THE ENTEROCYTE IN SMALL INTESTINAL ADAPTION
An experimental and clinicopathological study with special reference to the ultrastructure of the brush border

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som med vederbörligt tillstånd av rektorsämbetet vid Umeå Universitet för avläggande av medicine doktorsexamen kommer att offentligen försvaras i Tandläkarhögskolans sal B, 9 tr, onsdagen den 16 maj 1984

av

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UMEÅ 1984
ABSTRACT
Stenling, Roger; THE ENTEROCYTE IN SMALL INTESTINAL ADAPTATION. An exper­
imental and clinicopathological study with special reference to the
ultrastructure of the brush border. Umeå Univ, Med Diss, New Series 122
- ISSN 0346-6612.

The small intestine mucosa is known to be able to adapt itself to
several kinds of both physiological and pathological conditions. The
adaptive patterns of the structure of the enterocytes, particularly
their apical surface (brush border), were studied in three models: (1)
in rats, subjected to antrectomy or antral exclusion, combined with
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after long-term treatment with gluten-free diets; c) after long-term
challenge with dietary gluten following treatment; d) after short-term
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Differentiation of the rat enterocytes from the base to the crest
of the villi was structurally reflected by doubling of their apical cell
area, an increase in cell height, and a decrease of both nuclear and
mitochondrial volume densities. In mature normal rat enterocytes, high-
power SEM showed regularly arranged, nude microvilli in their apical
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Both in active coeliac disease and after long-term challenge with
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Additional key words: Scanning Electron Microscopy, Transmission
Electron Microscopy, Stereology.

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Both in active coeliac disease and after long-term challenge with dietary gluten, SEM analyses showed uniformly destructed villi. The apical surfaces of the enterocytes were frequently convex and irregular in size and delineation (the surface of the normal enterocytes was polygonal and flat). Ultrastructurally, the apical surfaces were severely damaged with a distortion of the glycocalyx and with marked irregularity of the microvilli. - After gluten elimination, the surface ultrastructure of the enterocytes in the coeliac gut mucosa generally showed a rapid, clear-cut restoration despite a remaining severe atrophy of the villi. Successful dietary treatment (after about one year of gluten-free diet) restored the small intestine mucosa to normal as assessed both by LM and low-power SEM. In contrast, high-power SEM often disclosed persisting lesions of the enterocytes. Another provocation with gluten for up to 9 days in clinically healed coeliac mucosa did not significantly alter the surface ultrastructure of the enterocytes.


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The present thesis is based on the following papers, which will be referred to in the text by their Roman numerals:


II. Stenling, R. and Helander, H.F.: Stereological Studies on the Rat Small Intestinal Epithelium

    III. Effects of alloxan diabetes. (Submitted for publication).


V. Stenling, R., Fredrikzon, B., Engberg, S. and Falkmer, S.: Surface Ultrastructure of the Small Intestine Mucosa in Children with Coeliac Disease

   Stenling, R. and Fredrikzon, B.: Surface Ultrastructure of the Small Intestine Mucosa in Children with Coeliac Disease
   II. Effects of short-term gluten elimination and challenge. (Submitted for publication).
CSA: Cobble-stone appearance
DM: Dissection microscopy
ECI: Enterocyte irregularity
GCD: Glycocalyx distortion
GCR: Glycocalyx restoration
LM: Light microscopy
MVI: Micro-villous irregularity
N: Normal
PVA: Partial villous atrophy
SEM: Scanning electron microscopy
SVA: Subtotal villous atrophy
TEM: Transmission electron microscopy
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INTRODUCTION

THE NORMAL SMALL INTESTINE MUCOSA

Some functional aspects

The terminal steps of the digestive processes take place close to the surface of the small intestine mucosa (30, 137). This process is catalyzed mainly by enzymes (e.g. dipeptidases and disaccharidases) located in the brush border region of the enterocytes (41, 46, 73). Absorption of the products of digestion predominates in the jejunum, whereas ileum provides an absorptive reserve capacity and also possesses specialized absorptive functions, e.g. for vitamin B and bile salts (34). The capacity of the small intestine to absorb nutrients depends on a variety of factors including intestinal motility, blood and lymph flows, the functional state of the enterocytes and the internal surface area of the small intestine (cf 78).

Some structural aspects

By tradition, the small intestine mucosa is divided into three distinct layers (Fig. 1): the muscularis mucosae, the lamina propria, and the epithelial cell layer. This cell layer covers both the villi that projects about 0.5 mm into the intestinal lumen, and the crypts of Lieberkühn which are simple tubules of about 0.1-0.2 mm in depth (102).

The architecture of the villi is usually characterized as follows:
1. Finger-shaped villi: Mainly cylindrical structures with a ratio of thickness to width that does no exceed 1:2 (31).
2. Leaf-shaped villi: Two or more finger-shaped villi fused together side by side (146).
3. Ridge-shaped villi: Two or more leaf-shaped villi fused together side by side (1, 114).

Normally, the architecture of the villi varies considerably. Thus, there are species, age and topographical variations. In the proximal small intestine mucosa, the villi are finger-shaped in the cat but leaf-shaped in the rat. In human adults, the villi of the proximal small intestine may be both finger- or leaf-shaped (31, 136,
Fig. 1. Three-dimensional schematic drawing of the architecture of the small intestine mucosa (94). With due permission from the publisher.

146) and occasionally ridge-shaped, whereas in children, ridged-shaped villi are common in the proximal small intestine mucosa, and may even predominate (102, 108, 114). In the distal part of the small intestine, the villi are predominantly finger-shaped both in adults and children (127, 136). There is also evidence that the architecture of the villi may be modulated by the nature of the diet; a high content of dietary fiber tends to broaden the villi (128, 136).

The height of the villi and the depth of the crypts of the human small intestine mucosa also varies with age and topography; the crypts are longer and the villi shorter in children (102). Similar age-depen-
dent variations are seen also in animals (74). In both animals and man, the villi become progressively shorter towards the distal part of the ileum (3).

In the epithelial layer that covers the villi enterocytes (absorptive cells) predominate. Additionally, there are mucus-secreting goblet cells, a few caveolated tuft cells, endocrine cells and so-called M-cells associated with the Peyer's patches (136). Also in the crypt epithelium, enterocytes and goblet cells prevail. In addition, there are undifferentiated precursor cells, endocrine cells and lysozyme-producing Paneth cells.

The internal surface area of the small intestine is believed to be of utmost importance for the absorptive capacity of the small intestine, and diseases that affect mucosal function often reduce its absorptive surface (136). Normally, the intestinal surface area is increased by the folds of Kerckring, the villi and the microvillous projections of the enterocytes (30, 37, 84, 110). The increment due to the folds of Kerckring has been estimated to about 3 times (94), that of the villi between 8 and 23 times (33, 100, 144), and that of the microvilli between 7 and 260 times (6, 20, 99, 104, 105, 154, 132).

The enterocyte

The term enterocyte is now commonly used and corresponds to the term "absorptive cell", rarely also to "epithelial cell". It was introduced by Booth, (1968), who compared the turnover of the absorptive cells of the small intestine mucosa with that of the cells of the hematopoietic system; the process of cell production and differentiation in the small intestine was referred to as "enteropoiesis".

The enterocytes are produced as undifferentiated enteroblasts in the lower two thirds of the crypts (151). Then, they migrate towards the tips of the villi where they are shed into the intestinal lumen (Fig. 1). During the process of migration they become more differentiated. A zone of rapid differentiation - known as the maturation compartment (151) - probably exists in proximity to the junction between the villi and the crypts. Functionally, the progressive differentiation of the enterocytes results in an increased capacity for digestion and absorption (132, 136). Biochemically, this is reflected by
Fig. 2. Schematic drawing of the ultrastructure of a normal enterocyte (79). With due permission from the publisher.

an increase of dipeptidase and disaccharidase activities (96), i.e. enzymes which are located in the apical membrane of the enterocytes (30, 41, 46). Morphologically, the differentiation is accompanied by an increase in cell height and cell volume, increase of rough and
smooth endoplasmic reticulum (132, 136), increase of mitochondrial volume (105, 117) and, above all, a dramatic increase of the apical cell surface (6, 20, 99, 104, 103, 132, 154, 136).

