FULL PAPER *Anatomy*

Distribution of the Pores of Epithelial Basement Membrane in the Rat Small Intestine

Takashi TAKEUCHI[1)] and Tatsuo GONDA[1)]

[1)]*Institute of Experimental Animals, Shimane Medical University, Izumo 693–8501, Japan*

(Received 25 July 2003/Accepted 19 January 2004)

ABSTRACT. The distribution and diameter of the pores of epithelial basement membrane in the intestinal villi and the lymph nodules of ileal Peyer's patches were investigated in the rat small intestine by scanning electron microscopy after the removal of the over lying epithelial cells with OsO_4 maceration. In the duodenum, jejunum and ileum, the pores were mainly distributed at the upper three fourths of the villi, but were scarce around the top of the villi. The diameter of some of the pores in the upper three fourths of the villi was larger than that of those in the lower portion. The protrusion of lymphocytes and the cytoplasmic processes of macrophages were also seen at the orifices of the pores. In ileal Peyer's patches, in contrast, pores were densely distributed in the lower one third of the follicle-associated epithelium (FAE) where M cells were mainly seen. Furthermore, these pores were larger than those found in the upper two thirds. Lymphocytes or cytoplasmic processes of macrophages were frequently seen in the lower one third of FAE. These results suggest that the pores at the basement membrane correspond to the passage of the immunocompetent cells which are in contact with M cells or villous columnar epithelial cells and that the abundance of pores is a sign of aggressive interaction between the particular epi thelial cells and the immunocompetent cells at the upper three fourths of intestinal villi and the lower one third of FAE in the rat small intestine.
KEY WORDS: antigen-presenting cell, M cell, Peyer's patch, rat, small intestine.

J. Vet. Med. Sci. 66(6): 695–700, 2004

The existence and distribution of pores in the epithelial basement membrane have been reported in mammalian intestines. It has been speculated that the density of pores indicates a degree of the traffic across the basement membrane [1, 3, 7, 9, 11, 15]. This hypothesis is based on the studies performed with scanning electron microscope (SEM) and transmission electron microscope (TEM) that revealed the porosity of the basement membrane or the transit of free cells, such as eosinophils, lymphocytes and macrophages, through the membrane [7, 9, 11]. Recently, it has been proposed that pores are formed by macrophages aggregating in the lamina propria near the tip of the villus, with penetration of the cytoplasmic processes into the intestinal epithelial cells to regulate their maturation or aging of the intestinal epithelial cells in murine small intestine [13]. Moreover, similar porosity has been recognized at the periphery of the follicle-associated epithelium (FAE) in ileal Peyer's patches (PP) [12]. Incidentally, it has been generally accepted that the luminal antigens are caught by M cells at the FAE in the PP and transported to lymphocytes or macrophages aggregating around M cells [2, 8]. In this report, to clarify the functional significance of the pores in the rat small intestine, the relationship between the distribution of the pores and the particular epithelial cells were determined after removal of the intestinal epithelium by OsO_4 maceration. Furthermore, the number and diameter of the pores were measured in every portion of the small intestine.

MATERIALS AND METHODS

Animals: Seven male Wistar rats aged more than 7 months were used in the present study. They were reared with a 12 light/dark cycle (light on at 8:00 am) at 23 ± 2°C and 50 ± 10% humidity and permitted free access to food and water. The animals were sacrificed by exsanguination under anesthesia with an *i.p.* injection of pentobarbital sodium.

Light microscopic and transmission electron microscopic (TEM) observations: For light microscopic observation, the duodenum, jejunum and ileum containing the ileal PP were perfused via cardia with 10% formalin for fixation and then embedded in paraffin. Thereafter, the sections of 2 μm in thickness were stained with periodic acid-methenamine-silver stain (PAM) to visualize the epithelial basement membrane.

For TEM observation, the animals were intracardially perfused with cold 2.5% glutaraldehyde-2% paraformaldehyde in 0.01 M phosphate buffered saline (PBS, pH 7.4). The ileal PPs were extracted and immersion-fixed in the same fixative for 2 hr before post-fixation with 2% OsO_4 in PBS (pH 7.4) for 2 hr. The specimens were then dehydrated and embedded in Epoxy resin. Thereafter, ultrathin sections counterstained with uranyl acetate and lead citrate were observed by using a TEM (JEOL, JEM 1,200 EX) at an accelerating voltage of 80 kV.

