

## Topographic Relations of Lymphatic Endothelial Cells in the Initial Lymphatic of the Intestinal Villus

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### *Summary*

The appearances of the joint areas where the lymphatic endothelial cells are in juxtaposition with one another in the initial lymphatic of the intestinal villus are described. The joint areas could be grouped into two main categories: simple valve-like and complicated. In the former group there were open, half-open and closed joint areas. The number of simple valves tended to increase during the distension of the lymphatic, whereas the complicated areas were numerous in compressed lymphatics. The joint areas were analysed by serial sectioning and with the successive sections their appearance was found to change from simple to complicated and vice versa. A few series of sections showed a change from a simple valve to a complicated one and to a simple one again. During this change in appearance the overlapping areas of the neighbouring endothelial cells changed their position in relation to one other and to the lumen of the lymphatic. So the originally abluminal cell was later found to be situated on the luminal side and vice versa. The change of sides seemed to occur at the complicated joint areas. The endothelial cells in the wall appeared to form large interdigitating cytoplasmic extensions between which the various types of joint areas were formed. The complicated joint areas with tight junction seemed to serve as points for effective fixing the endothelial cells together. The simple joint area between these, in addition to fixing cells together, produced valves through which interstitial fluid may drain into the lymphatic.

### *Introduction*

We know that there are valve-like joint areas\* in the walls of the initial lymphatics in various mammalian organs (1, 3, 4, 5, 6, 14, 16, 17, 18, 19, 21, 24, 28, 29). At these joint areas anchoring filaments which bind the abluminal surface of the lymphatic endothelium with the collagen network of the underlying lamina propria have been described (1, 13, 18, 26, 27, 29). In the intestinal villus we also know that muscle cells lie in the immediate vicinity of the initial lymphatics (14, 16, 23, 24, 32), which suggests that the function of the initial lymphatics is to collect interstitial fluid and pump it forward. We think that the valve-like joint areas can open to drain any excessive interstitial fluid, but when they close the fluid cannot leak into the intercellular space (7, 14, 23, 34). In addition to valves there are also other kinds of endothelial joint areas (7, 8, 14, 29). In this study we describe the appearance of the various types of these areas in the walls of lymphatics in the intestinal villi of the rat and clarify their relation with each other.

\*In this work "joint area" refers to the whole two-dimensional overlap between certain parts of two endothelial cells which are in juxtaposition with one another; and "junction" refers to the various types of cellular contacts (macula adherens or desmosome, zonula adherens or intermediate junction, zonula occludens or tight junction) in the junctional complexes described (20).

### *Materials and Methods*

17 male Sprague-Dawley rats (200-280 g) were used as experimental animals. Dilated and non-dilated lymphatics were produced by various means.

- 1) Lymphostasis for 20 minutes was produced in two animals by ligating the lymphatics at the base of the mesentery (22).
- 2) Venous stasis for 1, 10 or 20 minutes was produced (two animals in each group).
- 3) To increase capillary filtration of interstitial fluid 1 mg papaverin was injected intravenously into two animals and specimens were taken after 5 minutes.
- 4) In two animals specimens were taken after opening the mesenteric veins, thus decreasing venous pressure for five minutes.
- 5) There were 5 controls.

The samples were taken after cold (+4°C) phosphate-buffered (pH 7.3) 3% glutaraldehyde had been injected into the lumen of the gut and the fixed area had been cut into small pieces – which were then immersed in the same glutaraldehyde fixative. Three controls were fixed in this fashion and two controls by intra-arterial injection of the fixative. The specimens were post-fixed in 1% osmium tetroxide and embedded in Epon (30). The sections were cut with glass knives and stained with uranyl acetate in 50% ethanol and lead citrate (33), and studied in a Hitachi HS 7S electron microscope.

For the analysis of various types of joint areas numerous perpendicular and longitudinal sections of the villi were studied from all test animals.

