The Fate of Effete Epithelial Cells at the Villus Tips of the Human Small Intestine

Takeshi SHIBAHARA1,3, Noboru SATO1, Satoshi WAGURI1, Toshihiko IWANAGA2, Akira NAKAHARA3, Hisayuki FUKUTOMI3 and Yasuo UCHIYAMA1

Department of Cell Biology and Neuroanatomy1, Iwate Medical University School of Medicine, Morioka; Department of Anatomy2, Niigata University School of Medicine, Niigata; and Department of Internal Medicine3, Institute of Clinical Medicine, University of Tsukuba, Tsukuba, Japan

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Summary. Until recently, little has been known about the morphological features of dying enterocytes at the villus tips of the human small intestine. The present study aimed to show the exfoliating processes of effete enterocytes at the villus tips. Cellular elements of the duodenal lumen and jejunal tissue in humans were fixed and processed for DNA nick end labeling (TUNEL), and transmission and scanning electron microscopy (TEM and SEM). Most cellular elements in the duodenal lumen were enterocytes having TUNEL-positive nuclei. By SEM, protruding enterocytes were discerned at the villus tips. Using the SEM samples embedded in epoxy resin, protruding enterocytes were observed at the villus tips by TEM; they were shrunk by forming numerous clear and autophagic vacuoles, took dome-like profiles, and possessed nuclei with chromatin condensation. The intercellular spaces beneath these protruding or effete enterocytes were often occupied by large lymphocytes. By TUNEL reaction, positive stainings appeared in the epithelium not only at the tip of the villi but also around the site. The results suggest that effete enterocytes at the villus tips of human small intestine are first shrunk by forming clear and autophagic vacuoles, and showed that their nuclei exhibit chromatin condensation immediately before being exfoliated into the lumen.

It is generally believed that enterocytes (columnar epithelial cells) of mammalian guts are generated in crypts and move towards the villus tips, where they die by apoptosis, being exfoliated into the lumen (LEBLOND and MESSIER, 1958; MACDONALD et al., 1964; CHENG and LEBLOND, 1974; EASTWOOD, 1977; LEBLOND, 1981; WRIGHT and ALISON, 1984). According to the morphological criteria of apoptosis, dying cells show chromatin condensation and cell shrinkage, followed by heterophagocytosis (WYLLIE et al., 1980, 1984; KERR et al., 1987; CLARKE, 1990). Recently, GAVRIELI et al. (1992) have used in situ nick end labeling of dUTP mediated by terminal deoxytransferase (TUNEL reaction) to show that DNA fragmentation appears in epithelial cells of the rat and mouse small intestine and human large intestine at the villus tips. However, dying enterocytes with morphological features of apoptosis are hardly seen at the villus tips (KERR et al., 1987).

In the small intestine of guinea pigs, IWANAGA et al. (1993) have shown that intraepithelial lymphocytes and subepithelial macrophages are involved in removing effete enterocytes at the tips of villi. They demonstrated that the body of the effete enterocytes was removed by those cells, whereas their thin superficial layer was retained to be later exfoliated without destroying the barrier; these cell fragments could be recognized in the fluid collected from the ileal lumen. Until recently, however, there have been neither morphological nor biochemical reports on the fate of effete enterocytes in the human small intestine at the tip of villi. To investigate this problem, the present study examined the dying processes of effete enterocytes at the villus tips of the human small intestine.

MATERIALS AND METHODS

Collection of cellular elements in the duodenal lumen

Duodenal fluids were collected from five healthy male volunteers showing no abnormal findings in the duodenum (age: 26–30 years old), using a gastrofiberscope (Olympus P20, Tokyo, Japan). After inserting the fiberscope into the duodenum and confirming the presence of duodenal fluids, 20 ml physiological saline was slowly administrated through the catheter of the fiberscope, and then approximately 6 ml of the rinsed solution...
was gently sucked up through the catheter. The solution was immediately centrifuged at 3,000 rpm for 5 min. The pellets from each subject were further processed for electron microscopy, TUNEL reaction, and electrophoretic analysis of the genomic DNA.

