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Scanning Electron Microscopy of Crohn's Disease and Ulcerative Colitis of the Colon

H. Myllärniemi and J. Nickels

Second Department of Surgery, Department of Pathology and Department of Electron Microscopy, University of Helsinki, Helsinki, Finland

Summary. 12 patients with Crohn's disease (CD) and 9 with ulcerative colitis (UC) affecting the colon were studied by scanning electron microscopy (SEM). The macroscopically unaffected mucosa was distorted in both diseases by irregularities in the normal polygonal units. The individual absorptive cells were disorganized, having lost their pentagonal-hexagonal cell borders. In 7 cases of CD an increase of goblet cells was seen. In 4 cases of UC their number was decreased. One case of UC showed an increased number of goblet cells, the case possibly belonging to the sub-category of indeterminate colitis. The microvilli were normal in CD but affected in many cells from cases of UC. Re-epithelization was followed during conservative treatment in 2 UC patients.

It is suggested that with simplified SEM techniques the instrument can be a useful aid in the differential diagnosis of inflammatory colonic diseases.

Key words: Regional enteritis – Ulcerative colitis – Scanning electron microscopy.

Introduction

The surface epithelium of Crohn's disease (CD) differ from the epithelium of ulcerative colitis (UC) in the number of goblet cells (Price and Morson, 1975), amount of mucosubstances (Filipe and Dawson 1970) and structure of microvilli (O'Connor, 1972). We decided to study 21 cases of CD and UC with a scanning electron microscope (SEM) to see if any differences could be seen. Colon specimens were chosen because this part of the gastrointestinal tract most often gives rise to differential diagnostic problems between these two diseases. Attention was also paid to the possible effect of conservative treatment on UC.

Offprint requests to: J. Nickels, Department of Pathology, Haartmanink. 3, SF-00290 Helsinki 29, Finland

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Material and Methods

Specimens from 12 patients (5 male and 7 female) with clinically, roentgenologically and histologically verified CD and 9 patients (2 male and 7 female) with UC were studied. One control case underwent an operation for suspected Hirschprung's disease, showing a histologically normal colon.

Surgical specimens from 10 CD and 5 UC patients were studied. Colonoscopic biopsy specimens only were available in 2 CD and 4 UC patients. In 2 patients with UC serial biopsies were taken to see the possible effect of conservative treatment on the surface structure. 56 SEM specimens were studied. SEM biopsies were always taken from nonulcerated areas in addition to some biopsies from ulcerated areas and resection margins. To keep the tissues as nearly to their normal state as possible we did not wash the samples prior to fixation. The specimens were fixed overnight in 3 per cent phosphate-buffered (pH 7.2) glutaraldehyde at 4° C, dehydrated in a graded series of ethanol solutions and critical point dried. The surface of the dried tissue was coated with gold in a vacuum evaporator. A Jeol-U3 scanning electron microscope was used.

Histological sections were made from the adjacent tissue and stained with haematoxylin eosin, van Gieson and Alcian blue-PAS stains.

Results

Clinical Findings. The age at first onset of symptoms lay between 22 and 58 years (mean 35) for 12 patients with CD and between 22 and 75 years (mean 47) for 9 patients with UC. The duration of symptoms up to the time of SEM study varied from 1 month to 4 years in UC, being less than 2 years in the majority and from 1 month to 23 years in CD, being less than 4 years in the majority.

SEM Findings. The surface of normal colonic mucosa is arranged in regular polygonal units having a hole in the center corresponding to crypt opening (Fig. 1). The polygonal units are separated from each other by furrows. At higher magnification the individual pentagonal-hexagonal cells and cell borders can be seen like the microvilli on the surface of the absorptive epithelial cells (Fig. 1). The goblet cells can be identified as empty holes or by the mucus they discharge. These observations are similar to the earlier observations of normal human (Kavin et al., 1970; Fenoglio et al., 1975) and Rhesus monkey (Takeuchi and Zeller, 1972) colonic and rectal mucosa.

