

Disaccharidase activities in jejunal fluid

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SUMMARY Jejunal fluid and mucosal tissue were obtained simultaneously from the same jejunal site in a group of 29 children by a modified biopsy procedure. Lactase, maltase, and sucrase activities were measured in both fluid and mucosal specimens using the same analytical method. The fluid enzyme activities showed highly significant positive correlations with the same enzyme activity in the relevant tissue samples. Relative concentrations of disaccharidase enzymes represented by sucrase: lactase activity ratios also showed a highly significant positive correlation between fluid and tissue. This close relation suggests that the mucosa is the sole or predominant source of disaccharidase activity in the intestinal fluid. The results of kinetic studies comparing tissue and fluid enzyme characteristics also indicate a mucosal origin for the fluid enzyme activities. We conclude that disaccharidase activities in jejunal fluid reflect closely local tissue values and that these measurements may be useful in assessing mucosal enzyme activity in infants in whom jejunal biopsy is not possible.

The conventional method of studying intestinal disaccharidases is to measure enzyme activities in fragments of intestinal mucosa obtained by peroral biopsy. There are circumstances, however, where knowledge of brush border disaccharidase concentrations would be important but where jejunal biopsy is not feasible—in newborn infants for example, or during or after gastroenteritis. The collection of intestinal fluid offers less of an impediment than tissue biopsy and it is into the fluid that intestinal epithelial cells and their enzymes are shed. We aimed to determine whether disaccharidase activities in intestinal fluid reflect the activities of the same enzymes in the adjacent jejunal mucosa.

Materials and methods

Patients. Jejunal fluid and mucosal biopsy specimens were collected from 29 children aged 10 months to 14 years (mean 5.9 years, median 5 years) all of whom had gastrointestinal symptoms necessitating this diagnostic procedure. The appearances of the mucosal biopsy specimens on inspection by light microscopy enabled us to divide the children into 3 histological categories—normal mucosal structure, mild enteropathy, and sub-total villous atrophy (STVA).

Biopsy procedure. The small intestinal biopsy was done by a conventional procedure with the following modification. The tubing of the biopsy capsule ran

through a 12 FG oroduodenal tube with 7 to 10 additional holes cut in the distal end to facilitate inflow of fluid. The resulting increased rigidity gave the additional advantages of making the tube easier to advance and more resistant to biting. The progress of the capsule was followed by fluoroscopy and when it was correctly positioned at the duodeno-jejunal flexure gentle suction was applied to the oroduodenal tube until an adequate sample of fluid was obtained. The biopsy specimen was then taken in the usual way.

Specimen preparation. Jejunal fluid and biopsy tissue were prepared in the following manner.

Jejunal fluid

A small volume of each fluid was kept at room temperature for investigation for giardia. The rest was placed immediately into a vacuum container with solid carbon dioxide for transportation to the laboratory. On thawing for analysis, fluid specimens were homogenised by 10 strokes in a Potter-Elvehjem type homogeniser to disperse mucus or any tissue elements present.

Biopsy tissue

Specimens were divided into 2 pieces immediately on removal from the biopsy capsule. One piece was immersed in fixative for morphological studies and the second was enclosed in aluminium foil to prevent dessication and then placed in a vacuum container with solid carbon dioxide for transport to

the laboratory. Tissue weight was determined by weighing the foil before and after removal of the tissue and the tissue was homogenised by Dahlqvist's procedure.¹

Fluid and tissue homogenates were kept in water-ice baths at all times when thawed for analysis. Stored specimens were kept at -20°C .

Assay of disaccharidase activities. Lactase, sucrase, and maltase were measured in both tissue and fluid by the Dahlqvist method¹ with the modification that Trinder's² non-carcinogenic colour reagent was used for glucose (product) measurement. Measurements on the fluid were made using 100 μl volumes of undiluted fluid for lactase and sucrase activities and 10 μl for maltase measurement. All disaccharidase assays were done in duplicate and duplicate mean values were recorded.

