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Scanning Electron Microscopy of the Surface of Human Duodenum in Patients With and Without Duodenal Ulcer Disease

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Endoscopic biopsy specimens of normal human duodenum from patients with duodenal ulcer disease were studied by light microscopy and by scanning electron microscopy. For scanning, the tissue was dried by the critical point-drying technique. SEM criteria for both normal and abnormal duodenal surfaces were determined. SEM evidence for the presence of "tuft cells" similar to those found on the surface of rat duodenum was found in both the normal and abnormal human material. Although other reports have described the favorable effects of carbenoxolone sodium treatment on duodenal ulcer disease, our SEM studies of four patients found that this treatment had no effect on the duodenal surface.

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It has been shown previously by scanning electron microscopy (SEM) of forceps biopsy specimens of human gastric mucosa that there are striking morphologic surface differences from the normal in cases of both erosive and chronic gastritis[1] and in gastric surfaces before and after treatment with carbenoxolone.[2] This has raised the question as to whether or not there are surface differences between the normal duodenal mucosa and that from patients with duodenitis associated with duodenal ulcer disease.

Material and Methods

To answer this question and to determine SEM criteria for judging normality and abnormality in duodenal surface, forceps biopsies were obtained by fiber endoscopy from two patients without duodenal ulcer disease and from fifteen patients with duodenal ulcer found on endoscopy. Four of these fifteen had another endoscopy with forceps biopsy after four to five weeks of treatment with carbenoxolone sodium.

Two or more biopsies were taken from each duodenum in the series. Of these, one or more were paraffin-sectioned and H&E stained for conventional light microscopy (LM). The two controls were confirmed to be normal by light microscopy, and the fifteen patients with duodenal ulcer disease showed normal duodenal mucosa in two and duodenitis[3,4] in thirteen cases.

One specimen of each duodenum was immediately fixed in cacodylate-buffered, 2% glutaraldehyde solution. Contrary to some previous SEM studies on human duodenum and epithelium of the small intestine,[5-8] our specimens were not air dried but were handled by the more acceptable process of critical point-drying[9] in CO_2. Subsequently, they were sputter-coated with gold-palladium for SEM.

Results

Normal duodenum

In critical point-dried normal duodenum at low magnification (Figure 1), the numerous long, flattened villi are tonguelike or leaf-shaped and deeply creased. At higher magnification (Figure 2), the cell margins of individual epithelial cells at the luminal surface are often observed to be hexagonal in outline, with some pentagonal cell outlines also occurring. In some areas the luminal surfaces of individual epithelial cells are slightly convex, giving the surface a "cobblestone" appearance. In Figure 2 there is also evidence for the presence of underlying goblet cells (arrows) which are recognized as openings or dimples between surface cells. Although the gold-palladium sputter-coating tends to obscure most of the thick population of the microvilli of the brush border, at certain optimal angles or where the surface has been partly protected from the metal (Figure 3), the cells are observed to be heavily provided with stubby microvilli (solid arrows). There is also a deposit of fibrous substance (mucus) in isolated patches over the surfaces (clear arrows), which is not heavy enough to obscure the clarity of the surface structures underneath. Other cells are seen which possess sparsely microvillous and/or blebbed cell surfaces (Figure 4, arrows). There is measured evidence for approximately 2,000 goblet cells per mm^2 in the normal surface.

The following SEM criteria have been determined for normal human duodenal mucosa. Each of the criteria represents an average of the range of possible observations:

1. The presence of numerous, long, creased, tonguelike villi.
2. The relative smoothness (except for the creases) and homogeneity of the villi surfaces.
3. The presence everywhere of relatively flat-surfaced, heavily microvillous epithelial cells with intact luminal surfaces, and hexagonal or pentagonal luminal margins.
4. The occurrence of either dimpled depressions or blebbed areas between epithelial cells which represent underlying goblet cells (approximately 2,000 per mm^2).
5. The absence of prominent cobblestoning. Cells in the normal surface can be minimally raised with slightly depressed margins to produce an appearance of slight cobblestoning, particularly where the surface has maximum curvature.

Duodenal mucosa in duodenal ulcer disease

Four cases with duodenal ulcer disease were used to evaluate the surface of the duodenum for criteria of abnor-