In CD the normal polygonal units are disturbed (Fig. 2), being either irregular or totally absent (Fig. 3). The individual absorptive cells are deformed, having lost their normal polygonal shape. In 7 cases an increase in the number of goblet cells was seen in the diseased areas and resection margins (Fig. 3). In the other 5 cases the number of goblet cells was normal. No clear changes could be seen in the microvilli (Fig. 2). In areas where the epithelial cells were destroyed the denuded areas were coated by cellular debris, leucocytes and erythrocytes (Fig. 4).

In UC variations in the polygonal units were great. The individual cells were disorganized with irregular or inconspicuous cellular borders, giving the surface an uneven contour (Fig. 5). A clear difference from CD could be seen in the decreased number of goblet cells in 4 cases of UC (Fig. 5), being within normal limits in the other four. In one case the number of goblet cells was increased, however. This last case was also histologically difficult to classify but was treated as UC on clinical and roentgenological grounds. It belongs,

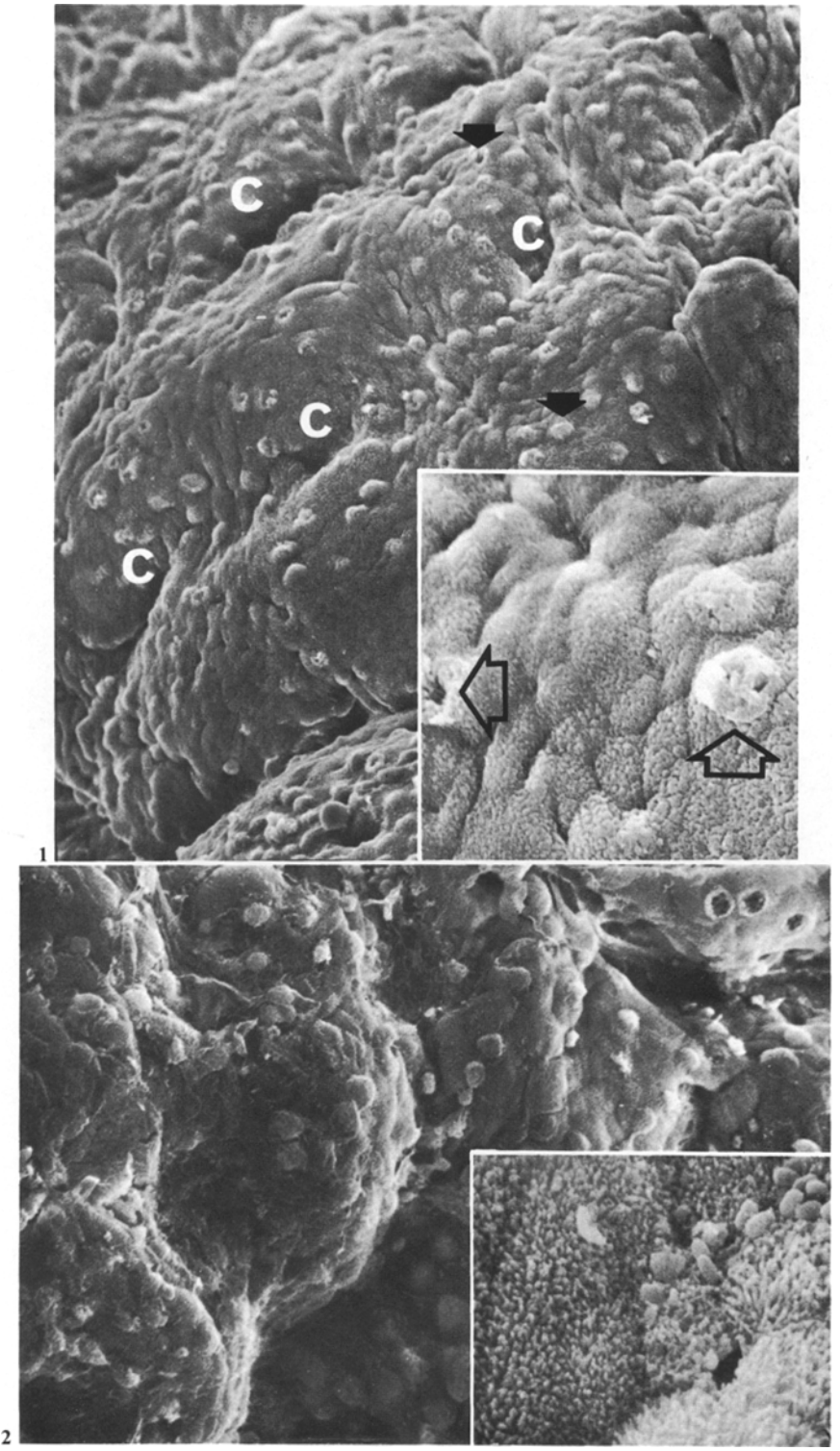


Fig. 1. Normal colonic mucosa, showing polygonal units separated by deep furrows. Arrows point to goblet cell or cell opening. C crypt opening. ×600. Inset: Individual cells with microvilli can be seen, arrows pointing to goblet cells. ×2,000

Fig. 2. Crohn's disease with irregularity and distortion of polygonal units. The number of goblet cells is increased. ×600. Inset: Normal microvilli and some bacteria (top right). ×6,000

