Lymphatic Network and Nerve Plexus in the Myenteric Layer of the Monkey Jejunum: A Topographic Study Using an Enzyme-Histochemical Method

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Received November 10, 1997

Summary. The topographic relationship between the lymphatic network and the nerve plexus in the myenteric layer of the monkey jejunum was studied by an enzyme-histochemical method. Identification of the lymphatics was achieved by a 5'-nucleotidase staining method, and the enteric neural components were visualized by acetylcholinesterase staining. A well-developed lymphatic network and a dense nerve plexus were demonstrated throughout the myenteric layer. Numerous segments of the initial lymphatics, with their blind endings at the apical parts, tended to gather toward the ganglion and run along the primary nerve strands. Elements of the tissue interstitium separated the lymphatics from the enteric nerves. Nerve terminals were often located closely beneath the endothelium of the initial lymphatics and were exposed to the subendothelial tissue on the side facing the abluminal surface of the lymphatic endothelium. These findings suggest that the lymph flow in the initial lymphatics might be regulated by the enteric nervous system, and that the transport of tissue fluid by the lymphatics might serve as a suitable microenvironment for the enteric nerves.

Several investigators have demonstrated an association of the nerve elements not only with collecting lymphatics (VAJDA, 1966; TODD and BERNARD, 1973; MCHALE et al., 1980; ALESSANDRINI et al., 1981; OHHASHI et al., 1982, 1983; ALLEN and MCHALE, 1986; ALLEN et al., 1988; GUARNA et al., 1991; SACCHI et al., 1994), but also with lymphatic capillaries (initial lymphatics) in the rat liver (MAGARI et al., 1979; MAGARI, 1983; ITO et al., 1990) and in the intestinal villi of the dog and monkey (ICHIKAWA et al., 1990, 1991a, b, 1993). These findings have indicated that the nerve fibers around the lymphatics may act as a mechanoreceptor or as a regulator of the lymph flow.

The myenteric nerve plexus of Auerbach has been investigated on its different aspects, but no reports seem available concerning its association with the initial lymphatics in the myenteric layer. We previously demonstrated intramural lymphatic networks in the rat and monkey small intestine by an enzyme-histochemical method with 5'-nucleotidase (5'-Nase) staining on whole-mount preparations of the laminar layers (SHIMODA et al., 1996, 1997). Acetylcholinesterase (AChE) staining, on the other hand, has been found useful for visualization of the nervous system of the intestinal wall (KOBAYASHI, 1987). The present study reveals the relationship between the initial lymphatic network and the myenteric nerve plexus of the monkey jejunum by light and electron microscopy using double staining for 5'-Nase and AChE activity.

MATERIALS AND METHODS

Ten young Japanese monkeys (*Macaca fuscata*) of both sexes, weighing 3–5 kg, were used in this study. They were kept under standard laboratory conditions and allowed standard laboratory animal chow and water *ad libitum*. All experiments were carried out in accordance with the Guidelines for Animal Experimentation, Oita Medical University.

Tissue preparation

After the animals were euthanized by exsanguination under deep anesthesia with sodium pentobarbital (25 mg/kg), a segment of the jejunum was placed in 0.1 M cacodylate buffer (pH 7.2) containing 7% sucrose at 4°C. Each segment was cut along the mesenteric border, and the intraluminal contents were flushed away with the same buffer. For light and scanning

electron microscope (SEM) study, the tissues were immersed in 4% paraformaldehyde in 0.1 M cacodylate buffer containing 7% sucrose for 4 h at 4°C. For transmission electron microscope (TEM) examination, small tissue pieces were fixed with 0.5% glutaraldehyde and 4% paraformaldehyde in the buffer mentioned above for 30 min and then kept in 4% paraformaldehyde for 4 h at 4°C. After the fixation, whole-mount specimens containing the myenteric layer, longitudinal muscle layer, and serosa were carefully prepared under a dissection microscope using watchmaker's forceps, and then rinsed in the same buffer. In addition, several pieces of the fixed intestine were embedded in OCT-compound (Miles, Diagnostic Division, Elkhart, IN, USA) after washing with the same buffer and then cut into 7 µm-thick sections with a cryostat. These preparations were processed for enzyme-histochemical staining for 5'-nucleotidase (5'-Nase) and acetylcholinesterase (AChE) activity.

