Specificity of ultrastructural changes of small intestinal epithelium in early childhood

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Araya, M., and Walker-Smith, J. A. (1975). *Archives of Disease in Childhood*, 50, 844. **Specificity of ultrastructural changes of small intestinal epithelium in early childhood.** Electron microscopical features of the small intestinal mucosa in 30 consecutive biopsies from 14 newly diagnosed coeliac patients, and 16 patients with chronic intermittent diarrhoea due to different causes, were studied. An attempt to define specific changes in each condition was made.

An abnormal brush border, increased number of free ribosomes, rich rough endoplasmic reticulum, poorly developed smooth endoplasmic reticulum, large Golgi complexes, and thickened basal lamina were the most frequent findings in the coeliac biopsies. These changes suggest abnormal cellular metabolism, but as they were also observed in children with a variety of clinical conditions, they could not be specifically related to coeliac disease. These observations suggest that in small children different causes leading to different degrees of abnormal small intestinal mucosa, as observed with the light microscope, produce nonspecific changes of the cellular ultrastructure, which cannot be related to the degree of abnormality observed at the light microscope level.

The ultrastructure of the small intestinal epithelium has now been studied by a number of observers, during normal and pathological conditions, in the search for typical features to characterize the different diseases (Hartman *et al.*, 1959; Palay and Karlin, 1959; Rubin *et al.*, 1960; Brown, 1962; Trier, 1963). Coeliac disease is one of the entities most extensively studied and abnormalities of the brush border, lysosomes, nuclei, and thickening of the basal membrane have been considered as typical of this disease (Hartman *et al.*, 1960; Shiner and Birbeck, 1961; Curran and Creamer, 1963; Trier and Rubin, 1965; Shiner, 1967; Biempica, Toccalino, and O'Donnell, 1968). The thickening of the basal lamina has been shown to be one of the first and more constant changes to occur after challenging a patient in remission with gluten (Biempica *et al.*, 1968; Shmerling and Shiner, 1969; Shiner and Shmerling, 1970). However, these types of changes have also been reported in recent years in other conditions, such as cow's milk protein intolerance (Kuitunen *et al.*, 1973), parasitic infestation (Ament, 1972), postgastroenteritis syndrome (Ament, 1972), and soya bean intolerance (Ament

and Rubin, 1972). There is little information about the specificity of the ultrastructural changes of the small intestine in young children when mucosal damage occurs due to coeliac disease and when it is secondary to causes other than coeliac disease.

The present study was planned to review the ultrastructure of the small intestinal mucosa of 30 consecutive small intestinal biopsies taken from children at the Royal Alexandra Hospital for Children. These 30 biopsies included children with probable coeliac disease, children who had nonspecific mucosal damage (partial villous atrophy), and children with normal small intestinal mucosa. We compare and contrast the ultrastructural appearances found in these types of children, with special emphasis on those signs thought to be 'typical' of coeliac disease.

Material and methods

Patients. 30 consecutive children, 18 males and 12 females, between the ages of 8 months and 12 years, were studied, details of whom are set out in Table I.

Fourteen children were considered to have probable coeliac disease, as the following criteria were fulfilled. (i) A suggestive clinical history. (ii) A flat or almost flat, convoluted pattern with visible crypt openings on

TABLE I

Jejunal biopsy appearances in 14 coeliac patients, and in 16 children with other forms of diarrhoea

