Mucus Release of Surface Mucous Cells of the Mouse Stomach with Special Reference to Cell Maturation Stages and Dietary Conditions

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Summary. The secretory activity of surface mucous cells was quantitatively studied in the mouse stomach under three different dietary conditions: ad libitum feeding, fasting for 15 hrs, and refeeding 1 hr after 15-hrs fast. Surface mucous cells were classified into isthmus cells, foveolar cells, surface cells and degenerating cells according to stage of maturation. The number of exocytosis and cytoplasmic granules was calculated in unit length of the apical plasmalemma for cells in each stage.

Foveolar and surface cells in fasted animals manifested higher exocytotic activity than the other two groups (P <0.01). This suggests that physical and chemical stimuli of the gastric content may greatly affect the secretory activity of the cell. Although the number of cytoplasmic mucous granules proved largest in the upper part of the foveola and less at the mucosal surface under any dietary condition, exocytotic activity did not differ significantly between the foveolar cells and surface cells. Degenerating cells very actively discharged mucus, regardless of dietary condition. It is reasonable to postulate that the cells secrete mucus in order to cover the surface and protect the mucosa from damage during degeneration.

Surface mucous cells of the stomach line the surface of the mucosa and gastric foveola. They are produced in the isthmus and mature with migration to the surface, where they are finally shed off several days later (STEVENS and LEBLOND, 1953; MESSIER and LEBLOND, 1960; HUNT and HUNT, 1962; MACDONALD et al., 1964; KAKU, 1966; HATTORI, 1974). Immature surface mucous cells in the isthmus contain a small number of secretory granules (KATAOKA and SAKANO, 1984). As the cells migrate toward the mucosal surface, the secretory granules become larger both in number and size. The granules are the most numerous in the cells lining the upper part of the foveola and thereafter they generally decrease at the mucosal surface (STEVENS and LEBLOND, 1953; DOIDGE et al., 1982; KATAOKA and SAKANO, 1984; KATAOKA et al., 1985). Several different mechanisms have been proposed for mucus release of the surface mucous cells such as usual exocytosis (KUROSUMI, 1961; OGATA and MURATA, 1969; ZALEWSKY and MOODY, 1979), a kind of diacrine release (STEGHENS and PFEIFFER, 1963), massive exocytosis during cell degeneration (KATAOKA et al., 1985), apical expulsion (ZALEWSKY and MOODY, 1979) and a kind of holocrine secretion accompanied with disruption of the extruded cells (ZALEWSKY and MOODY, 1979; HELANDER, 1981; TAMURA and FUJITA, 1983; TATSUMI et al., 1985; KATAOKA et al., 1985). Although the latter three processes evidently take place at the final stage of cell life, little is known about secretory activity during the different stages of cell maturation.
MATERIALS AND METHODS

A total of 15 adult ICR mice of both sexes were used in the present study. They were divided into three groups according to dietary conditions: a) ad libitum feeding, b) fasting for 15 hrs and c) feeding 1 hr after 15-hr fast. Surface mucous cells in each dietary condition were classified into isthmus cells, foveolar cells, surface cells and degenerating cells according to stages of maturation in the physiological renewal process.

Under Nembutal anesthesia, the animals were perfused with 2.5% glutaraldehyde fixative in cacodylate buffer (pH 7.4) from the left ventricle of the heart. Small pieces of the mucosa were removed from the body region of the stomach and immersed overnight in the same fixative. They were postfixed with 1% osmium tetroxide in cacodylate buffer (PH 7.4) for 2 hrs at 4°C. After dehydration by ethanol, the specimens were embedded in Epon 812 resin. For light microscopy, semi-thin sections of the specimens were stained with toluidine blue or trichromatic stain (malachite green-toluidine blue-basic fuchsin) after KUROTAKI (1972). For electron microscopy, thin sections were doubly stained with uranyl acetate and lead citrate.

Electron micrographs of the surface mucous cells were randomly taken at $\times 5,000$ in direct magnification and printed at $\times 10,000$ in final magnification. The length of apical plasmalemma was measured with an analyzer, Nikon Cosmo Zone. The number of exocytotic figures was counted on the same pictures. Thus, the number of exocytotic images in unit length of apical plasmalemma was calculated. Statistical comparison between groups differing in diet and maturation was carried out by using the Mann-Whitney test. The number of cytoplasmic granules in unit length of apical plasmalemma was also calculated to determine whether the frequency of exocytosis is affected by the total number of secretory granules.

RESULTS

Immature surface mucous cells, also called isthmus cells, were intermingled with parietal cells in the isthmus (Fig. 1, 2). They were cuboidal or low columnar in shape. A large nucleus was in the center of the cell. A small number of short microvilli were projected at the apical surface. Numerous free ribosomes, a few strands of rough endoplasmic reticulum and some mitochondria were scattered in the cytoplasm. The Golgi complex was in the supranuclear region, and dense mucous granules were formed in the complex. The mucous granules were present just beneath the apical surface and were smaller both in number and size than those in mature surface mucous cells (Fig. 3). Exocytotic release of the mucus was only occasionally found in any dietary group (Fig. 4).