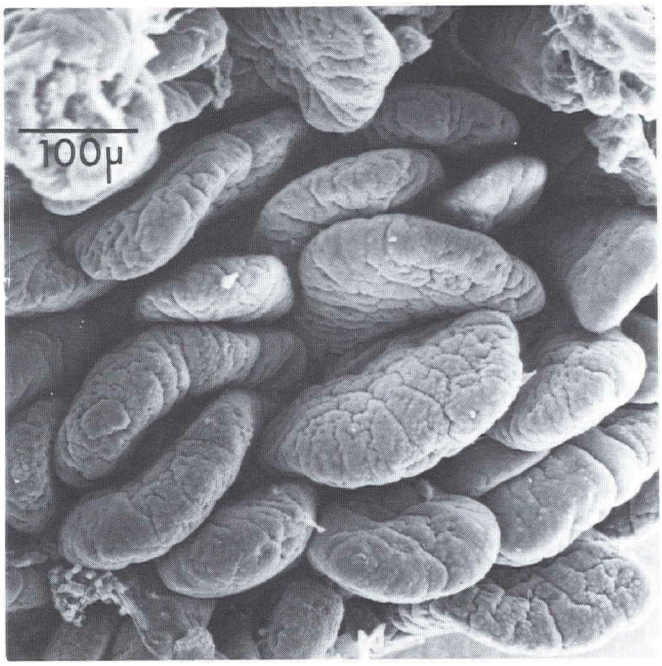

Fig. 1
Normal human duodenum showing tall, tonguelike, creased villi at low magnification (x160) (one $\mu = 10^{-4}$cms).

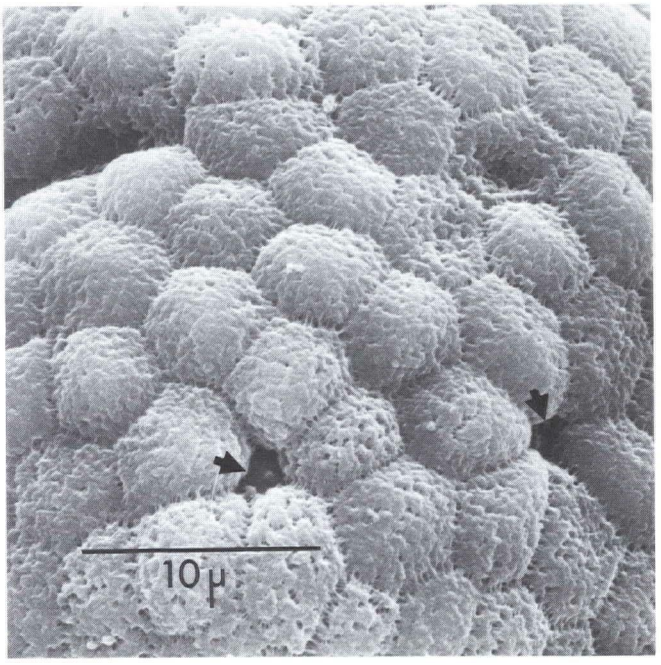

Fig. 2
The luminal surface of normal human duodenum, showing geometrical margins and slight cobblestoning. Openings to goblet cells at arrows (x3200).

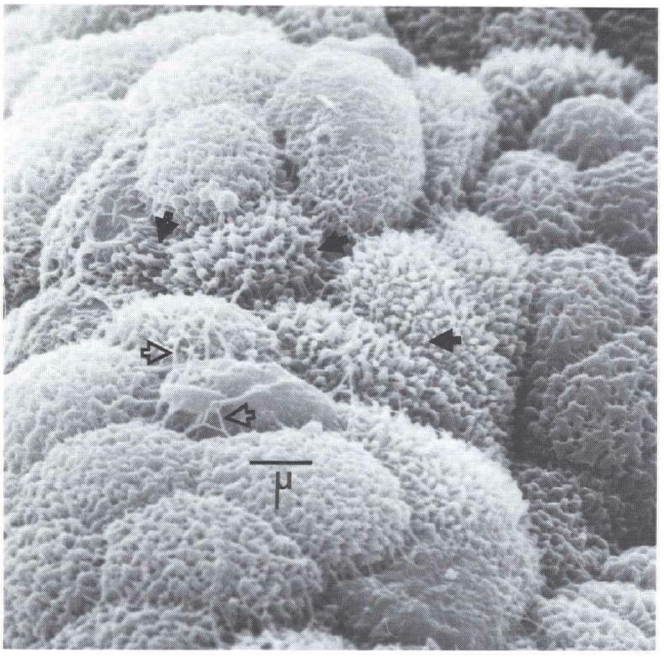

Fig. 3
Normal human duodenum surface, micrographed at a more oblique angle, showing microvilli (solid arrows) and mucus strands (clear arrows) (x8400).