Scanning electron microscopic (SEM) observation: Each portion of the small intestine was immediately extracted. Thereafter, tissues were fixed with a mixture of 1% glutaraldehyde and 1% paraformaldehyde in PBS (pH 7.2). The fixed tissues were cut into small pieces. After rinsing in PBS for 1 hr, they were macerated with 0.1% OsO_4 in PBS at 40°C for 24 hr. The specimens were agitated vigorously to remove epithelial cells efficiently. The degree of shedding of epithelial cells was checked under the dissecting microscope. The tissues were then conductive-stained by

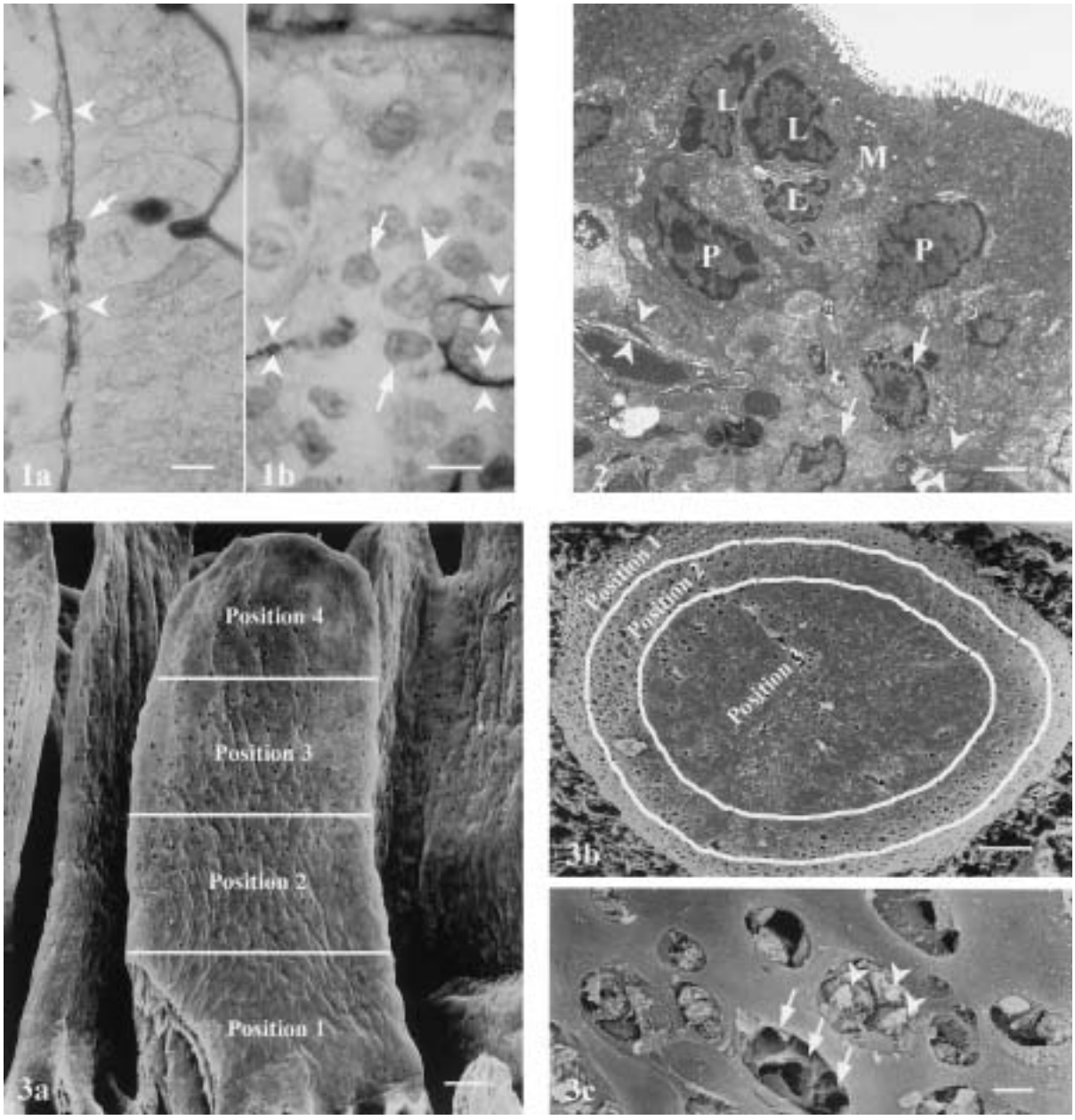


Fig. 1. Light micrographs of the epithelial basement membrane stained with periodic acid-methenamine-silver stain (PAM). a. Intestinal villi. The lymphocyte (arrow) passes through the basement membrane (small arrowheads). b. Follicle-associated epithelium (FAE) of ileal Peyer's patch (PP). Aggressive traffic of lymphocytes (arrows) and macrophage (large arrow head) through the basement membrane (small arrowheads) can be seen at the FAE. Bars =5 μm (a, b).

Fig. 2. Transmission electron micrograph of the FAE. A typical M cell (M) with sparse microvilli and the invasion of numerous migrating cells (lymphocyte: L, plasma cell: P) are seen. Lymphocytes (arrows) passing through the basement membrane (arrow heads) are frequently seen under the M cell. L: lymphocyte. P: plasma cell. Bar=2 μm.

Fig. 3. Scanning electron micrograph of intestinal villi and a lymph nodule. The epithelial cells were removed by OsO_4 maceration, and the basement membranes are exposed. a. Pores are mainly distributed at the upper three fourths (position 3) of a central vil lus. b. Whole lymph nodule of an ileal Peyer's patch. Pores are intensively distributed at the lower one third (position 1). c. Pores at the lymph nodule. The interior of the large pores was divided into small parts (arrows), and migrating cells were sometimes seen to project from the pores (arrowheads). Bars =50 μm (a, b) and 5 μm (c).