Enzyme-histochemistry

To identify the 5'-Nase activity of the lymphatics, the specimens were incubated in a standard medium for 30–40 min at 37°C as reported previously (KATO and MIYAUCHI, 1989; KATO, 1990a, b; KATO and GOTOH, 1990; KATO et al., 1991, 1993, SHIMODA et al., 1997). Briefly, the reaction medium contained 5'-adenosine monophosphate (AMP) (Sigma Chemical, St. Louis, MO, USA) as a substrate, lead nitrate (TAAB Laboratories Equipment, Berkshire, England) or cerium chloride (Sigma) as a capture agent and 2 mM L-tetramisole (Sigma) as an inhibitor of nonspecific alkaline phosphatase. Following incubations in the lead-based reaction medium, the preparation were rinsed with distilled water and immersed in 1% ammonium sulfide solution for 2 min at room temperature. All of the specimens incubated in the cerium-based medium underwent ultrastructural examination.

For 5'-Nase-AChE double staining, specimens treated with lead-based 5'-Nase staining were further incubated in the standard medium for AChE reaction, comprising 65 mM acetate buffer (pH 6.0), 4 mM sodium citrate, 3 mM cupric sulfate, 1 mM potassium ferricyanide, 2 mM acetylthiocholine iodide (Sigma), and 2 mM iso-octamethyl phosphoramidate (Sigma) as an inhibitor of nonspecific cholinesterase, according to the method by HEDREEN et al. (1985), with a slight modification. After being washed with distilled water, the specimens were treated with 1% ammonium sulfide solution for 1 min at room temperature, followed by five changes of 0.1 M sodium nitrate for 2 min each. They were then exposed to 0.1% silver nitrate for 1

min at room temperature, followed by a rinse in distilled water. The following controls for the reactions were used: 1) omission of the substrate (AMP or acetylthiocholine iodide) from the reaction medium; 2) addition of 50 mM NiCl₂ or 0.1 M eserine sulfide (Sigma) to the reaction medium; and 3) incubation of the preparations for 60 min at 60°C before incubation in the reaction medium.

After the 5'-Nase-AChE staining, some of the whole-mount preparations were mounted on glass slides with Crystal/mount (biomeda, Foster, CA, USA) and examined under a light microscope (Olympus BH-2, Tokyo, Japan). Other preparations were used for SEM examination in order to visualize more clearly the correlation between the lymphatics and the nerves.

Scanning electron microscopy (SEM)

Several whole-mount preparations which were treated with 5'-Nase and AChE staining were dehydrated in a graded ethanol series and dried according to the t-butylalcohol freeze-drying method described by INOUE and OSATAKE (1988). The dried specimens were mounted on an aluminium stub with double-sided tape and coated with carbon for backscattered electron images to a thickness of about 20 nm. The specimens were then examined with a scanning electron microscope (S-800, Hitachi) equipped with a GM type 30 backscattered electron detector. The backscattered electron micrographs were taken as reported previously (KATO, 1990b; KATO and GOTOH, 1990).

Transmission electron microscopy (TEM)

Following the cerium-based 5'-Nase staining, the specimens for TEM were postfixed in 2% osmium tetroxide in 0.1 M cacodylate buffer for 2 h at 4°C. They were dehydrated in a graded ethanol series, and embedded in Epok 812 (Oken, Tokyo). Ultrathin sections were cut on an Omu-4 Ultracut ultramicrotome (Reichert-Jung, Vienna, Austria), stained with uranyl acetate and lead citrate, and observed with a transmission electron microscope (JEM-1200 EX, JEOL, Tokyo).

