Clinical Applications of Scanning Electron Microscopy in Gastrointestinal Diseases

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Clinical Applications of Scanning Electron Microscopy in Gastrointestinal Diseases

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Abstract

We considered the role of scanning electron microscopy (SEM) in clinical investigation of different gastrointestinal diseases. The following clinical applications of SEM may be suggested on the basis of our original data and those reported in literature:

1. Peptic ulcer: assessment of the completeness of healing, by observing the mucosal surface architecture of the scars; identification of mucosal changes, namely enterocytic surface membrane alterations, predictive of recurrence;

2. Coeliac disease: early assessment of the response to gluten-free diet and follow-up of the patients by staging the process of mucosal repair in cerebriform, intermediate and villous patterns; in ulcerative and Crohn's colitis: enhancement of the diagnostic sensitivity of perendoscopic biopsy, by detecting differences in surface structure of mucosa surrounding ulcers in both diseases. This is subverted in ulcerative colitis and preserved in Crohn's colitis.

Finally the complementary role of SEM in relation to endoscopy and light microscopy is emphasized.

Introduction

The mucosal surface of the gut acts as a mediator between the external environment and the organism: it is, at the same time, a mucosal barrier and an absorptive and secreting surface whose morphology and function are closely correlated.

Scanning electron microscopy is the best morphological technique for the study of the mucosal surface of the gut; the magnification range available, in fact, permits the study of the mucosal architecture and details of the single epithelial cells. SEM therefore plays a fundamental role in deepening basic morphological knowledge and in complementing other techniques. On the other hand, in literature there is little evidence of its useful application in clinical investigation of the gastrointestinal tract (3, 6, 7).

We studied four different digestive diseases all of which are characterized by marked primitive or secondary alteration of the mucosal surface: 1. peptic ulcer 2. coeliac disease (gluten sensitive enteropathy) 3. ulcerative colitis 4. Crohn's disease of the colon (or with colonic involvement)

After having reviewed important contributions in literature we compared them with personal observations whose aim was to deepen our knowledge on mucosal repair as regards ulcer and coeliac disease and to find elements useful to the differential diagnostics of chronic inflammatory diseases of the colon.

Study population

Patients with peptic ulcer. Ten patients, 7 males and 3 females, mean age 40 years, with a range of 40-55 years, suffering from uncomplicated duodenal ulcer were studied. They underwent endoscopy and biopsy under base conditions and after 2 months of therapy with an H2-receptor antagonist.
(ranitidine, 300 mg daily). Periodic endoscopic controls had been performed even after 1, 2, 3, 4 weeks of therapy.

Two of these patients were submitted to a new endoscopic and biotic control 2 months after stopping the treatment, while the others after 6 months of maintenance therapy (ranitidine, 150 mg daily).

Multiple biopsies were taken from the edge of the ulcers, from the scars and from the adjacent uninvolved mucosa.

Controls consisted of 8 patients with gastrointestinal symptoms, but with no evidence of duodenal involvement.

Patients with coeliac disease. Thirty-six patients suffering from coeliac disease, 12 males and 24 females, aged from 12 to 62 years, mean age 36, underwent small intestinal biopsies. Single biopsies with the Crosby-Kugler capsule or multiple biopsies with the "multipurpose" were performed at the duodenal-jejunal flexure under base conditions and/or after a gluten-free diet. In all, 25 base-condition biopsies and 27 biopsies performed after a gluten-free diet were observed. Of the latter, 9 biopsies refer to a period of time ranging from 10 days to 3 months of diet, 11 biopsies relate to a period of time ranging from 6 to 18 months and 7 biopsies refer to a period of time ranging from 2 to 6 years of diet. A histopathological evaluation had been made both by subjective criteria and by morphometric measurement (8).

Controls consisted of 47 subjects investigated for gastrointestinal symptoms, who were free from any evidence of disease in the small intestine.

Patients with ulcerative colitis. Twelve patients, 8 males and 4 females, mean age 40 years, with a range of 29-61 years, were studied. The mean duration of the disease was 2.5 years, with a range of 0-10 years. The rectosigmoid colon was involved in all patients and in 4 also the descending colon.

