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Scanning electron microscopy study of small bowel biopsies in chronic diarrhoea in childhood

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Summary. In this study we have compared the results of Scanning Electron Microscopy (SEM) with Light- and Stereomicroscopy in a series of small bowel biopsies in children.

In 9 cases displaying features of partial or subtotal atrophy, Light and Dissecting-Microscopy yielded similar results. The distinction between coeliac and non-coeliac chronic diarrhoea was only possible on clinical grounds, and by the immunological detection of specific antibodies. On SEM however coeliac patients showed characteristic alterations consisting of: absence of villi; prominent crypt outlets resulting in a mosaic appearance; concentric furrows running all around the openings; and downy brush feature at high power. The microvilli were loosely distributed and had an irregular pleomorphic outline; they often displayed a drumstick swelling of the tip and were bent. In contrast, non-coeliac chronic diarrhoea cases were characterized by a thick mucous layer on the mucosal surface, that made it impossible to visualize further changes.

Peculiar vascular changes in lymphangiectasia and in sickle beta thalassemia could be detected only by Light Microscopy. In addition, in the lymphangiectasia case SEM allowed the detection of enteroadherent bacteria; and in the lambliasis case, of pseudomembranes.

Absence of glycocalyx was noted both in controls and in patients.

The results of this study point to a diagnostic utility of SEM particularly in the differential diagnosis of chronic diarrhoea; moreover they suggest that enteroadherent bacteria may not be pathogenic and that the absence of glycocalyx is not specific for allergic enteropathy as previously claimed.

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Introduction

The morphological evaluation of the small bowel intestinal mucosa largely relies upon Stereo- and Light-Microscopy.

Transmission Electron Microscopy (TEM) plays an essential role in the diagnosis of a few disorders, such as congenital and post-neonatal microvillus atrophy (Davidson et al., 1978; Schmitz et al., 1982; Phillips et al., 1985), and chronic diarrhoea due to enteroadherent bacteria (Ulsen and Rollo, 1980; Thoren et al., 1980; Rothbaum et al., 1982; Phillips et al., 1982). Moreover TEM allowed the identification of the majority of the viruses implicated in causing gastroenteritis (Madeley, 1986).

Due to its properties of three dimensional view, high resolution power and great depth of focus, the SEM could be an ideal instrument for morphological examination of large surface organs like the intestine.

In the last few years, the use of SEM has yielded promising results in some pathological conditions (Halter et al., 1982; Poley and Rosenfield, 1982; Carpino et al., 1985; Hardof et al., 1986; Poley, 1988).

To evaluate the diagnostic potential of this technique, we have studied by SEM a series of small bowel biopsies from patients with clinical evidence of malabsorption, and have compared the results with those obtained with the conventional techniques, i.e. Dissecting and Light-Microscopy.

Materials and methods

The material of the study comprised of small bowel biopsies from 13 patients (8 males, 5 females, age: 6

months to 17 years). The main clinical pathological data are given in Table 1.

Five patients suffered from coeliac disease: three were untreated and two were examined after a gluten diet.

Four patients had chronic diarrhoea, unresponsive to gluten withdrawal. Amongst these four patients, one, presenting autoantibodies against enterocytes, recovered after immunosuppressive treatment (autoimmune enteropathy) (Unsworth and Walker-Smith, 1985; Mirakian et al., 1986): one, having a Fanconi's Anemia, developed untreatable diarrhoea following a Salmonella infection, and one suffered from sickle beta thalassemia. Two patients respectively suffering from gastroesophageal reflux and chronic non-allergic gastritis, served as normal controls with regard to the intestinal morphology.

The remaining cases suffered from small intestinal lymphangiectasia and giardiasis respectively.

Biopsy specimens were obtained from the duodeno-jejunal junction by endoscopy or by a pediatric Watson capsule under fluoroscopic control; endoscopic biopsies comprised two specimens. An informed consent was obtained in all cases.

Each specimen was examined under the stereo-microscope and subsequently divided into two parts. One was fixed in 10% formalin and embedded in paraffin for routine histological diagnosis, the other was used for SEM processation. After washing in physiologic solution, the specimen was fixed in 2.5% glutaraldehyde in 0.1 M cacodylate buffer pH 7.4 for 24 hours at 4° C. After dehydration in alcohol, the specimen was prepared for critical point drying in liquid CO_2 (Anderson, 1951). The specimen was then dried, mounted on a metallic support with carbon and covered with a thin gold layer. Examination was carried out with a I.S.I.S.S. 40 SEM.

Stereomicroscopy and histological evaluations were carried out according to Thompson's criteria (Thompson, 1976). Digitate villi reflected the normal villous pattern; grade 1 abnormality was expressed by ridges, leaf and digitate villi, with occasional convolutions; grade 2 abnormality (partial villous atrophy) consisted of prevailing convolutions but ridges, leaf and digitate villi were also present; grade 3 abnormality (subtotal villous atrophy) corresponded to flat or convoluted mucosa with or without a mosaic pattern.

Results

Control cases (cases 1, 2)

Dissecting and Light-Microscopy revealed a normal jejunal mucosa, i.e. a normal villous pattern with digitate villi.

The low power view under SEM showed similar but better detailed features when compared with the stereomicroscope. Two main types of villi were identified: digitate, i.e. elongated with uniform diameter from the bottom to the top; leaf, with the bottom larger than the top. Clear cut indentations ran over the vast majority of villi. Crypt outlets were recognized as single openings on the basis of villi after careful searching.

Higher power revealed a polygonal contour of the enterocytes. These cells were closely packed (Fig. 1). The luminal pole was flat or slightly curved and covered with the round tips of microvilli. The globet cells showed a round profile and were easily recognized because of the focal mucus production. At very high resolution power the microvilli displayed a uniform appearance and were tightly packaged so that one could appreciate the convex tip. The glycocalix was not visible (Fig. 2).

Coeliac patients (cases 3, 4, 5, 6, 7)

