＜原　著＞

Relationship between Central Lacteals and Smooth Muscular Bundles in Small Intestinal Villi of Rats

OHKUBO Masato*

Summary

It is well known that small intestinal villi include smooth muscular bundles which produce some modes of villous movement. The piston-like retraction and extension of the villus in some species of animals is closely related to the intestinal lymph flow, whereas pendular movement is essential in the rat. This study was conducted to elucidate arrangement of intravillous smooth muscular bundles in the rat small intestine by light microscopy. There was a variation in the shape of the villus from the mountain range-like to finger-like configuration, starting from the aboral to the anal side of the small intestine. Several central lacteals were present in one villus, fusing to form a single lymphatic sinus at the base of the villus. The muscular bundles ran longitudinally from the base to the tip of the villus. The developmental degree of the muscular bundles gradually decreased from the aboral to the anal side of the small intestine. Regardless of the differences in the shape of the villus and the number and configuration of the central lacteal, the central lacteal was always surrounded by longitudinal muscular bundles in a palisading manner. Intimate association of the muscular bundles with the central lacteal was considered to function to excrete lymph via their contraction and relaxation regardless of the ostensible mode of the villous movement.

Key Words : Rat, Intestinal villi, Central lacteals, Muscular arrangement

Ⅰ. Introduction

The central lacteal of the small intestinal villus is the morphological and functional origin of the small intestinal lymphatic system. The villus has several modes of motility, and the piston-like retraction and extension of the villus in particular is closely related to intestinal lymph transport[1,2]. Villous movement is apparently induced by smooth muscular tissue in the villus, but little has been elucidated about its detailed configuration. The author conducted a preliminary investigation on the morphology of intravillous distribution of smooth muscular bundles in the villus of the canine small intestine, especially in relation to the central lacteal. As a result, the muscular bundle was found to be regularly arranged from the base to the tip of the villus, which appeared particularly to contribute to effective expression of the piston-like retraction and extension of the villus[3,4].

On the other hand, the villous morphology shows variation in accordance with the species of animal, developmental stage, and the site within the small intestine of the same animal; morphological variations range from knitting needle-like, through club-like to leaf-like shapes[5,6,7]. The canine villus appears club-like, and there are no findings of any other type of villus in term of smooth muscle. In order to morphologically analyze the mode of the small intestinal villous movement, it is indispensable to clarify the arrangement of the intramural muscular bundles of the villi with various configurations.

In the present study, the author observed the distribution of smooth muscular bundles in the villus

Department : *Department of Physical Therapy, and Center for Medical Science, International University of Health and Welfare

Received : March 13, 2002

using rat in which different types of villi are present in different sites of the small intestine. The villus of the rat essentially shows pendular movement. Since growth of the intravillous smooth muscular bundle is poor in the rat ileum, observation of its distribution is difficult in sections prepared by conventional histological staining. Therefore, in order to facilitate morphological observation, immunohistochemical staining using anti-desmin antibody was employed.

Ⅱ. Materials and Methods

Five male Wistar rats weighing 250-300g were used. The animals were anesthetized with ether at the time of sampling the material. Thoracotomy was performed to expose the heart, then a minor incision was made in the left ventricular wall, through which an indwelling catheter was inserted. Next, physiological saline was infused through the catheter without applying excessive pressure, in order to wash away the blood as much as possible, then the animal was irrigated with 4% paraformaldehyde in 0.1 M phosphate buffer solution for fixation. At that time, blood was drained outside through an indwelling catheter in the right ventricle. Materials were sampled at 4 sites: the duodenum, a proximal site of the jejunum (hereafter referred to as "jejunum"), the middle part of the small intestine, and a distal site of the ileum (hereafter referred to as "ileum").

