Cell Tissue Res (1993) 271:407–416

Cell & Tissue Research

Aggregation of macrophages in the tips of intestinal villi in guinea pigs: their possible role in the phagocytosis of effete epithelial cells

Hongxia Han[1], Toshihiko Iwanaga[1], Yasuo Uchiyama[2], Tsuneo Fujita[1]

[1] Department of Anatomy, Niigata University School of Medicine, Asahi Machi 1, Niigata, 951 Japan
[2] Department of Anatomy, Iwate Medical University School of Medicine, Uchimaru 19-1, Morioka, 020 Japan

Received: 4 June 1992 / Accepted: 14 September 1992

Abstract. Numerous macrophages were found aggregated in the lamina propria at the tips of villi in the small intestine of guinea pigs. These macrophages extended their pseudopodia into the epithelial lining and internalized fragments of effete enterocytes in their phagosomes. The epithelium of the villus tips was found to be infiltrated with numerous lymphocytes. They possessed electron-dense granules characteristic of natural killer cells, and actively interdigitated with the enterocytes. The latter were either fragmented or extensively lost in their basal cytoplasm, often leaving an attenuated apical cytoplasm of the cell. Immunohistochemical labeling using bromodeoxyuridine demonstrated that at 96 h after its administration, immunolabeled nuclei were encountered in the cytoplasm of macrophages in the lamina propria at the villus tips. These findings suggest that in the guinea pig, effete enterocytes are not simply exfoliated into the lumen, but are damaged by intraepithelial lymphocytes possessing a natural killer cytotoxicity, and subsequently phagocytosed by subepithelial macrophages.

Key words: Intestine, small – Intraepithelial lymphocytes – Macrophages – Ultrastructure – Guinea pig

Introduction

It is well known that macrophages, in addition to lymphocytes, plasma cells and granulocytes, are found in large numbers in the lamina propria of the gut (Deane 1964; Pabst 1987), reflecting the need for a powerful defense system in the intestinal mucosa due to its continuous exposure to pathogenic antigens. Aggregations of macrophages containing numerous phagosomes in the lamina propria have been documented in certain forms of chronic inflammation (Cohn 1968). Von Möllendorff (1925) and Maximow (1927) recorded that in the normal guinea pig, macrophages frequently gathered in the lamina propria at the tips of intestinal villi. This phenomenon seems to have been overlooked by later researchers, until Sawicki et al. (1977) reported concurrent data in the guinea pig. Von Möllendorff (1925) and Maximow (1927) assumed that the macrophages at the villous apices might take up certain substances from the intestinal lumen. In contrast, Sawicki et al. (1977) suggested that these macrophages might "play a role in the phagocytosis of some migrating cells of the intestinal mucosa, most probably of the sheath-fibroblasts and/or intraepithelial lymphocytes". These authors did not note the possibility that the epithelial cells (enterocytes) migrating to the villus tips might also be ingested by the macrophages.

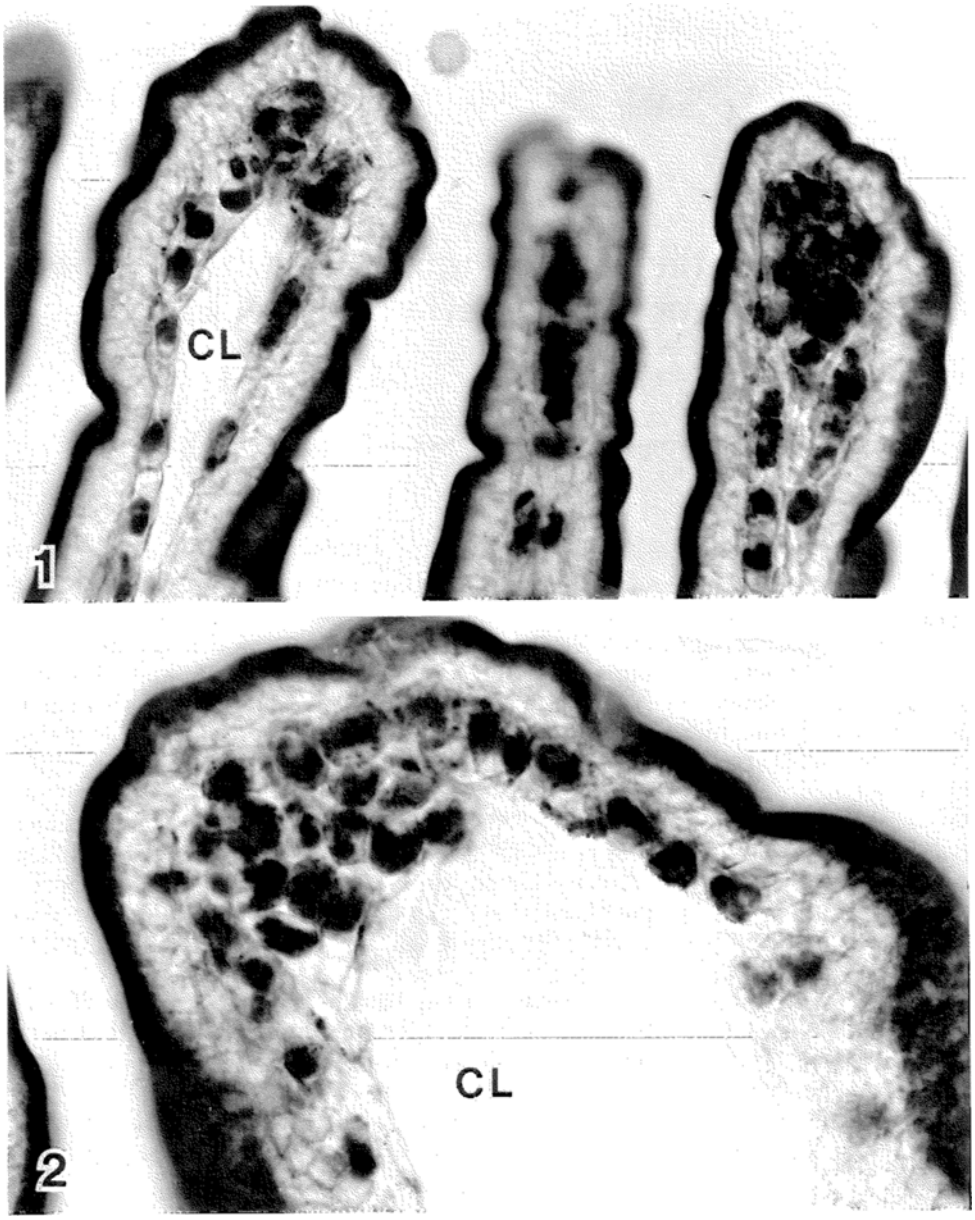
Meanwhile it has become a generally accepted view that the enterocytes, originating in crypts, move toward the villus tips eventually being extruded into the lumen (for reviews, see Eastwood 1977; Leblond 1981). This view has been based on autoradiographic studies using ^{3}H-thymidine in mice and rats (Leblond and Messier 1958; Quastler et al. 1959; Cheng and Leblond 1974a). In other mammalian species including the guinea pig, only limited information is available. Furthermore, very little morphological evidence has been presented for mammalian species indicating that aged enterocytes are actually exfoliated into the lumen.