Patients with Crohn's disease. Eleven patients, 8 males and 3 females, mean age 33 years, with a range of 20-60 years were studied. The mean duration of the disease was 3 years, with a range of 0-11 years. Two of these patients had no evidence of ileal involvement and the disease was located in the sigmoid colon.

Two patients with ileocolic disease who had required surgical therapy (ileo-transverse and ileo-sigmoid colostomy) had a recurrence in the rectum and in the sigmoid colon respectively.

Four patients with terminal ileitis (1 with previous ileo-transverse colostomy) had no evidence of colonic involvement. Three patients with terminal ileitis had a recurrence in the transverse, in the descending and sigmoid, and in the sigmoid colon respectively.

Both in ulcerative colitis and Crohn's disease multiple mucosal biopsies were taken from lesions and normal mucosa of the left colon. Controls consisted of 12 patients investigated for gastrointestinal symptoms who were free from any evidence of disease in the colon.

Methods

Specimens to be examined by SEM were immediately subjected to delicate washing in normal saline, in order to eliminate major impurities and blood residues. Fixation was achieved by means of 2.5% glutaraldehyde in phosphate buffer pH 7.3, for 1-2 hours, at 4°C. Some specimens were also post-fixed for 1 hour in 1% osmium tetroxide at room temperature. After washing in phosphate buffer, the biopsies were dehydrated in solutions with an increased concentration of acetone and critical-point dried using CO₂. After 20 nm gold sputtering, they were mounted on aluminum stubs and observed with a Philips SEM 505 Scanning Electron Microscope.

Fig. 1. - Normal human jejunum: leaf-like (l), tongue-like (t) and finger-like (f) villi. (SEM, bar = 100 µm)

Fig. 2. - Normal human duodenum (bulb): large leaf-like villi (l) and villous convolutions (c). (SEM, bar = 100 µm). Inset: detail of the lining epithelium. The enterocytes show polygonal profiles. (SEM, bar = 10 µm)

Fig. 3. - Edge of a duodenal ulcer: villi (v) are short, distorted and swollen. Arrows indicate an amorphous mucosal prominence in between them. (SEM, bar = 100 µm)

Fig. 4. - Detail of Fig. 3: three enterocytes with sparse microvilli and blebs (arrows) on the luminal surface are visible. Arrowheads indicate detached blebs. (SEM, bar = 1 µm)

Fig. 5. - Detail of Fig. 3: numerous blebs (arrows) are visible on the surface of anisocytic enterocytes covered by tightly packed microvilli. (SEM, bar = 1 µm). Inset: detail of microvilli. (SEM, bar = 1 µm)

Fig. 6. - Detail of Fig. 3: arrows indicate a small area with damaged or missing cells. b = bleb. (SEM, bar = 1 µm).
SEM in gastrointestinal diseases
Specimens to be examined by light microscopy (LM) were fixed in 10% buffered formalin, dehydrated and embedded in paraffin. Other specimens were fixed in glutaraldehyde, dehydrated and embedded in Araldite in order to obtain semithin sections.

Some of the biopsy or surgically resected specimens which had been fixed in formalin and embedded in paraffin for histopathological diagnosis were dewaxed in xylene, passed through absolute alcohol, then processed for SEM observation (5).

Results and Discussion

Peptic ulcer

Topographical changes in the duodenal surface must be kept in mind when evaluating accurately, by SEM, the duodenal mucosal surface in course of peptic disease. SEM studies of the small bowel mucosa mainly refer to the mid-distal duodenum which, due to its morphological analogies, is associated to the jejunum: in this part of the bowel the villi are predominantly leaf-like and tongue-like, with some finger-like villi (1, 2, 9, 19, 20, 36, 38, 39) (Fig. 1). Scarce attention has been paid to the normal mucosal surface architecture of the duodenal bulb, main site of peptic ulcers. The villous pattern of the duodenal bulb consists prevalently of widely spread leaf-like villi, often forming convolutions (Fig. 2). The lining epithelium is, instead, similar throughout the small bowel; it consists of absorptive cells, enterocytes, whose luminal surface has polygonal profiles and is covered by microvilli (Fig. 2). The luminal surface is plane or slightly convexed. Goblet cells are unevenly distributed in between the absorptive cells.

