Three-Dimensional Organization of Lymphatics and their Relationship to Blood Vessels in Rabbit Small Intestine. A Scanning Electron Microscopic Study of Corrosion Casts*

Osamu OHTANI and Aiji OHTSUKA

Department of Anatomy (Prof. T. MURAKAMI), Okayama University Medical School, Okayama, Japan

Received February 9, 1985

Summary. Casts of small intestinal blood vessels and lymphatics in the rabbit were made with methacrylate and observed under a scanning electron microscope (SEM). In casting the lymphatics, a specially diluted low viscosity medium was injected intraparenchymally into the intestinal submucosa. This parenchymal injection allowed good reproduction of fine lymphatics, including the initial lacteals in the villi. The central lacteals were completely surrounded externally by the subepithelial blood capillary networks of the intestinal villi. Individual villi in the lower intestine contained only one central lacteal that drained through a thin lymphatic in the glandular layer into the submucosal lymphatic plexus. Villi in the upper small intestine were broader than those in the lower small intestine, and contained two to five lacteals. They anastomosed with each other at the villous base and formed a markedly expanded sinus.

The cast submucosal lymphatics frequently showed constrictions strongly suggestive of valves. It was constantly observed that well-developed lymphatics in the submucosa ran in pairs and held an arteriole or artery between them. This close association of the lymphatics with arteries suggests that arterial vasomotion might provide an important hydrodynamic energy source for lymph formation and transport.

The study of the lymphatic system began in the 17th century with the discovery of the lacteal by ASELLI (1581-1626) (RÉNYI-VÁMOS, 1960; MAYERSON, 1963; LEEDS, 1977). Since then a variety of methods such as the parenchymal injection of India ink, the vascular injection of silver nitrate and the intra-arterial injection of India ink plus silver nitrate (MORI, 1969) have been used to demonstrate the structure of the lymphatics in various organs, including the small intestine. However, only a two-dimensional organization of the lymphatics was able to be demonstrated, because almost all of the above methods depended on either light microscopy (LM) or transmission electron microscopy (TEM), both with limited depth of field. As in the vascular corrosion casting/SEM technique (MURAKAMI, 1971) which greatly increased our knowledge about microvascular organization, the lymphatic corrosion casting/SEM technique yields a clearer definition of the lymphatic architecture than previously employed LM or TEM methods in certain

*Part of this study was presented at the 13th Medical SEM Symposium (Nov. 8-10, 1984, Karatsu, Japan).
organs such as lymph nodes (Irino et al., 1974; Kurokawa and Ogata, 1980) and the thyroid gland (Kobayashi et al., 1976). If the lymphatic corrosion casting/SEM technique can also be applied to other organs such as the small intestine—which possess many lymphatic capillaries starting with blind ends, our knowledge about the three-dimensional organization of the lymphatic system will be greatly broadened.

Thus, in this study the authors have elaborated on the lymphatic corrosion casting/SEM technique, and applied it toward the investigation of the lymphatic system of the small intestine. Furthermore, simultaneous casting of the lymphatic and blood vascular system was carried out to elucidate the relationship between them. SEM of intact tissues and LM of sections and India ink injected/cleared tissues were also conducted to investigate the relationship of the lymphatics to the surrounding tissue elements, and to reveal the luminal surface morphology of the lymphatics, particularly of the central lacteal.

MATERIALS AND METHODS

Ten rabbits (2.5-4.0 kg body weight) of both sexes were used. They were fed with combined solid food for experimental animals (ARC4, Oriental East, Tokyo) with free access to water. All the animals were anesthetized with an intravenous injection of pentobarbital sodium (Nembutal; 50 mg/100 g B.W; Abbot Labo., North Chicago) at the beginning of the experiments.

