Scanning Microscopy

Volume 5 | Number 4 Article 14

9-18-1991

The Scanning Electron Microscope: How Valuable in the Evaluation of Small Bowel Mucosal Pathology in Chronic Childhood Diarrhea?

J. Rainer Poley
Eastern Virginia Medical School

Scanning Microscopy, Vol. 5, No. 4, 1991 (Pages 1037-1063) 0891-7035/91$3.00+.00
Scanning Microscopy International, Chicago (AMF O'Hare), IL 60666 USA

THE SCANNING ELECTRON MICROSCOPE: HOW VALUABLE IN THE EVALUATION OF SMALL BOWEL MUCOSAL PATHOLOGY IN CHRONIC CHILDHOOD DIARRHEA?

J. Rainer Poley*

Department of Pediatrics, Eastern Virginia Medical School, and
Gerasimos S. Logaras Laboratory for Electron Microscopy, and
Children's Hospital of The King's Daughters, Norfolk, Virginia, USA

(Received for publication June 1, 1990, and in revised form September 18, 1991)

Abstract

Data are presented on scanning electron microscopy (SEM) on small intestinal biopsies of children with chronic diarrhea. In particular, there were 230 patients aged 3 months to 13 years with the following diagnoses: chronic nonspecific diarrhea, cow's milk protein intolerance, soy protein intolerance, giardiasis, cystic fibrosis, gluten-sensitive enteropathy, isolated lactase deficiency, isolated sucrase-isomaltase lactase deficiency, microvillus inclusion disease, rotavirus enteritis, protracted diarrhea of infancy, chylomicron retention disease, visceral myopathy and villous asthenia.

Examination of biopsied intestinal mucosa by SEM has yielded important new information and insights on structural pathology and ultrastructural topography. Many of the observed changes helped to better understand the pathophysiology of some of the diarrheal disorders. SEM was also able to detect new features such as mycoplasma-like microorganisms and the absence of the glycocalyx. To adequately assess small bowel mucosal pathology at the ultrastructural level, scanning electron microscopy is an indispensable tool.

Key Words: scanning electron microscopy, small bowel mucosa, chronic diarrhea, infants, children.

*Address for correspondence:
J. Rainer Poley,
Eastern Virginia Medical School, Pediatrics Dept.
800 West Olney Road,
Norfolk, VA 23507

Phone No.: (804) 628-7240
Fax No.: (804) 627-6541

Introduction

The standard tool for morphologic evaluation of biopsied small bowel mucosa has been, and probably always will be, the light microscope. This instrument gives information on morphology, it allows morphometric measurements, it gives information on cell migration within the lamina propria or within the epithelial layer, it recognizes special changes such as dilated lymphatics, and with special stains, such as periodic acid-Schiff (PAS), the brush border is made visible, or other diagnostic features are detected such as macrophages in Whipple's disease. As valuable as the light microscope may be, it only gives information on a very thin slice of tissue with the result that most events occurring on the surface cannot be assessed with the exception of obvious colonization of the mucosa with parasites or readily recognizable bacterial pathogens. Yet it is at the mucosal surface where all the interactions between host and environment take place, and the possibility to survey the mucosal surface for additional pathologic changes has always seemed desirable. The intestinal mucosa, with its very large surface area, is one of the ideal substrates for surface screening, which is nowadays accomplished with the scanning electron microscope.

Long used by industry, the scanning electron microscope was applied to small intestinal pathology in the 1960's and early 1970's [reviewed 13, 14]. In the 1980's this technique was used routinely for the investigation of biopsied small intestinal mucosa of infants and children with chronic diarrhea [69]. By virtue of its great depth of focus and the ability to survey large surface areas, the scanning electron microscope is ideally suited to detect, characterize, and survey changes on the mucosal surface. Terms such as "ultrastructural pathology" or "ultrastructural topography" have been applied to such changes [13,14].

To this author, scanning electron microscopy

(SEM), has become an indispensable tool for the evaluation of small bowel pathology. Findings have always been integrated with other investigational techniques, such as light microscopy (LM), measurement of disaccharidase activities, transmission electron microscopy (TEM), and microbiology. However, even from the point of view of diagnosis or differential diagnosis, SEM is now taking a large share of the overall assessment of small bowel mucosal pathology.

In this review, specific conditions will be discussed where more insight has been gained and more information has been obtained by SEM than from any other investigational tool. For instance, SEM has contributed to our understanding of intolerance to disaccharides (by test, clinically) despite normal disaccharidase activities and a structurally normal mucosa by LM. Furthermore, SEM explains why there may be generalized depression of disaccharidase activities despite a mucosa which is structurally normal by LM. Thirdly, in several conditions SEM demonstrates to the observer the presence of a physical barrier, which contributes to maldigestion and malabsorption, which is difficult to appreciate when LM shows a structurally normal mucosa. Fourthly, microorganisms which resemble *mycoplasma* species are easily visualized by SEM, while they are not seen by LM. Lastly, specific ultrastructural changes can be identified on the mucosal surface, such as the loss of glycocalyx; again, this would not have been possible, utilizing LM alone.

Methods and Materials

Preparation of Tissue

The tissue preparation techniques used in our laboratory have been published [70, 75]. All small bowel mucosal specimens were obtained under fluoroscopic control from the duodeno-jejunal junction using a double-port Crosby-Kuler biopsy capsule. Written and informed consent was obtained from the parents.

The preparation of intestinal mucosal specimens for SEM, as well as the scanning electron microscopes used, varies from laboratory to laboratory and a brief summary is presented in Table 1. Ideally, tissue preparation, small bowel mucosa in particular, should be standardized to minimize artefacts and to maximize informative yield and inter-observer comparison. Whilst it is possible that nuances in sample preparation, particularly fixation and dehydration procedures, could impart differences on various aspects of surface topography at high magnifications, there seems to be no appreciable alteration of structure and of mucosal surface at low and intermediate magnifications. The quality of photographic reproductions depends on instrument optics, on the skill of the operator and, perhaps also on the film used.

Patients

A total of 230 patients aged three months to 13 years with chronic diarrhea underwent small bowel biopsy. Representative samples from each major disease group were chosen at random, while there was only one patient each with microvillus inclusion disease, chylomicron retention disease, and visceral myopathy, owing to the rarity of these conditions. The majority of the patients had chronic nonspecific diarrhea (toddler's diarrhea). Carbohydrate intolerance was determined by glycemic and clinical response to oral lactose and sucrose tolerance tests. Disaccharidase activities were determined as described [71].

The intestinal mucosal specimens which served as "controls" were obtained from the following patient groups: (1) Children suspected of sucrase-isomaltase deficiency, after having been on a sucrose-free diet for one month and without diarrhea at the time of biopsy [69]; (2) Children with short stature biopsied to rule out celiac disease [15, 40, 67, 92, 93] since short stature may be the only manifestation of celiac disease [38].

This review deals mainly with the experience obtained by the author over a period of 10 years in reviewing small intestinal mucosal biopsies by SEM. Examination of the biopsies by SEM was part of a routine process and not done in a "blindly" controlled fashion. To be of benefit to the patient, it is advisable to review small bowel biopsies by SEM as soon as possible after they have been obtained. However, this may not always be possible.

Discussion

Normal Mucosa

By SEM and at low magnification (80-600x), the mucosa shows the following: the predominant pattern in infants consists mainly of ridges, whereas with increasing age, the pattern may change: more leaf- and tongue-shaped villi become visible, and after 3-5 years finger-shaped villi appear (Fig. 1-3). The proportion of finger-shaped villi in the distal duodenum of adults is perhaps greater [75, 93], and they are quite prominent in the adult jejunum [4]. However, the villi of the duodenum of adults may also be tongue- and leaf-shaped [12], which could be a variation of normal, or could denote subtle injury to the intestinal mucosa. The change in the villous pattern from infancy to childhood may be maturational as well as an adaptive phenomenon: the greater differentiation of villous structures may increase absorptive surface and is perhaps better apt to handle the increased quantity and perhaps, also quality of nutrients.

Table 1. Tissue Preparation for SEM.

Authors (Ref. #)	Saline rinse	Fixative	Time of fixation	Post-fixation in $OS O_4$	Dehydration	Critical point drying	Surface coating, SEM microscope
Toner & Carr, 1969 [94]	Yes	2% phosphate-buffered glutaraldehyde	4 hrs.	0.5%, 1 hour	Ethanol or acetone	Not mentioned	Carbon platinum, thickness not mentioned, Cambridge Stereoscan
Phillips et al, 1979 [67]	No	3% glutaraldehyde in 0.1M cacodylate buffer, pH 7.3	90 min.	1% in 0.1M phosphate buffer	Ethanol gradients	Yes	Gold-palladium; Cambridge S4-10
Halter et al, 1982 [40]	Not mentioned	3% glutaraldehyde in 0.1M cacodylate buffer, pH 7.4	24-72 hrs.	1%, in cacodylate buffer, pH 7.4	Ethanol gradients	Yes	Gold-palladium, 10 nm; Hitachi S-500
Poley and Rosenfeld, 1982 [75]	No	2% paraformaldehyde and 2.5% glutaraldehyde in 0.1M cacodylate buffer, pH 7.4	4 hrs.	1% in 0.1M cacodylate buffer, pH 7.4	Ethanol gradients	Yes	Gold-palladium, 300%A; JEOL JSM 35; Philips SEM/STEM 515
Schön et al, 1984 [84]	Not mentioned	Glutaraldehyde-formaldehyde solution	Not mentioned	Yes, no details	No details	Yes	Not mentioned; BS 300 Tesla
Stenling et al, 1984 [93]	After fixation	6.25% glutaraldehyde in 0.1M phosphate buffer, pH 7.2-7.4	4 days (human tissue), 1 day (animal tissue)	Not mentioned for SEM, yes for TEM specimens	Ethanol gradients and subsequently, iso-amyl acetate gradients	Yes	Gold, 20 nm; Bambridge S4
Carpino et al, 1985 [15]	Not mentioned	2.5% glytaraldehyde in 0.1M cacodylate buffer, pH 7.4	24-72 hrs.	Not mentioned	Acetone gradients	Yes	Gold, thickness not mentioned; Cambridge Stereoscan 150
Giorgi et al, 1985 [35]	Not mentioned	2.5% glutaraldehyde in Sorensen's buffer, pH 7.4	4 hrs.	Yes	Ethanol gradients	Yes	Gold, thickness not mentioned; Philips 505

The increase and the physical change of villous structures is most likely also associated with an increase in the "mass" of disaccharidase activities, to accommodate the greater amount of carbohydrates consumed as infants grow.

At intermediate magnification (800-2250x) more structural details of the mucosal surface become visible: mature enterocytes in the apical region of the villus show slight convexity towards the lumen [69, 74] and the cell borders are distinct (Fig. 4). This distinction is less conspicuous in the mid-portion of the villus and even less at the base, which is populated by younger and, hence, less mature enterocytes. Goblet cells are easily identified and, at the crest of the villi, occasional cell extrusion may be seen as a manifestation of cell renewal (Fig. 5). Cell extrusion in subapical regions is not a normal phenomenon, likewise so-called extrusion zones (see below).

At high magnification (6,600-19,000x), the fine details of the enterocyte surface are well appreciated: the meshwork of the glycocalyx covers the microvilli [69,93], so that their tips are not visible. The glycocalyx may appear more "dense" at times (Fig. 6), or may appear as a filigree-like network covering the microvilli (Fig. 7). Occasionally one sees cell borders which are raised, well defining the polygonal outline of the individual enterocytes (Fig. 8). It cannot be excluded that the latter finding may be a variation of the normal, or even an artefact.

Abnormal Findings and Pathologic Conditions

1. The "mucosal barrier": excess mucus covering the mucosal surface. (Structurally normal mucosa by LM, normal disaccharidase activities, clinical intolerance to carbohydrates). Situations such as the one mentioned above are encountered quite often and, because of the lack of appreciation of surface details with the light microscope, are ascribed to so-called patchy lesions. Certainly, the existence of patchy lesions has been documented in some pathologic conditions with a known etiology [60, 99], but patchy lesions cannot always be a major argument in situations where an obvious explanation for controversial findings is lacking. Let us consider the example where the mucosa appears structurally normal by LM as well as by TEM, and disaccharidases are normal (3 different mucosal specimens!), yet there is clinical as well as laboratory evidence of carbohydrate (lactose, sucrose) intolerance. How can these findings be reconciled? Certainly, one explanation is possible: the substrate does not or cannot make contact with the brush border, intimately associated with disaccharidases. Indeed, in such instances, the mucosal surface is covered with a rather thick layer of mucus, as can be clearly seen by SEM (Fig. 10), and by TEM (Fig. 11). The only correlate by LM is an increased number of goblet cells, also seen by SEM. This layer of mucus, when adhering closely to the mucosal surface, probably functions as a barrier to membrane digestion and most likely also to absorption. This mucosal barrier has been observed in chronic nonspecific diarrhea (CNSD) [69], in giardiasis [75], in food intolerance [72] and particularly in cystic fibrosis [71] where, with increasing age, the mucus layer becomes more pronounced and widespread (Fig. 12, 13). Although pancreatic exocrine insufficiency is the cause of maldigestion in CF, it is hypothesized that the "mucus barrier" interferes with membrane digestion and absorption.

The increased production of mucus can be seen as a host defense response. The presence of intestinal mucins has been important to zoologists, who have recognized its protective effect against parasitic disease in animals [2, 31, 57, 63]. Similar protective functions in relation to a variety of antigenic material, including microorganisms, may be important in man as well, and these have been recognized long ago [36]. For instance, mucins contained in secretory granules of goblet cells are potent inhibitors of certain lectins [7] that mediate the binding between oligosaccharide side chains of microbial structures to host cells. In experimental animals, elimination of mucus enhances bacterial growth in the small intestine [51]. The release of mucus by goblet cells is effected by bacterial toxins: it is dose-dependent and due to increased glycoprotein synthesis [29, 88]. Microbial proteases within the intestinal lumen

Figure 1: Normal mucosa. Infant. Predominant mucosal pattern is that of ridges (bar 0.1 mm).

Figure 2: Normal mucosa. Child. Short ridges, leaf- and tongue-shaped villi (bar 0.1 mm).

Figure 3: Normal mucosa. 15-year-old. Predominant pattern: finger-like villi (bar 0.1 mm).

Figure 4: Normal mucosa. Villous with well-delineated, slightly convex cells on the tip (mature and senescent cells). The cell outlines and convexities are less noticeable towards the mid- and lower portion of the villus (bar 100 μm).

Figure 5: Normal mucosa. Occasional extrusion of enterocytes on apical portion of villi (bar 10 μm).

Figure 6: Normal mucosa. Normal aspect of brush border, the glycocalyx appears more "dense" as in figure 7 (bar 1 μm).