There have been in literature few studies on duodenal ultrastructural pathology in course of treated and untreated peptic disease (4, 14, 25, 31, 32, 35). This is probably due to the fact that clinically it was not considered necessary to morphologically study in detail a lesion which does not tend to undergo malignant transformation and whose recovery may well be documented endoscopically or even assessed by clinical criteria only. On the other hand the natural history of the disease is recurrence. For this reason an accurate study of the mucosal surface could be necessary, utilizing a suitable technique such as SEM, in order to detect possible predictive morphological signs.

The mucosa surrounding the ulcer, in patients studied by us, shows marked alterations of the surface architecture: usually partial villous atrophy is evident; moreover the villi are sparse, distorted and swollen (Fig. 3).

Alterations of the lining epithelium are patchy: anisocytic cells with convoluted apical surfaces appear next to the normal ones; some of these bear sparse microvilli and show alterations of the apical membrane resulting in bleb formation (Figs. 4, 5). Small foci devoid of epithelial cells (Fig. 6) and areas of mucoid metaplasia may be observed (Fig. 7).

Mucus-secreting type cells similar to those of the gastric antrum were found adjacent to duodenal ulcers by Gregory et al. in a transmission electron microscopic (TEM) study (14). The authors suggested a protective function of these cells which transform to absorptive cells during healing.

In our patients the mucosa, far from the ulcers, endoscopically normal, shows hypotrophic villi as well as the same enterocytic surface membrane alterations observed on the edge of the ulcers.

Alterations of the microvilli of the enterocytes were evidenced at TEM by Pillay et al. (25) on the edge of deep and flat duodenal ulcers. Microvillar changes in the mucosa at a distance from the lesion were found only in case of flat ulcers. Shuman et al. (31), in determining the criteria for normal and abnormal human duodenum, included some areas of epithelial cells bearing sparse microvilli in duodenitis associated with peptic ulcer. No evidence was given of apical surface membrane alteration in form of blebs.
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Following the repair stages, during treatment with ranitidine full-dose, we observed a progressive return to normal morphological parameters. Areas of re-epithelialization are visible after a week of therapy (Fig. 6). After 4 weeks the ulcer is already endoscopically healed. However the rapidity with which the mucosa returns to normal is subjective, as viewed by SEM. In some patients, the mucosa which covered the site of the ulcer showed after 8 weeks of therapy a partial villous atrophy (Fig. 9).

In others the mucosal surface architecture was quite normal after 1 month (Fig. 10). After 8 weeks the alterations of the apical surface structure of the enterocytes had completely disappeared in all patients except 2: in these, apical surface alterations of the enterocytes were still present as blebs.

In patients which had suspended after 8 weeks the ranitidine full-dose therapy, enterocytic alterations reappeared after two months. The mucosa remained unaltered at SEM in all patients in maintenance therapy except in the two with alterations of the apical membrane of the enterocytes even 8 weeks after full-dose therapy; in these, in fact, there was recurrence.

On the basis of our observations we suggest three working hypotheses:

1. alterations of the apical surface membrane of the enterocytes represent the early lesion;
2. in the ulcerous patient there is a background of epithelial abnormality where a peptic lesion is formed;
3. monwitorization by means of SEM of enterocyte alterations facilitates individualization of patients prone to recurrence.

Coeliac disease

In coeliac disease there is a gluten-induced abnormality of the mucosa of the small intestine. The histopathology of the disease is well established: total (or subtotal) villous atrophy and crypt hyperplasia are the prominent features (23, 30). Studying the jejunal mucosa of untreated patients we observed that the flat mucosal surfaces with the same morphological appearance at light microscopy (LM) could be quite different when viewed by SEM.

In particular we were able to distinguish two types of flat mucosa: a) mucosa in which the crypt openings are numerous and almost regularly shaped and distributed. The epithelial cells which line them are arranged in a spiral pattern which forms a small cone with a wide base; at the top of the cone the crypts open up like a crater (Fig. 11). The surface of the enterocytes which cover the edges of the cryptic orifices and of those which internally cover the crypts very often appears to be fusiform. Such configuration conforms to the intercellular relationships. The epithelial cells which line the intercryptic spaces are quite anisocytic with respect to their luminal surface.

b) mucosa on which irregular cryptic orifices open up. The intercryptic areas are scarcely visible because of the presence of mucosal "flaps" which seem to "envelop" the crypts, thus reducing the lumen while, at the same time, connecting the intercrypt spaces to each other (Fig. 12).