SEM of lymphatic corrosion casts

Six rabbits were used for the lymphatic corrosion casting of the small intestine. The abdomen was cut open and the small intestine exposed. About 0.2-0.5 ml of methacrylate injection medium (Mercox, CL-2B or CL-2R, Japan Vilene Hospital, Tokyo) diluted to 40-50% (v/v) with methyl methacrylate monomer not containing hydroquinone (Oken Shyoji, Tokyo) to give a viscosity of 1.5-2.0 centistokes (cs; measured with a modified Ostwald viscometer at 20°C) was injected in and around the large lymphatics in the submucosa. Shortly before injection, a curing agent (MA, Japan Vilene Hospital, Tokyo) was added to the injection medium to give a concentration of 1% (w/v). One to 6 ml syringes with 23 gauge needles (Terumo, Japan Medical Supply, Hiroshima) were used. The injection pressure was not monitored by any special device, but was controlled with hand pressure while observing the injected medium spreading into the lymphatics. The injected parts of the small intestine were removed and placed in a hot water bath (60°C) for more than 2 hrs. They were put in concentrated NaOH (15-20%) at about 60°C, until tissue elements were completely corroded away (usually for 2 hrs or more). The lymphatic corrosion casts thus obtained were washed and then frozen in water and cut into appropriate blocks, and air-dried. The blocks of the lymphatic casts were mounted on the specimen-holders with silver paste (Dotite Type D-550; Fujikura Kasei, Sano, Japan), coated with gold in an ion coater (Eiko IB-3, Eiko Engineering, Tokyo) and observed under a SEM (JSM-U3, JEOL, Tokyo) with an accelerating voltage of 5 kV. Stereo-pairs of micrographs were frequently taken with a tilt separation of 4-6°.

SEM of lymphatic/blood vascular corrosion casts

Two rabbits were given the simultaneous injection into the lymphatic and blood vascular system. Under anesthesia, the abdomen was cut open and a cannula was inserted
into the superior mesenteric artery. The inferior vena cava was cut for outflow. The blood vascular bed was perfused with Ringer solution or physiological saline through the cannula. Approximately 0.2 ml of Mercox was injected intraparenchymally, as in the lymphatic corrosion casting/SEM technique, following the injection of Mercox (diluted with methyl methacrylate monomer to give a viscosity of 3-4 cs; OHTANI and MURAKAMI, 1978) into the blood vascular system. The injected small intestine was treated in the same way as in the lymphatic corrosion casting/SEM technique. The parts of the intestine where the Mercox was injected only into the blood vessels were processed for SEM of blood vascular corrosion casts.

**SEM of intact tissue**

One rabbit was perfused with Ringer solution through a cannula inserted into the superior mesenteric artery, followed by perfusion with 2% glutaraldehyde (GA) (Katayama Chemical, Osaka) in 0.1 M phosphate buffer solution (PBS; pH 7.4). The perfusion pressure applied was approximately 100 cmH₂O. During the perfusion with Ringer solution, the inferior vena cava was cut open just above the diaphragm and repeatedly held with forceps to interrupt the outflow for several sec. The luminal surface of the intestine was washed with a jet stream of Ringer solution. The small intestine perfusion-fixed with GA was cut into blocks, and immersed in the same fixative at room temperature for 4 hrs. After washing in PBS for 4 hrs, the blocks were put in 2% tannic acid in 0.1 M PBS at room temperature for 4 hrs (MURAKAMI, 1974). They were again washed in 0.1 M PBS for 4 hrs, and immersed in 1% OsS₄ in 0.1M PBS for 4 hrs. They were then dehydrated in a graded series of ethanol, and fractured in liquid nitrogen with a small chisel. The specimens were dried in a critical point dryer (HCP-2, Hitachi, Ibaragi) using liquid CO₂. They were mounted on the specimen- holders with silver paste, coated with platinum of 30 nm thickness in a SEM coating system (Polaron, Polaron Equipment Ltd., Watford), and observed under a SEM with an accelerating voltage of 15 kV.

**Light microscopy**

Part of the small intestine treated for the SEM of intact tissue was processed for light microscopy. Paraffin sections were stained with hematoxylin and eosin (H-E).