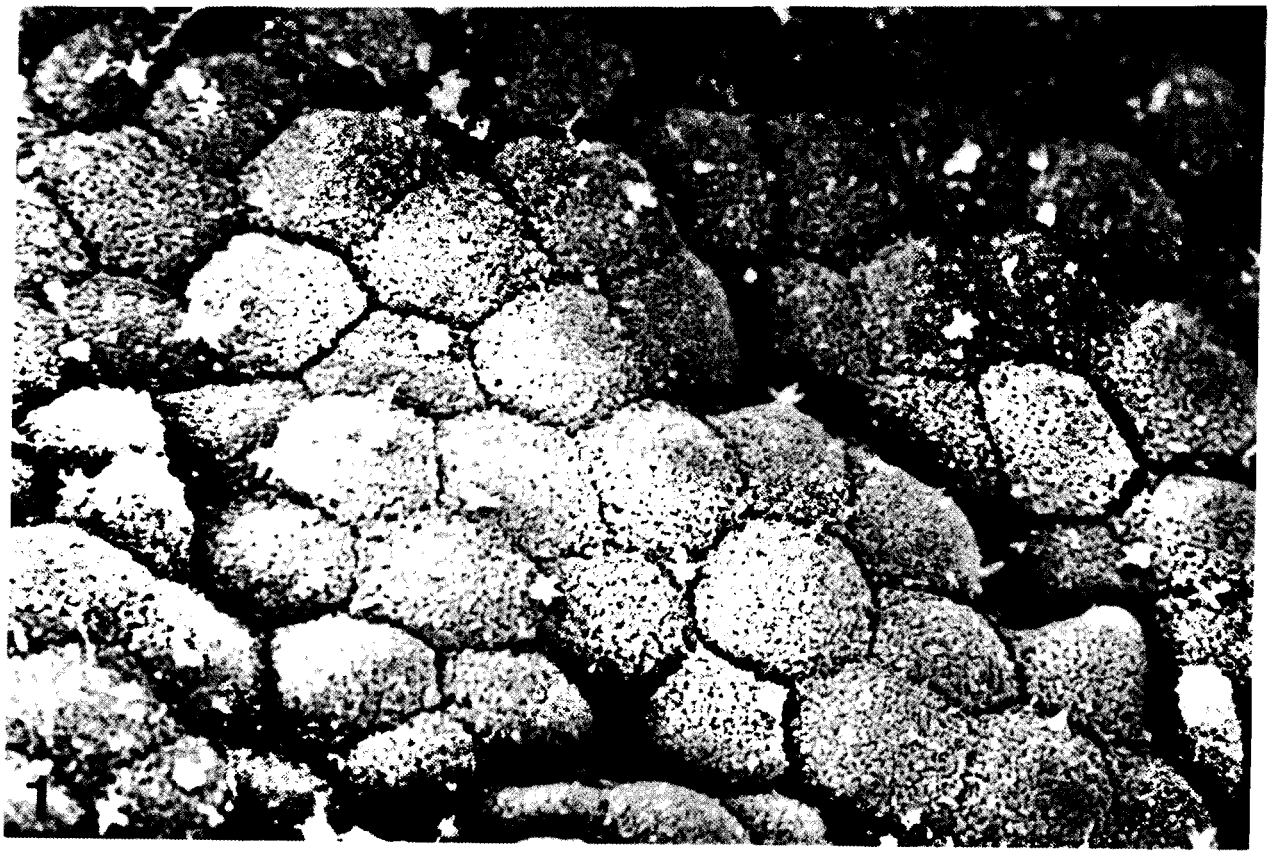
These five cases showed grade 3 abnormalities on Stereo and Light-Microscopy. The mucosal surface was flat with a mosaic appearance; villi were generally absent and when present, they were very short and abnormal. The lining epithelium was cuboidal or flat and hyper-cellular; there was marked accentuation of lymphocytic infiltration; the crypts were hyperplastic; inflammatory cells were prominent in the lamina propria (Fig. 3). At low power SEM the mucosal surface was flat. Due to the absence of villi, crypt outlets were prominent resulting in a mosaic appearance. Concentric furrows ran all around the openings (Fig. 4). The slightly curved elevations in between corresponded to residual atrophic villi. In areas with less severe atrophy, the intervening convolutions were more protuberant next to the openings, thus resulting in a «tench's mouth» appearance (Fig. 5). Elsewhere, they displayed a serpiginous profile resulting in a cerebriform pattern (Fig. 6). At times, the convolutions were connected by bridge-like structures resulting in a wrinkled appearance (Fig. 7).

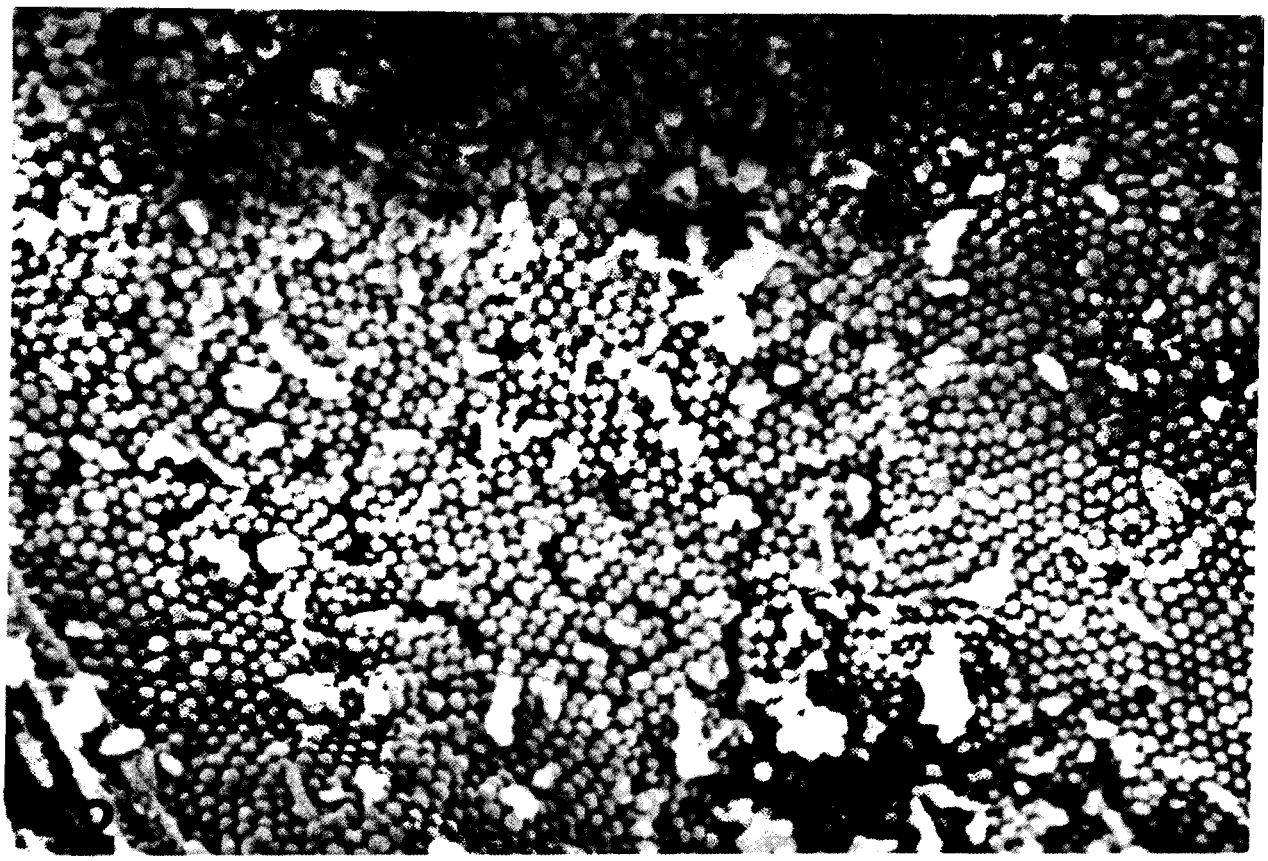
At higher power, the mucosa showed a downy brush appearance (Fig. 8). The microvilli were more loosely distributed compared with normal controls; furthermore they displayed an irregular pleomorphic outline with an extremely variable length; they often appeared as bent or with a drumstick swelling of the tip (Fig. 9). The glycocalyx was not visible.