In our preliminary observation of the guinea pig small intestine, we confirmed the early records on the aggregation of macrophages at the tips of villi and obtained findings supporting a hitherto undescribed view that these macrophages, in collaboration with lymphocytes, might be engaged in scavenging aged enterocytes. The present study aims at demonstrating events taking place at the tips of the villi in the guinea pig intestine, with special reference to the functional significance of the aggregated macrophages.

Materials and methods

Twenty male Hartley guinea pigs, weighing 250–300 g, were used in this study. The animals were anesthetized with pentobarbiturate

Correspondence to: T. Fujita



Figs. 1, 2. Histochemical staining for acid phosphatase activity in the ileum of a guinea pig. Macrophages with positive reaction for acid phosphatase are gathered at villus tips in the lamina propria near central lacteals (*CL*). Another site of intense activity is found in the striated border of enterocytes, but this reactivity was not inhibited by NaF in control experiments. **1**: ×350; **2**: ×500

and perfused via the aorta with physiological saline followed by 4% paraformaldehyde in 0.1 M phosphate buffer, pH 7.4. Various regions of the intestine from the gastric corpus to the rectum, at intervals of 5 cm, were obtained and immersed in the same fixative for an additional 6 h. The tissues were dipped in 30% sucrose solution overnight at 4° C and rapidly frozen in liquid nitrogen. Frozen sections, about 20 µm in thickness, were prepared in a cryostat (Coldtome CD-41, Sakura Inc., Tokyo, Japan) and stained for the detection of acid phosphatase activity, according to Burnstone (1958). Control experiments for acid phosphatase reactions were simultaneously carried out by incubating with the medium containing 10 mM NaF, a potent inhibitor for this enzyme. Paraformaldehyde-fixed specimens were also dehydrated via an ethanol-xylene series and embedded in paraffin. Paraffin sections, 4–6 µm thick, were stained with hematoxylin-eosin.

Localization of cathepsin B was immunocytochemically examined according to the avidin-biotin complex (ABC) method. Frozen sections were incubated with a rabbit antibody against rat cathepsin B diluted 1:1000 (0.6 µg/ml). Detailed characterization of the antiserum has been described elsewhere (Kominami et al. 1984, 1985). Specificity of the immunoreactions was confirmed by preincubation of the antiserum with rat liver cathepsin B.

For electron microscopy, another 10 animals were perfusion-fixed with 2.5% glutaraldehyde in 0.1 M phosphate buffer, pH 7.4. The jejunum and ileum were removed and dissected out into small pieces. After immersion in the same fixative for 2 h, the tissue blocks were postfixed for 1.5 h in 1% OsO_4 dissolved in phosphate buffer, dehydrated through a series of graded ethanol and embedded in Araldite via propylene oxide. The ultrathin sections were stained with uranyl acetate and lead citrate, and examined under a Hitachi H-7000 transmission electron microscope.

Twelve guinea pigs were injected peritoneally with 5-bromodeoxy-uridine (BrdU) (40 mg/kg body weight, saline solution, WAKO Chemicals, Tokyo). Subsequently, they were divided into four groups of 3 animals each, to be sacrificed after 12, 60, 72 and 96 h, respectively. The tissues obtained from the jejunum and ileum were fixed in absolute ethanol or Bouin's fluid for 6 h and embedded in paraffin. Dewaxed paraffin sections were incubated in 0.2 N HCl for 1 h at room temperature and neutralized with 0.1 M $Na_2B_4O_7$ (Moran et al. 1985; Sugihara et al. 1986). The

immunohistochemical detection of BrdU in the sections was carried out according to the avidin-biotin complex (ABC) method using a monoclonal antibody against BrdU (MAB-019, Chemicon International Inc., USA) diluted 1:200.

For the specificity control, an absorption test was performed with diluted antiserum pretreated with BrdU at a concentration of 10 µg/ml for 24 h at 4° C. After incubation in the control serum, no immunoreactive elements were recognizable.

Results

Distribution of intestinal macrophages

Numerous macrophages were found aggregated in the lamina propria near the apex of intestinal villi. They were easily identified in hematoxylin-eosin stained sections, due to their large (15–30 µm in diameter) eosinophilic cytoplasm, but could be unequivocally demonstrated by virtue of the acid phosphatase and cathepsin B reactions (Figs. 1–3). The activities for acid phosphatase (Figs. 1, 2) and cathepsin B (Fig. 3) were concentrated within small and large round bodies which were randomly dispersed in the cytoplasm.

An aggregation of macrophages was found in all villi throughout the small intestine, i.e., from the duodenum to the terminal end of the ileum. The cell aggregations were larger in size in the jejunum and ileum than in the duodenum. The major population of macrophages was located in the lamina propria at the tips of villi, occupying a rather well demarcated space between the epithelium and the domed end of the central lacteal (Fig. 2). The number of macrophages in each villus usually amounted to more than 10 per section. No special structures such as the intervention of blood vessels or reticular cells were recognized either in or outside the cell aggregations.

At the lower two-thirds of the intestinal villi, smaller macrophages with weaker acid phosphatase and cathepsin B reactivities were dispersed on the lateral sides of the central lacteals (Fig. 3). The macrophages were rare in the lamina propria around crypts.

Intense activity for acid phosphatase was also localized at the striated border of the epithelium (Figs. 1, 2). The activity in the striated border was not inhibited by preincubation of the medium with NaF, while that in the macrophages perfectly disappeared. A specific immunoreactivity for cathepsin B was also found in the cytoplasm of enterocytes, restricted to the villus tips; it was, however, fine granular and weak in reaction.

Ultrastructure of aggregated macrophages

The macrophages in the lamina propria at the tips of intestinal villi were often polygonal, being closely packed and pressed, and showing an epithelioid arrangement (Figs. 4, 8). The nucleus was usually round or oval and contained a distinct nucleolus. The cytoplasm possessed numerous phagosomes and residual bodies of varying sizes as well as smaller dense bodies, most of which were presumed to be lysosomes (Figs. 4, 5, 8). The phagosomes, especially large-sized ones (more than 3 µm in diameter), contained cytoplasmic fragments rich in mitochondria, Golgi apparatus and rough endoplasmic reticulum, which closely resembled the cell organelles of the enterocytes but distinctly differed from those of subepithelial fibroblasts or lymphocytes (Fig. 5). The cytoplasmic fragments in the phagosomes were occasionally accompanied by pyknotic nuclei (Figs. 4, 5), but the microvillous cell portion was never included. The contents of the phagosomes showed various stages of apparent digestion of cellular elements. Occasionally, large portions of cells maintaining an intact cell structure indistinguishable from that of the enterocytes were found internalized in the macrophages.

