Duodenal and gastric cell regenerating epithelia on margins of human duodenal ulcer and presence of *H. pylori* - An electron microscopic study

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Summary. Specimens from 22 cases of human duodenal ulcers obtained at surgery were studied by transmission and scanning electron microscopy. Observations were focused on the ulcer margins which always showed some evidence of healing by simple cuboidal epithelial cells migrating on the ulcer base. Two types of regenerating epithelia (RE) were found: the intestinal and the gastric cell types. The intestinal type RE originating from intestinal epithelium of the surrounding epithelium of the ulcer edge was composed of immature enterocytes, which differentiated into absorptive and goblet cells, and formed presumptive crypts and villi. The gastric type RE grew from adjacent metaplastic gastric mucosa at the edge of the ulcer and consisted of immature cells, which developed into mucous cells. Some ulcers had RE of both intestinal cell and gastric cell origin. In most margins, the RE was of only one cell type, but in others both intestinal and gastric type cells were present. In more developed regions both types formed presumptive glands. The basal lamina was frequently missing near the leading edge. This corresponded to degeneration and necrosis of RE, especially in areas of severe inflammatory foci. *Helicobacter (H.) pylori* colonization on gastric metaplastic epithelium was observed in about one third of the ulcer cases. In the surrounding epithelium of ulcers colonized with *H. pylori* there were degenerative changes, disruption of cell membranes, and massive cell exfoliation resulting in denuded lamina propria. Some gastric type RE was also colonized with *H. pylori*. No infection was found on intestinal epithelia. These findings suggest that *H. pylori* may be important in the development of duodenal ulcers as well as in the prevention or delay of ulcer healing.

Key words: Duodenum, Duodenal ulcer, Regenerating epithelium, Gastric metaplasia, *Helicobacter pylori*, Electron microscopy

Introduction

The healing process of the human duodenal ulcers has been reported by light and electron microscopy using endoscopic biopsy specimens (Gregory et al., 1982; Malfertheiner et al., 1985, 1989; Pan and Liao, 1990; Bode et al., 1991; Pan et al., 1991). However, surgically-resected duodenal ulcers have not been investigated specifically for evidence of ulcer healing by bordering cells. In a recent study of human chronic gastric ulcers (Ogata, 1995), regenerating epithelium (RE) was found along the margins of almost all ulcers even though complete repair did not occur. This study was undertaken to determine whether a similar process was present in duodenal ulcers. Observations focused on ulcer margins revealed the presence of both intestinal and metaplastic gastric mucosa cells forming the RE in duodenal ulcers.

In the present study, one third of the ulcer cases were found to be infected with *Helicobacter pylori*. These bacteria were encountered only in cases with metaplastic gastric mucosae and sometimes on gastric-type RE. They were not found on intestinal mucous or intestinal RE. These findings indicate that *H. pylori* infection may be an important factor in duodenal ulcers.

Materials and methods

Specimens from 22 human duodenal ulcers were obtained at surgery from 11 patients with chronic, incurable ulcers and 8 with perforated ulcers. Three ulcer scars (healed ulcers) were from resected duodenums of early gastric cancer patients. For TEM, each resected duodenum was immediately opened and Karnovsky's fixative (5% glutaraldehyde, 4% para-formaldehyde in 0.1M cacodylate buffer, pH 7.4) was poured on the ulcer. After 15 minutes, the ulcer and surrounding tissue was dissected from the duodenum and fixation continued in the same solution for 30 minutes. Appropriate areas were then cut into small blocks under a dissection microscope and fixation

continued for another hour. These blocks were osmicated in 1.2% osmium tetroxide in 0.1M cacodylate buffer (pH 7.4) for 1 hour, dehydrated in graded ethanol and embedded in Epon. Thin sections were stained with uranyl acetate and lead hydroxide and observed with a JEOL 1200 transmission electron microscope. Semithin sections were stained with toluidine blue for light microscopy.

For SEM specimens, the resected duodenum was immediately opened and the ulcer and its surrounding tissue was excised. The ulcer was bisected with a razor blade under a dissection microscope and one half was fixed in Karnovsky's fixative for TEM and the remainder gently washed in physiological saline solution to remove surface mucus, and fixed in 2.5% glutaraldehyde cacodylate buffer (pH 7.2) for 2 hours. The fixed specimens were cut into pieces of appropriate size, dehydrated through an ethanol series and critical-point-dried (HCP-2, Hitachi). The dry specimens were mounted on brass stubs and coated with 20 nm of gold in an ion-coater (IB-5, Eiko). These samples were observed with a Hitachi S-430 scanning electron microscope at 15 kV accelerating voltage.

Results

Microscopic examination of the margins of all duodenal ulcer specimens revealed varying degrees of cellular migration from the surrounding viable mucosae. These cells, the regenerating epithelia or RE which grew over the granulation tissue were of the intestinal or gastric cell type. The former arose from enterocytes and the latter from metaplastic gastric mucosae which was frequently observed in the duodenum. These two types of RE were frequently found as patches along the edge of the same ulcer (Fig. 1).

1. Intestinal cell type regenerating epithelium

Light microscopy of the intestinal type RE revealed a simple layer of cuboidal epithelium originating from intestinal mucosa at the edge of the ulcer. The leading cells were undifferentiated and formed a sheet which extended toward the center of the ulcer base (Fig. 1a-c). Differentiation of the cuboidal RE cells to columnar

absorptive cells was evident. They were devoid of mucous granules, which were typical of gastric surface cells, and mitotic cells were occasionally seen (Figs. 1b, 6). A few goblet cells were present among the developing enterocytes (Fig. 1c). In some regions the RE was pseudostratified epithelium and invaginated into the lamina propria or the granulation tissue to form presumptive immature crypts (Fig. 1c).

Electron microscopy of the intestinal-type RE cells confirmed their undifferentiated state. They had relatively large nuclei with prominent nucleoli (Figs. 3, 4). The cytoplasm was packed with free ribosomes and few elements of rough endoplasmic reticulum (Fig. 5). Mitochondria were scarce. In some areas of the RE there were cells in various stages of maturation towards absorptive cells (Figs. 3, 6). These young columnar absorptive cells had elongated nuclei and less prominent nucleoli. The microvilli were more numerous and longer than those of the undifferentiated cells. The basal lamina was discontinuous or lacking in many areas of the RE (Figs. 3, 4).

2. Gastric cell type regenerating epithelium

Light microscopy of the gastric cell type RE revealed a simple layer of cuboidal epithelium extending from the gastric metaplastic epithelium toward the center of the ulcer base (Figs. 1a,d,e, 16a,c). These undifferentiated cells had large nuclei with prominent nucleoli and were devoid of mucous granules. The undifferentiated RE cells occasionally underwent mitotic division (Fig. 1e). The cells adjacent to the undifferentiated cells contained a few mucous granules (Fig. 16c). These cells gradually increased in size and had more mucous granules.

