Electron Microscopic Observations of Surface Mucous Cells in the Mouse Gastric Mucosa during Physiological Degeneration and Extrusion*

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Summary. The gastric mucosa of adult mice was observed by electron microscopy, and the following findings were obtained. Surface mucous cells mostly undergo degeneration in situ before extrusion from the mucosal surface. Degenerating cells exhibit low electron density of the cytoplasmic matrix and interchromatin region of the nucleus. Some vacuoles can be seen in the cytoplasm. The rough endoplasmic reticulum and Golgi complex retain their normal configurations. Mitochondria are condensed. Lysosomes increase in number, and acid phosphatase activity is restricted within them. Massive exocytotic release of mucus is seen at the cell apex. The basolateral plasmalemma seems intact until the latest stage of extrusion. At the tight and gap junctions, the outer leaflets of apposing plasmalemmas remain fused. On the other hand, microfilaments and tonofilaments are dissociated from the intermediate junctions and desmosomes, respectively, during degeneration.

Massive discharge of mucus and well preserved basolateral plasmalemma of the degenerating cell may restrict the back-diffusion of gastric juice into the mucosa to a minimum level during the degeneration and extrusion processes.

Surface mucous cells of the gastric mucosa belong to the renewing cell population (MESSIER and LEBLOND, 1960). They are produced in the isthmus and migrate to the mucosal surface where they are finally extruded (STEVENS and LEBLOND, 1953; MESSIER and LEBLOND, 1960; HUNT and HUNT, 1962; MacDonald et al., 1964; KAKU, 1966; HATTORI, 1974). Their fine structural maturation also occurs concomitantly with migration (KATAOKA, 1970; TAMURA and FUJITA, 1983; KATAOKA and SAKANO, 1984). However, only a few studies have been available on their degeneration and extrusion processes in normal animals (PFEIFFER, 1970; HARDING and MORRIS, 1977; TATSUMI et al., 1985), and many questions remain to be solved.

MATERIALS AND METHODS

Adult male ICR mice, fed ad libitum, were sacrificed for this study between 10 and 11
K. Kataoka, Y. Takeoka and S. Hirano:

a.m. A 3-week-old mouse was also used for conventional electron microscopy. Under Nembutal anesthesia, the animals were perfused with 2.5% glutaraldehyde fixative (cacodylate buffer, pH 7.4) from the left ventricle, and the stomach was removed. The gastric cavity was opened by anterior wall incision, and the gastric contents were washed out by the same fixative. Tissue blocks, obtained from the body of the stomach along the greater curvature, were used for both conventional transmission electron microscopy and electron microscopic histochemistry of acid phosphatase.

For conventional electron microscopy, the tissues were fixed overnight with 2.5% glutaraldehyde fixative at 4°C, postfixed with 1% osmium tetroxide (cacodylate buffer, pH 7.4) for 2 hrs at 4°C, dehydrated by ethanol and embedded in Epon 812 resin. Ultrathin sections were doubly stained with uranyl acetate and lead citrate.

For electron microscopic demonstration of acid phosphatase activity, the tissues were fixed with 2.5% glutaraldehyde fixative for 1 hr at 4°C, and then rinsed overnight with 0.1 M cacodylate buffer (pH 7.4) containing 8% sucrose at 4°C. Frozen sections 20-100 μm in thickness were immersed in an incubation medium for 60-75 min at 20°C. The incubation medium was prepared after Barka and Anderson (1965) and consisted of 20 ml 0.05 M tris-maleate buffer (pH 5.0), 10 ml 1.25% sodium β-glycerophosphate and 20 ml 0.2% lead nitrate. The medium without β-glycerophosphate was used as control. After incubation, the sections were postfixed with osmium tetroxide and embedded in Epon. Ultrathin sections were lightly stained by lead citrate.

For light microscopic histochemistry of acid phosphatase, the animal was perfused with formol-calcium. Then the stomach was removed and further fixed overnight with formol-calcium at 4°C. The naphthol AS-BI phosphate technique after Barka and Anderson (1962) was used for demonstrating acid phosphatase activity. The incubation medium consisted of 36 ml buffered substrate solution (10 ml 1/7 M Michaelis veronal acetate buffer stock, 24 ml water and 2 ml 1% naphthol AS-BI phosphate) and 3.2 ml hexazonium pararosanilin solution (1.6 ml 4% pararosanilin hydrochloride and 1.6 ml 4% sodium nitrite). The incubation medium was adjusted to pH 5.0 with 1 N NaOH. The incubation medium without naphthol AS-BI phosphate was used as control. Frozen sections 5-20 μm in thickness were incubated in the incubation medium for 30 min at 37°C. Some sections were immediately mounted on a slide glass with glycerine. Other sections were dehydrated and embedded in paraffin. Paraffin sections 2-4 μm in thickness were used for light microscopic observation with or without counterstaining with methyl green. The glutaraldehyde-fixed frozen sections, incubated in Barka and Anderson’s medium (1965), were also used for light microscopic observation after treatment with ammonium sulfide.

