Panoramic Observation of the Mouse Gastric Mucosa by Superwide-Field Electron Microscopy*

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Received September 12, 1983

Summary. Transitional changes in cell population and ultrastructure in the gastric mucosa can be clearly demonstrated by superwide-field electron microscopy. A few undifferentiated cells as well as immature forms of the surface mucous, mucous neck and parietal cells are present in the lower part of the isthmus or the upper part of the neck. Surface mucous cells and mucous neck cells show maturer morphology with their migration to the mucosal surface or to the deep part of the neck, respectively. Parietal cells may migrate either upwards or downwards. Immature chief cells appear in the uppermost part of the base and mature as they migrate towards the gland bottom. A transition from mucous neck cells to chief and parietal cells is suggested. Collagen fibers and fibroblasts are more numerous in the foveola and isthmus regions than in deeper parts of the mucosa. Smooth muscle fibers are separated from the muscularis mucosae and run perpendicularly to the mucosal surface.

The fine structure of the four types of epithelial and glandular cells of the gastric mucosa, i.e., the surface mucous cells, mucous neck cells, chief cells and parietal cells, was intensively studied and greatly clarified in the 1960s (Ito, 1967). These cells do, however, belong to renewing cell populations (Messier and Leflond, 1960), and ultrastructural changes are noted in each cell type during their maturation (Kataoka, 1970), which occurs concomitantly with their upward or downward migration from their generation site (Hattori, 1974; Hattori and Fujita, 1976). Therefore, it is suspected that the cells are placed in the order of their maturation stages along the superficial-deep axis of the mucosa. The use of a superwide-field electron microscope enabled us to verify this hypothesis.

MATERIALS AND METHODS

Adult male ICR mice (10 weeks old), starved overnight, were used for this study. Under Nembutal anesthesia, the animals were perfused with 2.5% glutaraldehyde (cacodylate buffer, pH 7.4). The gastric body was cut into small pieces, fixed with 2.5% glutaraldehyde and postfixed with 1% osmium tetroxide. The specimens were dehydrated with ethanol and embedded in Araldite.

*This study was supported by General Research Grant and Cancer Research Grant from the Ministry of Education, Science and Culture, Japan.
Fig. 1. a and b. A panoramic view of the mouse gastric mucosa. (Figures a and b represent two parts continuous to each other). The area between the arrows is enlarged in Figure 2. The light micrograph of the corresponding area is shown in Figure 4. The asterisks (*)
indicate young parietal cells which are enlarged in Figure 3. F foveola, I isthmus, N neck, B base, arrow E an uppermost endocrine cell. × 540
For panoramic observation of the whole thickness of the gastric mucosa, a thin section with silver-golden interference color was mounted on a grid with a \(450 \times 620\ \mu m\) hole which had been made by cutting some of the bars under the binocular microscope. The full thickness of the mucosa could thus be seen without interruption by the grid bars. This section was doubly stained with uranyl acetate and lead citrate and examined in an Akashi LEM 2000 superwide-field electron microscope. Electron micrographs were taken at \(\times 360\) in direct magnification and enlarged photographically (Fig. 1-3). The consecutive thick section was mounted on a slide glass, and stained with periodic acid-Schiff (PAS) reaction and Mayer’s hematoxylin for light microscopic examination (Fig. 4).

For more precise observations of the isthmus-neck region, a silver section almost parallel to the mucosal surface was mounted on a 50/75 mesh grid and stained with uranyl acetate and lead citrate. Electron micrographs were taken at \(\times 430\) in direct magnification (Fig. 5, 6).

RESULTS

1. Preliminary observations on the thickness of the section and the resolution of the micrograph

When comparing a silver-golden section with a silver one, the former apparently resulted in poorer resolutions of the electron micrographs (Fig. 1–3) than the latter (Fig. 5, 6). Nevertheless, a silver section on a \(450 \times 620\ \mu m\)-holed grid could not be used, since it was too easily demaged mechanically as well as electronically. For panoramic observations of the whole thickness of the mucosa, electron micrographs were taken at \(\times 360\) in direct magnification for the following reasons: a) structures such as secretory granules of surface mucous, the mucous neck and chief cells as well as mitochondria, tubulovesicular system and intracellular canaliculi of parietal cells were easily demonstrated at this magnification; such observations enabled us to figure out the type and maturation stage of the cells based on our previous study (KATAOKA, 1970) as far as dealing with the normal adult mouse gastric mucosa; b) the resolution of the structures was not much different at slightly higher magnifications (\(\times 430\) or \(\times 540\)); and c) a further magnified observation creates difficulty in joining a number of pictures with each other. On a silver section, structures as small as ribosomes can be demonstrated at \(\times 430\) in direct magnification.