In some regions of the simple layered RE, there was pseudostratified epithelium which invaginated into the lamina propria or granulation tissue to form primitive glands (Fig. 1d). Underlying the RE there was usually granulation tissue with varying degrees of inflammation. The mucosa surrounding the duodenal ulcer was composed of intermingling patches of intestinal epithelium and gastric metaplastic epithelium; that is, the intestinal and the gastric type RE frequently coexisted in the same ulcer (Fig. 1). Evidence for the two types of

Fig. 1. Light micrographs of the RE of the duodenal ulcer stained with toluidine blue. **a.** Lower magnification photomicrograph of a section across a duodenal ulcer. On the left, the RE is continuous with adjacent intestinal epithelium (IE), while on the right the RE (GR) is extended from a patch of metaplastic gastric epithelium (GE). The surrounding epithelium of this ulcer is composed of a mixture of intestinal epithelium and metaplastic gastric epithelium. G: goblet cell. x 50. **b.** A higher magnification of the intestinal RE indicated by X in Fig. 1a. Intestinal type RE extends toward the ulcer base (upper right margin). Small cuboidal undifferentiated cells (U) with large nucleoli are continuous to the more matured columnar absorptive cells (A). The granulation tissue is seen under the RE. x 250. **c.** A higher magnification of a part of Fig. 1a indicated by Y. Pseudostratified area (P) of intestinal type RE extends into the granulation tissue forming the new crypt. G: goblet cell. x 220. **d.** A higher magnification of the right margin of the ulcer indicated GR in Fig. 1a. Note that the RE is gastric epithelial cell type. Arrow shows that the areas of the RE sprout into the granulation tissue forming immature glands. x 100. The inset shows an enlarged view of the boxed area with mucous granules containing surface mucous cells. Arrowhead shows matured gastric type mucous cell. x 300. **e.** A higher magnification of a part of the gastric type RE in Fig. 1d. The RE is composed of small polygonal undifferentiated cells with a large nucleus. Red blood cells are in the lumen. Arrow shows one of several mitosis. x 400.

Fig. 2. A duodenal ulcer scar (healed ulcer). Note the left side of the scar is composed of gastric metaplastic epithelium (GE), while the right half is of intestinal epithelium (IE). G: goblet cell. x 150
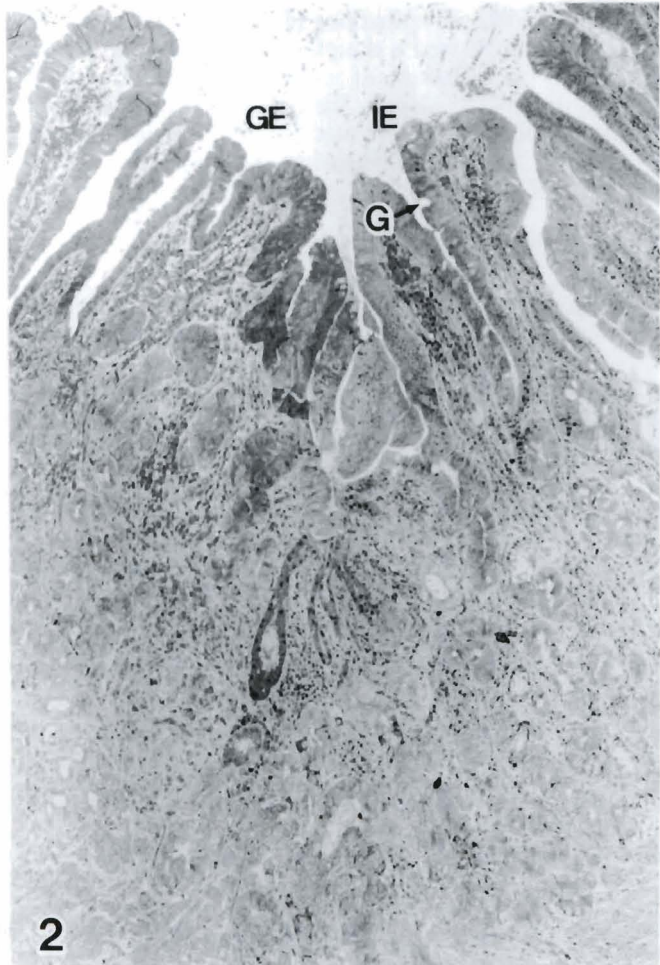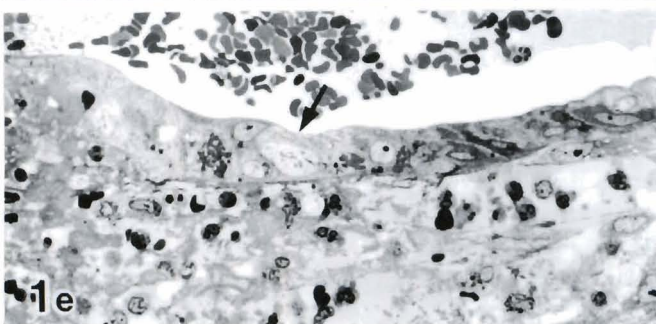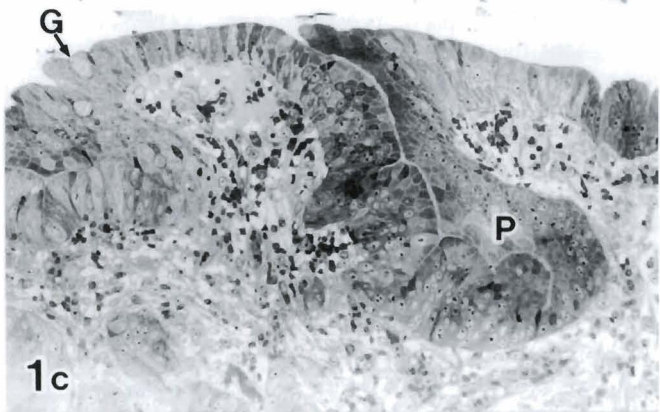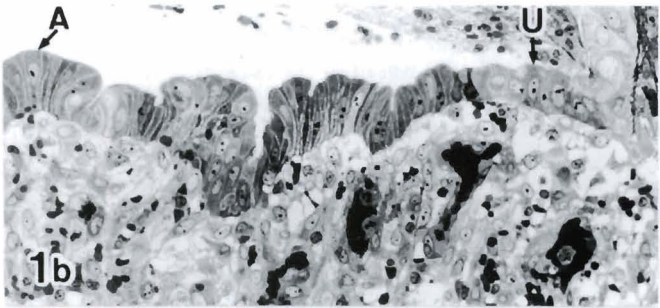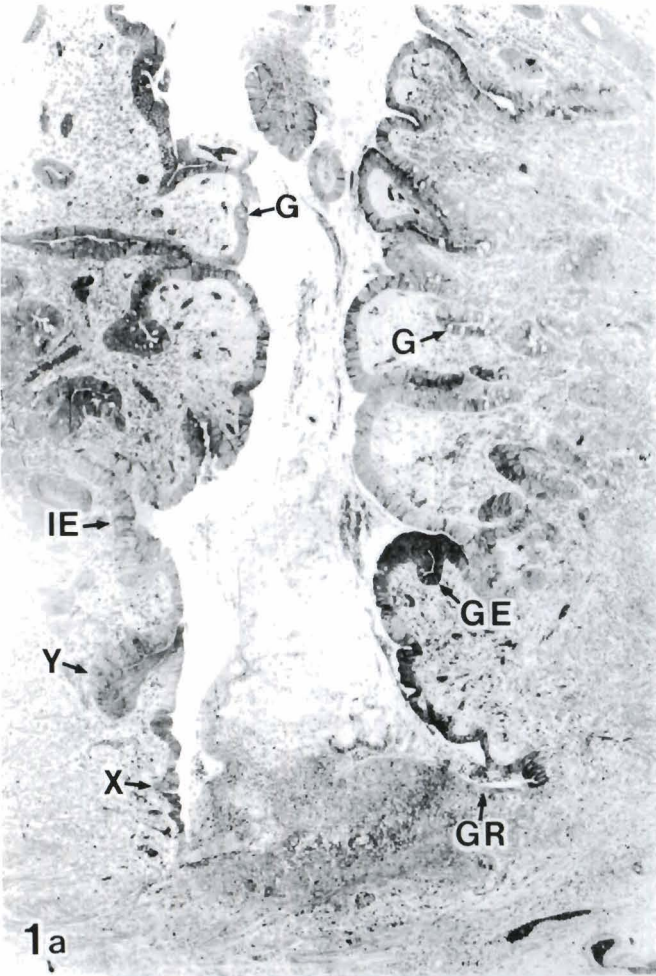
mucosae participating in ulcer healing was also seen in ulcer scars filled with matured glands (Fig. 2).