Sampled small pieces of the small intestine were immersed for one day in the same fixative. These samples were immersed in 10%, then 20% sucrose solution at 6℃ for 12 hours, respectively, then preserved in 30% sucrose solution for over 24 hours. After the excessive sucrose was wiped away, the tissues were embedded in O.T.C. compound (Miles Laboratories Inc.,Elkhart, IN, USA) and rapidly frozen in liquid nitrogen. These materials were then wrapped immediately with a wrapper and stored in a freezer at −90℃.

The tissue was thin-sectioned with a cryostat in a direction traversing the villus and cut into serial sections with a thickness of 15 μm. The sections were mounted on glass slides coated with poly-L-lysine. At staining, rabbit anti-desmin antibody (Sigma Chemical Co., St. Louis, MO, USA) was used as the primary antibody. Based on the result of an assay on the concentration of the antibody by dilution, the concentration designated for paraffin sections was further diluted at the ratio 1:50-100. Immunohistochemical staining was performed using commercially available kit (Histofine SAB-PO(R) kit, Nichirei Co., Tokyo, Japan) according to the Avidin-Biotin Complex (ABC) method. The immunoreaction was visualized by diaminobenzidine (DAB) and hydrogen peroxide in Trice-HCl buffer. In order to facilitate the observation, immunostained sections were briefly counter-stained with 1% methyl green. The stained sections were mounted after dehydration, and examined under the light microscope.

Another rat was perfused with 2% glutarardehyde and 4% paraformaldehyde in 0.1M phosphate buffer solution. Small pieces of the jejunum were taken and immersed in the same fixative for at least 12 hours. The specimens were postfixed for 2 hours in 2% osmium tetroxide in the same buffer, dehydrated through a graded series of ethanol, substituted with propylene oxide, and embedded in Quetol 651(Nissin EM, Tokyo, Japan). Ultrathin sections were cut with the direction of transversing the villus. These sections were stained with uranyl acetate and lead citrate and observed with a JEOL 100 CXⅡ electron microscope (Nippon Denshi Co, Tokyo, Japan).

Ⅲ. Results

1. Villous Shape

In the small intestine of the rat, the villous shape differed depending on the site in the small intestine. There was a wide variation in the shape of villi from mountain range-like to finger-like configuration, starting from the aboral to the anal side of the small intestine. In the duodenum and jejunum, the villi showed variations like mountain ranges. One villous mountain range had many peaks, and the free edge of the villus was rugged. In the middle of the small intestine, the villi appeared like a relatively small mountain range or a leaf, compared with that in the duodenum. The ileal villi were short compared with the villi at other sites, presenting a tongue-like or finger-like configuration.

2. Arrangement of the muscle cells of the vessels

The muscle layer of the blood vessels in the villus consists of single or multiple layers arranged concentrically. Myofilaments in the muscle cells are

arranged parallel to the long axis of the cells and, therefore, are also arranged transversely to the long axis of the blood vessels (Fig. 1-a). In contrast, although lacking the proper muscular layer, the central lacteal is closely surrounded by smooth muscular bundles. Since the myofilaments of these muscle cells are arranged in parallel to the long axis of the central lacteal, the smooth muscle cells are judged to be arranged in parallel to the long axis of the central lacteal (Fig. 1-b). This finding is closely associated with the histochemical observations. In the cross-section of the villus, it can be seen by light microscopy that, whereas the smooth muscle cells and myofilaments of the blood vessels are relatively small in number, the smooth muscle cells and myofilaments around the central lacteal are relatively large in number. Therefore, on immunostaining with the anti-desmin antibody, the muscle layer of the blood vessels is obscure, but the muscular bundles around the central lacteal can be seen clearly (Fig. 2).

3. Intravillous Smooth Muscular Bundles

At all sites in the small intestine, several central lacteals were present in one villus (Figs. 2, 3). The central lacteals had a longitudinal finger-like shape and they fused to form a single lymphatic sinus at the base of the villus (Figs. 3-b, -d, -f). Blood vessels also ran longitudinally in the villus.

