Ultrastructural Topography of Small Bowel Mucosa in Chronic Diarrhea in Infants and Children: Investigations with the Scanning Electron Microscope

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The term “ultrastructural topography,” or topographic histology, is relatively new. It has been introduced into the medical literature to mark a new dimension for the study of tissue surfaces by the scanning electron microscope. This tool is now enjoying more widespread application in the investigation of a variety of human tissues affected by pathologic processes (1,2). Although scanning electron microscopy (SEM) is limited to the study of surfaces, its advantages are obvious: it allows surveillance of large surface areas, and it has great depth of focus and a wide range of magnifications, both permitting high resolution images. Surface changes at the ultramicroscopic level can be recognized more precisely and can be described more accurately than would ever have been possible by the dissecting microscope (3) or by surface staining (4). An additional advantage of SEM is the rapid reproduction of the three-dimensional aspect of tissue structures and of individual cells, eliminating cumbersome and time-consuming reconstruction techniques (5). Lastly, the processing time for specimens is relatively short.

Studies of surface details of small bowel mucosa by SEM have been somewhat limited, although the great surface area of this organ system may be one of the principal domains of SEM (2). However, the first investigations of human tissue by SEM were done on small bowel mucosa (6–13). After these early communications, relatively few studies followed, all on the small bowel of adults (14,15). It was not until 1982 that major investigations of small bowel pathology by SEM in children were published (16–19).

This chapter reviews ultrastructural topography of small bowel mucosa in infants and children with several disease states associated with chronic diarrhea: chronic nonspecific diarrhea (CNSD); disorders characterized by marked changes in villous morphology, such as gluten-, cow’s milk protein-, and soy protein-sensitive enteropathies; intractable diarrhea of infancy; and other disorders, such as giardiasis, cystic fibrosis, and Crohn’s disease.

These studies of small bowel mucosa in infants and children with chronic
diarrhea were done primarily to seek new data utilizing SEM on a routine basis, and to complement those findings obtained by light microscopy, assays of disaccharidase activities, and microbiologic studies. Frequently, SEM was supplemented by transmission electron microscopy (TEM) to minimize pitfalls in the interpretation of findings by SEM (20).

PATIENTS

Chronic Nonspecific Diarrhea

A total of 62 patients, between 5 months and 11 years of age, underwent small bowel biopsy during the course of a workup for chronic diarrhea, while diarrhea was still present. The duration of the diarrhea varied from 4 to 20 months. Seven children (infants, young and older children, but not exceeding the age limits of the patient population) served as controls and, at the time of the biopsy, did not have diarrhea. A small bowel biopsy was done in this group of children to rule out sucrase-isomaltase deficiency. Sixty-five percent of the patients were boys. All had at least two stools examined for microbial enteric pathogens, *Giardia* and rotavirus. In addition, *Giardia* were never identified by mucosal imprint smear, light microscopy, or SEM. None of the patients had gluten, cow's milk, or soy protein intolerance, or a recognizable form of an allergic enteropathy. None had protracted diarrhea of infancy with failure to thrive (21–24), or familial diarrheal syndromes (25–28). A more complete account of mucosal ultrastructure in CNSD is given elsewhere (18).

Gluten-Sensitive Enteropathy

Three patients, aged 13, 16, and 28 months, had initial diagnostic biopsies and responded promptly to a gluten-free diet.

Milk Protein–Sensitive Enteropathy

There were two patients, aged 4 and 9 months, who were milk protein–sensitive. The diagnosis of milk protein intolerance was made by at least two positive challenges with cow's milk and by elimination of other causes of chronic diarrhea. Cow's milk (but not lactose) intolerance still persists at the age of 3½ years in one of the children.

Soy Protein–Sensitive Enteropathy

Two infants were studied, aged 5 weeks and 4½ months; the findings have been reported elsewhere in more detail (19). A second biopsy was done 6 weeks after the first one to study mucosal regrowth and regeneration. At the present, these children are 3 and 3½ years old, respectively, and have had no further signs of intolerance to either soy protein or to legumes.
Protracted Diarrhea of Infancy

Observations are described for one infant, 5 months of age, with failure to thrive and chronic diarrhea of 2 months' duration. Formula changes, including Pregestimil® (Mead Johnson & Co., Evansville, IN) did not prevent further deterioration. Small bowel biopsies were done at the ages of 7 and 12 months. The infant finally recovered after total parenteral hyperalimentation for 6 weeks, between 7½ and 9 months of age. No cause for the diarrhea could be identified.

Giardiasis

Four children, aged 15, 24, 26, and 30 months, were biopsied to obtain a diagnosis. Although by history three of them had had a positive exposure to well water, two to three stool examinations were negative for cysts. Two of these patients had an abnormal oral lactose tolerance test and showed evidence of malabsorption and failure to thrive. However, with one exception, the disaccharidase assays were normal, as was mucosal morphology by light microscopy. Two of these patients and their mucosal findings by SEM have already been reported (17). None of the four children had hypogammaglobulinemia.

Cystic Fibrosis

Three patients, aged 4 months, and 13½ and 23 years, underwent small bowel biopsy. The 23-year-old patient and the 13½-year-old patient had considerable problems with nutrition despite optimized pancreatic enzyme replacement. These patients, in particular the 13½-year-old, had evidence of malabsorption, i.e., steatorrhea, low serum iron, zinc, considerably decreased levels of serum vitamins A, E, and dihydroxy vitamin D₃, and essential fatty acid deficiency. The infant received Pregestimil and pancreatic enzyme supplements, and was doing well. The diagnosis of cystic fibrosis was made in all patients by at least three positive sweat chloride tests (29).

Crohn's Disease

Use of SEM to study the ileal mucosa in adults is reviewed and discussed elsewhere (30). Not included in this chapter are patients with isolated lactase and isolated sucrase–isomaltase deficiency.

METHODS

Scanning and Transmission Electron Microscopy

Biopsied mucosa was processed immediately ex vivo, as previously described (17): After immersion in a fixative containing 0.1 M cacodylate buffer, pH 7.4, 2% paraformaldehyde, and 2.5% glutaraldehyde, the specimens were postfixed
with 1% osmium tetroxide, then underwent stepwise dehydration in ethanol gradients. Specimens for SEM were further desiccated by critical point drying and then coated with gold–palladium. After mounting on aluminum stubs, the mucosa was examined with a JOEL Model JSM 35 scanning electron microscope, using an accelerating voltage of 15kV. In all specimens, standard views were obtained at low (54×, 150×),1 intermediate (540×, 1,500×), and high (4,400×, 7800×, 13,000×) magnifications. In addition, views at other levels of magnification were taken to highlight a particular finding.