RESULTS

Light microscopy

Under a light microscope, the enzyme-histochemical staining for 5'-Nase activity demonstrated intense lead-sulfide deposits of reaction products in the walls of the lymphatic vessels on both the whole-mount preparations and the tissue sections of the monkey intestine (Figs. 1, 2). Following AChE staining,

Fig. 1. Light micrograph of a cryostat section of the monkey jejunum stained with 5'-Nase-AChE double staining. The 5'-Nase-positive lymphatics (arrowheads) are identified in the myenteric layer of the jejunum, close to the nervous elements with intense AChE activity (arrows). *Lm* lamina propria, *Sm* submucosa, *Mc* circular muscle, *MI* longitudinal muscle, *Ss* subserosa. $\times 85$

Fig. 2. Light micrograph of a whole-mount preparation of the myenteric layer of the jejunum treated with 5'-Nase-AChE double staining. A well-developed network of 5'-Nase-positive lymphatic vessels is demonstrated in addition to a dense nerve plexus (Auerbach's plexus) with AChE activity. Some segments of the lymphatics run along the large fasciculi of AChE-positive nerves (arrows). Many blind endings (arrowheads) in the apical parts of the lymphatics cluster around the ganglia (G). 1 primary nerve strands, 2 secondary nerve strands, 3 tertiary nerve components. $\times 40$

significant silver precipitates of reaction products were localized in the nervous tissues along with the 5'-Nase-positive lymphatics (Figs. 1, 2). The AChE-positive nerve elements were distinguishable from the lymphatic vessels with 5'-Nase activity by their appearance and distribution under the light microscope, although both of the structures were colored the same dark brown. Further incubation in the reaction medium for AChE activity showed no remarkable changes in the intensity or localization of the reaction product of 5'-Nase activity compared with those specimens incubated in the medium for 5'-Nase alone. The control stainings showed neither 5'-Nase- nor AChE-positive structures.

The 5'-Nase and AChE double staining on the tissue

sections showed irregularly-shaped lymphatics close to AChE-positive enteric neural elements in the myenteric layer (Fig. 1). The whole-mount preparations, including the myenteric layer treated with the double staining, revealed a dense maze-like lymphatic network in addition to a nerve plexus consisting of ganglia, primary nerve strands (connectives), secondary nerve strands, and tertiary nerve components (FURNESS and COSTA, 1987; KOBAYASHI, 1987) (Fig. 2). The neural elements with AChE activity presented a well-developed mesh-like arrangement, which is known as a myenteric nerve plexus of Auerbach (Fig. 2). The initial lymphatics with 5'-Nase activity in the myenteric area appeared tubulo-saccular in shape and often revealed apical parts.

The apical aspect of the initial lymphatics was estimated to end blind when its contour was smooth with an intact wall. Initial lymphatics running close to the primary nerve connectives were often identified, and many blind endings at the apical parts of these lymphatics tended to gather around the Auerbach's ganglia (Fig. 2). Nerve fibers were sometimes found close to the lymphatic vessels (Fig. 2), although such dense innervations as the para- and perivascular nerve plexuses around the blood vessels (FURNESS and COSTA, 1987) were not recognizable around the lymphatic vessels.

Backscattered electron imaging by SEM

The backscattered electron imaging by SEM lucidly revealed the deposits of heavy metals due to the 5'-Nase (lead) and AChE (silver) reactions (Fig. 3). Observations of tissue sections well corresponded to the light microscopic distributions of the reactivities. The atomic number contrast of the metals clearly distinguished the 5'-Nase-positive lymphatics from the nerves with AChE activity. The backscattered SEM observation of the whole-mount preparations treated with 5'-Nase and AChE staining allowed more precise analysis of the relationship between the intramural lymphatics and nerves.

Many initial lymphatics in the myenteric layer tended to run along the primary nerve connectives and cluster around the ganglia with their blind endings (Fig. 3a). The direction of the major axis of the initial lymphatics often changed along the ganglia and the primary connectives (Fig. 3a). These initial lymphatics thus showed a maze-like distribution within a mesh formed by the ganglia and internodal strands of the myenteric nerve plexus. In addition, some segments of the initial lymphatics appeared to be attached with nerve fibers of the secondary or tertiary nerve components (Fig. 3b), and the blind endings of the initial lymphatics were sometimes intertwined with the nerve fibers (Fig. 3c).