**Tissue sampling of small intestine**

Jejunal samples of small intestine were obtained from surgically excised tissues of 10 patients with cancers of the stomach, pancreas, or biliary tract. Those tissue samples without pathological signs were immediately processed for light and electron microscopy.

**Tissue preparations for electron microscopy**

For transmission electron microscopy (TEM), the pellets from the rinsed fluids in the duodenal lumen and small pieces of the jejunal tissue were fixed with 2% paraformaldehyde (PA)-2% glutaraldehyde (GA) buffered with 0.1 M cacodylate-HCl, pH 7.4 (CB), at 4°C overnight. They were then postfixed with 2% OsO4 buffered with the same buffer at 4°C for 2 h, and treated with a 1% aqueous solution of uranyl acetate for 1 h. After dehydration using graded alcohols, they were embedded in Epon 812. Some pieces of the jejunal tissue were carefully processed for TEM. To obtain suitable thin sections containing villus tips, serial semi-thin sections were first cut at 2 μm with an ultramicrotome (Ultracut N, Reichert-Nissei, Tokyo, Japan) and appropriate semi-thin sections were chosen after staining with toluidine blue. Thin sections were cut from the semi-thin sections using an ultramicrotome, stained with uranyl acetate and lead citrate, and observed using a Hitachi H-7100 electron microscope (Tokyo, Japan).

For scanning electron microscopy (SEM), the jejunal tissue was cut into larger pieces (5 × 5 mm). Some samples were directly immersed in 2% PA-2% GA buffered with CB at 4°C overnight, while others were first washed thoroughly in 0.1M phosphate-buffered saline (PBS) by shaking sample bottles for 5 min and fixed with the same fixative. They were conductive-stained by the tannin-osmium method (MURAKAMI, 1974), dehydrated using graded alcohols, and transferred to 2-methyl-2-propanol. They were then critical point-dried using liquid-CO2, ion-coated with platinum by an ioncoater Hitachi E-1030, and observed using a Hitachi S-2300 scanning electron microscope.

To detect dying enterocytes by TEM, we used SEM samples exhibiting protruding enterocytes at the villus tips. After the SEM samples were trimmed to obtain the objective sites under a binocular microscope, they were re-dehydrated using graded alcohols and embedded in Epon 812. Serial thin sections were cut with an ultramicrotome and observed with an electron microscope with or without staining.

**DNA nick end labeling**

To check nuclear alterations in exfoliated enterocytes and epithelial cells of the jejunum, the terminal deoxynucleotidyl transferase (TdT)-mediate dUTP-biotin nick end labeling (TUNEL) reaction was applied to tissues according to the modified method by GAVRIELI et al. (1992). Parts of pellets from the rinsed solution in the duodenal lumen were re-suspended in 0.5 ml physiological saline and smeared on gelatin-coated glass slides. They were air-dried and fixed with 4% PA buffered with CB containing 4% sucrose, at 4°C for 15 min. The jejunal tissue, cut into larger pieces (5 × 5 mm), was immersed in the same fixative for 4 h and embedded in OCT compound (miles, Kankakee, IL) at −80°C. Serial sections of the tissue were cut at 10 μm with a cryostat (Sakura CM-501, Tokyo, Japan), and mounted on gelatin-coated glass slides.