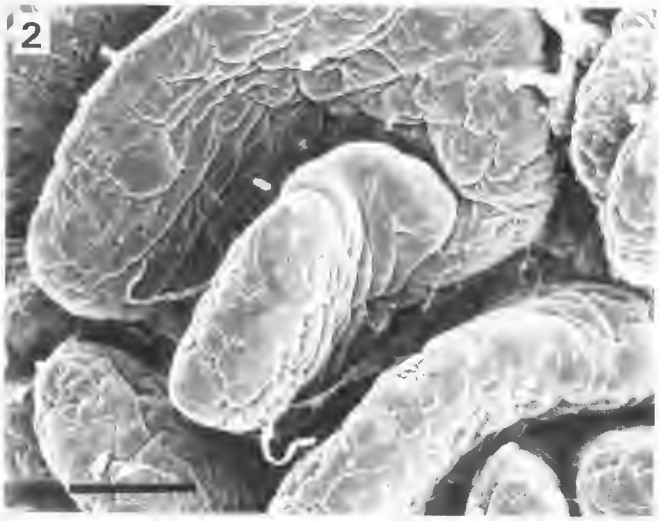
[19, 42, 43, 47, 76, 77] are able to degrade the peripheral oligosaccharide side chains of mucin glycoproteins, causing their destruction or inactivation and further facilitate microbial adherence and colonization on mucosal surfaces. It was of interest, therefore, to observe the presence of microorganisms in the juxtamucosal mucus layer in chronic nonspecific diarrhea [71, 73] (Fig. 14), and the mucoid pseudomembrane in giardiasis [75] (Fig. 15) .

The increased secretion of mucus also follows stimuli other than microbial activity, including interaction of the mucosa with certain plant lectins [30], the presence of antigen-antibody complexes [97], and of hypertonic solutions [100]. The latter condition arises in clinical situations where there is incomplete digestion and absorption of carbohydrates due to decreased availability of disaccharidases.

The increased production of intestinal mucus and its layering over the mucosal surface results in the formation of a physical barrier which should impair digestion and absorption of macro- and micronutrients, as well as of medications. All available evidence obtained through careful examination of biopsied intestinal mucosa by SEM, correlated with laboratory and clinical findings seems to support the association of the following: normal mucosa - normal disaccharidases - carbohydrate intolerance.

2. Increased cell shedding and extrusion of cytoplasm. (Structurally normal mucosa (by LM), clinical intolerance to carbohydrates, depressed disaccharidase activities). During the course of the study by SEM of small intestinal biopsies in children with CNSD, another interesting finding emerged: evidence of increased shedding of enterocytes (Fig. 16) as well as increased extrusion of cytoplasm (Fig. 17) from


1


2


3


4


5

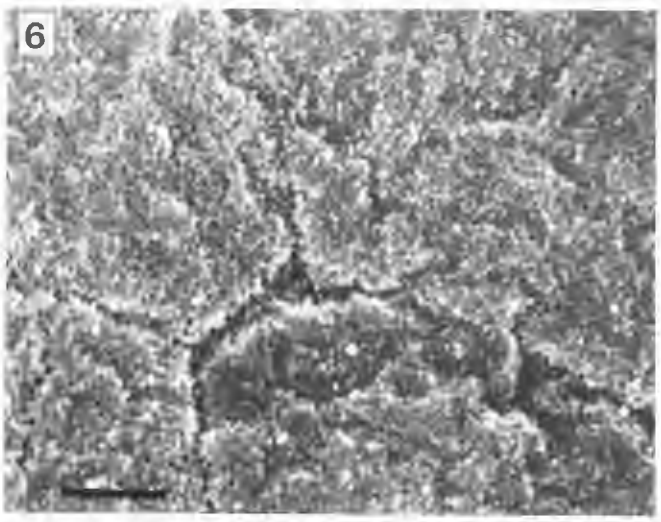

6

Table 2. Carbohydrate Intolerance in CNSD

1.	Normal disaccharidase activities, mucosal structure normal by LM	*Excess mucus on mucosal surface*
2.	Depressed disaccharidase activities, mucosal structure normal by LM	*Increased shedding of enterocytes and cytoplasm; microbial colonization of mucosal surface*
3.	Depressed disaccharidase activities, mucosal structure abnormal by LM	*Partial villous atrophy*
4.	Normal disaccharidase activities, mucosal structure abnormal by LM	*Probable: so-called patchy lesions*

presumably damaged epithelial cells [69, 71]. Cell extrusion could be widespread (Fig. 18) and the term "extrusion zone" [66] is well applicable . Cell extrusion did not occur only at the ridges of villi or their apical region, but also in subapical zones, which is distinctly abnormal.

Of further interest was the fact that light microscopy of biopsies from the same patients did not show changes in the villous architecture [69, 71], i.e., no villous damage, or any degree of villous atrophy. Villi were always of normal length, with only slight focal increases in cells in the lamina propria and perhaps focal edema. Crypts were occasionally hypertrophied with increased mitotic indices.

Despite that, the disaccharidase activities were always markedly depressed [69, 71], explaining the patient's clinically documented intolerance to sucrose and lactose. Several explanations for these findings may be put forward: (1) increased cell shedding and/or cell damage enhances enterocyte turnover by augmenting the production of crypt cells, which migrate with increased speed to the mid and upper portions of the villi. Such cells most likely carry a decreased charge of brush border hydrolases; (2) depression of disaccharidases by the action of dietary lectins [26]; (3) release of brush border hydrolases by pancreatic proteases [79]; (4) damage of brush border hydrolases by microbial proteases (see below); (5) differential glycosylation of disaccharidases [91]; and, (6) increased endocytosis of brush border enzymes.

Figure 7: Normal mucosa. Goblet cell surrounded by normal, mature enterocytes. Microvilli are covered by filigree-like meshwork of glycocalyx. Cell borders well outlined (bar 1 μm).

Figure 8: Normal mucosa. Elevated borders between enterocytes delineate the polygonal outline of cells (bar 100 μm).

Figure 9: 30-months-old child. Chronic nonspecific diarrhea (CNSD). Vast areas of the mucosal surface are covered by sheets of mucus. Normal disaccharidases, but clinical intolerance to sucrose and lactose (bar 0.1 mm).

Figure 10: 26-months-old child. CNSD. Vast areas of the mucosal surface are covered with sheets of mucus; outlines of villous structures not recognizable. Malabsorption of micronutrients, sucrose and lactose (bar 0.1 mm).

Figure 11: 26-months-old child. TEM. CNSD. Goblet cell actively discharging large amounts of mucus, which spreads over the brush border membrane of adjacent enterocytes (bar 10 μm).

Figure 12: Six-months-old infant with cystic fibrosis. Variable amounts of the mucosal surface are covered with what appears to be tenacious mucus (bar 1 μm).

Cell shedding and extrusion of cytoplasm (cytoplasmic blebs) which could be readily appreciated by SEM (Fig. 19), could be a mechanism of self-defense [9]; on the other hand, it could be due to the presence of microorganisms [100], their respective toxins [1, 22, 62] or other antigens [59]. On an experimental basis, increased cell shedding is also produced by axenic resins [17], anionic detergents [33, 39], plant fibers [18], or viruses [21, 95]. The cytoplasmic blebs indicate disintegration, most likely on the basis of injury to the cytoskeleton, which then reduces the cell's ability to maintain not only normal surface morphology and communication with neighboring cells, but also a normal glycocalyx.

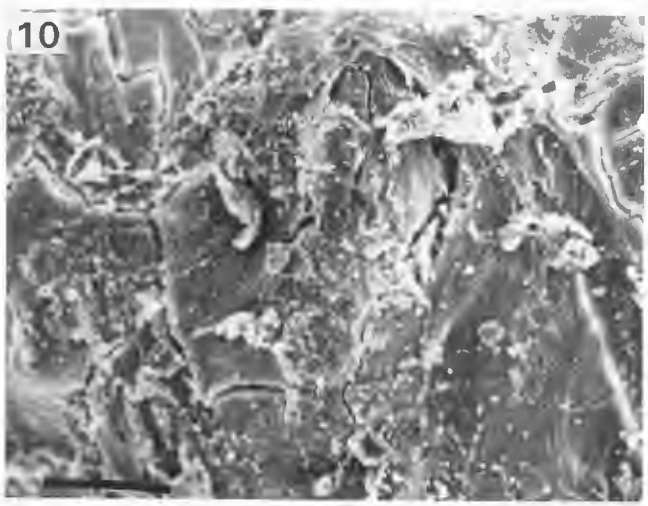
In Table 2, events are summarized which explain the various associations between cell morphology, disaccharidase activities and clinical intolerance to carbohydrates, as seen by SEM.

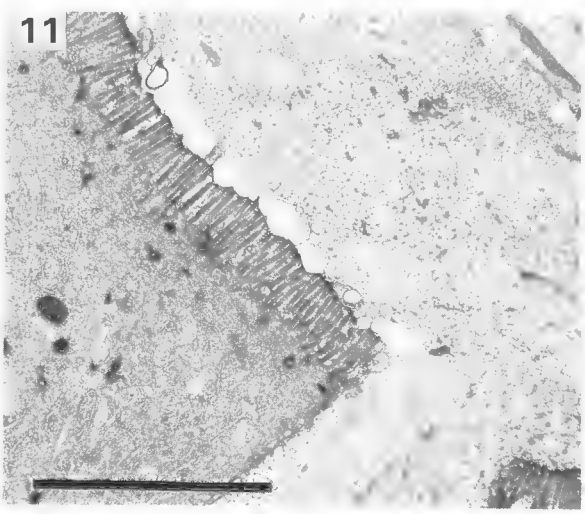
3. Presence of Microorganisms on the Mucosal Surface. Microorganisms (MO) in the small intestinal lumen, either commensal from the oropharynx or those usually present in the more distal portions of the digestive canal, are found in increased numbers in children with various types of chronic diarrhea [reviewed, 69]. With the help of SEM, colonies of MO attached to the mucosal surface have been identified in children with chronic nonspecific diarrhea [69, 73]. MO

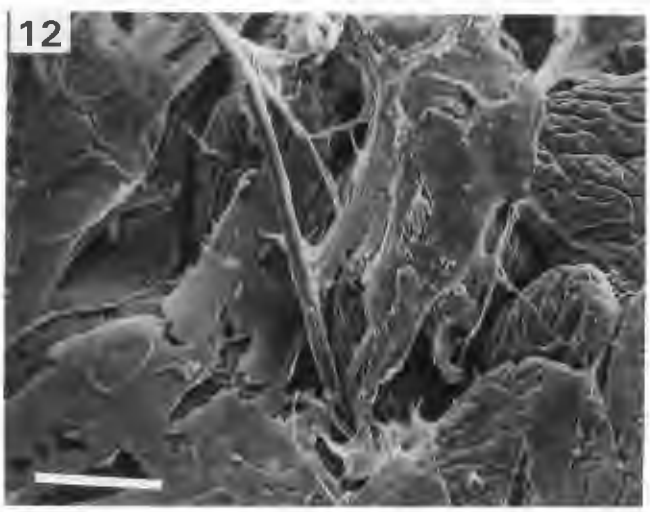

7


8


9


10


11


12

reach the intestinal tract by being swallowed and must avoid destruction by gastric acid. MO may also reach the intestine, as demonstrated by SEM, by attaching to non-keratinized cells of the oropharynx (Fig. 20), and such cells with their "load" stick to the mucosal surface [69]. MO are also seen in a juxta-membranous position (Fig. 21), enmeshed in mucus and in close proximity to the brush border membrane, but not attached to it, where they seem to thrive [69, 73].

The presence of MO could be of pathogenic importance also for CNSD, or the socalled contaminated (irritable?) small bowel [reviewed 69, 73]. Carbohydrate malabsorption is a frequent occurrence in this condition [25, 37], and the presence of undigested carbohydrate provides a stimulus for the proliferation of intraluminal MO: most bacteria indigenous to the human intestinal tract utilize carbohydrates as their primary energy source [19, 82].

Intolerance to carbohydrates as well as various degrees of disaccharidase deficiency is common in CNSD [69, 73]. In experimental animals, microbial proteases damage brush border hydrolases, such as lactase, sucrase and maltase [47, 48, 76], as well as alkaline phosphatase [83]. Damage to the latter enzyme may even be selective [8]. Proteases produced by bacteroides species selectively destroy sucrase [80].

Furthermore, commensal MO, considered nonpathogenic, as well as yeast strains recovered and cultured from duodenal fluid of children with chronic diarrhea, are able to degrade lactase [6]. Thus, there seems to be ample experimental and clinical evidence that microorganisms can induce changes in the activity of brush border hydrolases. This is most likely of importance in CNSD.

SEM was of particular help to identify, in infants and children with CNSD, microorganisms attached to the brush border membrane which morphologically resemble *mollicutes* or *mycoplasma* species (Fig. 21-23). This finding was of great interest, because *mycoplasma* spp. have not yet been identified in the gastrointestinal tract of humans. In general, *mycoplasmas* may appear in several morphologic forms [10, 11, 49, 50]: they may be spherical, or present as coccoid singlets or aggregates. Dependent on environmental conditions, they may elongate and become filamentous and terminate in a bulbous end. When, during replication, the cytoplasmatic division lags behind the division of the genome, coccoid chains are formed, which may branch [78]. Growth cycle, living conditions and environment [49, 78], are important determinants of their morphologic appearance: filamentous or coccoid forms in colonies or chains of variable length.

Despite their suggested presence in the human intestine as evidenced by SEM [73], it was not possible

Figure 13: 13-year-old with cystic fibrosis. Note deposition of sheets of mucus on the surface of intestinal villi. Clinically: malabsorption of macro- and micronutrient (bar 0.1 mm).

Figure 14: Three-year-old child. CNSD. Mixed microbial flora in mucus covering brush border. No attachment of micro-organisms to the mucosa (bar 10 μm).

Figure 15: 17-months-old child with giardiasis. Note pseudomembrane covering large area of mucosal surface. Arrows show impressions of suction discs; many trophozoites were seen in other portions of the biopsy. Child had malnutrition/malabsorption (bar 10 μm).

Figure 16: 19-months-old child. CNSD. Cell extrusion, extruded cell in the center of picture marked by arrow (bar 100 μm).

Figure 17: 22-months-old child. CNSD. Cell extrusion zone, with evidence of cell damage (cytoplasmic blebs) (bar 10 μm).

Figure 18: 26-months-old child. CNSD. So-called cell extrusion zone, with loss of enterocytes and cytoplasm [asterisk] (bar 100 μm).

to culture *mycoplasma* (J. Tully, J.R. Poley, unpublished data). Currently available serologic tests are unreliable for diagnosis, and only investigation of intestinal mucosa by SEM was able to document their presence on the small bowel mucosa of children with CNSD.

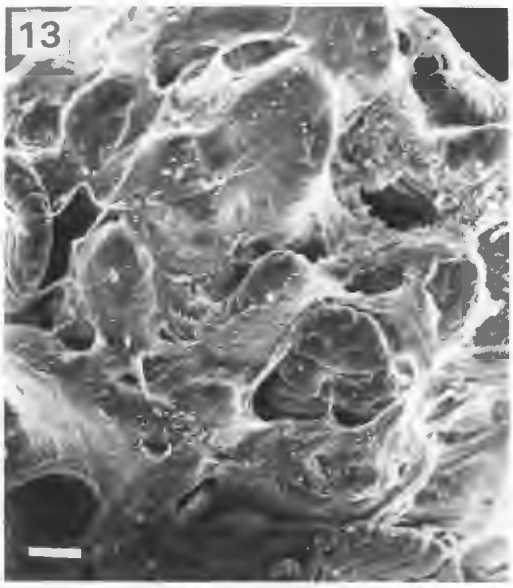
There seems to be a particular group of patients with CNSD who seem at risk for the colonization of small bowel mucosa by *mycoplasma* spp.: most are boys, aged between 15 and 36 months, are blond, with fair skin, a history of recurrent ear and upper respiratory tract infections, and have received multiple antibiotics (J.R. Poley, unpublished observations).