Electron microscopy confirmed that the undifferentiated cells in the gastric type RE did not contain mucous granules (Figs. 9, 11). These cells were small and cuboidal or low cuboidal (Fig. 11) and had relatively large nuclei with diffuse chromatin and prominent nucleoli (Figs. 9, 11). Microvilli were similar to those on the undifferentiated cells of the intestinal type RE, but were less numerous and shorter (Fig. 9). Tight junctions, desmosomes and cell interdigitations were well developed. Abundant free ribosomes were scattered throughout the cytoplasm, but mitochondria and rough endoplasmic reticulum were sparse (Fig. 9). In the RE, some cells contained a few mucous granules (Fig. 10). Other cells were larger and more differentiated, with numerous mucous granules and cell organelles (Figs. 12, 13). They appeared to be developing into matured gastric mucous cells. The basal lamina of the RE was often missing (Fig. 13).

3. Scanning electron microscopy

Observations of duodenal ulcers by SEM revealed a sheet of RE arising from the surrounding epithelium and extending toward the center of the ulcer base (Figs. 14, 15). In some RE, invaginations of epithelial cells around a small lumen formed a doughnut-like formation which resembled the openings of gastric pits (Fig. 15a-c). It was not possible to identify the type of RE growing over the ulcer base by SEM. Neither villi nor gastric pits were distinctive. However, extensive surface views of the ulcers could be examined.

4. Degeneration of the regenerating epithelium

Degeneration, necrosis and exfoliation of RE cells were frequent in both intestinal (Figs. 7, 8) and gastric types (Fig. 13), especially in areas of severe inflammatory foci. Extravasated red blood cells and infiltration of leukocytes (mostly neutrophils) in the lamina propria was common (Figs. 1, 7, 8, 13).

Detachment of the RE from their support or the lamina propria were also evident (Figs. 7, 13).

5. Helicobacter (H.) pylori infection

H. pylori colonization was observed around gastric metaplastic epithelium in 7 out of 22 duodenal ulcers examined. These bacteria were found only in areas of gastric metaplasia and not on intestinal mucosae. Light microscopy of gastric metaplastic epithelium colonized with *H. pylori* often showed severe degeneration, and necrosis of the mucous cells was frequently seen (Figs. 16a,b, 17, 18). Moreover, massive cell exfoliation causing denuded lamina propria was frequent in the surrounding mucosa (Figs. 16a,b, 18). Severe neutrophil infiltration was present in the lamina propria and granulation tissue (Figs. 16-18).

Electron microscopy of these infected areas showed degenerative changes such as cellular edema, vacuole formation and disruption of cell membranes and the dissolution of apical mucous granules (Figs. 19, 20). In addition, there was colonization of *H. pylori* on partially mature gastric type RE (Figs. 19c, 21) but not on the immature gastric type RE, containing few mucous granules (Fig. 16c). The RE colonized with *H. pylori* showed evidence of cytopathic changes, such as apocrine-like shading of apical cytoplasmic fragments and cellular vacuolization (Figs. 19c, 21).

Discussion

Previous studies have shown that during the healing process of experimental gastric ulcers, a monolayer of RE composed of immature cells migrates from the ulcer edge toward the center of the ulcer (Ferguson, 1928; Townsend, 1961; Ogata et al., 1970; Tarnawski et al., 1991). A similar process was also observed in the human chronic gastric ulcer margins where gastric RE, as well as metaplastic intestinal RE, were reported (Ogata, 1995). Rather similar findings were found in the present duodenal ulcer study. The undifferentiated cells of the intestinal type RE which migrate from adjacent intestine

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Figs. 3-8 are TEM images of the intestinal type RE.

Fig. 3. The cuboidal undifferentiated cells (U) seen at the right gradually mature into the tall immature absorptive cells (I). x 960. The inset shows an enlarged view of the boxed area. Note the basal lamina is absent. x 8,000

Fig. 4. An undifferentiated cell with rounded apical surface has a large nucleus with prominent nucleoli. The stubby microvilli are slightly longer and more numerous than on the undifferentiated cell of gastric type RE. A few mitochondria (M) are seen but there are no mucous granules. Note the basal lamina is absent. x 2,400

Fig. 5. A higher magnification of an undifferentiated cell. The cytoplasm contains numerous free ribosomes (arrows) and rough endoplasmic reticulum (ER). M: mitochondria. x 32,000

Fig. 6. A portion of the intestinal type RE. A mitotic figure is seen in the RE. SM: surrounding intestinal epithelium. x 1,700

Fig. 7. Leading edge (L) of an intestinal type RE. Some epithelial cells are degenerated (arrows). Numerous inflammatory cells are seen in the submucosa. x 1,000

