Microvillous surface area in secondary disaccharidase deficiency

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SUMMARY Electron microscopy was used to measure microvillous surface area in seven small intestinal biopsies showing secondary disaccharidase deficiency and in five biopsies having normal disaccharidase levels. There were significant reductions in microvillous surface area in the enzyme deficient group, although the microvilli were not severely damaged. Histological abnormality was not always present with disaccharidase deficiency. There was no significant difference in intra-epithelial lymphocyte counts between the two groups.

The majority of small intestinal disaccharidases are found in the brush border of the enterocyte (Crane, 1968), and have been shown to be specifically associated with the microvillous membrane (Johnson, 1967; Alpers and Seetharam, 1977). It would seem probable in view of this location that there should be a relationship between the amount of microvillous surface area and the level of disaccharidase activity in a small intestinal mucosal biopsy. Supporting this suggestion is the fact that in coeliac disease there is both a reduction in microvillous height of the mucosal enterocytes (Shiner, 1974), and a reduction in mucosal disaccharidase activity (Anderson, 1966; McNeish and Sweet, 1968).

This study was undertaken firstly to investigate whether the microvillous surface area and disaccharidase activity of the enterocytes of the small intestinal mucosa are interrelated; and secondly to ascertain the appearance of the microvilli in cases of secondary disaccharidase deficiency, as, though it has been widely assumed that the deficiency is due to the temporary damage of the microvilli of the enterocyte, this has not yet been satisfactorily documented.

Methods

PATIENTS

We studied 12 biopsies, taken from 10 children who had been admitted to the Queen Elizabeth Hospital for Children for investigation of a variety of gastrointestinal complaints. The clinical details of the

children are shown in Table 1. Most children were under three years of age, and all were Caucasian, except patient S.P. who was from the Indian sub-continent.

Biopsies were taken, after an overnight fast, under fluoroscopic observation using the double port modification of the paediatric Crosby capsule (Kilby, 1976). The site of biopsy was the fourth part of the duodenum or the duodenojejunal flexure. Each biopsy provided a sample for histological study, disaccharidase assay, and electron microscopy. Histological findings were described as normal (N) or near normal (+/-), or one of three grades of abnormality (+, ++, +++) (Harrison *et al.*, 1976).

ESTIMATION OF DISACCHARIDASE ACTIVITY

Immediately on removal from the capsule a small piece (0.5-4.0 mg) was cut from the intestinal biopsy, wrapped in about 1 cm² of tinfoil and frozen in liquid nitrogen. The sample was stored in a 3 ml screw-capped Sterilin tube at -60°C until estimation of disaccharidase activities could be performed (0-24 days).

On thawing, the piece was briefly dipped in sterile normal saline, blotted dry on filter paper, weighed and homogenised at 0°C in 80 volumes of water. This homogenate was used for lactase and sucrase estimations; it was further diluted to 1 in 480 for the maltase estimation. Disaccharidases were estimated by a modification of the method of Dahlqvist (1970), using 4-amino phenazone and phenol in the glucose oxidase step (Trinder, 1969). 20 µl of homogenate were incubated at 37°C for 40 minutes with 20 µl of 0.056 M lactose, maltose or sucrose in 0.01 M phos-

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Table 1 Clinical details

Biopsy no.	Patient	Sex	Age (months)	Diagnosis	Biopsy	Disaccharidases (normal or reduced)
1	S.T.	M	15	Post enteritis syndrome, CMPI	Pre milk challenge	N
2					Post milk challenge	R
3	J.O.	M	16	Multiple food intolerance	Pre milk challenge	N
4	S.P.	F	20	CMPI	Pre milk challenge	N
5	L.R.	M	34	Chronic diarrhoea	Investigation	N
6	M.R.	M	168	Acute inflammatory bowel disease*	Investigation	N
7	P.C.	M	8	Post enteritis syndrome, CMPI	Pre milk challenge	R
8					Post milk challenge	R
9	M.M.	F	10	Irritable colon syndrome	Investigation	R
10	S.H.	F	12	Post enteritis syndrome	Investigation	R
11	G.S.	M	16	Failure to thrive, ? coeliac	3 month gluten challenge	R
12	L.H.	M	20	Cystic fibrosis, CMPI, ? coeliac	Pre milk challenge	R

CMPI: cow's milk protein intolerance.