The differentiated enterocytes are tall columnar cells with an ellipsoid nucleus in the basal half of the cytoplasm (Fig. 2), (131, 132, 136). In close proximity to its apical pole there is a prominent Golgi complex. The cytoplasm contains a relatively high amount of mitochondria, occupying about 13% of the cytoplasm (105) suggesting a high oxidative metabolism in the enterocytes. The most characteristic feature of the enterocytes is the 0.5-1.5 μm wide striated border (136). At the electron microscopical level, it displays closely packed, regular microvilli, about 0.1 mm in width and 0.5-1.5 μm in length, first demonstrated by Granger and Baker (51).

The microvillous membrane (the apical cell membrane) is wider (10 to 11 nm) than the baso-lateral cell membrane (7 to 9 nm) (44, 124), which is also reflected by a different biochemical composition (136). The core of the microvilli contains a bundle of actin filaments that projects into the terminal web; this is a fibrillar plate, which is believed to stabilize the apical part of the enterocyte (136).

Connected with the microvillous membrane, and probably a part of it, is the glycocalyx ("surface coat", "fuzzy coat"), a mucopolysaccharide complex which differs structurally and biochemically from the mucus secreted by the goblet cells (65, 66, 67, 134, 136). The glycocalyx is produced by the enterocytes (11, 12). It cannot be removed from viable enterocytes by proteolytic or mucolytic substances, and it is remarkably resistant to mechanical forces (65, 67). In contrast, it is decreased and fragmented in degenerated cells (65) and easy to remove from brush border preparations (95). Several functions have been ascribed to the glycocalyx, such as terminal digestion, absorption and protection, and some of the disaccharidases have their enzymatically active part within the glycocalyx (24). In OsO₄-fixed specimens, the glycocalyx appears to consist of fine filaments, attached directly to the outer leaflet of the microvillous membrane (65, 67, 136). Its thickness in sections from fixed small intestine mucosa specimens varies considerably from species to species: in man and cat it is thick, in rat it is thin (65, 67).
ADAPTATION OF THE SMALL INTESTINE MUCOSA

Dowling (39) has launched two major types of response by the small intestine mucosa (Fig. 3); (a) A "physiological" response ("type I") with adaptive hyper- or hypoplasia including corresponding altera­tions of digestive and absorptive function; (b) A "pathological" response ("type II") representing repair processes following mucosal injury, affecting structure as well as absorptive and digestive function.

"Physiological" adaption

"Physiological" adaption has been extensively studied, especially after resection of small intestine segments and following by-pass operative procedures. Although many adaptive phenomena have been described, the underlying mechanisms are still not clear. Three major influences are discussed, viz. luminal nutrition, pancreatobiliary secretions and hormonal factors (39, 147, 148).

Increased functional capacity of the small intestine mucosa may be found after partial resection, by-pass operations, in hyperphagic conditions such as hypothermia, lactation and experimental diabetes, after pancreato-biliary diversion, and in cases with increased serum levels of cholecystokinin, secretin, entero-glucagon or gastrin (39, 147, 148). As a rule, these changes are more pronounced in the distal than in the proximal portions of the small intestine (39). Structurally, the increased functional capacity is reflected by increase in small intestinal weight, and by increase in the mucosa surface enlargement due to villi; they become longer than normal. Moreover, there is an accelerated cell proliferation, structurally seen as an increase in crypt depth and in the number of mitotic enteroblasts (39, 147).

The results from studies on the structure of individual enterocytes are conflicting. Following partial resection, the length of the microvilli has been observed to be increased (129), unchanged (54, 149), or decreased (49, 153) in the remaining parts of the jejunum or ileum. In acute experimental diabetes, no qualitative alteration of the microvilli has been found (81). In general, the activities of brush border enzymes such as disaccharidases and dipeptidases increase per unit length of the small intestine, while specific activities
either are increased, unchanged or decreased (81, 147). Results of recent studies demonstrate that after resection of proximal small intestine, these activities seem to decrease if expressed per unit mucosa surface indicating a reduced functional capacity of the indivi-
dual enterocytes (91).

**Decreased functional capacity** of the small intestine mucosa has been observed in a wide variety of experimental conditions such as self-emptying blind loops after by-pass operations, prolonged fasting, parenteral nutrition, hypophysectomy, and in the jejunum after ileojejunal transposition (39, 147). The structural and biochemical alterations of the small intestine mucosa, observed under these conditions are, as a rule, opposite to those under conditions of hyperfunction (see above). Thus, decrease in small intestinal weight, cell proliferation, crypt depth, and villous length are commonly seen. There are, however, but few observations of the structure and the functional capacity of the individual enterocytes. In a study of self-emptying jejunal blind loops Toskes et al. (133) found that the enterocytes were indistinguishable from those in non-operated controls. Recently, Menge et al (91) have pointed out that the absorptive capacity, expressed per unit area of the small intestine, is significantly increased in self-emptying jejunal blind loops indicating enhanced functional capacity of individual enterocytes.

"Pathological" adaption

The structural response of the small intestine mucosa in various pathological conditions is known to be rather uniform and non-specific, irrespective of the etiological factor (7, 76). It is characterized by increased loss of enterocytes into the intestinal lumen and often also by increased rates of enteroblast proliferation and migration of enterocytes (32, 139, 140). By light microscopy (LM), i.e. in conventional histopathological sections, the villi appear shorter and broader than normal (partial villous atrophy) (PVA), and in severe cases the villi are absent or almost so (subtotal villous atrophy) (SVA) (4, 5, 28, 39, 77, 98, 121). There is also an increase in crypt depths and an increase of inflammatory cells, mainly plasma cells, within the lamina propria (4, 5, 98, 121). In addition, the enterocytes at the mucosa surface often show a decreased height (98), and increased amount of intraepithelial lymphocytes is often found (60, 88). Dissecting microscopy (DM) and low-power SEM both reveal an architecture of the villi characterized by low convolutions, correspon-
ing to PVA, or a flat mucosa with numerous visible crypt openings, corresponding to SVA (1, 55, 86, 114).

Pathological conditions where this type of adaptive reaction has been described include coeliac disease, dermatitis herpetiformis, tropical sprue, postinfective malabsorption, milk- and soy-protein intolerance, intestinal lymphoma, and Zollinger-Ellison syndrome (cf. 76). In coeliac disease the lesions are mainly diffuse, whereas other diseases such as postinfective malabsorption, milk- and soy-protein intolerance and dermatitis herpetiformis, may show a patchy distribution (76).

In this context [coeliac disease] has been studied extensively. The etiologic factor is a fraction of wheat gluten exerting the deleterious effect on the small intestine mucosa. The pathogenesis of coeliac disease is still unknown (28, 29, 75). Several theories and hypotheses have been proposed including protease or peptidase deficiencies (29), a lectin-like mediated damage (143) and various immunological mechanisms (cf 75). As the structural lesion of the small intestine mucosa is caused by the presence of gluten, manipulation of the diet followed by structural adaption of the mucosa is used for diagnostic purpose (89). The most severe structural lesions are seen in the active, untreated phase of the disease. Following long-term exclusion of gluten from the diet, the mucosa structure is restored, and after about one year most biopsy specimens in children have been completely normalized as assessed by DM and LM (150). With subsequent reintroduction of gluten, the lesions reappear. The structural alterations of the mucosa are most pronounced in the upper small intestine, whereas the distal ileum is less affected (39, 127). It should also be emphasized that the same amount of gluten may produce quite different degrees of intestinal damage in different patients (56).

The ultrastructural lesions of the enterocytes in coeliac disease, as visualized by means of TEM (Fig. 4), include irregularly formed nuclei with variable position, decrease in the amount of endoplasmic reticulum with dilatation of its cisternae, increased amount of free ribosomes, swelling of mitochondria with disrupted cristae, increase in the amount of lysosomal bodies and fat globules, and an incomplete terminal web. The microvilli are shorter, irregularly for-
Fig. 4. Schematic drawing of the ultrastructure of an enterocyte in active coeliac disease (79). With due permission from the publisher.
from the upper parts of the crypts appear to be rather normal. There is only sparse information in the literature about the structural alterations of the glycocalyx in coeliac disease (64, 103, 119).

AIMS OF THE STUDY

The overall objective of the present study was to contribute to the understanding of the structural reactions of the enterocytes during "physiological" adaption and in "pathological" response of the small intestine mucosa.