the tannin-osmium method, dehydrated through a graded series of ethanol and critical-point dried by liquid CO_2. The dried specimens were coated with platinum-palladium in a ion-sputter coater (Hitachi E-1030, Japan) and observed in a SEM (Hitachi S-800, Japan). Some tissue blocks of an ileal PP were observed without maceration.

The intestinal villus was equally divided into 4 parts from the base to the villous tip, and these parts were termed position 1 to 4. In the PP, FAE was divided into 3 parts, which were termed position 1 to 3 from the periphery to the top. For an analysis of the number of pores in each position, 5 villi and lymph nodules whose basement membranes were exposed, were selected randomly from 5 rats. For each position in the villus and PP, the number of pores in an area of 2,500 μm^2 was measured by using the Scion Image (Scion, U.S.A.). The diameter and major axis of 25 randomly selected pores were also measured. The mean diameter of the pores in each position was calculated and compared among positions. The data were statistically examined by analysis of variance (ANOVA) followed by Fisher's PLSD test. All data were expressed as means ± SD, and statistical significance was defined as the 5% level.

RESULTS

Light microscopic and TEM observations: Lymphocytes and macrophages passing through the epithelial basement membrane were seen at the intestinal villi in every part of the small intestine (Fig. 1a). At the intestinal villus, the cellular transit across the basement membrane was frequently found at position 3, whereas numerous lymphocytes were located all over the intraepithelial region and lamina propria. In addition, the aggregations of macrophages were seen in the lamina propria near the villous tip. On the other hand, at the FAE of an ileal PP, the transit of migrating cells was observed particularly at position 1, where M cells were mainly located. In this position, the basement membrane was fragmented because of an aggressive infiltration by numerous lymphocytes, plasma cells and macrophages (Fig. 1b). Under TEM, lymphocytes passing through the epithelial basement membrane were observed frequently at the basal sides of M cells (Fig. 2). The migrating cells often entered the basal concaves of M cells.