The specimens for TEM were processed as follows after drying in ethanol gradients (17): The tissue was treated with propylene oxide and Polybed 812. After embedding in fresh resin for 36 hr at 30°C, 14 p.s.i., silver sections were prepared on an LKB Ultratome III and stained with uranyl acetate and lead citrate, and tissue sections were examined with a Philips EM 301 transmission electron microscope.

None of the specimens was rinsed or washed with saline before immersion in the first fixative so as not to distort or to destroy the situation in vivo.

Peroral Suction Biopsy

Mucosal specimens were obtained with a double port Crosby-Kugler capsule under fluoroscopic control and exclusively from the duodeno-jejunal junction, with a success rate of 100%. Written, informed consent had been obtained from the parents and from the 23-year-old patient after it was ascertained that they understood the reasons for and the technique and potential risks of the procedure. There were no biopsy-related incidents or complications.

Microbiology

Duodenal fluid was collected before biopsy whenever possible for routine aerobic and anaerobic microbiology (not quantitative). Because only routine methods were employed, strict anaerobes could not be cultured. A mucosal imprint smear was always prepared for gram and/or Wright and trichrome stains.

Disaccharidase Activities

These were measured on mucosal specimens from all patients by a modification (31,32) of the original method of Dahlqvist (33).

RESULTS AND DISCUSSION

Normal Mucosa

Before reviewing pathologic changes as defined by ultrastructural topography, the findings in the control population will be discussed.

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1 These are original magnifications. Magnifications in legends reflect size change in reproduction.
At low magnification (54X, 150X; Figs. 1 and 2), the prevalent mucosal pattern at the duodeno-jejunal junction in infants and children up to 5 to 6 years of age consisted chiefly of villous ridges of various lengths that were close together, not permitting visualization of crypt openings. Tongue- or finger-like villi became more prevalent with increasing age, but finger-like villi were never the sole mucosal pattern in children (of any age) at the location of biopsy. After 6 or more years of age, the villous pattern approximated that of adults (5-10,12,13,33a). The mucosal pattern was always uniform, i.e., there were no shortened villi or ridges—they were always of uniform height.

At intermediate magnifications (450X, 1,500X, Figs. 3 and 4), the individual cell borders became distinctly visible. As in adults, the confines of individual enterocytes were characterized by hexagonal or pentagonal outlines. Toward the upper third of the villus (ridge), the cells projected with a flat dome–like convexity toward the lumen (the normal appearance of mature enterocytes), whereas this was not present in the middle or lower third of the villous structure. There, the cells appeared flat and their borders impressed by slight elevations, similar to findings reported in adult duodenum (9,12,13). The cell borders on the top of the villi appeared less coherent, perhaps in anticipation of the process of cell shedding. Openings of goblet cells were easily identified. Goblet cells were either empty or in a state of discharging small amounts of mucus, which

![Image of mucosal surface](image.png)

**FIG. 1.** Mucosal surface at the duodeno-jejunal junction (patient's age 3½ years). There are villous ridges of varied length, and occasional tongue- and finger-shaped villi. All villi appear of uniform height. The mucosal surface is free of mucus. (SEM; X57.)
FIG. 2. Villous ridges of varied length and close together. The corrugated folds may be a preparation artefact, but most likely allow for villous expansion during digestion. Crypt openings are not visible. The surface is clear and free of mucus. (SEM; ×150.)

FIG. 3. Portion of a villous ridge. The outlines of the enterocytes are clearly visible, and the cells of the upper third of the villus show a flat dome–like projection toward the lumen. Note occasional small tufts of mucus on the surface. (SEM; ×560.)
was appreciated as small, whipped-cream like tufts. Extrusion of cell cytoplasm or of enterocytes as a process of cell renewal was occasionally seen on the crest of villous ridges or of individual villi, but in health this was never conspicuous, nor was there cell extrusion in subapical regions or at the base of villi (34).

At high magnifications (4,400×, 7,800×, 13,000×; Figs. 5–7), the surface details of enterocytes were clearly visible, as were the cell borders. The tips of the microvilli could not be seen because they were covered by a fine, filigree-like meshwork of the glycocalyx. This meshwork was regular, without condensation, breaks, or other disarray of its architecture. The glycocalyx became more and more indistinct toward the middle and lower third of the villus, where it appeared more homogeneous. Since the lower parts of the villi are populated by cells which are not fully mature, their appearance was somewhat different: the cell surface was flat, the cell borders slightly raised but tight-appearing, and there was evidence of “condensation” of the glycocalyx. The difference in the surface structure between mature and immature enterocytes is mirrored quite well by TEM studies (34). Microorganisms (MO) were never identified on the cell surface.
FIG. 5. Several enterocytes surround a goblet cell, which is not discharging mucus. The flat dome-like projection of the enterocyte surface and the cell borders are clearly visible. Not visible are the tips of microvilli, which are covered by the glycocalyx. (SEM; ×4,600.)

FIG. 6. Better view of the cell surface. Again, tips of microvilli cannot be seen because they are covered by the glycocalyx. (SEM; ×8,200.)
FIG. 7. Cell borders appear as slight depressions. The fine meshwork of the glycocalyx can be recognized; it covers the tips of the microvilli. (SEM; X13,650.)

FIG. 8. Several colonies of MO adhere to the mucosal surface. Although the cell borders are still distinct, the surface relief is flattened and the surface shows a "condensation effect," compared with Fig. 5. (SEM; X4,600.)
Chronic Nonspecific Diarrhea

Scanning Electron Microscopy

A previous study of mucosa in infants and children with CNSD utilizing SEM (18) was done to investigate events on the mucosal surface, a place characterized by interactions between host and potential antigens (dietary, microbial). An attempt was made to look for ultrastructural changes and to correlate SEM findings with those of light microscopy, disaccharidase assays, and microbiology. Histologic (21,22,24,35) or TEM (36) investigations of small bowel mucosa in chronic diarrhea in children were limited to protracted diarrhea of infancy or to cow's milk protein intolerance (37). No detailed information on small bowel pathology in CNSD is otherwise available.