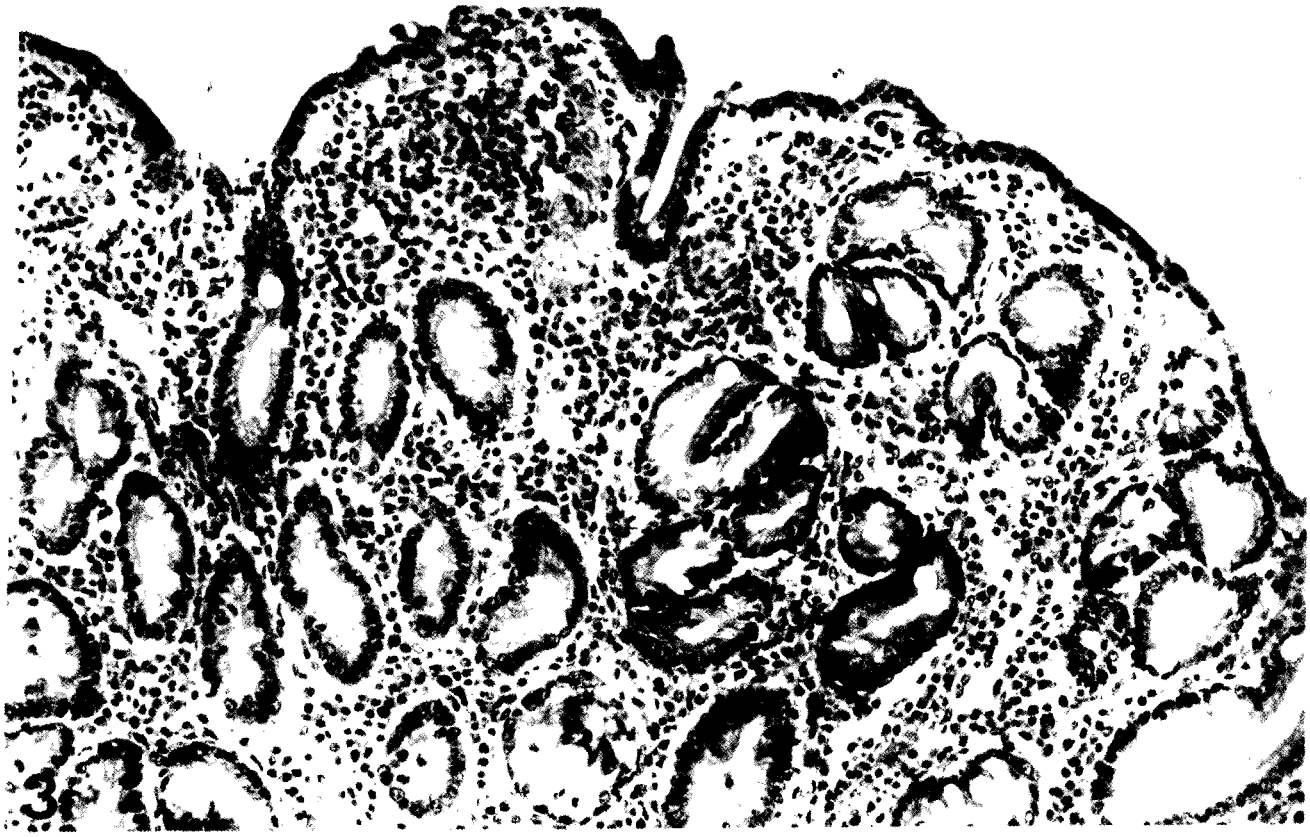
Chronic diarrhoea patients (cases 10, 11, 12, 13)

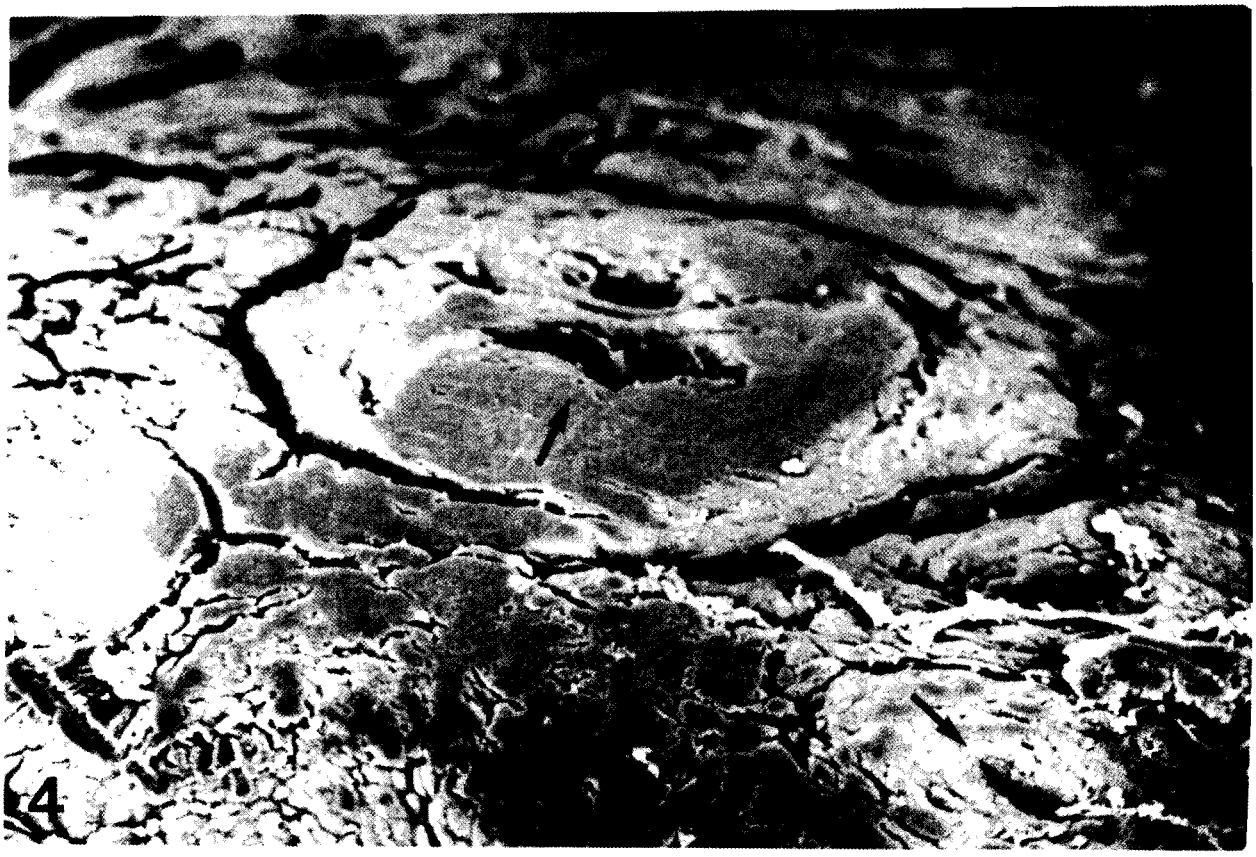
Under the Stereo and Light-microscope 3 cases displayed substantial features of subtotal villous atrophy (grade 3 abnormality), while the fourth (case 12, Sickle Beta Thalassemia) showed partial villous atrophy (grade 2 abnormality).