Development of the intravillous smooth muscular bundle varied in accordance with the site in the small intestine. The muscular bundle was thick in the duodenum and jejunum, with an equivalent degree of development. However, the developmental degree of the muscular bundles gradually decreased from the aboral to the anal side of the small intestine, becoming extremely thin in the ileum. The muscular bundle extended longitudinally from the base to the tip of the villus in any site of the small intestine, but the bundle thickness and cross-sectional shape differed between the base and the tip. At the base, the smooth muscle bundle ran longitudinally and closely around the central lacteal, surrounding it in a palisading manner. At sites distal from the central lacteal, muscular bundles were rare. Cross-sections at about the lower 1/4 level of the villus show that most muscle bundles ran to form a palisading configuration around the central lacteal, but, although rare, there were (longitudinal) bundles which are parallel to the long axis of the villus, apart from the central lacteal (Figs. 2, 3-a, -c, -e). In the upper half of the villus, there were two types of muscle bundle: one which surrounded the central lacteal in a palisading manner and one which was located apart from the central lacteals. At the upper 1/3 level of the villus, the muscular bundle showed the greatest thickness compared to the bundles at other levels.

Ⅳ. Discussion

It is well known that the small intestinal villi include smooth muscle bundles, which produce villous movement. The degree of villous movement has a positive close correlation with the volume of intestinal lymph flow. There are 3 patterns in the villous movement: piston-like retraction and extension, pendular movement and tonic contraction. It is said that the piston-like retraction and extension of the villus in particular is closely related to intestinal lymph flow[1,2].

The configuration of the intestinal villus and central lacteal varies in different animal species and in different sites of the small intestine in the same animal[5,6,7,8,9,10]. The appearance of the villus varies widely from leaf-like through tongue-like, knitting needle-like, club-like and their respective transitional shapes. Furthermore, the number of central lacteals in one villus differs from one animal to another, and one or several central lacteals may exist in one villus. In canine, rabbit and feline intestine, a single central lacteal exists in one club-like villus[3,4,8,11,13]. Previously, the author demonstrated that the developmental degree of intravillous smooth muscle gradually decreases from the aboral to the anal side of the small intestine in dogs[4]. Intestinal villus is essentially composed of reticular tissues[14] and has no particular apparatus for lymph transport. The piston-like movement gradually decreases from the aboral to the anal side of the small intestine[1]. This suggested that the difference in the developmental degree of the intravillous smooth muscle is related to the regional difference in the kinetic volume of the villus and the volume of the intestinal lymph flow[4].

The intestinal villus is leaf-shaped or tongue-shaped and has plural central lacteals in rats[10,12,13,15]. However, as in dogs, the intravillous smooth muscle

tissue consists only of longitudinal muscular bundles running from the base to the free edge of the villus, and no muscular bundles with circular or transverse distribution are observed. The smooth muscular bundles are relatively thick in the duodenum and jejunum but poor and thin in the ileum. The muscular bundles of the rat, other than the bundles in close contact with the central lacteal, are relatively rich in the duodenum and jejunum, decrease in the middle part of the small intestine, and become rare in the ileum. This suggests a gradual decrease in the development of the intravillous smooth muscle from the aboral to the anal side of the small intestine. The relation of the arrangement of the intravillous smooth muscle to the central lacteal is basically similar in dogs and rats.

On the other hand, it has been reported that the villous movement in rats is not piston-like retraction and extension as in dogs but essentially pendular movement[2]. These two villous kinetic patterns may be caused by the difference in the contraction of the intrinsic smooth muscular bundle. That is, simultaneous contraction and relaxation of all the muscular bundles may cause piston-like movement of villi. On the contrary, if intravillous muscular bundles are separated into two groups delimited by the line of the central lacteals and it each bundle group contracts and relaxes in an antagonistic manner just like the flexor and extensor muscles of the extremities, the villus would show pendular movement.

