Correlative Scanning Electron Microscopy in the Study of Human Gastric Mucosa

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CORRELATIVE SCANNING ELECTRON MICROSCOPY IN THE STUDY OF HUMAN GaSTRIC MUCOSA

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Abstract

We studied two aspects of the human gastric mucosa: - the surface morphology of mucous cells, as viewed by scanning electron microscopy (SEM); - the glycosidic components of intracellular mucins, characterized by means of lectins. The latter were conjugated with fluorescein isothiocyanate and with colloidal gold-silver for the visualization of the reaction products in light microscopy (LM) and in SEM (backscattered mode) respectively.

The surface morphology of mucous cells appears to be correlated to the secretory state. In gastric ulcers we found a prevalence of non-secreting cells. A decrease in glycosidic receptors for fucose-binding lectin and galactose-(1-3)-N-acetyl-galactosamine-binding lectin was also observed. This suggests the presence of an impaired mucus secretion which may play a role in the pathogenesis of gastric ulcer. Spiral bacteria, supposed to be aetiologically related to peptic ulcer and gastritis, were easily detected by SEM. Intestinal metaplasia defined "complete" in LM showed surface morphology and glycosidic components different from those of true intestinal mucosa. This implies the necessity of taking into account also these parameters when classifying this lesion. The same applies to polyps.

Our data indicate that correlative SEM may contribute further information on the pathogenesis and pathology of gastric diseases.

KEY WORDS: correlative scanning electron microscopy, gastric mucosa, lectins, colloidal gold-silver staining, ulcer, intestinal metaplasia, polyps, Campylobacter-like organisms.

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Introduction

The gastric mucosa represents a barrier against numerous aggressive agents from the external environment or even from its own secretion. This barrier function is based on the integrity of the lining epithelium and on mucus secretion (10).

The gastric mucosa has been largely studied by light microscopy (LM) and transmission electron microscopy (TEM) in various physiological and pathological conditions, both in animals and humans (17,18,22,23). These techniques, however, provide only bi-dimensional information on tissue sections. Scanning electron microscopy (SEM) is the only morphological technique which makes it possible to study the mucosal surface architecture and, at the same time, to have a detailed view of the lining epithelial surface directly exposed to noxious agents: the "luminal" surface.

Nevertheless only a few reports deal with the application of SEM in the study of human gastric mucosa, in particular with regard to pathological conditions (4,20,21).

With regard to mucus secretion, gastric mucins have been the object of numerous histochemical studies based on dyes with different affinities and almost exclusively performed at light microscopy level (11,19).

We attempted a new approach to the study of gastric mucosa using correlative morphological techniques capable of providing information on: mucosal architecture, surface morphology of mucous cells and composition of their mucin secretions.

For this purpose we studied the human gastric mucosa by SEM in normal conditions and in diseases characterized by an alteration of the mucosal barrier or in any case by marked modifications of the surface structure: ulcer, intestinal metaplasia, polyps.

In the same conditions we studied the intracellular gastric mucins by means of lectins; proteins which recognize specific glycosidic residues (12). Fluoresceinated lectins were used for
light microscopy and gold labelled lectins for visualization of the glycosidic binding sites by means of scanning electron microscopy in the backscattered mode.

Materials and Methods

The gastric mucosa of eight patients (40-77 years) suffering from ulcer disease (untreated ulcer of the angulus), of seven patients (47-74 years) with chronic atrophic gastritis and intestinal metaplasia and of one 87 years old patient with antral polyps was studied.

Controls consisted of eight patients (42-58 years) investigated for upper abdominal pain and without any evidence of gastrointestinal disease.

Two adjacent mucosal biopsies were taken at endoscopy both from the site of the lesion, within the area of disease activity, and at a distance from it in the uninvolved mucosa (body). Of the two contiguous biopsies one was processed for scanning electron microscopy (SEM), the other for light microscopy (LM). The latter was reprocessed for scanning electron microscopy.