The smears and cryosections were pretreated with or without 20 μg/ml proteinase K (Sigma) at room temperature (RT) for 15 min. After treatment with 0.3% H2O2 in methanol for 30 min, they were incubated with 100 U/ml TdT and 10 nmol/ml biotinylated 16-2'-dUTP (Behringer-Manheim-Yamanouchi, Osaka, Japan) in TdT buffer (100 mM sodium cacodylate, pH 7.0, 1 mM cobalt chloride, 50 μg/ml gelatin) in a humid atmosphere at 37°C for 60 min. Further incubation with peroxidase-conjugated streptavidin (Nichirei, Tokyo, Japan) was carried out for 60 min at RT. Staining for peroxidase was performed with 0.0125% diaminobenzidine (DAB) and 0.002% H2O2 in 0.05 M Tris-HCl buffer (pH 7.6) for 10 min. The sections were viewed with a Nomarski differential interference contrast (DIC) microscope (Olympus, Tokyo, Japan).

Using serial sections, the number of TUNEL-positive nuclei in the epithelium of each villus was counted.

**Agarose gel electrophoresis**

The genomic DNA from the pellets of the rinsed fluids in the duodenal lumen was prepared as previously described (SAMBROOK et al., 1989). The sample was subjected to electrophoresis on a 1.8% agarose gel. To detect DNA fragments, the gel was stained with etidium bromide.

**Immunohisto/cytochemistry**

Antibody: Rabbit antibody against rat cathepsin B was prepared and purified by affinity chromatography as reported previously (KOMINAMI et al., 1985; UCHIYAMA et al., 1989, 1990).

For light microscopy, the jejunal tissue cut into
small pieces, was frozen by Freon 22 cooled by liquid nitrogen and stored in liquid nitrogen until use. According to the method by Grube (1980), all frozen samples were freeze-dried at −35°C for 72 h, fixed by vapor-phase PA at 80°C for 1 h, and embedded in Epon 812. Serial semi-thin sections were cut at 1 μm with an ultramicrotome and mounted on clean glass slides by heat. The resin was removed with sodium methoxide (Mayor et al., 1961). Sections were treated with 0.3% H₂O₂ in methanol for 30 min and incubated with 2% normal goat serum (NGS) in PBS containing 0.05% Tween 20 (Sigma, U.S.A.) (TPBS) for 20 min at RT. They were incubated with anti-cathepsin B (4 μg/ml) diluted with TPBS for 72 h at 4°C. Further incubations were performed with biotinylated goat anti-rabbit IgG and then with streptavidin-peroxidase (Histofine, Nichirei) at RT for 30 min. After each step, the sections were rinsed thoroughly in 0.01 M phosphate-buffered 0.5 M NaCl (pH 7.2), containing 0.1% Tween 20. Staining for peroxidase was performed in the same manner as stated above and immunostained

Fig. 1. TEM image of luminal cell elements obtained by rinsing the duodenal lumen. Most collected cells have microvilli on the cell surface and nuclei (N) with distinct chromatin condensation. Small or large clear vacuoles (arrowheads) and dense bodies (arrows) are seen in the cytoplasm, while mitochondrial profiles appear intact. ×2,400

Fig. 2. Light micrographs of rinsed duodenal cell elements nick end labeled with biotinylated dUTP introduced by terminal deoxytransferase (TUNEL). The nuclei of most collected cells are positively stained by the reaction. Differential interference contrast. ×960
sections were viewed with a DIC microscope.

For TEM, tissues cut into small pieces were fixed with 4% PA-0.1% GA buffered with CB at 4°C for 30 min and with 4% PA buffered with CB for 1 h. The samples were dehydrated with graded alcohols and embedded in LR White. Thin sections were cut with an ultramicrotome and mounted on nickel grids.

Thin sections were incubated with 5% NGS for 20 min at RT. They were incubated with anti-cathepsin B (38 μg/ml) at 4°C for three days. Then they were treated with gold-labeled goat anti-rabbit IgG for 1 h; the gold particles were coated and their size (15 nm in diameter) adjusted according to the method by Slot and Geuze (1985). Between each step, the grids were rinsed in 0.02 M Tris-HCl-buffered 0.5 M NaCl, pH 8.2, containing 0.1% bovine serum albumin (BSA; Sigma).