The small intestine mucosa of coeliac disease has been described by various authors (1, 15, 18, 20, 37). In particular, Poley (26) emphasized the possible different appearances of a flat mucosa when viewed at SEM: described three stages of severity (I, II, III), the latter being the most severe lesion and corresponding to the mucosa described in point a) by us. The type I mucosa which is constituted by ridges was detected by us only in treated patients.

In coeliac disease the response to gluten-free diet represents a crucial point in the diagnosis and clinical management of the disease. With the light microscope we can observe the evolution of the repairing mucosa from total (or subtotal) villous atrophy to partial villous atrophy. The intermediate mucosal patterns representing the earliest stages of repair cannot be detected.

Yardley et al. (40) indicate that the early mucosal change consists of the increase in height of the surface epithelium, detectable at LM after 6 to 10 days of gluten-free diet. This parameter varied considerably in our patients both under base conditions and after gluten withdrawal.

We attempted to identify all the stages of mucosal repair by studying, at SEM, the jejunal mucosa of coeliac patients after 10 days to 6 years of gluten-free diet. For this purpose we also used specimens previously fixed in formalin, embedded in paraffin and sectioned for routine histopathology. In these specimens, reprocessed for SEM, we directly compared the mucosal surface and the transected edge (Fig. 13).

In the first stage of repair the mucosa appears slightly elevated from the crypt plane: this is due to a pericryptic arrangement of the proliferating enterocytes (Figs. 14, 15). Mucosal bridges interconnecting the crypts are also present. The corresponding aspect in light microscopy is shown by the transected edge and it is that of an unmodified flat mucosa.

In the successive stages of repair the following mucosal patterns are detectable by SEM: convoluted ridges, which give the mucosa a cerebriform pattern (Figs. 16, 17); a transition mucosa which includes convoluted ridges, convoluted
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Fig. 13. - Jejunal mucosa of untreated coeliac disease. Dewaxed paraffin block. Surface: flat mucosa with numerous crypt openings (co). Transected edge: total villous atrophy; c = longitudinally cut crypts. (SEM, bar = 100 µm).

Fig. 14. - Treated coeliac disease. Repair of the jejunal mucosa after 3 months of gluten-free diet. Surface: the mucosa appears elevated from the crypt plane. Transected edge: total villous atrophy. co = crypt opening; mb = mucosal bridge interconnecting the pericryptic enterocytes; arrow = baso-lateral surface of crypt; c = longitudinally cut crypt. (SEM, bar = 100 µm).

Fig. 15. - Same specimen of Fig. 14 observed after "tilting". co = crypt opening; mb = mucosal bridge. (SEM, bar = 100 µm).

Fig. 16. - Treated coeliac disease. Repair of the jejunal mucosa after 1 year of gluten-free diet. Transected edge: partial villous atrophy. co = crypt opening; c = longitudinally cut crypt. (SEM, bar = 100 µm).

Fig. 17. - Same specimen of Fig. 16 after "tilting". Surface: cerebriform mucosa. cr = convoluted ridges; co = crypt opening. (SEM, bar = 100 µm).
villous structures and villi (Figs. 18, 19). The latter are still short and often have a common base.

Both cerebriform and transition mucosa are classified by light microscopy as partial villous atrophy, with no distinction between them except the height of the villous structures.

Our findings suggest a higher sensitivity of SEM with respect to light microscopy in:
1. detection of early surface mucosal changes.
2. evaluation of the "maturity" of the repairing mucosa, by identification of the different patterns (cerebriform, transitional, villous).

It is well known that the mucosal restoration can occur at different times after starting the treatment, depending on the response of the individual patient and on the completeness of the gluten withdrawal (23). In our cases the first stage of repair was observed by SEM in a period ranging from 1 to 3 months. In the period from 6 to 18 months the mucosa ranged from convoluted ridges to a villous pattern, being the villi less than normal in height and with a marked exfoliation at their tips (Figs. 20, 21).