Five serial sections were cut from two specimens with dilated lymphatics and open valves. Three of these (over a distance of 0.9, 1.0 and 0.9  $\mu\text{m}$ ) were cut from an animal with venous stasis that had lasted for one minute. Two serial sections (over a distance of 0.8 and 0.7  $\mu\text{m}$ ) were cut from an animal treated with papaverin. The sections were cut perpendicular to the long axis of the intestinal villi at the central parts of the villi. The sections were collected on grids, each section on a grid of its own. Each serial section had a light microscope section (0.5-1.0  $\mu\text{m}$  thick) cut before and after the serial section. The light microscope sections were stained with 1% methylene blue in 1% sodium borate (10). These sections were used in the search for corresponding places in the serial sections (9). A preselected lymphatic was photographed in each section at a low (2 100 x) magnification and the joint areas of endothelial cells at higher (7 700 x) magnification). Joint areas could not be found in all sections because bars of the grid occasionally covered parts of the lymphatic. Matching of the findings in various sections was easy because the distances between the sections were small in relation to the size of the cells around the lymphatic (11, 12).

### *Results*

The basic structure of a rat's intestinal initial lymphatics is shown in Fig. 1. The types of joint area we found in these lymphatics are shown in Fig. 2. We found various combinations of joint areas in the walls of the lymphatics. Except where the valve-like joint areas were open – with the two endothelial cells not touching each other – the joint areas contained junctions, mostly with the appearance of zonulae adherentes (3, 20, 29) between the two endothelial cells. The number of these varied from 1 to 6 in each joint area. The simplest closed joint areas had only 1-2 junctions, while the more complicated had more. The joint areas often seemed to connect with a perilymphatic channel (2) over which the endothelial cell bulges into the lumen of the lymphatic.

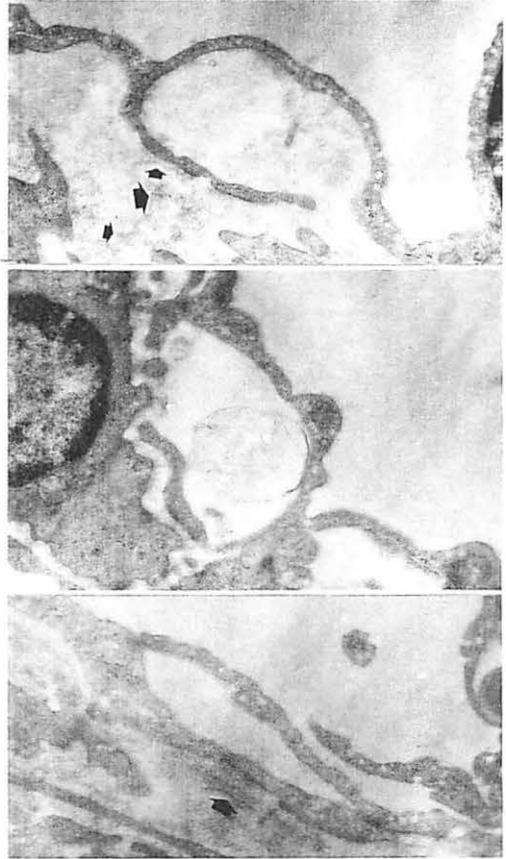


Fig. 1. A partly dilated and partly compressed lymphatic in the central part of the intestinal villus. The lymphatic is surrounded by groups of muscle cells (M) and in the lamina propria there are lymphocytes (L), plasma cells (P), fibroblasts (F), eosinophils (E) and macrophages (Ma). Note the appearance of perilymphatic channels (PC) in the wall of the dilated part of the lymphatic. On the right, two perilymphatic channels and one simple joint area with separation of the endothelial cells are shown. The uppermost perilymphatic channel contains hazily staining intercellular material. Collagen fibrils at the arrows. The other perilymphatic channel contains a rounded body composed of membrane-like material.