As the cells migrated along the foveolar wall to the mucosal surface, they became taller and maturer (Fig. 5). The nucleus was located in the basal part of the cell and microvilli became longer and more numerous. The cytoplasmic structures tended to be arranged zonally. Numerous dense granules were accumulated in the apical cytoplasm. Mitochondria gathered just beneath them. The well developed rough endoplasmic reticulum was in the supra- and perinuclear region. The large Golgi complex was located in the supranuclear cytoplasm and mucous granules were actively formed in it.
The cells became extremely tall at the mucosal surface (Fig. 6). The nucleus was located at a level from a half to the upper one third of the cell height. Microvilli decreased in number and length, and the cell surface appeared somewhat convex. The number of mucous granules varied considerably from cell to cell, but it was generally smaller than that in the cells lining the foveola. As in the foveolar cells, a zonal arrangement of mucous granules, mitochondria, rough endoplasmic reticulum and Golgi complex was evident. A few strands of the endoplasmic reticulum and some mitochondria were scattered in the tapered basal cytoplasm.

The frequency of exocytosis of the mucus varied among foveolar and surface cells; some cells exhibited up to six exocytotic figures but others did not. The cells adjacent to the degenerating cells tended to release mucus more actively. Among the different dietary groups, foveolar and surface cells in fasted animals manifested a higher exocytotic activity than those in animals fed ad libitum or refed after fasting (P < 0.01) (Fig. 4).

Degenerating cells were present at the opening of the foveola to the mucosal surface and at the interfoveolar mucosal surface. The cells were characterized by the extremely low density of the cytoplasmic matrix in contrast to dense ribosomes and membranous organelles (Fig. 6). The content of the mucous granules exhibited various electron densities. Although some degenerating cells exhibited scanty exocytosis, most of them exocytosed mucus very actively regardless of dietary condition (Fig. 4). Multigranular exocytosis, in which second and third granules open into the first granule undergoing exocytosis, was frequently seen (Fig. 6b). The released mucus...
formed a thick layer over the degenerating cell surface. In some cases, the apical plasmalemma of the degenerating cell was ruptured and mucus granules flowed out to the gastric lumen with other cytoplasmic components. In addition, extruded cells with remaining mucous granules were degraded in the gastric lumen. In the latter two cases, the mechanism of mucus release could be considered a kind of holocrine secretion. When estimating exocytotic activity, these cases of “holocrine secretion” were not counted (Fig. 4).

**DISCUSSION**

Surface mucous cells of the stomach can be grouped into isthmus cells, foveolar cells, and mucosal surface cells according to their positions. These groups eventually represent maturational stages of the cells, since the cells are formed in the isthmus and migrate along the foveola to the 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). The present electron microscopic observation confirms findings in previous studies (KATAOKA, 1970; KATAOKA and SAKANO, 1984) that surface mucous cells mature ultrastructurally with migration. The isthmus cells
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Exhibit the typical morphology of immature cells with their high nucleo-cytoplasmic ratio, scanty membranous organelles, numerous free ribosomes and a few mucous granules, and they acquire abundant cytoplasm with well developed secretory machinery during migration along the foveola.

It has been the impression by light and electron microscopic observations that mucous granules increase from the isthmus to the upper part of the foveola, but decrease at the mucosal surface (Stevens and LeBlond, 1953; Doidge et al., 1982; Kataoka and Sakano, 1984; Kataoka et al., 1985). The present study quantitatively confirms this phenomenon in all dietary conditions. Increasing numbers of mucous granules from the isthmus to the upper part of the foveola suggest that the mucus is synthesized faster than it is secreted in the foveolar cell (Doidge et al., 1982). There may be three possibilities for the decrease of mucous granules in surface cells. The first possibility is that the surface cell discharges mucus more actively. In the present study, however, no significant increase of mucus release was observed either at the opening of the foveola or at the mucosal surface when compared with the other parts of the foveola. The second possibility is that the mucous production decreases at the mucosal surface due to the aging of the cells. Changes in mucous production are conceivable during the lifespan of the surface mucous cells in view of the facts that the uptake of amino acids is decreased in surface cells (Watteil et al., 1979), carbohydrate composition is modified during cell life (Gerard et al., 1968; Watteil et al., 1979; Sato and Spicer, 1980), and lysosomes increase in surface cells (Kataoka et al., 1985). The third possibility is that the cell apex is stretched at the mucosal surface and the granules appear less numerous in a section of a cell. In fact, the surface cells are funnel-shaped and the microvilli are remarkably fewer and shorter than those of the foveolar cells. The contribution of the latter two possibilities must be estimated in future studies by analyses of mucus production and absolute mass of intracellular mucus during cell migration, maturation and aging.