The more specific aims of the investigation were the following:
1. To study the fine structure of the duodenal and jejunal enterocytes during states of normal differentiation and desquamation.
2. To try to experimentally evaluate the influence of the stomach's antrum, a self-emptying duodenal blind loop, duodenal by-pass and short-term diabetes mellitus on the fine structure of the duodenal and jejunal enterocytes.
3. To investigate the surface ultrastructure of the human enterocytes normally and in untreated coeliac disease, and their adaption to long- and short-term elimination and challenge of gluten.
4. To evaluate whether a routine SEM analysis can improve the morphological assessment in the diagnosis of coeliac disease.

MATERIALS AND METHODS

ANIMALS (I, II, III)
Male Sprague Dawley rats (ALAB, Sollentuna, Sweden), female inbred R-strain rats (bred at the University of Umeå) (63) and male European short-haired cats (bred at the University of Umeå) were used for this investigation. At the beginning of the experiments, the Sprague Dawley rats weighed about 300 g and were 2 to 3 months old. The R-strain rats were about three months old and weighed about 200 g. All rats were reared under normal laboratory conditions with free access to water and a standard pellet diet (Astra-Ewos, Södertälje, Sweden). The cats were adult, weighing about 3 kg. They were either kept in large cages or allowed to move about freely in a room, and regularly fed a stan-
standard diet (commercially available mink diet; Umeå, Sweden). They were vaccinated and treated with vermicides, appeared healthy, ate well and had no signs of gastrointestinal disease.

Operations (II)
Following an overnight fast in wire-mesh bottom cages with free access to water, the rats were anaesthetized intraperitoneally with sodium pentobarbital (40 mg/kg). The following operations were carried out:

Antrectomy: The resection included the entire length of the lesser curvature up to the cardia, the greater curvature up to a point half-way between the pylorus and the fore-stomach, and the proximal 3 to 5 mm of the duodenum. Particular care was taken to leave the blood supply to the remaining parts of the stomach as intact as possible. Gastrointestinal continuity was restored, either by an end-to-end anastomosis between the stomach and the duodenum (Billroth I procedure), or by an end-to-side anastomosis between the stomach and the jejunum about 10 cm distally to the ligament of Treitz (Billroth II procedure).

Antral exclusion: The distal half of the antrum was isolated from the rest of the stomach and converted into an antral pouch which emptied through the pylorus into the duodenum (2). Gastrointestinal continuity was then restored by end-to-side gastrojejunostomy as in the rats antrectomized according to the Billroth II procedure.

Sham operation: Duodenum was transected 3 to 5 mm distally to the pylorus and the jejunum about 10 cm distally to the ligament of Treitz. Continuity was then restored by end-to-end anastomoses.

Postoperative care: The operated animals were kept fasting for 2 to 3 days while receiving daily subcutaneous injections of about 10 ml of Tyrode solution (87). They were then given a normal diet. At the time of sacrifice, there were no significant weight differences between the groups of the operated animals.

Experimental diabetes
Following a 48 hours' fast, a single injection of alloxan monohydrate (55 mg/kg body weight) was given in the inferior caval vein. The renal pedicles were clamped during the injection and for further 5 min.
Control rats were injected with physiological saline, and none of the rats were given insulin afterwards. After the injection, the animals were kept in plastic cages and their diabetic condition was checked after 2 weeks. For this purpose, the 24-hour-urin-output and the non-fasting blood glucose were assayed. In addition, the amount of consumed food during 24 hours was also quantified. All alloxan-injected rats were found to be diabetic (blood glucose level above 300 mg/100ml; urinary output above 15 ml/100 g body weight/24 h). They were also hyperphagic with a mean food intake of 14.6 g/100 g body weight/24 h compared to the saline-injected control rats (mean 6.3 g/100 g body weight/24 h). No differences in weight change were observed between the diabetic rats and the control rats during the period of observation.

Collection of specimens (I, II, III, IV)
Fasted as well as non-fasted animals were used. Perfusion fixation (I, II) was carried out as follows: Under anaesthesia (sodium barbital, intraperitoneally) the abdominal cavity was opened and the aorta cannulated in retrograde direction, just above its bifurcation. By means of a peristaltic pump a modified Tyrode buffer solution (87) was then infused (20 ml/min) into the vascular system. The portal vein was opened to permit escape of blood and perfusate, and when the blood vessels of the duodenum appeared empty of blood, the rinsing fluid was substituted by the fixative. The fixative consisted of rinsing fluid to which 4 % glutaraldehyde had been added. After perfusion for 8 minutes, a portion from the oral end of the horizontal part of the duodenum and a portion of the jejunum, about 10 cm (I, III) or 15 cm (II) distally to the ligament of Treitz, were excised and fixed for another 3 hours in ice-cold 4 % glutaraldehyde, post-fixed in 1 % OsO₄ in Tyrode solution for 1 hour, dehydrated in rising concentrations of ethanol, and embedded in Epon.

For immersion fixation (IV) the abdominal cavity of the rats and the cats was opened under anaesthesia (sodium barbital, intraperitoneally). A portion of the proximal jejunum was opened antimesenterically, excised and immediately immersed in a fixative consisting of 6.25 % glutaraldehyde in phosphate buffer (113), and then stored in
the fixative in a refrigerator for about 24 hours before further preparation.

**MAN (IV, V, VI)**

**Controls (IV)**

Biopsy specimens of the small intestine from 12 healthy children of both sexes, aged 14 months to 14 years, with constitutional short stature, and 6 adult healthy volunteers of both sexes, aged 31 to 42 years, were studied. In the children, the biopsies were made to exclude a clinically silent intestinal disorder (52). All persons could eat conventional food without adverse reactions, and there were no signs or symptoms of any kind of gastrointestinal disease.

**Coeliac disease (V, VI).**

The small intestine mucosa from 28 children, aged 10 months to 15 years, that fulfilled the diagnostic criteria for coeliac disease as recommended by the European Society for Gastroenterology and Nutrition (89), were studied. Biopsy specimens were not taken from children with cow's milk protein intolerance (138), intestinal infections, fecal excretion of rotavirus, or from children in whom dietary lapses were suspected.

**Dietary variations (V, VI)**

Dietary variations with respect to gluten were instituted in two different ways, and the dietary instructions were given to the patients and their families by a specially trained, professional dietician.

1. Conventional long-term elimination and challenge with dietary gluten were both carried out to elucidate the relationship between the gluten intake and the morphological alterations as observed by LM and low-power SEM (V). For this purpose, small intestine mucosa biopsy specimens were collected at three occasions. The first biopsy was taken in the active phase of the disease while gluten was still in the diet. The second biopsy specimen was obtained after about one year of gluten elimination at a time when there were no clinical signs or symptoms of coeliac disease. The third biopsy was taken after another 4 to 6 months; during that time period dietary gluten was reintroduced; about 10 g gluten was given daily, i.e. corresponding to 4 slices of white wheat bread. Totally, 48 biopsy
specimens from 27 patients were available for SEM analysis.

2. Short-term gluten elimination and challenge were carried out in parallel with the long-term gluten alterations (VI). Elimination was performed 2 to 30 days prior to biopsy No. 1 or 3, and gluten challenge 1 to 20 days prior to biopsy No. 2. Totally, 26 biopsy specimens from 15 children were examined. In 14 of these 15 children, biopsy specimens subjected to conventional long-term variations of dietary gluten (see above) were also analyzed (V).

Collections of specimens (IV, V, VI)

Following an over-night fast, small intestine biopsy specimens were collected from the level of the ligament of Treitz under fluoroscopic control. In all the children and in 3 of the adults, standard suction biopsy instruments were used. In the remaining 3 adults, the biopsy specimens were taken by a Watson capsule, attached to a fibre gastro­scope.

The biopsy specimens were fixed at room-temperature in phosphate buffered 6.25 % glutaraldehyde within 30 sec after the biopsy capsule had been fired. After about 30 min, while the specimens were still in the fixative, they were preliminarily assessed under a dissection micro­scope (DM). Then, they were divided in two halves, one for LM and one for ultrastructural examinations. For the ultrastructural examination, they were kept in the fixative in a refrigerator at +4° C for at least 4 days before further preparation.