Case no.	Age (yr) (m)	Diagnosis	Dissecting microscopy	Light microscopy
1	8	Coeliac disease	Flat	Subtotal villous atrophy
2	1 4	"	"	"
3	1 5	"	"	"
4	1 6	"	"	"
5	1 6	"	"	"
6	1 6	"	"	"
7	2	"	"	"
8	2 2	"	Convoluted pattern with visible crypt openings	Severe partial villous atrophy
9	4 2	"	Flat	"
10	4 8	"	Flat	"
11	5 6	"	Convoluted pattern with visible crypt openings	"
12	6	"	Convoluted pattern with visible crypt openings	"
13	6 6	"	Flat	Subtotal villous atrophy
14	10	"	Flat	Severe partial villous atrophy
15	9	Failure to thrive, chronic intermittent diarrhoea	Normal	Normal
16	9	Failure to thrive, chronic intermittent diarrhoea	"	Normal
17	1	Postgastroenteritis syndrome	"	Mild partial villous atrophy
18	1 1	Chronic intermittent diarrhoea	"	Mild partial villous atrophy
19	2	Steatorrhoea of unknown origin	"	Normal
20	2 3	Giardiasis	"	Mild partial villous atrophy
21	2 3	Postgastroenteritis syndrome	"	Normal
22	2 6	Chronic intermittent diarrhoea; ?irritable colon syndrome	"	"
23	2 9	Cow's milk intolerance	"	"
24	3 9	Failure to thrive, chronic intermittent diarrhoea	"	"
25	4	Chronic intermittent diarrhoea	"	"
26	5	Normal child, sib of child with vitamin B ₁₂ malabsorption	"	"
27	5 3	Failure to thrive, chronic intermittent diarrhoea	"	"
28	8	Chronic intermittent diarrhoea; mental retardation	"	"
29	11	Normal child, sib of child with vitamin B ₁₂ malabsorption	"	"
30	12	Chronic intermittent diarrhoea; ? irritable colon syndrome	"	"

dissecting microscope examination of the small intestinal biopsy. (iii) Subtotal or severe partial villous atrophy with hypertrophic crypts and increased cellularity of the lamina propria on light microscope examination of the biopsy. (iv) Good clinical response to a gluten-free diet.

As this study was carried out prospectively, these children have not yet been challenged with gluten after a period of thriving on a gluten-free diet. Therefore, the diagnosis of coeliac disease still remains to be

proven, but nonetheless is highly probable. For comparison they will hereafter be referred to as coeliacs.

Sixteen children were biopsied because they had chronic intermittent diarrhoea and failure to thrive. Two were sibs of a child with vitamin B₁₂ malabsorption. Dissecting microscope examination of all these biopsies was regarded as being within normal limits (i.e. tongue-like, leaf-like, and long thin ridge-like villi). Light microscopy was normal in 12 cases and showed a

moderate degree of partial villous atrophy in four biopsies (Table I). In three (Cases 15, 22, and 30, Table I) an abnormal sociopsychological history was obtained and a motility disorder related to this was diagnosed (? irritable colon syndrome). In one child (Case 23) there was a history strongly suggestive of cow's milk intolerance. In 2 children (Cases 17 and 21) there was an antecedent history of acute vomiting and diarrhoea, and the diagnosis of postgastroenteritis syndrome was made. In the remaining cases there was no definite diagnosis made.

Biopsies. All biopsies were taken at the duodeno-jejunal junction with a paediatric Crosby biopsy capsule (Watson modification) and cut into two. One half was

fixed in formalin for routine light microscope study. The other half was cut into two again and processed as follows.

The biopsy was fixed in freshly prepared (a) bicarbonate buffered 2% OsO_4 , pH 7.4 for one hour, and/or (b) cacodylate buffered 2.5% glutaraldehyde, pH 7.4 for one hour, and postosmicated in 2% buffered OsO_4 , pH 7.4 for one hour. The biopsies were then treated with uranyl acetate (Watson, 1958), dehydrated in graded ethanols and propylene oxide, and afterwards embedded in Epon 812. Thick sections were obtained and stained with toluidine blue (Trump, Smuckler, and Benditt, 1961). A middle third villus area was selected in one block and a deep crypt area in the other block. Numerous thin silver sections were cut from these areas,

Morphological appearance of Golgi apparatus, free ribosomes and polysomes, rough endoplasmic reticulum