CNSD is by far the most common cause of diarrhea in an otherwise healthy child (38–40). The great majority of these children do not show evidence of failure to thrive and, superficially, CNSD seems more a nuisance than a bona fide disease entity. The term "irritable colon of childhood" (38,39,41) has been

FIG. 9. Various types of MO adhere to the mucosal surface. A larger colony is in the right upper corner. The cell surface appears morphologically intact. (SEM; ×9,800.)
FIG. 10. Two colonies of MO adherent to the mucosal surface, designated by arrows. Morphologically, the MO resemble *E. coli*. The large pieces of debris are probably nonkeratinized cells from the oropharynx. (SEM; ×4,600.)

FIG. 11. MO morphologically resembling *Mycoplasma (pneumoniae?)*. There are multinucleated chains (arrows) and several asymmetric coccoid forms (arrowheads). All adhere to the cell surface. (SEM; ×9,800.)
used as a descriptive one, but it is unsatisfactory since it does not explain mechanisms responsible for either etiology or pathogenesis.

The diarrhea in children with CNSD is often watery, suggesting the presence

FIG. 12. MO morphologically resembling *Mycoplasma* (genus?). Note extensive colonization with mainly filamentous forms, inserted with one end between the microvilli, and showing beading, branching, and bulbous ends (SEM; ×9,100.)

FIG. 13. MO morphologically resembling *Mycoplasma* (?). Some are plump with bulbous projections; some seem bottle-shaped (arrows) and show beading. The latter, as well as single filamentous forms with terminal bulbs, are inserted between the microvilli. (SEM; ×18,900.)
of secretory mechanisms in either the small bowel, the colon, or both. The water phase of stools is appreciably increased in CNSD, to an extent similar to that in children with bacterial overgrowth in the small bowel (42). Similarly, increased fecal passage of water occurs in adults with bacterial overgrowth syndromes (43). Entero-adherent MO have been identified on the mucosal surface of children with chronic diarrhea (36,44–46).

Investigations of small bowel mucosa in CNSD have afforded several new findings that were not observed in mucosa of controls. These were the presence of MO on the mucosal surface; increased mucus covering the mucosal surface; increased cell shedding; damage to the microvillar surface; and partial villous atrophy. The latter was seen in only four patients.

**Presence of Microorganisms**

The finding of MO was very common, and entero-adherent MO were seen in 4 of 5 specimens studied. These MO had morphological characteristics of cocci, bacteria, rods (Figs. 8–10), and resembled *Mycoplasma pneumoniae* in
SEM IN CHRONIC DIARRHEA

various stages of their growth cycle (35,47–50) (Figs. 11–14). *Mycoplasma* have thus far not been identified in the intestinal tract of humans. However, there is a resurgent interest in the colonization by *Mycoplasma* of the intestinal tract of animals (51,52) and of humans (J. G. Tully and S. Razin, personal communication).

In healthy adults (53–57) and children (58,59), the uppermost portions of the small bowel (duodenum and upper jejunum) either do not contain any or only small numbers of commensal gram-positive MO, whereas gram-negative MO are absent (59,60). On the other hand, there is now abundant evidence of increased microbial activity in the small intestine of children (61–69) and adults (55,56,70) with various types of chronic diarrhea. Gram-negative, entero-adherent MO (36,44,45,46,61,71,72) are emerging as potential pathogenic agents in infantile diarrhea, and increased colonization usually parallels the severity of the diarrhea (61).

To exert pathogenic effects in the small intestine, MO have to be swallowed; they must then avoid destruction by gastric acid and adhere to the surface of

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**FIG. 15.** MO adhering en masse to the surface of a nonkeratinized cell of the oropharynx. There are mainly cocci and plump rods, and occasionally slender rods. The cell is adherent to an enterocyte. (SEM; ×9,800.)
FIG. 16. Cocciform MO actively dividing on the surface of a nonkeratinized epithelial cell from the oropharynx; this cell is adherent to the surface of an enterocyte. (SEM; ×13,650.)

FIG. 17. MO colonizing the dense layer of mucus covering the microvilli. There are cocci and bacilli, some actively dividing; furthermore, dividing cocciform MO are visible, which have a capsule (TEM; ×11,550.)
enterocytes. There is usually a postprandial increase of MO in the stomach (53), but little or no growth of potentially harmful MO in the stomach occurs below a pH of 3 (53,71,73,74). However, infants with chronic diarrhea often have an elevated gastric pH (74) which should, theoretically, permit growth of undesirable MO (53,75). The reasons for the decrease of gastric acidity are unclear, but certain microbial enterotoxins depress gastric acid production (76). On the other hand, acid-resistant MO, such as lactobacilli, Candida and streptococcal species are carried into the small bowel (71,77) and, if survival conditions are good, may even become pathogenic (78). Commensal MO, which inhabit the naso- and oropharynx and the upper small bowel, are considered nonpathogenic, but could become a menace to the host in a different environment, by large numbers (79), and/or by positive interaction with potential pathogens (80). Finally, studies with SEM (18) have provided evidence that MO are carried into the small bowel by adhering in substantial numbers to nonkeratinized epithelial cells of the oropharynx (Figs. 15 and 16).

Many questions remain unanswered, particularly those that relate to control mechanisms that mediate between the intestinal microbial ecosystem and the host (81). MO that attach themselves to a variety of epithelial cells seem to have the potential to adhere also to the surface of enterocytes (44,46,80,
FIG. 19. Large areas of the mucosal surface are covered by mucus and debris; the villous pattern is almost invisible. The larger pieces of debris (arrowheads) are clumps of nonkeratinized cells from the oropharynx, many of which carry adherent MO. Adherent cells from the oropharynx were commonly seen in children with recurrent rhinitis and serous otitis, suggesting allergic problems of the upper respiratory tract. This 18-month-old child had clinical intolerance to carbohydrates (lactose, sucrose), but had normal disaccharidase and brushborder alkaline phosphatase activities. (SEM; X150.)

FIG. 20. Mucosal surface covered with mucus, having the aspect of a fine veil; cell outlines are still visible. Disaccharidase activities were normal. (SEM; X1540.)
82–88). Once adherent, MO may deliver metabolic products or toxins into the cell. To adhere, MO must penetrate the defense barrier of the host (82), a very complex system involving humoral and cell-mediated immunity, and physicochemical barriers (89) such as the protective mucin layer on the mucosal surface and cell-surface proteins like fibronectin (90). Microbial adherence is influenced by polarity of the microbial surface (91) and is afforded by capsular antigens, lectins, or lectin-like substances (92–94) on the microbial surface, which interact with target sugars on host tissue. For instance, mannose residues on the cell surface are important for the adherence of *Escherichia coli* (82,94,95) and for *Candida* (96).