Fig. 8. Severely degenerated intestinal type RE. A necrotic absorptive cell (arrow) detaches from the basal lamina. Inflammatory cells in the lamina propria. x 1,200
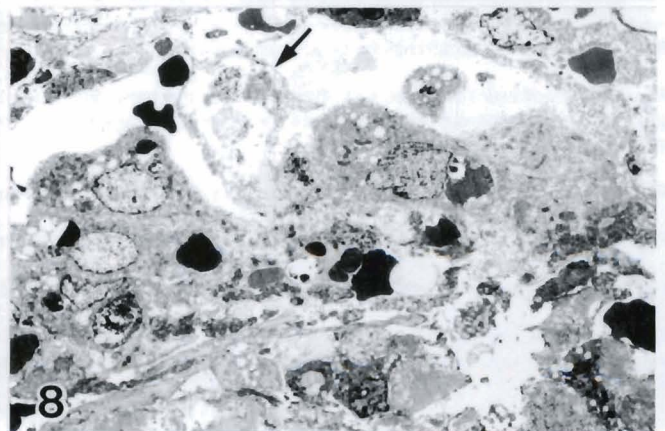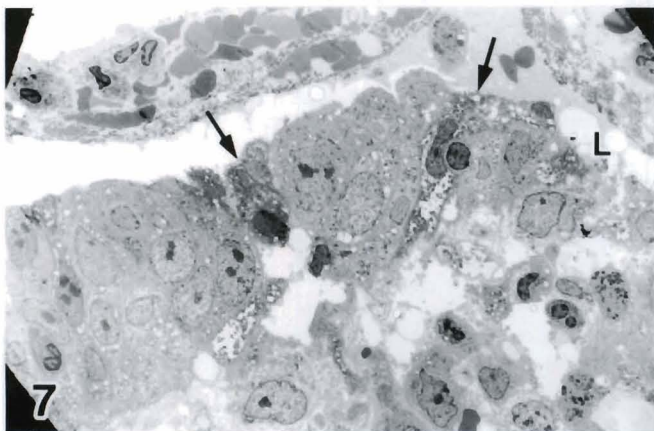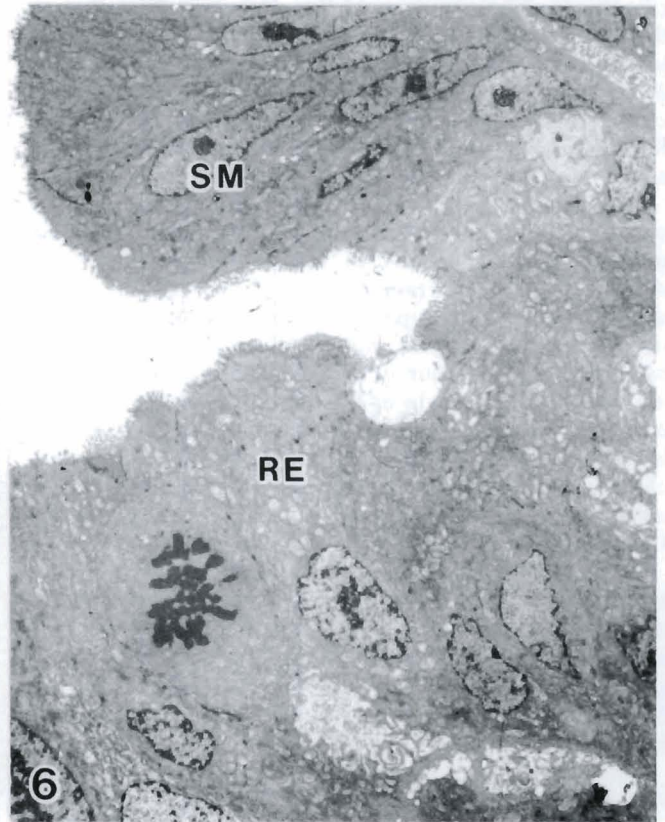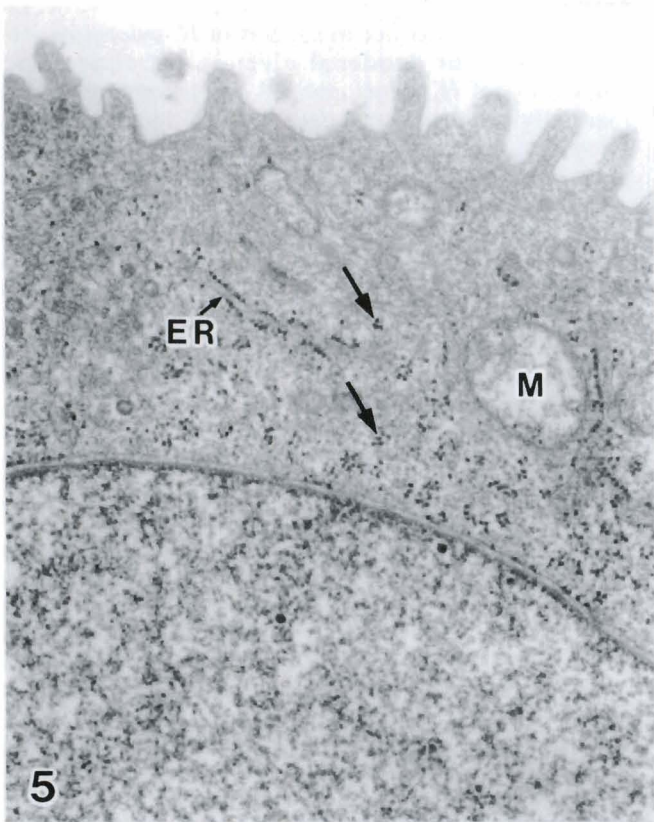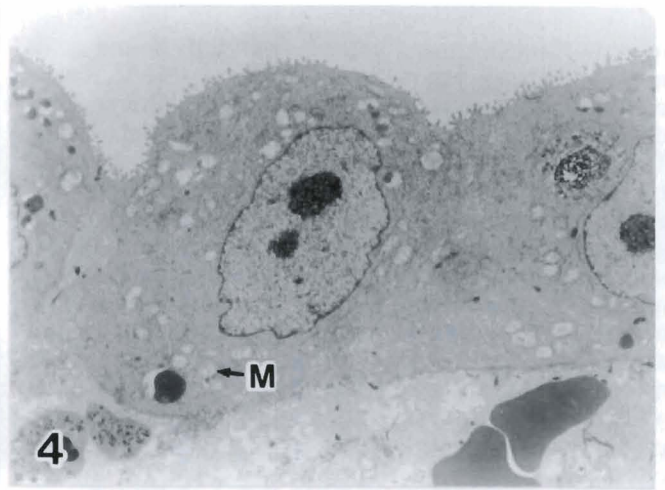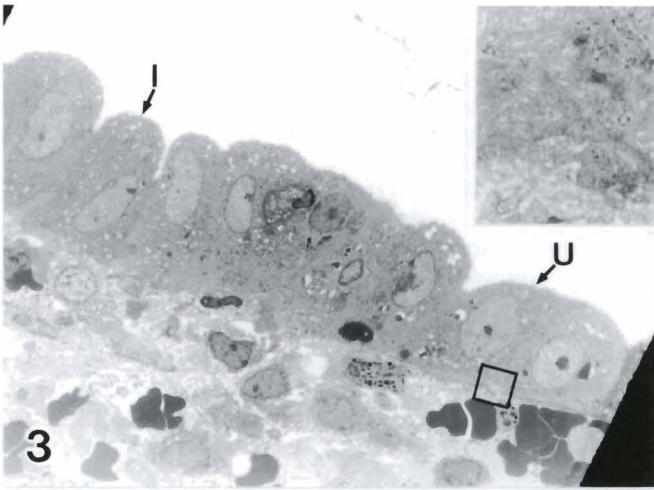
mucosa resemble principal cells normally found at or near the base of intestinal crypts (Trier, 1963). These cells are also similar to primitive intestinal epithelial cells in human embryonic intestine (Toyota et al., 1989). The undifferentiated cells of both intestinal and gastric type RE have large nuclei with prominent nucleoli, abundant free ribosomes, few organelles and no mucous granules. However, the undifferentiated cells of the intestinal type RE have more numerous and longer microvilli than does the gastric type RE. These undifferentiated cells gradually mature into young absorptive cells which are columnar and do not contain mucous granules nor become typical goblet cells filled with mucous granules. The undifferentiated cells in gastric type RE have no mucous granules and only a few cell organelles and their cytoplasm is packed with free ribosomes. The structure of this cell resembles that of the granule-free cells in the cell proliferation zone of the gastric gland (Lee and Leblond, 1985) and the undifferentiated cells in the embryonic stomach (Kataoka et al., 1984). The undifferentiated cells of gastric type RE matured by increasing their mucous granule content and developing into surface mucous cells.

During embryonic histogenesis, the simple pseudo-stratified intestinal epithelium forms primitive crypts and intestinal villi (Dongen et al., 1976; Toyota et al., 1989). A similar process was observed in the present study, where the intestinal type RE attempts to heal duodenal ulcers. In the gastric type RE, some parts of the pseudostratified portion of the RE budded or invaginated into the lamina propria or into the granulation tissue to form primitive gastric pits and glands. A similar process has been observed during rat experimental gastric ulcer healing and its resemblance to normal gastric morphogenesis has been recognized (Tarnawski et al., 1991).

The SEM micrographs of duodenal ulcers confirm and demonstrate in three dimensions the extensive RE at the edge of the ulcers. These whole-mount specimens also show the bulging of the epithelial cells around gastric pit or crypt openings. Wide expanses of the RE that are not seen in section of ulcers are seen to good advantage in the SEM micrographs.

The frequent degeneration and necrosis of the RE in both the intestinal and gastric type RE may be indicative of the failure of complete ulcer healing. Although it is not known why this occurs, the immature RE cells may be more fragile than fully mature cells. Local aggressive factors, especially hydrochloric acid, which is present in very high concentrations in duodenal ulcer patients, may play a role in RE cell damage. Inflammatory reactions in the ulcer base seem to accompany RE cell disruption. Moreover, the absence of a basal lamina under the RE cells no doubt hinders the migration of the RE cells and healing of human duodenal ulcers.