RESULTS

Surface mucous cells were columnar in the gastric foveola (Fig. 1). The lateral cell membranes were in close apposition to each other. The nucleus was located in the basal cytoplasm. Flattened cisternae of the rough endoplasmic reticulum were seen in the perinuclear and supranuclear region. The Golgi complex was located apically and laterally to the nucleus. Mucous granules were gathered in the apical cytoplasm, and small mitochondria crowded beneath them.

At the mucosal surface, surface mucous cells became tall and columnar with the tapered basal cytoplasm (Fig. 1, 2). The lateral cell surfaces were separated by a wide intercellular space except for cell junctional regions. Some vesicular profiles were
often seen in the intercellular spaces, but they were never surrounded by the unit membrane (Fig. 3). The nucleus was located at a level from half to the upper 1/3 of the cell height. The Golgi complex was often seen lateral to the nucleus. It was also located in the supranuclear region, and less frequently in the infranuclear region. Mucous granules, which were fewer than those in foveolar cells, were gathered in the apical cytoplasm while mitochondria crowded beneath them. Lysosomes occurred frequently and lipid droplets occasionally.

One or a few degenerating surface mucous cells were sometimes intercalated among normal surface mucous cells at the mucosal surface (Fig. 1, 2). The degenerating cell was characterized by the low electron density of its cytoplasmic matrix. In the nucleus, dense chromatin and nucleoli contrasted with the clear interchromatin region. However, more severe nuclear injuries, such as pyknosis, karyolysis and karyorrhexis, were not observed. Dense ribosomes, still in the form of polyribosomes, were prominent in the clear cytoplasmic matrix. The configuration of rough endoplasmic reticulum

Fig. 1. An electron micrograph of the gastric mucosa of the adult mouse. Surface mucous cells are columnar and are closely lined up along the gastric foveola (right). At the mucosal surface, they are tall and tapered, and are separated by the intervening intercellular space except for the junctions. Also note the different levels of the nuclei (N) between the foveolar and surface cells. G Golgi complex, D degenerating cell, S smooth muscle in the lamina propria. ×2,400. All figures except for Figure 8 are of an adult animal.
Fig. 2. a and b. Degenerating cells are conspicuous by their low electron density of the cytoplasmic matrix and interchromatin region of the nucleus. The rough endoplasmic reticulum and Golgi complex (G) exhibit normal configurations, mitochondria look denser (arrows M), and secretory granules exhibit variable electron density of their content. A vacuole (V) and a lipid droplet (L) are also seen. a: ×7,000, b: ×5,000
and Golgi complex appeared to be normal. Mitochondria usually did not swell but looked denser. Secretory granules exhibited wide variations in the electron density of their content. Many secretory granules were open to the apical plasmalemma of the degenerated cell, releasing their mucous content by exocytosis, while neighboring normal cells rarely exhibited exocytosis (Fig. 4). The released mucus formed a layer covering the epithelial surface. Lysosomes, clear vacuoles and lipid droplets were seen in the cytoplasm of the degenerating cell.

In addition to the inevitable exocytotic release of mucus, the apical plasmalemma was sometimes ruptured and a certain amount of cytoplasmic elements flowed out into the gastric lumen, depending on the extent of the rupture (Fig. 5c). On the other hand, the continuity of the basolateral plasmalemma was well preserved during the degeneration process (Fig. 1–3, 5). At the early stage of degeneration, the tight junction, intermediate junction and desmosomes exhibited a normal configuration (Fig. 5a). Felt-like materials, including microfilaments, were attached along the cytoplasmic surface of the tight and intermediate junctions of the early degenerating cell. The attachment plaque and tonofilaments were also attached to the cytoplasmic side of desmosomes. The felt-like materials, attachment plaque and tonofilaments disappeared with the progress of the degeneration (Fig. 5b, c). The lateral plasmalemma itself,
however, remained intact; this included the membrane fusion at the tight junction. At the site of the gap junction, the outer leaflets of the membranes remained fused between the normal and degenerating cells so that the pentalaminar structure of the gap junctional membranes could be seen (Fig. 6). In some regions, the lateral plasmalemmas of the degenerating and normal cells were very closely apposed, with the intervening intercellular space (less than 10 nm) being narrower than the usual intercellular space between normal cells (15–20 nm).

The basal lamina of the epithelium sometimes exhibited a small region of discontinuity and an overlap beneath the degenerating surface mucous cell (Fig. 3). The underlying lamina propria contained fine collagen fibers and filaments of the extracellular matrix. In addition to fibroblasts and macrophages of the lamina propria, smooth muscle fibers were seen adjacent to the epithelial basal lamina (Fig. 1).