One rabbit was used for the puncture injection of India ink into the lymphatics. Under anesthesia the abdomen was opened and the small intestine was exposed. India ink was injected into the parenchyma in the vicinity of the submucosal lymphatics. The injected tissue was fixed in 10% formaldehyde and dehydrated through a graded series of ethanol. Part of the tissue was processed for paraffin sections, and the remainder was cleared by immersion in methyl salicylate.

**RESULTS**

None of the results obtained in the present study showed any difference between male and female rabbits.

**SEM of lymphatic corrosion casts**

Injection of the resin with a viscosity of 1.5-2.0 cs filled the lymphatics well, including the central lacteals, without filling the blood vascular system (Fig. 1-4).
Fig. 1. A scanning electron micrograph of a lymphatic corrosion cast of the ileum. Numerous rod-like central lacteals (cl) with blind ends are seen draining through thinner lymphatic vessels (approx. 15-30μm) into the submucosal lymphatic plexus (sl). ×135

Fig. 2. Stereo scanning electron micrographs of a lymphatic corrosion cast of the ileum. The central lacteals and their vessels draining into the submucosal plexus are seen threedimensionally. ×75
Fig. 3. The submucosal lymphatic plexus of the ileum. Note that two thick lymphatics run parallel and form a coarse network, each of which is filled with a lymphatic network consisting of smaller vessels. Compare with the light micrograph of India ink injected and cleared tissue (Fig. 14) which gives a less clear image than this SEM picture. \( \times 55 \)

Fig. 4. A higher magnification of Figure 3. Numerous oval or fusiform impressions of the endothelial nuclei (n) are seen on the surface of the lymphatic corrosion cast. \( \times 850 \)

Fig. 5. A scanning electron micrograph of a corrosion cast of a lymphatic in the mesentery. A bicuspid valve is replicated. Imprints of the endothelial nuclei (n) are also seen. \( \times 150 \)
In the lower part of the small intestine the central lacteals were replicated as rod-like structures (70–110 \( \mu m \) in diameters and 400–500 \( \mu m \) in length) (Fig. 1–3). They started with blind ends and, at the level of the villous base, they abruptly reduced their diameters to 15–30 \( \mu m \) and drained into the submucosal lymphatic plexus (Fig. 1, 2). Two to five central lacteals with interconnections were frequently observed in the upper small intestine (see below). In the submucosal layer, thin lymphatics of averaging 50 \( \mu m \) in diameter (D) were interconnected, forming a two-dimensional network which filled in the mesh of the network formed by thick lymphatics averaging 170 \( \mu m \) D (Fig. 1–3). Usually two thick lymphatics ran side by side each other with frequent interconnections and formed a coarse network; the distance between adjacent intersections ranged from 1 to 2 mm (Fig. 3). The mucosal lymphatics were connected with both thin and thick lymphatics in the submucosa. The surface of the lymphatic casts showed oval or fusiform indentations (Fig. 4). The efferent lymphatics which arose

Fig. 6. A scanning electron micrograph of a lymphatic/blood vascular corrosion cast of the jejunum viewed from the luminal side. The central lacteals (yellow) starting with blind ends are clearly seen surrounded by the villous subepithelial capillary networks. \( \times 80 \)
from the thick vessels of the submucosal plexus pierced obliquely through the muscularis externa and connected with the lymphatics in the mesentery. The casts of the lymphatics in the mesentery showed marked constrictions characteristic of bicuspid valves as well as imprints of endothelial nuclei (Fig. 5).