In literature there is only a recent paper by Halter et al. (15) attempting a systematic study of villous regrowths after gluten-free diet. The authors, referring to a pediatric population, point out the utility of SEM, mainly in early assessment of dietary response.

Ulcerative colitis and Crohn's disease

Ulcerative colitis and Crohn's disease are two well recognized disease entities. The differential diagnosis between the two is however very difficult and not even with clinical, radiological, endoscopic and biotic information is it possible to separate the two diseases. Frequently, in fact, a correct diagnosis is only obtained "after" by examining the histopathological characteristics of resected segments allowing the study of the whole thickness of the colon wall. The development of endoscopic techniques has facilitated the study of the mucosal surface of the whole colon from which biopsies may be taken. Perendoscopic biopsy often fails in the diagnosis of Crohn's disease because the specimen is taken from the mucosal layer only, while the basic lesion of this disease, the granuloma, is predominantly localized in the submucosa and inflammation is transmural (21).

The aim of the application of SEM in the study of the colon mucosa in ulcerative colitis and Crohn's disease is to provide new information in order to increase diagnostic accuracy and reliability of perendoscopic biopsy. In literature there are few contributions to this regard (10, 11, 16, 17, 22, 28).

We have tried an approach to the problem by studying at SEM the colonic mucosal surface of perendoscopic biopsies and surgical resections in patients with well-established diagnosis. SEM was not only applied to damaged areas but also to the uninvolved mucosa in order to detect possible minimal changes endoscopically and histologically undetectable.

The colonic mucosal surface of the left colon of normal subjects appears subdivided into polygonal units. These units are outlined by furrows more or less shallow. The surface of the crypt unit may be flat or project above the intercrypt area: each contains a central crypt opening with a diameter ranging from 12 to 25 \( \mu m \) (Figs. 22,23). Goblet cells are in a crown shaped arrangement around the crypt openings and are sparse in the intercrypt areas.

The surface of the absorptive cells may be more or less convex and have polyhedric profiles. We may sum up our results concerning the SEM appearance of colonic mucosa in ulcerative colitis and Crohn's disease in the following way: Active ulcerative colitis. a) mucosal surface structure near the ulcers (Fig. 24): crypt openings: markedly decreased in number; unevenly distributed; of different diameter; crypt units: if present, extremely irregular in size and shape;
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goblet cells: patchy distribution; emptied, enlarged orifices. b) mucosal surface structure of pseudopolyps (Fig. 25): crypt openings: decreased in number; slightly irregular distribution; crypt units: polymorphism; goblet cells: markedly decreased in number; emptied enlarged orifices. Ulcerative colitis in remission. Mucosal surface structure (Fig. 26): crypt openings: decreased in number; slightly irregular distribution; crypt units: present, but not well delineated; goblet cells: decreased in number; emptied, enlarged orifices. The mucosal surface structure of uninvolved mucosa shows the same remission alterations but to a lesser extent.

Crohn's disease. a) mucosal surface structure near the ulcers (Fig. 27): crypt openings: enlarged (25-35 µm); crypt units: present, but not well delineated; goblet cells: normal or increased in number, filled with mucus; b) surface structure of cobblestone mucosa (Fig. 28): crypt openings: enlarged (50-60 µm); crypt units: well delineated; goblet cells: increased in number, filled with mucus (Fig. 29).

Our results, together with those reported in literature (12, 16, 24, 29) regarding the colonic mucosa, viewed at SEM, demonstrated that the definition of normality of the mucosal surface must keep in mind the variability of certain parameters; in particular the "prominence" of the crypt units, the depth of the furrows which delimit the crypt units and the patency of the crypt openings. This aspect was also emphasized by Siew (33) in a recent review on SEM of the normal and pathological colon. Our observations too, which only refer to the left colon, show the variability of the parameters mentioned-above, while the subdivision of the mucosal surface in crypt units remains constant.