PREPARATIVE PROCEDURES

Light microscopy (LM)

Routine histopathological preparative techniques were used to examine LM serial sections. They were cut parallel to villi and crypts from paraffin embedded tissue specimens and stained with hematoxylin-eosin and according to the periodic-acid-Schiff (PAS) procedure (IV, V, VI). For stereological and morphometrical LM investigations (I, II, III), 2 μm sections from glycol-metacrylate-embedded tissue samples were stained with hematoxylin-eosin.

Scanning electron microscopy (SEM)

The fixed specimens were rinsed with about 5 ml of cold 0.9 % NaCl, using a syringe to flush the mucosa surface. Then, they were dehydrat-
ed in rising concentrations of ethanol, transferred to isoamyl acetate in rising concentrations and dried from liquid carbon dioxide in a critical-point drying apparatus (Polaron E 3000). After dehydration, the specimens were coated with approximately 20 nm gold in a modified vacuum unit (Edwards Vacuum Coating Unit, Mod E12-E14) and finally examined in a Cambridge S-4 SEM.

Each specimen was studied at three different magnifications during the same sequence.

1. At low-power magnification (x 100), the general architecture of the small intestine mucosa was assessed, particularly the shape of the villi.

2. At medium-power (x 1,000), the structure of the surface of the villi, the crypt-openings and areas between them were studied. In particular, the shape and delineations of the enterocytes and the size and distribution of the extrusion zones were examined.

3. At high-power magnification (x 10,000), the surface ultrastructure of the enterocytes was analyzed, particularly their glycocalyx layer and the underlying microvilli.

Transmission electron microscopy (TEM)
For stereological measurements (I, II, III), about 60 nm thick sections were cut on an LKB microtome and placed on formvar-coated 150 mesh copper grids. Section thickness was estimated by the method described by Small (125). After contrasting with uranyl acetate and lead citrate, the sections were studied either in a Siemens IA or in a Zeiss 109 Electron Microscope. Some specimens, primarily analyzed in the SEM, were subsequently embedded in Epon® and sectioned for TEM (90).

Alternative methods of fixation and preparation (IV)
To explore how the biopsy procedure and the fixation techniques may influence the surface structure of the small intestine mucosa, pilot experiments were carried out using gut mucosa samples from cats and rats (IV). These procedures included delay of fixation, rinsing of the mucosa surface before and after fixation, and variations in composition of the fixative and in the duration of fixation.
STEREOLOGICAL ANALYSES (I, II, III)

Light microscopical analyses

To calculate the enlargement of the intestinal surface area due to villi, stereological analyses were carried out using a square grid inserted into one of the eyepieces of the microscope. From each sample, five sections were cut perpendicularly to the length axis of the small intestine; they were also roughly perpendicular to the mucosa surface. The number of intersections between the grid lines and the epithelial surface \( I_C \) was counted and compared to the number of intersections \( I_t \) of a reference line drawn through the border between the villus bases and the crypt lumina. Sections parallel to the intestinal length axis were also used \( (1) \).

Morphometric analyses using an eyepiece micrometer were also carried out \( (III, IV) \). In each specimen the length of villi and of crypts, was measured. The height of the enterocytes at the crest region of the villi was also measured.

Electron microscopical analyses

Electron micrographs at a primary magnification of about 4,000 were taken of epithelial regions at the crests \( (I, II, III) \) and at the bases \( (I) \) of the villi. Such epithelial regions were micrographed only when the microvilli were sectioned length-wise or close to length-wise. Micrographs were selected at random from the crests and from the bases of the villi. Paper prints of these micrographs at a final magnification of about 11,000 were used for the subsequent morphometric procedures. Calibration of the magnification was performed using a carbon grating with 54864 lines per inch.

A transparent square lattice \( (I,II) \) or a Merz' lattice \( (III) \) test grid was placed over each paper print; its intersections served as test points (roughly 120 per 100 \( \mu m^2 \)). The regions used for morphometry were limited by two randomly placed parallel lines, about 10 \( \mu m \) apart, the apical plasma membrane and the basal plasma membrane. The number of test points falling over the entire epithelial region, over the nucleus, over the paracellular spaces and over the mitochondria, respectively, were counted. The number of intersections between the test lines and the apical cell membrane \( I_a \), and between the test
lines and a straight reference line drawn through the bases of the microvilli ($I_s$), was also counted. Using these values, the nuclear volume density ($V_{vn}$) was calculated in reference to the cell volume, the mitochondrial density ($V_{vm}$) in reference to the cytoplasmic volume, and the paracellular volume density ($V_{vp}$) in reference to the total epithelial volume (141). The surface density of the apical plasma membrane was determined in reference to the cell volume. The enlargement of the apical cell area ($E_{em}$) due to microvilli was calculated by dividing $I_a$ by $I_s$.

Errors associated with the stereological methods
Since the measurements of the apical surface density of the enterocytes were carried out mainly in regions where the microvilli had been cut length-wise, a systematic error has been introduced. This type of error has been discussed by Sitte (123), and in order to reduce it, the square lattice was tilted to an angle of about 20° with the apical cell surface (I, II).

Stereological formulas are valid only when the sections studied are infinitely thin. Assuming a section thickness of 60 nm, a microvillous length of 1.4 µm and a microvillous diameter of 0.13 µm, the values for the apical plasma membrane density ($I$) have been overestimated by some 25% (142).

Further errors are caused by the fixation, dehydration, embedding, sectioning and the preferred orientation of the sections (142). No attempts have been made to correct for these errors and, as a consequence, more attention was paid to the comparison between the different experimental groups than to the absolute figures. The personal error of the stereological measurements was estimated (I) and expressed as percentage of the corresponding mean values (42). The error was 6.8% for the nuclear volume density, 8.3% for the paracellular channel volume density, 6.7% for the mitochondrial volume density, and 4.0% for the surface density of the apical cell membrane.

Statistical analyses
Significances of difference between the mean values was calculated either using the two-tailed students t-test (I, II, III) or the
Mann-Whitney test for unpaired samples (IV) (126). The prerequisite for using the parametric test, viz. that the numerical series were normally distributed, was checked graphically only.

**NOMENCLATURE**

**LM:** The classification was made according to a three-graded scale (82) using the terms: Normal villous structure or slight alteration only (N), partial villous atrophy (PVA), and subtotal villous atrophy (SVA). This nomenclature corresponds to "grades I-II", "grade III" and "grade IV", respectively, of the classification of Alexander (1). The reason for classifying biopsy specimens with minor structural deviations only as N, was that such alteration of the small intestine mucosa do not significantly discriminate children with symptoms of gut disease from controls (102, 47).

**SEM:** The terms N, PVA and SVA was also applied to low-power SEM. They were developed from those originally used in DM and LM classifications (1, 114). As a rule, PVA corresponds to the term "low convolutions" and SVA to the classical picture of a "flat mucosa". The N was used when the structure of the biopsy specimens did not differ from that of the controls. In medium- and high-power SEM, no adequate terms to describe the structural alterations found are available. Some descriptive terms have been introduced. These are:

1. **Cobble-stone appearance (CSA),** indicating a convexity of the apical surface of the enterocytes.
2. **Enterocyte irregularity (ECI),** indicating evident variation in size and shape of the apical surface of the enterocytes.
3. **Microvillous irregularity (MVI),** indicating that the microvilli are irregular with respect to their length and frequency.
4. **Glycocalyx distortion (GCD),** indicating that the apical surface of the enterocytes is altered with disrupted and decreased glycocalyx. This alteration has been semiquantitatively graded in GCD+ and GCD++. 
RESULTS

ANIMAL RECOVERY AFTER THE OPERATIONS (II) AND AFTER ALLOXAN ADMINISTRATION (III)

The mortality was high after antral exclusion (about 50\%) with most of the deaths occurring within the first few days after the operation. Also in the antrectomized groups, the mortality was rather high (about 25\%); somewhat higher for the rats operated according to the Billroth II procedure. There were no fatalities after the sham operations or after the induction of diabetes by means of alloxan.