Transmission electron microscopy (TEM)

In the specimens including the myenteric layer treated

with cerium-based 5'-Nase staining, dense granular reaction products could be seen on the surface of the lymphatic endothelial cells, but not on the blood vessels. TEM examination revealed the fine structure of the nervous tissue in the myenteric layer of the monkey small intestine. Therefore, the lymphatic vessels and each component of the myenteric nerve plexus were easily identified under the TEM.

The individual ganglia of the myenteric plexus in the monkey intestine were not encapsulated, although a discontinuous framework consisting of fibroblasts and their processes lay over their surface (Fig. 4a). In those tissue specimens including the myenteric ganglia, the apical parts of the 5'-Nase-positive lymphatics often approached the ganglia, leaving spaces of the tissue interstitium measuring 2–5 μm (Fig. 4a).

Many initial lymphatics were confirmed by TEM to run along the primary nerve strands for a long distance (Fig. 4b), as demonstrated by light microscopy and by SEM. The nerve connectives were in proximity to the lymphatics (at a distance of 1–4 μm) and separated from the endothelium of the lymphatic vessels by some of the tissue interstitium. The surface of the nerve connectives was partly surrounded by fibroblasts and their processes (Fig. 4b).

Small nerve bundles of unmyelinated fibers, which probably arose from the secondary or tertiary nerve components, were often located closely beneath the endothelial lining of the initial lymphatics, leaving a narrow space measuring only 50–300 nm (Fig. 5a). Some nerve fibers of these bundles with terminal-like appearances were directly exposed to the interstitial space facing the abluminal aspect of the lymphatic endothelium, while others were ensheathed by enteroglial cells (Fig. 5b, c). Most of the axon profiles of the nerve bundles contained numerous small clear vesicles (30–60 nm in diameter) and large vesicles with a dense core (60–140 nm in diameter). The population of these vesicles within each neurite varied widely (Fig. 5b, c). No distinct membrane specialization such as a synaptic contact was recognizable between the subendothelial nerve fibers and the lymphatic endothelium in the present TEM study.

Fig. 3. Backscattered imaging scanning electron micrographs of whole-mount preparations from the myenteric layer of the jejunum treated with 5'-Nase-AChE double staining. **a.** The 5'-Nase-positive lymphatics are strongly highlighted and the AChE-positive nerves are slightly less bright. Some segments of the initial lymphatics appear to run along thick nerve strands (*arrows*) and gather with the blind endings around the ganglia (*arrowheads*), corresponding to the light microscopic profile shown in Figure 2. *L* lymphatics, *G* ganglia. *1* primary nerve strands, *2* secondary nerve strands, *3* tertiary nerve components. $\times 90$. **b.** AChE-positive nerves attaching to the wall of a 5'-Nase-positive lymphatic (*arrowheads*). $\times 170$. **c.** The apical part of a lymphatic vessel with 5'-Nase activity is intertwined with AChE-positive nerves (*asterisks*). $\times 530$

Fig. 3. Legend on the opposite page.

Fig. 4. TEM views of the myenteric layer of the jejunum stained for 5'-Nase. **a.** The apical part of a 5'-Nase-positive lymphatic (*L*) is in the vicinity of a myenteric ganglion (*G*). *NC* nerve cells. $\times 3,000$. **b.** A 5'-Nase-positive lymphatic vessel (*L*) is accompanied by a primary nerve strand of the myenteric nerve plexus (*P*). *SM* smooth muscle. $\times 5,000$