2. Panoramic observation on the gastric mucosa

The epithelium and the gastric gland proper

The gastric epithelium invaginates to form numerous tiny pits, the gastric foveolae, into whose bottom the tubular gastric gland proper opens. The free surface and foveolae are lined with a simple columnar epithelium consisting of surface mucous cells. The gastric gland proper can be divided into three portions—the isthmus, neck and base—on the basis of differential population in cells (PLENK, 1932; STEVENS and LEBLOND, 1953) (Fig. 1). In the isthmus, so-called isthmus cells are intermingled with parietal cells. The neck and the base are characterized by the presence of mucous neck cells and chief cells, respectively, in addition to parietal and a few endocrine cells.

The so-called isthmus cells are primarily an immature form of surface mucous cells, and contain a few dense mucous granules just beneath the apical plasma membrane.
The cells become taller, with mucous granules increasing in number, as the cells migrate from the bottom of the isthmus towards the upper part of the foveola (Fig. 2, 4). At the mucosal surface, the number of mucous granules decreases. Lysosomes increase in the cells at the upper part of the foveola and the mucosal surface. A few migrating lymphocytes are found between the surface mucous cells lining the foveola and the free surface.

At the transitional region between the isthmus and the neck, a very few undifferentiated cells are found (Fig. 5, 6b). They are small cuboidal cells containing a nucleus of large karyo-cytoplasmic ratio with a few nucleoli. Cytoplasmic organelles are poorly developed except for numerous free polyribosomes. They neither contain secretory granules nor show the numerous microvilli which are specific to parietal cells.

Mucous neck cells appear at the upper end of the neck (Fig. 2, 3b). The granules increase in number and show some changes in the internal structure as the cell migrates towards the base. At the uppermost part of the neck, the cells contain somewhat smaller, and electron denser granules as compared to typical mucous neck cells, which appear just beneath this region and contain numerous moderately dense mucous granules (Fig. 2, 3). At the lower part of the neck and occasionally in the base, some cells exhibit an intermediate morphology between the mucous neck and chief cells: Secretory granules are about the same size as zymogen granules and have a heterogeneous content which is weakly stained with PAS reaction (Fig. 3a, 4).

Chief cells first appear at the top of the base (Fig. 2, 3a). Immature chief cells are relatively small cells containing electron dense zymogen granules. As they migrate towards the bottom of the base, the cells increase in size, and zymogen granules increase both in number and in size. The interior of most zymogen granules appears less dense in typical mature chief cells.

Immature parietal cells are occasionally present from the lower part of the isthmus to the upper part of the neck (Fig. 2, 3, 6a). The youngest parietal cells can be identified by their characteristic microvilli (KATAOKA, 1970) (Fig. 6a). The intracellular secretory canaliculi then gradually develop, and tubulovesicular elements increase in number. Some immature parietal cells contain mucus-like granules resembling those in the mucous neck cells near the cell apex and the intracellular canaliculi (Fig. 5, 6c). Mature parietal cells are found in the isthmus, neck and base. The uppermost parietal cells in the isthmus usually show dilated intracellular canaliculi and a few tubulovesicular elements (Fig. 1, 2). Degeneration figures of parietal cells are scattered at the upper end of the isthmus, lower part of the neck and the base (Fig. 1–3).

A few endocrine cells of several types are scattered in the neck and the base (Fig. 2). The uppermost endocrine cell observed in this study was present in the transitional region between the isthmus and neck (Fig. 1).

The stroma of the lamina propria

The stroma of the mucosa is the loose connective tissue containing blood capillaries, collecting venules, smooth muscle fibers and thin unmyelinated nerve fibers (Fig. 1–3).