THE NORMAL SMALL INTESTINE MUCOSA (I, III)

Low and medium magnification (LM, SEM)

Assessments at low magnifications (LM, SEM) confirmed previously known species and age dependent differences in the architecture and the height of the villi and the depth of the crypts (31, 102, 108, 114, 136, 146). Thus, the villi of the rat duodenal and jejunal mucosa were regularly leaf-shaped, whereas the cat jejunal mucosa presented finger-shaped villi. In man, the architecture of the villi varied. Both finger-shaped and leaf-shaped villi occurred in the duodenal and jejunal mucosa, together with a variable proportion of ridge-shaped villi. In specimens from adults, finger-shaped or leaf-shaped villi predominated; occasionally a few ridge-shaped ones were also seen. In children, especially in those below two years of age, ridges were more frequent and often prevailed.

The surface enlargement due to villi was similar in the rat duodenal and jejunal mucosa. In man, there was a significant difference between the adult small intestine mucosa and that of the children: the villi were higher and the crypts were shorter in the adults.

The general structure of the surface of the villi was similar in all specimens studied, both from the animal and the human mucosa. Thus, the surface appeared smooth, interrupted by circular or irregular furrows. At the tip of most villi a demarcated extrusion zone was present, often displaying a few enterocytes with convex, almost domeshaped apical surfaces. Apart from the proximity of the extrusion zones, where the apical surface of the enterocytes could be slightly...
convex, they were generally flat.

High magnification (SEM, TEM)
The ultrastructure of the apical surface of the enterocytes was essentially the same in cats and man (IV). No differences were found between adults and children. In these specimens, the apical surfaces were covered with a prominent glycocalyx that obscured the underlying microvilli. The glycocalyx appeared smooth but with slight, irregular elevations and small pits. These pits tended to increase in size towards the tip of the villi and to partly anastomose in enterocytes with slightly convex apical surfaces. In contrast to the feline and human enterocytes, those of the rat displayed a thin glycocalyx on their apical surface and regularly distributed microvillous tips were easily discernable. In all species, the apical surface of the enterocytes became disorganized within the extrusion zones with fragmented and decreased glycocalyx (cats and man) and with irregularly formed microvilli that were loosely packed or even absent.

Stereologically, the density of the apical cell surface area of the enterocytes increased during their migration from the base to the crest of the villi, both in the fasted and non-fasted rats and both in duodenum and jejunum (I). This was also the case for the volume density of the paracellular channels and the height of the enterocytes. In contrast, the volume density of the nucleus and of the mitochondria decreased towards the crest of the villi.

The 24-hour fasting of the rats made the enterocytes slightly shorter compared to the non-fasted rats, both in duodenum and jejunum. In addition, the surface density of the apical plasma membrane was increased in jejunum (33%). Differences between duodenum and jejunum were found in fasted animals: both the surface density of the apical plasma membrane (28%) and the surface enlargement due to microvilli (41%) were larger in jejunal enterocytes.

Effects of variations in fixation and other preparative procedures (IV)
Delay of fixation resulted mainly in an increased size of the extrusion zones, and after 10 min, large portions of the villi showed severe
degeneration of the enterocytes, including desquamation. Mechanical forces operating during the biopsy procedure often damaged villi and enterocytes in and near the periphery of the biopsy specimens. OsO₄, used either as a primary fixative or during postfixation, dramatically altered the surface ultrastructure of the enterocytes: the pits and the low irregular elevations disappeared and the surface appeared fibrillar. In addition, following primary fixation with OsO₄, considerable amount of mucus clots remained at the surface of the villi.

Rinsing before or after fixation, use of 4% or 6.25% glutaraldehyde as a primary fixation, immersion of the mucosa specimens in the content of the gastric cavity, or prolonged fixation for up to 20 days, did not produce any obvious ultrastructural alterations at any of the magnifications studied. Even phosphate buffered 4% formaldehyde seemed to give a preservation of the fine structure of the mucosa surface quite similar to that obtained by means of the glutaraldehyde fixatives.

ADAPTION OF THE SMALL INTESTINE MUCOSA TO ANTRECTOMY AND ANTRAL EXCLUSION (II)

After creation of a duodenal blind loop by antrectomy according to the Billroth II procedure, the duodenal villi changed to finger-shaped, while the jejunal villi remained leaf-shaped. LM morphometric measurements revealed significant shortening of both the height of the villi (19-37 %) and the depth of the crypts (16-34 %) in the duodenal blind loop, both after antrectomy and after antral exclusion.

Stereologically, alterations were confined mainly to the size of the apical cell area. After antrectomy with gastroduodenostomy the jejunal enterocytes lost almost 40% of their apical cell surface, while no significant alterations occurred in the duodenum. After antrectomy with gastrojejunostomy there was a decrease in the apical cell area of the enterocytes in the jejunum by some 30%, and in contrast to antrectomy with gastroduodenostomy the apical cell area increased in the duodenum by almost 80%.

After antral exclusion with gastrojejunostomy a similar decrease of the apical cell area of the enterocytes occurred in the jejunum, and an increase in duodenum, although to a minor degree than
after antrectomy with gastro-jejunostomy.

The morphometric analyses of the mitochondria revealed a significant increase of their volume density in jejunum of antrectomized (B-II) and antrally excluded rats. No other changes were registered.

ADAPTATION OF THE SMALL INTESTINE MUCOSA TO ALLOXAN DIABETES
The structural alterations of the small intestine mucosa were confined to the jejunum. Light microscopically, both the height of the villi (31 %), the depth of the crypts (34 %) and the height of the enterocytes (13 %) increased in the diabetic rats. Ultrastructurally, the apical cell area of the jejunal enterocytes decreased slightly (12 %). In addition, their nuclear volume density increased with some 20 %.

ADAPTATION OF THE SMALL INTESTINE MUCOSA IN COELIAC DISEASE (V, VI)
Active disease and after conventional long-term gluten challenge (V)
The LM and low-power SEM results confirmed the well known mucosal response to long-term influence of dietary gluten. SVA prevailed in biopsy specimens from untreated children, whereas the frequency of PVA were more common in biopsy specimens taken after the long-term gluten challenge following treatment. As a rule, the mucosa structure assessed by means of low-power SEM, agreed well with the picture obtained by means of LM in cut sections.

By medium-power SEM (x 1,000), the size and shape of the apical surface of the enterocytes varied considerably. This appearance was assessed as enterocyte irregularity (ECI) and was most obvious in SVA specimens. In addition, the apical surface of the enterocytes appeared convex giving the mucosa surface a cobble-stone appearance (CSA). This appearance was present particularly at the crests of the villi in PVA specimens. The extrusion zones seemed to be increased in size but, as in the controls, they were sharply demarcated. In PVA specimens, they were found at the crests of the villi, in SVA specimens between the collar-like area that surrounded the crypt openings.

High-power SEM and supplementary TEM analyses revealed that the glycocalyx was disrupted and decreased (GCD) and that the microvilli were irregular and shorter than normal (MVI). In biopsy specimens showing PVA, the surface ultrastructure abnormalities were particular-
ly obvious at the crests of the villi and along the upper parts of their sides. In SVA-specimens, they were spread all over the mucosa except for within the crypt openings where more normal conditions prevailed.

After conventional long-term gluten elimination (V)

After long-term treatment with gluten-free diets, the children appeared clinically healed. In general, this was also the case for the small intestine mucosa structure as assessed by LM and low-power SEM. In the two specimens where LM and low-power SEM disagreed, SEM analysis presented normal leaf- and ridge-shaped villi in spite of that LM fulfilled criteria for PVA.

In contrast to the assessments by low-power magnifications, medium-power SEM showed persistent CSA rather frequently (15 out of 21). On the other hand, the apical borders of the enterocytes were regular with respect to their size and delineations, i.e. no obvious ECI remained. The CSA was mainly limited to the crest of the villi. Also by high-power SEM, surface ultrastructural alterations were found in a rather high frequency (12 out of 21 biopsy specimens). These alterations resembled those seen in the mucosa specimens from children with untreated coeliac disease, and consisted of both GCD and MVI. As with CSA, GCD was limited to the crest of the villi, and the severity of the alterations varied among the biopsy specimens.