Fig. 3. The number of cytoplasmic mucous granules in unit length of apical plasmalemma of the cells of each maturation stage under different dietary conditions. Data are expressed as mean ± standard error. In each dietary group, foveolar cells contain more numerous granules than isthmus cells and surface cells (P<0.01).
The present study has shown that the frequency of mucus release of the surface mucous cells increases in fasted animals and decreases in refed animals. Though the frequency in the isthmus was too low for statistical analysis, foveolar and surface cells in fasted animals manifested a remarkably higher frequency of exocytosis than those in animals either fed ad libitum or refed after fasting ($P<0.01$). The results suggest that the decrease of gastric content stimulates the mucus release directly or indirectly through the nervous system and/or the endocrine system. In the present study the number of mucous granules in the surface mucous cells did not change significantly under different dietary conditions. On the other hand, in the bat, Ito (1981) described a marked reduction in intracellular mucus content after feeding. Grant et al. (1953) reported an increase in extracellular mucus in the stomach of dogs, cats and kittens 2 to 7 hrs after feeding. Similar observations have been made by Gerard et al. (1968) in the dog 3 and 20 hrs after feeding, while the amount of mucus in surface cells was either reduced or unchanged, varying within specimens from the same animal. The discrepancies among the various investigators might depend upon the criteria of secretory activity (amount of intracellular mucus, exocytotic activity or amount of extracellular mucus), the length of fasting and feeding time, and species differences. The volume of the extracellular mucus does not exactly correspond to the exocytotic activity of the surface mucous cells nor to the amount of secreted mucus, since a
certain part of the secreted mucus might be removed from the mucosal surface and dietary stimulation may affect cell degeneration accompanying massive mucus release.

Exocytosis (Kuroumi, 1961; Ogata and Murata, 1969; Zalewsky and Moody, 1979) and a kind of diacrine secretion (Stephens and Pfeiffer, 1968) have been proposed as the mechanism of mucus release. The present study has shown that exocytosis is the only mechanism of mucus release in non-degenerating surface mucous cells under physiological conditions. In degenerating cells, exocytosis is extremely active and, in addition, multigranular exocytosis is often seen. In accordance with the varying extent of physico-chemical injuries, the apical plasmalemma of the degenerating cell seems either intact (Eastwood and Kirchner, 1974; Eastwood and Erdmann, 1978) or disrupted (Eastwood, 1975; Tatsumi et al., 1985). In the latter, cytoplasmic components including mucous granules flowed out to the gastric lumen (Eastwood, 1975; Tatsumi et al., 1985; Kataoka et al., 1985). A similar phenomenon was described by Zalewsky and Moody (1979) and was called apical expulsion. Finally, mucus release is accompanied by the destruction of extruded cells in the gastric lumen (Zalewsky and Moody, 1979; Helander, 1981; Tamura and Fujita, 1983; Tatsumi et al., 1985; Kataoka et al., 1985). The last case can be called "holocrine secretion".

Fig. 5. Surface mucous cells lining the foveola (foveolar cells). Numerous dense granules are packed in the apical cytoplasm. $\times 5,800$. Inset: Exocytotic figures (arrows) in the foveola. $\times 17,000$
Fig. 6.  

a. Surface mucous cells at the mucosal surface (surface cells) and degenerating cells (D). Degenerating cells exhibit many exocytotic figures (arrows). $\times 10,000$.

b. Degenerating cells exocytose mucus very actively. Arrows: multigranular exocytosis. $\times 23,000$
The present study quantitatively confirms the qualitative results of KATAOKA et al. (1985) that degenerating cells very actively discharge mucus by exocytosis regardless of dietary condition. In either usual or multigranular exocytosis, the apical plasmalemma and fused limiting membrane of the granules remain continuous to effectively seal off the cytoplasm from the gastric lumen. This mechanism along with persistence of intercellular junctions (KATAOKA et al., 1985) may prevent the back diffusion of gastric content to the mucosa during physiological degeneration. As KATAOKA et al. (1985) have suggested, it is a quite reasonable mechanism for a large volume of secreted mucus to cover the surface to protect the mucosa from damage during degeneration.

The cell adjacent to the degenerating cell tends to release mucus more frequently than other non-degenerating cells. Some possible explanations may be offered regarding the increased exocytotic activity of the former. The first possibility is that the two cells are functionally coupled by a gap junction, as an electron microscope study has shown the persistence of a gap junction between non-degenerating and degenerating cells (KATAOKA et al., 1985). However, coupling between non-degenerating and degenerating cells seems doubtful, since intercellular communication channels are closed by slight changes in intracellular pH and free Ca++ levels (ALBERT et al., 1983). The second possibility is that the non-degenerating cell is activated by microenvironmental changes caused by degeneration of the neighboring cell or by certain local mediators released from the neighboring degenerating cell. Whether such mediators are actually present or not remains to be solved. Finally, the surface mucous cell migrates by a pipeline system, which means, in short, "first produced, first migrates" (HATTORI and FUJITA, 1976). Thus, the neighboring cells are about the same age and share a similar microenvironment. This might make it possible for both degenerating and adjacent non-degenerating cells to have high exocytotic activity. Regardless of the mechanism, their mucus secretion seems favorable for mucosal protection.

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