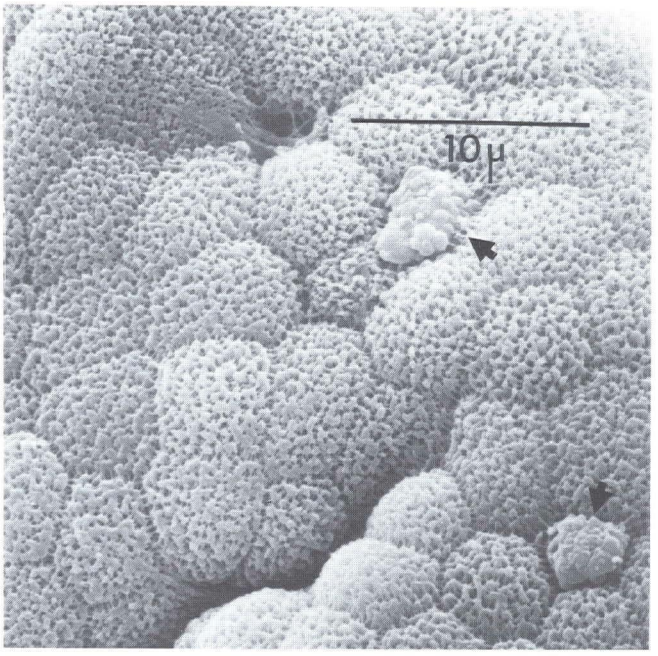

Fig. 4
Normal human duodenum surface, showing goblet cells (arrows) with blebbed surfaces, protruding into the lumen (x3200).

mality. The villi are as long as they are in the normal duodenum but are swollen at the tips, much less tonguelike, and fewer in number (Figure 5). Perhaps due to their swollen nature, their surfaces are not creased. Some areas of epithelial cells appear different from the normal epithelial cells in that their surfaces bear fewer and more discrete microvilli (Figure 6). Many goblet cells are represented by structureless openings (arrows, Figure 7) in the surfaces, while others have an intact, smooth, slightly swollen membrane protruding into the lumen (Fiugres 6, 7). The goblet cells are measurably more numerous here than in the normal duodenum, i.e., approximately 6,500 per mm.^{2} Although some areas are excessively cobblestoned by raised luminal cell surfaces, cobblestoning in other areas can be similar to that in the normal.

The following SEM criteria are suggested for *abnormal* human duodenal mucosa in duodenitis associated with duodenal ulcer disease. Each again represents an average of the observations.

1. Shorter, somewhat fewer villi.
2. Clubbed or swollen villi.
3. Lack of normal creasing of villi.
4. Prominent cobblestoning in most areas.
5. Some damaged epithelial cells.
6. Many more goblet cells
7. Some areas of epithelial cells bearing sparse microvilli.

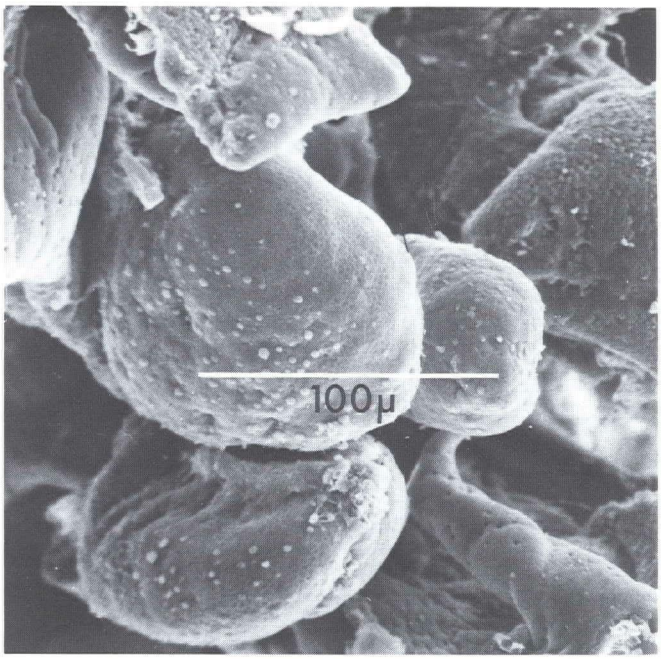

Fig. 5
Human duodenum in duodenal ulcer disease, showing swollen villi at low magnification (x400).

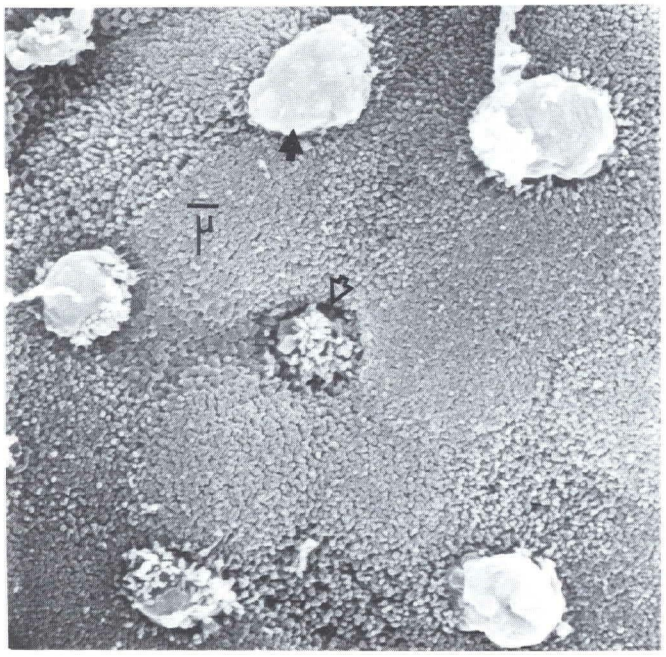

Fig. 6
Human duodenum in duodenal ulcer disease showing a possible tuft cell at the clear arrow, and six goblet cells represented at the solid arrow (x4300).