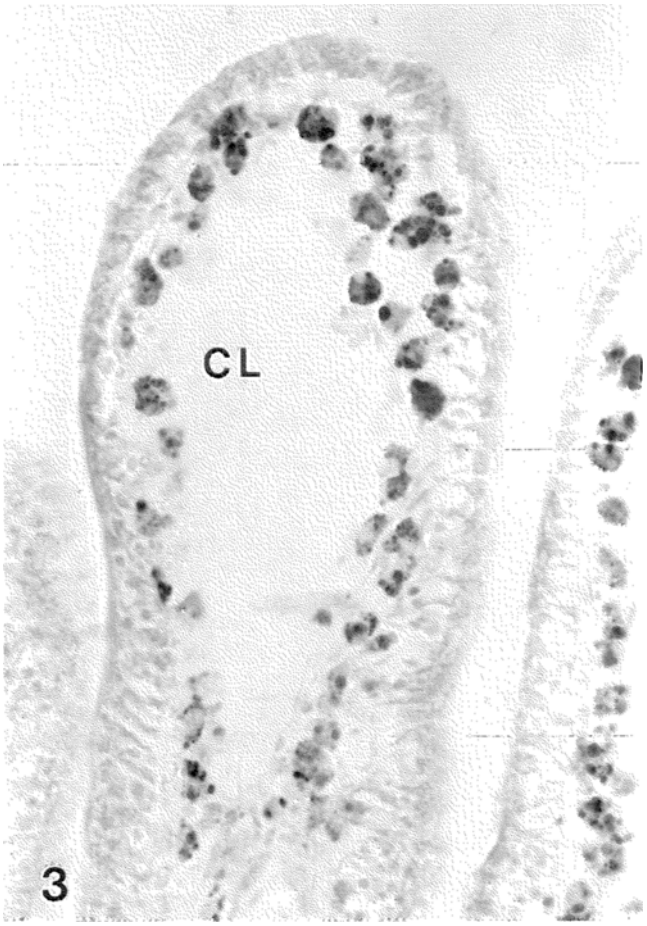
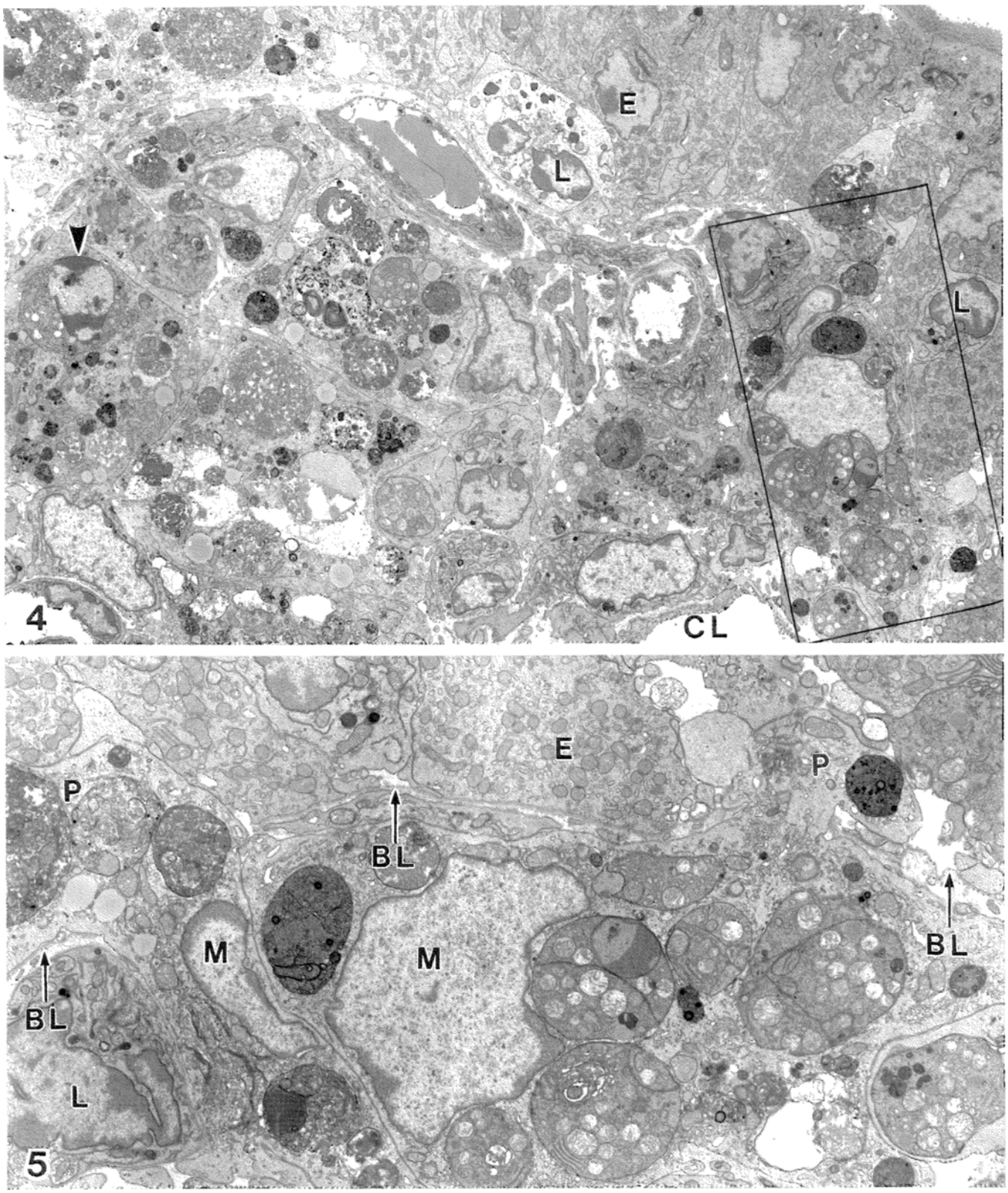
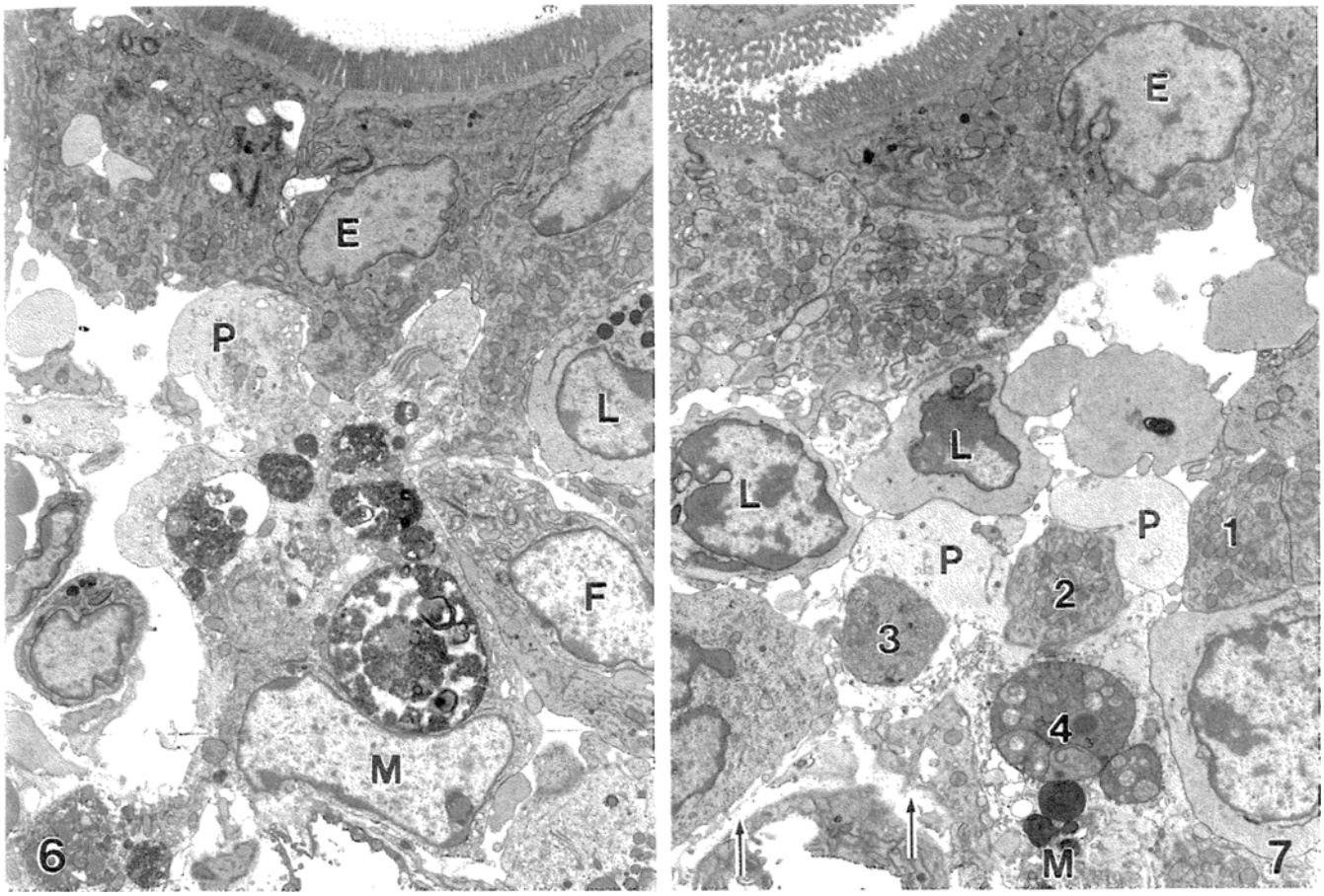