The cytoplasm of degenerating cells occasionally protruded into the gastric lumen (Fig. 7a). The continuity of the basolateral plasmalemma and the tight junction persisted even in such cells. The process of extrusion could not be observed in the adult animals in this study. The extruded cell, which still maintained its cellular integrity, was sometimes seen in the gastric lumen (Fig. 7b).

In a 3-week-old animal, we occasionally found exfoliating cells, whose degeneration and exfoliation processes seemed somewhat different from those seen in adult animals (Fig. 8). The decrease in electron density of the interchromatin region of the nucleus was not so evident as in adult degenerating cells. The density of the cytoplasmic matrix was almost normal, especially in the perinuclear and infranuclear
Cell Loss in Gastric Mucosa

regions. The cytoplasm contained many vacuoles. Mitochondria were not condensed but tended to be swollen. The exocytotic release of mucus was seen at the cell apex. There were no evident intercellular junctions between the exfoliating and neighboring normal cells, and the basal lamina seemed to be exposed to the gastric lumen through the intercellular space. The neighboring normal cells extended ameboid processes containing felt-like materials, small vesicles and some glycogen particles toward the exfoliating cell.

By light microscopic histochemistry of acid phosphatase, dotty reaction products increased according to cell migration from the isthmus to the mucosal surface by using either incubation media, naphthol AS-BI phosphate technique (BARKA and ANDERSON, 1962) or β-glycerophosphate technique (BARKA and ANDERSON, 1965) (Fig. 9a). By electron microscopy, the reaction products were precipitated only in lysosomes of both normal and degenerating cells, and the cytoplasmic matrix was completely free from

Fig. 5. The junctional complex between normal and degenerating (D) cells. × 66,000. a. In the early stage of degeneration, the tight junction, intermediate junction and desmosome look normal. b. At a more progressive stage of degeneration, felt-like materials along the cytoplasmic surface of the tight and intermediate junction, as well as attachment plaque and tonofilaments of the desmosome, disappear in the degenerating cell. On the other hand, the lateral plasmalemmas are well preserved in both normal and degenerating cells, and the outer leaflets remain fused at the tight junction (arrow). c. With rupture of the apical plasmalemma, cytoplasmic elements of the degenerating cell flow out to the gastric lumen. Nevertheless, the lateral plasmalemma is preserved.
reaction products even in the degenerating cell (Fig. 9b). With the substrate-free control media, no reaction was seen in either light or electron microscopy.

**DISCUSSION**

Immature surface mucous cells undergo proliferation in the isthmus and become mature with their upward migration along the foveola (Kataoka, 1970; Tamura and Fujita, 1983; Kataoka and Sakano, 1984). At the mucosal surface, they become taller and tapered. The occurrence of a wide intercellular space between the surface mucous cells may be disputable. We consider that it is not an artifact arising from tissue
Cell Loss in Gastric Mucosa

preparation but an actually existent structure in the living animal, since it is never seen in the isthmus and foveola and is constantly recognized on the mucosal surface of well preserved specimens. As Helander (1981) has described, the expanded intercellular space may represent a channel for transepithelial transport of water and electrolytes. However, it should be noted that this space is always separated from the gastric lumen by the tight junction of the epithelium. Vesicular profiles seen in this space are never surrounded by the unit membrane. It is unknown whether they represent debris of degenerated cell components or artifacts formed during tissue preparation.

With cell migration from the foveola to the mucosal surface, the position of the nucleus changes from the cell base to the half or upper 1/3 of the cell height as demonstrated by the present as well as previous studies (Tatsumi et al., 1985). The infranuclear position of the Golgi complex, described by Tatsumi et al. (1985), may not be an essential change during cell migration. In the present study, the Golgi complex is in a supranuclear and perinuclear position in the isthmus and foveolar cells, and is located mainly in the perinuclear, sometimes in the supranuclear, and only occasionally in the infranuclear regions of the mucosal surface cell.

The present study demonstrated two types of cell loss. The predominant type, involving the loss of electron density of the cytoplasmic matrix and of the interchromatin region of the nucleus, reveals common ultrastructural features with in situ degeneration described in the normal gastric mucosa (Pfeiffer, 1970; Tatsumi et al., 1985) as well as in the mucosal injuries induced by ethanol (Eastwood and Kirchner, 1974; Eastwood and Erdmann, 1978), bile salt (Eastwood, 1975) and stress (Harding and Morris, 1977). Although mucus release was previously only briefly mentioned by Eastwood and Erdmann (1978), exocytotic release of mucus is always accompanied with cell degeneration in the normal mouse. A large quantity of secreted mucus
covers the epithelial surface and protects the mucosa from damage during the degeneration and exfoliation processes. Exocytosis suggests an increased intracellular Ca++ level in the degenerating cell (Alberts et al., 1983). This is worthy of attention since disintegration of microfilaments and tonofilaments, which was observed in this study during degeneration, may also be related to high cytoplasmic Ca++ levels (Alberts et al., 1983). The gap junction seems to persist between the normal and degenerating cells. However, it may not be actively engaged in intercellular communication, since the communication channel is closed by decreased cytoplasmic pH values and elevated cytoplasmic Ca++ levels (Alberts et al., 1983).