All connective tissue fibers are of the thin collagenous variety. They are abundant beneath the epithelium lining the mucosal surface and around the foveola and the isthmus. On the other hand, the stroma around the neck and the base of the gland contains only scanty fibers. Connective tissue cells are also more numerous around the foveola and the isthmus than in the deeper part of the gland. Fibroblasts are the most common cellular elements of the loose connective tissue in the gastric mucosa. Macrophages are often found among the free cells, with lymphocytes less regular in
Fig. 2. a and b. (The bottom of Figure a continuous to the top of Figure b). Surface mucous cells (S) show a gradual change in number of secretory granules from the isthmus towards the surface (cf. Fig. 4). Parietal cells show various stages of maturation: immature (asterisks) and mature (P) cells, a parietal cell with dilated secretory canaliculi (arrow P) and degenerating cells (D). L gastric lumen, arrow L migrating lymphocyte in the epithelium, M mucous neck cell, C chief cell, E two different types of endocrine cells, Ca blood capillary, arrows: smooth muscle fibers, MM muscularis mucosae, V collecting venule. The enclosed areas are enlarged in Figure 3a, b. × 900
Fig. 3. Legend on the opposite page.
appearance. Other cell types, such as granular leukocytes, plasma cells and mast cells, are rather infrequent.

The gastric mucosa receives a rich blood supply by fenestrated capillaries. The thin portion of the endothelium, where numerous fenestrations open, are in the most intimate relation to the basal bulge of parietal cells than any other epithelial as well as glandular cell type. Collecting venules are often found near the muscularis mucosae.

Thin smooth muscle bundles, separated from the muscularis mucosae, run parallel to the gland, reaching just beneath the basal lamina of the epithelium lining the mucosal surface.

DISCUSSION

The gastric mucosa is a physiologically renewing tissue (Messier and Leblond, 1960). Frequent mitotic figures in the isthmus were noted by early histologists (Plenk, 1932). Following the advance of H-thymidine autoradiography, it has been clarified that most proliferative cells are present in the isthmus (generative cell zone), and surface mucous cells migrate upwards to the mucosal surface (Messier and Leblond, 1960; Hunt and Hunt, 1962; MacDonald et al., 1964; Kaku, 1966; Hattori, 1974). Electron microscopically, the cells capable of thymidine incorporation and proliferation in the isthmus are mostly immature forms of surface mucous cells containing some secretory granules in the apical cytoplasm (Kataoka, 1970).

The upward migration of surface mucous cells in a pipe-lines system (Hattori and Fujita, 1976) concurs with their ultrastructural maturation, so that the maturing cells are orderly placed from the bottom of the isthmus towards the mucosal surface, as shown in this study. The gradual increase in number of secretory granules towards the top of the gland was shown in this study.

Fig. 3. a. The lower neck region to the base. A typical mucous neck cell (M) and a probable transitional form from the mucous neck to chief cell (M') correspond to PAS-positive and weakly PAS-positive cells in Figure 4 (arrows), respectively. Compare immature (C') and mature (C) chief cells. D a degenerating parietal cell, E an endocrine cell. x 1,700. b. The transitional region between the isthmus and the neck. An immature surface mucous cell (S) and a typical mucous neck cell (lower M) correspond to arrow heads in Figure 4, respectively. Mucous neck cells in the upper end of the neck (upper M) contain granules denser than the typical cell (lower M). Asterisk: an immature parietal cell. x 2,300. c and d. Very young forms of parietal cells (asterisks). A secretory canaliculus (arrow) is seen above the nucleus in d. x 2,300.

Fig. 4. A light micrograph of the consecutive thick section corresponding to the area between the arrows in Figure 1, and Figure 2. PAS-hematoxylin stain. Cf. Figure 3 for arrows and arrow heads. x 380.
of the foveola and decrease at the luminar surface were already noted by Stevens and Léblond in 1953 as a change in PAS-positive content. The carbohydrate synthesis must be modified during the maturing and migrating process of surface mucous cells, since Sato and Spicer (1980, 1982) found sulfated glycoprotein in isthmus cells but neutral glycoprotein in foveolar and surface cells. An increase in number of lysosomes at the upper foveola and the surface may be a preceding sign of cell degeneration and extrusion at the mucosal surface.

Hattori (1974) designated the presence of undifferentiated cells at the lower end of the isthmus as "the constriction." The present study supports his view, because a few morphologically undifferentiated cells are present at this site and maturing cells arrange orderly in either upward or downward direction from this point. However, undifferentiated cells in the present as well as previous studies (Corpron, 1966; Kataoka, 1970) are identified as such by merely ultrastructural criteria: the absence of specific secretory granules, among others. Truly undifferentiated or pluripotential stem cells are difficult to identify by their morphology, and the contribution of such cells to new cell formation in the physiological renewal may be small, since morphologically identified undifferentiated cells are extremely scarce (Fig. 5), and the majority of 3H-thymidine-incorporating cells belong to immature forms of surface mucous and mucous neck cells (Kataoka, 1970).