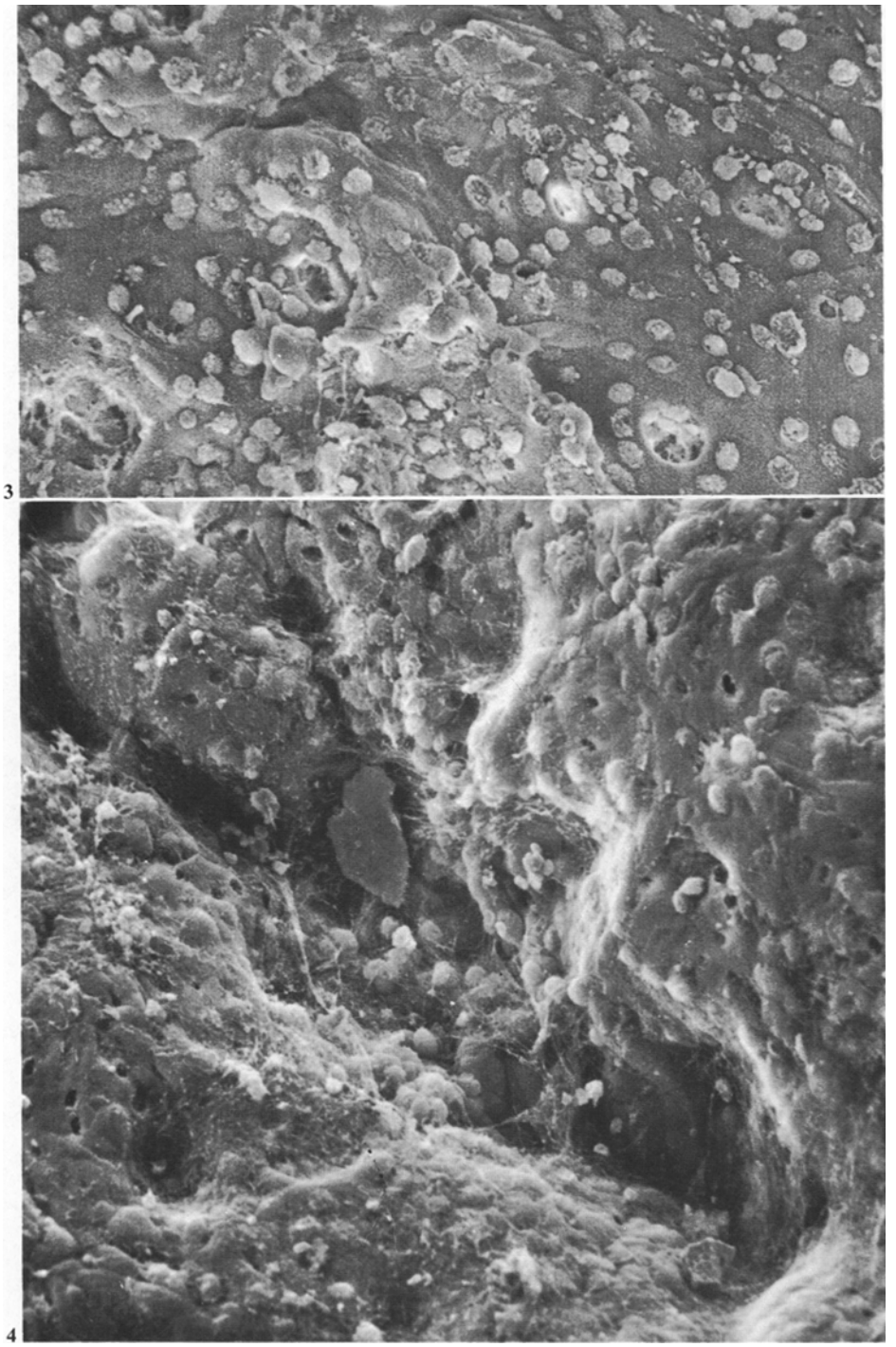
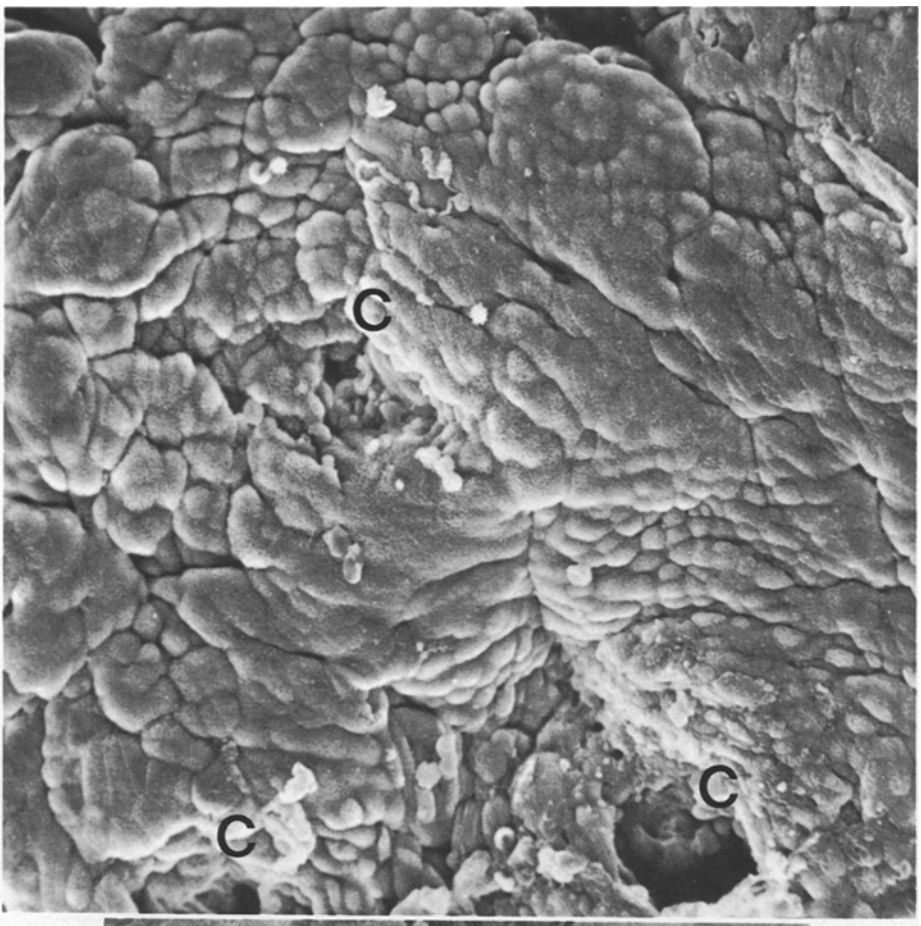


Fig. 3. Crohn's disease, in which the polygonal units have almost disappeared. Plenty of goblet cells can be seen with active extrusion of mucus. ×600

Fig. 4. Crohn's disease. The center area is denuded of epithelial cells and covered with a meshwork of fibrin, mucus and debris. An increased amount of goblet cells is visible. ×600