Fig. 3. Immunohistochemistry for cathepsin B in the jejunum of a guinea pig. Immunoreactive macrophages are numerous in the subepithelial region of the villus tip. Macrophages in the lower part of the villi are smaller and less intensely immunoreactive. *CL* Central lacteal. ×300

Phagocytotic activity of macrophages upon enterocytes could be demonstrated more clearly at the base of the epithelium. Macrophages beneath the basal lamina very often extended one or more pseudopodia deep into the epithelial layer (Figs. 5–7). It was not rare for the cell bodies of macrophages to be located in the epithelial lining between enterocytes. Fragments of enterocytes as depicted below appeared to be internalized by pseudopodia of macrophages. Various phases of interna-



Figs. 4, 5. Aggregation of macrophages at the villus tip in the ileum. Numerous macrophages are closely packed in the lamina propria. Phagosomes in the macrophages vary in their contents, some including almost intact cellular elements (*arrowhead*). **5** Higher magnification of the *boxed area* in **4**. Two macrophages (*M*) beneath the basal lamina (*BL*) extend their cytoplasmic processes (*P*) into the epithelial layer. Phagosomes in the cytoplasm contain mitochondria-rich cellular elements. *CL* Central lacteal; *E* enterocytes; *L* lymphocytes. **4**: ×3000; **5**: ×6800



Figs. 6, 7. Enterocytes at villus tips in ileum of guinea pigs. Pseudopods (*P*) of macrophages (*M*) extend into the epithelial layer; in **6**, they contact directly the excavated aspect of the enterocytes (*E*). In **7**, cytoplasmic fragments of enterocytes found in the intercellular space are presumably being engulfed by the pseudopods (*P*) of a macrophage. Numbers *1–4* indicate possible orderly sequential engulfment of these fragments. *Arrows* indicate the basal lamina showing the original boundary of the epithelium now infiltrated with lymphocytes (*L*) and macrophages; *F* fibroblast. **6**: ×3700; **7**: ×4700

lization by pseudopodia could be seen: some pseudopodia directly contacted the fragmented cytoplasm, while others enveloped or engulfed it (Fig. 7).

Intraepithelial lymphocytes at the villus tips

Numerous lymphocytes were found to invade the epithelium at the tips of villi (Figs. 4, 7, 8). Intraepithelial lymphocytes were less frequent in the lower portion of intestinal villi. The Golgi apparatus of lymphocytes was comparatively well developed in the perinuclear region, but the rough endoplasmic reticulum was scanty. Many of the intraepithelial lymphocytes contained electron-dense granules, 300–600 nm in diameter (Fig. 8), and were identifiable as large granular lymphocytes (LGLs). It was rare that the lymphocytes possessed rod-cored vesicles which represent another type of granular elements frequently observed in the rat LGLs (Kaneda 1989). The electron-dense granules tended to gather in paranuclear portions of the cytoplasm; together with mitochondria they were usually located close to the Golgi apparatus.

The lymphocytes extended irregular-shaped cell processes, deeply interdigitating with the enterocytes (Figs. 8, 9). Both cells were thus tightly juxtaposed without any intervening spaces. Many irregular-shaped spaces, with no structural contents, were dispersed in the epithelium. Dome-shaped cavities were occasionally formed, leaving an attenuated apical cytoplasm with microvilli (Figs. 8, 9). Enterocytes at the villus tips were characterized by fragmentation or a pinching-off of the cytoplasm (Figs. 7–9), presumably due to the invasion of lymphocytes into the epithelium. Such fragmentation was remarkable in the basal half of the epithelial lining. Thus, membrane-bounded cytoplasmic fragments with or without a nucleus were distributed close to the basement membrane. The plasma membranes and organelles in the cell fragments remained essentially intact in ultrastructure, not showing any marked morphological signs of degeneration. The apical portion of the epithelium, despite such fragmentation and loss of the basal cell portion, was still intact, although its microvilli were irregular in arrangement and extended in length.

