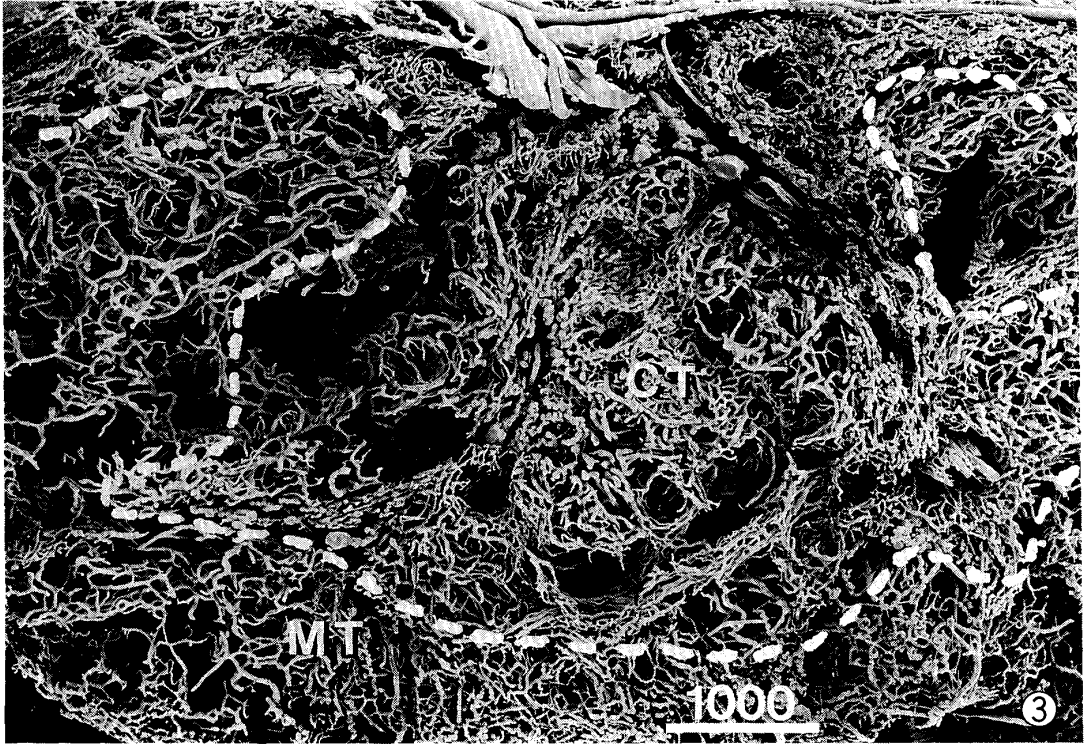
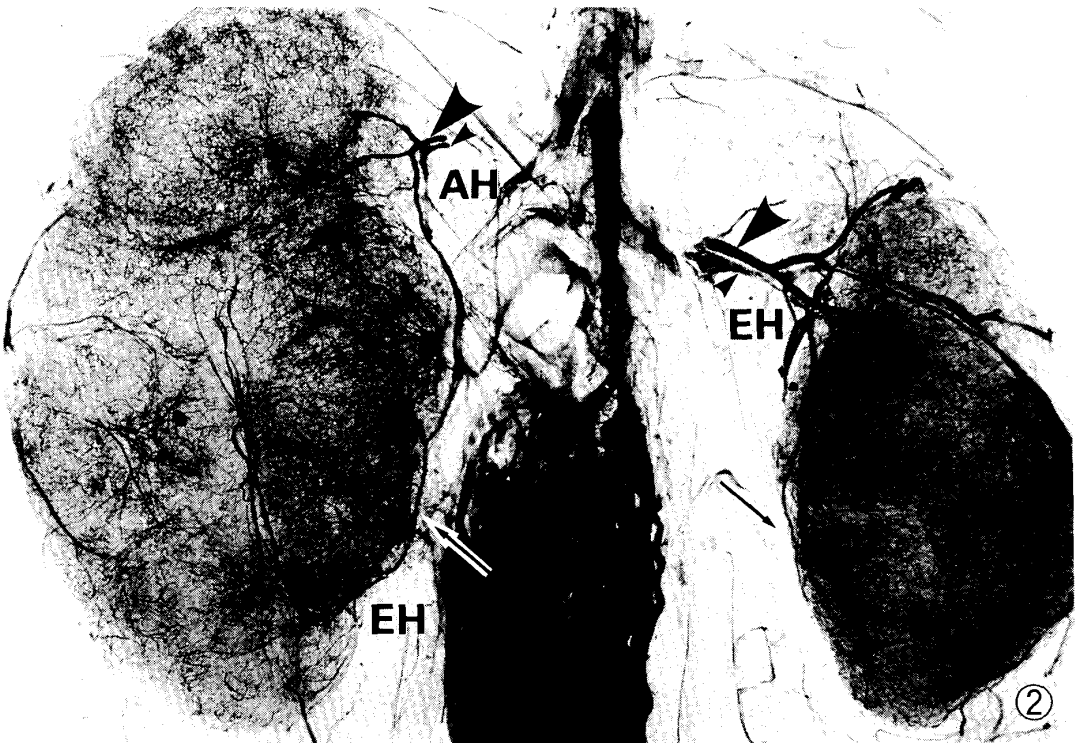
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## EXPLANATION OF FIGURES