Fig. 5. TEM views of the myenteric layer of the jejunum treated for 5'-Nase histochemistry. **a.** Unmyelinated nerve bundles with terminal-like appearances (*NT*) are located closely beneath the endothelial cells of a 5'-Nase-positive lymphatic vessel (*L*). *S* a secondary nerve strand of the myenteric nerve plexus. $\times 12,000$. **b** and **c.** Higher magnifications of the areas indicated with asterisks (*) and (***) in **a**. Nerve fibers beneath the 5'-Nase-positive lymphatic (*L*) have lost the entero-glial covering on the side facing its endothelium (*E*). Notice the vesicles (arrowheads) contained in various amounts in these terminal-like nerve profiles. $\times 22,000$

DISCUSSION

The present histochemical results in the myenteric layer of the monkey jejunum demonstrated a tendency for the initial lymphatics to approach both the ganglia and the primary connectives of the myenteric nerve plexus. This suggests that the transport of the interstitial tissue contents by the initial lymphatics extending close to the ganglia or primary connectives might play an important part in the maintenance of a suitable microenvironment for the myenteric neurons. This hypothesis is supported by previous findings using the corrosion cast/SEM technique. OHTANI and MURAKAMI (1987) showed that the myenteric nerve plexus could be replicated (as could the lymphatic network) by an intra-arterial injection of low viscosity methacrylate in the stomach of young rats; they proposed that the enteric neurons were housed in interconnected channels in which fluid might be able to flow.

Most of the previous studies on the contribution of nerves to lymphatic vessels evaluated the association of nerve fibers with such large lymphatics with a developed muscular coat (collecting lymphatics) as mesenteric lymphatics, predominantly from the point of view of their possible regulation of the contractile activity of the lymphatics (VAJDA, 1966; TODD and BERNARD, 1973; MCHALE et al., 1980; ALESSANDRINI et al., 1981; OHHASHI et al., 1982; ALLEN and MCHALE, 1986; ALLEN et al., 1988; GUARNA et al., 1991; SACCHI et al., 1994). Conversely, a few reports have been available dealing with nerves associated with lymphatic capillaries lacking in smooth muscle cells, such as the central lacteals (MAGARI et al., 1979; MAGARI, 1983; ITO et al., 1990; ICHIKAWA et al., 1990, 1991a, b). The present study demonstrated a topographical proximity of nerve bundles towards the initial lymphatics in the myenteric layer of the monkey intestine. It was further shown by TEM that the nerve fibers in the subendothelial layer of the initial lymphatics appeared like nerve terminals in ultrastructure.

The endothelial cells of the initial lymphatics have been thought to play an important role in the regulation of lymph formation and/or flow (LEAK and BURKE, 1966, 1968; LEAK, 1976; HOGAN and UNTHANK, 1986). Several studies raised the possibility that the lymphatic endothelium might be one of the targets of some bioactive substances including neurotransmitters (MAGARI et al., 1979; MAGARI, 1983; UNTHANK and HOGAN, 1987; ITO et al., 1989, 1990; ICHIKAWA et al., 1990, 1991a, b). The existence of the subendothelial terminal-like nerve fibers found in the present study supports the hypothesis that the nerves may

regulate the above-mentioned lymphatic endothelial activities by certain substances released from them.

It seems also possible that the nerves in the subendothelial layer of the initial lymphatics might be sensory in nature. The results of the previous electron-microscopic studies dealing with the collecting or initial lymphatics suggested that the associated nerves might function as receptors to detect not only tension changes in the lymphatic wall, but also chemical information in the lymphatic endothelial environment (MAGARI et al., 1979; MAGARI, 1983; ITO et al., 1990; ICHIKAWA et al., 1990, 1991a, b; SACCHI et al., 1994). Also in the myenteric layer of the monkey intestine, it seems reasonable to postulate that certain subendothelial nerves may act as mechano- and chemoreceptors. These possibilities remain to be explored in future studies.

Acknowledgement. The authors wish to thank Mr. T. KAJIWARA for his expert technical assistance.