The lymphocytes frequently showed an intimate topographical relationship with macrophages in and beneath the epithelium; many intraepithelial lymphocytes with electron-dense granules were in contact with the

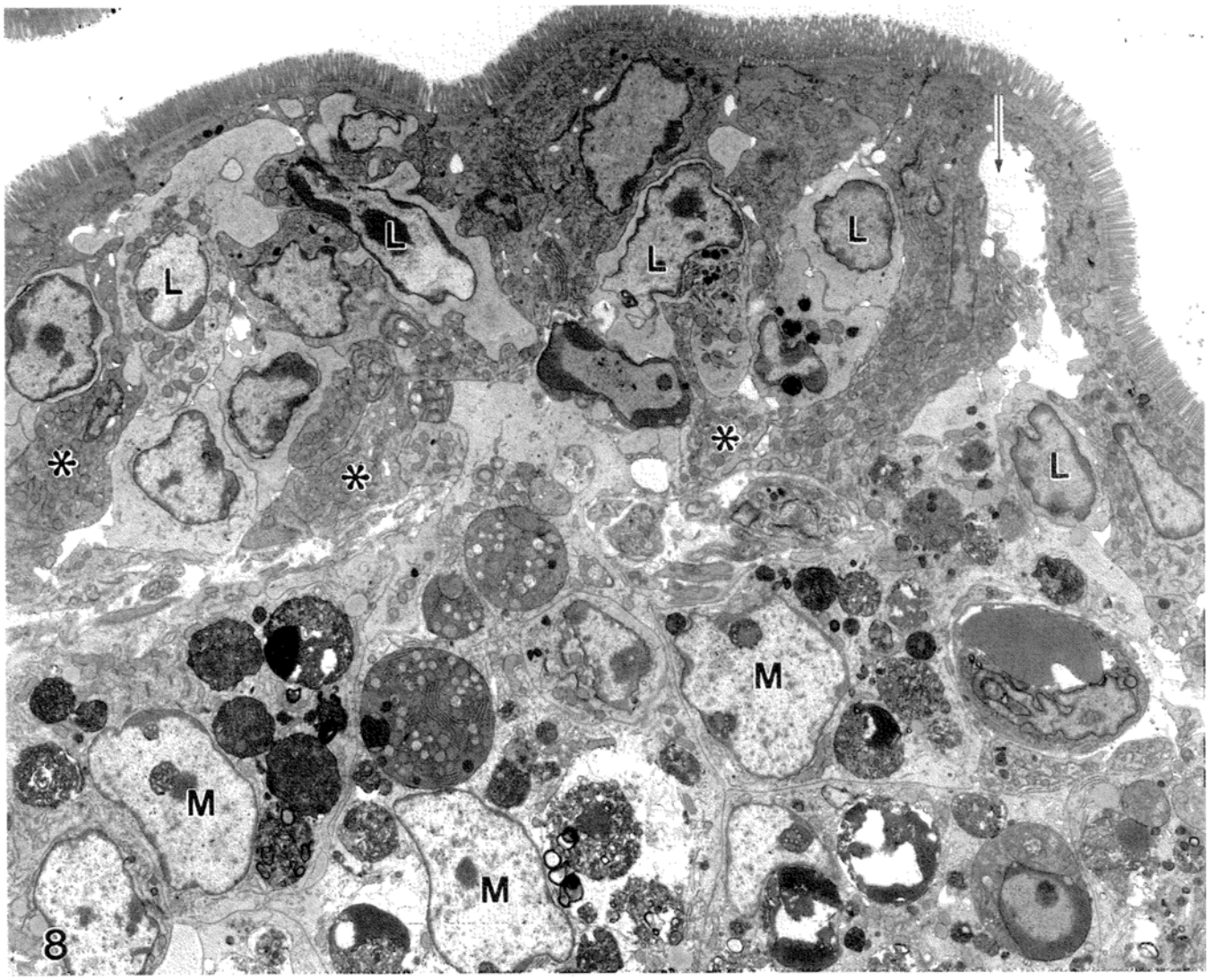


Fig. 8. Numerous lymphocytes (*L*) invade the epithelial lining at the villus tip in the ileum of a guinea pig. These lymphocytes are characterized by irregularly shaped cell processes and electron-dense granules. The cytoplasmic fragments of enterocytes (*asterisks*) are numerous in the basal portion of the epithelium. *Arrow* Space likely due to loss of cytoplasm. The subepithelial region is occupied by a dense accumulation of macrophages (*M*) containing large phagosomes and lysosomes. ×3500

pseudopodia of macrophages or intervened between epithelial cells and macrophages. They were intermingled with groups of macrophages in the lamina propria.

Cell kinetics of BrdU-labeled epithelial cells

Twelve hours after the administration of BrdU, immunoreactivity for BrdU was recognized in the nuclei of epithelial cells lining the upper half of the crypts. The BrdU-immunoreactive epithelial cells shifted with time along the villi, accumulating in the apical region of villi after 60 and 72 h (Fig. 10a). At 96 h, only a few labeled cells could be found within the epithelial lining, these being restricted to the villus tips. Simultaneously, immunoreactivity for BrdU was recognized in coarse cytoplasmic bodies in some of the macrophages present in the lamina propria of the villus apices (Fig. 10b, c). On morphological grounds, the BrdU-immunoreactive bodies represent labeled nuclei or their fragments enclosed in phagosomes of macrophages. Macrophages containing these BrdU-labeled bodies accounted for 20% or less of all macrophages in the lamina propria.

Discussion

The present study indicates that in the small intestine of normal guinea pigs, conspicuous aggregations of large macrophages occur in the lamina propria at the tip of every villus. In other mammalian species, accumulations of macrophages in the tips of intestinal villi have been noted under experimental or pathological conditions. LeFevre et al. (1978, 1979) reported that in mice to which latex had been given orally, latex-containing macrophages migrated from Peyer's patches to the tips of