The apical plasmalemma of the degenerating cell seems either intact (Eastwood and Kirchner, 1974; Eastwood, 1975; Eastwood and Erdmann, 1978) or disrupted (Eastwood, 1975; Tatsumi et al., 1985), and this difference is probably due to the varying degrees of physico-chemical injuries to the apical surface of individual cells. When the apical plasmalemmal rupture is extensive, most of the cytoplasmic elements flow out into the gastric lumen, leaving the intact basolateral plasmalemma (Tatsumi et al., 1985). The basolateral plasmalemma, including the tight junction, remains well preserved during degeneration (Eastwood, 1975; Eastwood and Erdmann, 1978). It may serve as a barrier against the back-diffusion of the gastric content to the mucosa.

Fig. 8. An unusual type of the exfoliating cell in a 3-week-old mouse. The electron density of the perinuclear cytoplasm and the nucleus of the exfoliating cell is almost normal. Many vacuoles and the exocytotic release of the mucus (arrow) are seen. Junctions are not evident between the exfoliating cell and ameboid lateral processes (P) of neighboring cells, and the basal lamina covering the lamina propria (lower right corner) seems to be exposed to the gastric lumen through the intercellular space. × 4,900
Cell Loss in Gastric Mucosa

along with a rather dense extracellular matrix beneath the epithelium (Tatsumi et al., 1985).

The extrusion mechanism of the degenerated cell was not clarified in this nor in previous studies. The extruded cell first maintains its integrity as an individual cell, and then is broken in the gastric lumen. The remaining mucous granules are discharged at the same time, so that this can be regarded as a type of holocrine secretion (Helandner, 1981; Tamura and Fujita, 1983; Tatsumi et al., 1985). In the gastric mucosa of the normally fed mouse, however, the exocytotic release of mucus during degeneration is more extensive and thus may be more important. The re-sealing mechanism of the site of cell loss is not known. As Tatsumi et al. (1985) have suggested, the compressive force may be derived from cell migration and/or contraction of smooth muscle cells just beneath the basal lamina. In addition, ameboid lateral cell processes of neighboring cells, seen in the second type of cell loss, must be taken into account, although they were not evident in the present study.

The second type of cell loss was occasionally found in a 3-week-old mouse, but not in an adult. The exfoliating cell contains many vacuoles, but it exhibits almost normal cytoplasmic density and the nucleus appears normal. Exocytosis of mucous granules is evident again in this case. The neighboring normal cells project ameboid processes serving to fill up the intercellular space. A similar type of cell extrusion has been briefly described in the food-deprived rat by Harding and Morris (1977), although they observed the extrusion of a normal cell instead of a vacuolated cell noted in the present study.

These two mechanisms of cell loss, degeneration in situ and extrusion of an almost normal cell, may not be alternative, but both occur in normal animals under various conditions. Physical stimuli of ingested food (mechanical, osmotic, temperature, etc.) and the chemical nature of the gastric content (pH, peptic activity, chemical nature of

Fig. 9. a. A light micrograph showing acid phosphatase activity demonstrated by the naphthol AS-Bi phosphate technique. The activity, represented by dark spots here, increases with cell migration from the isthmus to the foveola and finally to the mucosal surface. × 580. b. By electron microscopy, acid phosphatase activity is restricted within lysosomes in either normal (lower right) or degenerated (D) cells. ×14,000.
the ingested food, and probably backflow of the bile in certain circumstances) may greatly affect the nature of exfoliating cells and cell loss. In the normally fed adult, degeneration in situ seems to prevail. In developing animals, where the gastric gland is still immature (KATAOKA et al., 1984) and the peptic activity is lower (FURIHATA et al., 1973), some exfoliating cells may not be severely degenerated, and even normal-appearing cells could be extruded in food-deprived animals (HARDING and MORRIS, 1977).

The primary cause of cell degeneration and loss is not known. Leakage of lysosomal enzymes into the cytoplasm seems unessential, since acid phosphatase activity is restricted within lysosomes even in a degenerating cell. The physical and chemical stimuli by the gastric content may play an important role in the process of cell degeneration in situ, because the fine structure of the degenerating cell is similar between normal animals and animals which have received an intragastric administration of ethanol (EASTWOOD and KIRCHNER, 1974; EASTWOOD and ERDMANN, 1978) or bile salt (EASTWOOD, 1975).

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