Scanning electron microscopy

The specimens were washed in saline, then fixed in 2.5% glutaraldehyde in phosphate buffer pH 7.3 for 2 h at 4°C and post-fixed in 1% osmium tetroxide for 1 h at room temperature. They were successively subjected to centrifugation (3 min at 3500 g) in 1% HCl. After dehydration in an ascending concentration of ethanol and critical point drying with CO₂, the specimens were mounted on aluminium stubs, coated with gold by sputtering and observed with a Philips 505 SEM.

Light microscopy

Specimens were fixed in 10% buffered formalin, embedded in paraffin and observed with a Zeiss Photomicroscope III.

Lectin-histochemistry

Specimens were processed for SEM. The blocks were dewaxed in xylene, passed through ascending concentration of ethanol and critical point dried. Blocks and sections were processed for SEM, mounted on aluminium stubs, coated with carbon and observed in a Philips 505 SEM in the backscattered electron (BSE) mode.

Results

Normal gastric mucosa (Figs. 1-11)

SEM. The gastric mucosal surface appears fairly flat throughout the stomach. Shallow furrows subdivide it into areas of different size in which the gastric pits (or foveolae) open. In the gastric fundus, body and antrum, the pit openings appear roundish and regularly distributed (Fig. 1), the interfoveolar areas are flat; whereas in the vicinity of the pylorus, the pit openings become elongated and the interfoveolar areas look like low convolutions (Fig. 2).

The mucous cells of the lining epithelium show polygonal outlines and a slight convexity of the luminal surface (Fig. 3). Their surface morphology may be extremely variable within the same specimen: the microvilli, which are short and stubby, like little knobs, may be distributed all over the surface or mainly localized at the periphery (Fig. 4); in this case the central portion of the apical surface bulges towards the lumen bearing the impressions of the underlying mucus granules. The rarer and more peripheralized microvilli, along with the progressive bulging of the apical surface are generally associated with the presence of mucus granules (approximately 1 μm in diameter). These granules appear about to be extruded. Holes of the same diameter may be also found. They may represent the space left by the secreted granules (Fig. 5). Sometimes it is possible to observe cells in which all the apical microvilli are present and the underlying granules are therefore visible (Fig. 6).

Lectin-histochemistry. Surface mucous cells are labelled by all the lectins used, except ConA (Figs. 7, 8, 9). DBA binding was absent in 3 out of 8 normal biopsy specimens. Mucous neck cells are labelled by LTA, WGA and PNA. PNA labels also the luminal surface of chief cells, DBA the
Correlative SEM of human gastric mucosa

Canalicular surface of parietal cells (Fig. 10). The cytoplasm of chief cells is labelled by LTA and WGA. A positive reaction was observed for all lectins on antral glands as well as on surface mucus.

At ultrastructural level, an evident labelling was obtained only with WGA. The reaction products are well demonstrated in the BSE mode. They correspond to the site of the mucus granules, as shown by the tri-dimensional image, and are also detectable on the luminal surface of the cells (Fig. 11).

Gastric ulcer: edge of the ulcer (Figs. 12-21)

SEM. The mucosal surface shows alterations consisting of:
- loss of the "foveolar pattern" (Fig. 12), and possible presence of intestinal metaplasia (Figs. 12, 13);
- changes in the lining epithelium (Fig. 14): anisocytosis, ballooning cells with a smooth surface or completely covered by microvilli, enhanced exfoliation and prevalence, among the cells with a normal appearance, of those bearing numerous microvilli (Fig. 15).

Light microscopy showed various degrees of chronic atrophic gastritis in an active phase. A certain degree of mucin depletion was also present (Fig. 17). In 1 out of 8 patients dysplastic changes were observed. In this case SEM showed cells covered by microvilli with an intermediate aspect between the gastric and intestinal type. (Fig. 16).