After the immunoreactions, the sections were stained with a saturated aqueous solution of uranyl acetate and lead citrate and observed with an electron microscope.

Control experiments: Control semi-thin sections were incubated with anti-cathepsin B adsorbed by rat liver cathepsin B or with a non-immune rabbit serum diluted to 1:1,000, followed by incubation with biotinylated goat anti-rabbit IgG and streptavidin-peroxidase. Some sections were directly incubated with the second antibody without any preceding incubation with the first antibody. For immuno-electron microscopy, thin sections were incubated with the adsorbed antibody or with the non-immune rabbit serum diluted to 1:200, followed by the gold-labeled second antibody. Some sections were directly incubated with the gold-labeled second antibody without pre-treatment with the first antibody.

All controls confirmed the specificity of the immunoreactivity in light and electron microscopy.

RESULTS

Luminal cell elements in the rinsed duodenal fluids

To examine whether exfoliated enterocytes have nuclei, luminal cell elements in the duodenum were morphologically observed. Most cells found in the rinsed fluids had microvilli on the cell surface and nuclei (Fig. 1), indicating that they were enterocytes with nuclei and had been exfoliated from the duodenal epithelium. They contained numerous clear vacuoles and autophagic vacuoles in the cytoplasm, while their nuclei had the marginally condensed chromatin which is typical of apoptosis. DNA nick end labeling of dUTP was then applied to smears of the rinsed duodenal fluids; most cellular elements were positively stained by the TUNEL reaction (Fig. 2). Simultaneously, genomic DNA extracted from the pellets of the rinsed duodenal fluids was analyzed by agarose gel electrophoresis. As shown in Figure 3, genomic DNA was fragmented into oligonucleosomes. These results suggest that effete epithelial cells in the human small intestine are exfoliated totally as a whole cell body, while exfoliated enterocytes have apoptotic features.

Electron microscopy

It has been believed that dying enterocytes possessing nuclei with chromatin condensation are scarcely seen at the villus tips of the small intestine (Kerr et al., 1987). Therefore, to examine the complete profiles of effete enterocytes at the villus tips, we prepared two
Fig. 4. SEM image of the villus tips of the human small intestine. A and B. SEM samples not rinsed before fixation. Numerous protruding enterocytes (arrows) having microvilli on the cell surface can be seen at the villus tips (A). A protruding enterocyte (PE) forms a dome-like profile (B). C and D. SEM samples thoroughly rinsed before fixation. No protruding cells are detected at the villus tips (C), whereas a small pit (arrow) is seen at a villus tip (D). A: ×730, B: ×7,300, C: ×640, D: ×2,800
Fig. 5. Legend on the opposite page.
types of SEM samples: one was fixed with 2% PA-2% GA immediately after intestinal samples were obtained, while the other was first washed thoroughly with PBS, and then fixed with the fixative. In the non-rinsed SEM samples, protruding enterocytes from the epithelial layer of the small intestine were found at the villus tips (Fig. 4A). These protruding enterocytes, appearing independently and generally having microvilli on the cell surface, seemed to form dome-like profiles (Fig. 4B). In the rinsed SEM samples, almost no protruding enterocytes were discerned at the tip of villi, but small pits were often found at the tip (Fig. 4C, D). These pits seemed to be the traces where exfoliated enterocytes lay.