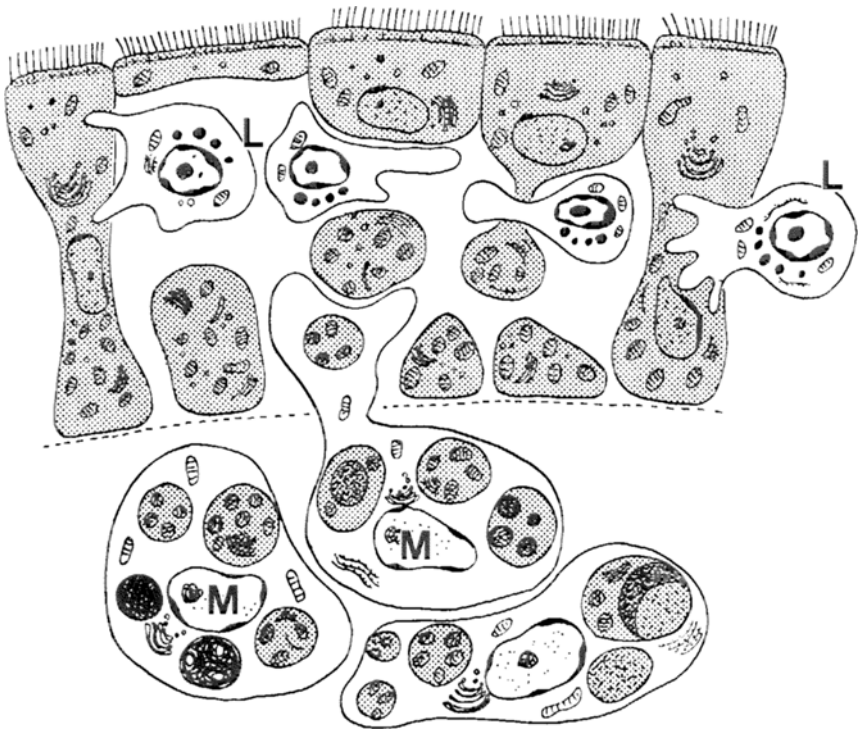


Fig. 9. Schematic drawing showing the proposed actions (see this study) of macrophages (*M*) and lymphocytes (*L*) upon aged epithelium at the villus tip in the small intestine of the guinea pig. The basal portions of epithelial cells are largely lost and fragmented, and subsequently phagocytosed by macrophages

neighboring villi. Astaldi et al. (1966) demonstrated, in the lamina propria of villus tips of the human, iron-loaded macrophages and their increase in number in hemosiderosis. Such macrophages sequestering waste material were considered to be eventually shed into the lumen.

With regard to the intestine of guinea pigs, however, it seems unlikely that inflammation or pathological conditions might induce such extensive and consistent occurrence of macrophage aggregations as described in the present paper. The cause of this phenomenon should be sought in more common and normal processes in the intestinal mucosa. Moreover, the selective localization of the aggregation at the villus tips leads us to link their functional significance to a specific condition at this locus. Some researchers may object to regarding the macrophage aggregation as a normal structure, but we were able to find similar aggregations of macrophages with a specific acid phosphatase activity in the intestine of newborn guinea pigs which were free from germ invasion and inflammation (unpublished data).

The present electron-microscopic study has revealed that the macrophages in question contained degraded enterocytes in their phagosomes, suggesting that the macrophages digest the effete epithelial cells at the villus apex, which represents the terminal site for epithelial cell migration. This view is in accordance with the results obtained by cell-labeling with BrdU. In the guinea pig small intestine, Sawicki et al. (1977) demonstrated Feulgen-positive, DNA-containing structures in the cytoplasm of macrophages gathered in the lamina propria. However, the conclusion reached in their electron-microscopic and autoradiographic study was that the macrophages may phagocytose subepithelial fibroblasts and/or intraepithelial lymphocytes. This view of Sawicki et al. (1977) could not be supported in the present investigation. That a small amount of subepithelial elements, including flattened pericryptal fibroblasts which are known to move upwards to the villus tips (Parker et al. 1974), might be phagocytosed by the macrophages at the villus tip cannot be excluded, but the major component represents the epithelial enterocytes. Our conclusion is strongly supported by ultrastructural images corresponding to different stages of the macrophage pseudopodia engulfing the cytoplasmic and nuclear elements of the enterocytes.

The turnover and renewal of the intestinal epithelial cells have been studied by previous investigators mainly in the mouse and rat, using ^{3}H-thymidine autoradiography. Different types of epithelial cells proliferate in the crypts and migrate upward to reach the villus tips (Cheng and Leblond 1974b). The enterocytes are known to have a life span of 2 to 3 days in the mouse and rat, and 6 days in the human (Leblond and Messier 1958; MacDonald et al. 1964; Quastler et al. 1959). The cell turnover time obtained from the present BrdU method in the guinea pig is in agreement with the findings of previous autoradiographic studies in the mouse and rat.

Effete or dying cells at the villus tips have been believed to exfoliate from the epithelial lining into the lumen (Padykula 1962; Cheng and Leblond 1974a; Eastwood 1977). In the mouse small intestine, Leblond and Messier (1958) reported the free occurrence of heavily labeled nuclei of epithelial cells in the lumen 72 h after the administration of ^{3}H-thymidine. In contrast, the present BrdU study demonstrated the possibility that labeled fragments of epithelial cells moving upwards to