**SEM of lymphatic/blood vascular corrosion casts**

The central lacteals were surrounded by the subepithelial capillary network in the villus (Fig. 6–8). The number and shape of the central lacteals varied, depending upon the shape of villi. In the upper part of the intestine where the villi had a flattened conical shape, each villus usually contained two to five lacteals with interconnections (Fig. 7). Thus, a sinus was formed near the villous base. The sinus of each villus drained into two or three lymphatics that descended through the glandular layer to lead into the submucosal lymphatic plexus (Fig. 7). Towards the lower parts of the intestine, the villi progressively became narrower, and the number of the central

![Fig. 7. A scanning electron micrograph of a lymphatic/blood vascular corrosion cast of the jejunum. In this picture are seen the villous subepithelial capillary networks (c) each of which surrounds one to three central lacteals with interconnections (cl). The lacteals are connected at their bottom with thin lymphatics (arrowheads) that descend to join the submucosal lymphatics (sl). The lymphatic system is colored yellow in this picture. Between the villous capillary networks and the central lacteals is some resin which leaked and formed networks, possibly representing tissue channels. × 60](image-url)
Fig. 8. A scanning electron micrograph of a vascular corrosion cast showing villous microvasculature in the ileum. A villous arteriole (a) connects near the villous tip with the subepithelial capillary network (c) that drains at various levels of the villus into venules (v). Halfway down the villus, a transverse mucosal venule (tv) is seen collecting capillaries of the lower half of the villus as well as those of the upper half. Note that the arteriole (a) terminating in a T-junction below the villous tip gives rise to two marginal vessels (arrows), one of which travels back down the apical margin of the villus, while the other passes up and around the tip. Both of the marginal vessels, giving off branches to the subepithelial capillary network en route, ultimately connect with the villous venules. These marginal vessels seem to represent preferential pathways from the arterial to the venular side. Note also that each villous capillary network is supplied with several thin arterioles (arrowheads) in addition to the main villous arteriole (a). Comparing this picture with Figures 6 and 7, one may easily differentiate the lymphatics from the blood vessels. × 65

Fig. 9. A submucosal view of the lymphatic/blood vascular corrosion cast of the ileum. Capillaries are not replicated in this preparation. Note that two thick lymphatics (sl) running parallel hold a submucosal artery (a) between them. Arrowheads indicate constrictions suggestive of the presence of valves. × 40
lacteals in each villus decreased. In the ileum each villus had only one rod-like central lacteal (see above).

When a high injection pressure was applied, resin leaked out of the central lacteal and formed a close-meshed network between the villous subepithelial capillary network and the central lacteal (Fig. 7).

In the submucosal layer, arterial arcades and their primary branches were held by thick lymphatics running along both sides of the arteries (Fig. 9). These thick lymphatic casts frequently showed marked constrictions suggestive of the presence of valves at these sites (Fig. 9).

SEM of intact tissue

The villi of the lower intestine in the rabbit were flattened finger-like projections. Towards the upper part of the intestine they became broader and took on a flattened conical shape. SEM of a fractured ileum showed lacteals positioned in the very center of the villi (Fig. 10). Each villus in the ileum possessed only one central lacteal. In the broader villi in the upper intestine, two to five central lacteals were observed (see above). The luminal aspect of the central lacteal showed many bulges reflecting underlying endothelial nuclei (Fig. 11). Many lymphocytes existed in the lumen of the central lacteal (Fig. 11). Between the villous epithelium and the lacteals existed many spaces or channels that were neither occupied by tissue elements nor lined with endothelium (Fig. 11).

![Fig. 10 and 11. Scanning electron micrographs of fractured ileum. Fig. 10. The central lacteals (cl) are seen at the center of the transversely fractured villi. ×295. Fig. 11. A longitudinally fractured villus showing the luminal view of the central lacteal (cl); a lymphocyte (l) and endothelial nuclear bulgings (n) are seen. Note the tissue channels (tc) between the epithelium (E), capillary (c) and the central lacteal. ×4,850](image)
Light microscopy

Light microscopy of the small intestine fixed by perfusion with GA also showed expanded central lacteals lined with a thin endothelium (Fig. 12). The observation of

---

**Fig. 12.** A light micrograph of the ileum perfused with GA and stained with H-E. A central lacteal (cl) is clearly seen. ×160