To confirm the morphological features of these protruding enterocytes by TEM, the SEM samples were embedded in Epon 812 and serial thin sections having appropriate villus tips were examined. Enterocytes at the villus tips possessed numerous clear vacuoles and vacuoles containing dense materials or membranous structures (autophagic vacuoles) in the cytoplasm (Fig. 5A). These enterocytes often lacked...
a basal cytoplasm and/or extended their thin cytoplasmic processes to the basal lamina, so that a wide intercellular space was formed in the basal part of the epithelium. In the intercellular space or along the baso-lateral membranes of these enterocytes, no bleb-like structure or protrusion was seen. These enterocytes contained round or irregularly shaped nuclei with dispersed chromatin, but the nuclei mostly appeared intact (Fig. 5A, B). The enterocytes were connected with adjacent enterocytes via junctional complexes and had microvilli on the luminal surface (Fig. 5C). Some enterocytes protruded into the lumen, forming bridge or dome-like structures (Figs. 5B, 6A). These dome-like enterocytes connecting with the adjacent enterocytes possessed irregularly shaped nuclei with or without marginally condensed chromatin. Some protruding enterocytes showing shrinkage and nuclear chromatin condensation displayed a loose connection with adjacent enterocytes only on one side (Fig. 6B). In such cases, the connection between the adjacent enterocytes was detected beneath the protruding enterocytes. No enterocytes exhibited nuclear chromatin condensation before they protruded into the lumen and formed dome-like profiles. Moreover, the intercellular spaces formed widely in the basal part of the epithelium at the villus tips were often occupied by large lymphocytes. As far as we examined, these lymphocytes did not contain specific granules in the cytoplasm (Fig. 5A), while they always contacted effete enterocytes, often extending their cytoplasmic processes along the lateral surface of effete enterocytes.

Thus, the protruding enterocytes at the villus tips had features typical of apoptotic cells, consisting of cytoplasmic and nuclear chromatin condensations before they were exfoliated. In the rinsed SEM samples, we failed to find protruding enterocytes, suggesting that these cells are easily lost during sample preparation. We then counted these protruding enterocytes at the villus tips using the non-rinsed SEM samples of the small intestine; the incidence of the protruding cells per villus was 6.8±4.5 (mean±standard deviation) (Fig. 7).

To confirm the presence of effete or protruding enterocytes at the tip of villi using carefully processed TEM samples, we prepared serial semi-thin sections stained by toluidine blue and chose appropriate sections that were further processed for thin sections. Enterocytes at the villus tips mostly possessed irregularly shaped nuclei with dispersed chromatin (Fig. 8A). They contained numerous mitochondria, rough endoplasmic reticulum, and dense bodies in the apical cytoplasm, with an often reduced basal cytoplasm. Therefore, the intercellular space became wide in the basal part of the epithelium. Some enterocytes contained clear vesicles in the cytoplasm, while they elongated their slender cytoplasmic processes to the basal lamina to enclose the intercellular space. The intercellular space was also seen in the upper part of the epithelium, though it was narrow. In these villus...
tips existed protruding enterocytes that possessed nuclei with chromatin condensation and contained clear and autophagic vacuoles in the cytoplasm (Fig. 8B, C). Their basal cytoplasm was reduced and often occupied by adjacent enterocytes. In the intercellular space, large lymphocytes were often seen, while neither a bleb nor apoptotic body was seen in the epithelial layer or in the subepithelial region (Fig. 8A).

Fig. 8. TEM image of a villus tip of the human small intestine. The sample was carefully processed for TEM. A. Enterocytes at the villus tip have a reduced basal cytoplasm, so that the intercellular space (*) widens in the basal part of the epithelium, where large lymphocytes (L) are seen. Some enterocytes possess clear vesicles in the cytoplasm (arrows), while they elongate their slender cytoplasmic processes to the basal lamina. B. A protruding enterocyte contains a nucleus with chromatin condensation and reduces a basal part of the cytoplasm. The cell possesses numerous clear vesicles and autophagic vacuoles. C. In the apical part of the effete enterocyte, numerous autophagic vacuoles, which often contain membranous structures and parts of the cytoplasm, can be seen (arrows). A: ×2,400, B: ×4,500, C: ×13,000
TUNEL reaction and lysosomal alterations of effete enterocytes

As SEM and TEM confirmed that apoptotic enterocytes appear at the villus tips of the small intestine, the TUNEL reaction was applied to cryosections of the small intestine. As shown in Figure 9, positive staining of the TUNEL reaction was detected in the epithelium of all villi when serial cryosections stained were examined. The positively stained enterocytes appeared not only at the tips but also around the tips. This indicates the possibility that DNA fragmentation occurs in effete enterocytes which do not always have morphological features of apoptosis. Therefore, we counted the number of TUNEL-positive enterocytes per villus using serial cryosections; it was $28.9 \pm 14.3$ (mean ± standard deviation) (Fig. 10).