Despite the differences in species and the site within the small intestine between the dog and the rat, it is a common finding that the central lacteal is surrounded by only longitudinal smooth muscular bundles in palisade-like arrangement. When these muscular bundles contract, they strongly compress the central lacteal. This compression causes excretion of lymph from the central lacteal into mucosal and submucosal lymphatic vessels. This is not necessarily correlated with the ostensible kinetic pattern of the villus.

On the other hand, some studies have demonstrated the presence of nerve fibers and terminals, which are considered to be sensory, in contact with the endothelial cells of the central lacteal[16,17,18]. If this nerve is involved in the absorption and excretion of lymph, it may also be involved in the contraction and relaxation of the smooth muscle. However, this hypothesis remains unconfirmed at present.

In conclusion, the findings of the present morphological study suggest that regardless of the differences in the shape of the villus and the number and configuration of the central lacteal, substantial development of longitudinal smooth muscular bundles is observed around the central lacteal, and these muscular bundles function to excrete lymph via their contraction and relaxation.

Acknowledgement

The author is grateful to Yoichi Nakamura Ph.D. of the Department of Anatomy, Tokyo Medical University for his assistance in electron microscopic observation. The author is also indebted to Prof. J. Patrick Barron of the International Communications Center of Tokyo Medical University for his review of this manuscript.

References

1. Womack WA, Barrowman JA, Graham WH. Quantitative assessment of villous motility. Am. J. Physiol., 252 (Gastrointest. Liver Physiol. 15, G250-256 (1987).
2. Womack WA, Tygart PK, Mailmanet D. Villous motility: Relationship to lymph flow and blood flow in the dog jejunum. Gastroenterology, 94, 977-983 (1988).
3. Ohkubo M, Yoshii M, Uchino S. Arrangement of intravillous smooth muscles of the canine duodenum (in Japanese with English abstract). Journal of Tokyo Medical College, 49, 3-8 (1991).
4. Ohkubo M. Arrangement of intravillous smooth muscle and its relationship to the central lacteal (in Japanese with English abstract). Kanae Igaku Joseikin Jushousha Gyousekishu, 19, 159-172 (1992).
5. Hilton WA. The morphology and development of intestinal fold and villi in vertebrates. Amer. J. Anat., 1, 459-504 (1902).
6. Nakamura K, Komuro T. A three-dimensional study of the embryonic development and postnatal maturation of rat duodenal villi. J. Electron Microsc., 32, 338-347 (1983).
7. Komuro T, Hashimoto Y. Three-dimensional

structure of the rat intestinal wall (mucosa and submucosa). Arch. Histol. Cytol., 53, 1-21 (1990).
8. Yamada Y, Araki, K, Ogata, T. Three-dimensional organization of lymphatics in the dog small intestine: a scanning electron microscopic study on corrosion casts. Arch. Histol. Cytol., 58, 465-474 (1995).
9. Shimoda H, Kato S, Kudo T. Enzyme-histochemical demonstration of the intramural lymphatic network in the monkey jejunum. Arch. Histol. Cytol., 60, 215-224 (1997).
10. Fukushima K, Sasaki I, Masuda T, et al. Three-dimensional structure of mucosal and submucosal lymphatics in rat small intestine. Tohoku J. Exp. Med., 185, 9-14 (1998).
11. Ohtani O, Ohtsuka A. Three-dimensional organization of lymphatics and their relationship to blood vessels in rabbit small intestine. A scanning electron microscopic study of corrosion casts. Arch. Histol. Jap., 48, 255-268 (1985).
12. Ohtani O. Three-dimensional organization of lymphatics and its relationship to blood vessels in rat small intestine. Cell Tissue Res., 24, 365-374 (1987).
13. Unthank JL, Bohlen HG. Lymphatic pathways and role of valves in lymph propulsions from small intestine. Am. J. Physiol., 25 (Gastrointest. Liver Physiol. 17), G390-G398 (1988).
14. Takahashi-Iwanaga H, Fujita T. Lamina propria of intestinal mucosa as a typical reticular tissue. Cell Tissue Res., 242, 57-66 (1985).
15. Ushiki T. The three-dimensional organization and ultrastructure of lymphatics in the rat intestinal mucosa as revealed by scanning electron microscopy after KOH-collagenase treatment. Arch. Histol. Cytol., 53, Suppl., 127-136 (1990).
16. Ichikawa S, Ohkubo M, Uchino S et al. 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).
17. Ichikawa S, Shiozawa M, Iwanaga T, et al. Immunohistochemical demonstration of peptidergic nerve fibers associated with the central lacteal lymphatics in the duodenal villi of dogs. Arch. Histol. Cytol., 54, 241-248 (1991).
18. Ichikawa S, Kasahara D, Iwanaga T, et al. Peptidergic nerve terminals associated with the central lacteal lymphatics in the ileal villi of dogs. Arch. Histol. Cytol., 54, 311-320 (1991).