Another factor inhibiting duodenal ulcer repair may be *H. pylori* infection. Recent studies have shown this bacterial infection to be closely associated with duodenal ulcers (Johnston et al., 1986, 1988; Andersen et al., 1987; Bode et al., 1987, 1988; Wyatt et al., 1987, 1990; Goodwin, 1988; Carrick et al., 1989; Graham, 1989; Malfertheiner et al., 1989; Noach et al., 1993). However, morphological evidence in support of *H. pylori* infection as the cause of duodenal ulcer is not clear. After elimination of *H. pylori*, patients are reported to have a markedly reduced recurrence rate of duodenal ulcers (Coghlan et al., 1987; Marshall et al., 1988). It is generally believed that acid induces gastric metaplasia and that this allows *H. pylori* to colonize the duodenal mucosa to produce duodenitis (Wyatt et al., 1987, 1990; Goodwin, 1988; Carrick et al., 1989). These inflammatory foci may be vulnerable for aggressive luminal factors and may cause the duodenal ulcers. However, there is still no convincing morphological evidence that there is a direct relationship between duodenal ulcers and *H. pylori* infection.

H. pylori infection is very high, 95% (Lambert et al., 1985) and 100% (Marshall and Warren, 1984) in the antral mucosa of duodenal ulcer patients, but relatively low, 28% (Bode et al., 1987) and 28% (Satoh et al., 1993) in the duodenal mucosa. In the present study, the bacterial infection was identified morphologically in 32% (7/22) of cases. This finding is based on the very limited areas of each ulcer that were examined morphologically. If the entire duodenal mucosa of 22 cases had been examined in great detail, a higher rate of *H. pylori* infection could probably be applied.

In previous electron microscopic studies of duodenal ulcers, *H. pylori* was observed only on gastric type metaplastic mucous cells (Malfertheiner et al., 1989;

**Figs. 9-13.** TEM micrographs of the gastric cell type RE.

Fig. 9. An undifferentiated cell with large nucleus and prominent nucleoli and few short microvilli. Note the scant cytoplasm contains a few cell organelles and numerous free ribosomes, but no mucous granules. D: desmosome; G: Golgi apparatus; M: mitochondria; R: rough endoplasmic reticulum; T: tight junction. x 6,000

Fig. 10. A higher magnification of immature mucous cell. The mucous granules (M) are arranged in several layers. x 7,200

Fig. 11. A flattened undifferentiated cell. The cytoplasm contains few cell organelles and no mucous granules. x 3,000

Fig. 12. Moderately mature mucous cells are elongated and contain numerous mucous granules. x 2,400

Fig. 13. The leading edge of gastric type RE consisting of moderately mature mucous cells. The cell at the leading edge (L) has many vacuoles. Note the presence of neutrophils (N) in the lamina propria. x 2,600. The insert shows an enlarged view of the boxed area. Note the lack of the basal lamina. x 4,500
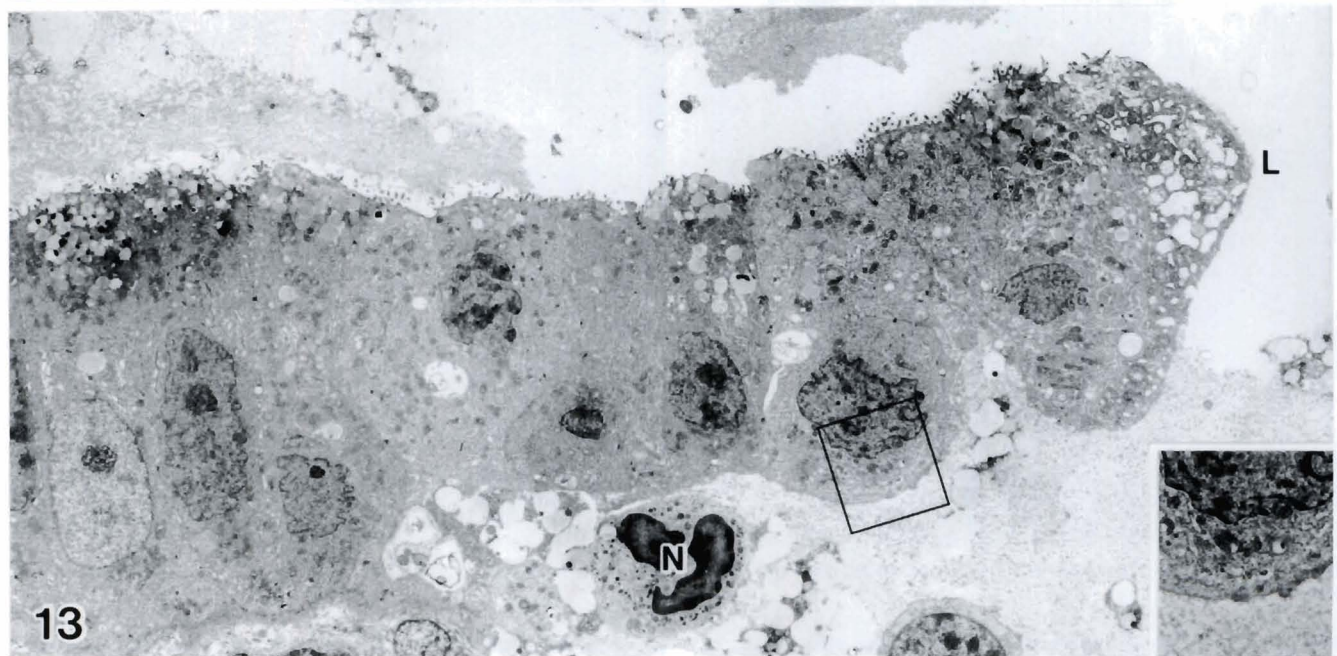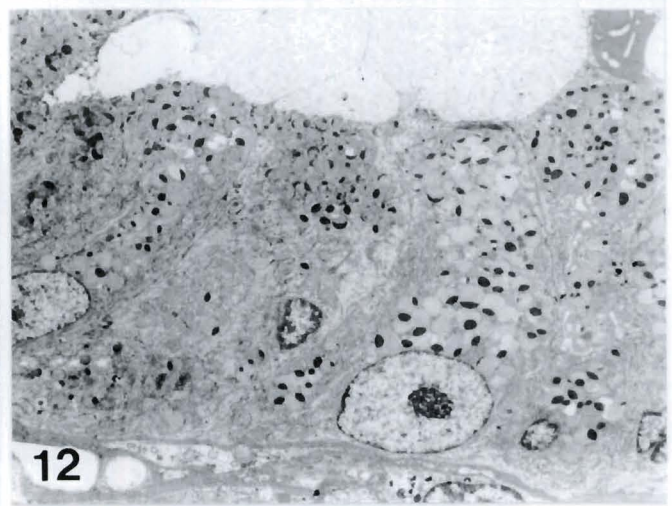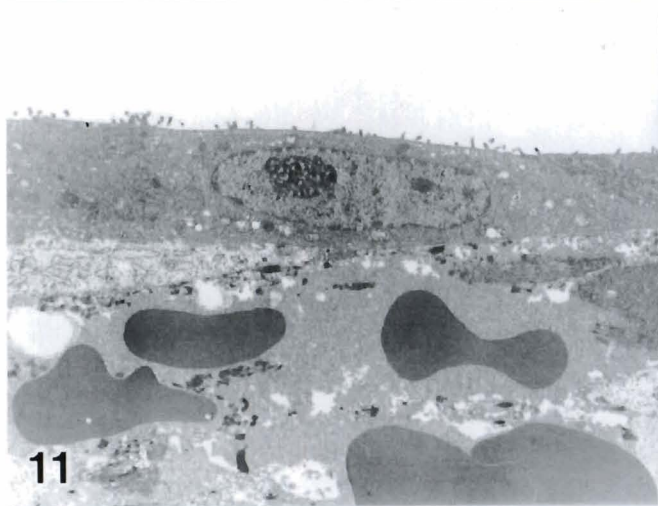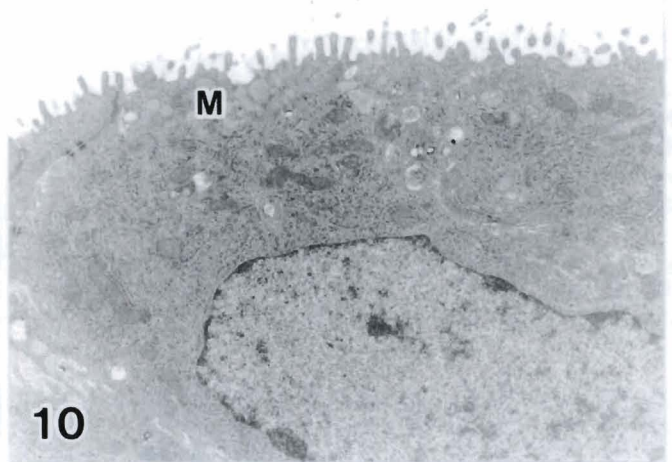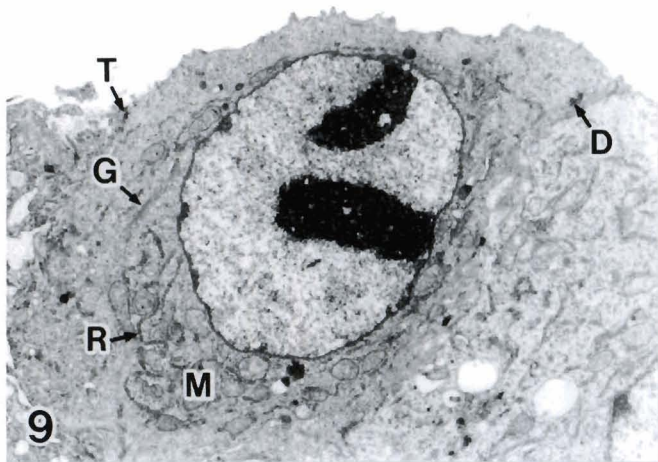
Bode et al., 1991). Ultrastructural changes of the gastric mucosa infected with *H. pylori* have been reported by several authors (Tricottet et al., 1986; Chen et al., 1986; Hazelle and Lee, 1986; Bode et al., 1988; Caselli et al., 1993). These studies showed the widening of intercellular spaces, depletion of mucous granules and cellular degeneration.