Case no.	Golgi	Polysomes and free ribosomes		Rough endoplasmic reticulum
		Villus	Crypt	
1	Prominent	Numerous	Very numerous	Normal
2	Prominent	Few	Numerous	Very abundant
3	Prominent	Very numerous	Very numerous	Poor
4	Prominent	Few	Very numerous	Normal
5	Prominent	Numerous	Very numerous	Abundant
6	Prominent	Very numerous	Very numerous	Normal
7	Normal	Normal amount	Numerous	Normal
8	Prominent	Very numerous	Very numerous	Abundant
9	Prominent	Numerous	Very numerous	Normal
10	Prominent	Very numerous	Very numerous	Poor
11	Normal	Very numerous	Numerous	Abundant
12	Prominent	Normal amount	Very numerous	Abundant
13	Prominent	Numerous	Very numerous	Abundant
14	Prominent	Very numerous	Very numerous	Abundant
15	Prominent	Very numerous	Very numerous	Abundant
16	Normal	Very numerous	Very numerous	Normal
17	Normal	Normal amount	Numerous	Abundant
18	Prominent	Normal amount	Very numerous	Poor
19	Prominent	Normal amount	Numerous	Poor
20	Prominent	Numerous	Numerous	Normal
21	Prominent	Normal amount	Very numerous	Abundant
22	Prominent	Normal amount	Few	Abundant
23	Prominent	Normal amount	Numerous	Abundant
24	Prominent	Normal amount	Very numerous	Abundant
25	Prominent	Very numerous	Very numerous	Normal
26	Normal	Very numerous	Very numerous	Abundant
27	Normal	Few	Numerous	Poor
28	Prominent	Normal amount	Numerous	Normal
29	Prominent	Numerous	Very numerous	Poor
30	Prominent	Numerous	Very numerous	Poor

stained with lead citrate (Reynolds, 1963) and studied with a Philips 200 electron microscope. The prints obtained were studied and coded by one of the authors (M.A.) so that they could be restudied and reclassified blindly by another observer.

Results

Clinical. Relevant clinical details of children studied are shown in Table I.

Electronmicroscopy (Table II). The brush border appeared completely disorganized with a poor or absent glycocalyx covering in all 14 coeliac

cases (Fig. 1a). In one coeliac patient (Case 4), however, extensive patchy areas of normal brush border were found in villus and crypt areas (Fig. 1b). There was no gluten withdrawal before the biopsy. Normal brush border was the most common finding in the 'noncoeliac' group (Fig. 1c). In most of the 16 noncoeliac biopsies there were small patchy areas along the villi which showed shorter, looser, and sometimes branched microvilli. The glycocalyx was always normal and pinocytotic vesicles were frequent.

The terminal web was well developed in most cases and occasionally presented with disrupted

ticulum, smooth endoplasmic reticulum, and basal membrane in villus and crypt areas

Crypt	Smooth endoplasmic reticulum		Basal membrane	
	Villus	Crypt	Villus	Crypt
Abundant	Poor	Poor	Normal	Normal
Poor	Poor—nil	Poor	Normal	Normal
Rich	Poor—nil	Poor	Normal	Normal
Abundant	Absent	Poor	Normal	Normal
Abundant	Poor	Poor	Normal	Normal
Poor	Poor	Poor—nil	Normal	Thickened
Normal	Poor	Poor	Normal	Thickened
Abundant	Poor	Poor—nil	Thickened	Normal
Abundant	Poor	Poor—nil	Normal	Thickened
Poor	Poor	Poor—nil	Normal	Normal
Normal	Poor	Dilated abnormal vesicles	Normal	Normal
Poor	Rich, dilated, vesicles	Poor	Thickened	Normal
Poor	Poor	Poor	Thickened	Normal
Abundant	Poor	Poor	Normal	Thickened
Abundant	Poor	Poor	Thickened	Normal
Abundant	Poor	Poor—nil	Thickened	Normal
Abundant	Rich	Normal	Thickened	Normal
Abundant	Poor	Poor	Normal	Normal
Normal	Rich, dilated	Normal	Thickened	Thickened
Abundant	Rich, dilated	Rich, dilated,	Normal	Normal
Abundant	Rich, dilated	Poor	Normal	Normal
Abundant	Rich, dilated	Rich, dilated	Thickened	Normal
Abundant	Rich, dilated	Rich, dilated	Thickened	Normal
Poor	Rich, dilated	Rich, dilated	Normal	Normal
Poor	Rich, dilated	Rich, dilated	Normal	Normal
Normal	Poor	Poor	Normal	Normal
Normal	Rich	Normal	Normal	Thickened
Normal	Normal	Poor	Thickened	Normal
Normal	Poor	Poor	Normal	Normal
Normal	Rich	Normal	Thickened	Normal

(a)

(b)

FIG. 1.—Different appearances of the brush border. Osmium fixed. Magnification 21 600 $\times$. (a) Case 10. Coeliac patient. The main features are disorganized microvilli (MV), moderately developed smooth endoplasmic reticulum (small arrows), and numerous free ribosomes (large arrows). (b) Case 4. Coeliac patient. Normal brush border in a villus area. MV, normal microvilli. (c) Case 20. Child with giardiasis. Normal microvilli (MV) and rich smooth endoplasmic reticulum (large arrows) are the main features.

fibres in some coeliac biopsies. Lateral membranes and intercellular spaces showed a normal profile in all cases.