SEM observation: In the duodenum, ileum and jejunum, the pores on the basement membrane were mainly found in position 3 of the intestinal villi in comparison with the other

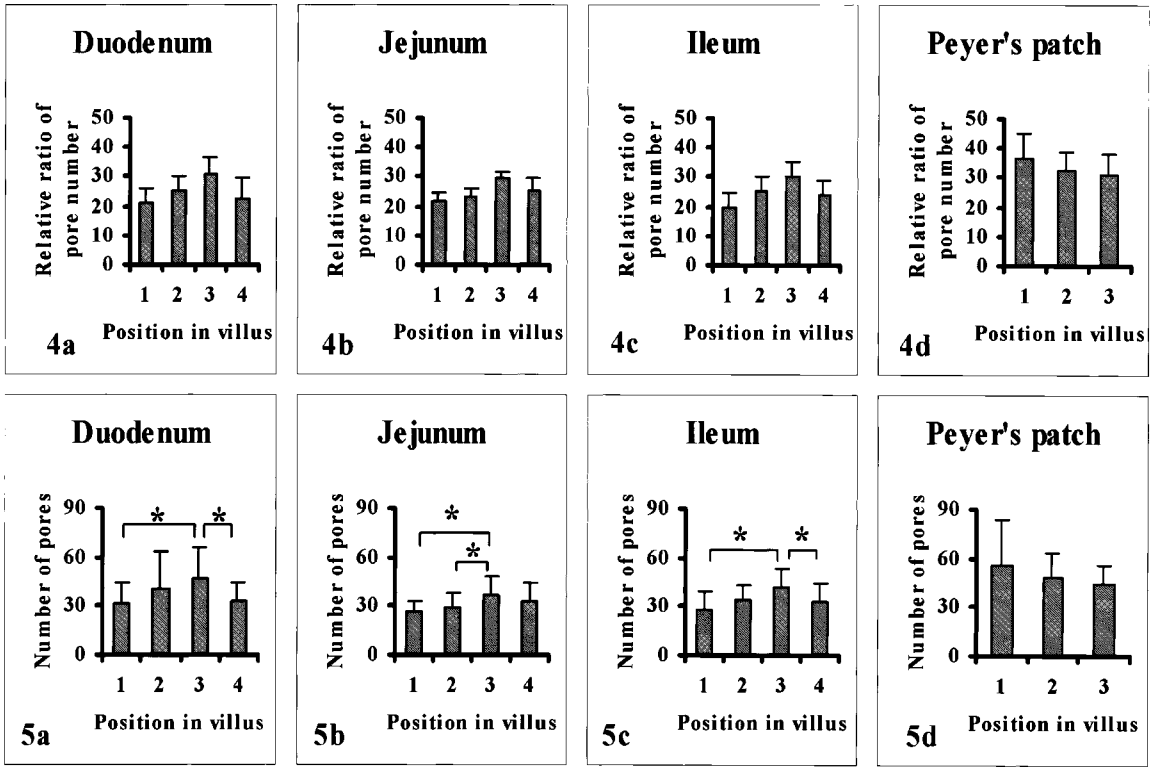


Fig. 4. The number of pores in each position of the intestinal villi and lymph nodules in the duodenum (a), jejunum (b), ileum (c) and ileal Peyer's patch (d). Each column expresses the percentage of the mean number of pores and the SD.

Fig. 5. The number of pores in each position of the intestinal villi and lymph nodules in the duodenum (a), jejunum (b) and ileum (c) and ileal Peyer's patch (d). Each column expresses the mean number of pores and the SD. Asterisks indicate significant differences compared to position 3 ($P<0.05$).