After short-term gluten elimination (VI)

By LM, low- and medium-power SEM, no fundamental differences were found in comparison with the results obtained from the assessments of biopsy specimens from untreated children or those subjected to long-term gluten challenge and where short-term gluten elimination was not performed. In contrast, assessment by high-power SEM, revealed a clear-cut restoration of the ultrastructure of the apical surface of the enterocytes (GCR) in not less than 12 out of 13 biopsy specimens taken from 2 to 7 days after initiation of gluten elimination; in one case even as early as after 2 days. The restoration consisted mainly of a normalization of the glycocalyx covering the mirovilli.
After short-time gluten challenge (VI)

At low magnifications, LM revealed PVA in 5 specimens while 6 were assessed as N. By low-power SEM one specimen showed low convolutions (PVA) while the remaining 10 had a normal appearance.

The general structure of the surface of the villi (medium-power SEM) and the ultrastructure of the apical surface of the enterocytes (high-power SEM) did not differ from biopsy specimens of the treated children not subjected to short-time gluten challenge. Thus, CSA was found in 6 out of 11 specimens and surface ultrastructural lesions (GCD, MVI) in 7 out of 11 specimens. It should be noted however, that there was a tendency for the ultrastructural lesions to aggravate with the time of gluten provocation, and after 7 days of provocation or more GCD++, MVI was observed in 3 out of the 4 specimens examined.

DISCUSSION

SEM PREPARATIVE PROCEDURES (VI)

The main steps in preparing biological tissues for SEM include fixation, dehydration and coating. Different procedures may give variable results (50), and a true picture of the in vivo surface ultrastructure can probably not be obtained. Therefore, attention should be focused on comparisons of the surface morphology after the different experimental and dietary procedures, using adequate controls.

Considerable experimentation preceded the choice of the SEM fixative of 6.25% glutaraldehyde in phosphate buffer solution where the ionic strength of the buffer was about 200 mOsm. The active buffer osmolarity has been shown to be of great importance for SEM analysis of certain cell types (22, 23). This seems not to be the case for the enterocytes, and buffer osmolarities of 150 to 300 mOsm gave roughly the same ultrastructural appearance of the apical surface of the enterocytes. It should also be noted that osmotic forces operating within the lamina propria vary considerably from the bases to the crests of the villi (69).

Shrinkage of cells and distortion of cell surfaces following SEM preparation is influenced by stabilization of the cell membranes during fixation (8, 17, 18, 19). Using cell culture techniques, it has
previously been pointed out that an adequate cell membrane stabilization can be achieved by postfixation in OsO₄ (8, 19, 48) after initial glutaraldehyde fixation, or by increase of the fixation time to several days when only glutaraldehyde solutions are used (17, 19). The influence from fixation times and type of fixative are not known in detail for stabilized tissue such as the epithelial layer of the small intestine. Current reports on small intestine mucosa structure have used glutaraldehyde fixation times, varying from "over night" to 30 days (10, 25). Our results show comparable surface ultrastructure of the apical surfaces of the enterocytes when fixed in only glutaraldehyde for 1 to 20 days (IV). Therefore, OsO₄ was avoided in the preparative procedure. It should also be noted that simplicity of the preparative procedures is advantageous when dealing with assessments of material for clinical routine diagnostic purposes.

It is almost a truism to state that rapid fixation is important for adequate preservation of the ultrastructure. For TEM studies of animal tissues, perfusion fixation is usually preferred. Since immersion fixation is the only possible procedure for biopsy specimens from human beings, meticulous care should be taken not to delay fixation since autolytic processes rapidly destroy the structure of the cells. It should be stressed, that the commonly used initial DM assessment of the small intestine mucosa prior to fixation should be avoided, since this delays fixation, and structural alterations could be misinterpreted as actual pathological lesions by means of SEM. It might, however, be performed after immersion of the specimen in the fixative.

Structural artifacts are produced by the suction biopsy procedure and by instrumental manipulation of fresh tissue samples. Our observations (IV) indicate that this is the case, and peripheral parts of the suctioned biopsy specimens should therefore not be included in the structural analyses.

It has been pointed out that metal coating of specimens for SEM may obscure surface ultrastructural features (40), but in the present study this has not hampered the observations.

THE STEREOLOGICAL TECHNIQUE (I)

The stereological technique and its errors have been discussed in
detail in the Material and Method section (page 24).

THE NORMAL ENTEROCYTE AND ITS DIFFERENTIATION (I, IV)

Our stereological data on enterocytes from the base and from the crests of the villi (I) confirm previously noted structural differences between these cells (6, 20, 104, 132, 154). These differences reflect changes in age and functional state of the enterocytes as they move up towards the crest of the villi (105, 117). In particular, the increase of the apical surface area of the enterocytes towards the crests of the villi corresponds to the increase in the disaccharidase and the dipeptidase activities located to the apical plasma membrane (96).

The decrease in the volume density of the nuclei and of the mitochondria towards the crest of the villi seems mainly to reflect an increase in cell volume rather than an actual change in absolute figures. This suggestion gets support from the increase of the height of the enterocytes. It is also supported by Plattner and Klima (105), who found no significant nuclear volume changes between the enterocytes of the crypts and the villi.

Studies on gall-bladder epithelium (14, 35), have shown that the paracellular spaces represent an important compartment for water transport. These spaces are large during high transport but collapsed when transport is inhibited. The small intestine epithelium apparently reacts in a similar fashion (130), and a comparison between our base and crest values of the paracellular spaces shows significantly higher values for the crest epithelium, presumably indicating a greater water transport at the crest of the villi.

The interspecies variations of the glycocalyx layer, observed by means of SEM in this study (IV), confirm those made previously by means of TEM (65, 67). Previous SEM studies (9, 55, 86, 106, 107) have inferred that the microvilli are difficult to visualize in man, and Phillips (103), using preparative techniques avoiding coating procedures, suggested that the substance obscuring the microvilli probably represents the glycocalyx. This view is supported by the following SEM observations in cat, rat and man: (1) Microvilli are clearly observed in the rat enterocytes in contrast to those seen in both the human and
the feline small intestine mucosa where they are covered by a layer making the microvilli invisible; (2) The SEM appearance of this layer varies with different fixation procedures (glutaraldehyde or OsO₄); this is in agreement with previous TEM studies (109); (3) Disruption and decrease of this layer of the apical surfaces of degenerated enterocytes occurs in the extrusion zones; this is also in agreement with previous TEM observations (45, 65, 95); (4) A concordance exists between the results of the SEM and the supplementary TEM analyses from corresponding regions in this study.

EFFECTS OF "PHYSIOLOGICAL" ADAPTION (I, II, III)

"Physiological" adaption of the small intestine is structurally reflected by alteration of its absorptive surface (39, 147). Luminal nutrition, pancreato-biliary secretions and hormonal factors probably represent major factors. Adaption of the small intestine mucosa following experimental procedures such as resection, by-pass and experimental diabetes are rapidly initiated (39, 147). In hyperplastic adaption, the primary event seems to be an increased proliferation of the enterocytes with a probable imbalance between their production and extrusion. Within 2 weeks a new steady state develops and after 4-5 weeks, the period of observation in this study, morphological adaption seems to be fully established (39, 147).

The changes in the architecture of the villi (finger-shaped villi instead of leaf-shaped), and the decrease both in depth of the crypts and height of the villi, observed in the duodenal blind loop after antrectomy, are similar to observations made in jejunal and ileal self-emptying blind loops and associated with a hypoplastic condition (57). A slower migration and turnover of enterocytes occurs in bypassed segments of the jejunum and ileum (147), and it is quite conceivable that this also occurs in a duodenal blind loop. In jejunal blind loops, the absorptive capacity per unit length of the small intestine is decreased. In contrast, it has been noted that the absorptive capacity of the mucosa is increased when expressed per unit area of the mucosa surface (91). This might reflect a larger amount of enterocytes per unit area or increased amounts of transport sites of the apical plasma membrane. The latter alternative is compatible with
our findings concerning the apical cell area of the enterocyte: The increase in the microvillous surface area, observed in enterocytes on the crest of the villi from the rats with duodenal blind loops, most likely provides a structural basis for such an increased absorptive capacity. This in turn, could be linked to a localized alteration in the maturation and ageing processes.

The conditions of a self-emptying duodenal blind loop connected with operative procedures on the antral part of the stomach are quite complex. No food passes through it. The pancreato-biliary secretions are more concentrated, although the secretory activity of the liver and of the pancreas may be changed (an increase in secretory granule density is observed in pancreatic exocrine cells following Billroth II operation; Emdin and Stenling, preliminary data). In addition, the presence of luminal microorganisms might be altered. It could be speculated that the difference in apical cell area between the antrally excluded and the Billroth-II-operated rats could be due to different serum gastrin levels. Increased serum gastrin levels after antral exclusion (2) may result in an increased rate of enterocyte migration (71), compared to antrectomy with gastro-jejunostomy (62, 97); after antral exclusion the absorptive cells at the villous crests would then be less differentiated, and their apical cell area less developed.