In a previous electron microscopic study of human gastric ulcers infected with *H. pylori* (Ogata and Araki, 1996), we illustrated the process of apocrine-like mucous granule release from mucous cells as well as the

degeneration and necrosis of these cells. A similar loss of apical mucous areas and severe degeneration and necrosis of mucous cells were also observed in the gastric metaplastic epithelium of duodenal ulcers when *H. pylori* were present.

Erosion of the gastric mucosa infected with *H. pylori* has been reported (Chan et al., 1992; Hui et al., 1992; Ogata and Araki, 1996). Recently, Krakowka et al. (1995) showed that microulcers were formed in the gastric mucosa of gnotobiotic piglets infected with *H. pylori*. This model excludes all known exogenous
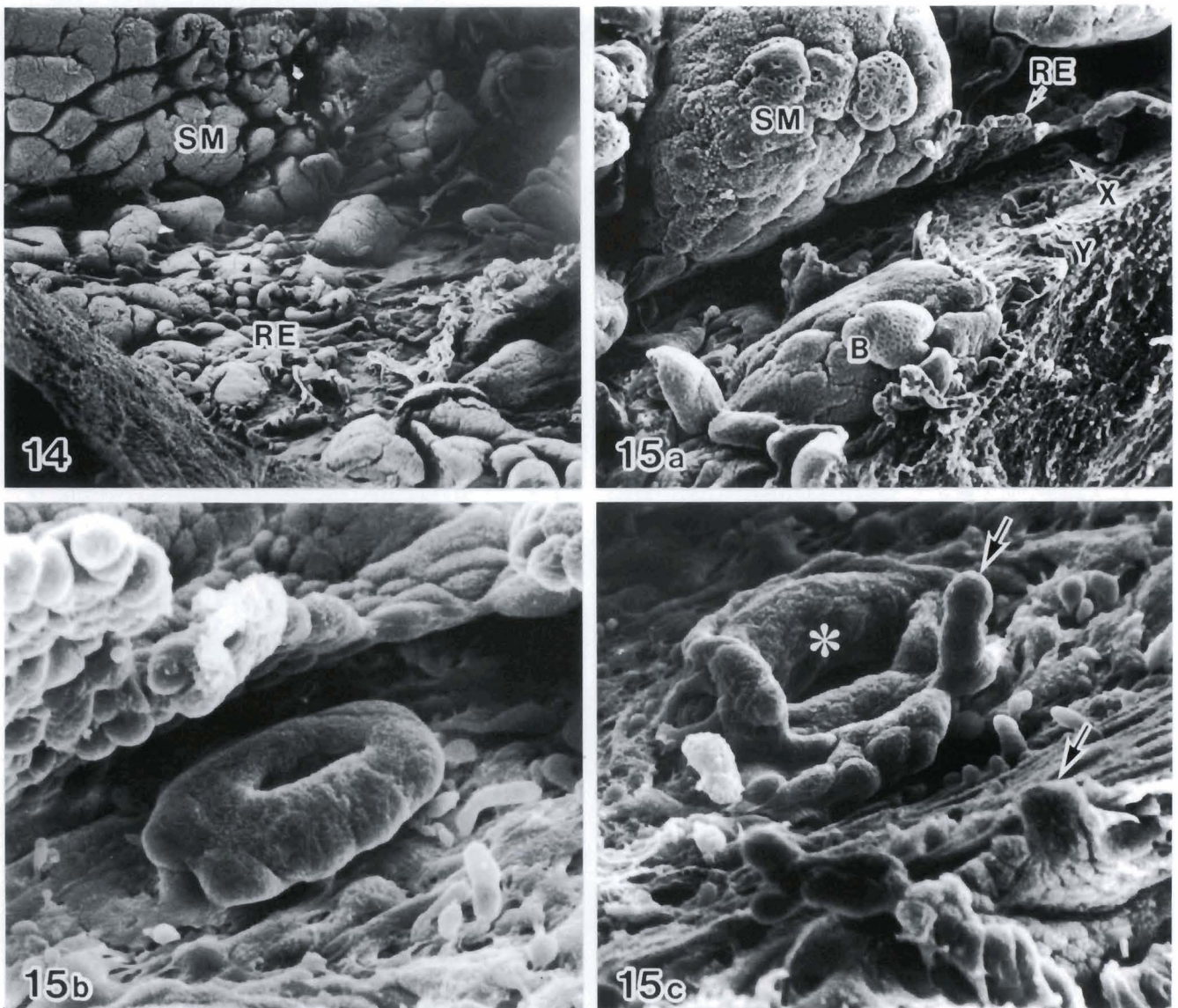

Fig. 14. SEM image of a duodenal ulcer. A layer of RE extends from the edge of the surrounding mucosa (SM) toward the center of the ulcer base. x 100