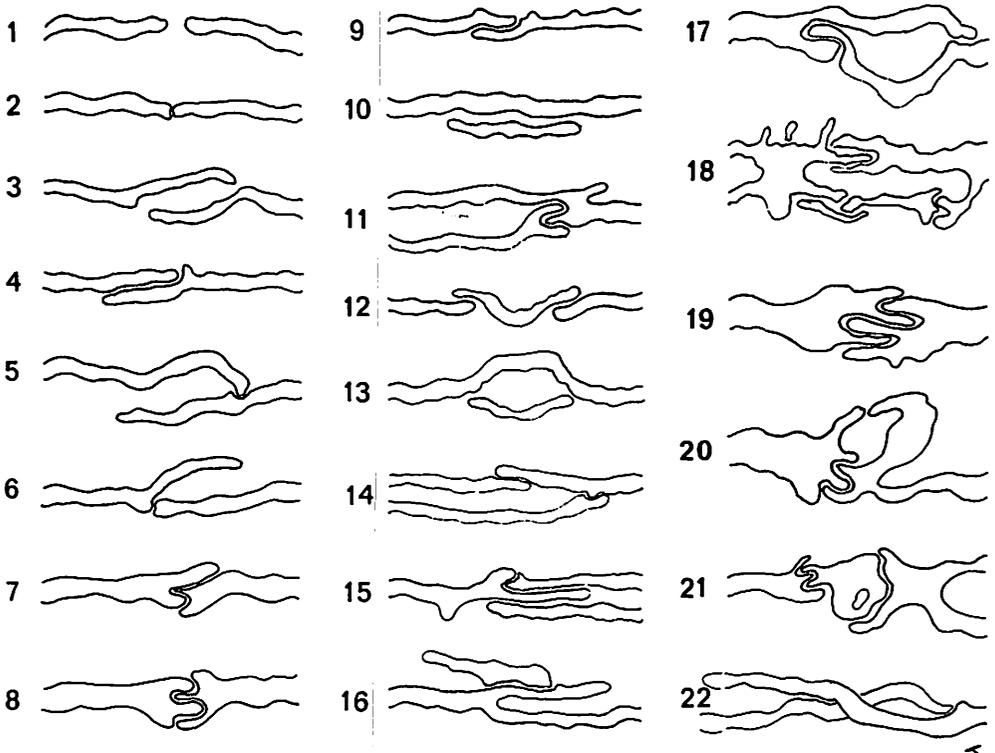


Fig. 2. Various types of joint areas between two endothelial cells drawn from electron micrographs. In interpretation the fact that the view is two-dimensional should be carefully considered. Moreover, a few sections through perilymphatic channels without a valve are included (13). This study aims to explain the appearance of simple valve-like joint areas and of more complicated ones in the same model. Simple joint areas are shown in Figures 1-6, 9, 12 and 14. Of these Figure 3 shows an open, Figures 5 and 6 half-open and Figures 1, 12 and 14 a closed simple joint area. Typical complicated joint areas are shown in Figures 7, 8, 11, 17-21. Figures 10, 13 and 16 show no valve-like formations, but are probably sections from the vicinity of a simple joint area and their appearance can be explained by the level of sectioning. Figure 22 shows a joint area where the two endothelial cells seem to interdigitate (c.f. Fig. 3 E).

The joint areas were grouped into simple and complicated ones (Fig. 2). The former were valve-like with two overlapping leaves of cytoplasm. They were "open" when the leaves were distinctly separated, "half-open" when their inner overlap was bulging into the lumen of the lymphatic (due to a neighbouring dilated perilymphatic channel) and only connected with the leaf underneath at one point, or closed when the two leaves lay intimately against each other. In the "half-open" and closed simple valves junctions with darkening and thickening of the opposing cell membranes could be seen (Fig. 3). Complicated joint areas always contained more than two layers but were not composed of more than two cells and contained one or more cellular junctions (Fig. 4). We formed the impression that the simple valves were commoner in dilated lymphatics than in compressed ones (15, 25).