The unit of disaccharidase activity used is defined as that activity which hydrolysed 1 μmol of substrate per minute at pH 5.9 and 37°C . Tissue activities were expressed as U/g of tissue, wet weight. Fluid activities were recorded as U/l of fluid. The total protein content of the fluid was measured by the Lowry procedure³ so that fluid activities might also be expressed per g of protein contained.

Enzyme kinetics investigation. Linearity and proportionality of the fluid disaccharidase enzyme assays were determined using the 2 stage, end point procedure that Dahlqvist¹ described for tissue enzyme measurement. Linearity of enzyme activity was investigated by end point determinations with substrate hydrolysis stages of 10, 20, 30, 40, 50, and 60 minutes duration—the latter being the duration of the substrate hydrolysis stage of the Dahlqvist¹ procedure.

Proportionality was assessed by enzyme measurements made on dilutions of fluid up to 1:6. The upper limit of enzyme activity in the proportionality experiments was restricted to that activity which liberated a quantity of glucose in 60 minutes not greater than that contained in the top glucose standard (0.32 μmol). Fluids with activities in excess of this limit were diluted appropriately with 0.15M saline.

Linearity and proportionality experiments were also made using a Beckman DU-8B microprocessor controlled recording spectrophotometer in the kinetics mode, recording optical density increments at 2 minute intervals for 100 minutes. The single stage disaccharidase procedure of Messer and Dahlqvist⁴ was used for the latter experiments.

The determinations of apparent Michaelis constant (Km) values were made using the Dahlqvist¹ 2 stage, end point procedure with substrate con-

centrations of 56, 28, 14, 7, and 3.5 mmol/l followed by conventional Lineweaver-Burk reciprocal plots. Unweighted regression analysis was used to calculate values of Km.

Fluid Km values were determined on fluid specimens after centrifugation at 100 000 *g* for 1 hour at 4°C . Fluid Km values were also determined for lactase and sucrase isolated from pooled fluid by preparative isoelectric focusing.⁵

Statistical methods. The significance of differences in the direct data for the 3 enzymes was determined with the Mann-Whitney U test. The degree of correlation between tissue and fluid values and tissue and fluid sucrase:lactase ratios was determined using Spearman's rank correlation procedure. The Wilcoxon matched pairs, signed ranks test was used for determining the significance of differences between the fluid:tissue ratios of the 3 enzymes in the normal histology group.

Results

Histology. The structure of the duodenal mucosa was normal in 17 of the 29 children investigated. Seven of the remaining 12 children had STVA with a diagnosis of coeliac disease confirmed in 6 according to the criteria of the European Society for Paediatric Gastroenterology and Nutrition.^{6,7} Four children had a mild enteropathy only—2 after episodes of gastroenteritis, 1 caused by coeliac disease, and 1 in whom coeliac disease was suspected but not proved. In the remaining child a non-specific enteropathy accompanied infestation with giardia.

Enzyme activities. All 17 children with normal mucosal structure had tissue sucrase and maltase activities above the lower limit of the paediatric control range published by Townley⁸ who used the same assay procedure as that used in this study. Two of the 17 children, however, had tissue lactase activities that were below Townley's⁸ range. Fig. 1 shows the tissue and fluid values for the 3 enzymes in the 15 children with both normal histology and normal enzyme values and in the 7 children with STVA. The activities of all 3 tissue enzymes were, as expected, appreciably lower in the children with STVA.

The fluid enzyme activities in the 2 groups were similarly related—the STVA children again having values appreciably lower than the children with normal histology. The 2 children with low tissue lactase activities but normal mucosal structure also showed fluid lactase activities that were lower than the rest of the children with normal histology.