Whereas most MO attach themselves to cell surfaces, there may be others which do not attach but rather colonize the mucin layer or mucus covering the surface of cells (55,97). An example of MO colonizing the mucus layer is shown in Fig. 17.

**FIG. 21.** Goblet cell actively discharging mucus underneath a layer of mucus containing debris from extruded enterocytes. According to the length of the microvilli (34), the location of this goblet cell is in the midvillus region. Such goblet cell activity was never observed in control mucosa (TEM; ×8,000.)
FIG. 22. Thick layer of mucus on the surface of enterocytes. A cell about to be extruded is visible: it has lost a great amount of its cytoplasm and appears less electron-dense than the surrounding cells. A small number of mitochondria remains, and there is vesiculation of the endoplasmic reticulum. The microvilli show bleb formation and vesicular degeneration. Four multivesicular bodies (lysosomal origin?) are designated by arrowheads. (TEM; ×4,900.)

FIG. 23. Mucus overlying the brushborder area. Within the mucus are cellular constituents from extruded enterocytes. Portions of the endoplasmic reticulum adhere to the brushborder surface (white arrows); portions of the Golgi apparatus (arrow) and mitochondria are visible as well (TEM; ×20,300.)
FIG. 24. Marked extrusion of cell cytoplasm (bleb formation; cytoplasmic injury) in a subapical region of a villus. Several enterocytes show frayed microvilli. Similar processes extended over other areas of the mucosa. There was a generalized depression of disaccharidase activities; villous morphology by light microscopy was normal. (SEM; ×2,100.)

FIG. 25. Cell extrusion zone involving a large number of enterocytes. The cytoplasmic injury is characterized by the appearance of blebs of various sizes (arrows). Injury to the cell surface is also characterized by the elongation and fraying of microvilli. (SEM; ×2,500.)
Excessive Mucus on the Mucosal Surface

Excessive mucus on the mucosal surface was found in over half of the specimens examined, and mucus covered between (estimated) 20 and 50% of the mucosal surface. By light microscopy, the number of goblet cells was always increased, whereas the height of the villi was normal. However, this mucus is usually not appreciated using light microscopy unless it is preserved by special techniques (98).

The increased secretion of intestinal mucus may be explained by several factors: increased microbial activity (55,99), as in experimental blind loops, in which there is also a marked increase in the number of goblet cells. The stimulation of mucus release by goblet cells is effected by microbial toxins [dose dependent and due to increased glycoprotein synthesis (100,101)]; by antigen–antibody complexes (102); and by the presence of hypertonic solutions (103) resulting from incomplete digestion and absorption of carbohydrates.

The MO-induced increased production of goblet cell mucus may, in part, be generated in self-defense. The presence of intestinal mucin was recognized by
zoologists as affording protection against parasitic disease in animals (104–107),
whereas elimination of mucus enhances bacterial growth in animal intestine
(108). On the other hand, the structural breakdown of intestinal mucin is afforded
by microbial glycosidases (109–114) or by secondary bile acids (115). These
actions could then pave the way for microbial adherence. Protective functions
of intestinal mucin against microbial as well as mechanical and chemical injury
(116, 117) may also be important for humans and need to be explored. Mucin
contained in secretory granules of goblet cells is a potent inhibitor of certain
lectins (118), which mediate the binding between MO and the cell.

Excessive production of intestinal mucus (Figs. 21–23) and its interaction
with the enterocyte surface may result in the formation of a barrier to nutrients.
This has recently been described in human giardiasis (17), and a similar barrier
possibly exists on the mucosal surface in certain patients with cystic fibrosis
(see below). Excessive mucus on the mucosal surface inhibits contact of car-
bohydrates with disaccharidases, resulting in clinical intolerance of lactose and/

FIG. 27. Zone of cell damage, many cells with frayed and elongated microvilli. These zones were
seen on all villous ridges. The surface morphology of the other enterocytes had the aspect of
"immaturity" (see text). There was generalized disaccharidase deficiency; morphology by light
microscopy was normal. (SEM; ×1,260.)
or sucrose, whereas the respective disaccharidase assays show normal activity (see below).

Increased Extrusion of Cell Cytoplasm and Enterocytes (Increased Cell Shedding) (Figures 24–28)

Considerable extrusion of cell cytoplasm and/or of enterocytes was seen in half of the mucosal specimens (18). In contrast to mucosa in healthy subjects, the cell shedding process was greatly amplified, involving cell shedding in sub-apical regions down to the middle or lower third of the villus, and with the appearance of cell extrusion zones (46). Increased cell shedding, extrusion zones in particular, was more common in mucosal specimens with entero-adherent MO, and this suggests some cause and effect relationship, i.e., damage by mi-

**FIG. 28.** Transmission electron micrograph of a damaged enterocyte to compare with Fig. 27. The microvilli are frayed and elongated and show features of degeneration (blebs; arrows). There is condensation of the terminal web. Most of the cytoplasm has been lost; there is minimal residual endoplasmic reticulum and most of the cell space is occupied by large and abnormal mitochondria (TEM; ×7,700.)
FIG. 29. Mucosal surface with "cracked clay" appearance secondary to clumping of microvilli. (SEM; ×13,650).

FIG. 30. These enterocytes, here surrounding a goblet cell, are stripped of glycocalyx, exposing the tips of the microvilli. Since this phenomenon was observed over wide areas, the absence of the glycocalyx may have been responsible, in part, for a depression of disaccharidase activities. (SEM; ×13,650.)
FIG. 31. Control view for Fig. 30. The fine meshwork of glycocalyx covers the tips of the microvilli. (SEM; \( \times 13,650 \).)

FIG. 32. A very unusual finding: "strip-mining" of the cell surface, mainly involving the glycocalyx and the uppermost portion of the microvilli. Portions of the mucosal surface (not shown) were colonized by microorganisms resembling *Mycoplasma*. The biopsy was done after this 18-month-old boy received treatment with cephalaxin for 7 days because of an otitis media. The diarrhea responded promptly to erythromycin and did not recur. (SEM; \( \times 4,800 \).)
FIG. 33. Enterocytes with cytoplasmic injury and surface necrosis. (SEM; ×2,300.)