**Fig. 13.** A light micrograph of the ileum injected with India ink into the lymphatics and stained with H-E. Two submucosal lymphatics (sl) and an artery (a) are seen confined in a triangular compartment of the submucosal space. G intestinal gland, M muscularis externa, V villus. ×80

**Fig. 14.** A light micrograph of the ileum injected with India ink and cleared in methyl salicylate. The submucosal lymphatic plexus (sl) can be seen only two dimensionally. ×10
several consecutive sections revealed that the mucosal lymphatics passed straight or slantwise through the muscularis mucosae and emptied into the submucosal lymphatic plexus. Smooth muscle cells oriented perpendicularly to the submucosal plane and loosely associated with the central lacteals were observed in the interstices of the villi. In the submucosa, two lymphatics running closely on either side of an artery were confined in a triangular compartment of the submucosa (Fig. 13). LM of India ink-injected and cleared tissue showed such large lymphatics forming a coarse network, whose every mesh was filled with a dense network of thin lymphatics as observed by the SEM of lymphatic corrosion casts (Fig. 14).

DISCUSSION

The present study using the lymphatic corrosion casting/SEM technique was able to demonstrate the three-dimensional organization of the small intestinal lymphatics, including the central lacteals previously visualized much less clearly by conventional LM and TEM. Furthermore, the combination of the SEM of lymphatic corrosion casts with that of lymphatic/blood vascular corrosion casts allowed the examination of the spatial relationship between the lymphatics and the blood vessels as well as the clear distinction between the two.

As is well known, intraparenchymal injection is a long-established technique for the study of lymphatic organization. HYRTL (1860; cited by RUSNÝÁK et al., 1967) described the usefulness of retrograde injection into the lymphatics. On the other hand, BARTLES (1909; cited by RUSNÝÁK et al., 1967) stressed that intraparenchymally injected substances might fill tissue spaces other than lymphatics where the injected substances met the least resistance, and thus disputed the reliability of the results obtained by the injection method. This view is shared also by many later authors (e.g., RÉNYI-VÁMOS, 1956). The intraparenchymal puncture-injection of resin into small pieces of such organs as the kidney and liver was reported to replicate blood vessels (MURAKAMI, 1976). In the present study, injection into the parenchyma was made in such a way that the tip of the needle entered the lymphatics. Immediately after the tip of the needle appeared to break or tear the wall of the lymphatics, the injected resin was observed flowing rapidly into the lymphatics. The SEM images of our lymphatic corrosion casts show good correlation with those obtained by SEM of fractured tissue. The surface of the lymphatic corrosion casts shows indentations which correspond to the bulges of the endothelial nuclei. Thus, it is evident that our lymphatic corrosion casts produced by partly retrograde and partly orthograde injection represent the actual lymphatic lumen, including the central lacteals which start with blind ends.

Using an intraparenchymal injection, attempts have been made to make corrosion casts of the thyroid lymphatics (KOBAYASHI et al., 1976), the appendix (BOCKMAN, 1983a, b), and the tongue (CASTENHOLZ, 1984) for SEM observation. However, as high-viscosity plastic was used in those studies, the lymphatics were either too expanded or only partly filled. In the present study, Mercox was diluted with methyl methacrylate monomer to give a viscosity of 1.5–2.0 cs, as was done in making vascular corrosion casts in which the viscosity of Mercox was adjusted to 3–4 cs (OHTANI and MURAKAMI, 1978). This adjustment of the injectant viscosity seems to be crucial in filling lymphatics including the initial lymphatics.

The present study has clearly shown that each slender villus in the lower small intestine possesses one central lacteal, while broader villus in the upper intestine has
two to five interconnected lacteals. This fact suggests that the ratio of the volume of
the interstice versus that of the central lacteal would be constant in a given physi-
ological condition, although a detailed analysis has not been done.

The sinus formed by interconnections of two to five central lacteals at the base of
the broad villi in the upper intestine seems to serve as a reservoir for fluid propelled
into the central lacteals.