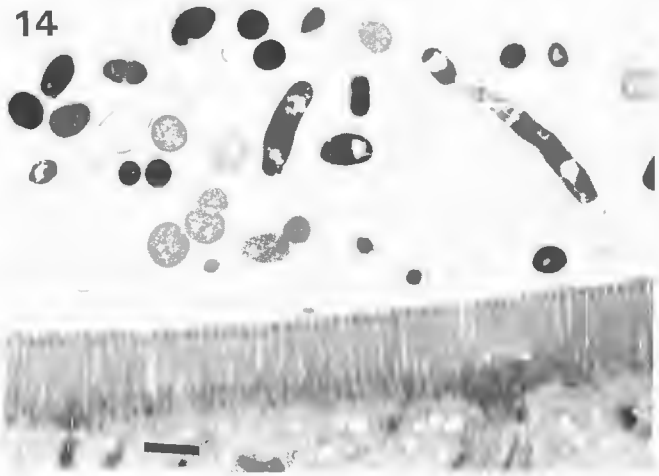
By LM, structural changes of the mucosa are absent except for slight focal cellularity in the lamina propria as well as mild focal edema, changes which are nonspecific.

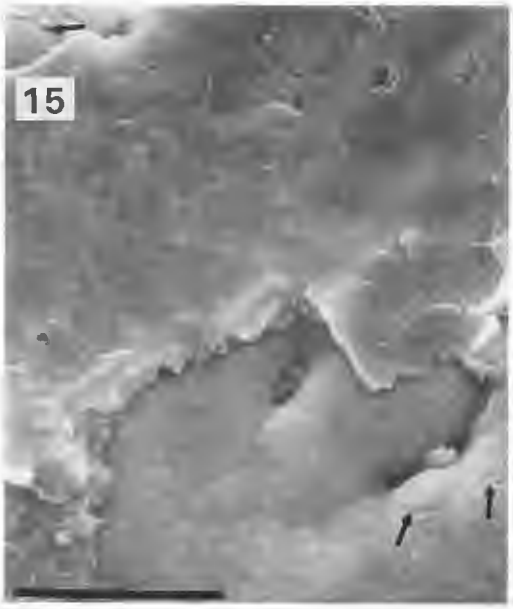
Disaccharidase activities are variably depressed. Attachment of mycoplasma to cell surfaces is always associated with damage to the host cell [65], and damage to brush border enzymes could be mediated through the discharge of the metabolic products of *mycoplasmas*, primarily ammonia and hydrogen peroxide [78].

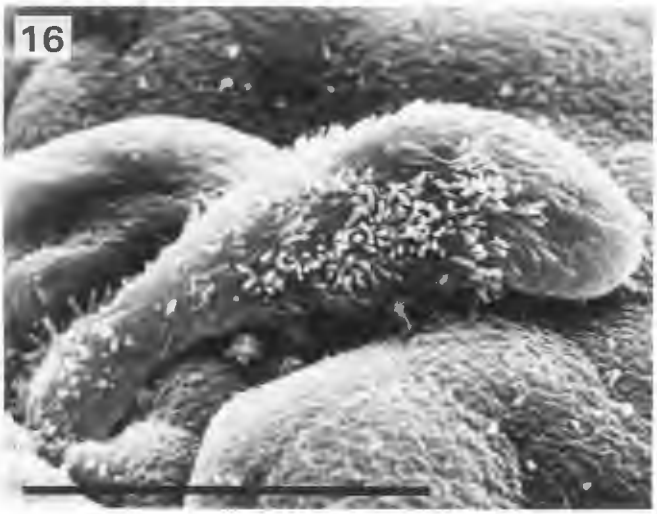
More studies are needed for the identification of *mycoplasma* in human small intestine, as well as for the investigation of their pathogenic potential.

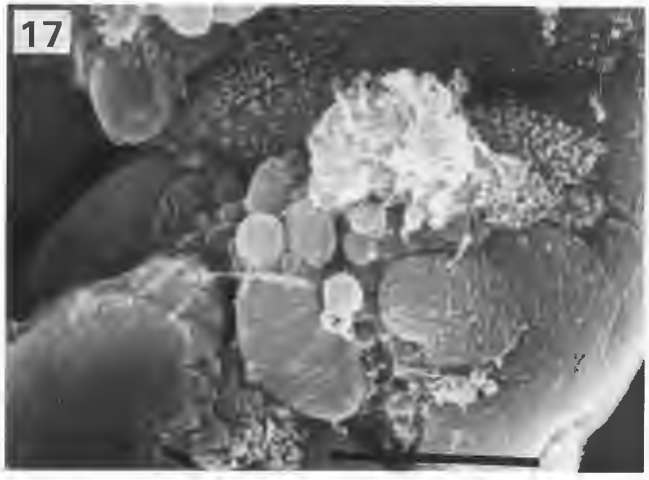
4. Structural Changes. In CNSD of childhood, SEM of biopsied small bowel mucosa could demonstrate

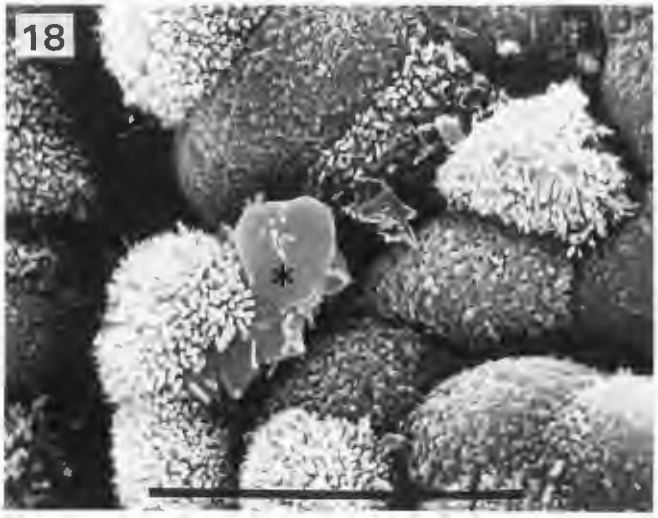

13


14


15


16


17


18

occasional morphologic changes, which are mentioned only briefly, because they are uncommon. There is occasional partial villous atrophy (Fig. 24), or focal villous atrophy (Fig. 25) exposing crypt openings [69, 71, 73]. There may be partial ablation of microvilli with their glycocalyx (Fig. 26) and such changes have been found in the vicinity of *Candida* species [73]. Lastly, clumping of microvilli (Fig. 27) was observed [69], which gives the surface the appearance of "cracked clay", similar to observations in experimental animals after perfusion of the intestine with hydroxy fatty acids [32] . The possibility of an artefact cannot be excluded.

5. Loss of Glycocalyx. Without the help of SEM, this newly described and interesting lesion could probably not have been found [72]. In a series of 10 children with intolerance to a variety of dietary proteins, cow's milk protein in particular, a widespread loss of the glycocalyx was identified, which was seen on all villous structures of the biopsy. Infants with cow's milk protein intolerance (CMPI) often show failure to thrive in addition to chronic diarrhea, and as a morphologic correlate, significant damage to the intestinal mucosa, such as partial or subtotal villous atrophy, has been identified by light [27, 53], and transmission electron microscopy [90].

In the report regarding older infants and children with CMPI and intolerance to other dietary protein [72], the mucosal structure was normal, but the feature of particular interest was the loss of the glycocalyx (Fig. 28, 29). As a corollary, disaccharidase activities were greatly depressed (Fig. 30), which is not astonishing, since disaccharidases and other brush border hydrolases are intimately associated with the glycocalyx or fuzzy coat [46].

The pathogenesis of the loss of the glycocalyx is not understood. The glycocalyx, composed of weakly acid mucopolysaccharides produced by the enterocyte, is an integral component of the cell and is intimately associated with the brush border membrane [45]. In vitro, it cannot be removed by enzymes or detergents, and usually only extruded cells or dead cells lose their glycocalyx. However, in the mucosa of children with food protein intolerance evidence of obvious cell damage is lacking, as seen by TEM. Once the offending dietary protein was removed, the glycocalyx slowly reformed and brush border enzymes returned to normal levels, but the latter process can take weeks or months [72].

The discrepancy between a normal small bowel mucosa in the presence of disaccharidase deficiency and/or abnormal carbohydrate tolerance tests in patients with CMPI and normal histology mentioned by Harrison and Walker-Smith [41] by Iyngkaran et al [44] and by Morin et al [64], seems now explained by the loss of glycocalyx.

Figure 19: 27-months-old child. CNSD, extrusion of cytoplasmic blebs (bar 10 μm).

Figure 20: 33-months-old child. CNSD. Aggregate of sloughed nonkeratinized epithelial cells from the oropharynx with bacteria. Single cells or aggregates stick to the surface of enterocytes (bar 100 μm).

Figure 21: Six-year-old child. CNSD. Micro-organisms resembling *mycoplasma* spp., arranged in beaded chains and short filaments with bulbous ends. Micro-colonies marked by arrows (bar 1 μm).

Figure 22: 36-months-old child. CNSD. Mycoplasma-like micro-organisms on mucosal surface arranged in beaded chain. Few coccoid elements on the surface (arrows) (bar 1 μm).

Figure 23: Three-year-old child. CNSD. Mycoplasma-like micro-organisms arranged in several beaded chains, which also divided (bar 10 μm).

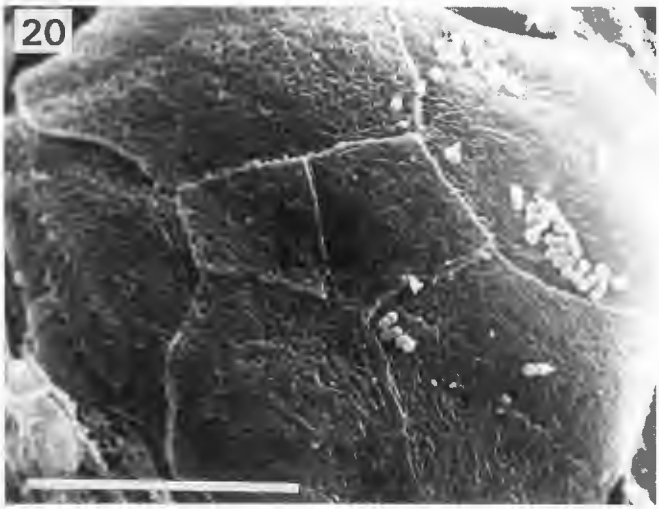
Figure 24: 18-months-old child. CNSD. Villous structures of decreased height, very narrow intervillous spaces - partial villous atrophy (bar 0.1 mm).

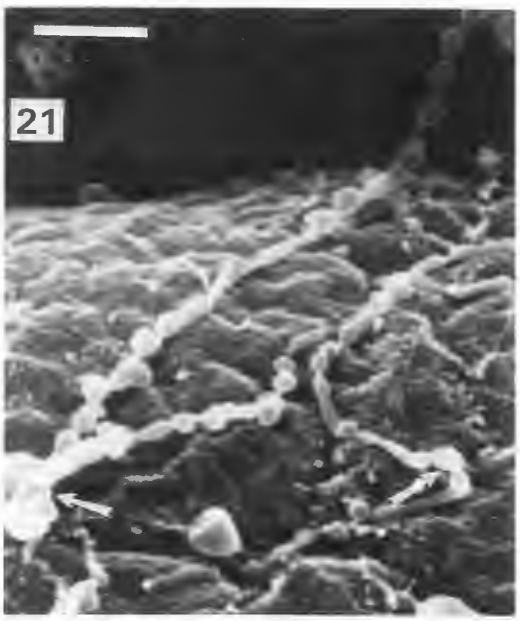
6. Surface Ultrastructure of the "Flat Mucosa". Several conditions associated with chronic diarrhea in children have been identified, common to all of which is a "flat mucosa" as seen by LM. These conditions are celiac disease, severe CMPI, soy protein intolerance (toxicity), and microvillous inclusion disease. Whereas by LM, vague nuances in the appearance of enterocytes may be distinguished between these conditions, along with changes in the lamina propria, SEM is able to define many more ultrastructural details on the mucosal surface which readily help to separate these conditions from one another.

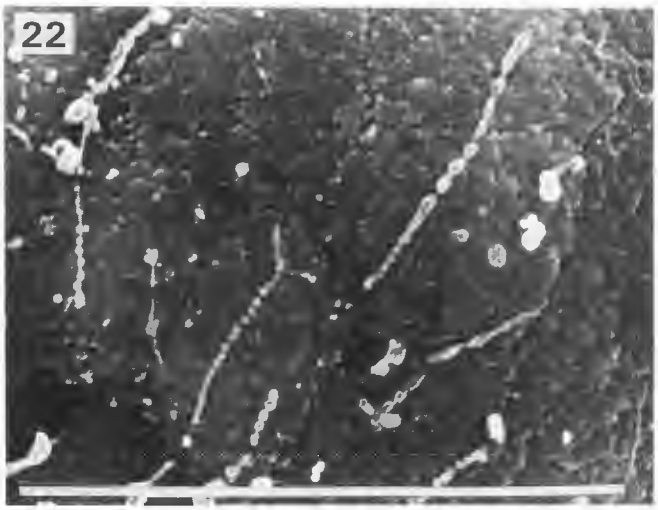
6.1. Celiac disease (gluten-sensitive enteropathy). The classical lesion, as seen by light microscopy, is a "flat mucosa", with crypt hyperplasia and significant infiltration of the lamina propria by immunocytes, and increased presence of intraepithelial lymphocytes. Whereas the intestinal lesion in celiac disease has been extensively studied by TEM [89], accounts of investigations by SEM are few.