Fig. 15. SEM images of a duodenal ulcer. RE is presumably gastric type because the surrounding epithelium (SM) is gastric metaplastic epithelium. **a.** Bulging of epithelium (B) and new gland opening (X,Y) are seen on the ulcer base. x 150. **b.** A higher magnification of a part of Fig. 15a labeled X. A doughnut-like gland opening is seen on the ulcer base. x 1,200. **c.** Higher magnification of a part of Fig. 15a labeled Y. RE form a new gland opening (*). Some epithelial cells of the RE project upward (arrows). x 800
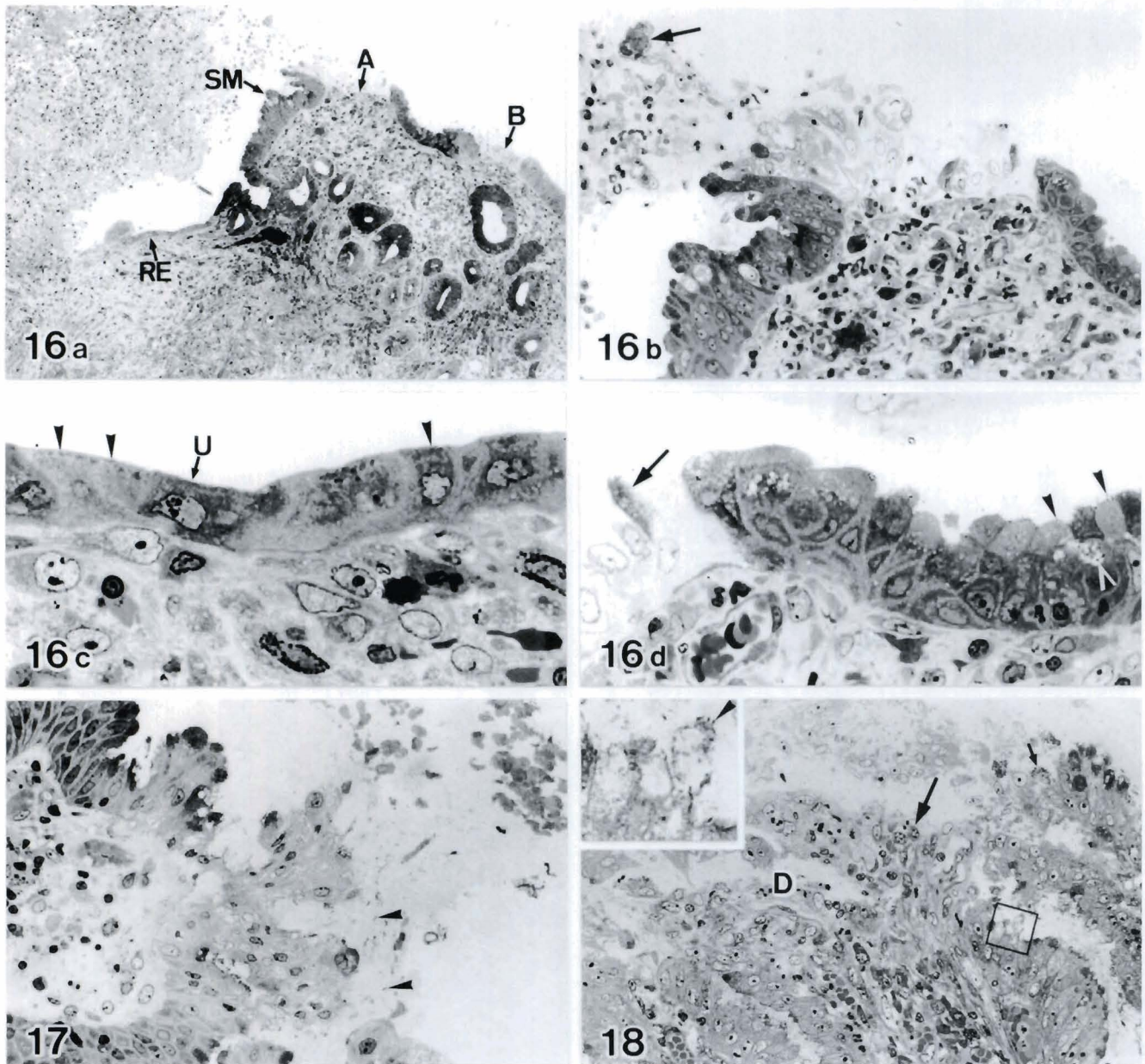
Fig. 16. Light micrographs of the duodenal ulcer colonized with *H. pylori* stained with toluidine blue. **a.** Low magnification of metaplastic gastric epithelium of surrounding mucosa with gastric type RE migrating over the ulcer. Two erosion sites (A, B) in the surrounding epithelium (SM). x 50. **b.** A higher magnification of the eroded area labeled A in Fig. 16a. Severe degeneration and exfoliation of mucous cells. Arrow points to a cluster of degenerated epithelial cells. x 160. **c.** A higher magnification of the RE area of Fig. 16a. Epithelial cells adjacent to the undifferentiated cell (U) have a few mucous granules (arrowheads). Note *H. pylori* colonization is absent around this immature RE. x 800. **d.** A higher magnification of a part of Fig. 16b. Degenerated cells and detached cells (arrow) are seen. Arrowheads show *H. pylori*. x 700

Fig. 17. The surrounding mucosa of the duodenal ulcer colonized with *H. pylori* (arrowheads). The loss of apical area and marked degeneration of metaplastic mucous cells are seen. x 300

Fig. 18. Gastric metaplasia epithelia in an area with severe inflammation. A chain of degenerated epithelial cells which have lost their apical mucous area (arrow) is detached from the basal lamina, and the lamina propria is denuded (D). Severe neutrophil infiltration of the lamina propria. x 200. The insert shows an enlarged view of the boxed area. Arrowhead shows *H. pylori*. x 800
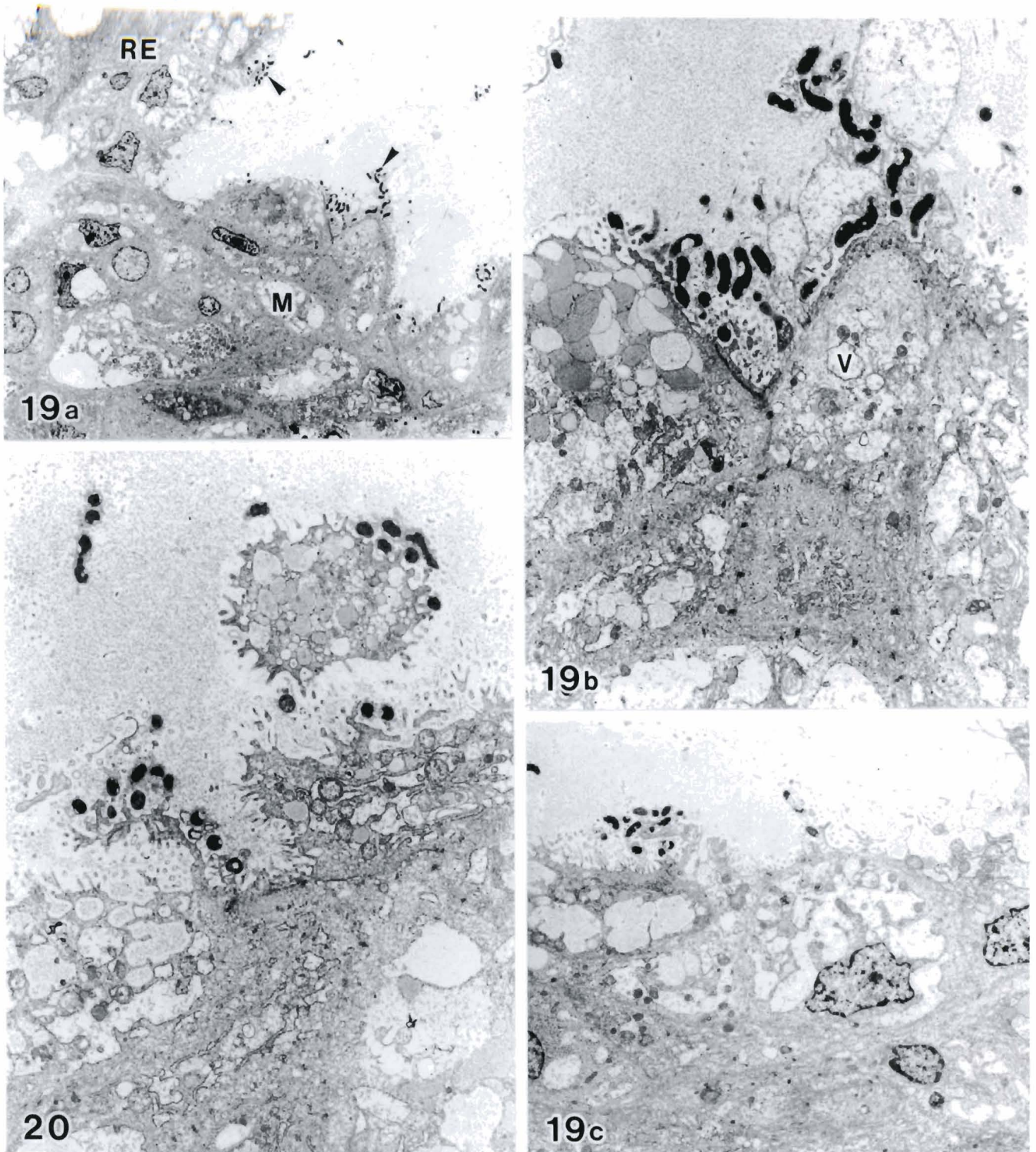
Fig. 19. TEM micrograph of duodenal ulcer colonized with *H. pylori*. **a.** Gastric type RE is continuous with the gastric metaplastic epithelium (M) of the surrounding mucosa. Note *H. pylori* are colonizing the surrounding mucosa as well as the RE. Arrowheads: *H. pylori*. x 1,300. **b.** A higher magnification of a part of the surrounding epithelium in Fig. 19a. There is a loss of cytoplasmic matrix density, cytoplasmic vacuolation (V). Numerous *H. pylori* appear to be causing apical blebs which become detached. x 5,000. **c.** A higher magnification of the RE region in Fig. 19a. Numerous large vacuoles in degenerating mucous cells. x 2,800