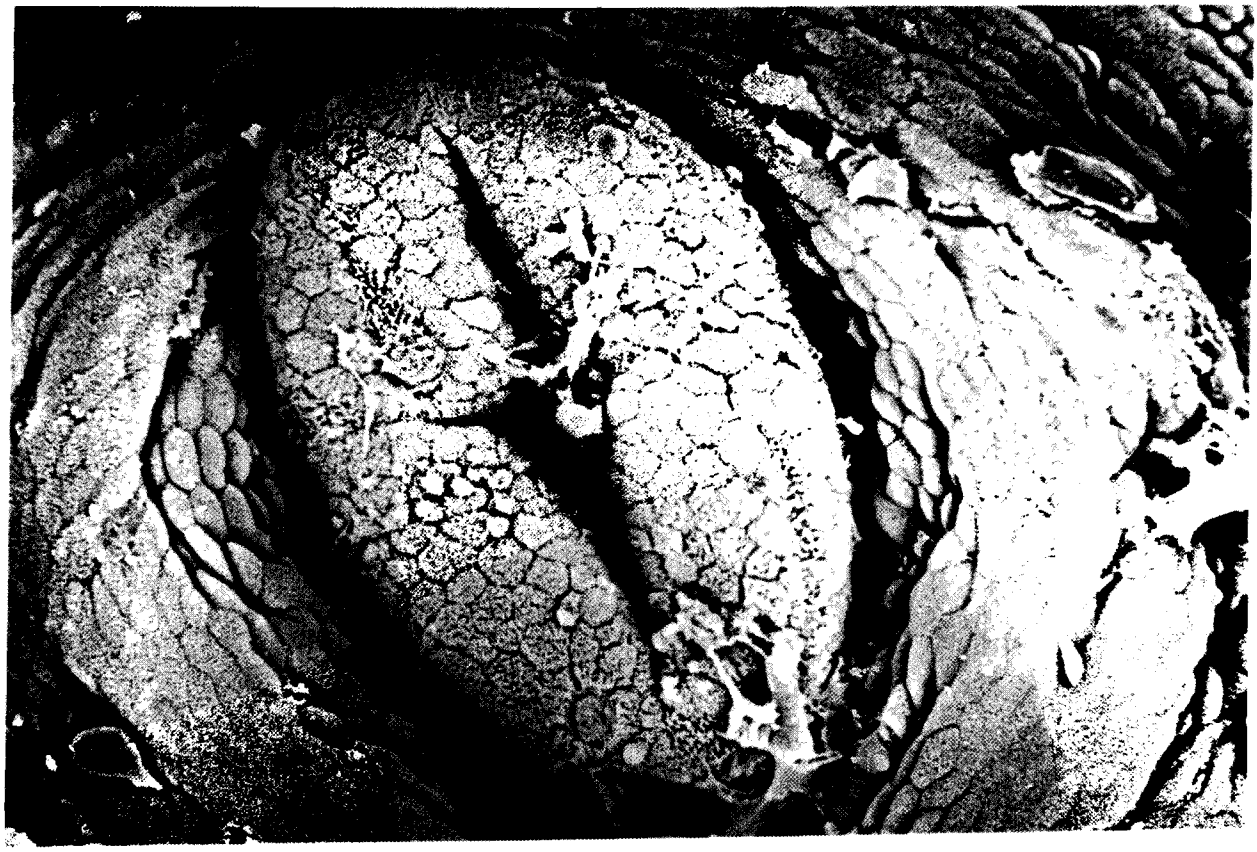
In addition, the latter case was characterized by a marked dilatation of blood capillaries whose lumen were filled up by «rouleaux erythrocytes» (Fig. 10). On SEM, a thick mucous layer covered the surface, thus preventing the visualisation of enterocytes (Figs. 11, 12). On very high power a fine network could be appreciated within the mucous layer. In exposed areas one could see stumpy protrusions corresponding to abortive villi and a mucus secretion extruding from goblet cells. Microvilli could not be appreciated.