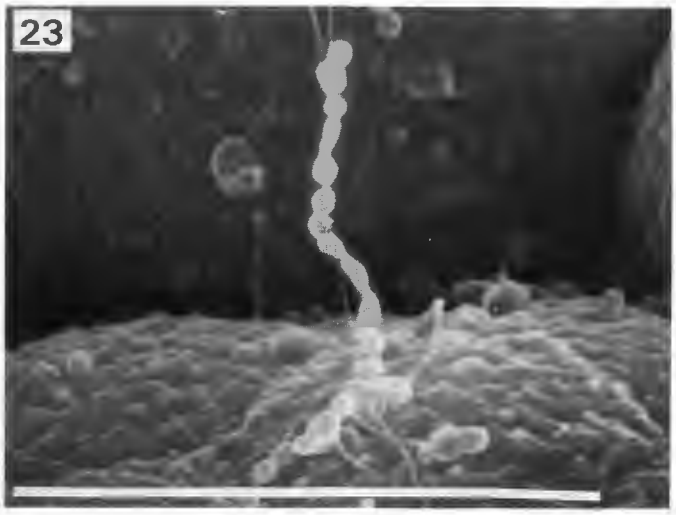
Examination of flat celiac mucosa (not partial villous atrophy) by SEM suggested initially three grades of severity of mucosal damage [70, 79]. However, it now seems apparent that this classification can be extended to four gradations (SEM grade 1 to 4): **SEM grade 1**: broad, convoluted, meandering villous structures of markedly decreased height and with only occasional "crypt wells" [80] (Fig. 31); **SEM grade 2**: a fairly uniform picture of "crypt wells" [89], composed of circular collar-like villous structures, again of markedly decreased height (Fig. 32); **SEM grade 3**:

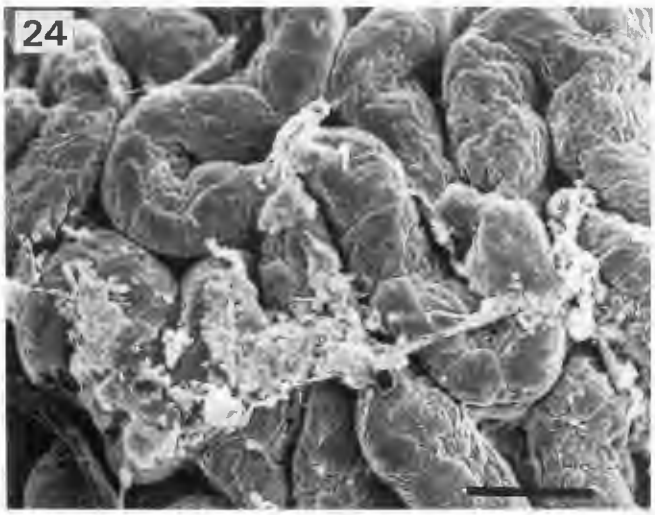

19


20


21


22


23


24

markedly flattened villous structures with rows of enterocytes arranged in circular fashion around crypt openings, which are situated on mound-like elevations (Fig. 33). Enterocytes of all these mucosae are still covered in part with some glycocalyx and this is in opposition to the mucosal injury found in protein intolerance of other cause, where villous structures are preserved (CMPI in infants excepted), whereas the glycocalyx had disappeared [72]; **SEM grade 4**: a totally flat lesion also by SEM, without any recognizable structural arrangements of enterocytes, only crypt openings are seen (Fig. 34). This is also confirmed by TEM.

In addition, and in the SEM grade 2 celiac mucosa, peculiar changes have been identified on the mucosal surface: well circumscribed, punched-out lesions of variable size but of uniform depth, which are due to ablation of the entire brush border membrane (Fig. 35). The etiology and pathogenesis of this lesion is unexplained and it is not known whether it is unique to mucosal damage in celiac disease. Disaccharidase activities are depressed in all four grades of mucosal damage, most notably in grades 3 and 4.

The investigation of celiac mucosa by SEM has attracted the interest of several groups [15, 67, 93], who monitored mucosal rebuilding during treatment with a glutenfree diet: whereas fairly good correlation between SEM and LM was documented by Halter et al [40], and by Stenling et al [93], this was not confirmed by Carpino et al [15]. This reforming of villous structures is an interesting process which occurs at variable speed [15, 67, 93] whereby velocity of regrowth seems independent of the severity of the initial lesion. Factors, such as strict adherence to a gluten-free diet or lack thereof and other yet unknown events, are probably responsible. Furthermore, like with most conditions producing small intestinal mucosal disease, there exist also in celiac disease "patchy lesions" [87], which make interpretation difficult not only in regard to the extent and severity of the initial lesion, but also with reference to the dynamics of villous regrowth.

It is not known whether the different degrees of mucosal injury in celiac disease, as identified by SEM, represent the individual vulnerability of the mucosa, the duration and/or intensity, or both of gluten exposure, or exposure to various genetic brands of wheat. It seems fairly certain that genetic determinants are important in toxicity-type interactions between host mucosa and grain lectins, believed to play a role in the pathogenesis of this disease [5, 52, 98].

6.2. Soy Protein Intolerance. Infants with intolerance (toxicity?) to soy protein may have a flat mucosa by LM [3, 74] and failure to thrive is the consequence. The extent and variability of the mucosal lesions are better demonstrated by SEM than by LM [74]: it seems that, the younger the infant, the more vulnerable the mucosa, and the more extensive the ultrastructural changes (Fig. 36). Older infants, although still with a severe lesion, may have more patchy changes, even within a given biopsy specimen: remnants of villous ridges alternate with ultrastructurally flat areas and visible crypt openings [74] (Fig. 37). Changes of the surface ultrastructure in soy protein intolerance differ from those seen in celiac disease or in severe CMPI. At a younger age, the villous structures are not recognizable as much as there is significant damage to the enterocytes. Later, the glycocalyx thins out, but does not disappear. Following the withdrawal of the soy protein, the villous structures re-grow within a few weeks [74], probably faster than in celiac disease. However, further studies are needed to delineate more fully the process of recovery.

6.3. Intolerance to Cow's Milk Protein (CMP). Intolerance to CMP in infants has been associated with a flat mucosa by LM [27, 53], and with ultrastructural changes as seen by TEM [90]. By SEM, the infantile small bowel mucosa does not appear as severely damaged as in soy protein intolerance or in celiac disease, although the height of the villous ridges is depressed to a significant extent [73] . The remnant villous structures are crowded together and there is little intervillous space (Fig. 38). The enterocytes on the surface show "crowding", giving the villi a corn-cob-like appearance. "Cell crowding" is probably due to a loss of

Figure 25: 16-months-old child. CNSD. Partial villous atrophy with crypt wells (bar 1 μm).

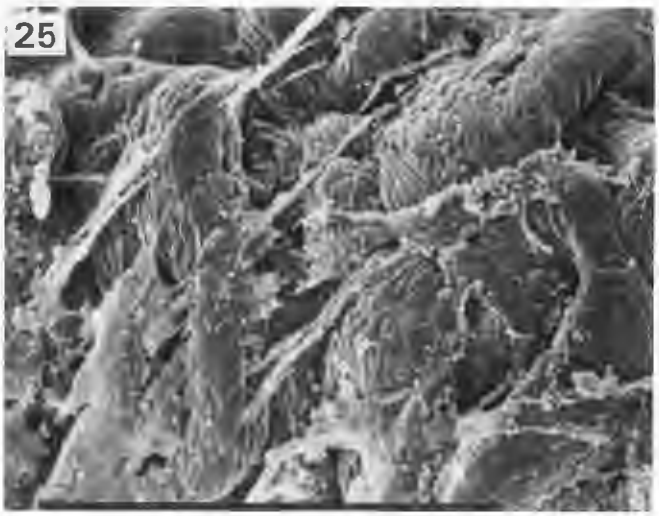
Figure 26: 29-months-old child. CNSD. Presence of *Candida* with partial ablation of brush border membrane. Frequent ear infections, frequent antibiotic therapy (bar 1 μm).

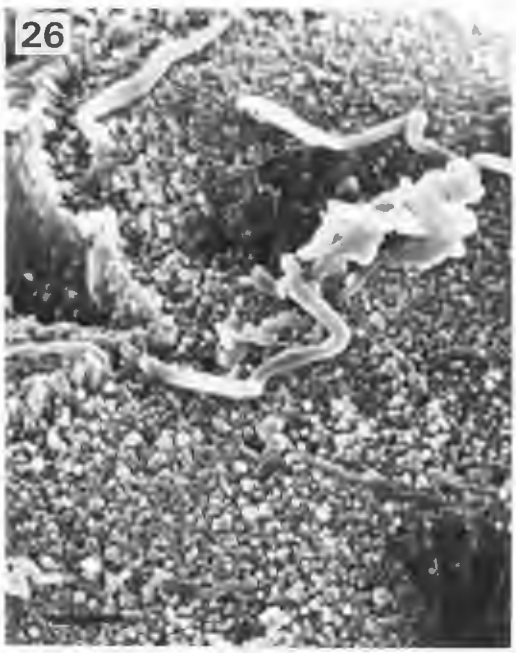
Figure 27: 20-months-old child. CNSD. Clumping of microvilli, which involved large areas of the surface (bar 1 μm).

Figure 28: 17-months-old child. Food protein intolerance (cow's milk). Complete loss of glycocalyx making tips of microvilli well visible. Center: goblet cell (bar 1 μm).

Figure 29: 15-months-old child. Food protein intolerance (cow's milk). Loss of glycocalyx: tips of microvilli are well visible. Cell borders are indistinct (bar 1 μm).

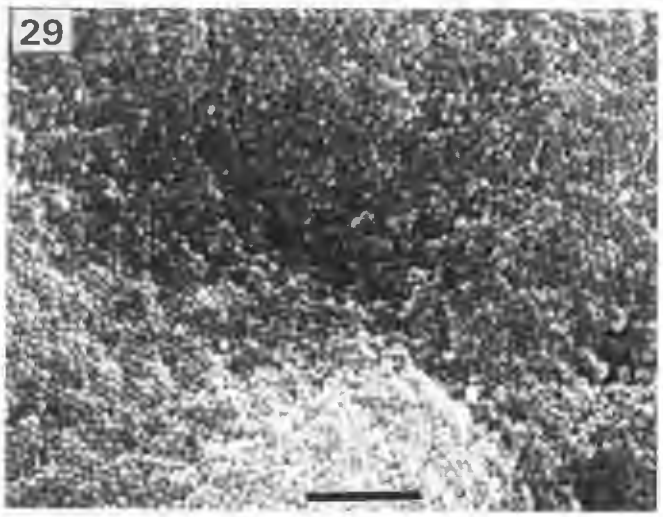
Figure 30: Disaccharidase activities in 10 patients with intolerance to dietary protein, particularly cow's milk protein. Two patients were re-biopsied after omission of offending dietary protein.

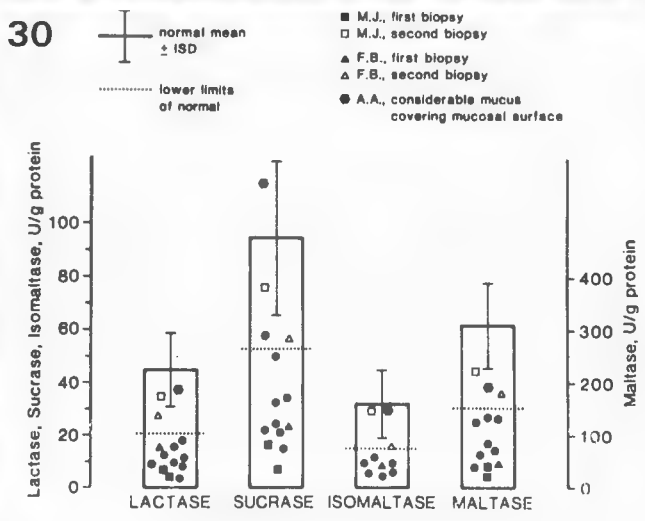

25


26


27


28


29


30
normal mean
± ISD
lower limits
of normal
M.J., first biopsy
M.J., second biopsy
F.B., first biopsy
F.B., second biopsy
A.A., considerable mucus
covering mucosal surface
Lactase, Sucrase, Isomaltase, U/g protein
Maltase, U/g protein
0
20
40
60
80
100
0
100
200
300
400
LACTASE
SUCRASE
ISOMALTASE
MALTASE

matrix or cytoplasm, or due to changes in the cytoskeleton. Widespread loss of the glycocalyx is seen as well, distinguishing this lesion from the flat mucosa of other cause.

6.4. Microvillus Inclusion Disease. This rare condition is characterized by secretory diarrhea which develops in early life [23, 24]. By LM there is a flat mucosa with hypoplastic crypts and in the apical portion of enterocytes, PAS-positive material may be seen, which is not associated with macrophages. Characteristic and diagnostic findings are seen by TEM [23, 68, 73], microvillous inclusions in particular, which may be found in subapical regions, in the space between basolateral membranes of adjacent cells, as well as in the cell interior (Fig. 39, 40). In the cell interior, the microvillus inclusions may be associated with lysosomal structures [23, 73].

By SEM, the appearance of the surface architecture of the villous remnants is quite unique and unlike that seen in any other entity associated with a flat mucosa (Fig. 41-44). The villous ridges are stunted and show a marked cellular disarray. Many enterocytes appear "bald", i.e., without any or with only a very scarce crop of microvilli. Occasional cells still have decent microvilli, however these are stubby and short. About 85% of all enterocytes bearing microvilli lack a glycocalyx.

The natural history of microvillous inclusion disease indicates that most patients do not survive, since the process of mucosal disease seems to be progressive and later on (J.R. Poley, unpublished data), the mucosa consists only of basal membrane with interspersed, nest-like remnants of glandular structures (Fig. 45) . Analysis of structural mucosal proteins in microvillous inclusion disease is quite different from that in health [16, 23]. This finding, as well as the secretory diarrhea, suggest that the underlying pathology in this condition may be failure of enterocyte differentiation, i.e., the differentiation from a secretory (crypt) to absorbing (villous) epithelium.

7. Isolated Disaccharidase Deficiencies. Isolated lactase and sucrase-isomaltase deficiencies are inherited disorders of carbohydrate digestion, characterized by a marked decrease of the respective brush border enzyme level in the presence of a normal histology by LM. Whereas in isolated lactase deficiency, other disaccharidases are normal, maltase and gluco-amylase activities are depressed to a variable extent in sucrase-isomaltase deficiency. Preliminary data on structural topography in these disorders have been published [73].

7.1. Isolated Lactase Deficiency. The mucosal pattern as seen by SEM is normal; however, there is more mucus on the mucosal surface (Fig. 46), as compared with the situation in health, and this may be related to events discussed earlier, i.e., the increased secretion of mucus in presence of hypertonic solutions, such as undigested lactose. Individual cells on the villous surface have a normal aspect and arrangement and the cell borders are easily distinguishable. The appearance of the brush border and glycocalyx is normal. Locally, there may be increased extrusion of cells and cytoplasm, which could also be due to the osmotic effects of undigested lactose.

7.2. Sucrase-Isomaltase Deficiency. By SEM, villous architecture appears normal, yet fairly large areas (more so than in isolated lactase deficiency) of the mucosal surface are covered by mucus (Fig. 47). The reasons for the markedly increased presence of mucus on the surface are not clear, but may relate to similar phenomena of increased mucus secretion seen in some patients with CNSD and sucrose intolerance. Furthermore, the hypothesis is advanced that the muco-tractive effect of undigested sucrose is more pronounced than that of lactose. The brush border and glycocalyx appear more "compact" than controls, or with isolated lactase deficiency. However, this finding could also be a variation of normal. On rare occasions microorganisms resembling filamentous forms of *mycoplasma* may be seen attached to the mucosal surface. The latter finding has no influence on the pathogenesis or expression of this enzyme deficiency.

8. Rotavirus Enteritis. Rotavirus, of which several serologic types are known [34], is the most common cause of acute gastroenteritis in infants and children worldwide. It may cause transient lactase deficiency and/or malabsorption of those hexoses requiring active

Figure 31: 13-months-old child. Gluten-sensitive enteropathy (GSE). GSE grade I. Markedly depressed villous structures, arranged in linear fashion. Occasional crypt well (not shown). Some mucus covering the surface (bar 0.1 mm).