Riddell and Levin (29) classified the crypts which open in the centre of the polygonal units as "secondary" and as "primary" those representing longer and deeper furrows which subdivide the mucosa in territories containing 20-100 crypt openings. The authors, studying uninvolved marginal areas of resected specimens for carcinoma, detected mucosal surfaces lacking in crypt unit pattern, in which the crypts open into transversally running grooves. Similar aspects have also been described in normal rectal mucosa (17). These variable aspects were kept in mind when studying the mucosal surface of the colon in patients with ulcerative colitis and Crohn's disease. Our observations too, which only refer to the left colon, show the variability of the parameters mentioned-above, while the subdivision of the mucosal surface in crypt units remains constant.

The most interesting data regarding our study are the following:
1. the mucosa close to the ulcers of ulcerative colitis is subverted.
2. the mucosa close to the ulcers of Crohn's disease is preserved.
3. there was never reduction in the number of goblet cells in Crohn's disease.

Other aspects may be pointed out even if not constant:
1. the reduction in number of goblet cells in ulcerative colitis and above all the empty aspect of these cells which seem to be functionally exhausted. In some cases we observed, in active ulcerative colitis, a patchy distribution of goblet cells rather than a decrease in number.
2. the increase in number of goblet cells in Crohn's disease and their aspect of cells in active phase of secretion. Kavin et al. (16) studied with SEM the rectal mucosa of 2 patients with ulcerative colitis. The authors emphasized the presence of corrugations

Fig. 24. - Active ulcerative colitis. The mucosal surface structure near the ulcers is subverted. cu = crypt unit; co = crypt opening; gc = goblet cell with emptied, enlarged orifice. Arrowhead: regenerating epithelial cells. (SEM, bar = 100 µm).

Fig. 25. - Active ulcerative colitis. Mucosal surface structure of a pseudopolyp. cu = crypt units well delineated of different size and shape; gc = goblet cell with emptied enlarged orifice. (SEM, bar = 10 µm).

Fig. 26. - Ulcerative colitis in remission: slightly irregular distribution and decrease in number of crypt openings (co). Decreased number of goblet cells (gc). m = mucus. (SEM, bar = 10 µm).

Fig. 27. - Crohn's disease of the colon: the mucosal surface structure near ulcerated areas is preserved. co = enlarged crypt orifice; arrows = goblet cells filled with mucus. (SEM, bar = 100 µm). Inset: detail of goblet cells. (SEM, bar = 10 µm).

Fig. 28. - Crohn's disease of the colon. The surface structure of cobblestone mucosa is well preserved. Crypt openings are large and filled with mucus (m). (SEM, bar = 100 µm).

Fig. 29. - Crohn's disease of the colon. Cobblestone mucosa. Goblet cells (arrows) are increased in number and appear in active phase of secretion. (SEM, bar = 10 µm).
adjacent to the furrows which delimit the crypt units and interpreted them as signs of regenerating epithelial cells. We also observed this aspect (Fig. 24), not only in ulcerative colitis but also in cases of Crohn's disease.

Mylärniemi and Nickels (22) studied 9 cases of ulcerative colitis and 12 of Crohn's disease and in the latter also they pointed out an alteration of the mucosal surface structure with disappearance of the crypt units. We believe that these data are not in contrast with ours. We, in fact, did not study, at SEM, in ulcerative colitis and in Crohn's disease the ulcerative areas but those immediately adjacent to them.

Rickert and Carter (28) described the appearance at SEM of the so-called aphtoid ulcers which are considered the early lesion of Crohn's disease. In the colon the mucosa surrounding aphtoid ulcers shows a normal surface architecture. The changes in number and morphology of the goblet cells detected by us during ulcerative colitis and Crohn's disease are generally in agreement with those reported in literature. These changes have also been detected in histopathological studies in light microscopy (27). SEM permits better evaluation of the goblet cells distributed on the mucosal surface surrounding the crypt openings and of their secreting state. Dvorak et al. (10) detected an increase in number of goblet cells, apparently in the secretion phase, on altered villi in Crohn's disease at ileal location. This hyperplasia was also found on the borders of grossly and histologically normal resected segments.

The authors hypothesized that mucus has a role in the pathogenesis of this disease. In normal conditions the mucus is a barrier against antigenic molecules present in the lumen of the intestine. In Crohn's disease excess mucus, which is not removed as a consequence of altered motility, remains with the entrapped antigens thus facilitating contact and penetration of these in the mucosa.