FIG. 34. Partial villous atrophy. The ridges are of decreased height (as evidenced also by light microscopy) and they are spaced farther apart. There is considerable secretion of mucus, which obscures openings of crypts. There was generalized disaccharidase deficiency as well as colonization of the mucosal surface with MO (SEM; ×154.)
croval activity similar to that observed in experimental intestinal bacterial overgrowth (103). Increased extrusion of cell cytoplasm or cells may result from the action of microbial toxins (119–121) or other antigens (122), and is viewed as a self-defense mechanism (82). On the other hand, cell damage with increased cytoplasmic injury (blebs) (Fig. 22) and increased cell shedding is probably not totally specific to action by MO (123), since it can also be produced in experimental animals by exposure of jejunal mucosa to anionic detergents (124), axenic resins (125), plant fibers (126), or viruses (127,128). In regard to the latter, none could be identified in our patients. The formation of cytoplasmic blebs appears before cell death, and seems to be related to changes in the

![Graph showing activities of disaccharidases in controls and 56 children with CNSD.](image)

**Fig. 35.** Activities of disaccharidases in controls and 56 children with CNSD. There seems to be no trend in age with regard to single or multiple enzyme deficiencies. ▲ = partial villous atrophy.

FIG. 37. Mucosa with some recognizable arrangement of villous structures; SEM stage I lesion. Note the circular or semicircular arrangement of depressed villous ridges, which appear uniformly tall. (SEM; ×154.)
FIG. 38. Mucosa with marked depression of villous ridges, on which individual cell outlines are still visible, whereas small neighboring areas appear more flat; crypt openings are visible; SEM stage II lesion. There was flat mucosa by light microscopy and depression of disaccharidase activities (particularly lactase and sucrase), which was more marked than that of the specimen in Fig. 37. (SEM; ×210.)

FIG. 39. Mucosa without recognizable villous structure or cell outlines. Only crypt openings can be recognized; SEM stage III lesion. Flat mucosa was seen by light microscopy. The specimen is from the same patient as in Fig. 38. (SEM; ×154.)
concentration of extramitochondrial calcium ions (129), which reduce the ability of the cytoskeleton to maintain normal surface morphology.

**Damage to the Microvillar Surface and Brushborder**

In several mucosal specimens, changes on the microvillar surface are worthy of mention. First, there was a characteristic clumping of microvilli, giving the surface a "cracked clay" appearance (Fig. 29), changes quite similar to those produced in the intestine of experimental animals after perfusion with hydroxy fatty acids (130). Indeed, increased amounts of hydroxy fatty acids are found in the stools of children with CNSD (J. R. Poley and J. B. Thompson, unpublished data). Hydroxy fatty acids are synthesized from unsaturated dietary fatty acids by MO (131,132), and are potent secretagogues (133,134).

Second, removal of the glycocalyx (Figs. 30 and 31) exposes the microvilli and the brushborder membrane, which are intimately associated with disaccharidase activity (135). Depression of disaccharidase activity was observed in such specimens.

Third, breaks in the surface coat of microvilli were common in the vicinity of MO (Fig. 32). These breaks could function as entry points for macromolecules, which then could initiate cellular changes and/or damage to the mucosa by direct toxicity or by immediate or delayed hypersensitivity.

**FIG. 40.** Mucosal surface from an area of an SEM stage III lesion. Microvilli have practically disappeared and the cells are flattened. There are rare and enlarged mitochondria and increased endoplasmic reticulum. Microvillar degeneration is marked by bleb (arrow). (TEM; X15,700.)
Fourth, micro-ulcerations and necroses of the enterocyte surface were occasionally seen (Fig. 33), but the cause for these was unclear. A variety of factors, including microbial toxins, may be responsible.

**Partial Villous Atrophy**

Partial villous atrophy (Fig. 34) was seen in only four patients, all of whom were over 1 year old. MO were usually present, as well as increased secretion of mucus and increased cell shedding.

**Microbiology**

Gram stains of mucosal imprint smears have been studied in 28 of 56 patients (18). Most commonly seen were many gram-positive cocci in pairs, groups, or short chains; many gram-negative rods or pleomorphic gram-negative rods; gram-positive and gram-negative diplococci; and gram-positive rods. Frequently, numerous epithelial cells were identified as well; there were occasional white blood cells and yeasts.

Duodenal fluid was obtained from 20 of 56 patients. The predominant MO which could be cultured were α-streptococci, group D (*Streptococcus viridans*); β-streptococci, group A; *Peptostreptococcus anaerobius*; *Staphylococcus epider-
midis and Staphylococcus aureus; as well as E. coli, Veillonella parvula, CDC group F-2 fusobacteria, Pseudomonas spp., Bacteroides saccharolyticus, and Moraxella spp.

In either gram stains or cultures, multiple organisms in a variety of combinations were usually present. Strict anaerobes could not be cultured because only routine techniques were employed.

Since biopsied mucosa was not cultured, it cannot be known if any or all of the MO identified above were adherent to enterocytes. MO of a similar nature were often found in the contents of the upper intestinal tract in children with chronic diarrhea (61,66,67). The numbers of MO were usually greater with a longer duration of the diarrhea (66).

Disaccharidase Activities

Determinations of disaccharidases (lactase, sucrase, maltase) were done for all 62 mucosal specimens. Figure 35 shows the distribution of disaccharidases in a previously published series of 56 patients (18). A depression of one or more enzymes was observed in 64% of patients, irrespective of age. The additional 6 patients included in this article did not alter this distribution. All children with

FIG. 42. Wide areas of the mucosal surface are in disarray. There is extrusion of cytoplasm, and the individual cells seem only loosely connected to each other. Microvilli are recognizable, but are fraying and elongated; there is loss of the glycocalyx. This is the same patient as in Fig. 41. (SEM; ×4,600.)
partial villous atrophy had a depression of all disaccharidases, which is to be expected because of the abnormal villous morphology. Surprisingly, a depression of disaccharidase activities was also observed in specimens which showed normal villous morphology by light microscopy. By SEM, there was an increased presence of MO and/or increased cell shedding. Damage to the brushborder was evidenced by a depression of disaccharidase activities and is attributable to the action of MO, which, in experimental animals, was caused by microbial glycosidases able to degrade brushborder enzymes such as lactase, sucrase, maltase (112,113,136), and alkaline phosphatase (97). Of considerable interest, therefore, is the report that nonpathogenic bacteria and yeasts cultured from duodenal fluid of children with chronic diarrhea were able to degrade lactase (78), an observation which supports findings made in children with CNSD (18).