Lectin-histochemistry. We found a decrease in the intensity of fluorescence with regard to the fucose-binding lectin (LTA) and the galactose-(1-3)-N-acetyl-galactosamine-binding lectin (PNA) on surface mucous cells (Fig. 18). No major differences were found in the binding pattern of the other lectins studied (Fig. 19). Figs. 17 to 21 show a correlation between FITC-PNA and FITC-WGA labelling on paraffin sections and WGA-colloidal gold-silver on the corresponding reprocessed block (Fig. 20). Looking at the surface of the reprocessed block it is also possible to appre-
Fig. 4. - Normal gastric mucosa: short and stubby microvilli (mv) are localized at the periphery of mucous cell surface. The latter bulges towards the lumen. arrows = underlying mucus granules. SEM. Bar = 1 µm.

Fig. 5. - Normal gastric mucosa: progressive bulging of the apical surface of mucous cells. arrows = mucus granules in phase of extrusion. arrowhead = hole left by an extruded granule; mv = microvilli. SEM. Bar = 10 µm.

Fig. 6. - Normal gastric mucosa: mucous cells with loss of the apical surface. arrows = mucus granules; arrowheads = holes. SEM. Bar = 10 µm.

Fig. 7. - Histological section of normal gastric mucosa: FITC-LTA labels surface mucous cells (mc), neck mucous cells (nc), and chief cells (cc). Parietal cells (arrows) are unlabelled. LM. Bar = 100 µm.

Fig. 8. - Histological section of normal gastric mucosa: FITC-WGA shows the same binding pattern of FITC-LTA. mc = surface mucous cells; nc = neck mucous cells; cc = chief cells. LM. Bar = 100 µm.

Fig. 9. - Histological section of normal gastric mucosa: FITC-PNA binding is visible prevalently on surface mucous cells (mc) and neck mucous cells (nc). LM. Bar = 100 µm.

Fig. 10. - Histological section of normal gastric mucosa: parietal cells (arrows) labelled by FITC-DBA are visible. LM. Bar = 100 µm.

Fig. 11. - Histological section of normal gastric mucosa: a bright signal coming from the WGA-colloidal gold-silver reaction is visible both on intracellular mucus (m) and luminal surface of mucous cells (arrows). BEI. Bar = 100 µm.

Fig. 12. - Edge of an antral ulcer: no pit openings are visible. The mucosal surface is corrugated, with a cobblestone appearance of the lining epithelium. Villous structures representing intestinal metaplasia (im) are also visible. SEM. Bar = 100 µm.

Fig. 13. - Detail of Fig. 12: intestinal metaplasia. The microvilli are of intestinal type. No fuzzy coat is visible. gc = goblet cell in phase of secretion. SEM. Bar = 1 µm.
Fig. 14. - Detail of Fig. 12: anisocytosis, exfoliating ballooning cells (b). SEM. Bar = 10 µm.

Fig. 15. - Detail of Fig. 12: mucous cells covered by numerous microvilli. SEM. Bar = 10 µm.

Fig. 16. - Edge of an antral ulcer with dysplasia: microvilli with an intermediate aspect between gastric and intestinal type are visible on surface mucous cells. mc = lateral portion of mucous cells. SEM. Bar = 1 µm.

Fig. 17. - Edge of an antral ulcer (histological section): active chronic gastritis, mucin depletion and intestinal metaplasia (arrows) are visible. *= tissue from the base of the ulcer. LM. Bar = 100 µm.

Fig. 18. - Section serial to that of Fig. 17: FITC-PNA binding is almost completely absent in surface mucous cells (mc) and in intestinal metaplasia (arrows). The bright fluorescence belongs to intraluminal mucus. LM. Bar = 100 µm.

Fig. 19. - Section serial to those of Figs. 17 and 18: FITC-WGA binding is present in surface mucous cells (mc) and in intestinal metaplasia (arrows). LM. Bar = 100 µm.

Fig. 20. - Reprocessed paraffin block corresponding to sections of Figs. 17-19. The WGA-colloidal gold-silver reaction is also visible on the luminal surface (arrow) of mucous cells. BEI. Bar = 100 µm.