As effete enterocytes at the villus tips possessed numerous membrane bounded vacuoles containing dense materials or membranous structures, we further examined the immunohistochemical features of lysosomes in the epithelium of the small intestine using a monospecific antibody against cathepsin B. In semi-thin sections, fine granular immunodeposits for cathepsin B were localized in the apical part of the epithelial cells (Fig. 11A). These cathepsin B-immunopositive granules in the enterocytes became coarse at the villus tips and increased in number (Fig. 11A, B). In the lamina propria, cathepsin B-immunopositive macrophages were present throughout the villi, though in some cases these macrophages clustered near the

![Fig. 9](image_url)  
**Fig. 9.** TUNEL staining of the human small intestine. A and B. Positive staining is demonstrated in epithelial cells located not only at the villus tips but also at the upper region of the villi. Differential interference contrast. ×400

![Fig. 10](image_url)  
**Fig. 10.** Histogram of TUNEL positive epithelial cells in human small intestinal villi. The number of TUNEL positive epithelial cells per villus was counted using serial cryosections. mean ± standard deviation.
Effete Epithelial Cells in Human Intestinal Villi

villus tips (Fig. 11). By electron microscopy, immunogold particles indicating cathepsin B were localized in homogeneously dense lysosomes located in the apical cytoplasm of most enterocytes. In enterocytes at or near the villus tips, these immunogold particles labeled membrane-bounded vacuoles containing dense materials, which were often scattered in the lumenal part of the cytoplasm (Fig. 12), indicating that the vacuolar structures in effete enterocytes near the villus tips were autolysosomes.

DISCUSSION

The main findings of the present study on the exfoliating processes of effete enterocytes at the villus tips of the human small intestine are the following: 1) Exfoliated enterocytes in cellular elements obtained by rinsing the duodenal lumen showed cell shrinkage, nuclear chromatin condensation, and DNA fragmentation. 2) Exfoliating or protruding enterocytes showed nuclear chromatin condensation at the villus tips when they were shrunk and formed dome-like profiles. 3) Junctional complexes with adjacent enterocytes were present until exfoliation. 4) The intercellular space formed by the shrinkage of effete enterocytes was often occupied with lymphocytes.

The total process of renewing enterocytes in the small intestine involves the proliferation, differentiation, migration and eventual loss or death of the cells (LEBLOND and MESSIER, 1958; MACDONALD et al., 1964; CHENG and LEBLOND, 1974; EASTWOOD, 1977; LEBLOND, 1981; WRIGHT and ALISON, 1984). Effete enterocytes die by apoptosis, although exfoliating enterocytes...
showing typical features of apoptosis are hardly seen at the villus tips (KERR et al., 1987). In fact, [3H] thymidine-labeled epithelial cells have been demonstrated in gastric washings from normal persons in a study on renewal of gastrointestinal epithelial cells (MACDONALD et al., 1964), although there is no direct evidence for the fate of effete epithelial cells in the human small intestine. In the guinea pig small intestine, however, no epithelial cells are seen in cellular elements obtained by rinsing the intestinal lumen, but only small apical cytoplasmic pieces of enterocytes with microvilli are found (IWANAGA et al., 1993). In the present study, therefore, we examined morphological features of exfoliated enterocytes obtained by rinsing the human duodenal lumen; cellular elements in duodenal rinsing were mostly enterocytes showing features typical of apoptosis: cytoplasmic condensation, nuclear chromatin condensation and DNA fragmentation into oligonucleosomes. This suggests that senescent enterocytes exfoliated from the villus tips of the small intestine probably have apoptotic features.