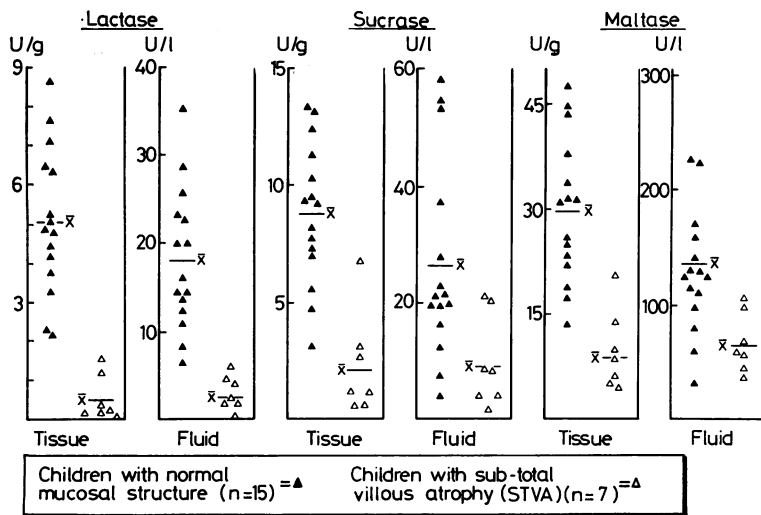


Fig. 1 Disaccharidase activities in tissue and fluid showing the results in children with normal mucosal structure and with sub-total villous atrophy (STVA).

Tissue and fluid activities of all 3 enzymes were significantly lower in the STVA children (Mann-Whitney U-Test, $P < 0.01$ in all cases).

Tissue:fluid enzyme relations. Fig. 2 shows the relation between disaccharidase activities in tissue and fluid for all 29 children studied. All 3 enzymes showed highly significant positive correlations between tissue and fluid enzyme concentrations. Tissue maltase activity was also compared with fluid maltase activities expressed as units of enzyme

per g of fluid protein. This alternative method of expressing fluid enzyme concentrations did not appear to be advantageous. A poorer correlation was shown producing a lower value for the Spearman rank correlation coefficient.

Fluid: tissue ratios for all 3 enzymes were calculated for the children with normal mucosal structure. The ratios for maltase were significantly higher than the ratios for lactase and sucrase (Wilcoxon's matched pairs, signed rank test, $P = < 0.01$ in both cases).

The relative concentrations of enzyme in tissue and fluid were also closely related producing a highly significant positive correlation of the tissue sucrase:lactase ratio with the fluid sucrase:lactase ratio (Fig. 3).

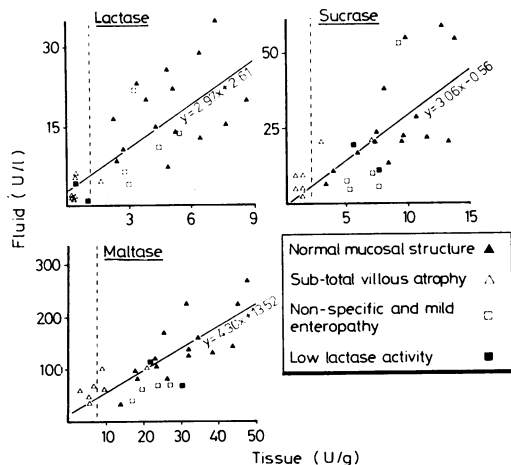


Fig. 2 Correlation of tissue and fluid enzyme activities showing the results in all 29 children studied.

Spearman's rank correlation coefficients for lactase, maltase, and sucrase were 0.78, 0.81, and 0.77. Statistical evaluation gave values of $P < 0.001$ in each case. The vertical dashed lines on the tissue axes show the lower limit of control values for children.⁸

Enzyme properties. The enzymes in the jejunal fluid exhibited the following properties.

Solubility

Fluid specimens from 10 children with normal mucosal structure were ultracentrifuged (1 hour at 100 000 g at 4°C) to remove insoluble materials including any brush border attached enzyme from desquamated enterocytes or their debris. Maltase activity was determined in the supernatant fluid (soluble enzyme) and in the pellet material resuspended in water to the original volume (particle bound enzyme). Particle bound enzyme represented a minor part of the activity in each fluid, values ranging from 3.4–36.2% of the total fluid maltase

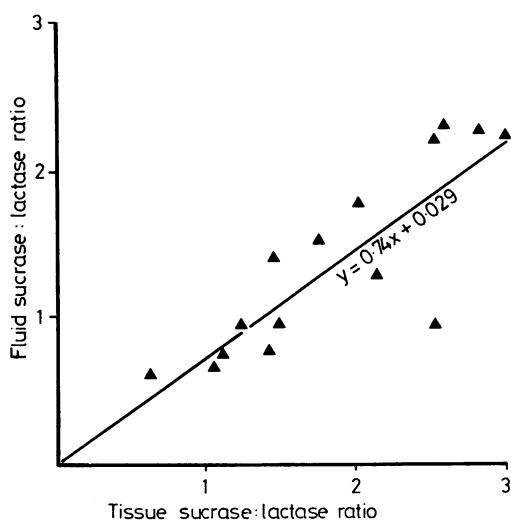


Fig. 3 Correlation of the tissue and fluid sucrose:lactase ratios in the children with normal histology.