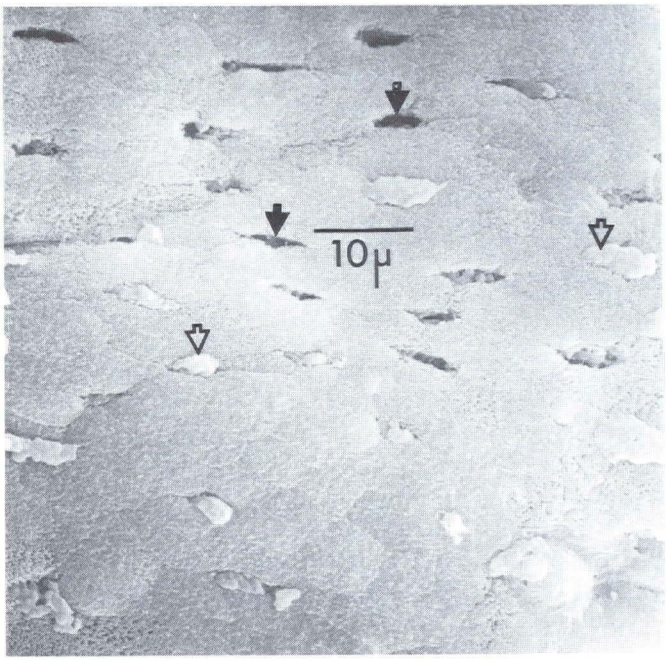

Fig. 7
Human duodenum in duodenal ulcer disease (x1300). A variety of many goblet cells are represented, ranging from structureless openings (arrows) to those with intact surface membranes (clear arrows) protruding into the lumen. Cobblestoning is not pronounced because of the flat viewing angle.

The patients with duodenal ulcer disease treated with carbenoxolone sodium (50 mg carbenoxolone sodium 4X a day) showed no consistent effects measurable or describable by SEM before and after treatment, nor was there consistent agreement between SEM and light microscope observations (see table). As far as efficacy of treatment was concerned, the weight of the evidence suggested that there was no effect on the duodenal mucosal surface.

Discussion

The first SEM study[10] of normal human duodenal mucosa, using air-dried specimens and a low magnification of 75x, described only sigmoid-shaped surface folds and clefts partially covered by mucus. Thirteen years later, the SEM technique has improved to a point where it is possible to observe individual epithelial cells as well as the microvilli covering them. This higher degree of resolution has solved some problems but has introduced new ones. For example, in both normal and abnormal human duodenal mucosa, cells with microvillus projections rising relatively high into the lumen have been found. Whether these are goblet cells or the "tuft" cells as observed on the apical surfaces of rat duodenum[11] cannot be as yet completely determined by SEM alone. Similar cells have been seen in human gastric mucosa,[12] but further investigation is required to prove that "tuft" cells are present in the villi of the human duodenum.

The differences in the findings of both SEM and LM before and after treatment with carbenoxolone sodium probably result from the small number of patients studied and from the tiny amount of sample material obtainable by forceps biopsy. It was not considered safe to use the suction biopsy technique to obtain larger biopsy specimens blindly from the duodenal bulb of ulcer patients.

Human duodenal biopsy in patients with duodenal ulcer disease, before and after treatment with carbenoxolone sodium (duogastrone®)

Patients	Pretreatment		Posttreatment	
	SEM	LM	SEM	LM
1	Not Usable	N	A	N
2	N	A	A	A
3	N	A	N	Missing
4	A	A	A	A

N = Normal
A = Abnormal
SEM interpretation according to criteria outlined; LM according to usual criteria.[3,4]

Thus, in only four patients was the mucosa considered adequate for comparative SEM studies before and after treatment with target endoscopic biopsies. It is not surprising that in one instance (patient 2) the pretreatment SEM biopsy was normal and the posttreatment abnormal, since the pattern of duodenitis may well be patchy rather than diffuse.

In a double-blind study[13] of 152 duodenal ulcer patients treated with carbenoxolone sodium, clinical evaluation suggested a significant difference ($P<0.01$) in favor of the drug as opposed to placebo. Furthermore, after 6-12 months of carbenoxolone therapy, only 2 of 23 patients showed persistent duodenal ulcer as opposed to 7 of 14 control patients.[14]

Acknowledgment

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