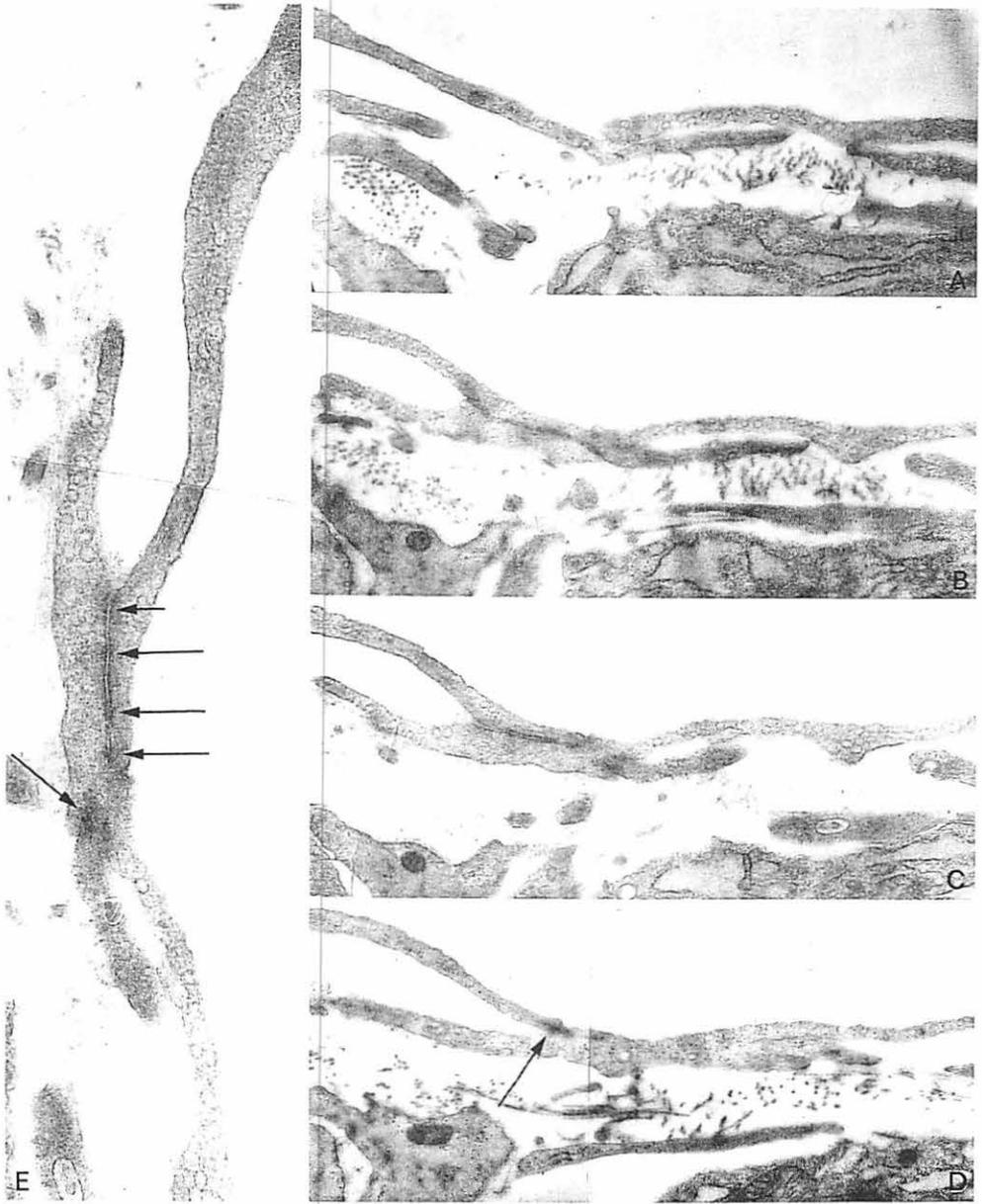


Fig. 3 A-D. Four sections cut in series from one and the same endothelial cell joint area. The distances between the upper surfaces of the sections (from A to B, from B to C and from C to D) are about 0.36, 0.18 and 0.54  $\mu\text{m}$  (each section about 0.09  $\mu\text{m}$  thick). Note the change of the luminal leaf to the abluminal side of the lymphatic wall. In A there is a simple closed joint area with the cell on the right situated on the luminal side of the wall and in D there is a simple half-open joint area with the cell on the left situated of the luminal side. A junction at the arrow. In the E site of interdigitation in C is shown in detail. Arrows point to the sites where a junction at the cell membrane may be seen. At those sites the cell leaves are more intimately fixed together than elsewhere in the wall of the lymphatic.

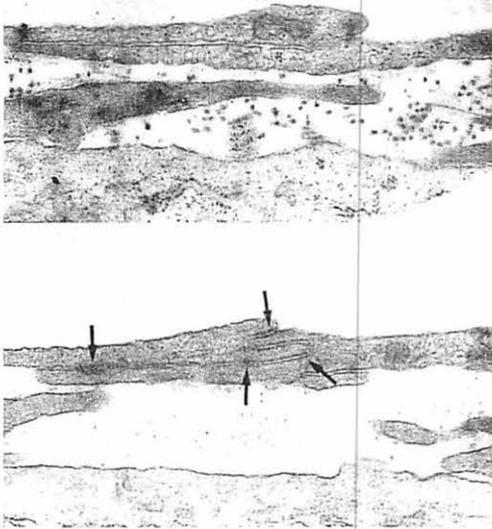


Fig. 4. The joint area of two endothelial cell leaves shown in two serial sections. The distance between the upper surface of the sections A and B is about  $0.45 \mu\text{m}$ . In A the joint area is simple, in B complicated. In later sections the area was seen simple again, but the cell leaf of the right side was then situated lumenally in relation to the other leaf. In B the arrows show the sites of junctions.