To our knowledge, there has been no report on the presence of exfoliating enterocytes with morphological features typical of apoptosis, particularly nuclear chromatin condensation at the villus tips of the small intestine. The present results using SEM and TEM indicated that exfoliating enterocytes with the characteristic figures of apoptosis are easily lost from the villus tips during the tissue preparation, especially by rinsing the intestinal tissues before fixation. In our observations of the carefully processed intestinal tissues for electron microscopy, outstanding events appearing in effete enterocytes at the villus tips as follows: 1) the cells were shrunken in the basal cytoplasm, resulting in the enlargement of the intercellular spaces at the villus tips; 2) the cell shrinkage further proceeded and the cells protruded into the lumen, forming dome-like profiles; and 3) chromatin was marginally condensed in nuclei immediately before exfoliation (Schematic presentation in Fig. 13).

Autophagy, consisting of the sequestration of intracellular components and their degradation by lysosomal enzymes, usually occurs in normal cells to maintain cellular turnover, while it is known to greatly increase in cells under pathological conditions which cause cell dysfunction such as hypoxia, ischemia, endotoxin shock, and metabolic inhibitors (GLAUMANN et al., 1981). The present electron microscopic and immunohistochemical examinations demonstrated that enterocytes located around the villus tips possessed numerous clear vacuoles and autophagic vacuoles immunopositive for cathepsin B, a representative cysteine proteinase in lysosomes. It has been shown that autophagic dying cells undergo intense endocytosis, which can be thought of as inward blebbing and serves as well to reduce the area of the cytoplasm (CLARKE, 1990). In the present study, no outward blebbing was observed around effete enterocytes at the villus tips. Therefore, it seems likely that the presence of numerous clear vacuoles in effete enterocytes is attributable to inward blebbing of the baso-lateral membranes of the cells, resulting in the shrinkage of the effete enterocytes. From the present study, it remains unknown whether the formation of clear vesicles in enterocytes precedes that of autophagic vacuoles. However, it is inevitable that the reduced baso-lateral membranes of enterocytes subsequently alter cellular turnover, an event which may facilitate the formation of autolysosomes. In the process of autophagic alternations frequently occurring in dying cells, the autophagy is believed to protect the cells from death (CLARKE, 1990).

In the guinea pig small intestine, the greater parts of effete enterocytes are phagocytosed by subepithelial macrophages which cluster in the villus tips (IWANAGA et al., 1993; HAN et al., 1993; HAN, 1993). The present

**Fig. 12.** Immunocytochemical localization of cathepsin B in an enterocyte located at a villus tip of the human small intestine. Immunogold particles indicating cathepsin B are localized in lysosomes containing heterogeneously electron dense materials. ×30,000
immunohistochemical and electron microscopic studies showed that macrophage clusters were not always found at the villus tips of the human small intestine, but macrophages were usually dispersed in the lamina propria. This type of macrophage localization in the lamina propria of the human small intestine is similar to that in rats and mice whose intestinal epithelial cells are suggested to be exfoliated from the villus tips (LEBLOND, 1981). The results indicate that macrophages in the lamina propria of the human small intestine do not participate in the exfoliating process of effete enterocytes. This notion is compatible with the present findings that the outward blebbing of effete enterocytes and apoptotic bodies were not detected at the villus tips, supporting the hypothesis that the shrinkage of effete enterocytes is mediated by inward blebbing, reflecting the presence of numerous clear vesicles in the cells.