Mucosa colonized by MO morphologically resembling Mycoplasma never showed morphologic damage using light microscopy, but some measure of damage to the brushborder was common, since 8 of 10 specimens studied showed a depression of disaccharidases. A generalized depression of brushborder enzymes was more common in younger individuals and with heavy colonization. Colonization of other epithelial cells by Mycoplasma (137) was always associated with cell damage. It is hypothesized that damage to the brushborder of enterocytes,
and the resultant depression of disaccharidases, could be the result of the release from *Mycoplasma* of toxic metabolic cell products such as ammonia and hydrogen peroxide (138).

A generalized depression of disaccharidases was also found in mucosal specimens exhibiting increased cell shedding, as seen with SEM. The villous morphology by light microscopy was unremarkable. Increased cell shedding is followed by increased cell production to maintain a normal numerical population of enterocytes on the villi. However, these enterocytes may not be fully mature functionally and, therefore, may not possess normal disaccharidase activities (139). With SEM, enterocytes on the top and middle third of villi had an "immature" aspect. The finding of disaccharidase deficiency in mucosal specimens without morphologic or histologic abnormality in this study is in keeping with observations made by others (140).

Oral tolerance tests with lactose and sucrose were always abnormal when there was increased microbial activity and/or increased cell shedding, because of the decrease of brushborder enzymes. Other explanations for the reduction of brushborder enzymes may be the presence of a patchy enteropathy (141,142) or the above-mentioned brushborder damage by microbial glycosidases (112,113, 136) and/or pancreatic proteolytic enzymes (135).

Disaccharidase activities were normal in most mucosal specimens, the surfaces

**FIG. 44.** Mucosal surface with flat profile and unrecognizable cell borders. Colonization of surface with cocciform MO, arranged singly, in pairs, and in short chains. This is the same patient as in Fig. 43. (SEM; X4,600.)
of which were covered by considerable mucus. Whenever they were done, oral carbohydrate tolerance tests (lactose, sucrose) were abnormal in these patients, who also had clinical intolerance to dietary carbohydrates, which aggravated the diarrhea (J. R. Poley, unpublished observations). Carbohydrate intolerance probably resulted from the inability of the sugars to make contact with the respective brushborder enzymes, and so they remained undigested in the lumen, causing osmotic water retention. Furthermore, undigested carbohydrates are an important energy source for MO. Relationships between the SEM aspects of mucosa and disaccharidase activities are shown in Fig. 36.

The evidence thus far presented strongly suggests, but does not yet conclusively prove, that a variety of entero-adherent or juxta-mucosal MO may play a role in the pathogenesis of CNSD and the pathophysiology of intestinal secretion. All the changes on the mucosal surface seen with SEM in children having CNSD and detailed above probably can be ascribed to the action of MO, and all of these findings compare favorably with those obtained in experimental animals, the intestines of which were exposed to MO or to their toxins (99,112, 113,119–121,143,144) and from studies in infants with E. coli and diarrhea (36,65).

The majority of children with CNSD were between 10 and 28 months of age, an age at which active exploration of the environment and of the body occurs, affording ample opportunity for the ingestion of MO. Aggravation of

**FIG. 45.** Marked depression of villous ridges (compare with Fig. 2). There was generalized depression of disaccharidase activities. (SEM; X154.)
CNSD has been noted with infections of the respiratory tract (41), and antimicrobial agents have been used with some success in CNSD (39,41,145). Of substantial interest was the possibility that MO, morphologically resembling *Mycoplasma* and present in various stages of their growth cycle (35,47–50), could emerge as intestinal pathogens, but this needs additional study.

The extent to which genetic influences may render subjects liable to develop CNSD should be explored: 65% of our patients were boys and 85% of the white children were blond and blue-eyed. It is possible that genetic traits of the host affect microbial colonization of the intestine in humans (146) through the increased ability of MO to degrade the epithelial defense barrier, such as the oligosaccharide moieties of mucin glycoproteins. These glycoproteins carry ABH (O) blood-group determinants (147,148) and may be involved in modifying microbial adherence to cell surfaces (149).

Despite all these mucosal alterations, most patients with CNSD did not exhibit failure to thrive in the strict sense. Malabsorption of nutrients does occur in children with chronic diarrhea due to microbial contamination of the small bowel (150). By conventional and accepted definition, CNSD of children in developed countries is not associated with failure to thrive or with failure to gain appropriate weight. Although no absorption studies were done in the patient

**FIG. 46.** Marked alterations of the mucosal surface. Villous ridges are not recognizable, although the cell outlines appear distinct. Adjacent to these areas, the surface is more flat and crypt openings are visible. Other portions of the biopsy show occasional normal-appearing ridges (not shown), suggesting a patchy enteropathy. There was generalized depression of disaccharidase activities. (SEM; ×250.)
population reported, 12% showed increased weight acceleration and a few even showed increased height acceleration after appropriate therapeutic intervention (to be reported elsewhere) that led to the prompt cessation of the diarrhea. In contrast, children with chronic diarrhea and a contaminated small bowel who live in third world countries frequently show failure to thrive (64) due to malabsorption of nutrients (65), which may not be in adequate supply in the first place.

Conditions Associated with Villous Atrophy

Gluten-Sensitive Enteropathy (Celiac Disease)

A recent publication (16) indicates that SEM is superior to the conventional light and dissecting microscopes for the assessment of mucosal damage in gluten-sensitive enteropathy (celiac disease). By light microscopy, the histologic aspect of mucosa in celiac disease is characterized by subtotal villous atrophy, and the overall impression is that of a "flat" mucosa. In contrast, flat-appearing mucosal lesions can further be classified using SEM into several stages of severity, which are tentatively designated as SEM stages I, II, and III, the latter being the most severe lesion. Examples of these stages are shown in Figs. 37–39, and a transmission electron micrograph of SEM stage III is shown in Fig. 40.
An SEM stage I lesion shows mucosa with recognizable organizational arrangement: depressed villous ridges arranged in cuff- or collar-like fashion, forming a crypt well (151). Several crypt openings are found in the interior of such a well. The SEM stage II lesion shows a marked depression of villous ridges, which are only barely recognizable as such and may be compared to ruffled mounds. Finally, the SEM stage III lesion shows a flat surface, devoid of any elevated structures, and individual crypt openings are seen with ease. Stage II and III lesions may be seen together on one mucosal specimen, whereas mucosal changes in the stage I lesions seem uniform.