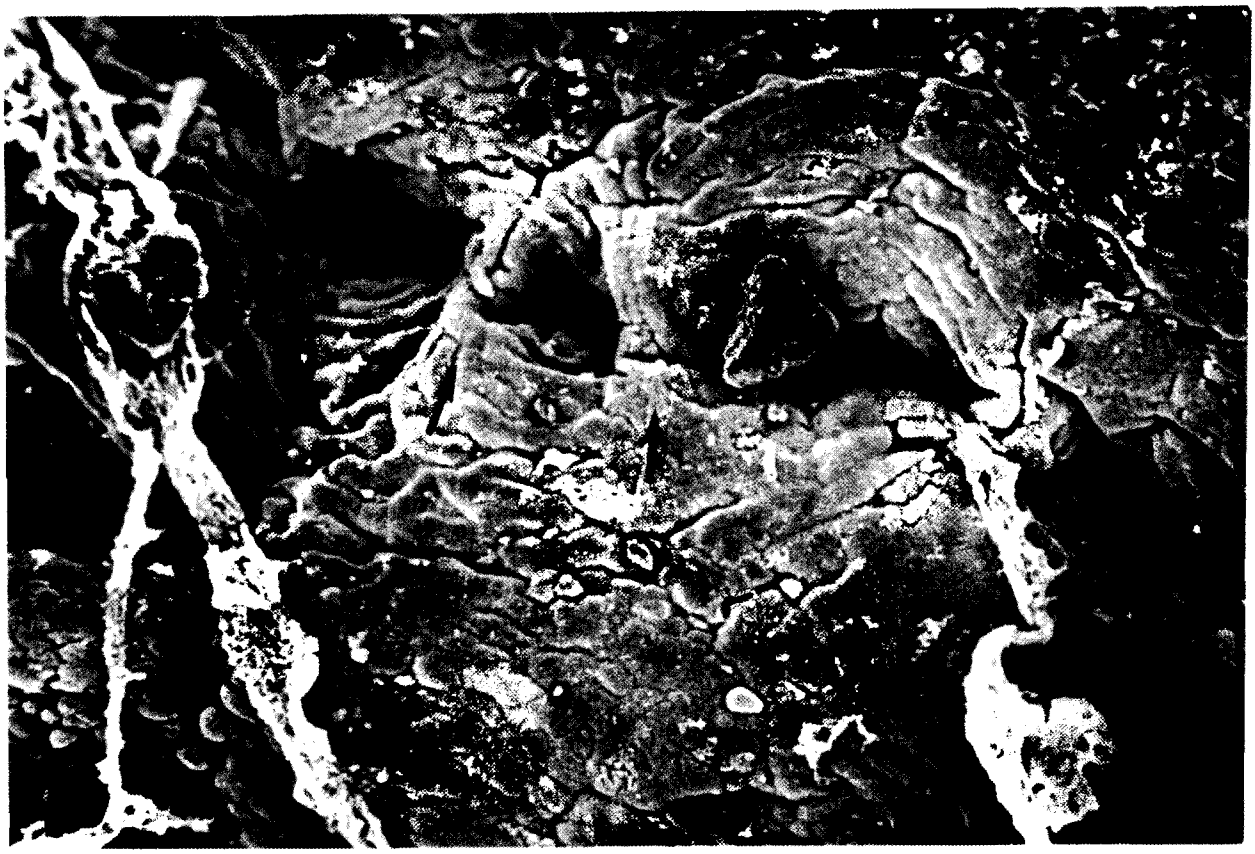


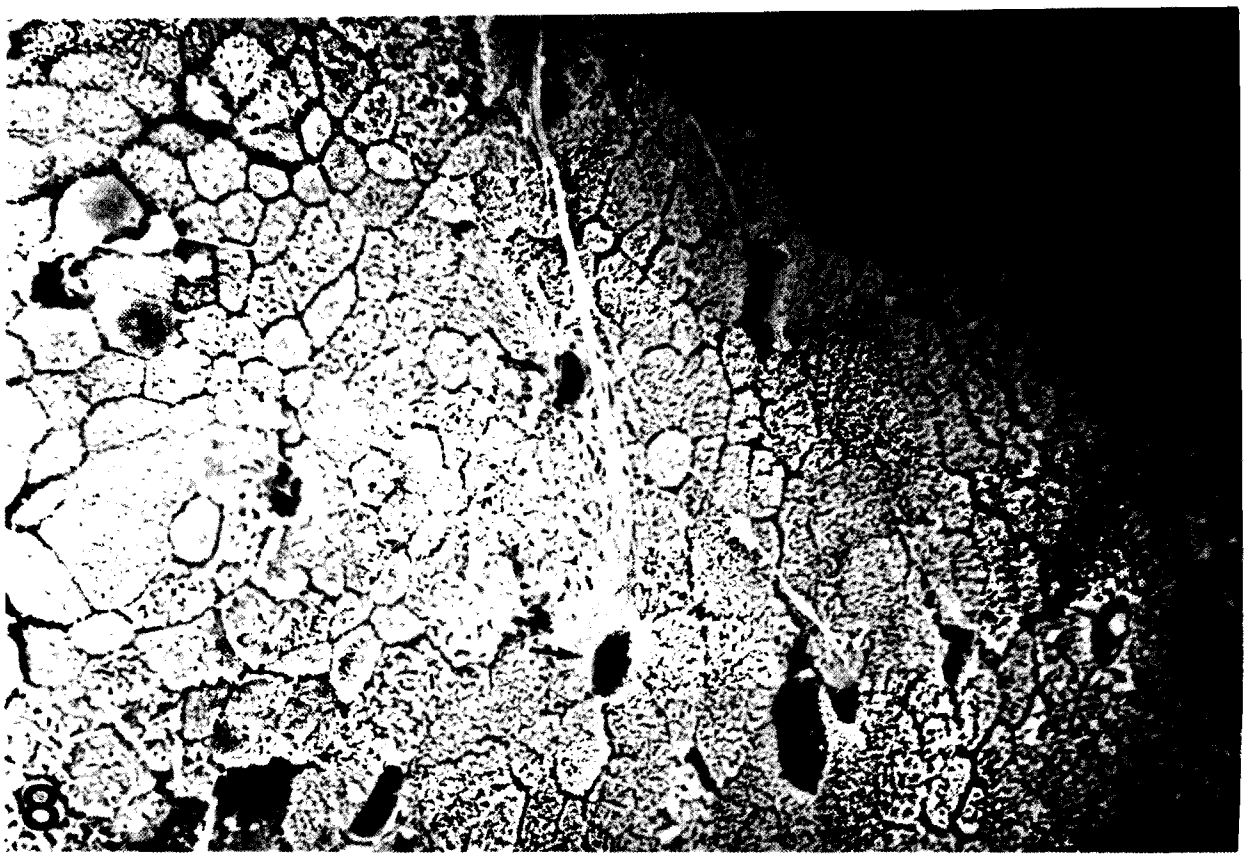


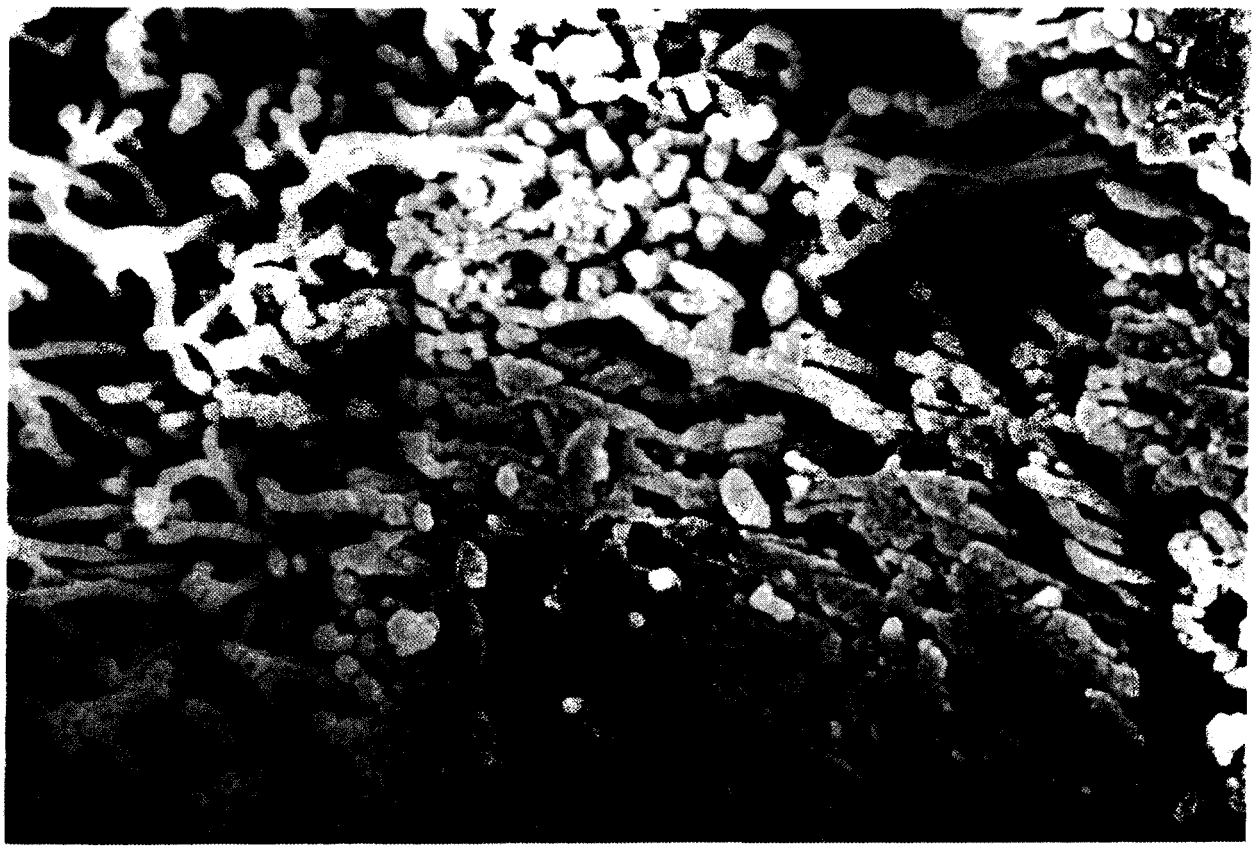


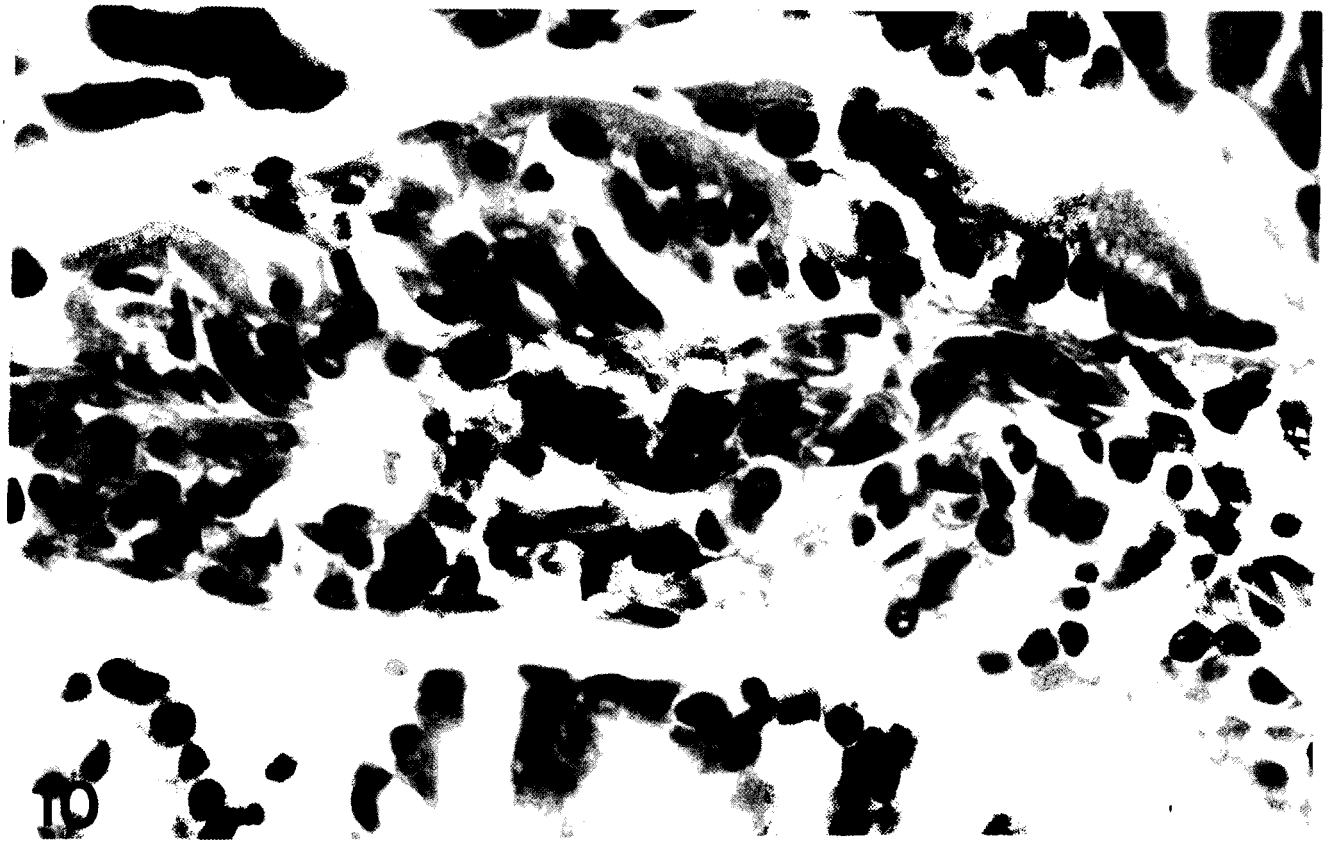


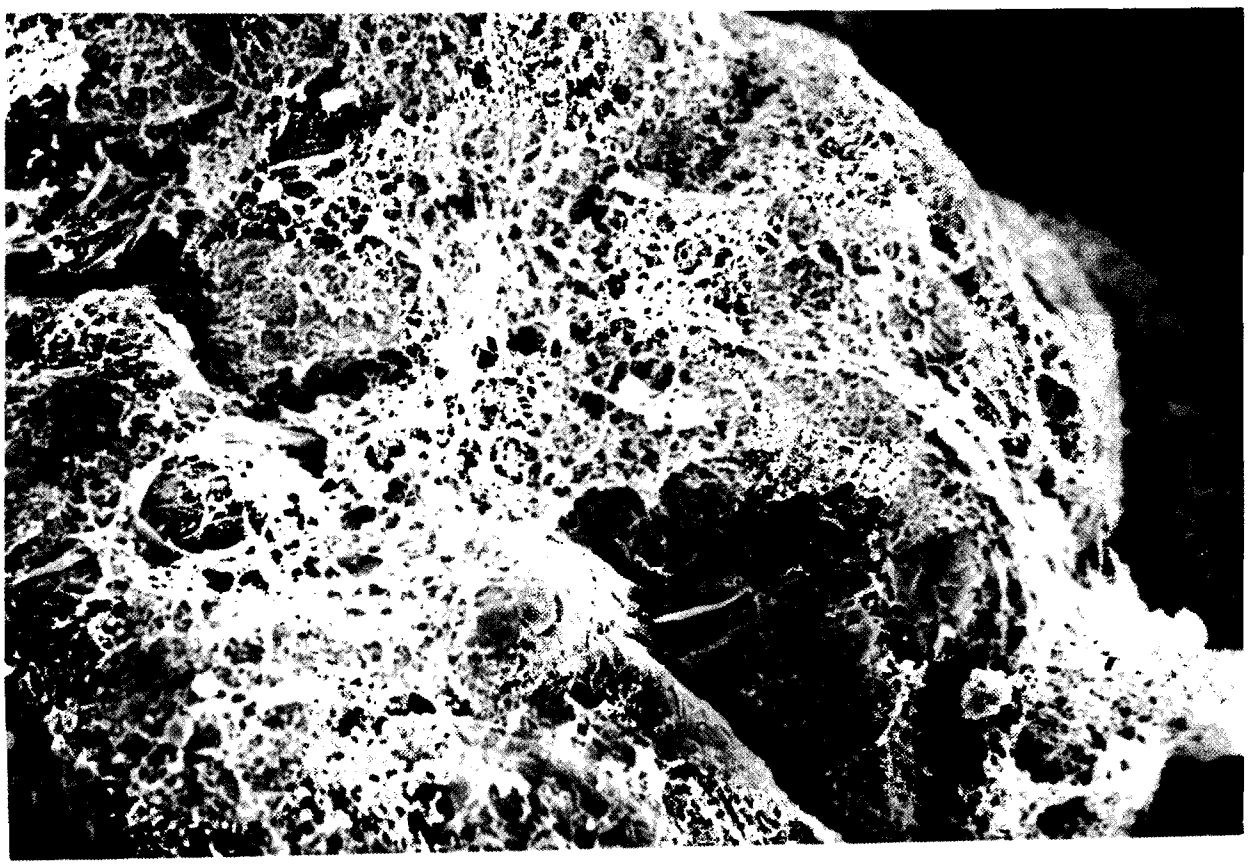


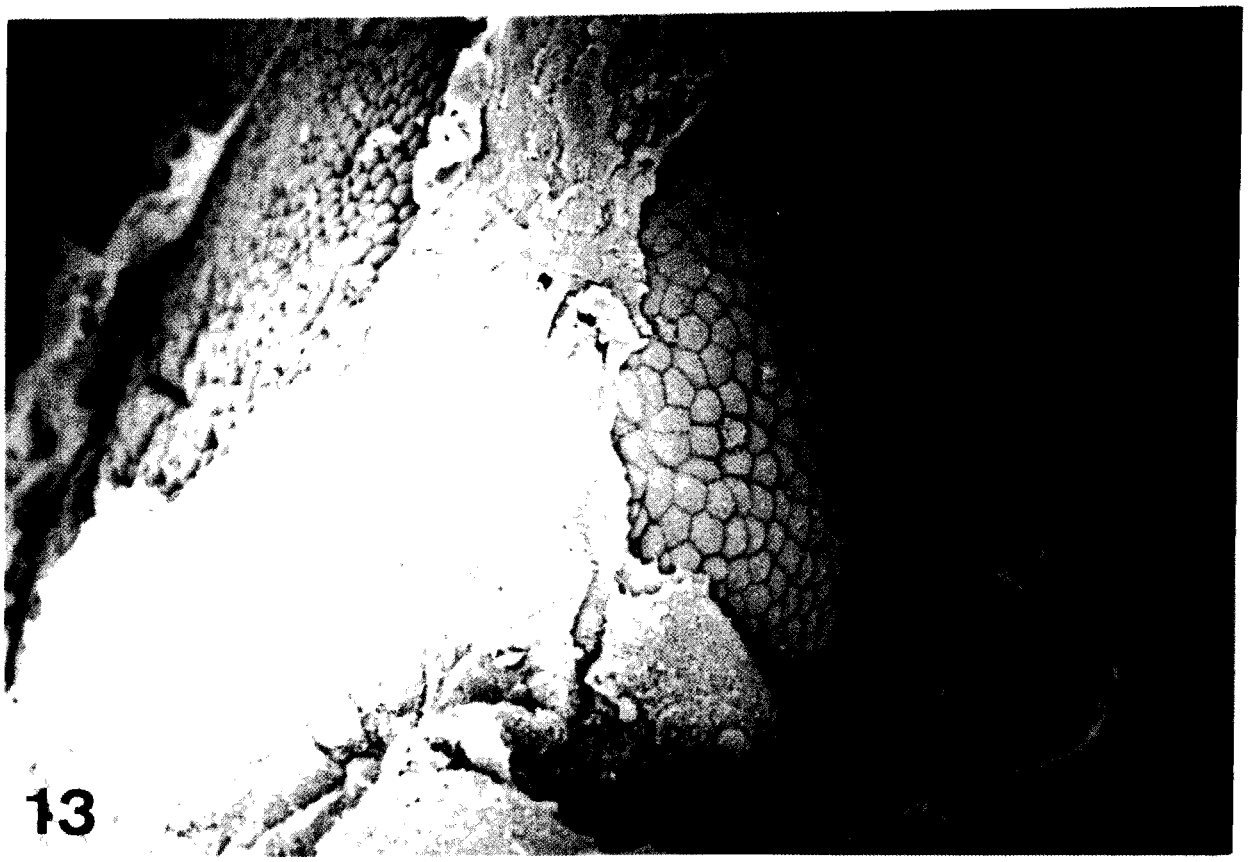








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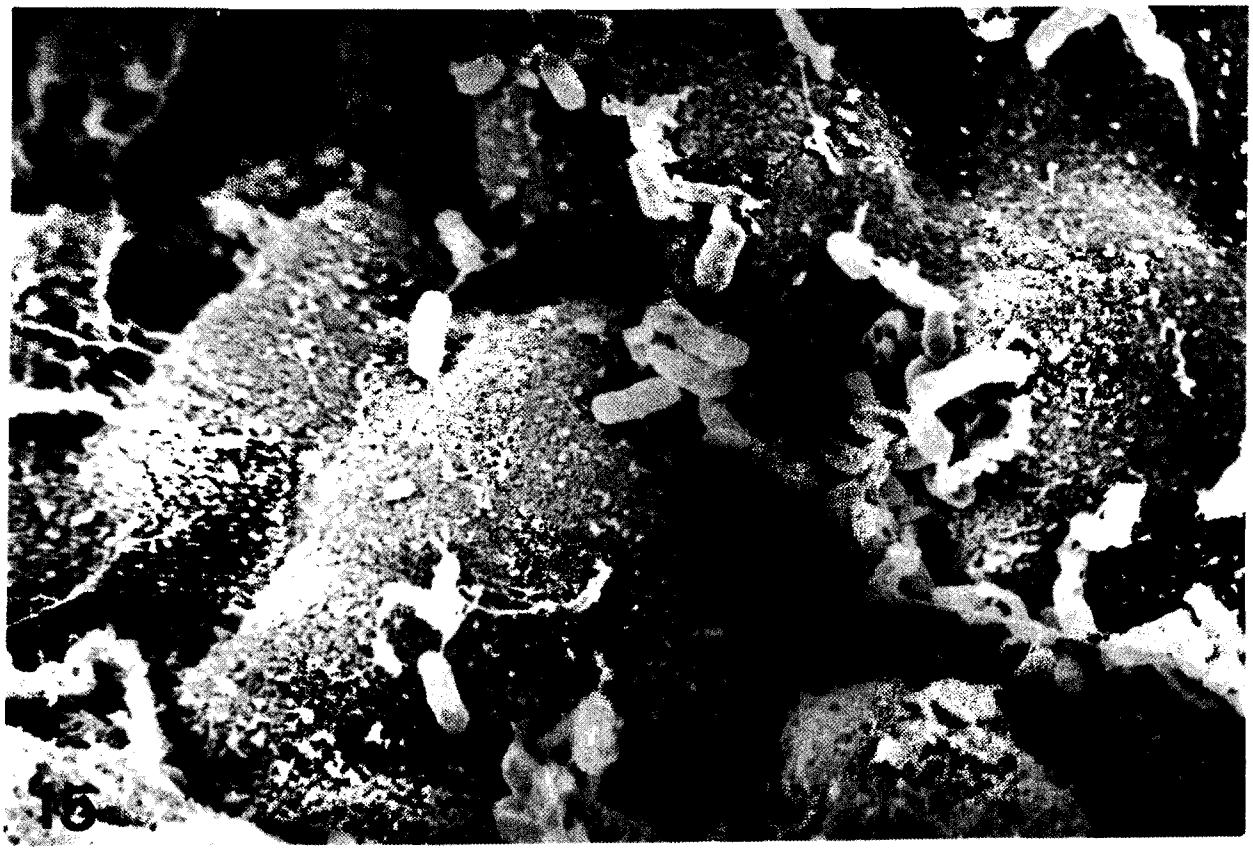

Fig. 1. (control): normal appearance of enterocytes with regular polygonal contour. × 5,280

Fig. 2. (control): Small bowel normal mucosal surface devoid of glycocalyx. Tips of microvilli are clearly visible. × 30,580