The present study by means of the lymphatic/blood vascular corrosion casting/
SEM technique has clearly demonstrated that the central lacteals lie deep in the villous
subepithelial capillary network. Such an anatomical relationship between the lymph-
atic and blood capillaries has already been proposed by Mori (1979) based on their
extensive studies in a number of organs. According to Mori, in organs with a free
surface, like the intestine and skin, the lymphatic capillaries always are located deeper
than the blood capillaries.

The close-meshed network formed by the resin which leaked out of the central
lacteals between the subepithelial capillary network and the central lacteals seems to
 correspond to the interstitial spaces without tissue elements observed by the SEM of
fractured tissue. This structure may represent tissue channels that can provide pas-
 sage for fluid and dissolved substances from the capillaries and epithelium to the central
lacteals. These interstitial spaces perhaps correspond to the “tissue channels” demon-
strated by Casley-Smith and Vincent (1978) and to the “extravascular fluid pathway”
described by Kihara (1956).

Noteworthy is that valves exist in the submucosal lymphatic plexus, and that the
submucosal arteries accompanied by lymphatics of larger diameter are confined in a
triangular compartment surrounded by the muscularis mucosae and the muscularis
externa. This anatomical relationship probably has functional importance. Because
of the extreme thinness of the lymphatic wall, the pulsation or fluctuation of the
arteries in response to intravascular pressure could easily deform the lymphatics, and
thereby change the lymphatic vessel volume. Since the normal lymph flow is uni-
directional because of the presence of valves, vasomotion accompanied by the lymph-
atic volume change would force lymph to flow towards the efferent lymphatics. Our prelimi
nary intravital microscopic study showed vasomotion of the arteriolar
vessels in the small intestine in anesthetised rabbits. Thus, blood vascular vasomotion
may represent an important hydrodynamic energy source aiding lymph formation and
transport in the rabbit small intestine as proposed by some authors studying skeletal
muscle (Skalak et al., 1984). However, our preliminary study did not reveal any dis-
tinct compartments confining the lymphatics and arteries in the rat small intestine as
seen in the rabbit (details to be reported elsewhere). Thus, the hypothesis proposed
here may not be extrapolated to other animals like the rat. Other mechanisms such
as the mechanical motion of the organ (Clough and Smaie, 1978) and an osmotic pres-
sure gradient (Casley-Smith, 1977) could also be responsible for lymphatic filling.

The smooth muscle fibers loosely associated with the central lacteal (Papp et al.,
1962) also may facilitate lymph propulsion by contracting the lacteal on its long axis.
Spontaneous contractions of the central lacteal have been suggested (Papp et al., 1962).
On the other hand, the intestinal villus during its elongation may expand the contained
central lacteals with the aid of the lymphatic anchoring filaments (Leak and Burke,
1968; Böck, 1978), and thus create a relative pressure which results in its filling (Allen,
1967). The broad sinus at the bottoms of the central lacteals observed at the base of
the villi in the upper intestine might also serve to create a pressure gradient.
Acknowledgements. The authors are grateful to Dr. Akio Kikuta for his advice. Thanks are also due to Mr. Nobuo Hayashi and Mr. Noboru Kishimoto (Central Research Laboratory of Okayama University Medical School) for their help in scanning electron microscopy.