#### *Serial Section Study*

Serial sections showed that as the sections advanced the appearance of the joint areas changed. First, closed joint areas might change into open ones (Fig. 5), both for simple joints (with or without junctions) and for more complicated ones. Similarly complicated joint areas might appear simple in further sections and vice versa (Fig. 4). In a few cases a simple valve appeared complicated after a few sections and then simple again. But the relationship between the luminal and abluminal cytoplasmic leaf was reversed after the complicated joint was passed. Occasionally this change of sides occurred without an intervening complicated joint area (Fig. 3). It became obvious that the complicated joint areas were the points of intersection of two cytoplasmic flaps of the neighbouring cells. These flaps were interdigitating and apparently produced valves between the complicated joint regions.

The perilymphatic channels (Fig. 1) were also traced in the serial sections. They caused bulging of the cytoplasm in the lumen of the lymphatic. The lateral borders of the channels were usually formed by the sites of the anchoring filaments which attached the cells to the lamina propria. Serial section studies showed that most channels were situated in the immediate neighbourhood of a simple valve or of several valves.

#### *Discussion*

The probable relationship between the two endothelial cells in the wall of the lymphatic is shown in Figs. 6 A and B. This model explains how the various kinds of joint areas which appear in single sections come about (6, 14, 19, 24, 31, 32). This study also suggests that the perilymphatic channels are closely connected with the function of initial lymphatics (2). Channels filled with interstitial fluid often seem to lead to the simple



Fig. 5. Three sections cut in series of one and the same place in the wall of the lymphatic. The distance between A and B is  $0.27 \mu\text{m}$ . Note the absence of an apparent joint area in A. Nevertheless, fibrillary areas (arrow) in the endothelial cells suggest *en face* sectioned junctions, although the endothelial cell leaves seem to originate from one cell. Note the occurrence of a simple contact area in the abluminal endothelial cell layer in A – an unusual finding (double arrow). This suggests that there are parts of three endothelial cells in this figure. In B an open valve appears. In C ( $0.45 \mu\text{m}$  from section B) an open connection between the lumen and the lamina propria is well established.

joint areas in the endothelial wall of lymphatics. The fluid from these channels can drain into the lymphatic lumen through the simple valves when they open. After the valves close there would be no back flow. This is not to say that considerable loss of water cannot occur out again through the wall of the lymphatic to produce the concentration of lymph which calculations of protein concentrations in and outside the initial lymphatic indicate (7, 34).

The variation of the appearance of the joint areas seemed to depend on the state of filling of the lymphatic (15, 24). The serial section study offers an explanation to this. If the endothelial cell borders interdigitate as we suggest then possibly during distension the complicated joint areas occupy a relatively shorter distance of the cellular borders in the lymphatic wall than in non-dilated states. On the other hand, during distension the simple valves and especially those without overlapping of the cells (Fig. 1/2), should occupy a larger proportion of the borders in the lymphatic endothelial cell wall. In the latter it looked as though the integrity of the wall had been lost, but the coherence was preserved