Fig. 3. (coeliac patient): the microphotograph shows subtotal villous atrophy, epithelial changes, inflammation and hyperplastic crypts. × 240

Fig. 4. (coeliac patient): flat mucosal surface with concentric furrows running all around the crypt outlets (arrows). × 1,055

Fig. 5. (coeliac patient): the photograph shows a «tench's mouth» configuration of the crypt outlets. × 1,450

Fig. 6. (coeliac patient): the picture shows convolutions with a cerebriform pattern. × 550

Fig. 7. (coeliac patient): the parallel ridges are connected by bridge-like structures (arrows). × 925

Fig. 8. (coeliac patient): mucosal surface with downy-brush feature. The goblet mucus-secreting cells are clearly distinguishable (arrows). × 2,080

Fig. 9. (coeliac patient): The microvilli are sparse with irregular length. Some others with a «drumstick» swelling of the tip are seen. × 28,380

Fig. 10. (chronic diarrhoea in Sickle Beta Thalassemia patient): the villous pattern shows grade 2 abnormality. Capillaries in the lamina propria are filled up with rouleaux red blood cells. × 600

Fig. 11. (chronic diarrhoea): mucosal surface is covered by thick layer of mucus. Only goblet cells are recognizable as round openings. × 3,500

Fig. 12. (autoimmune enteropathy): mucosal ridges are covered by thick layers of mucus and poorly recognizable.