Fig. 21. - Detail of Fig. 20 after "tilting". The WGA-colloidal gold-silver complexes are visible on the apical membrane of mucous cells (arrows). SEM. Bar = 1 µm.

Fig. 22. - Mucosa at a distance from the ulcer (body): the pit openings (arrows) are irregularly shaped and distributed. Corrugated mucosal surface and cobblestone epithelium are visible. SEM. Bar = 100 µm.

Fig. 23. - Detail of Fig. 22. Exfoliating ballooning (b) cells next to normal ones are visible. SEM. Bar = 10 µm.

Gastric ulcer: mucosa at a distance from the ulcer SEM. Alterations of the mucosal surface architecture are represented by disarrangement of the foveolar pattern: the pit openings are irre-
gularly shaped and distributed. The mucosal surface appears corrugated and irregular, with a cobblestone appearance of the lining epithelium (Fig. 22), as is seen in the edge of the ulcer. The surface of mucous cells also shows the same alterations as observed in ulcers, except that in this instance the cells covered by microvilli do not prevail (Fig. 23).

Light microscopy showed the presence of a mild atrophic gastritis.

Lectin-histochemistry. We observed the same lectin binding pattern as in the edge of the ulcer.

Intestinal metaplasia (Figs. 24-35)

SEM. In the cases that we studied, the gastric mucosa which underwent intestinal metaplasia showed different SEM appearances:
- flat mucosa subdivided by shallow furrows into areas irregular in size and shape (Fig. 24). The lining epithelium is constituted by cells with the same surface appearance as enterocytes: in fact they are provided with long, tightly-packed microvilli whose tips are not distinguishable due to the presence of the fuzzy coat. Goblet cells are interspersed between the "enterocytes" (Figs. 24, 25);
- mucosa in which structures resembling short and stubby villi (Figs. 26, 34) are close to irregularly shaped foveolae (Figs. 27, 28); the microvilli show an intermediate aspect between gastric and intestinal cells (Figs. 29, 30, 31). In some cases the lining epithelium is formed by intestinal type cells in which the fuzzy coat is scarcely visible (Fig. 35).

Light microscopy showed the presence of a complete intestinal metaplasia in all the cases that were studied (Fig. 32).

Lectin-histochemistry. The binding pattern of LTA, WGA and DBA was the same as in the small intestine, even if the labelling intensity was lower: the lectin binding sites were localized both on the brush border and goblet cells. Changes in ConA and PNA binding pattern were found in intestinal metaplasia with respect to small intestine mucosa. Goblet cells positive for ConA, which are never present at intestinal level, were, in fact, observed. Moreover, with regard to PNA, areas of positive goblet cells were found in the same specimen next to negative ones. The brush border was always unlabelled (Figs. 32, 33). Figs. 34 and 35 show the corresponding reprocessed paraffin block.

Polyps (Figs. 36-43)

SEM. The surface of the antral polyps showed leaf-like villous structures resembling those of the small intestine. At the top of these villi the normal exfoliation area is replaced by epithelial "crests" (Fig. 36). The lining epithelium has a cobblestone appearance. The luminal surface is covered by microvilli with an intermediate appearance between gastric and intestinal cells (Fig. 37).

Light microscopy applied to the adjacent biopsy specimen showed the presence of a tubular adenoma (Fig. 38). However the corresponding reprocessed paraffin block showed the presence of two types of structures, one villous, the other formed by low convolutions (Figs. 42, 43).

Lectin-histochemistry. The strongest reaction was found for PNA on the supra-nuclear cytoplasm of cells with residual mucus granules. These were also labelled by WGA and, less, by ConA. PNA and WGA also labelled the microvillar membrane. The binding of LTA was almost exclusively limited to this site. No DBA binding was observed. Figs. 38-43 show a correlation between light microscopy and scanning electron microscopy in secondary electron (SE) and backscattered electron (BSE) mode.
Correlative SEM of human gastric mucosa
Fig. 30. - Detail of Fig. 29 showing that the lining epithelium is constituted by cells with an intermediate aspect between gastric and intestinal type. SEM. Bar = 10 µm.