*Manuel *et al.* (1978).

phate buffer, pH 6.0. To stop disaccharidase activity and develop glucose colour, 600 µl of 'colour reagent' (see appendix) was added to each tube and incubation continued for another 40 minutes. Suitable homogenate and substrate blanks, a reagent blank, and a standard containing 100 nmol glucose were treated in a similar fashion. After colour development, tubes were centrifuged and the absorbance at 515 nm was read in 1 cm cuvettes on a Unicam SP 500 spectrophotometer. Disaccharidase activities were calculated from these results. Control ranges (Table 2) were calculated using the disaccharidase activities from the biopsies of children who had no gastroenterological symptoms at the time of biopsy or whose symptoms were subsequently attributed to non-gastroenterological causes. All of these control specimens came from the fourth part of the duodenum or the duodenojejunal flexure, and were histologically normal.

ELECTRON MICROSCOPY

The samples for electron microscopy were processed as described previously (Phillips *et al.*, 1979). The surface area of microvilli per square micron of cell surface—that is, the increase in surface area afforded by the microvilli—was also calculated as described earlier (Phillips *et al.*, 1979), with the following modifications: each villus was split into five equal regions (Fig. 1) and a minimum of two microvillous surface area measurements were performed at each

level for each biopsy, the means being presented in the results; on average, microvilli were measured along 2 µm of cell surface for each microvillous surface area calculation; mean microvillous height in the mid villous region of each biopsy was recorded.

Intraepithelial lymphocytes, per 100 epithelial cells, were counted as described by Ferguson *et al.* (1976).

The biopsies under study were divided into two groups: (1) normal, when the activities of the three disaccharidases were within the normal ranges, and (2) reduced, when one or more of the disaccharidase activities were low.

Student's *t* test, modified for small numbers, was applied to the results.

Fig. 1 Diagrammatic representation of the five villous regions.

Table 2 Disaccharidase enzyme activities: control data

	Enzyme		
	Lactase	Maltase	Sucrase
Mean activity	4.5	25.7	6.9
Mean $\pm$ 2 SD	1.7–11.7	13.1–50.6	3.2–14.9
Number of biopsies	16	16	15

All activities are expressed as µmol substrate cleaved per minute per gram wet weight.

Note: A log normal distribution has been assumed.

Table 3 Patients with normal and with reduced disaccharidase enzyme activities: details of histology, cell surface area increase afforded by microvilli, microvillous appearance, and intraepithelial lymphocyte levels

Biopsy no.	Disaccharidase assay			Histology	Increase in cell surface area provided by the microvilli					Microvillous height in mid region (μm)	Description of microvilli	IEL
	L	M	S		Low	Low- Mid	Mid	Upper	Tip			
Group 1—Normal disaccharidase activities												
1	3.8	28.2	8.4	N	25.9	26.5	45.4	35.1	14.1	1.4	Normal, some branching in mid villous region	18.9
3	2.5	39.6	14.5	N	16.1	27.7	39.9	35.6	15.4	1.2	Normal	32.5
4	5.2	37.2	8.7	N	18.2	26.3	26.5	24.4	14.0	1.3	Normal	16.5
5	3.2	18.3	5.6	N but $\uparrow$ IEL	26.1	34.8	27.7	22.6	15.6	1.0	Normal, some branching in upper villous region	60.1
6	2.2	31.5	8.1	N	17.4	26.1	25.4	31.2	14.3	1.7	Normal, slightly uneven in places	31.0
Mean					20.7	28.3	33.0	29.8	14.7	1.3		32.1
SD					4.9	3.7	9.1	6.0	0.8	0.3		16.6
Group 2—Reduced disaccharidase activities												
2	0	8.4	2.1	+ / N	17.2	17.6	18.8	17.2	12.1	0.8	Short over all villus	21.5
7	1.4	7.8	2.1	+ / -	20.0	16.1	19.7	17.6	9.9	0.8	Short in mid villous region	9.9
8	0.6	10.1	1.9	+ - + + / N	13.8	17.9	21.3	13.1	12.4	0.9	Short over all villus, branching in low villous region	16.9
9	2.0	10.8	2.8	N	19.2	18.0	19.1	23.3	14.3	0.6	Short and branching in mid villous region—some damage	29.5
10	0	3.8	0.8	+	11.5	11.6	14.0	10.8	8.4	0.6	Short over all villus	28.0
11	0	0.3	0	+	13.7	16.0	17.3	11.4	5.2	0.7	Short and irregular in upper villous region	15.1
12	1.5	15.0	4.8	+ / -	17.4	37.1	19.7	15.1	7.4	1.0	Normal	9.4
Mean					16.1	18.7	18.6	15.5	10.0	0.8		18.6
SD					3.2	8.4	2.3	4.3	3.2	0.2		8.1

L: lactase, M: maltase, S: sucrase, IEL: intraepithelial lymphocyte count.