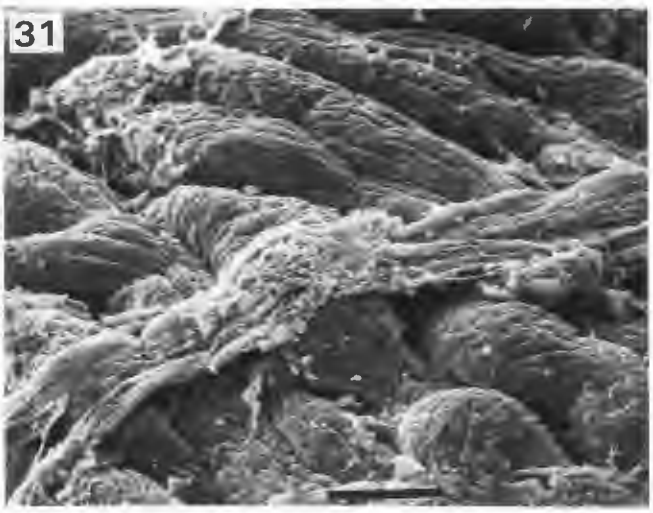
Figure 32: 17-months-old child. GSE. Mucosal atrophy with predominant crypt wells (SEM Grade II) (bar 0.1 mm).

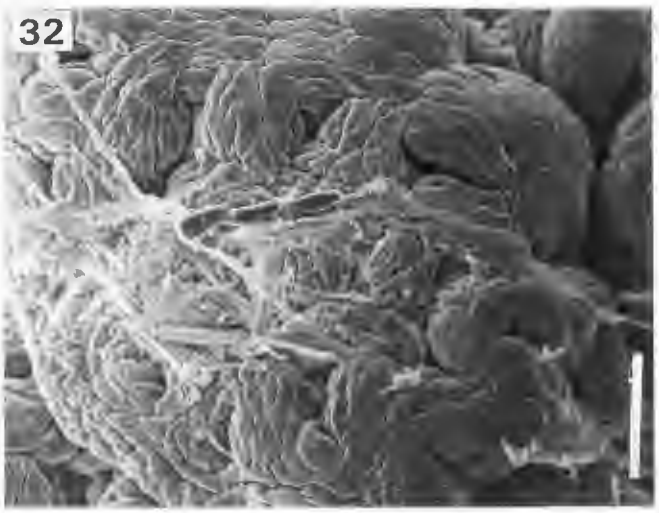
Figure 33: 17-months-old child. GSE (SEM Grade III). Flat mucosa. Circular arrangement of enterocytes around crypt openings, which appear mound-like (bar 0.1 mm).

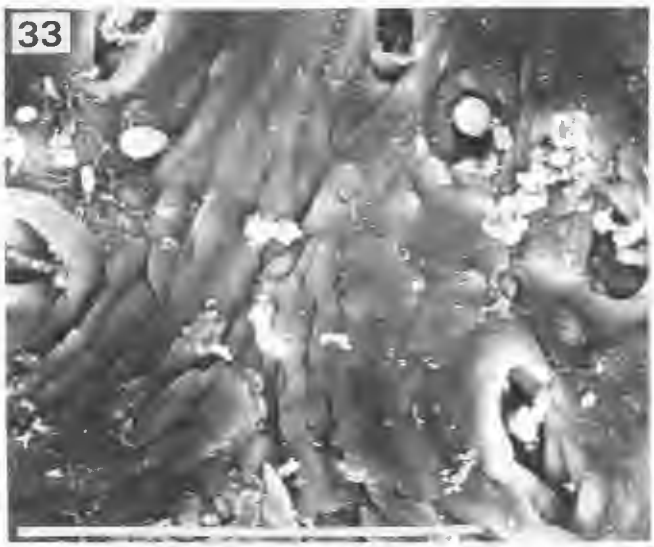
Figure 34: 20-months-old child. GSE. (SEM Grade IV). Visible are crypt openings (bar 0.1 mm).

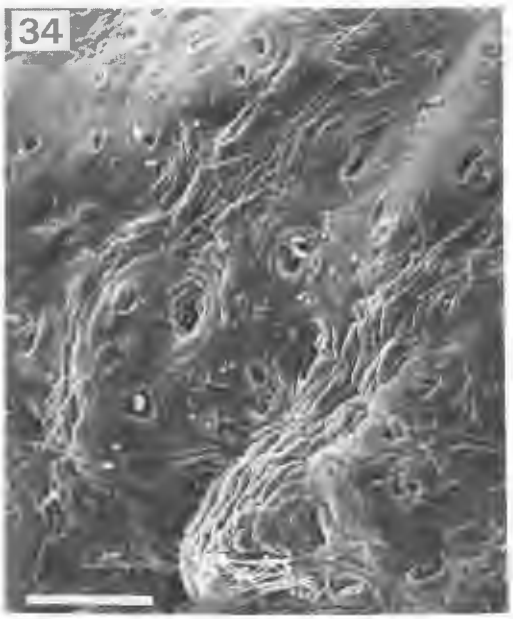
Figure 35: 10-year-old child. GSE (GSE grade II). Focal ablation of brush border membrane, exposing plasma membrane of enterocytes (bar 1 μm).

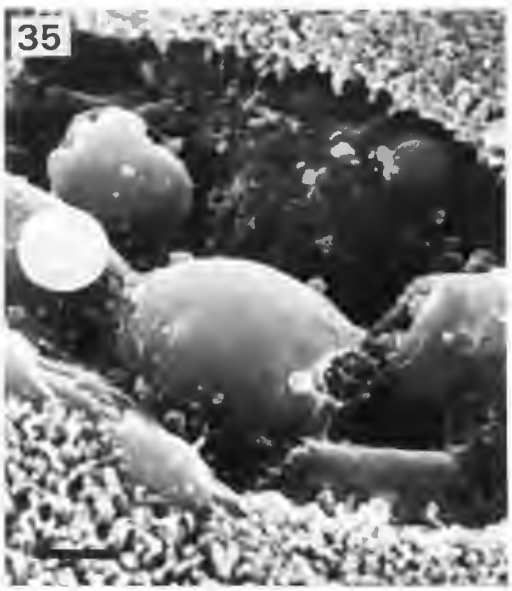
Figure 36: Three-months-old infant with intolerance to soy protein, malabsorption, failure to thrive and villous atrophy (bar 0.1 mm).

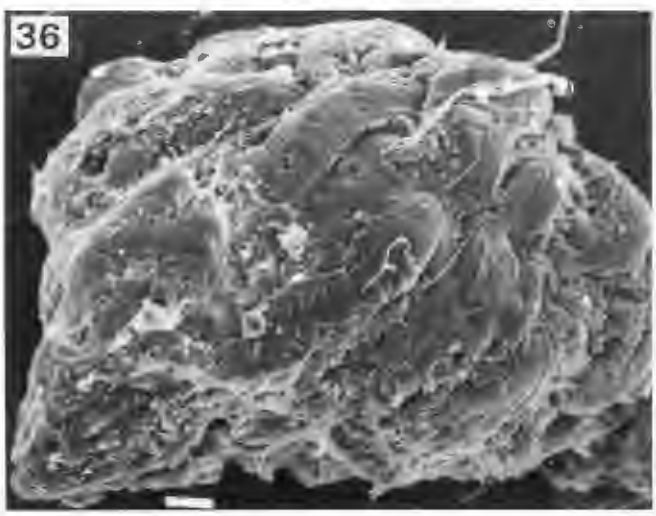

31


32


33


34


35


36

transport for their absorption, i.e., glucose and galactose.

As in experimental studies in piglets [95], there is depression of villous height, akin to flat mucosa, which is readily appreciated by SEM (Fig. 48, 49). There is also significant deposition of mucus on the mucosal surface. In areas not covered by mucus, cell outlines are irregular and enterocytes are of variable size, appearing "crowded" together, probably due to loss of cell plasma. The variable size of enterocytes also suggests disturbances of the integrity of the cytoskeleton (Fig. 50).

Borders of individual enterocytes are well outlined, and there is focal loss of glycocalyx and fraying of microvilli. In analogy to studies in piglets [95], and in suckling mice [21], the abnormal appearing enterocytes are probably infected cells and may not function properly, particularly in regard to carbohydrate digestion and absorption. Enterocytes covered by mucus do not permit identification of surface details. Resolution of infection restores villous architecture to normal.

As seen by SEM, infection of the small bowel with rotavirus causes more widespread structural damage as well as damage to the surface of enterocytes. Although possibly patchy in distribution, the ultrastructural changes may explain the problem with carbohydrate digestion and absorption, clinically manifest as the "post-viral enteritis syndrome".

9. Protracted Diarrhea of Infancy. Protracted diarrhea of infancy may have a well-defined etiology [54], yet in about 1/4 of infants, no specific diagnosis is obtained. Failure to thrive is common and the diarrhea may not be amenable to conventional therapy. Treatment with total parenteral nutrition is often life-saving.

The appearance of the mucosa by SEM suggests partial villous atrophy and the surface is invariably covered with mucus, suggesting increased secretory activity. In addition, there may be focal villous atrophy with appearance of crypt openings (Fig. 51). The volume of enterocytes seems to be reduced, with poor cell-to-cell contact, making cell borders irregular. Numerous microvillar blebs indicate degeneration of microvilli. On the mucosal surface, microorganisms may be seen either as small coccoid chains or as filamentous forms, bearing morphologic resemblance to *mycoplasma* species, but remaining otherwise unidentified.

Resolution of the pathologic process leads to mucosal recovery with reappearance of normal morphology and glycocalyx. The examination of the intestinal mucosa by SEM in protracted diarrhea of infancy does not provide clues as to the cause of this disorder; however, colonization with certain microorganisms, not seen by light microscopy, may be a consequence of mucosal damage and such damage could be further enhanced by the action/presence of such microorganisms.

Figure 37: Infant with intolerance to soy protein. "Micropatchy" lesion with marked mucosal atrophy on the right side, and moderate mucosal damage on the left (bar 0.1 mm).

Figure 38: Six-months-old infant with milk protein intolerance. Villous atrophy with cell crowding, "corn-cob" appearance (bar 0.1 mm).

Figure 39: Nine-months-old infant with microvillus inclusion disease. Microvillus inclusion body in center of cell (arrow) associated with lysosomal structure. Immature secretory granules towards lumen. Short microvilli (bar 10 μm).

Figure 40: Microvillus inclusion disease. Microvillus inclusions in apical portions of mucosal cells (bar 10 μm).

Figure 41: Microvillus inclusion disease. Villous ridges of decreased height, "hobnail"-like surface because of varying size of cells and loss of cell matrix (bar 0.1 mm).

Figure 42: Microvillus inclusion disease. "Enterocytes" without microvilli (bar 1 μm).

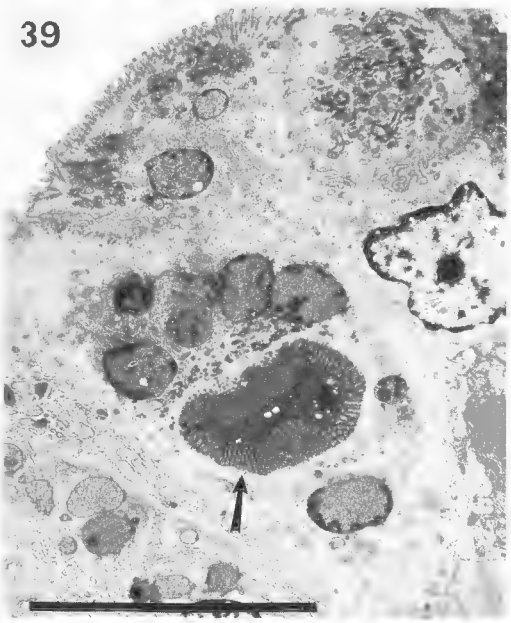
10. Chylomicron Retention Disease. This is a recently described disorder, where chylomicrons are formed within the intestinal mucosa, but cannot be released from the cell into the lymphatics [81]. By SEM, chylomicrons of variable size may be seen in the cell interior (Fig. 52) or on the mucosal surface, but they could be extruded with or without cytoplasm. The increased presence of chylomicrons in the cell interior is confirmed by TEM (Fig. 54). Presence of chylomicrons on the cell surface may not only suggest chylomicron retention disease, but could also be seen in intestinal lymphangiectasis.

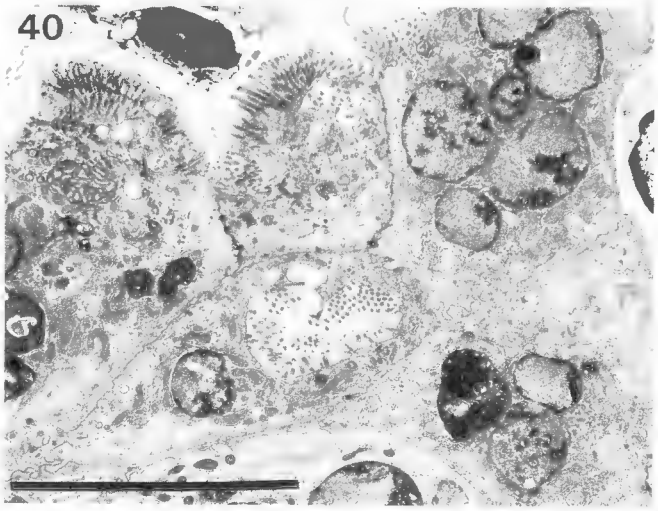
11. Gastric Metaplasia of the Small Intestine. Gastric metaplasia or gastric heterotopia of the small intestine has been described in celiac disease of adults [96], and has also been seen in children with the same disorder (J.R. Poley, unpublished observation). Gastric heterotopia has also been seen in necrotizing enterocolitis [28], in Crohn's disease [56] and in peptic ulcer disease [58]. Conversely, intestinal metaplasia of the gastric mucosa has also been observed in peptic disorders [55]. The etiology of gastric heterotopia is unclear, but is probably a nonspecific response of the intestinal mucosa to damage by a variety of noxious stimuli.

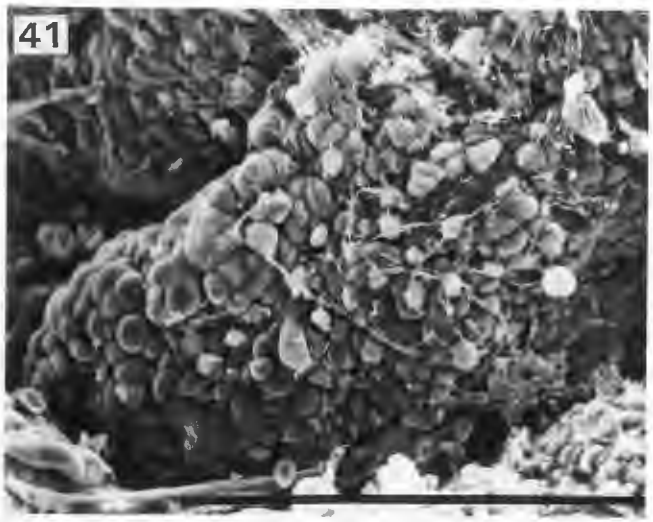
By SEM, the intestinal mucosa with gastric heterotopia may appear in two different aspects. The first is a flat mucosa with gastric cells on the surface,


37


38


39


40


41


42

and its appearance is similar to that of gastric mucosa in a Meckel's diverticulum (Fig. 55). The flat mucosa and gastric glands are also easily identified by LM. The second, villous form, of gastric heterotopia is more difficult to detect by LM, because there are "intestinal" villi, covered with enterocyte-like cells, but their surface details escape detection. It is particularly here that SEM is helpful in identifying changes on the cell surface, i.e., gastric-type cells (Fig. 56, 57). It is not known whether the villous form of gastric heterotopia is a forerunner to the more "classic form", where villi have disappeared or are much decreased in height.