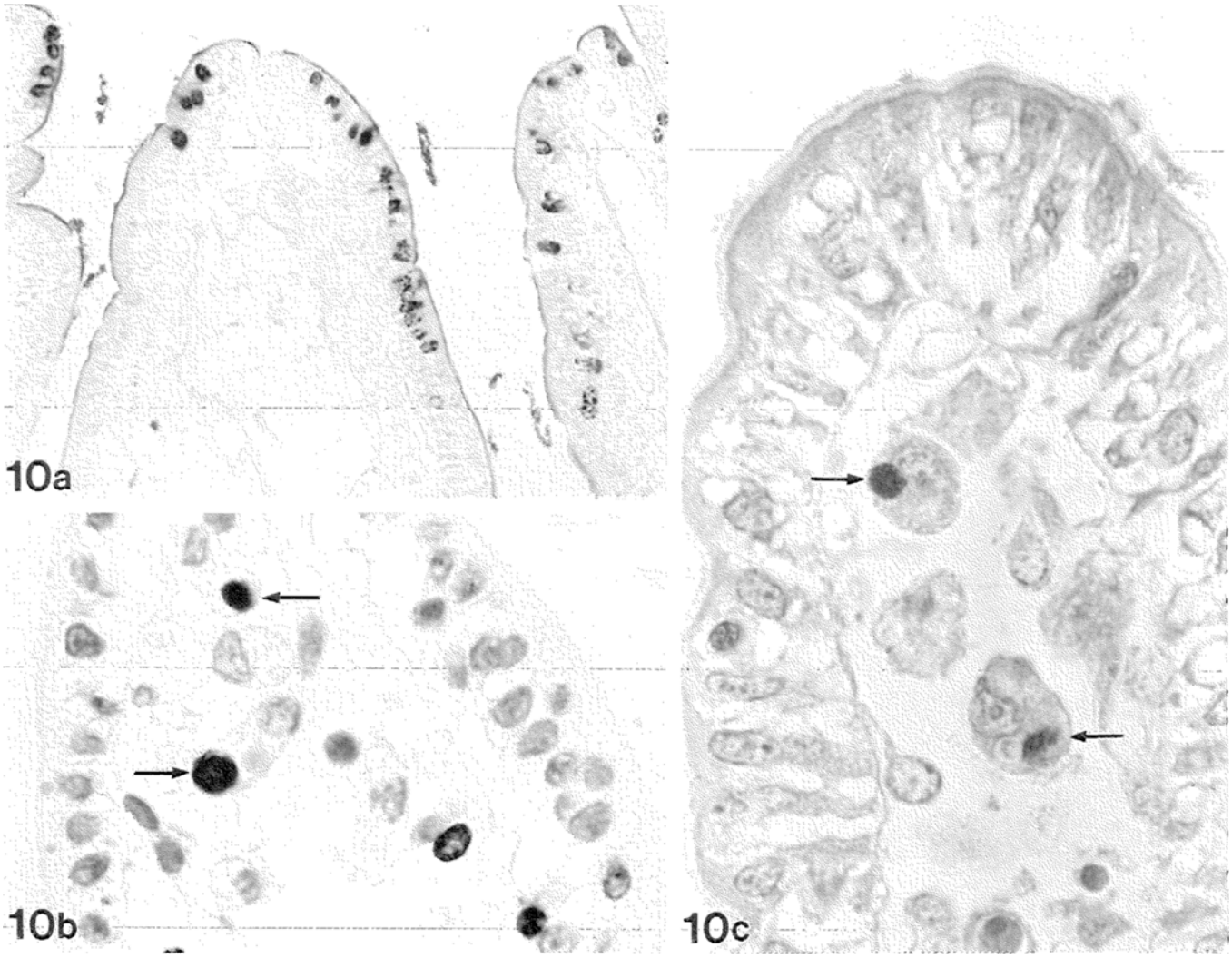


Fig. 10. Immunohistochemistry of the ileal villi in guinea pigs given 40 mg/kg bromodeoxyuridine. Labeled cells can be recognized in the epithelial lining of the villus tips after 72 h (**a**). After 96 h, round immunoreactive bodies (*arrows*), which most probably correspond to labeled nuclei, are seen in the cytoplasm of macrophages in the lamina propria (**b**, **c**). In **b**, four nuclear elements immunoreactive for bromodeoxyuridine are found in the lamina propria; two of them (*arrows*) are localized in the cytoplasm of macrophages. **a** ×320; **b** ×1000, **c** ×1260

the villus apices could later be found in the lamina propria. Neither the light- nor the electron-microscopic observations in the present study indicated exfoliation of degenerated epithelial cells. The frequent extension of macrophage pseudopodia into the epithelium and the inclusion of enterocytes suggest that enterocytes within the epithelial lining are engulfed by macrophage processes and then transported into the lamina propria. Both the lack of microvilli derived from enterocytes in the phagosomes of macrophages and the presence of numerous cytoplasmic fragments in the epithelium lead us to assume that macrophages in the villus tips engulf only the basal portions of enterocytes rather than the whole cell. This is also supported by the occurrence of a smaller population of BrdU-labeled macrophages in the lamina propria, since BrdU is incorporated only by the nucleus of enterocytes which was perhaps not always included in the engulfed cellular fragments.

Kerr et al. (1972) proposed that certain cells undergoing normal cell death are condensed into membrane-bounded globules with maintenance of organelle integrity, and designated this process "apoptosis". The membrane-bounded apoptotic bodies ultrastructurally resemble cell fragments observed in the phagosomes of macrophages. In epithelia, apoptotic bodies were recorded to be dispersed in intercellular spaces and either extruded into the lumen or phagocytosed, mainly by adjacent epithelial cells and partly by macrophages (for review, see Wyllie et al. 1980). Harmon et al. (1984) described that in the intestinal epithelium of rat fetuses (the caecum and colon), apoptotic bodies were phagocytosed by neighbouring enterocytes, some of them being extruded into the lumen. The occurrence of apoptosis in intestinal villi has not been dealt with in previous literature; apoptosis in the intestinal epithelium has been observed predominantly in the crypts (Searle et al. 1975). In contrast to the above-mentioned features of the hitherto recorded apoptotic bodies, our cell fragments in the intestinal villi

are not intensely condensed in structure and not phagocytosed by epithelial cells. The uptake of apoptotic bodies by epithelial cells has been reported to be accompanied by increased activity of lysosomal enzymes in these cells (Wyllie et al. 1980). The present study demonstrated that intense immunoreactivity for cathepsin B, a major lysosomal enzyme (Katunuma and Kominami 1983), was located in the macrophages, and not in epithelial cells. Although the cell fragments in the villus tips may be classified as apoptotic bodies, they are characterized by being phagocytosed extensively by macrophages.

The migration of lymphocytes into the intestinal epithelium is a common phenomenon (cf. Viney 1990). The intraepithelial lymphocytes have been shown by immunologists to represent a special type of T cell bearing $\gamma\delta$ receptors, which are able to detect and destroy damaged epithelial cells (Janeway et al. 1988). The existence of dense granules in the intraepithelial lymphocytes enables us to identify them as large granular lymphocytes (LGLs) (Kaneda 1989), which are generally regarded as a special type of T lymphocyte with natural killer activity (NK cells) (Saksela et al. 1979; Reynolds et al. 1981; Cerf-Bensussan et al. 1985). In the mouse small intestine, 30–45% of the intraepithelial lymphocytes have been reported to be LGLs (Guy-Grand et al. 1978). In the rat colon, over 90% of the intraepithelial lymphocytes have been identified as NK cells (Nauss et al. 1984). It is most reasonable to suggest that NK cells are disposed at the villus apices of the guinea pig and are engaged in killing the effete epithelial cells. The interdigitation of these lymphocytes with their target cells as demonstrated in the present study has been pointed out to be an important sign of cytotoxic activity in cells (Sanderson and Glauert 1979).

The present paper has dealt with a peculiar aggregation of macrophages at the villus tips in the guinea pig intestine. Our preliminary survey, at present, has demonstrated similar aggregations of phagosome-rich macrophages in the monkey and in horse (unpublished data). In the mouse and rat, the animals which have been most frequently used for cell-dynamic analysis of the intestine, the macrophages at the same position of intestinal villi are inconspicuous in appearance; this may be one of the reasons why this peculiar phenomenon has escaped the attention of researchers in the past.

Acknowledgements. This work was supported by a grant from the Research Foundation of Tachikawa General Hospital. The authors wish to thank Professor Toru Abo at the Department of Immunology, Niigata University School of Medicine, Professor Katsuko Kataoka at the Department of Anatomy, Hiroshima University School of Medicine, and Professor Kenjiro Wake at the Department of Anatomy, Faculty of Medicine, Tokyo Medical and Dental University, for their helpful discussion and advice. They also thank Professor Eiki Kominami at the Department of Biochemistry, Juntendo University School of Medicine, for the generous gift of the antibody against cathepsin B and the antigen.