Fig. 13. (giardiasis): thick mucoid pseudomembrane covers wide areas of the mucosal surface. × 1,000

Fig. 14. (small intestinal lymphangiectasia): the villous pattern shows grade 1 abnormalities. Lymph vessels in the tip axis of villi are dilated. × 240

Fig. 15. (small intestinal lymphangiectasia): the picture shows bacteria adhering to enterocyte surface devoid of glycocalyx. × 7,500

Giardiasis patient (case 8)

Grade 2 abnormality was observed under the Stereo- and Light-Microscope. Villi were short and abnormally shaped, crowded and folded. The surface epithelium was cylindrical and hypercellular. The luminal pole of enterocytes had a dome-shaped appearance and a shedding tendency; stratified mucus was adherent to the surface. An increase of inflammatory cells including lymphocytes, plasma cells and eosinophils was noted in the lamina propria.

No parasites were seen under light microscope, while jejunal juice and stools examination was positive.

On SEM, large areas of the surface were covered with a thick pseudomembrane which prevented the visualisation of villi (Fig. 13). At high power a close net covered the microvilli; a beaten appearance (impression of suction disks) was not appreciated on the surface of the pseudomembrane.

Table 1.

patient	age	sex	disease	dissecting-microscope appearance
1)	12 m	M	gastro-oesophageal reflux	normal
2)	2 y	M	gastritis	normal
3)	4 y	M	coeliac disease	grade 3 abnormality
4)	6.5 y	F	"	"
5)	6 m	M	"	"
6)	5.5 y	F	"	"
7)	11.5 y	M	"	"
8)	2 y	F	giardiasis	grade 2 abnormality
9)	2 y	M	small intestinal lymphangiectasia	normal
10)	5.5 y	F	chronic diarrhoea	grade 3 abnormality
11)	18 m	M	chronic diarrhoea (Fanconi's Anemia)	"
12)	2 y	M	chronic diarrhoea (Sickle Beta Thalassemia)	grade 2 abnormality
13)	17 y	F	chronic diarrhoea (autoimmune enteropathy)	grade 3 abnormality

M = male F = female m = month y = year

Small Intestinal Lymphangiectasia patient (case 9).

The Stereomicroscope revealed a nearly normal mucosa; on Light-Microscopy slight changes consisting in segmental hypotrophy were noted.

Dilated lymphatics were present in the villous axis (Fig. 14), beneath the epithelium and in the deep lamina propria. There was an increase in inflammatory cells.

On SEM the picture was similar to that of normal controls, except for the presence of bacteria which were in close contact with microvilli, in the absence of demonstrable glycocalyx (Fig. 15).

Discussion

The small intestinal biopsy remains a crucial step in the diagnostic investigation of malabsorption and in monitoring the response to treatment.

Dissecting Microscopy is a simple and fast means to evaluate the villous pattern, as stereomicroscopic features correlate fairly well with light microscopic observations (Thompson, 1976; Walker-Smith, 1988).

In addition Light-Microscopy allows a more detailed appreciation of the villous structure, the morphology of the lining epithelium including the brush border, and of the lamina propria and its content. In a large proportion of cases, Stereo- and Light-Microscopy suffice to demonstrate the pathological substrate of malabsorption, such as villous atrophy.

Per sé, however, the microscopic features of villous atrophy are neither specific nor pathognomonic. The serological detection of specific antibodies, either antigliadin antibodies or enterocyte autoantibodies, is helpful in recognizing either gluten-sensitive (Savilahti et al., 1983; Unsworth et al., 1983) or autoimmune enteropathy (Unsworth and Walker-Smith, 1985; Mirakian et al., 1986). These clinical entities however can be definitely diagnosed only by the clinical course; the former by remission and relapse at gluten withdrawal and with gluten; the latter by the lack of response to diet, and possible remission with immunosuppresive therapy.

TEM plays an essential role in the diagnosis of congenital and post-neonatal microvillous atrophy and in enteropathy due to enteroadherent bacteria.

The diagnostic potential of SEM has not been extensively investigated. SEM has provided useful information on the sequence of villous regrowth in coeliac disease after gluten withdrawal (Halter et al., 1982; Carpino et al., 1985). Moreover SEM has been shown to be more sensitive than Stereo- and Light-Microscopy in detecting early signs of improvement or deterioration of the villous pattern.

In the present study SEM has proven to be useful in differentiating coeliac disease from other conditions associated with villous atrophy which share similar light microscopic features.

In chronic non-coeliac diarrhoea a thick mucous layer invariably covered the mucosa surface, thus preventing the visualisation of the enterocyte contour and the microvilli.

In contrast, the mucous layer was not present in coeliac disease; thus SEM seemed to allow the detection of the characteristic coeliac changes, i.e. flat mucosa, downy-brush surface, thin pleomorphic drumstick microvilli with a bent appearance.

On SEM, the mucous layer in chronic non-coeliac diarrhoea looked different from that observed in giardiasis. In the latter condition it is presented as a

pseudomembrane. Other authors have observed a beaten appearance of the pseudomembrane, attributed to the suction disks of the lamblia (Poley, 1984). The pseudomembrane seems to represent a lamblia-associated feature, being useful in refining an usually laborious diagnosis.

Over-production of mucus represents a non-specific response to different noxious stimuli. That may contribute to the malabsorption by acting as a physical barrier between the intestinal luminal content and the absorbent epithelium.

As a rule, the diagnosis of intestinal lymphangectasia is possible by Light-Microscopy. In only a single case from our series with proven lymphangectasia have we observed on SEM bacteria adherent to the microvilli surface. This coincidental finding would suggest caution in attributing a phathogenetic role to enteroadherent bacteria.

Thus, the absence of glycocalyx on the mucosal surface at the duodenojejunal junction has been reported as a typical feature in infants with chronic diarrhoea and intolerance to dietary proteins (Poley, 1988).

We have observed small bowel surfaces devoid of glycocalyx in our control patients and in a patient with intestinal lymphangiectasia. This discrepancy may be related to technical factors.

A further observation of interest in the present study refers to the Sickle Beta Thalassemia case. Light-Microscopy revealed dilatation of capillaries whose lumen were filled up with «rouleaux erytrocytes». This picture might reflect a slowing down of blood circulation and result in enterocyte ischemic changes.

In conclusion, SEM proves to be a useful technique for monitoring the morphodynamics of villous regrowth and to detect very subtle morphological alterations. Moreover, it allows the differential diagnosis between coeliac disease and other conditions presenting villous atrophy.

The SEM findings in lambliasis and lymphangectasia need further confirmation in additional cases. Nonetheless, our observations might be relevant, because the presence of pseudomembrane seems to be specific for lambliasis, whilst the presence of adherent bacteria would not necessarily imply a pathogenetic role.

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