12. Visceral Myopathy. Visceral myopathy is rare congenital disorder which is characterized by the clinical picture of chronic idiopathic pseudo-obstruction of the entire intestinal tract, often associated with similar anomalies of the urinary collecting system [85, 86]. The enteropathy is a myopathic disorder caused by degeneration of the smooth muscle. The ganglion cells and neuronal system seem to be intact morphologically.
Since these children cannot be fed, they have to be sustained by total parenteral nutritional administered via a central venous line. The intestinal mucosal pathology is of interest, since one is looking at an "unfed" (no enteral nutrition) gut.

Studies by SEM show that the villous structures are short, plump and show very little surface relief or other characteristics (Fig. 58, 59). In particular, the cell borders are not visible and one encounters a decreased number of goblet cells. The glycocalyx is very ill-defined.

13. Villous Asthenia. There are occasional patients presenting with chronic diarrhea, malabsorption and poor weight gain, where light microscopy fails to give a clue as to the pathophysiology of this disorder. By contrast and by SEM, the mucosal ridges appear very narrow, thin and frail (asthenic), but are of normal height, the intervillous space is increased and per square unit of measurement, less mucosal surface is available for absorption (Fig. 60). Morphologically, the surface architecture of the enterocytes is intact, and the glycocalyx appears normal as well. It is postulated, but has to be proven, that in some children with diarrhea and failure to thrive, villous asthenia or simply a reduced mass of mucosa (cause unknown) may be responsible for the symptoms.

Summary

In reviewing the application of SEM to routine use in pathology and biomedical research, Carr and Toner (1) have made two important statements: first, "SEM has provided the role of teacher and illustrator, as well as diagnostic pathologist, technologist and biologic scientist". Secondly, they have at last corrected the

Figure 43: Microvillus inclusion disease. Surface of enterocytes with an abnormal appearance. Note cells with absent microvilli, marked by asterisks (bar 1 μm).

Figure 44: Microvillus inclusion disease. Several cells have microvilli, some even with a glycocalyx (arrow). Not excessively long rootlets of some microvilli (arrowheads). "Tubular" material stains PAS positive [asterisk] (bar 1 μm).

Figure 45: Microvillus inclusion disease. Progressive mucosal destruction. Villous structures consist merely of basement membrane (asterisk), surrounding cellular aggregates in crypt-like arrangement identified by arrows (bar 0.1 mm).

Figure 46: 11-year-old child with isolated lactase deficiency. Normally structured mucosa; however, there is some mucus on the surface (effect of undigested lactose ?) (bar 10 μm).

Figure 47: Four-year-old child with sucrose-isomaltase deficiency. As in some cases of chronic nonspecific diarrhea, increased mucus on mucosal surface. Undigested sucrose has some mucotractive effect (bar 0.1 mm).

Figure 48: 21-months-old child with rotavirus enteritis. Villous atrophy, with visible crypt openings (bar 10 μm).

---

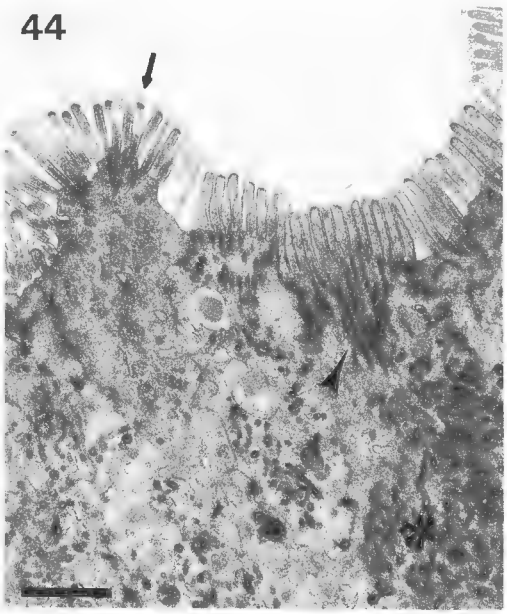
the widespread view that SEM was only giving "images of lunar landscapes which evoke an impression of unreality and expensive irrelevance".

Much beyond providing "pretty pictures", SEM has definitely come of age and has been eminently useful when applied **routinely** in the study of small bowel mucosa. It is likely that further studies on the ultrastructural topography of various other human tissues may be somewhat limited for some time, because of the limited availability of facilities with the appropriate hardware, and because of the relative scarcity of experienced scanning electron microscopists. More cooperative efforts between clinicians and basic scientists should further the cause for the routine use of SEM in diagnostic small intestinal pathology.

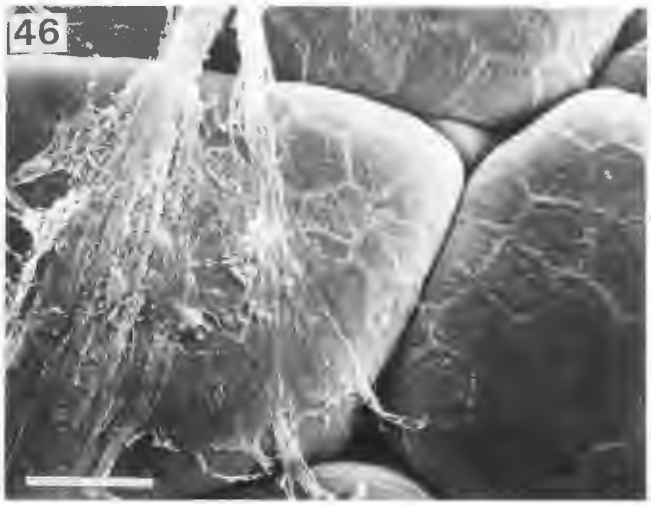
SEM is vastly superior to LM and the dissecting microscope in the examination of surface and structural details of biopsied small intestinal mucosa. SEM has identified hitherto unknown and/or unsuspected changes involving structure and surface architecture of enterocytes and has also been useful as a diagnostic tool.

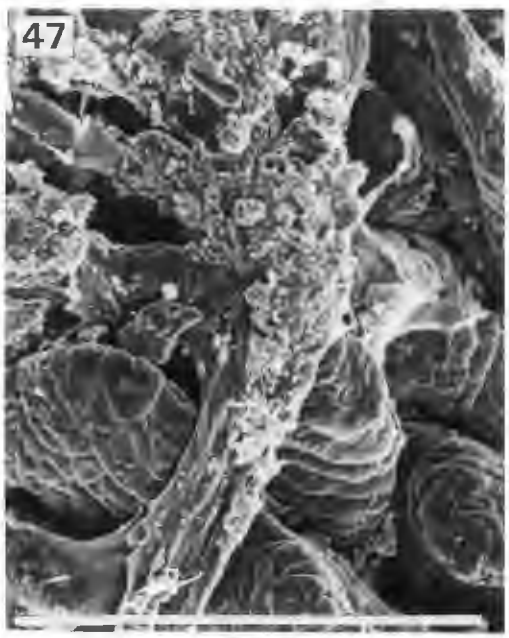
In any work with SEM for the study of biologic specimens, and in small bowel in particular, it is important to combine work by SEM, with LM, TEM, brush border enzymes and other techniques to avoid pitfalls in interpretation and to distinguish pathologic


43


44


45


46


47


48

changes from artifacts.

What about the future of SEM and intestinal mucosal pathology? New scanning electron microscopes are equipped with additional features, such as x-ray microanalysis and back-scattered electron imaging with computer-enhanced image processing. If properly used in relevant and appropriate studies, SEM seems to have a bright and almost unlimited future for the study of the pathology of the gastrointestinal tract and of other organ systems.

Acknowledgements

This work was supported in part by NIH grant RR-09028-03 and by the Chairman's Fund, Department of Pediatrics. The skillful technical assistance of Ms. Anna Shore and of Mr. J. H. Slusser, Jr., the typing of the manuscript by Ms. Lilian Orlina, and by Ms. Frances L. Patterson is gratefully acknowledged. Thanks go to Mr. Anthony W. Walker, Jr., for the determination of disaccharidase activities.

Figures 1, 3, 8, 14, 15, 17, 18, 22, 26, 28, 30, 34, 36, 41, 46, 50, 52, 53 and 54 and Table 1 are reproduced by permission (J.R. Poley: Scanning Electron Microscopy and Small Bowel Pathology in Children with Chronic Diarrhea and Malabsorption: a Review and Presentation of New Data. (In: Textbook of Gastroenterology and Nutrition in Infancy. E. Lebenthal, Ed. Raven Press Ltd., 1989. pp 1207-1253.)

References

1. Abrams GD, Allo M, Rifkin GD, Fekety R, Silva J Jr (1980) Mucosal damage mediated by clostridial toxin in experimental clindamycin-associated colitis. Gut **21**:493-499.

2. Ackert JE (1942) Natural resistance to helminthic infections. J. Parasitol **28**:1-24.

3. Ament ME, Rubin CE (1972) Soy protein - another cause of the flat intestinal lesion. Gastroenterology **62**:227-234.

4. Asquith P, Johnson AG, Cooke T (1970) Scanning electron microscopy of normal and celiac jejunal mucosa. Amer J Dig Dis **15**:511-521.

5. Auricchio S, DeRitis G, DeVincenzi, Mancini E, Minetti M, Sapora O, Silano V (1984) Agglutinating activity of gliadin-derived peptides from *bread* wheat: implications for coeliac disease pathogenesis. Biochem Biophys Res Comm **121**:428-433.

6. Bampoe V, Sapsford RJ, Avigad S, Shiner M (1979) Lactose degradation by human enteric bacteria. Lancet **2**:125-127.

7. Barondes SH (1981) Lectins: their multiple endogenous cellular functions. Annu Rev Biochem **50**:207-231.

8. Batt RM, McLean L (1987) Comparison of the biochemical changes in the jejunal mucosa of dogs with aerobic and anaerobic bacterial overgrowth. Gastroenterology **93**:986-993.

9. Beachey EH (1981) Bacterial mediating the attachment of bacteria to mucosal surfaces. J Infec Dis **142**:325-345.

10. Biberfeld G, Biberfeld P (1970) Ultrastructural features of *Mycoplasma pneumoniae*. J Bacteriol **102**:855-861.

11. Boatman ES, Kenny GE (1971) Morphology and ultrastructure of *Mycoplasma pneumoniae* spherules. J Bacteriol **106**:1005-1015.

12. Bonvicini F, Zoli G, Maltarello MC, Bianchi D, Pasquinelli G, Versura P, Gasbarrini G, Laschi R (1985) Clinical applications of scanning electron microscopy in gastrointestinal diseases. Scanning Electron Microsc 1985; III:1279-1294.

13. Carr KE, Toner PG (1981) Scanning electron microscopy in biomedical research and routine pathology. J Microsc **123**:147-159.

14. Carr KE, Chung P, McClay ALC, Toner PG, Wong AL (1981) The role of scanning electron microscopy in diagnostic pathology. Diagn Histopathol **4**:237-244.

15. Carpino F, Ceccamea A, Magliocca FM, Familiari G, Lombardi ME, Bonamico M (1985) Scanning electron microscopy of jejunal biopsies in

Figure 49: 13-year-old child with rotavirus enteritis. Marked depression of villous structures with focal atrophy, visible crypt wells and crypt openings (bar 0.1 mm).

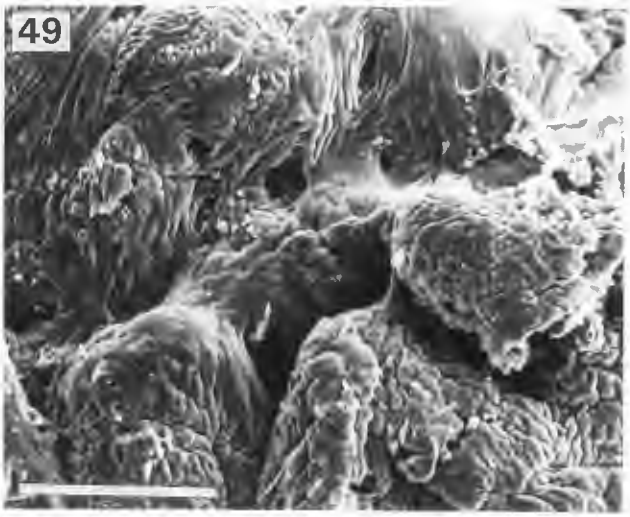
Figure 50: 13-year-old child with rotavirus enteritis with marked changes of enterocytes: unequal size and frayed microvilli, suggesting damage to the cytoskeleton (bar 10 μm).

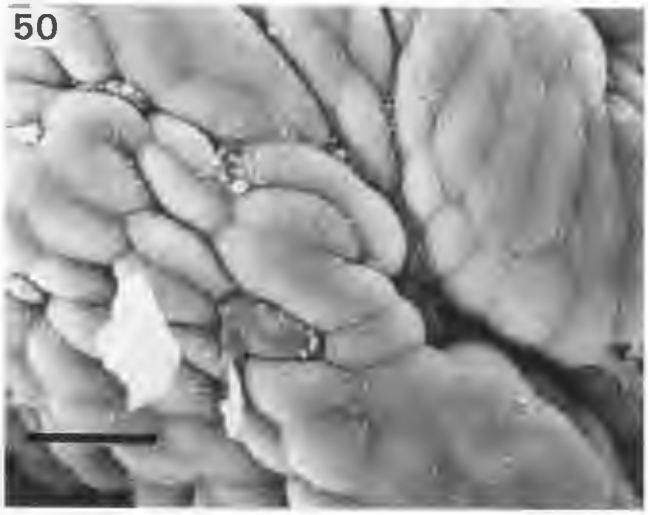
Figure 51: Four-months-old child with protracted diarrhea of infancy. Focal partial villous atrophy, with visible crypt openings (bar 0.1 mm).

Figure 52: Three-and-a-half-year-old child. Chylomicron retention disease. Fracture of portion of the villous shows individual enterocytes with retained chylomicrons, appearing as round spheres of variable size. Asterisk: cell surface (bar 10 μm).

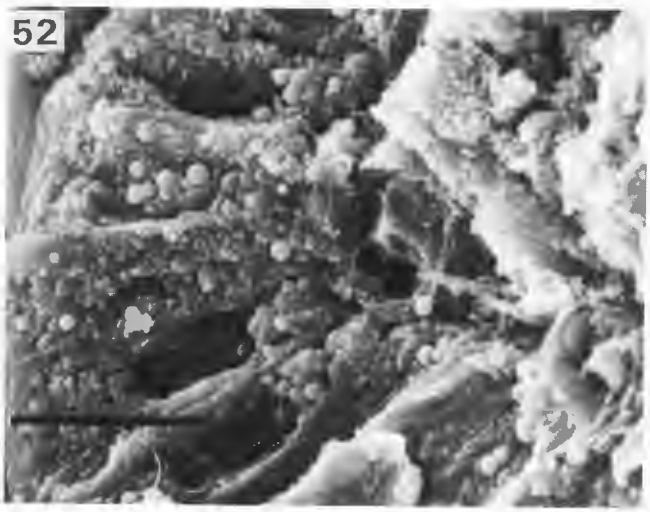
Figure 53: Chylomicron retention disease. Next to extruded cell, two small spheres representing chylomicrons (bar 10 μm).