Legends

Fig. 1 Electron micrographs showing muscle cells in the muscular layer of the blood vessel (a) and those surrounding the central lacteal (b) of rat intestinal villus sectioned transversely. The blood vessel has a thin single muscle layer arranged circularly, whereas the central lacteal is closely surrounded by thick muscular bundles arranged longitudinally. E: endothelium of the blood vessel (a) or the central lacteal (b), LC: lumen of the central lacteal, LB: lumen of the blood vessel, M: smooth muscle cell, P: plasma cell. X 30,000

Fig. 2 Light micrograph of a transverse section of the rat duodenal villus. Central lacteal (arrow head) is closely surrounded by muscular bundles in a palisade-like arrangement, which are distinguished from the blood vessel (arrow). Immunostaining of the muscle bundle using anti-desmin antibody. X 1,000.

Fig. 3 Transverse sections of villi at different sites of rat small intestin. Central lacteals (arrow heads) are distinguished from blood vessels (arrows) by surrounding thick muscular bundles. a, c and e: at the upper 1/3 level of the villus and b, d and f: at the base of the villus. Despite the differences in position of the small intestine, the central lacteal is commonly surrounded by muscular bundles in a palisading arrangement (a and b: duodenum, c and d: middle of the small intestine, e and f: ileum). The central lacteals fuse to form a single lymphatic sinus (LS) at the base of the villus (b, d and f).
X 500.