REFERENCES

Allen, L.: Lymphatics and lymphoid tissue. Ann. Rev. Physiol. 29: 197–224 (1967).
Bock, P.: Histochemical staining of lymphatic anchoring filaments. Histochemistry 58: 343–345 (1978).
Bockman, D. E.: Scanning electron microscopy of dome and cupola development and of lymphatic sinus architecture in rabbit appendix. (Abstract). Anat. Rec. 205: 19A–20A (1983a).
———: Functional histology of appendix. Arch. histol. jap. 46: 271–292 (1983b).
Casley-Smith, J. R.: Lymph and lymphatics. In: (ed. by) G. Kaley and B. M. Altura: Microcirculation. University Park Press, Baltimore, 1977 (Vol. 1, p. 423–502).
Casley-Smith, J. R. and A. H. Vincent: The quantitative morphology of interstitial tissue channels in some tissue of the rat and rabbit. Tiss. Cell 10: 571–584 (1978).
Castenholz, A.: Morphological characteristics of initial lymphatics in the tongue as shown by scanning electron microscopy. Scanning Electron Microscopy/1984/III: 1343–1352 (1984).
Clough, G. and L. H. Smaje: Simultaneous measurement of pressure in the interstitium and the terminal lymphatics of the cat mesentery. J. Physiol. (Lond.) 283: 457–468 (1978).
Irino, S., T. Ono, K. Hiraki and T. Murakami: A study of the lymphatics and lymph nodes by injection replica scanning electron microscope method. (In Japanese). Ketsueki to Myakkan 5: 513–516 (1974).
Kihara, T.: The extravascular lymph system considered as the reticuloendothelial system. (Abstract, in Japanese). Acta. anat. nippon. 31: 116–117 (1956).
Kobayashi, S., H. Osatake and Y. Kashima: Corrosion casts of lymphatics. Arch. histol. jap. 39: 177–181 (1976).
Kurokawa, T. and T. Ogata: A scanning electron microscopic study on the lymphatic microcirculation of the rabbit mesenteric lymph node. A corrosion casts study. Acta anat. 107: 439–466 (1980).
Leak, L. V. and J. F. Burke: Ultrastructural studies on the lymphatic anchoring filaments. J. Cell Biol. 36: 129–149 (1968).
Leeds, S. E.: Three centuries of history of the lymphatic system. Surg. Gynecol. Obstet. 144: 927–934 (1977).
Mayerson, H. S.: The physiologic importance of lymph. In: (ed. by) E. F. Hamilton and P. Dow: Handbook of physiology. Waverly Press, Baltimore, 1963 (p. 1035–1073).
Mori, K.: Identification of lymphatic vessels after intra-arterial injection of dyes and other substances. Microvasc. Res. 1: 268–274 (1969).
———: Morphology of peripheral lymphatic vessels—A review. (In Japanese). Acta anat. nippon. 54: 1–20 (1979).
Murakami, T.: Application of the scanning electron microscope to the study of the fine distribution of the blood vessels. Arch. histol. jap. 32: 445–454 (1971).
———: A revised tannin-osmium method for non-coated scanning electron microscope specimens. Arch. histol. jap. 36: 189–193 (1974).
———: Puncture perfusion of small tissue pieces for scanning electron microscopy. Arch. histol. jap. 39: 99–103 (1976).
Ohtani, O. and T. Murakami: Peribiliary portal system in the rat liver as studied by the injection replica scanning electron microscope method. Scanning Electron Microscopy/1978/II: 241–244 (1978).
Papp, M., P. Röhlich, I. Rusznyák and I. Törö: An electron microscopic study of the central lacteal in the intestinal villus of the cat. Z. Zellforsch. 57: 475–486 (1962).
Rényi-Vámos, F.: Das Lymphgefäsßsystem des Dünnarm und seine Rolle im Fettttransport. Acta Med. Acad. Sci. Hung. 9: 153-164 (1956).

Rényi-Vámos, F.: Das inner Lymphgefäsßsystem der Organe. Akadémiai Kiadó, Budapest, 1960.

Rusznyák, I., M. Földi and G. Szabó: Lymphatics and lymph circulation. Physiology and pathology. 2 ed. Pergamon Press, Oxford, 1967.

Skalak, T. C., G. W. Schmid-Schonbein and B. W. Zweifach: New morphological evidence for a mechanism of lymph formation in skeletal muscle. Microvasc. Res. 28: 95-112 (1984).

大 谷 修
〒700 岡山市備田町2-5-1
岡山大学医学部
第二解剖学教室

Dr. Osamu OHTANI
Department of Anatomy
Okayama University Medical School
Shikatacho 2, Okayama, 700 Japan