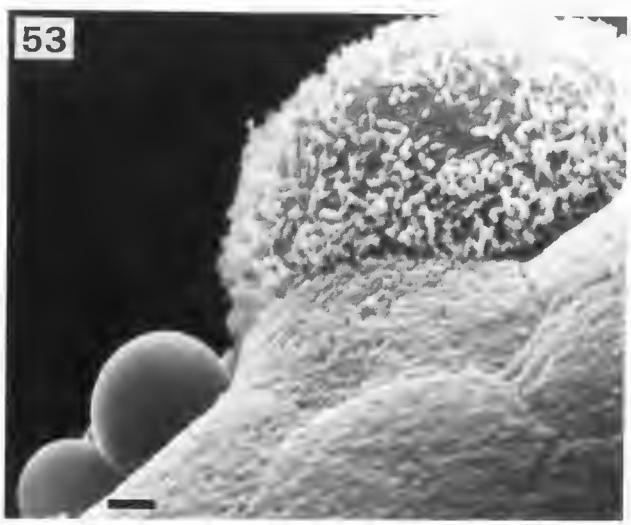
Figure 54: Chylomicron retention disease. Enterocytes filled with small and large chylomicrons (TEM) (bar 10 μm).

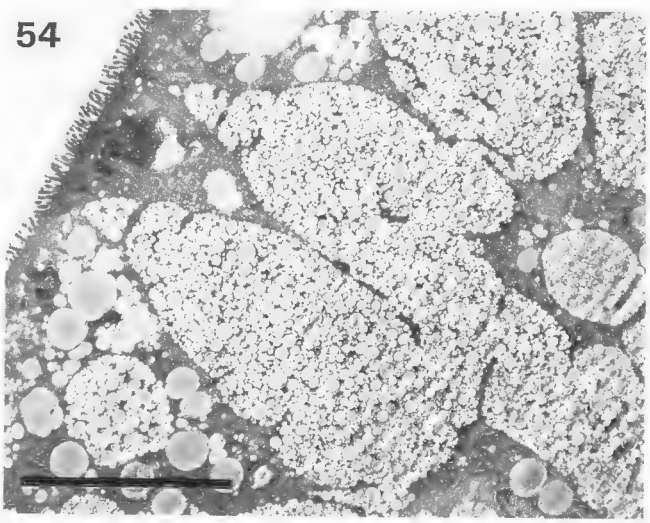

49


50


51


52


53


54

patients with treated and untreated coeliac disease. Acta Paediatr Scand **74**:775-781.

16. Carruthers L, Phillips AD, Dourmashkin R, Walker-Smith JA (1985) Biochemical abnormality in brush border membrane protein of a patient with congenital microvillous atrophy. J Pediatr Gastroenterol Nutr **4**:902-907.

17. Cassidy MM, Lightfoot FG, Grau LE, Roy T, Story JA, Kritchevsky D, Vahouny GV (1980) Effect of bile salt-binding resins on the morphology of rat jejunum and colon. A scanning electron microscopic study. Dig Dis Sci **25**:504-512.

18. Cassidy MM, Lightfoot FG, Grau LE, Story JA, Kritchevsky D, Vahouny GV (1981) Effect of chronic intake of dietary fibers on the ultrastructural topography of rat jejunum and colon: a scanning electron microscopic study. Amer J Clin Nutr **34**:218-228.

19. Clamp JR, Allen A, Gibbons RA, Roberts GP (1978) Chemical aspects of mucus. Br Med Bull **34**:25-41.

20. Clausen CR, Christie DL (1982) Chronic diarrhea in infants caused by adherent enteropathogenic Escherichia coli. J Pediatr **100**:358-361.

21. Coelho KIR, Bryden AS, Hall C, Flewett JH (1981) Animal model. Pathology of rotavirus infection in suckling mice: a study by conventional histology, immunofluorescence, ultrathin sections, and scanning electron microscopy. Ultrastruct Pathol **2**:59-80.

22. Creamer B (1967) The turnover of the epithelium of the small intestine. Br Med Bull **23**:226-230.

23. Cutz E, Rhoads JM, Drumm B, Sherman PM, Durie PR, Forstner GG (1989) Microvillus inclusion disease: an inherited entiation. New Engl J Med **320**:646-651.

24. Davidson GP, Cutz E, Hamilton JR, Gall DG (1978) Familial enteropathy: a syndrome of protracted diarrhea from birth, failure to thrive, and hypoplastic villous atrophy. Gastroenterology **75**:783-790.

25. Davidson GP, Robb TA, Kirubakaran CP (1984) Bacterial contamination of the small intestine as an important cause of chronic diarrhea and abdominal pain: diagnosis by breath hydrogen test. Pediatrics **74**:229-235.

26. Erickson RH, Kim J, Sleisenger MH, Kim YS (1985) Effect of lectins on the activity of brush border membrane-bound enzymes of rat small intestine. J Pediat Gastroenterol Nutr **4**:989-991.

27. Fontaine JL, Navarro J (1975) Small intestine biopsy in cow's milk protein allergy in infancy. Arch Dis Child **50**:357362.

28. Ford WDA, Phillips GE, Davidson GP, Douglas BS (1986) Neonatal necrotizing enterocolitis: a cause of gastric metaplasia in the mid-ileum. Aust NZ J Surg **56**:449-453.

29. Forstner JF, Roomi MW, Fahim REF, Forstner GG (1981) Cholera toxin stimulates secretion of immunoreactive intestinal mucin. Amer J. Physiol **240**:G10-G16.

30. Freed DLJ, Buckley CH (1978) Mucotractive effect of lectin. Lancet **1**:585-586.

31. Frick LP, Ackert JE (1949) Further studies on duodenal mucus as a factor in age resistance of chickens to parasitism. J. Parasitol **34**:192-206.

32. Gaginella TS, Lewis JC, Phillips SF (1977) Rabbit ileal mucosa exposed to fatty acids, bile acids and other secretagogues. Amer J Dig Dis **22**:782-790.

33. George KJ, Specht W, Nell G, Rummel W, Schulz L (1982) Effects of deoxycholate on the perfused rat colon. Scanning and transmission electron microscopic study of the morphological alterations occurring during the secretagogue action of deoxycholate. Digestion **25**:145-154.

34. Gerna G, Passarini N, Sarasini A, Battaglia M (1985) Characterization of xenotypes of human rotavirus strains by solid-phase immune electron microscopy. J Infec Dis **152**:1143-1151.

35. Giorgi PL, Catassi C, Sbarbati A, Cinti S (1986) Ultrastructural findings in the jejunal mucosa of children with IgA deficiency. J. Pediat Gastroenterol Nutr **5**:892-901.

36. Goldsworthy NE, Florey H (1930) Some properties of mucus with special reference to its antibacterial functions. Br J Exp Pathol **11**:192-208.

Figure 55: "Flat-type" gastric heterotopia in a 12-year-old patient with Gluten-sensitive enteropathy (GSE). Flat mucosal surface with pit-like openings (bar 0.1 mm).

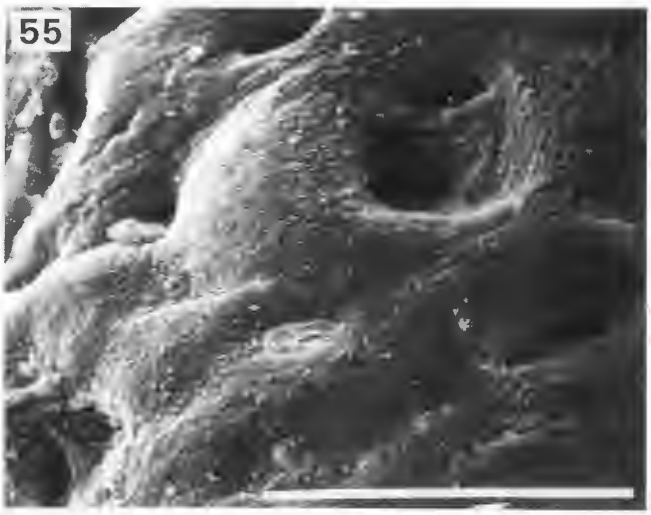
Figure 56: "Villous-type" gastric heterotopia of small intestine in a 32-month-old child with GSE. Note tongue- and leafshaped structures resembling small intestinal villi. Gastric cells, shown in greater detail in figure 57, are marked by arrows (bar 0.1 mm).

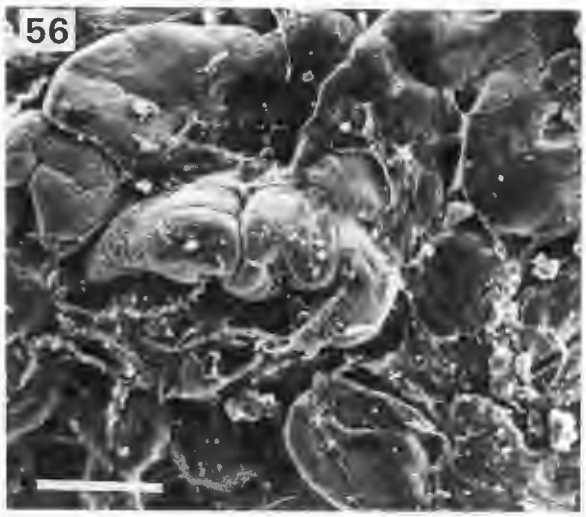
Figure 57: Gastric heterotopia of small intestine in child with GSE (same as in Fig. 56). Gastric type cells with stubby microvilli. Deep cell borders (bar 1 μm).

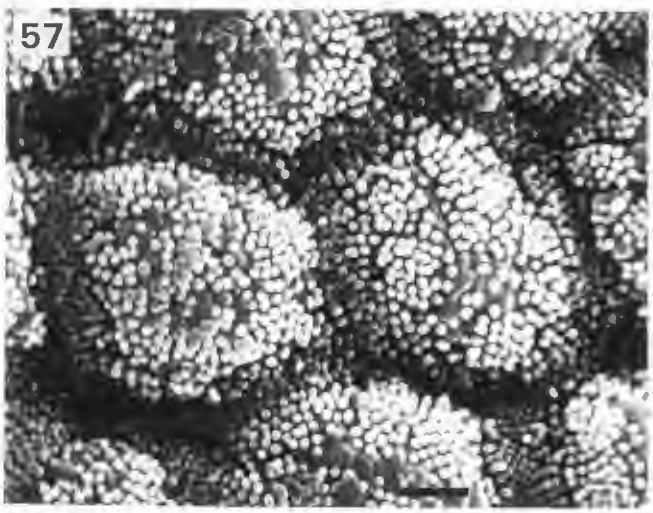
Figure 58: Six-months-old infant with visceral myopathy. Mucosal surface with stubby villous structures, which are poorly differentiated (bar 0.1 mm).

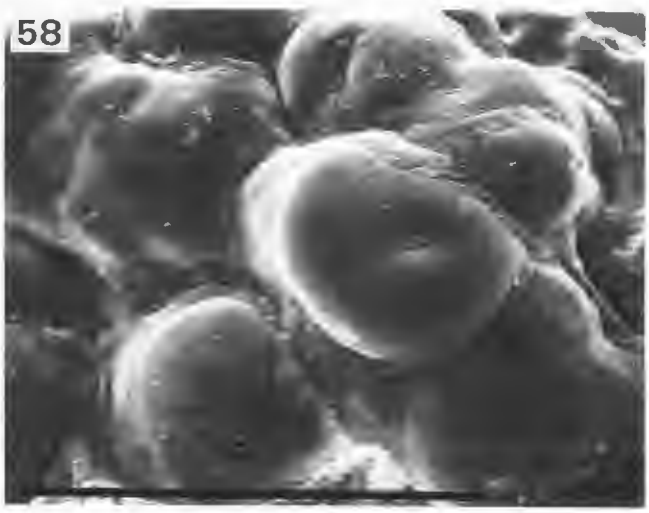
Figure 59: Visceral myopathy. Mucosal surface without recognizable structure, i.e., glycocalyx. Cell outlines barely visible; goblet cell in center [arrow] (bar 10 μm).

Figure 60: "Villous asthenia." 23-months old child with chronic diarrhea of otherwise unknown cause. Villous ridges are very narrow ("asthenic") and are separated by large intervillous spaces (bar 0.1 mm).

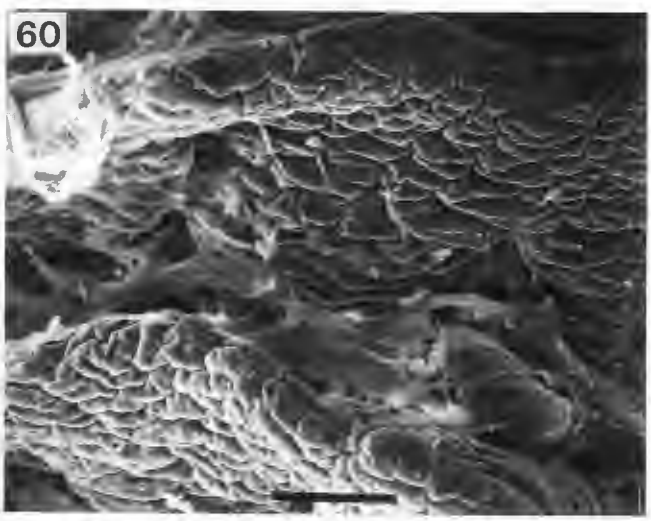

55


56


57


58


59


60

37. Gracey M (1979) The contaminated small bowel syndrome: pathogenesis, diagnosis, and treatment. Amer J Clin Nutr **32**:234-243.

38. Groll A. Candv DCA. Preeze MA, Tanner JM, Harries JT (1980) Short stature as primary manifestation of coeliac disease. Lancet **2**:1097-1099.

39. Gullikson GW, Cline WS, Lorenzsonn V, Benz L, Olsen WA, Bass P (1977) Effects of anionic surfactants on hamster small intestinal membrane. Gastroenterology **73**:501-511.

40. Halter SA, Greene HL, Helinek G (1982) Gluten-sensitive enteropathy: sequence of villous regrowth as viewed by scanning electron microscopy. Hum Pathol **2**:811-818.

41. Harrison M, Walker-Smith JA (1977) Reinvestigation of lactose-intolerant children: lack of correlation between continuing lactose intolerance and small intestinalmorphology, disaccharidase activity and lactose tolerance. Gut **18**:48-52.

42. Hoskins LC, Boulding ET (1981) Mucin degradation in human colon ecosystems. Evidence for the existence and role of bacterial sub-populations producing glycosidases as extracellular enzymes. J Clin Invest **67**:163-172.