References

Astaldi G, Meardi G, Lisino T (1966) The iron content of jejunal mucosa obtained by Crosby's biopsy in hemochromatosis and hemosiderosis. Blood 28:70–82

Burnstone MS (1958) Histochemical demonstration of acid phosphatases with naphtol AS-phosphates. J Natl Cancer Inst 21:523–539

Cerf-Bensussan N, Guy-Grand D, Griscelli C (1985) Intraepithelial lymphocytes of human gut: isolation, characterization and study of natural killer activity. Gut 26:81–88

Cheng H, Leblond CP (1974a) Origin, differentiation and renewal of the four main epithelial cell types in the mouse small intestine. I. Columnar cell. Am J Anat 141:461–480

Cheng H, Leblond CP (1974b) Origin, differentiation and renewal of the four main epithelial cell types in the mouse small intestine. V. Unitarian theory of the origin of the four epithelial cell types. Am J Anat 141:537–562

Cohn ZA (1968) The structure and function of monocytes and macrophages. Adv Immunol 9:163–214

Deane HW (1964) Some electron microscopic observations on the lamina propria of the gut, with comments on the close association of macrophages, plasma cells, and eosinophils. Anat Rec 149:453–474

Eastwood GL (1977) Gastrointestinal epithelial renewal. Gastroenterology 72:962–975

Guy-Grand D, Griscelli C, Vassalli P (1978) The mouse gut lymphocyte, a novel type of T cell. Nature, origin, and traffic in normal and graft-versus-host conditions. J Exp Med 148:1661–1677

Harmon B, Bell L, Williams L (1984) An ultrastructural study on the "meconium corpuscles" in rat foetal intestinal epithelium with particular reference to apoptosis. Anat Embryol 169:119–124

Janeway CA, Jones B Jr, Hayday A (1988) Specificity and function of T cells bearing $\gamma\delta$ receptors. Immunol Today 9:73–76

Kaneda K (1989) Liver-associated large granular lymphocytes: morphological and functional aspects. Arch Histol Cytol 52:447–459

Katunuma N, Kominami E (1983) Structures and functions of lysosomal thiol proteinases and their endogenous inhibitor. Curr Top Cell Regul 22:71–101

Kerr JFR, Wyllie AH, Currie AR (1972) Apoptosis: a basic biological phenomenon with wide-ranging implications in tissue kinetics. Br J Cancer 26:239–257

Kominami E, Bando Y, Wakamatsu N, Katunuma N (1984) Different tissue distributions of two types of thiol proteinase inhibitors from rat liver and epidermis. J Biochem 96:1437–1443

Kominami E, Tsukahara T, Bando Y, Katunuma N (1985) Distribution of cathepsins B and H in rat tissues and peripheral blood cells. J Biochem 98:87–95

Leblond CP (1981) The life history of cells in renewing systems. Am J Anat 160:113–158

Leblond CP, Messier B (1958) Renewal of chief cells and goblet cells in the small intestine as shown by radioautography after injection of thymidine-^{3}H into mice. Anat Rec 132:247–259

LeFevre ME, Olivo R, Vanderhoff JW, Joel DD (1978) Accumulation of latex in Peyer's patches and its subsequent appearance in villi and mesenteric lymph nodes. Proc Soc Exp Biol Med 159:298–302

LeFevre ME, Hammer R, Joel DD (1979) Macrophages of the mammalian small intestine: a review. J Reticuloendothel Soc 26:553–573

MacDonald WC, Trier JS, Everett NB (1964) Cell proliferation and migration in stomach, duodenum and rectum of man. Gastroenterology 46:405–417

Maximow A (1927) Bindegewebe and blutbildende Gewebe. In: Möllendorff W von (ed) Handbuch der mikroskopischen Anatomie des Menschen, vol. II/1. Springer, Berlin, pp 232–583

Möllendorff W von (1925) Beiträge zur Kenntnis der Stoffwanderung bei wachsenden Organismen. IV. Die Einschaltung des Farbstofftransportes in die Resorption bei Tieren verschiedenen Lebensalters. Histophysiologische Beiträge zum Resorptionsproblem. Z Zellforsch 2:129–202

Moran R, Darzynkiewicz Z, Staiano-Coico L, Melamed MR (1985) Detection of 5-bromodeoxyuridine (BrdUrd) incorporation by

monoclonal antibodies: Role of the DNA denaturation step. J Histochem Cytochem 33:821–827
Nauss KM, Pavlina TM, Kumar V, Newberne P (1984) Functional characteristics of lymphocytes isolated from the rat large intestine. Response to T-cell mitogens and natural killer cell activity. Gastroenterology 86:468–475
Pabst R (1987) The anatomical basis for the immune function of the gut. Anat Embryol 176:135–144
Padykula HA (1962) Recent functional interpretations of intestinal morphology. Fed Proc 21:873–879
Parker FG, Barnes EN, Kaye GI (1974) The pericryptal fibroblast sheath. IV. Replication, migration, and differentiation of the subepithelial fibroblasts of the crypt and villus of the rabbit jejunum. Gastroenterology 67:607–621
Quastler H, Bensted JPM, Lamerton LF, Simpson SM (1959) Adaptation to continuous irradiation. observation on the rat intestine. Br J Radiol 32:501–512
Reynolds CW, Timonen T, Herberman RB (1981) Natural killer (NK) cell activity in the rat. I. Isolation and characterization of the effector cells. J Immunol 127:282–287
Saksela E, Timonen T, Ranki A, Häyry P (1979) Morphological and functional characterization of isolated effector cells responsible for human natural killer activity to fetal fibroblasts and to cultured cell line targets. Immunol Rev 44:71–123
Sanderson CJ, Glauert AM (1979) The mechanism of T-cell mediated cytotoxicity. VI. T cell projections and their role in target cell killing. Immunology 36:119–129
Sawicki W, Kucharczyk K, Szymanska K, Kujawa M (1977) Lamina propria macrophages of intestine of the guinea pig. Possible role in phagocytosis of migrating cells. Gastroenterology 73:1340–1344
Searle J, Lawson TA, Abbott PJ, Harmon B, Kerr JFR (1975) An electron-microscope study of the mode of cell death induced by cancer-chemotherapeutic agents in populations of proliferating normal and neoplasmic cells. J Pathol 116:129–138
Sugihara H, Hattori T, Fukuda M (1986) Immunohistochemical detection of bromodeoxyuridine in formalin-fixed tissues. Histochemistry 85:193–195
Viney J, MacDonald TT, Spencer J (1990) Gamma/delta T cells in the gut epithelium. Gut 31:841–844
Wyllie AH, Kerr JFR, Currie AR (1980) Cell death: the significance of apoptosis. Int Rev Cytol 68:251–306