Intestinal segments in continuity after by-pass operations experience hyperplasia with increased proliferation of enterocytes (39, 147). Such cell kinetic alterations are associated with increased rates of migration of the enterocytes (145). However, their turnover time, i.e. the time from the production to the final extrusion of the enterocytes, might not be altered (147). In the present study (II), the intestinal segments that remained in continuity after the operations showed slightly longer villi and crypts than the control rats but not when compared to the sham-operated rats. Intraluminal factors provide the most likely explanation for the decrease in the apical surface enlargement of the enterocytes: the jejunum is filled more rapidly since the pylorus no longer regulates the gastric emptying. Gastrin does not appear to play any major role since no difference was found between the antrally excluded rats and those with antrectomy. It is also possible that the morphological transformation observed - de-
crease in apical cell area and increase of mitochondrial density - reflects a "duodenalization" of the jejunal mucosa.

In addition to intestinal resection and small intestinal segments in continuity after by-pass operations (39, 147), experimental diabetes mellitus also is known to evoke hyperplasia of the small intestine mucosa (39, 93, 147). Several factors seem to contribute to this hyperplasia, including both increased food intake and an altered hormonal milieu (93, 147). Our results (III) - increased length of both the villi and the crypts - support previous observations of the presence of a hyperplasia as regards the jejunal mucosa. Moreover, this mucosal hyperplasia was associated with structural alterations of the enterocytes. The slightly decreased apical cell area and increased nuclear volume density indicates that the differentiation of the enterocytes might be altered.

Phillips et al. (104), studying the jejunal enterocytes in children, observed a decrease of their apical surface area in the crest-region compared to the mid-region of the villi. They suggested that this decrease might occur as an adverse luminal effect due to the exposure of a more concentrated luminal content to the enterocytes at the crest of the villi. In the present study (III), the diabetic rats ate more food than the control rats, and it might be possible that the decrease in apical surface area of the enterocytes in the diabetic rats, can be influenced by such an adverse luminal effect. It is also of interest to note that segments in continuity after gastrojejunostomy in rats (II) also display a decreased apical surface area of their enterocytes, and that a decrease in apical surface density and in height of the jejunal enterocytes occurs in non-fasted rats compared to those starved for 24 hours.

EFFECTS OF "PATHOLOGICAL" ADAPTION (V, VI)

Most authors agree that sensitivity to gluten results in a diffuse, generalized, structural alteration of the proximal small intestine mucosa although a few patients with coeliac disease have been said to present a patchy mucosal lesion (83). The diffuse, generalized character of the lesion is consistent with the observations made in the pre-
sent study, both concerning the architecture of the villi and the fine structure of the enterocytes (V, VI).

Previously, both ECI and CSA of the small intestine mucosa have been reported to occur in coeliac disease (9, 85, 86). These lesions seem, however, to represent rather unspecific cellular alterations; they have also been found in duodenitis (115) and in several experimental conditions (68, 101). Toner et al. (131) has pointed out that CSA could be of artificial origin and mainly due to the preparative procedures. With the technique used in this study, this possibility seems unlikely since neither CSA nor ECI were found in control specimens (IV, V, VI). It should also be noted that the terminal web of the enterocytes and its connected intracellular fibrils, which are thought to stabilize the apical part of the enterocytes, seem to be defective in coeliac disease. Such a lesion in the cytoskeleton of the enterocytes may contribute to the formation of CSA.

That presence of MVI of the enterocytes represent a common feature in untreated coeliac disease is well-known from previous TEM reports by others (112, 119). Distortion of the glycocalyx (GCD) has been suspected (9) and also inferred from TEM studies (64). In addition, Madara and Trier (80) pointed out that there is a decrease in the number of intramembrane particles of the apical plasma membrane. With the SEM technique used in this study (V, VI), both the alterations of the microvilli (MVI) and those of the glycocalyx (GCD) could be identified. Since both GCD and MVI were also observed in the apical surface of the degenerated enterocytes that were being extruded from the villous tips in the control specimen, our findings support the hypothesis of Araya and Walter-Smith (7) that the structural lesions of the enterocytes in coeliac disease are unspecific.

As shown in the Results, despite treatment with gluten-free diets for extended periods of time, persisting, ultrastructurally detectable lesions of the apical surface of the enterocytes were found in not less than 12 out of 21 cases in spite of a LM normalization in 17 out of the 21 specimens. This observation could be explained by: (1) Commercially available "gluten-free" flour contains small amounts of gluten (27); (2) Variations occur in the response of the small intestine mucosa to gluten exposure (56); (3) Difficulties exist to persuade
patients to maintain the gluten-free diet over long periods of time (53).

From the distribution of the surface ultrastructural lesions observed by means of SEM - more extensive at crests in PVA specimens, mainly limited to crests after long-time gluten elimination; confined to the whole mucosa surface except within the crypts openings in SVA specimens - it can be speculated that noxious gluten fractions initially adhere to the enterocytes. Results obtained by others in vitro (38) may indicate such an initial mechanism. It should also be noted that the biopsy specimens of the small intestine mucosa were taken about 12 hours after the last meal; during this time the effects of gluten on the enterocytes that migrate from the crypts is not known.

The time needed for the structure of the enterocytes to normalize on gluten withdrawal in patients with active coeliac disease is still not clear. Since the enterocytes are rapidly renewed - about every 24 hours (135) - it might be expected that a considerable normalization of the enterocytes would take place within a few days. This has been indicated with respect to the height of the enterocytes (152) and in vitro studies also support such an assumption (21, 61, 72, 139). However, conflicting results exist with respect to the importance of gluten on the restoration of the enterocytes (59). The present study (V) shows that the surface ultrastructure of the enterocytes is rapidly normalized (within a week), reflecting a structural normalization of the enterocytes. The slight alterations that persist, i.e. irregularities of the microvilli as indicated by TEM, probably reflect an inadequate maturation of the enterocytes due to their rapid turnover in the flat mucosa.

The time needed for gluten to induce a structural lesion of the enterocytes of treated patients with coeliac disease is not known (26, 36, 127). The amount of gluten given and a variable sensitivity to gluten obviously influence the results reported (56), and conflicting observations have been made both in vitro (43, 61) and in vivo (36, 119, 122). From the results of the present study no further conclusions can be drawn.

From a diagnostic point of view (IV, V, VI), the SEM technique provides a simple and reproducible routine procedure for the assess-
ment of small intestine mucosa samples in children adding further structural variables to be analyzed compared to light and dissecting microscopy. By including SEM in the morphological assessments it may be possible: (1) To enhance the diagnostic precision of those biopsy specimens where LM is not conclusive and where a considerable variation in the LM assessments exists; (2) to dramatically shorten the time needed for an adequate information on the structural response to gluten withdrawal in children; (3) to differentiate coeliac disease from other diseases affecting the small intestine mucosa where LM is inadequate. These include cow's milk protein intolerance, post enteritis syndrome in children and dermatitis herpetiformis, where an irregular or patchy alteration is claimed to exist (138), also at the cellular level (106, 107).

GENERAL SUMMARY WITH CONCLUDING REMARKS

Summary
Both on altered physiological demands and in pathological states related to excess trauma, the small intestine mucosa has a large ability to alter its absorptive capacity with related adaption of its structure, enzyme activity and cell kinetic conditions. By applying SEM and quantitative LM and TEM morphological methods, the overall objective of this study was to contribute to the understanding of the structural reaction of the enterocytes in both "physiological" and "pathological" adaption of the small intestine mucosa.

The normal small intestine mucosa
The main structural features of the small intestine mucosa comprises the villi, the crypts of Lieberkühn and the lamina propria. The structure of the villi is known to show marked interspecies variations; this was illustrated using rats, cats, and man. In man, age variations are also known to occur; the villi were found to be shorter and the crypts longer in children; small children presented more ridge-like villi.