Crohn's disease, after resection of the segments affected, may recur in any part of the gastrointestinal tract, and, in fact, it should be considered a multifocal disease. There is evidence in literature (13) which shows that rectal mucosa apparently spared in patients with Crohn's disease has an increased plasmacellular infiltration and an increased activity of the glucosamine-synthetase, the first enzyme in the biosynthesis of mucus. Kaye et al. (17), studying uninvolved rectal mucosa at SEM did not find any differences between controls and Crohn's disease.

We also searched for possible changes in the mucosa far from the areas affected. The only finding was the constant increase of the diameter of the crypt openings.

It is possible that this is due to the presence of abundant mucus secretion in the lumen of the crypts, not always visible because removed during specimen processing for SEM.

In conclusion it is evident how alterations observed at SEM of the colonic mucosa in ulcerative colitis and Crohn's disease are not diagnostic in an absolute sense. It is sufficient to recall, in fact, how the increase in goblet cells was important, for example, in the Clindamycin-associated colitis (34) and in the irritable bowel syndrome (33). We believe, however, that information provided by SEM, used in a global clinical, radiological, endoscopical and histopathological evaluation may contribute considerably to solve the specific problem of a differential diagnosis between these two chronic inflammatory diseases of the colon.

General Comments

The contribution of SEM to the study of the digestive disease taken into consideration in this paper is based fundamentally on two points: 1. possibility to detect minimal changes: this is useful in order to know the real extension of the lesion, to detect precociously its evolution and to evaluate its complete regression. 2. complementarity regarding histopathological investigation: this is facilitated by the suitability of reprocessed paraffin blocks for observation in SEM. Moreover it appears very important from a clinical point of view that SEM may integrate usefully endoscopic information.

References

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Discussion with Reviewers

Reviewer 1: What evidence do the authors have that patients with duodenal ulcers have "a background of epithelial abnormality where a peptic lesion is formed"?

Authors: We have no evidence, this is merely a hypothesis based on certain observations: even the mucosa far away from the ulcers is altered. The alterations are only submicroscopic (blebs) but they reflect a "generalised disease" of duodenal mucosa: this is not always correlated with acid hypersecretion.

Reviewer 1: Do the "subverted" areas near the ulcers in active ulcerative colitis represent crypt abscesses?

Authors: Not always. In fact, the mucosal surface structure is subverted also in the absence of crypt abscesses. We can confirm this finding since we directly correlated the histopathological features with the SEM features on the same reprocessed paraffin block.

S. Siew: Could you please define precisely what you mean by subverted mucosa?

Authors: We applied the term "subverted" to alterations of the colonic mucosal surface consisting of loss of the crypt unit pattern or in any case of a regular distribution of crypt orifices (see the text with regard to active ulcerative colitis).

S. Siew: How do you differentiate the blebs which are demonstrated in Figs. 4 and 5 from mucoid metaplasia of Fig. 7?

R. H. Riddell: In Fig. 4 could the sparse microvilli be due to overheating of the specimen during coating? No control is illustrated at this magnification (see also Fig. 21). Were you able to perform TEM to ensure that these were not mucus droplets? (The cells arrowed have long but scant microvilli and are in a minority and appear to be producing similar structures. See also Fig. 7).

Authors: In Fig. 4 we can observe 3 enterocytes with rarer microvilli showing a swollen apical surface with blebs; the bleb indicated by two arrows bears microvilli on its surface: this does not occur on mucus granules. The blebs on the
epithelial surface, visible both in Figs. 4 and 5, probably correspond to the vesicles described by Potten and Allen (41) as microvillar breakdown. We found similar aspects on thin sections of the same specimen (Figure 30). However, we are obviously not sure we have "caught" the same cells viewed by SEM.

In Fig. 7, mucoid metaplasia is represented by exfoliating ballooninng cells with a smooth surface. Semithin sections confirmed this finding. Overheating of the specimens (Figs. 4 and 21) would also have damaged the neighbouring cells.

S. Siew: Do Figs. 9 and 10 represent the mucosal surface of an area of actual scarring or of a healed ulcer? It is rather surprising to find perfect mucosal regeneration in a scar.