Results

The histological findings, the results of the disaccharidase assay, the increase in surface area afforded by the microvilli, a subjective description of the microvillous appearance, the microvillous height in the mid region, and the intraepithelial lymphocyte count are shown in Table 3.

In the normal disaccharidase group there was a

trend for the cell surface area provided by the microvilli to increase from the low to the mid region of the villus, where it reached a maximum, and to decrease from the mid region towards the tip where it reached a minimum, as shown previously (Phillips *et al.*, 1979). In those biopsies where the disaccharidase activity was low, this pattern did not always exist, although the microvillous surface area per square micron of cell surface always decreased towards the

Fig. 2 The mean increase in cell surface area provided by microvilli in the various villous regions from patients with normal disaccharidase activities (hatched line) and patients with reduced disaccharidase activities (solid line). Vertical bars = $\pm$ one standard deviation.

Fig. 3 Microvillous appearances in relation to disaccharidase activity in patient S.T. (a, c) pre-milk challenge (biopsy number 1), microvilli from the mid (a) and tip (c) villous regions. (b, d) post-milk challenge (biopsy number 2), microvilli from the mid (b) and tip (d) villous regions. $\times 30,300$.

tip. Mean microvillous height in the mid region was significantly shorter ($P < 0.005$) in the reduced disaccharidase group.

The mean microvillous surface areas per square micron of cell surface of the two groups were compared at the different villous levels (Fig. 2). The mean microvillous surface areas of the normal disaccharidase group were higher than those of the reduced disaccharidase group at the same villous level. Statistically significant differences (Fig. 2) were found at all levels; the most significant differences were found when comparing the mid and upper villous regions of the two groups ($P < 0.005$).

An example of the microvillous appearance in biopsies with normal and reduced disaccharidase activities is shown in Fig. 3. The biopsies were from the same child (S.T.) before and after challenge with cow's milk.

The mean intraepithelial lymphocyte count of the normal disaccharidase group was higher than the mean intraepithelial lymphocyte count of the reduced disaccharidase group, although the difference was not statistically significant, being primarily due to a very high count in one of the normal disaccharidase biopsies (L.R.) (Table 3).

Discussion

In the children we have studied there was an inter-relationship between microvillous surface area and disaccharidase activity—that is, when disaccharidase activity was reduced then the microvillous surface area was also reduced. This is consistent with the observation that disaccharidase enzymes are localised on the microvillous membrane (Alpers and Seetharam, 1977). Height is the major contributory factor to the surface area of a microvillus and alterations in this dimension account for most of the changes in surface area. Thus, in the mid region of the villus, the microvilli are shorter in the reduced disaccharidase group and they present less surface area.

The occurrence of a reduction in surface area, rather than generalised microvillous disruption, suggests an alteration in the turnover or synthesis of microvillous membrane, perhaps as a result of a restriction in the supply of necessary metabolites, or a case of demand exceeding supply when turnover of microvillous membrane is high.

In contrast with our findings of limited instances of microvillous degeneration in secondary disac-

charidase deficiency, Klish *et al.* (1978) reported severe distortion and fragmentation of microvilli, as well as a reduction in microvillous surface area, in acute acquired monosaccharide intolerance. Secondary monosaccharide intolerance is a more complete manifestation of sugar intolerance than just intolerance to disaccharide, which occurs in the same clinical condition (Anderson and Burke, 1975) and the extent of microvillous damage may determine the extent of carbohydrate intolerance.

Although there was a reduction in microvillous surface area in the disaccharidase deficient group, the histological gradings (Tables 3 and 4) did not show a uniform abnormality—indeed, three of the seven biopsies were normal or near normal. This supports Harrison and Walker-Smith's (1977) and Morin *et al.*'s (1979) findings that, in childhood, reduced disaccharidase activity and overt mucosal damage are not always related and demonstrates that a normal histology coupled with disaccharidase deficiency is not restricted to primary disaccharidase disorders.

The interrelationship between intraepithelial lymphocyte levels and disaccharidase activities in children with coeliac disease having a gluten challenge has been described by Egan-Mitchell *et al.* (1978). Although this may be true for coeliac disease, it does not seem to be a general rule, as no statistical difference was found between the normal and reduced disaccharidase groups in this study.

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Appendix

COLOUR REAGENT

- 30 mg 4-amino-phenazone; 4500 units horseradish peroxidase (Schwartz Bio-research Inc.) 1500 units glucose oxidase (Sigma Type V); 100 mg sodium azide in 100 ml 0.5M Tris buffer pH 7.2. Stored at 4°C.
 - 0.1% phenol in water. Stored in the dark at 4°C.
- Mix 3 volumes of (a) with 1 volume (b) just before use.