Lysosomes appeared in clusters and were larger and of unhomogenous material in the coeliac cases. Multivesicular bodies were frequent in all cases. Autophagosomes and residual bodies were seen in some noncoeliac biopsies, but not in the coeliac ones.

Prominent Golgi, occupying large supranuclear areas as shown in Fig. 2 were observed in 24 of the 30 biopsies; 12 of the 14 coeliacs and 12 of the 16 noncoeliacs (Table II). No lipidic particles were seen in relation to the Golgi. Both the canalicular and vesicular components appeared equally developed, and no difference between villus and crypt areas was found.

Numerous free ribosomes and polysomes (Fig. 3a) were found in the epithelium in the villus areas of 17 of the 30 biopsies, 14 of the 17 were associated with a poorly developed smooth endoplasmic reticulum. This was found 10 times in coeliacs and 7 times in noncoeliacs. Rough endoplasmic reticulum was in general well defined, and little difference was observed between villus and crypt areas and coeliac and noncoeliac biopsies.

Smooth endoplasmic reticulum was in general poorly developed in coeliac biopsies, there being a clear predominance of rough endoplasmic reticulum over smooth endoplasmic reticulum in 6 coeliac biopsies. In noncoeliacs well-developed systems of rich smooth endoplasmic reticulum with numerous and dilated membranes was observed in the villus areas of ten biopsies (Fig. 3b).

FIG. 2.—Case 10. Supranuclear area showing multiple images of Golgi apparatus (G). Osmium fixed. $\times 17\,800$.

FIG. 3.—Free ribosomes, rough endoplasmic reticulum, and smooth endoplasmic reticulum in villus areas of coeliac and 'noncoeliac' biopsies, respectively. Osmium fixed. $\times 16\,300$. (a) Case 10 coeliac patient. Numerous free ribosomes (small arrows), minimal amounts of smooth endoplasmic reticulum and rough endoplasmic reticulum (RER). Some dilated cistaernae in the Golgi apparatus (G) and some dilatation of the intercellular spaces (ICS) are also seen. M, mitochondria. (b) Case 19 noncoeliac patient. Predominance of smooth endoplasmic reticulum (large arrows) over rough endoplasmic reticulum (small arrows), and a few ribosomes in a biopsy from child with steatorrhoea of unknown origin. M, mitochondria.

In 2 patients with chronic intermittent diarrhoea of no proven cause, loss of the normal relation of mitochondria/rough endoplasmic reticulum was observed (Fig. 4).

A thickened basal lamina (Fig. 5 a, b, c) was found in 16 of the 30 biopsies; 7 times in coeliac patients and 9 times in the noncoeliacs. Only one case (Case 14) showed a thickened basal lamina clearly similar in extent and appearance to that described previously by Shmerling and Shiner (1969) (Fig. 5a). In 15 cases there was a thickened basal lamina in either crypts or villi and in one case, both in crypts and villi. No significant difference was found between the coeliac group and the noncoeliac group with regard to age, or cause of the chronic intermittent diarrhoea, and presence of a thickened basal lamina. Thickening around small capillary vessels (Fig. 5a), as described by Shiner (1970), was found in some coeliacs, but not in the noncoeliac biopsies.

Discussion

Disorganization of the brush border was characteristic in all 14 coeliac biopsies. However, the presence of extensive areas of normal microvilli in one patient (Case No. 4) is contrary to the accepted concept that in coeliac disease there is a universal lesion at least at light microscope level.

Images of prominent Golgi of normal profile were present in most biopsies. There is some controversy about Golgi complexes in coeliac disease. Biempica *et al.* (1968) found 'very small or only remnants' of Golgi in coeliac patients, while Shiner (1967) has reported 'an increase in number and size' of Golgi complexes under the same circumstances. Our present results agree entirely with this latter description.