Authors: Both specimens were taken from "white" (inactive) scars.

S. Siew: Would you consider that the loss of the normal jejunal villous morphology in coeliac disease (Figs. 11, 13) gives rise to a mucosal pattern resembling that of the colon? This may be a basic reaction on the part of the gastrointestinal tract and the counterpart of intestinalizations of damaged gastric mucosa?

Authors: The loss of villi certainly gives rise to a flat mucosa with visible crypt orifices as the colon mucosa. However, this is due rather to mucosal atrophy than to colonic metaplasia. In fact, on the flat mucosal surface of coeliacs the goblet cells are very rare, contrary to what is seen in the colonic mucosa.

R. H. Riddell: In peptic ulcer were biopsies for patients and controls taken from the same site each time?

Authors: In control subjects the biopsies were taken both from the duodenal bulb, as this was the site of peptic ulcers in our patients, and from the mid-cistal duodenum.

R. H. Riddell: In Fig. 3 villi are bifid - Is this normal?

Authors: No. However we did find bifid villi not only in damaged but also in regenerating mucosas.

R. H. Riddell: Please define the term hypotrophic (villi) and the criteria used. Can you illustrate this?

Authors: By hypotrophic villi we mean villi shorter than normal. When compared with histological morphometric measurement hypotrophic villi fall between normal mucosa and partial villous atrophy.

R. H. Riddell: Was there any correlation between the length of time to healing in duodenal ulcer and the likelihood of recurrence of the ulcer? (i.e. Do ulcers healing quickly take longer to recur?).

Authors: In our patients we could only find a correlation between incompleteness of healing (presence of blebs), full-dose treatment interruption and recurrence.

R. H. Riddell: Figs. 14 - 19 show very poor villous formations after fairly extensive periods of gluten-free diet. Are these representative of all patients examined? While the length of time for response to occur is notoriously variable, do you think there was inadvertent gluten ingestion? Did the changes in microvilli in TEM and numbers of intraepithelial lymphocytes on light microscopy suggest that this might have been the case?

Authors: The mucosal patterns shown were representative of most of the patients studied. We agree about possible inadvertent gluten ingestion. Changes in microvilli and numbers of intraepithelial lymphocytes are not specific for gluten sensitivity.

R. H. Riddell: While the differential diagnosis between ulcerative colitis and Crohn's disease may be difficult, in most patients the parameters mentioned allow a correct diagnosis to be made. Further, the basic lesion is not simply a granuloma, although this may of course be present. Haggitt lays out good criteria for separating these diseases on a biopsy in the absence of granulomas (in Norris, Pathology of the Colon, Rectum and Anus, Churchill-Livingstone, 1983, pp 21-59). If the authors really
think this, what were their criteria for separating patients into Crohn's disease or ulcerative colitis?

Authors: The histopathological criteria for the evaluation of endoscopic biopsies were those suggested by Morson (see reference 21). With SEM we only studied patients with a well established diagnosis. When the biopsy was not adequate for diagnosis, subsequent examination of resected colonic segments and/or clinical, radiological and endoscopic findings allowed us to distinguish the two diseases. We did not study patients with "indeterminate" bowel disease although it is commonly accepted that these represent 25-30% of the patients in which the inflammatory process is limited to the colon and rectum.

Additional Reference


R.H. Riddell: Most of Figs. 22-28 show "holes" in the sites where goblet cells might be expected, including the normal controls. Are the authors convinced that this is not an artefact of preparation? Also, inflammatory (pseudo) polyps can be found in both diseases. Do the authors think that these polyps occurring in ulcerative colitis can be distinguished from those occurring in Crohn's disease?

Authors: The "holes" represent goblet cell orifices which may be more or less large and more or less filled with mucus depending on the functional stage (see also the detail of Fig. 27). In pseudo polyps of Crohn's disease we would expect a preserved surface structure.

R.H. Riddell: In Fig. 29, are the authors certain that the surface cells are not part of an inflammatory exudate?

Authors: When we found leucocytes they showed a well preserved surface morphology, completely different from the amorphous aspect of the cells mentioned, in some of which little mucus granules are also visible.