Fig. 20. *H. pylori* infection of gastric epithelium causes many surface outfolds and a large cell fragment is detached. Note numerous *H. pylori* present on the cell surface. x 5,000
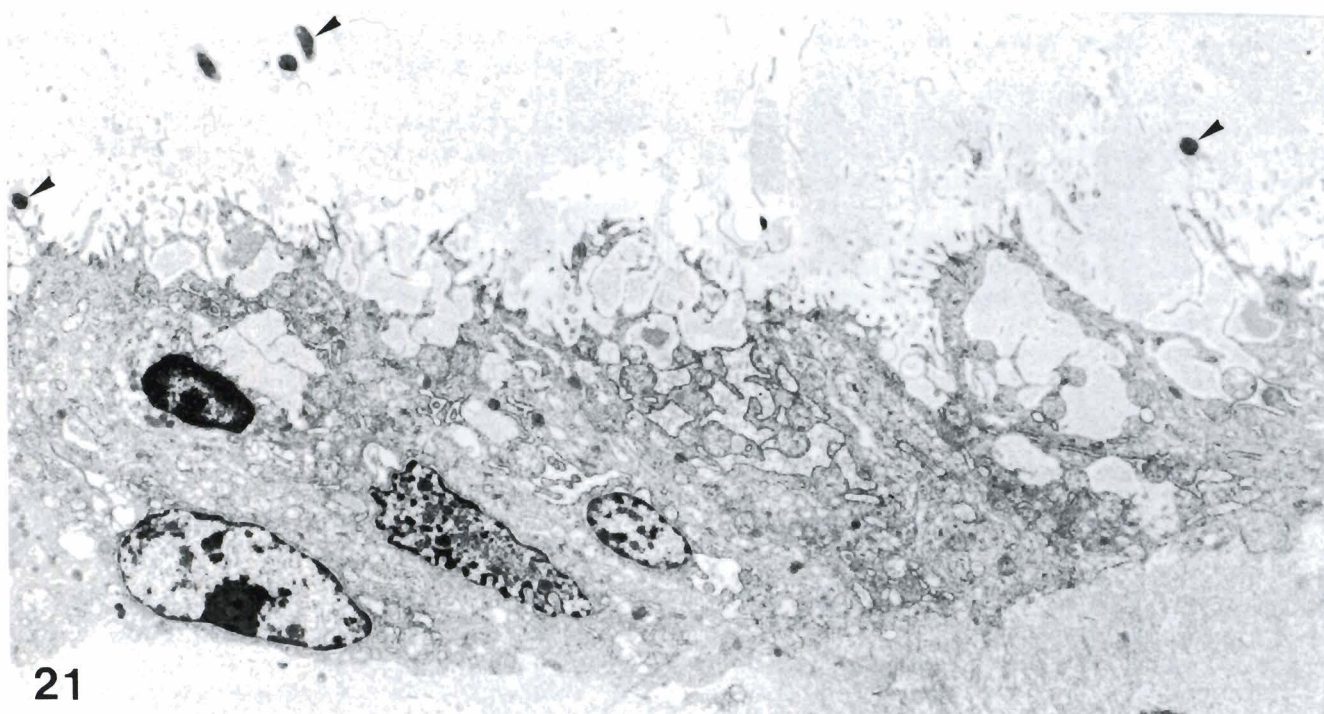
Fig. 21. Part of gastric type RE detached from the substrate and colonized with *H. pylori* (arrowheads). Apical cell membranes are disrupted and numerous large vacuoles are seen in the cytoplasm of these moderately mature mucous cells. Some nuclei are pyknotic or show degenerative change. Note the lack of basal lamina under the RE. x 3,000

ulcerogenic agents, such as tabacoo and alcohol, etc. In a previous study, we showed that in areas adjacent to human gastric ulcers colonized with *H. pylori* there were detachment of sheets of epithelial cells from the lamina propria resulting in denuded areas (Ogata and Araki, 1996). Similar areas were also observed in duodenal ulcers in this study. These sites may be the most vulnerable areas for new ulcer formation or for enlarging the original ulcer.

In the same study (Ogata and Araki, 1996), we observed the colonization of *H. pylori* on moderately mature gastric type RE but not on immature RE cells with few mucous granules. In duodenal ulcers, the colonization of *H. pylori* on gastric type RE was also observed. In addition, there were degenerative changes of the moderately mature mucous cells of the moderately of RE colonized with bacteria, suggesting that there was some interference with ulcer healing. The presence of duodenal *H. pylori* appears to enhance the degeneration of gastric metaplastic epithelium and to cause cell exfoliation resulting in the denuded areas of the lamina propria. These may be the predisposed areas for new ulcer formation. In addition, this bacterial infection appears to hinder the regeneration process of the mucosal defect. Therefore, the elimination of this infection should reduce or prevent the gastric metaplastic epithelium and the RE from the pathology caused by *H. pylori* and thus enhance duodenal ulcer healing.

This study reveals a constant and dynamic effort of duodenal musocae, whether normal or metaplastic, to form RE even in chronic ulcer cases. The failure of complete ulcer healing appears to be due to an imbalance between damaging forces, and mucosal regenerative activities. Therefore it seems probable that if damaging factors are eliminated and repair processes enhanced, ulcer healing would be promoted.

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