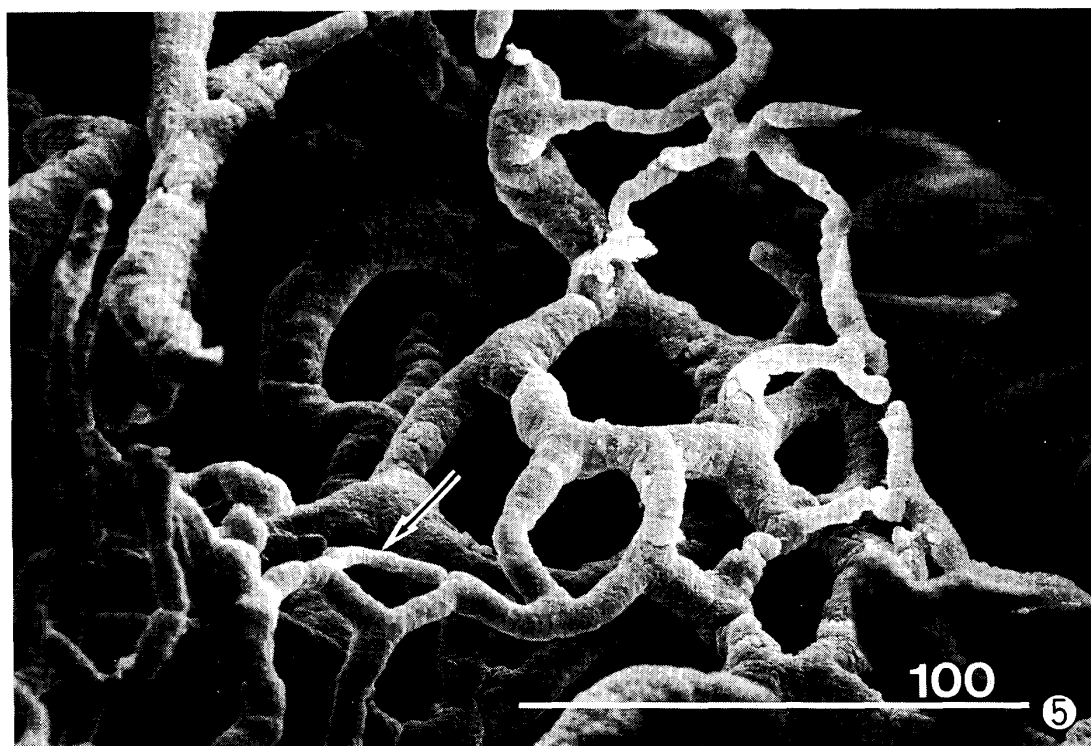
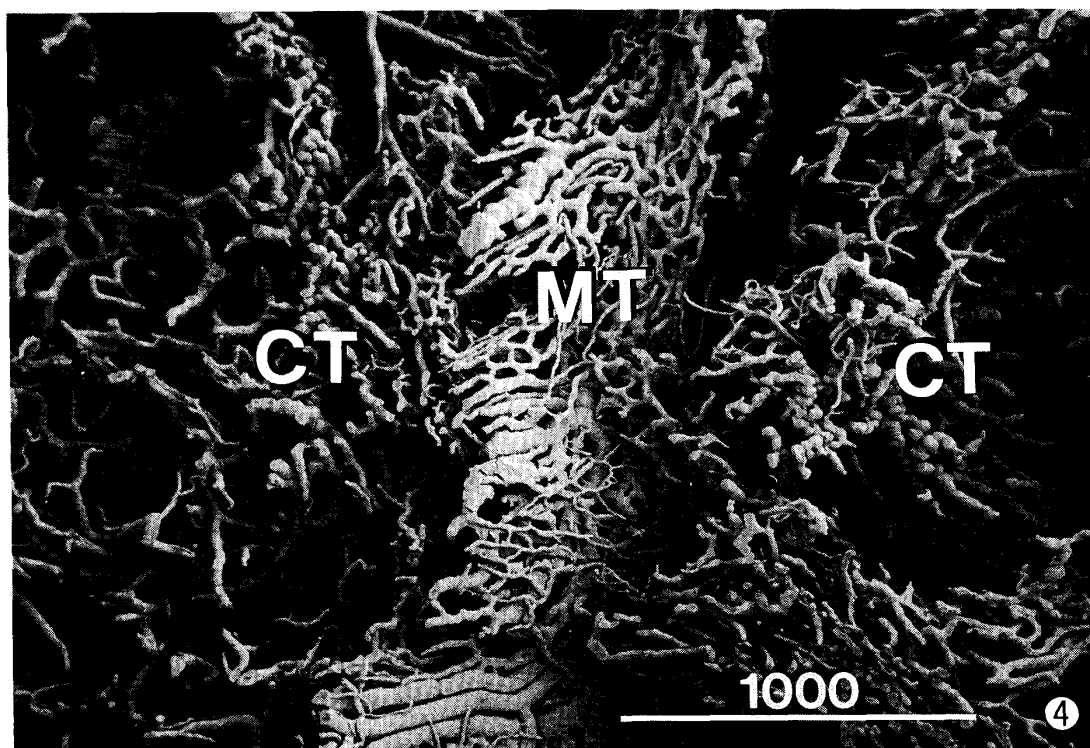
## PLATE I