REFERENCES

- ALESSANDRINI, C., R. GERLI, G. SACCHI, L. IBBA, A. M. PUCCI and C. FRUSCHELLI: Cholinergic and adrenergic innervation of mesenteric lymph vessels in guinea pig. *Lymphology* 14: 1-6 (1981).
- ALLEN, J. M. and N. G. MCHALE: Neuromuscular transmission in bovine mesenteric lymphatics. *Microvasc. Res.* 31: 77-83 (1986).
- ALLEN, J. M., J. G. MCCARRON, N. G. MCHALE and K. D. THORNBURY: Release of 3H noradrenaline from the sympathetic nerves to bovine mesenteric lymphatic vessels and its modification by alpha-agonist. *Brit. J. Pharmacol.* 94: 823 (1988).
- FURNESS, J. B. and M. COSTA: Arrangement of the enteric plexuses. *Cytoarchitectonics of the enteric nervous system*. In: *The enteric nervous system*. Churchill, Livingstone, 1987 (p. 6-54).
- GUARNA, M., A. M. PUCCI, C. ALESSANDRINI, N. VOLPI, M. FRUSCHELLI, D. D'ANTONA and C. FRUSCHELLI: Peptidergic innervation of mesenteric lymphatics in guinea pigs: An immunocytochemical and pharmacological study. *Lymphology* 24: 161-167 (1991).
- HEDREEN, J. C., S. J. BACON and D. L. PRICE: A modified histochemical technique to visualize acetylcholinesterase-containing axons. *J. Histochem. Cytochem.* 33: 134-140 (1985).
- HOGAN, R. D. and J. L. UNTHANK: The initial lymphatics as sensors of interstitial fluid volume. *Microvasc. Res.* 31: 317-324 (1986).
- ICHIKAWA, S., M. OKUBO, S. UCHINO and Y. HIRATA: The intimate association of nerve terminals with the lacteal endothelium in the canine duodenal villi observed by transmission electron microscopy of serial sections. *Arch. Histol. Cytol.* 53, Suppl.: 137-146 (1990).