Mucous neck cells seem to exhibit transitional forms at both the upper and lower ends of the neck, with typical cells present between them. Mucous neck cells with relatively dense granules shown by the present study at the upper end of the neck may

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**Fig. 5.** A panoramic view of the isthmus (I) and neck (N) showing the distribution of undifferentiated cells (crosses) and the cells (asterisks) with transitional morphology between parietal and mucous neck cells. The enclosed area, and the cells indicated by arrows b and c are enlarged in Figure 6. × 320
correspond to transitional cells between isthmus cells and mucous cells with rim granules (corresponding to mucous neck cells in mice). Such was described by SATO and SPICER (1980) by the coexistence of mucous granules containing glycoproteins specific to isthmus cells as well as to mucous cells with rim granules. Cells with weakly PAS-

Fig. 6. a. In the lower part of the isthmus, many immature forms of surface mucous cells (S) are present. A very young parietal cell (P) shows some characteristic microvilli. ×3,100

b. The cell shown here may be called undifferentiated, since some apical vesicles (arrow head) and a few small basal granules (arrow) are found in any cell type, and the cell shows no differentiated signs. ×6,200.

c. The cell has an intracellular canaliculus (arrow) characteristic to a parietal cell and mucus-like granules which look like the secretory granules of a mucous neck cell (M). ×3,700
positive, heterogeneous granules in the lower neck region seem to correspond to the transitional form from mucous neck to chief cells described by Sato and Spicer (1980). The transition from mucous neck to chief cells was also supported by a cell kinetic study (Hattori and Fujita, 1976). Further cytochemical studies are required to clarify the exact nature of these transitional cells.

Chief cells are restricted to the base of the gland, and arranged according to their maturation stage from the top to the bottom of the base. The arrangement of mucous neck cells, probable transitional forms between mucous neck and chief cells, and immature and mature forms of chief cells, is somewhat disordered probably due to their migration in the stochastic flow system (Hattori and Fujita, 1976).

The youngest, immature forms of parietal cells (Kataoka, 1970) are present from the lower end of the isthmus to the upper part of the neck. The presence of mucous-like granules in some of these cells suggests the possibility of transformation from mucous neck to parietal cells which has already been stated by Hunt and Hunt (1962) in their light microscopic study. Mature parietal cells are present throughout the isthmus, neck and base. Degeneration of parietal cells was found at the upper part of the isthmus, lower part of the neck and the base. These facts give ultrastructural evidence to the theory of Hattori (1974) and Hattori and Fujita (1976) that new parietal cells are formed at "the constriction" and migrate either upwards or downwards. By the extrusion of degenerated parietal cells, the mechanism of which is still unknown, the foveolar and surface epithelium is rendered free from parietal cells, and the base of the gland contains fewer parietal cells than the neck.

Ito and Schofield (1974) described the abundance and depletion of tubulovesicular elements in resting and stimulated parietal cells, respectively. In such observations, ultrastructural changes in maturing parietal cells must be taken into account. It is not clear whether the depletion of tubulovesicular elements and dilatation of intracellular canaliculi in the uppermost parietal cells in this study have any relation to the functional state of the cell.

In the stroma of the lamina propria, the difference in the amount of collagen fibers is demonstrated between the superficial and deeper parts of the mucosa. Abundant collagen fibers in the surface, foveolar and isthmus region may contribute partly in forming the stromal sheath enclosing the generative cell zone (Hattori, 1974) and partly in giving mechanical support to the mucosal surface. Thin smooth muscle fibers perpendicular to the mucosal surface were noticed by early histologists (Plenk, 1932), but have been neglected by most electron microscopists. They may play an important role in squeezing out the glandular secretion into the gastric lumen.

In conclusion, this study clearly demonstrates the orderly arrangement of maturing epithelial and glandular cells and the difference in stromal structure along the superficial-deep axis of the mucosa. In addition, superwide-field electron microscopy is useful to examine the occurrence and distribution of rare cell types, such as undifferentiated cells and possible transitional elements between mucous neck and parietal cells.

Acknowledgements. The authors are grateful to the staff of Akashi Precision Inc., Hachioji, Tokyo, Japan, for kindly allowing us use of their Akashi LEM 2000 superwide-field electron microscope, and for their technical help.
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