- Fig. 2 Angiography of a sliced specimen with barium injection. Nodal arteries (large arrowheads) divided from jejunal artery run parallel to venules (small arrowheads). Arrows show that the nodal arteries penetrate into the node via the peripheral convex surface.
- AH: A-type hilus, EH: E-type hilus, Jejunal lymph node  $\times 5$
- Fig. 3 A vascular cast of the whole aspect of a nodular unit in the jejunal lymph node (cut surface). The upper part shows the A-type hilus. CT: cortex-like tissue, MT: medulla-like tissue. Bar in  $\mu$



## PLATE II

- Fig. 4 A vascular cast of the CT (cortex-like tissue)-MT (medulla-like tissue) junctional area in jejunal lymph node (cut surface). Arterioles run parallel to venules and dense microvasculatures are seen in both areas. Bar in  $\mu$
- Fig. 5 A vascular cast of the CT in jejunal lymph node (cut surface). Many anastomoses between arterioles and arteriovenous communication (arrow) are shown. Bar in  $\mu$



## PLATE II

Fig. 6 A vascular cast of the surface view in the jejunal lymph node.  
Nodal artery (A) runs parallel to nodal vein (V).

Note the arteries penetrating into the node at the peripheral convex surfaces (arrowheads) and the capillaries converging into veins (arrows) Bar in  $\mu$

Fig. 7 A vascular cast of the CT (cut surface).

Note the postcapillary venules (arrows) surrounding the germinal centers (GC). They communicate with the capillaries within the GC. Bar in  $\mu$