Fig. 31. - Detail of Fig. 26. Cells covered by microvilli with an intermediate aspect between gastric and intestinal cell. SEM. Bar = 1 µm.

Fig. 32. - Antral mucosa with intestinal metaplasia indicated by small and large arrows (histological section). LM. Bar = 1 mm.

Fig. 33. - Section serial to that of Fig. 32. FITC-PNA binding is absent in intestinal metaplasia indicated by small arrows. Positive goblet cells are indicated by large arrow. Pyloric glands are intensely labelled. LM. Bar = 1 mm.

Fig. 34. - Reprocessed paraffin block corresponding to sections of Figs. 32, 33. Arrows = goblet cells. SEM. Bar = 100 µm.

Fig. 35. - Detail of Fig. 34, after "tilting". Intestinal type microvilli without evidence of fuzzy coat. SEM. Bar = 1 µm.

Fig. 36. - Antral polyp: villous structures with apical "crests" (arrows). SEM. Bar = 100 µm.

Fig. 37. - Detail of Fig. 36: the microvilli have an intermediate aspect between gastric and intestinal cells. SEM. Bar = 10 µm.

Fig. 38. - Antral polyp (histological section of a biopsy specimen adjacent to that of Fig. 36): tubular adenoma. LM. Bar = 100 µm.

Fig. 39. - Reprocessed paraffin block corresponding to section of Fig. 38. s = mucosal surface; * = transected edge. SEM. Bar = 100 µm.
Correlative SEM of human gastric mucosa
Fig. 40. - Same block of Fig. 39: the WGA-colloidal gold-silver reaction is demonstrated in BSE mode both on surface (s) and transected edge (arrows). BEI. Bar = 100 µm.

Fig. 41. - Detail of Fig. 40 showing the reaction products on the luminal surface of a tubule (arrow). BEI. Bar = 10 µm.

Fig. 42. - Same block of Fig. 39 after "tilting": Villous structures (v) and low convolutions (c) are visible. * = transected edge. SEM. Bar = 100 µm.

Fig. 43. - Detail of Fig. 42 showing that the preservation of surface structure in the reprocessed block is comparable to that of the specimen of Fig. 36. The lining epithelium has a cobblestone appearance. SEM. Bar = 100 µm.

Presence of bacteria

In cases of gastric ulcer and chronic gastritis we observed the presence of spiral bacteria (Campylobacter-like organisms) mainly disposed at the edges of the cell surfaces (Fig. 44). They bear a polar flagellum (Fig. 45).
Correlative SEM of human gastric mucosa

Discussion

Our study provided essentially the following contributions: 1) Morpho-functional correlations, with regard to the mucus secreting cells. 2) A pathogenetic hypothesis of gastric ulcer. 3) A "working" hypothesis for a deeper knowledge of the pathological basis of the gastric diseases studied.

1) With respect to the first point, we can distinguish three types of cells in the lining epithelium: - "resting" cells, that is cells not in a phase of secretion and corresponding to those covered by many microvilli; - cells in a "pre-secretory" state, corresponding to those with peripheralized microvilli and bulging apical membrane; - "secreting" cells are those in which the mucus granules appear to detach from the apical surface. SEM clearly shows that gastric mucus secretion occurs by a merocrine mechanism: our observations indicate that the aspects of loss of the entire apical portion of the cell are artefactual.

2) In ulcers we observed a prevalence of "resting" cells. These appear in LM with a certain degree of mucin depletion. The latter is probably a secondary event due to acute inflammatory infiltration of the mucosa. In fact, at a distance from the ulcer, the three types of mucous cells were equally represented as in normal mucosa and the morphological signs of mucin depletion were not prominent, in spite of the presence of a chronic gastritis. The histochemical study of mucins by lectins indicate some changes in the glycosidic components of intracellular mucus, which could be responsible for failure in gastric protection.