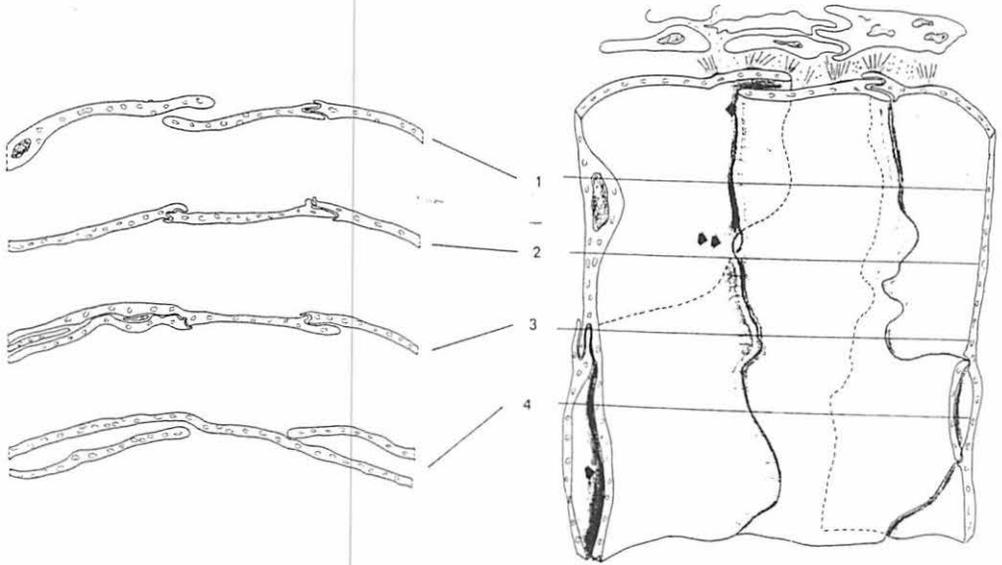


Fig. 6 A. The appearance of the wall of the lymphatic based on eleven serial sections cut over  $4.2 \mu\text{m}$ . On the left four sections of the series are shown. The intersection point at two arrows is shown as a hole between the cytoplasmic leaves. In reality, however, the intersection is the point of tightest attachment of the two neighbour endothelial cells. (Preliminary presentation at the 4th International Congress of Lymphology, Tucson, Arizona 1973).

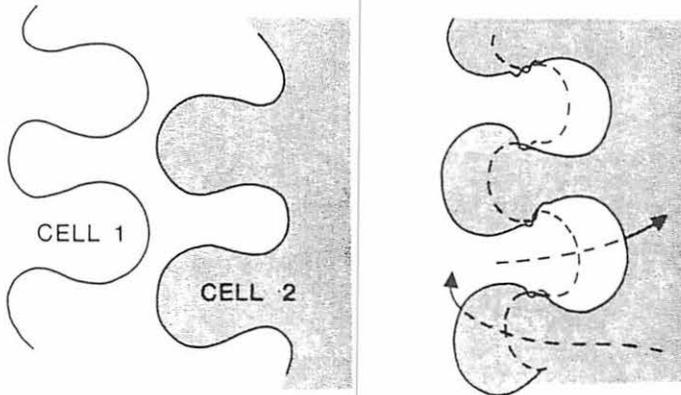


Fig. 6 B. Schematic drawing showing how the cytoplasmic leaves of the two neighbouring endothelial cells interdigitate in the wall of the initial lymphatic of the intestinal villus. On the left the cells are shown apart; on the right joining each other. The view on the right is from inside the lumen of the lymphatic and the white and grey cytoplasmic leaves correspond to the white and grey cells on the left. The cell on the luminal side of the wall is shown in its real colour, the cell borders under the luminal cell leaves are sketched in by dashes. Note the points of interdigitation, which seem to be able to bind the cell more firmly together than the sites where the cytoplasmic leaves simply lie on each other. The form of the interdigitating cell borders is arbitrary and probably it varies largely in practice. The appearance described in the figure would provide a firm structural support for the wall of the lymphatic. Arrows are seen to pass through the simple joint areas where interstitial fluid can enter the initial lymphatic.

the areas (appearing as closed simple or complicated joint areas in the electron micrographs) where the cell borders were in touch with each other.

The earlier theory of the structure of the lymphatic wall did not explain all the variations in the appearance of the joint areas in the lymphatic wall. Interdigitating cell borders, with each cell having flaps on the luminal and abluminal side of the lymphatic, are a possible further explanation to the variation. Most probably the coherence of the lymphatic wall depends on the organization of the endothelial cells in this fashion. For the final proof the application of freeze-fracture replication and scanning electron microscopic methods could be necessary.

In conclusion, this study suggests that the apparent variation of joint areas depends on two factors. First, the natural structure of the lymphatic creates joint areas with various appearances. Second, the functional status of the lymphatic, i.e. whether it is dilated or compressed, also changes the proportion of various types of joint areas in tissue sections. Nevertheless, the latter variation is possibly produced by an increase or decrease in the widths of simple and complicated joint areas of two adjacent endothelial cells in initial lymphatics of intestinal villi.

#### Acknowledgements

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