Plate 1

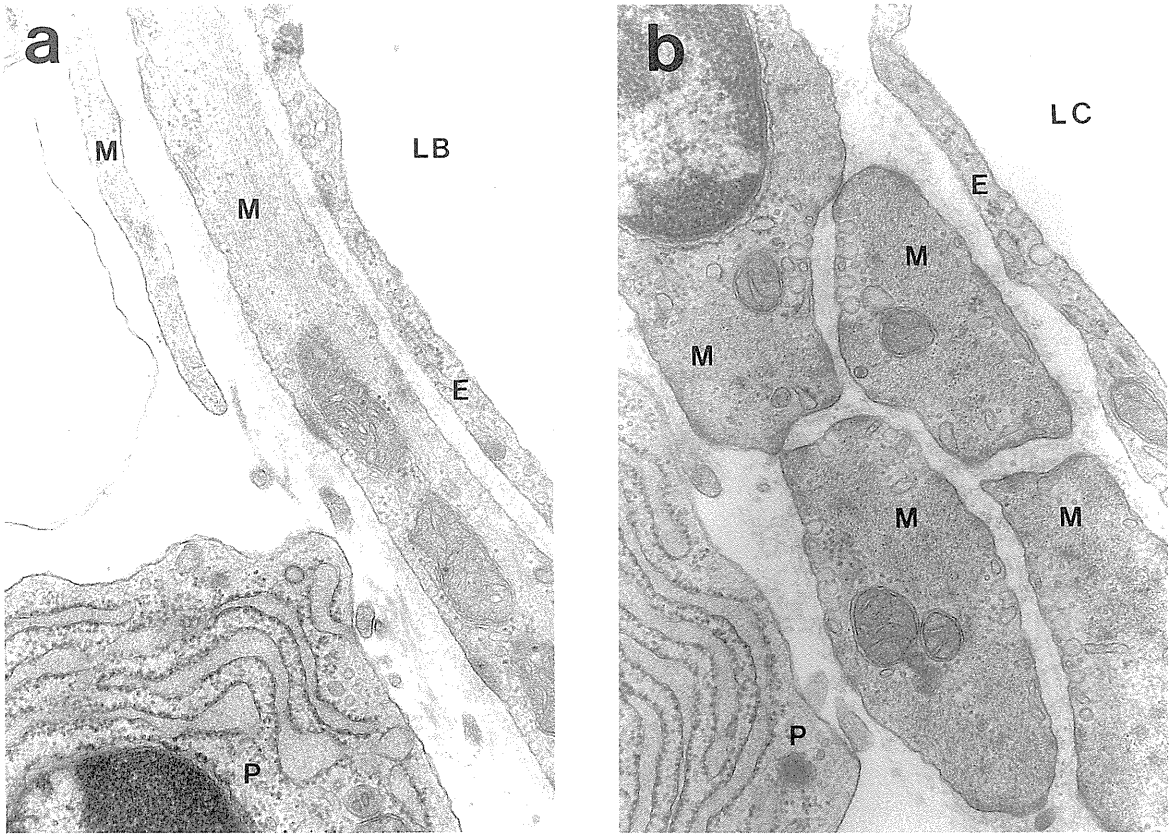

Fig. 1

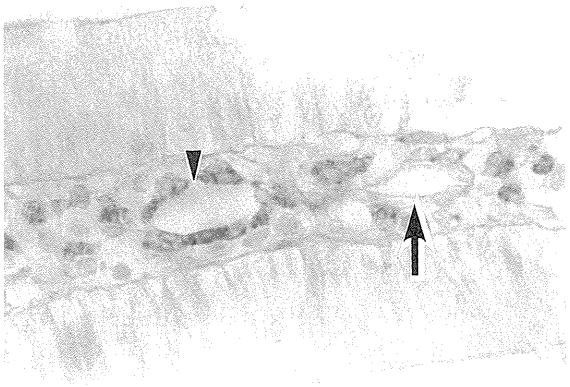

Fig. 2

Plate 2

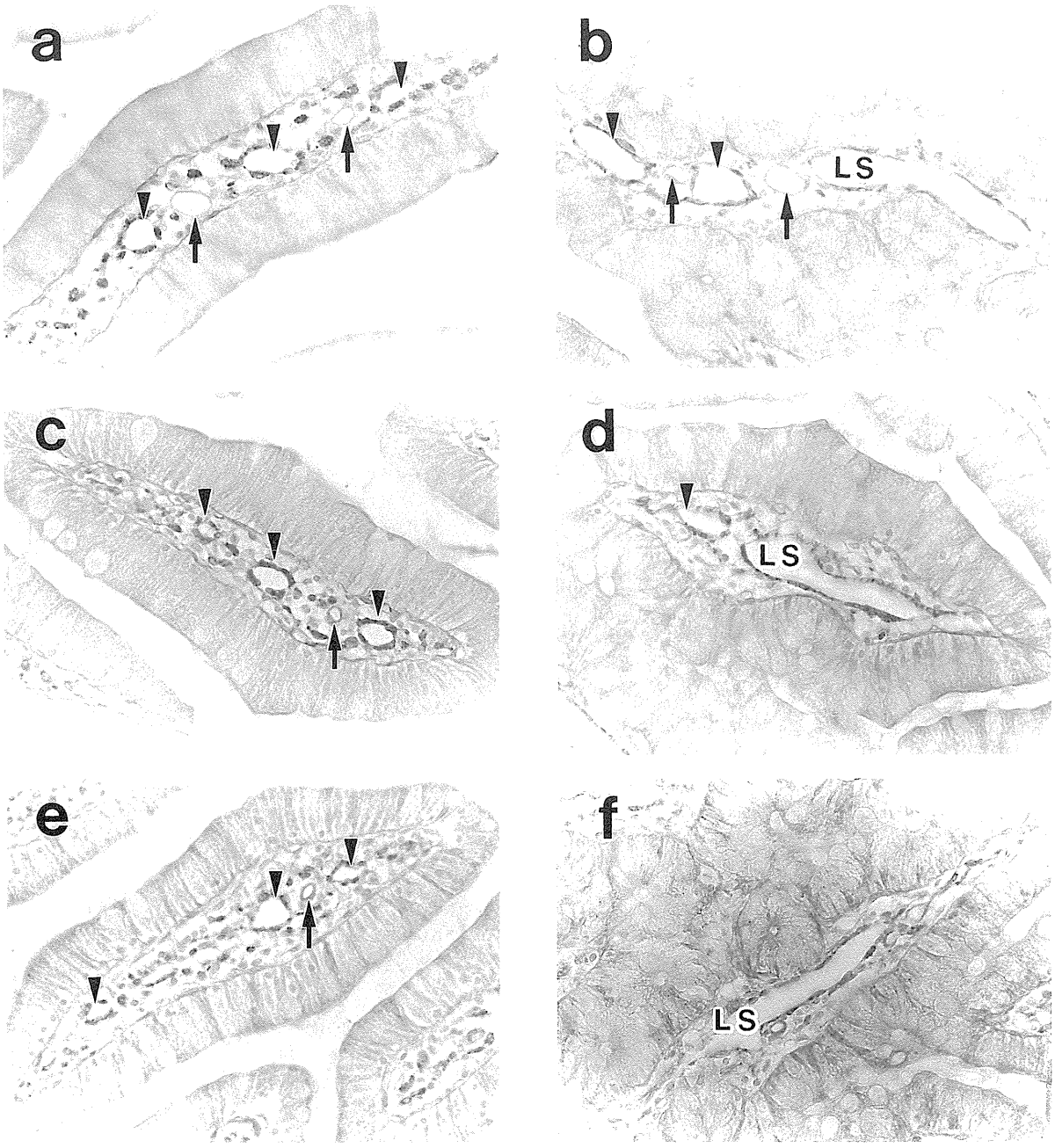

Fig. 3

ラットの小腸絨毛における中心リンパ管と平滑筋束の関係について

大久保　眞　人
国際医療福祉大学　理学療法学科，基礎医学研究センター

要　旨

小腸壁内のリンパ輸送は一般に絨毛のピストン様往復運動によるが、ラットでは特異的に振り子運動である。絨毛運動は内在する平滑筋によるが、その分布形態は不明である。本研究では免疫組織化学的手法を用いてラット小腸絨毛の平滑筋の分布を観察した。ラット小腸絨毛は小腸の吻側から肛門側に向かうに従って山脈状から指状に変化する。中心リンパ管は単一絨毛内に複数存在し、基底部で集合して一本になる。平滑筋束の発達程度は吻側から肛門側に至るにつれて貧弱になる。平滑筋束は絨毛内を縦走し、中心リンパ管の全長に亘ってその周囲を柵状に取り囲む。中心リンパ管と平滑筋束の関係は著者らが先に明らかにした他の動物における場合と同じである。動物種の違いによって絨毛の表面的な運動様式および形態、中心リンパ管の数と形は異なるものの、中心リンパ管とその周囲の平滑筋束の形態学的関係は基本的に一致している。

キーワード：ラット、小腸絨毛、中心リンパ管、平滑筋束