3) With respect to the other lesions investigated, namely, intestinal metaplasia and polyps, our data indicate that they too could be well correlated by SEM studies with more functional observations by histochemical methods. To date many efforts have been done to achieve a classification of intestinal metaplasia which could provide useful indications on the possible evolution of the disease; in fact, the so-called "incomplete" metaplasia is considered to be a precancerous lesion (14). SEM studies of intestinal metaplasia allow both to better define the real extension of this mucosal alteration due to the possibility of observing a large mucosal surface and to characterize the lining cells on the basis of their microvillous pattern. In fact, we found cells lacking in the typical surface features of the enterocytes in cases of metaplasia which have
been defined "complete" by LM. Moreover, in the same cases, some glycosidic components of mucous cells were different from those of true intestinal mucosa (1). For this reason, we feel that a systematic study of these two parameters, surface morphology and lectin histochemistry, could improve knowledge and definition of this lesion. The same considerations apply to polyps: a tri-dimensional evaluation of their structure which, as we have shown, may vary within the same biopsy specimen appears important. Moreover, the study of the luminal surface of the lining epithelium may also be particularly interesting. In the polyps that we studied, the epithelial cells shared common surface features with those described in other lesions which undergo mucin depletion: dysplastic cells of the ulcer edge, cells of "intermediate" type in intestinal metaplasia. These features consist mainly of elongation and increased number of microvilli. The lectin binding pattern was similar to that described by some authors in hyperplastic and neoplastic conditions (2, 6).

Lectin histochemistry is a technique which has only recently been applied to human gastric mucosa in particular with regard to pathological conditions (2, 6, 15). In fact, few data are available in the literature with respect to the other "classical" histochemical techniques (5, 9). For this reason all the knowledge that has been acquired is far from being well established. Equivocal results may be due to differences in fixation procedures or in the type of marker to which lectins are conjugated. Our observations showed that fluorescence on formalin fixed-paraffin embedded sections was the most reproducible technique. Fluorescence offers the advantage of using material routinely processed and to also perform retrospective studies. On the other hand the colloidal gold-silver technique allows the observation of the reaction products in SEM resulting in the following advantages: better knowledge of the topographic distribution of the reaction products due to tri-dimensional images; enhanced resolution. For these reasons we feel that these techniques, when applied to the same specimen, may be complementary to each other.

The finding of spiral bacteria on the surface of mucous cells in cases of gastric ulcer and chronic gastritis is in agreement with that reported by other authors who claimed a fundamental role for these microorganisms in the pathogenesis of gastric inflammatory diseases and peptic ulcer (13, 16). Drugs with bactericidal properties seem to change the natural history of peptic ulcer, mainly preventing the recurrence of the disease. SEM appears to be a very reliable technique for detecting these bacteria and for recognizing them as Campylobacter-like.

In conclusion, we think that all our observations give further support to the importance of introducing SEM parameters in the study of the pathology of gastric lesions with some direct implication in clinical practice.

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References


Correlative SEM of human gastric mucosa


Discussion with Reviewers

J.R. Poley: In what way do the results of your study clarify or shed more light on the pathogenesis of "gastric ulcer" and of "intestinal metaplasia"?

authors: With regard to gastric ulcer, our data indicate that there is an alteration of intracellular mucins. This does not seem to be secondary to the ulcerative lesion. In fact, if this were the case, we would expect the alteration of the mucins to be limited or at least to be mainly in the area of the ulcerative lesion. The alteration of the mucins is however present in other areas of the stomach too. Recently it has been suggested that intracellular mucins play a major role in cytoprotection with respect to the external mucous coat. For this reason we think that the qualitative change in intracellular mucins may be one of the causes of the impaired mucosal defence which is now attributed with a pathogenetic role for the gastric ulcer.