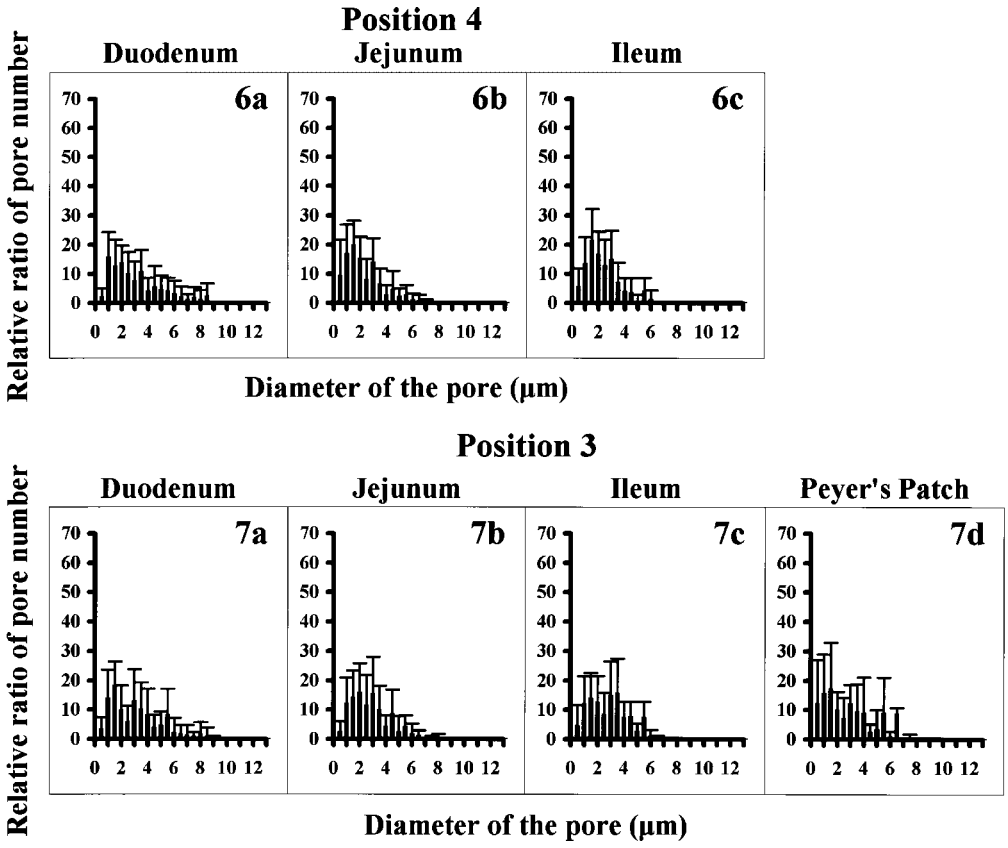


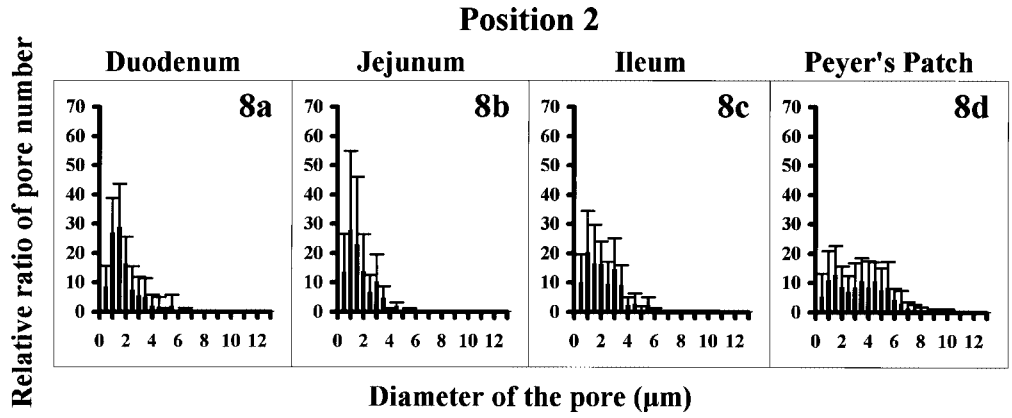
Fig. 6. The number of pores classified with their diameter in position 4 of the intestinal villi in the duodenum, jejunum and ileum. Each column indicates the presence of the mean number of pores and the SD.
Fig. 7. The number of pores classified with their diameter in position 3 of the intestinal villi and lymph nodules in the duodenum, jejunum, ileum and ileal Peyer's patch. See Fig. 6 for detail.

positions (Figs. 3a, 4a, b, c, 5a, b, c). The pores were rarely found around the tops of villi in accordance with the results of the light microscopic observation (Fig. 3a). Some migrating cells were remained at the orifice of the pore. On the other hand, pores over 5 μm in diameter were rarely found at positions 1 and 2, but they were frequently observed at positions 3 and 4 (Figs. 6–9). Pores of approximately 1 μm in diameter were commonly seen at every position of the villi, whereas the number of pores with a large diameter increased toward the top of the villi (Figs. 6–9).