By means of SEM, the normal enterocytes were found to present flat apical surfaces with regular polygonal delineations. Evidently,
the ultrastructure of their apical surface is influenced by the thickness of the covering glycocalyx layer; microvilli were easily seen in rat absorptive cells but were obscured by the glycocalyx in cat and human enterocytes. No differences in the surface ultrastructure were found to exist between human adults and children. Extrusion of the enterocytes from the crests of the villi is structurally represented by distinct extrusion zones, comprising degenerated cells with irregular, convex apical surfaces and with distorted surfaces ultrastructure and cytoplasmic disorganisation.

By means of a TEM stereological analysis of the mucosa of the proximal small intestine in the rat, the duodenal enterocytes were found to differ from their jejunal counterparts by a lower apical cell area and a higher mitochondrial volume density indicating a functional difference.

In rats, 24 hours' fast was found to shorten the total length of the small intestine by some 20% and to slightly decrease the height of the enterocytes both in the duodenum and in the jejunum.

The normal differentiation of the absorptive cells in the fasted rat, as they move up along the villi, was found to be reflected structurally by increase (64 - 103%) of the apical cell area, increase (169 - 216%) of the paracellular channels and increase (19 - 24%) in cell height. The increment of the apical cell area and the paracellular channels is compatible with an enhanced capacity for terminal digestion and transport of water and electrolytes. The decrease in mitochondrial volume density and nuclear volume density from the bases to the crests of the villi was supported to be mainly a consequence of an increased cell volume.

Self-emptying duodenal blind loop ("hypoplastic" adaption)

Creation of a self-emptying duodenal blind loop in the rat, either by antrectomy or by antral exclusion, both combined with gastrojejunostomy, was found to produce structural changes of the villi and the crypts associated with hypoplasia of the mucosa. The related alterations of the structure of the enterocytes consisted of an increase in their apical surface area by 78% (antrectomy) and 28% (antral exclusion), indicating an increased functional capacity of the brush border.
It was speculated that gastrin might indirectly influence on the structure of the duodenal enterocytes.

Direct continuity of jejunum with the gastric cavity
Creation of a direct continuity of the jejunum with the gastric cavity (gastrojejunostomy) resulted in a decrease of the apical surface area of the absorptive cells of the crests of the villi by about 30 - 40 % and an increase of their mitochondrial volume density by about 20 - 30%, indicating a "duodenalization" of the jejunal mucosa. Intraluminal factors seem to play a major role for this structural adaption of the enterocytes.

Experimental diabetes mellitus ("hyperplastic" adaption)
Short-term alloxan diabetes in the rat increased the length of the villi (31 %) and the depth of the crypts (34 %) in the jejunum. The related alterations of the enterocytes to this hyperplastic adaptive condition consisted of a slight decrease in their apical cell area (12 %) and increased density of their nuclear volume (23 %). Cell kinetic alterations and/or luminal factors are presumably responsible.

Coeliac disease (adaption to "excess trauma")
The results of this study confirm the well-known DM and LM structural characteristics of the small intestine mucosa following long-term traumatic influence of gluten with excessively increased extrusion of enterocytes, i.e. absence of villi (SVA), or presence of only short villi (PVA), together with an increased depth of the crypts. The three-dimensional surface picture of the mucosa lesion, as assessed by SEM, was found to be of diffuse character without any patchy alterations.

The related fine structural alterations of the enterocytes, observed by means of SEM, were found to be represented by a change of their apical surface from a flat, regularly polygonal form to an irregular and often convex shape. Their glycocalyx layer was usually distorted, combined with irregularity of the microvilli. The distribution of these fine structural alterations of the enterocytes within the mucosa surface is consistent with the view that they represent lesions caused by the traumatic influence of gluten and not just the presence
of immature enterocytes, that are rapidly migrated from the crypts.

After treatment with long-term dietary gluten elimination, most of the biopsy specimens examined became normal when assessed by conventional LM and low-power SEM. In contrast, medium and high-power SEM often showed persisting abnormalities of the mucosa surface. Thus, by including SEM in the assessment of biopsy specimen from children with coeliac disease, the diagnostic precision was increased. It was discussed that influence of minor amounts of gluten and difference in sensitivity to gluten of the small intestine mucosa might be of importance for the ultrastructural lesions observed by SEM.

Short-term treatment with gluten-free diets did not alter the severe lesions of the mucosa structure as observed by conventional LM and low-power SEM. In contrast, high-power SEM revealed a clear-cut restoration of the surface ultrastructure of the enterocytes, in one biopsy specimen as early as after 2 days. It was concluded that SEM analysis of surface ultrastructural features can dramatically shorten the time needed to measure an adaptive effect on the structure of the small intestine mucosa after gluten withdrawal.

Short-term gluten challenge of children with coeliac disease after long-term treatment did not significantly affect the surface ultrastructure of the enterocytes.

Concluding remarks
Considering the enterocyte in adaption of the small intestine mucosa, it is concluded that:
1. The structure of the enterocytes, in particular their apical surface, is involved both in "physiological" and "pathological" adaption of the small intestine mucosa.
2. The SEM picture of the apical surface of the normal enterocytes in man, cat and rat, is in agreement with previous knowledge obtained by means of TEM. In particular, the glycocalyx seems to be important for the character of the SEM appearance of their surface ultrastructure. Quantitatively, the apical surface area of the normal enterocytes increased during their differentiation from the base to the crest of the villi.
3. In "physiological" adaption, the apical surface area of the entero-
cytes increases in conditions associated with gut mucosa hypoplasia (self-emptying duodenal blind loop) and decreases in conditions associated with gut mucosa hyperplasia (short-term experimental diabetes, small intestine in continuity after by-pass procedures). The mechanism seems to be complex and both altered differentiation of the enterocytes mediated by hormonal and intraluminal factors as well as a direct effect of intra luminal factors on the enterocytes may influence.

4. In "pathological" adaption, the presence of structural lesions of the enterocytes due to traumatic influence of gluten in coeliac disease in children can be visualized by means of SEM analysis of their apical surfaces. These structural lesions of the enterocytes might serve as a more sensitive indicator of influence from gluten than the structure of the villi since they were observed in small intestine mucosa specimens where conventional LM was normal. Therefore, addition of SEM analysis to routine histopathological assessment of the small intestine mucosa in coeliac disease in children seems to improve the diagnostic precision.

5. In contrast to the structure of the villi that remains severely damaged, elimination of gluten from the diet rapidly (within a week) produce a clear-cut adaptive restoration of the surface ultrastructure of the enterocytes in children with coeliac disease. Thus, the surface ultrastructure of the enterocytes can be used as a rapid structural indicator of adaption of the small intestine mucosa to gluten withdrawal.
ACKNOWLEDGEMENTS

This work was supported by grants from the Swedish Medical Research Council (Projects Nos. 12X-718 and 12X-2298), from Tore Nilsson's Fund for Medical Research, from the Group Insurance Co. "Förenade Liv", from the Swedish Diabetes Association and from the Medical Faculty, University of Umeå.

In addition, I wish to thank all those who have guided, assisted and supported this work, which was performed at the Departments of Pathology, Anatomy, and Pediatrics, University of Umeå. In particular, I wish to express my sincere gratitude to:

My supervisors, Professor Sture Falkmer, Professor Herbert F. Helander and Docent Bo Fredrikzon. They introduced me to this field of science, and without their enthusiasm and their mild pressure, this work would probably not have been finished.

Professor Frank Bergman, Head of the Department of Pathology, for valuable discussions.

Professor Axel Bergenholz for stimulating cooperation
My colleagues, Dr Henry Nyhlin, Doc Erik Hägg and Dr Stefan Engberg for valuable criticism and for their work with the patients.
Mrs Kerstin Näslund, Mrs Ewa Wikman, Mrs Sigrid Kilter, and Miss Ulla Hedlund for skilful technical assistance.
Mr Bengt Carfors for excellent photographic work, never failing in enthusiasm.
Mr Per Hörstedt, for his knowledge, guidance and assistance in the field of SEM.
Mrs Inga Greta Nilsson and Mrs Birgitta Forsgren for typing this Thesis.
All my friends and colleagues at the Departments of Pathology, Anatomy, Pediatrics and Internal Medicine.
My family; Hjördis, Fredrik, Daniel, Maria, Henrik and Johannes for not allowing me to spend most of my time with this project.
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