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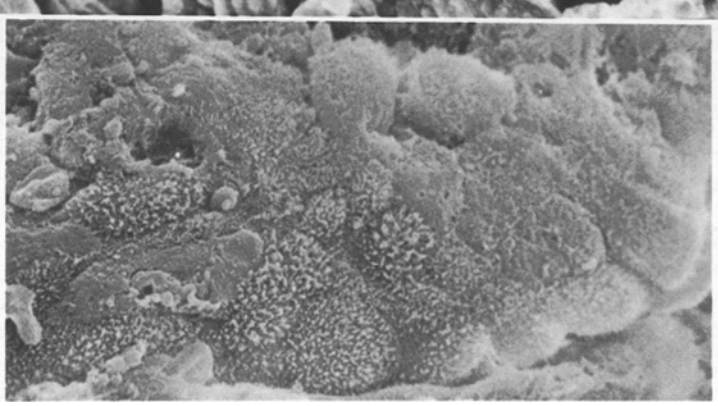

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Fig. 5. Ulcerative colitis. The polygonal territories show great variations. Goblet cells are very few. C crypt opening. ×600

Fig. 6. Ulcerative colitis showing variations in the microvilli between neighbouring cells. ×2,000

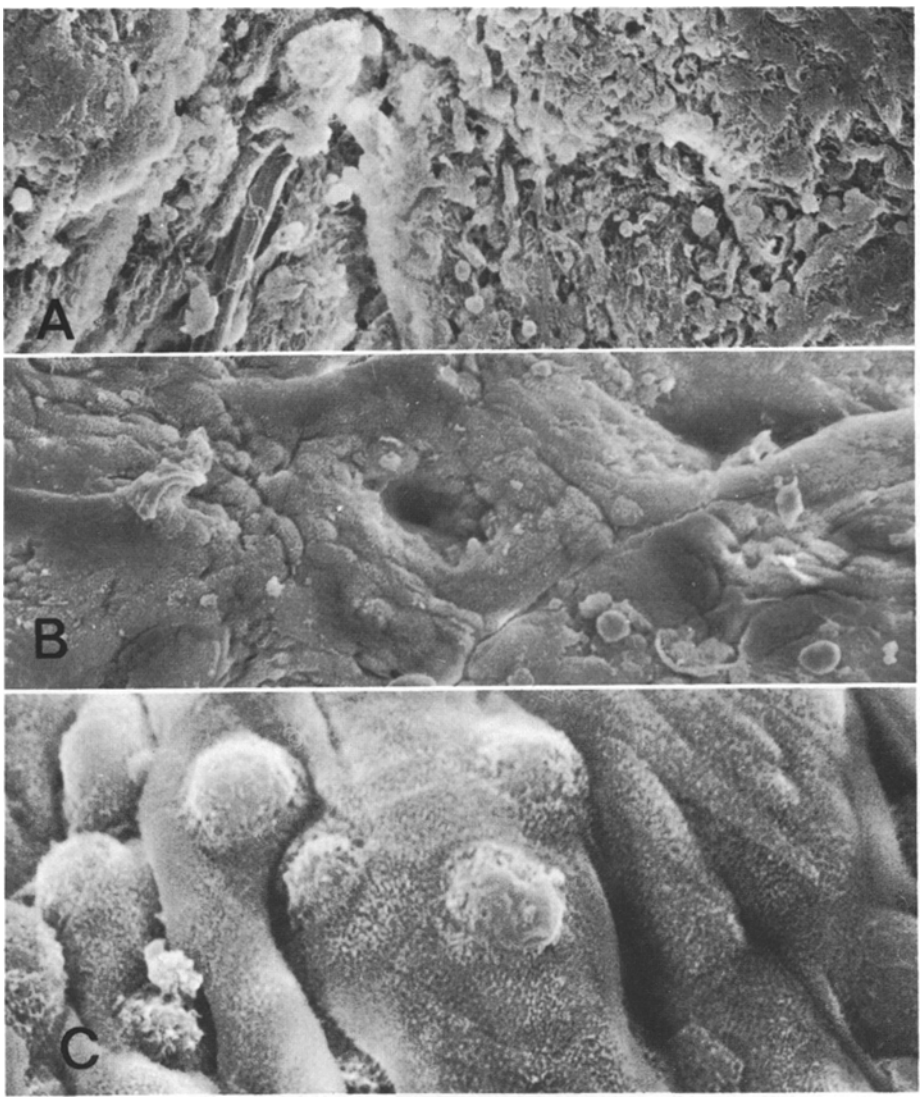

Fig. 7A–C. Ulcerative colitis. **A** Acute state with denuded epithelium. ×600. **B** The same patient 1 month later after conservative treatment; irregular re-epithelization with few goblet cells. ×600. **C** Same patient 3 months later showing normal population of goblet cells. ×2,000