In the ileal PP, a large number of pores were found throughout the FAE as compared with the number in the villi (Figs. 3a, b). These pores were distributed intensively at position 1 of the dome area, and the number of pores tended to be smaller toward position 3, in contrast to the other small intestinal portions (Figs. 4a, b, c, 5a, b, c). On the other hand, pores 1 to 4 μm in diameter were seen throughout the FAE, but the number of pores over 8 μm in diameter increased toward position 1 (Figs. 7–9). The interior regions of the large pores were divided into several small parts, and migrating cells were occasionally seen to project from the pores (Fig. 3c).

DISCUSSION

Several lines of studies with TEM and SEM have been performed to determine the existence and role of pores at the epithelial basement membrane in mammalian small intestines. From TEM studies in rats, the epithelial basement membrane is crossed frequently by different types of migrating cells, such as eosinophils, lymphocytes and macrophages [1, 7, 15]. SEM studies also revealed that pores are mainly found in the upper portion of the intestinal villi, and that the cellular processes of migrating cells are observed through pores in the rat [3, 7, 9, 11]. These studies suggest that the pores of the basement membrane of the intestinal villi play a role in the passage of migrating cells from the lamina propria to the intestinal epithelium. On the other hand, the presence of pores was also reported in the basement membrane of ileal PPs [12]. In this report, pores were remarkably abundant in ileal PPs in comparison with the adjacent villi, and they increased centrifugally from the top to the periphery of the FAE in rats and monkeys. In the present study, the number of pores was statistically dominant in the upper three fourths of intestinal villi in the small



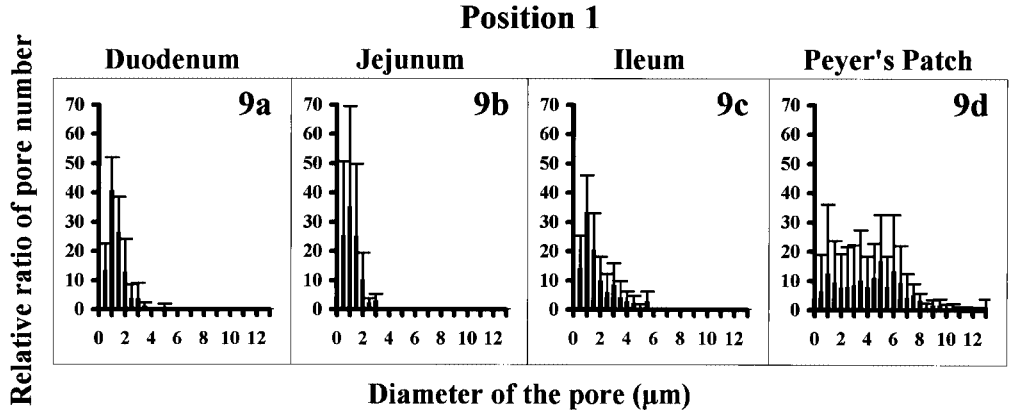


Fig. 8. The number of pores classified with their diameter in position 2 of the intestinal villi and lymph nodules in the duodenum, jejunum, ileum and ileal Peyer's patch. See Fig. 6 for detail.

Fig. 9. The number of pores classified with their diameter in position 1 of the intestinal villi and lymph nodules in the duodenum, jejunum, ileum and ileal Peyer's patch. See Fig. 6 for detail.

intestine or in the lower one third of the FAE. Moreover, an active transit of lymphocytes, plasma cells or macrophages through pores was found at the corresponding position. These results suggest that the pores of both the intestinal villi and FAE are formed by a similar process.

Macrophages, which aggregate in the lamina propria near the tips of the villi, were reported to form pores and insert cytoplasmic processes into enterocytes in order to complete the cellular life and regulate the maturation or aging of enterocytes in the rat, mouse and guinea pig small intestine [4, 13]. In our study, the transit of lymphocytes through the pores was found frequently in the upper part of the villi. In addition, the transit was also seen extremely in the lower position of the FAE. In chicken cecal tonsils, microvillous epithelial cells disappeared near the top of the FAE [6], and DNA fragmentation of epithelial cells, which is a sign of apoptosis, has already started at the middle portion of the intestinal villi and FAE in the chicken cecum [14]. These findings suggest that most pores not only indicate the sign of the extension of cytoplasmic process of macrophages, but also the passage of migrating cells.