By freeze-fracture and light and electron microscopic studies, effete enterocytes at the villus tips of rat and hamster small intestine have been shown to extrude into the lumen, during which junctional complexes of extruding enterocytes with neighboring cells are maintained by proliferating junctional elements along the lateral margin of the cells (MADARA, 1990). As mentioned above, the apical cytoplasmic pieces at the villus tips of the guinea pig small intestine maintain junctional complexes with adjacent enterocytes, while the adjacent enterocytes form new junctional complexes beneath the exfoliating apical cytoplasmic pieces when they are exfoliated from the villus tips (IWANAGA et al., 1993). As stated in the Results, the maintenance of junctional complexes during the exfoliating process of effete enterocytes in the human small intestine is similar to that in the guinea pig small intestine, although exfoliating enterocytes in the human small intestine always contain nuclei. Villus tip extrusion zones are widely cited as potential sites for transepithelial macromolecular leaks (WALKER, 1981). From morphological observations in various mammals, however, an epithelial barrier mediated by junctional complexes is always maintained at the villus tips during the exfoliation of effete enterocytes.

IWANAGA et al. (1993) have hypothesized that the disposal of effete enterocytes is not simply attributable to the gene expression (programming) for apoptosis in the enterocytes. They have shown the presence of large granular lymphocytes which closely contact effete enterocytes, and that their cytoplasmic processes often penetrate effete enterocytes at the villus tips of the guinea pig small intestine (IWANAGA et al., 1993; HAN, 1993), suggesting that these lymphocytes may participate in the activation of an endogenous suicide program in the cells. In the present

Fig. 13. A schematic presentation of the exfoliating process of effete epithelial cells at a villus tip of the human small intestine. E enterocyte, L lymphocyte.
study, we also found large lymphocytes closely associated with effete enterocytes at the villus tips. Most lymphocytes found at the tip of villi contained no dense granules. According to Cerf-Bensussan et al. (1985), intraepithelial lymphocytes isolated from the human small intestine express the phenotype associated with cytotoxic-suppressor T cells, but they do not show any cytotoxic activity when stimulated with known potentiators of natural killer activity. At present, it remains unknown whether the large lymphocytes found at the villus tips can induce apoptosis of enterocytes. Further studies are required to define the functional characteristics of these lymphocytes.

In the present study, enterocytes showing cell shrinkage and nuclear chromatin condensation were limited to the cells that protruded into the lumen at the villus tips and formed dome-like profiles. The number of these protruding enterocytes per villus was 6.8 ± 4.5 when counted using SEM. On the other hand, TUNEL-positive enterocytes were widely distributed in the epithelium of the upper villus portion, and counted 28.9 ± 14.3 per villus. From these results, it seems likely that DNA fragmentation in enterocytes occurs prior to morphological alterations in the nuclei of the cells when examined by the TUNEL method. At present, it is difficult to define what reversible or irreversible alterations lie in the cells. According to an in vitro study of neonatal sympathetic neurons, DNA fragmentation of the neurons occurs close to the neuronal death (Deckwerth and Johnson, 1993). This may indicate that TUNEL-positive enterocytes are closely linked to cell death. Therefore, it may be important to reveal the morphological features of these TUNEL-positive enterocytes, when establishing an accurate definition of morphological features of cell death. Moreover, the fact that the exfoliating processes have not been shown until the present study may suggest that the exfoliating processes occur within a short duration after the initiation of DNA alterations.

From the present results, it is concluded that enterocytes in the human small intestine are exfoliated into the lumen at the villus tips without destroying the epithelial barrier, showing features typical of apoptosis—cell shrinkage and nuclear chromatin condensation—before exfoliation. During the exfoliating processes, no heterophagocytosis of dying enterocytes is mediated by subepithelial macrophages, but intraepithelial lymphocytes are closely associated with effete enterocytes at the villus tips.

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Prof. Yasuo UCHIYAMA
Department of Anatomy
Osaka University Medical School
2-2 Yamadaoka, Suita
Osaka, 565 Japan

内 山 安 男
565 大阪府吹田市山田丘 2-2
大阪大学医学部
解剖学第一講座