perhaps, to the sub-category of indeterminate colitis, (Lee et al., 1979) which forms 10% to 15% of colectomy specimens operated on for inflammatory bowel disease. Microvilli were seen clearly in some cells but were almost invisible in the neighbouring areas (Fig. 6).

In 2 patients the effect of conservative treatment with Salazopyrine and cortisone enemas was studied. The ulcerated mucosa was re-epithelized, but at 1 month still showed some irregularity of the polygonal units (Fig. 7a and b). The Goblet cell population was normal by 3 months and the surface was still normal about 1 year later (Fig. 7c).

Discussion

Very few previous SEM studies of colonic CD and UC have been made. Dvorak et al. (1979) like us, found goblet cell hypertrophy, hyperplasia and increased secretion of mucus in 12 patients having CD in the ileum, and in one patient having the disease in the colon. The authors discuss the possible enhanced barrier function of this increased coat of mucus in areas of early involvement of CD. The overall surface contour seen in the present study resembles the changes seen for example, in SEM studies of colonic adenomas (Fenoglio et al., 1975) and the epithelium seen in chronic cholecystitis (Myllärniemi and Nickels, 1975) with anastomosing cords of epithelium separated by deep clefts. Fenoglio et al. (1975) also demonstrated an increased amount of goblet cells in hypersecretory villous adenomas.

Of special interest is the present finding that resection margins can show distinct changes on SEM, being macroscopically and light microscopically normal. This finding has clinical importance when the conservative treatment of the disease is considered. This fact, also demonstrated by Dvorak et al. (1979), sustains the theory that CD really affects the entire gut (Dunne et al., 1977). It has also been shown that the histologically normal mucosa in CD contains elevated glucosamine-synthetase levels (Goodman et al., 1976), an epithelial enzyme connected with regenerative activity of the gastrointestinal tract and suggesting that the epithelium is always abnormal in CD. The microvilli in our cases showed no SEM changes, a finding supported by the transmission electron microscope studies of O'Connor (1972).

CD does not necessarily show SEM changes in the whole colonic epithelium. Kaye et al. (1979) studied 8 patients with CD affecting the ileum, ascending and transverse colon. No clear SEM changes could be seen in sigmoidoscopically and histologically normal rectal mucosa. On the other hand, only 2 CD diagnoses were made from surgical specimens.

In UC the most interesting difference from CD was the decreased number of goblet cells, seen in four cases. Kavin et al. (1970) studied two patients with UC during clinical remission by SEM and found the surface architecture to be distorted by irregular corrugations and micropolyps with very few goblet cells. Filipe and Dawson (1970) report a marked decrease of mucosubstances in patients with active UC but not in CD. The microvilli in our UC specimens were partly degenerated or atrophic in many cells, which is in accordance with the transmission electron microscopic findings of O'Connor (1972). Otto et al. (1975) also found electron microscopically destroyed and decreased number of microvilli and alterations in the glycocalyx in UC.

The preparation techniques for SEM have been simplified. It is not necessary to evaporate any heavy metal surface layers after specimen fixation prior to examination with SEM. The specimen can easily be coated with osmium by the thiocarbohydrazide technique before critical point drying (Malick and Wilson, 1975). We have also used air dried specimens after osmium treatment, with good preservation of microvilli when studied with even 10,000-fold magnifications (Kalima et al., 1979). No difficulty with charging effects was noticed. With simplified SEM techniques the instrument can be a useful supplement in the routine studies of gastrointestinal inflammatory diseases, in both the

differential diagnosis of CD and UC and follow-up studies on the effect of conservative treatment.

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