As regards intestinal metaplasia, we have not made any pathogenetic hypothesis; we only suggest that this lesion would be better characterized by applying combined SEM and lectin-histochemistry. By applying these techniques we were able to find that the so-called "complete metaplasia" has different morphological and histochemical characteristics from true intestinal mucosa.

S. Siew: You have postulated that the mucin depletion of "resting cells" is probably a secondary event due to acute inflammatory infiltration of the mucosa. What is the pathogenesis?

authors: Mucin depletion was observed only in correspondence to the ulcer, where the neutrophilic infiltrate predominated, and not at a distance from the lesion. Mucin depletion is present in cases of reactive and regenerative epithelial changes. The latter may occur on the edge of an ulcer as well as in other types of mucosal lesions complicated by acute inflammation.

J.R. Poley: In patients with intestinal metaplasia, was there bile reflux into the stomach? Is anything known about the pH of the gastric contents?

authors: We did not find a consistent bile reflux during endoscopy. We have no data about the pH.
J.R. Poley: Have any of the patients you reported received treatment and, if so, to what extent did treatment influence your findings?
Authors: Patients were all untreated.

S. Siew: In Fig. 17 you state that there is active chronic gastritis. What are your criteria for saying that?
Authors: For the classification of chronic gastritis we referred to that of Whitehead R. (1979) (Mucosal biopsy of the gastrointestinal tract, W.B. Saunders, Philadelphia, pp. 11-29). In particular we evaluated the presence of neutrophils as a marker of "acute activity", the infiltrate of chronic type, the grade of gland atrophy and the presence of intestinal metaplasia. In particular, the mucosa of Fig. 17 showed a chronic atrophic gastritis in phase of "acute activity".

S. Siew: You have performed a detailed SEM study of the gastric mucosa and mucin secretion. You have not documented the presence of round tipped extensions of cytoplasm, which were described by Watson J.H.L. et al. (1975) (Some observations on the ultrastructure of the surface of normal human stomach mucosa. Proc Electron Microsc Soc Amer, 470-471). Would you please comment on your experience in this connection.
Authors: We cited in reference n. 4 another paper in which Watson is co-author and in which these structures are described. The "bulbous projections" correspond to what we called microvilli. The cited authors also described lateral rod-like necks of different length connecting the projections. The TEM study performed on gold-palladium coated reembedded material did not explain these aspects which were not relevant in our specimens. An artefact due to metal-coating cannot be excluded.

S.A. Halter: How did backscattered electron imaging contribute to the lectin histochemistry that was not evident by fluorescence microscopy?
S.A. Halter: What is the evidence that fluorescence microscopy is the "most reproducible technique"?
Authors: The advantages of the detection of a histochemical reaction in BSE are: better resolution with respect to LM and as a consequence higher sensitivity; better definition of the topography of the reaction, especially if it is applied to the entire block. The disadvantages with respect to fluorescence microscopy are: less reproducible results as concerns the intensity of the reaction; this may limit semi-quantitative studies.

J.R. Poley: Has lectin labeling "become of age" for the routine study of gastric mucosal pathology, or is it still in the development stages?
Authors: We think that we are still in the development stage. Only a systematic application of lectins will reveal the potential of this technique. Uniformity of methods is also required to achieve homogeneous and comparable results. In fact data available in literature were obtained with different methods (type of fixation, type of marker for the lectin) by the different authors.

S.A. Halter: Does your study have any direct application for clinical practice and/or diagnosis?
Authors: The search for Campylobacter-like organisms which SEM can reveal better than any other technique, in our opinion constitutes a valid reason for routine application of SEM in clinical practice. The presence of these bacteria should be monitored in all patients with ulcers before and after treatment. Interesting data may emerge, for example, in non-responders or in patients with ulcer relapses. As concerns the other parameters studied we think that systematic application of the described techniques could help in identifying patients with different mucosal backgrounds within the limits of the same gastric pathology.