An increased number of free ribosomes have been reported by Trier and Rubin (1965) and Biempica *et al.* (1968) in coeliac patients, and by Wartiovaara

FIG. 4.—Case 24. Supranuclear area showing loss of the normal spatial relation of mitochondria (M) to rough endoplasmic reticulum. N, nucleus; ▲, smooth endoplasmic reticulum; g, golgi. $\times 6350$.

and Tarpila (1973) after a certain dose of irradiation in the small intestine of rats. All these changes, together with the changes in smooth and rough endoplasmic reticulum, suggest an abnormal cellular activity, but with our present data we cannot relate them more specifically to any pathological entity.

A thickened basal lamina in 9 of the 16 children who did not have coeliac disease indicates that this is not a primary or essential feature of early child-

hood coeliac disease. It may occur in cases of nonspecific small intestinal mucosal lesion.

Only one case (Case 14) was thought to have, during the blind study, the typical appearance of adult coeliac disease. Whether age could influence the production of a thickened basal lamina in a more specific manner, we cannot say, because this study included 30 children consecutively studied and only one case (the above mentioned one) was older than 6½ years.

FIG. 5.—Different appearances of the basal lamina. Osmium fixed. (a) Case 14. Coeliac disease. Thickened basal lamina (BL) with abnormal amounts of collagen (C), and some 'white fibres'. The thickening is also seen around a capillary vessel (CV), which contains a red blood cell. EC, epithelial cell. $\times 13\,600$.

(b)

(c)

FIG. 5.—(b) Case 23. Patient with cow's milk intolerance. The basal lamina (BL) is thickened with abundant collagen (c). EC, epithelial cell, F, fibroblast projection. $\times 13\,600$. (c) Case 17. Patient with postgastroenteritis syndrome. Moderate amounts of collagen ($\blacktriangle$) thicken the basal lamina (BL) without major involvement of the capillary vessel (CV) wall. EC, epithelial cell. $\times 13\,200$.

Kuitunen *et al.* (1973) presented evidence that infants with cow's milk protein intolerance and malabsorption syndrome during the first months of life present coeliac-like ultrastructural changes in the intestinal epithelium, describing in one child 'accumulation of undulating and whorled collagen fibres' at the basal lamina. This is in agreement with our findings (Fig. 5b).

All the evidence available seems to show that changes of the fine structure of the epithelial cell of the small intestine are nonspecific.

Lindberg and Meeuwisse (1973) have recently reported that 8 out of 40 children diagnosed as having coeliac disease, because they fulfilled the same criteria we used to select our patients, did not reproduce a flat mucosa after 2 years of challenge by being on a gluten-containing diet.

A flat small intestinal mucosa, or a mucosa with severe partial villous atrophy, has been described in

a number of disorders other than coeliac disease. These include acute gastroenteritis (Ament, 1972), cow's milk protein intolerance (Kuitunen *et al.*, 1973), giardiasis (Ament, 1972), and soy protein intolerance (Ament and Rubin, 1972). While a flat mucosa still most often suggests the diagnosis of coeliac disease, it may be due to other causes.

As mentioned above, the children presented here as coeliacs cannot be diagnosed with certainty until each child has been investigated by gluten challenge to show permanent intolerance to gluten. It is thus possible that the morphological changes shown in this study will prove with time to be in some cases related not to coeliac disease alone but rather to mucosal damage from any cause. If this is the case, it would provide an explanation for the lack of specificity in the ultrastructural changes observed.

We conclude firstly that in small children who have a severely abnormal mucosa, where the diagnosis of coeliac disease is probable, abnormal brush border and lysosomes, increased number of free ribosomes, poorly developed smooth endoplasmic reticulum, large Golgi complexes, and thickened basal lamina are the most frequent findings. However, there is no evidence to support the fact that these morphological changes are specifically related to this disease. Secondly, in small children who present with various clinical conditions (such as cow's milk protein intolerance, postgastroenteritis syndrome, irritable colon syndrome, or chronic intermittent diarrhoea of unknown cause) and in whom the light microscope appearance of the mucosa is normal or shows a mild degree of partial villous atrophy, the same type of electron microscope changes as described above can be observed: the relation between the severity of the changes shown in light microscopy and those observed in electron microscopy is poor.

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