Since the mucosal damage of an SEM stage I lesion is less advanced, it would follow that recovery of the mucosal lesion and repair should proceed at a more rapid pace than that of an SEM stage III lesion. Correspondingly, disaccharidase activities are probably depressed to a greater extent in SEM stage III lesions, and recovery of their activities to normal should take longer as well. However, this hypothesis needs to be tested. Whereas SEM stage I lesions may be found during the initial biopsy, they are also seen in the recovery phase in children (16) and adults (6,9,152).

Sequential events during mucosal regeneration after introduction of a gluten-free diet have been described (16) but need to be studied in more detail, if feasible. It seems that during mucosal reconstruction enterocytes become arranged

**FIG. 48. Giardia lamblia** (duodenalis) trapped in mucus on the small bowel surface. The ventral sides of the parasites are visible, exposing the suction discs with the asymmetric spiral ridge and the dorsal pair of flagellae. (SEM; ×3,780.)
around crypt openings in a cuff- or collar-like fashion, providing a surface aspect similar to the topography of gastric mucosa around gastric pits or foveolae (153). Subsequently, mucosal reconstruction would proceed to produce a more organized surface relief, as seen in SEM stage I lesions.

**Soy Protein Intolerance**

Mucosal biopsies from infants with soy protein intolerance have been studied with light microscopy (154) and with SEM (19). Two examples of soy protein-induced mucosal damage, as seen with SEM in infants, are demonstrated. In a 5-week old infant (Fig. 41), the mucosal damage was quite extensive, showing great disarray (Fig. 42) in the arrangement of enterocytes. This aspect was similar to the appearance of fetal jejunal mucosa (not shown). In contrast, the mucosa in a 4½-month-old infant (Fig. 43) still showed some recognizable villous architecture on one side of the mucosal specimen, whereas adjacent areas appeared more flat and resembled the SEM stage II lesion seen in celiac disease. Of interest was the colonization of vast areas of the mucosa in the older infant (Fig. 44) by cocciform MO, which were adherent to the mucosa in pairs and short chains but which could not be identified further. It is hypothesized that the soy protein-induced mucosal damage may have unmasked “receptor sites,” permitting microbial adherence. The contribution of the microbial colonization to the mucosal damage cannot be assessed.
Both infants were biopsied again after 6 weeks, and the mucosal reconstruction was more advanced, and disaccharidase activities had recovered to a greater extent in the older as compared with the younger infant. It seems that age, rather than duration of exposure to soy protein, is more important for the degree of initial mucosal damage. That damage, however, may then determine the speed of recovery or mucosal reconstruction.

Examination of mucosa with SEM in celiac disease, or soy or milk protein-induced enteropathy does not shed more light on the pathogenesis of the severe mucosal lesions seen in these conditions. The lesions may be produced either by cell-mediated immunity (155,156), or by lectin-induced (157,158) and saponin-facilitated (159) cytotoxicity.

**Intolerance to Cow’s Milk Protein**

Severe lesions of small intestinal mucosa of infants having cow’s milk protein enteropathy have been observed with light microscopy and TEM (37), but not

![Fig. 50. Mucoid pseudomembrane. Trophozoites were located above the top border of this picture (not shown), but impressions of suction discs are visible and marked by arrows. Such impression marks have not been identified previously in human giardiasis. This child had evidence of malabsorption (17). (SEM; ×2,500.)](image)
with SEM. By light microscopy, intestinal lesions, even severe ones, may show a patchy distribution.

In our two patients, whose mucosa could be studied by SEM, moderate villous damage was demonstrable (Fig. 45). By light microscopy, these findings corresponded to a "partial villous atrophy." Disaccharidase activities were depressed, probably due to damage to the brushborder and/or a reduction of the total enzyme mass secondary to a decrease of villous height.

Of interest is the intestinal colonization with enteric pathogens in infants having cow's milk protein intolerance (148); this is reminiscent of microbial adherence in the one patient with soy protein–induced mucosal damage.

**Intractable Diarrhea of Infancy**

We have observed one patient with intractable diarrhea of infancy, and findings by SEM (Figs. 46 and 47) are available from an initial small bowel biopsy and from a follow-up study 5 months later, after the child had received total parenteral

*Fig. 51. Mucosa from a 13½-year-old child with evidence of malabsorption and carbohydrate intolerance. Vast portions of the mucosa were covered by a dense layer of mucus. Determination of disaccharidase activity showed isolated lactase deficiency. (SEM; X380.)*
hyperalimentation. The initial biopsy (Fig. 46) demonstrated focal villous atrophy, as well as evidence of increased production of mucus and excessive cell shedding. MO were seen on the mucosal surface but could not be identified. There was also a generalized disaccharidase deficiency. The subsequent biopsy (Fig. 47) showed good recovery of villous morphology, but increased cell shedding was still present. However, the infant has shown good catch-up growth and weight gain.

Giardiasis

Observations using SEM of intestinal infection of adults (160), and children (17) with giardiasis have been published (Figs. 48 and 49), as well as studies using SEM in experimental animals (161,162). However, with one possible exception (17), none of these reports could satisfactorily explain the occurrence of malabsorption in giardiasis. The mucoid barrier or mucoid pseudomembrane apparent in Fig. 50 has been associated with the malabsorption of lactose and other nutrients (17). Thus far, this mucoid pseudomembrane has not been found

FIG. 52. Mucosal biopsy from a 23-year-old patient. Mucosal surface is not recognizable (compare with Fig. 4) because it is covered by a thick and viscid-appearing layer of mucus, which was not visible by light microscopy. Embedded in the mucus are old erythrocytes (arrows), some of them with a spiny surface. Fresh erythrocytes (arrowhead) adhere to the surface of the mucus layer. Clinically, there was no history of bleeding from either the upper gastrointestinal tract or the respiratory tract. The mucus layer covered large areas of the mucosal surface. Disaccharidase activities were normal (SEM; X1,540.)
in other conditions associated with chronic diarrhea. Certainly, further documentation of this mucoid pseudomembrane is needed.