- ICHIKAWA, S., M. SHIOZAWA, T. IWANAGA and S. UCHINO:** Immunohistochemical demonstration of peptidergic nerve fibers associated with the central lacteal lymphatics in the duodenal villi of dogs. *Arch. Histol. Cytol.* 54: 241-248 (1991a).
- ICHIKAWA, S., D. KASAHARA, T. IWANAGA, S. UCHINO and T. FUJITA:** Peptidergic nerve terminals associated with the central lacteal lymphatics in the ileal villi of dogs. *Arch. Histol. Cytol.* 54: 311-320 (1991b).
- ICHIKAWA, S., K. KYODA, T. IWANAGA, T. FUJITA and S. UCHINO:** Nerve terminals associated with the central lacteal lymphatics in the duodenal and ileal villi of the monkey. *Acta Anat.* 146: 14-21 (1993).
- INOUE, T. and H. OSATAKE:** A new drying method of biological specimens for scanning electron microscopy: The t-butyl alcohol freeze-drying method. *Arch. Histol. Cytol.* 51: 53-59 (1998).
- ITO Y., S. MAGARI and M. SAKANAKA:** Prostaglandin I₂ synthase in the lymphatic endothelium of rat liver as revealed by preembedding immunoelectronmicroscopy. *Lymphology* 22: 51-55 (1989).
- : Immunoelectron-microscopic localization of peptidergic nerve fibers around lymphatic capillaries in the rat liver. *Arch. Histol. Cytol.* 53, Suppl.: 199-208 (1990).
- KATO, S.:** Enzyme-histochemical demonstration of intralobular lymphatic vessels in the mouse thymus. *Arch. Histol. Cytol.* 53, Suppl.: 87-94 (1990a).
- : Enzyme-histochemical identification of lymphatic vessels by light and backscattered image scanning electron microscopy. *Stain Technol.* 65: 131-137 (1990b).
- KATO, S. and M. GOTOH:** Application of backscattered electron imaging to enzyme histochemistry of lymphatic capillaries. *J. Electron Microsc.* 39: 186-190 (1990).
- KATO, S. and R. MIYAUCHI:** Enzyme-histochemical visualization of lymphatic capillaries in the mouse tongue: Light and electron microscopic study. *Okajimas Fol. Anat. Jap.* 65: 391-404 (1989).
- KATO, S., A. YASUNAGA and Y. UCHIDA:** Enzyme-histochemical method for identification of lymphatic capillaries. *Lymphology* 24: 125-129 (1991).
- KATO, S., M. MIURA and R. MIYAUCHI:** Structural organization of the initial lymphatics in the monkey mesentery and intestinal wall as revealed by an enzyme-histochemical method. *Arch. Histol. Cytol.* 56: 149-160 (1993).
- KOBAYASHI, S.:** Enteric endocrine and nervous systems (In Japanese with English summary). *Endosc. Forum Dig. Dis.* 3: 1-18 (1987).
- LEAK, L. V.:** The structure of lymphatic capillaries in lymph formation. *Fed. Proc.* 35: 1863-1871 (1976).
- LEAK, L. V. and J. P. BURKE:** Fine structure of the lymphatic capillary and adjoining connective tissue area. *Amer. J. Anat.* 118: 785-809 (1966).
- : Electron microscopic study of lymphatic capillaries in the removal of connective tissue fluid and particulate substances. *Lymphology* 1: 39-52 (1968).
- MAGARI, S.:** Peripheral and autonomic nerves, sympathetic ganglia, and the lymphatic system. In: (ed. by) M. FÖLDI and J. R. CASLEY-SMITH: *Lymphangiology*. F. K. Schattauer Verlag, Stuttgart-New York, 1983 (p. 519-533).
- MAGARI, S., K. FUJIKAWA, Y. MIZUTANI and A. NISHI:** Morphological studies on liver lymphatic. *Lymphology* 12: 14-17 (1979).
- McHALE, N. G., I. C. RODDIE and K. D. THORNBURY:** Nervous modulation of spontaneous contractions in bovine mesenteric lymphatics. *J. Physiol.* 309: 461-472 (1980).
- OHASHI, T., S. KOBAYASHI and S. TSUKAHARA:** Innervation of bovine mesenteric lymphatics: from the histochemical point of view. *Microvasc. Res.* 24: 377-385 (1982).
- OHASHI, T., A. OLSHOWKA and D. M. JACOBOWITZ:** Vasoactive intestinal peptide inhibitory innervation in bovine mesenteric lymphatics. *Circulation Res.* 53: 535-538 (1983).
- OHTANI, O. and T. MURAKAMI:** Lymphatics and myenteric plexus in the muscular coat in the rat stomach: A scanning electron microscopic study of corrosion casts made by intra-arterial injection. *Arch. Histol. Jap.* 50: 87-93 (1987).
- SACCHI, G., E. WEBER, M. AGLIANÒ and L. COMPARINI:** Subendothelial nerve fibers in bovine mesenteric lymphatics: An ultrastructural and immunohistochemical study. *Lymphology* 27: 90-96 (1994).
- SHIMODA, H., S. KATO, T. KUDO and T. USUI:** Enzyme-histochemical demonstration of lymphatic network in the rat small intestinal wall (In Japanese with English summary). *Jap. J. Lymphol.* 19: 65-71 (1996).
- SHIMODA, H., S. KATO and T. KUDO:** Enzyme-histochemical demonstration of intramural lymphatic network in the monkey jejunum. *Arch. Histol. Cytol.* 60: 215-224 (1997).
- TODD, G. L. and G. R. BERNARD:** The sympathetic innervation of the cervical lymphatic duct of the dog. *Anat. Res.* 177: 303-316 (1973).
- UNTHANK, J. L. and R. D. HOGAN:** The effect of vasoactive agents on the contractions of the initial lymphatics of the bat's wing. *Blood Vessels* 24: 31-44 (1987).
- VAJDA, J.:** Innervation of lymph vessels. *Acta Morphol. Acad. Sci. Hung.* 4: 197-208 (1966).

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