M cells are abundant in the lower to middle portion of the FAE in several mammalian and chicken intestines [2, 5, 6, 8]. It has been generally accepted that M cells transport antigens from the lumen to antigen-presenting cells, such as macrophages and dendritic cells that have close contact with M cells, and that M cells initiate an immune response or oral immunological tolerance [2, 8]. Therefore, the abundance of pores at the lower portion of the FAE suggests aggressive interaction between M cells and antigen-presenting cells. Furthermore, the major histocompatibility complex class II molecules are expressed in villous columnar epithelial cells in the rat small intestine [10]. From these findings, it is possible that the large pores in the upper portion of the intestinal villi also signify an aggressive interaction between epithelial cells and immunocompetent cells as well as in the lymph nodule in the rat small intestine.

REFERENCES

1. Donnellan, W. L. 1965. The structure of the colonic mucosa. The epithelium and subepithelial reticulohistiocytic complex.

Gastroenterology **49**: 496–514.
2. Gebert, A., Rothkoette, H. J. and Pabst, R. 1996. M cells in Peyer's patches of the intestine. *Int. Rev. Cytol.* **167**: 91–129.
3. Highison, G. J. and Low, F. N. 1982. Microdissection by ultrasonication after prolonged OsO_4 fixation: a technique for scanning electron microscopy. *J. Submicrosc. Cytol.* **14**: 161–170.
4. Iwanaga, T., Hoshi, O., Han, H., Takahashi-Iwanaga, H., Uchiyama, Y. and Fujita, T. 1994. Lamina propria macrophages involved in cell death (apoptosis) of enterocytes in the small intestine of rats. *Arch. Histol. Cytol.* **57**: 267–276.
5. Jepson, M. A., Simmons, N. L., Hirst, G. L. and Hirst, B. H. 1993. Identification of M cells and their distribution in rabbit intestinal Peyer's patches and appendix. *Cell Tissue Res.* **237**: 127–136.
6. Kitagawa, H., Hosokawa, M., Takeuchi, T., Yokoyama, T., Imagawa, T. and Uehara, M. 2002. The cellular differentiation of M cells from crypt undifferentiated epithelial cells into microvillous epithelial cells in follicle-associated epithelia of chicken cecal tonsils. *J. Vet. Med. Sci.* **65**: 171–178.
7. Komuro, T. 1985. Fenestrations of the basal lamina of intestinal villi of the rat. Scanning electron microscopy. *Cell Tissue Res.* **239**: 183–188.
8. Kraehenbuhl, J. P. 2000. Epithelial M cells: differentiation and function. *Annu. Rev. Cell Dev. Biol.* **16**: 301–332.
9. Low, F. N. and McClugage, S. G. 1984. Microdissection by ultrasonication: Scanning electron microscopy of the epithelial basal lamina of the alimentary canal in the rat. *Am. J. Anat.* **169**: 137–147.
10. Mayrhofer, G. and Spargo, L. D. J. 1990. Distribution of class II major histocompatibility antigens in enterocytes of the rat jejunum and their association with organells of the endocytic pathway. *Immunology* **70**: 11–19.
11. McClugage, S. G. and Low, F. N. 1984. Microdissection by ultrasonication: Porosity of the intestinal epithelial basal lamina. *Am. J. Anat.* **171**: 207–216.
12. McClugage, S. G., Low, F. N. and Zimny, M. L. 1986. Porosity of the basement membrane overlying peyer's patches in rats and monkeys. *Gastroenterology* **91**: 1128–1133.
13. Takahashi-Iwanaga, H., Iwanaga, T. and Isayama, H. 1999. Porosity of the epithelial basement membrane as an indicator of macrophage-enterocyte interaction in the intestinal mucosa. *Arch. Histol. Cytol.* **62**: 471–481.
14. Takeuchi, T., Kitagawa, H., Imagawa, T. and Uehara, M. 1999. Apoptosis of villous epithelial cells and follicle-associated epithelial cells in chicken cecum. *J. Vet. Med. Sci.* **61**: 149–154.
15. Toner, P. G. and Ferguson, A. 1971. Intraepithelial cells in the human intestinal mucosa. *J. Ultrastruct. Res.* **34**: 329–344.