It is hypothesized that excessive mucus, containing entrapped debris and constituents from extruded enterocytes and dead parasites, may adhere to the mucosal surface. Additional host factors, as well as environmental factors, must be sought to explain the pathogenesis of the mucoid pseudomembrane. In analogy to experimental animals (104-107), the increased secretion of intestinal mucus in human hosts is probably stimulated by infection with the parasite and could serve as a defense mechanism by trapping and immobilizing the parasites, rather than providing a “friendly” habitat.

Cystic Fibrosis

No information on the ultrastructural topography of small bowel mucosa in cystic fibrosis has been published. Although studies of tracheal epithelium, pan-

FIG. 53. Mucosal surface covered with a dense layer of mucus. Outlines of a goblet cell are visible to the left. This is the same patient as in Fig. 51. (TEM; ×11,550.)
creas, and liver have received a great deal of attention, there has been little focus on events occurring at the level of the small intestine, on its surface in particular. The small bowel is, after the pancreas, the most important organ in digestion, and is the principal organ for the absorption of nutrients. Despite optimized replacement therapy with pancreatic enzymes, many patients still exhibit malnutrition and biochemical evidence of malabsorption.

Part of the poor digestion in exocrine pancreatic insufficiency may be corrected by feeding the patient adequate amounts of pancreatic enzymes and by protecting these supplemental enzymes from acid inactivation (163), but yet malabsorption continues to be a problem.

Investigation using SEM of biopsied jejunal mucosa from a 13½-year-old patient (Fig. 51) and a 23-year-old patient (Fig. 52) with cystic fibrosis and malabsorption of nutrients, trace elements, and vitamins, as well as essential fatty acid deficiency, demonstrated that large areas of the bowel surface were covered with sheets of mucus (also shown in Fig. 53), which probably function as a diffusion barrier to nutrients. Early in life, as shown on small bowel mucosa in a 4½-month-old infant (Fig. 54), there seems to be evidence of increased secretion of mucus, but the mucus is not yet adherent to the cell surface. It seems of great importance to further substantiate the presence and adverse affects of a mucous barrier on small bowel mucosa in patients with cystic fibrosis, and to find appropriate remedies.

FIG. 54. Extrusion of mucus at the cell borders. Occasional areas of the mucosal surface were covered by a thin veil of mucus. Mucosal morphology by light microscopy was normal. Disaccharidase activities were normal. This is a 4½-month-old child. (SEM; x13,850.)
Crohn’s Disease

Reports on mucosal pathology as seen with SEM in the small bowel of patients with Crohn’s disease are limited to studies of the ileum of adults (30). These studies were also complemented by TEM (164). The gross villous architecture of normal ileum as seen with SEM does not differ a great deal from that observed in the jejunum, and the mucosal surface is comparable to that of the duodenum: a rather flat surface relief with elevated cell margins and visible orifices of goblet cells. Detailed views of brushborder or microvilli were not available.

In Crohn’s disease, the size and shape of the villi are changed markedly (30). Their height is decreased and they are broadened and club-shaped. There is fusion of villi or connections between villi by epithelial bridges. Another important feature is the marked increase in the number of goblet cells, which are actively discharging mucus and, thus, most likely contribute to the intestinal protein loss which accompanies this disease.

SUMMARY

In this chapter, changes of ultrastructural topography of small bowel mucosa in children with various types of chronic diarrhea have been reviewed. Routine use of SEM, coupled with TEM, light microscopy, assays of brushborder enzyme, and microbiologic studies, has provided new information that seems to be clinically relevant.

For instance, this study provides suggestive evidence that the presence of a variety of MO in the upper intestinal tract may be involved in the pathogenesis and/or pathophysiology of CNSD in children (“irritable colon of childhood,” so-called contaminated small bowel without failure to thrive). Furthermore, among these MO seem to be forms, as seen by SEM, which morphologically resemble *Mycoplasma*, genus unidentified. *Mycoplasma* have hitherto not been described in human intestine and further evidence is needed for appropriate identification. It also remains to be seen whether or not *Mycoplasma*, if present in the small intestine, cause or contribute to the diarrhea.

Disaccharidase deficiencies may be present despite a normal-appearing small bowel morphology by light microscopy; by SEM, and in CNSD, there is frequent evidence of markedly increased cell shedding (followed by stepped-up cell production and rapid migration onto the villi) and/or marked damage to the brushborder that explain disaccharidase deficiencies. However, the possibility also has to be entertained that so-called patchy lesions may be responsible for the phenomenon of disaccharidase deficiency in the presence of normal mucosal morphology.

In mucosa showing villous atrophy by light microscopy, additional studies using SEM demonstrated the feasibility of grading mucosal damage more accurately and in more detail. The presence of more severe or more advanced mucosal damage, as seen with SEM, may have some bearing on the individual’s response to dietary antigens (toxins) and/or on the rate of recovery. SEM probably
cannot distinguish between mucosal damage due to gluten, milk or soy protein intolerance. More information has to be obtained about this as well.

Lastly, the finding of a mucous or mucoid barrier is of considerable interest. In addition to the mucoid pseudomembrane reported in giardiasis, a similar mucoid barrier seems to exist on the surface of the small bowel mucosa in patients with cystic fibrosis. This may explain, in part, why continued malabsorption is present despite optimized enzyme replacement and antacid enzyme protection. A mucous barrier is also seen in a fairly large number of patients with CNSD who clinically demonstrate carbohydrate intolerance but whose disaccharidase activities are normal. The excess mucus has to be viewed as an unstirred layer of infinite thickness, which may be a formidable barrier to the absorption of nutrients.

In conclusion, most findings reported in this chapter are new and need confirmation by additional studies. However, the abnormalities of the ultrastructural topography of the mucosal surface seen in various diarrheal states may be helpful in developing new thoughts on research into pathophysiologic processes and to develop new avenues of therapy. SEM should be very useful in the examination of host–environment interactions on the mucosal surface in patients with a variety of intestinal disorders characterized by diarrhea and malabsorption. SEM may in the future be employed as a diagnostic tool, but more experience is needed. Lastly, SEM should give insight into ultrastructural topographic changes on the surface of the developing intestine. It is expected that more effort will be devoted in the future to studies using SEM of the gastrointestinal tract, particularly the small intestine. In this context, it has been suggested (2) that the gastrointestinal tract is one of the principal domains for investigations using SEM.

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REFERENCES

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122. Lipkin M. Physiol Rev 1973;53:891-95.