43. Hoskins LC, Zamchek N (1968) Bacterial degradation of gastrointestinal mucins. I. Comparison of mucus constituents in the stool of germ-free and conventional rats. Gastroenterology **54**:210-217.

44. Iyngkaran N, Abdin Z, Davis K, Bocy CG, Prathap K, Yadav M, Lam SK, Puthucheary SD (1979) Acquired carbohydrate intolerance and cow milk protein-sensitive enteropathy in young infants. J Pediatr **95**:373-378.

45. Ito S (1969) Structure and function of the glycocalyx. Feder Proc **28**:12-25.

46. Johnson CF (1969) Hamster brush border surface particles and their function. Feder Proc **28**:26-29.

47. Jonas A, Flanagan PR, Forstner GG (1977) Pathogenesis of mucosal injury in the blind loop syndrome. Brush border enzyme activity and glycoprotein degradation. J Clin Invest **60**:1321-1330.

48. Jonas A, Krishnan C, Forstner G (1978) Pathogenesis of mucosal injury in the blind loop syndrome. Release of disaccharidases from brush border membranes by extracts of bacteria obtained from intestinal blind loops in rats. Gastroenterology **75**:791-795.

49. Kammer GM, Pollack JD, Klainer AS (1970) Scanning-beam electron microscopy of *Mycoplasma pneumoniae*. J Bacteriol **104**:499-502.

50. Klainer AS, Pollack JD (1973) Scanning electron microscopy techniques in the study of the surface structure of *mycoplasmas*. Ann NY Acad Sci **225**:236-250.

51. Knehans AN, O'Dell BL (1980) Intestinal microflora in the guinea pig as observed by scanning electron microscopy. Effect of fibrous dietary supplements. J Nutr **110**:1543-1554.

52. Kolberg J, Sollid L (1985) Lectin activity of gluten identified or wheat germ agglutinin. Biochem Biophys Res Comm **13**:867-872.

53. Kuitunen P, Rapola J, Savilahti E, Visakorpi JK (1973) Response of the jejunal mucosa to cow's milk in the malabsorption syndrome with cow's milk protein intolerance. A light and electron microscopic study. Acta Paediatr Scand **62**:585-595.

54. Larcher FV, Shepherd R, Francis DEM, Harries JT (1977) Protracted diarrhoea in infancy. Analysis of 82 cases with particular reference to diagnosis and management. Arch Dis Child **52**:595-605.

55. Laschi R, Pasquinelli G, Versura P (1987) Scanning electron microscopy application in clinical research. Scanning Microsc **1**:1771-1795.

56. Lechago J, Black C, Smaloff IM (1976) Immunofluorescence studies of gastric heterotopia of the small intestine in Crohn's disease. Gastroenterology **70**:429-432.

57. Lee GB, Ogilvie BM (1981) The mucus layer in intestinal nematode infection. In: Ogra PL, Bienenstock J, eds. *The Mucosal Immune System in Health and Disease*. Proc 81st Ross Conf Pediat Res. Columbus, OH, Ross Laboratories, pp. 69-73.

58. Lessels AM, Martin DF (1982) Heterotopic gastric mucosa in the duodenum. J Clin Pathol **35**:591-595.

59. Lipkin M (1973) Proliferation and differentiation of gastrointestinal cells. Physiol Rev **53**:891-915.

60. Manuel PD, Walker-Smith JA, France NE (1979) Patchy enteropathy in childhood. Gut **20**:211-215.

61. Marsh MN (1981) Studies of intestinal lymphoid tissue: the cytology and electron microscopy of gluten-sensitive enteropathy with particular reference to immunopathology. Scand J Gastroenterol **70**(Suppl):87-106.

62. McDonel JL (1979) The molecular mode of action of *Clostridium perfringens* enterotoxin. Amer J Nutr **21**:210-218.

63. Miller HRP, Nawa Y (1979) Immune regulation of intestinal goblet cell differentiation: specific induction of nonspecific protection against helminths. Nouv Rev Fr Hematol **21**:31-45.

64. Morin CL, Weber A, Buts JP, Roy CC, Brochu P (1979) One-hour blood xylose test in diagnosis of cow's milk protein intolerance. Lancet **1**:1102-1104.

65. Muse KE, Powell DA, Coller AM (1976) *Mycoplasma pneumoniae* in hamster tracheal organ

culture studies by scanning electron microscopy. Infec Immunity **13**:229-237.

66. Phillips AD (1981) Small intestinal mucosa in childhood in health and disease. Scand J Gastroenterol Suppl **70**:65-85.

67. Phillips AD, France NE, WalkerSmith JA (1979) The structure of the enterocyte in relation to its position on microscopical study. Histopathology **3**:117-130.

68. Phillips AD, Jenkins P, Raafat F, Walker-Smith JA (1985) Congenital microvillous atrophy: specific diagnostic features. Arch Dis Child **60**:135-140.

69. Poley JR (1983) Chronic nonspecific diarrhea in children: investigation of the surface morphology of small bowel mucosa utilizing the scanning electron microscope. J Pediatr Gastroenterol Nutr **2**:71-94.

70. Poley JR (1983) The small bowel mucosa in disease states characterized by chronic diarrhea: observations by scanning electron microscopy. Scanning Electron Microsc 1983; III:1293-1306.

71. Poley JR (1984) Ultrastructural topography of small bowel mucosa in chronic diarrhea in infants and children: investigations with the scanning electron microscope. In: *Chronic Diarrhea in Children*, E. Lebenthal, ed. Nestle/Vevey-Raven Press/New York. pp. 57-105.

72. Poley JR (1988) Loss of the glycocalyx of enterocytes in the small intestine: a feature detected by scanning electron microscopy in children with gastrointestinal intolerance to dietary protein. J. Pediatr Gastroenterol Nutr **7**:386-394.

73. Poley JR (1989) Scanning electron microscopy and small bowel pathology in children with chronic diarrhea and malabsorption: a review and presentation of new data. In: *Textbook of Pediatric Gastroenterology and Nutrition*, E Lebenthal, ed. 2nd Edition, Raven Press, NY. pp. 1207-1253.

74. Poley JR, Klein AW (1983) Scanning electron microscopy of soy protein induced damage of small bowel mucosa in infants. J Pediatr Gastroenterol Nutr **2**:271-287.

75. Poley JR, Rosenfield S (1982) Malabsorption in giardiasis: presence of a luminal barrier (mucoid pseudomembrane). A scanning and transmission electron microscopic study. J. Pediatr Gastroenterol Nutr **1**:63-80.

76. Prizont R (1981) Glycoprotein degradation in the blind loop syndrome. Identification of glycosidases in jejunal contents. J Clin Invest **67**:336-344.

77. Prizont R, Konigsberg N (1981) Identification of bacterial glycosidases in rat cecal contents. Dig Sid Sci **26**:773-777.

78. Razin S (1981) Mycoplasmas: the smallest pathogenic prokaryotes. Isr J Med Sci **17**:510-515.

79. Riby JE, Kretchmer N (1985) Participation of pancreatic enzymes in the degradation of intestinal sucrase-isomaltase. J Pediatr Gastroenterol Nutr **4**:971--979.

80. Riepe SP, Goldstein J, Alpers DH (1980) Effect of secreted *bacteroides* proteases on human intestinal brush border hydrolases. J Clin Invest **66**:314-322.

81. Roy CC, Levy E, Green PH, Sniderman A, Letarte J, Buts JP, Orquin J, Brochu P, Weber AM, Morin CL (1987) Malabsorption, hypocholesterolemia, and fatfilled enterocytes with increased intestinal apolipoprotein B. Gastroenterology **92**:390-399.

82. Salyers AA (1979) Energy sources of major intestinal fermentative anaerobes. Amer J Clin Nutr **32**:158-163.

83. Savage DC (1972) Associations and physiological interactions of indigenous microorganisms and gastrointestinal epithelia. Amer J Clin Nutr **25**:1372-1379.

84. Schön E, Müller B, Klimmt G, Lorenz A (1984) Bemerkenswerte microbielle Befunde an kindlicher Dünndarmschleimhaut (Interesting bacteriological findings in the small intestinal mucosa of children). Dt Z Verdau Stoffwechselkr **44**:40-54.

85. Schuffler MD (1981) Chronic intestinal pseudo-obstruction syndrome. Med Clin NA **65**:1331-1357.

86. Schuffler MD, Lowe MC, Bill AH (1977) Studies of idiopathic intestinal pseudo-obstruction. I. Hereditary hollow visceral myopathy: clinical and pathological studies. Gastroenterology **73**:327-338.

87. Scott BB, Losowsky MS (1976) Patchiness and duodenal-jejunal variation of the mucosal abnormality in coeliac disease and dermatitis herpetiformis. Gut **17**:984-992.

88. Sheer HP, Mertens RB (1979) Choleratoxin-induced glycoprotein secretion in rabbit small intestine. Gastroenterology 77:18-25.

89. Shiner M (1974) Cell distribution in the jejunal mucosa in coeliac disease. In: *Coeliac Disease*, Proceedings of the Second International Symposium, Hekkens WTJM, Pena AS, Eds. Stenfert Kroese, Leiden. pp. 121-137.

90. Shiner M (1981) Ultrastructural features of allergic manifestations in the small intestine of children. Scand J Gastroenterol **16**(Suppl):49-64.

91. Sjöström H, Norén O, Danielsen EM (1985) Enzymatic activity of "high-mannose" glycosylated forms of intestinal microvillar hydrolases. J Pediatr Gastroenterol Nutr **4**:980-983.

92. Stenling R, Fredrikzon B, Engberg S, Falkmer S (1984) Surface ultrastructure of the small intestine mucosa in children with celiac disease. I. Untreated disease and long-term elimination and challenge. Ultrastruct Pathol **6**:295-305.

93. Stenling R, Fredrikzon B, Nyhlin H, Helander

HF, Falkmer S (1984) Surface ultrastructure of the small intestine mucosa in healthy children and adults: a scanning electron microscopic study with some methodological aspects. Ultrastruct Pathol **6**:131-140.

94. Toner PG, Carr KE (1969) The use of scanning electron microscopy in the study of the intestinal villi. J Pathol **97**:611-617.

95. Torres-Medina A, Underdahl NR (1980) Scanning electron microscopy of intestine of gnotobiotic piglets infected with porcine rotavirus. Can J Comp Med **44**:403-411.

96. Trier JS, Moxey PC, Fordtran JS, McDermott RP (1973) Ectopic gastric mucosa in celiac sprue. Gastroenterology **65**:712727.

97. Walker WA, Abel SN, Wu M (1976) Intestinal uptake of macromolecules. V. Comparison of the *in vitro* uptake by rat small intestine of antigen-antibody complexes prepared in antibody or antigen excess. J Immunol **117**:1028-1032.

98. Weiser MM, Douglas AP (1976) An alternative mechanism for gluten toxicity in coeliac disease. Lancet 1:567-569.

99. Welsh JD, Rohrer GV, Drewry R, May JC, Walker A (1966) Human intestinal disaccharidase activity. II. Diseases of the small intestine and deficiency states. Arch Int med **117**:495-503.

100. Yardley JH, Bayless TM, Luebbers EH, Halsted CH, Hendrix TR (1972) Goblet cell mucus in the small intestine. Findings after net fluid production due to cholera toxin and hypertonic solutions. Johns Hopkins Med J **131**:1-10.

Discussion with Reviewers

P.G. Toner: In section 2, the author suggests that certain changes may be due to "loss of cell plasma" or "disturbances of the integrity of the cytoskeleton". Would he care to justify or expand upon these statements?
Author: The appearance, by SEM, of the changes of enterocytes are made by inference and on the basis of other studies documenting such cell damage (Science 1982; 217: 1257-1259).

P.G. Toner: Where descriptions and illustrations of ultrastructural findings in particular clinical conditions are based on the author's personal observations, would he indicate the numbers of cases examined on which these statements are based?
Author: All conditions (patients) described are based on the author's personal observations. In particular, the conditions and numbers of patients are listed below:

Chronic nonspecific diarrhea	170
Milk protein intolerance	14
Soy protein intolerance	2
Giardiasis	8
Cystic fibrosis	6
Gluten-sensitive enteropathy	12
Isolated lactase deficiency	6
Isolated sucrase-isomaltase defic	2
Microvillus inclusion disease	1
Rotavirus enteritis	3
Protracted diarrhea of infancy	2
Chylomicron retention disease	1
Visceral myopathy	1
Villous asthenia	2

P.D. Millikin: In Figure 4, is it possible that the less prominent cell convexities at the base of the villus are a matter of focus?
Author: Yes. Immature enterocytes in lower portion of the villus are less prominent and have less clearly defined cell borders.

P.D. Millikin: In Figures 6 and 7, how do we know that the glycocalyx is actually obscuring the tips of the microvilli, and that the pattern is not an artefactual change? Is there corroborative evidence from TEM, for example?
Author: There is excellent corroborating evidence by TEM that the tips of the microvilli are indeed covered by glycocalyx or the fuzzy coat. Hence, by SEM and in health, the tips of the microvilli cannot be seen.

P.D. Millikin: Similarly, in Figure 8, although the cell borders are raised, the microvilli appear markedly flattened. What is the evidence that this is not an artefact?
Author: One cannot say that the microvilli are flattened on this view. Unclear is why the cell borders are raised - it is probably a variation of normal, or could be a fixation artefact.

P.D. Millikin: Figures 9, 10, 12, 13 show the mucosal surface covered with mucus. Did you make the usual efforts to remove the mucus and find it resistant, or did you simply leave the mucus in situ to illustrate a point?
Author: No effort was made to remove the mucus, since documentation of its presence was considered important. A thick layer of mucus can be a formidable barrier to absorption.

P.D. Millikin: When the mucosa is normal but there is clinical carbohydrate intolerance, is there any information about the quality of the mucus? Is it chemically abnormal in some way, or is there just

hypersecretion of normal mucus?
Author: In conditions of clinical carbohydrate intolerance, there is little if any information on the chemical composition of mucus. Most likely, however, there is hypersecretion of normal mucus.

P.D. Millikin: In Figures 28-29, is there corroborative evidence that the loss of glycocalyx is real and not an artefact of preparation? TEM evidence, for example?
Author: The loss of the glycocalyx as demonstrated in these figures is most likely not an artefact. Such loss has only been demonstrable in mucosae from patients with intolerance to dietary protein. In over 250 mucosal biopsies from children with other causes of chronic diarrhea, as well as from controls, a loss of glycocalyx was never seen.

P.D. Millikin: In Figure 35, is it possible that the "ablation of the brush border membrane" may actually represent separation between 2 adjacent enterocytes, exposing cells in the underlying lamina propria? Any evidence, one way or the other?
Author: It is believed very unlikely that the phenomenon of "ablation of portions of the brush border membrane" represents separation between 2 adjacent cells. By contrast, the cells remain in close contact with each other which can be verified by TEM.

P.D. Millikin: At the clinical level, how was intolerance to carbohydrates determined?
Author: Clinical intolerance to carbohydrates (lactose, sucrose) was determined by routine carbohydrate tolerance tests.

P.D. Millikin: How was disaccharidase insufficiency determined?
Author: Disaccharidase insufficiency was determined by assay of brush border enzymes (lactase, sucrase, maltase, isomaltase).