Spearman's rank correlation coefficient = 0.89, $P < 0.001$.

activity. Only 2 values exceeded 10% (17.5 and 32.6%) and the median value was 6.7% of total fluid maltase activity.

Kinetic characteristics

The results of a comparison of apparent K_m values for fluid and tissue disaccharidases are shown in the Table. The values for fluid and tissue maltase

Table Apparent Michaelis constant (K_m) values (mmol/l) for fluid and tissue in children with normal mucosal structure

Case No	Sucrase		Maltase		Lactase	
	Tissue	Fluid	Tissue	Fluid	Tissue	Fluid
1	23.3	8.4	8.7	7.0	20.0	7.0
2	33.8	37.4	11.8	10.9	15.2	3.6
3	17.5	6.8	11.7	10.7	15.5	6.7
4	25.9	29.2	8.2	10.1	18.4	11.7
5	13.0	9.7	9.2	13.4	16.6	21.8
6	18.1	21.0	11.4	12.4	26.6	22.4
Mean	21.9	18.7 (24.4)*	10.1	10.7	18.7	12.1 (23.0)*

Each tissue/fluid comparison was made with tissue and fluid collected simultaneously from the same patient.

*Apparent K_m values determined for enzymes isolated by preparative isoelectric focusing using fluid pooled from 12 children with normal mucosal structure.

Conversion: SI to traditional units—sucrose 1 mmol/l \approx 34.2 mg/dl; maltase 1 mmol/l \approx 34.2 mg/dl; lactase 1 mmol/l; \approx 34.2 mg/dl.

covered a similar narrow range with similar means. Values for sucrase were less homogeneous but with similar fluid and tissue mean values. Five of the 6 fluid lactase K_m values were lower than the relevant tissue K_m and the mean was also appreciably lower, though not significantly so. The fluid lactase K_m value on enzyme isolated by isoelectric focusing was higher, however, and closer to the mean tissue K_m value. All but 1 of the fluid K_m values were below the substrate concentration chosen by Dahlqvist¹ for his tissue disaccharidase measurements confirming that the same substrate concentration was equally appropriate to the measurement of the enzyme in solution in intestinal fluid.

Proportionality was shown for the 3 enzymes within the upper activity limit defined in the methods section. The linearity experiments done with the 2 stage, end point Dahlqvist¹ procedure showed losses of approximately 10% of activity at 60 minutes compared to the initial 10 minute velocity rate. Linearity experiments on the recording spectrophotometer using the Messer and Dahlqvist⁴ single stage procedure produced similar evidence of linearity but also showed an initial lag phase not seen with the 2 stage, end point procedure.

Discussion

Enzyme activities hydrolysing lactose, maltose, and sucrose were found in all specimens of intestinal fluid investigated. Whether expressed as direct concentrations or in relative terms as concentration ratios there were statistically significant positive correlations between tissue and fluid enzyme activities. Assessing relative changes in disaccharidase activities from changes in the enzyme ratios rather than the direct data has the advantage of being unaffected by changes in concentration produced by fluid movement into or out of the intestine.

We are aware of only 1 other clinical study using luminal enzyme activities. Torres-Pinedo⁹ investigated a group of infants with clinical evidence of lactose intolerance after diarrhoea caused by *Escherichia coli*. His measurements of sucrase and lactase in luminal material recovered by a wash out procedure¹⁰ showed that both enzyme activities were reduced.

In our studies the sucrose:lactase ratio in fluid was lower than in tissue (Fig. 3). This may be explained in part by a different susceptibility of the 2 enzymes to proteolysis but may also be the result of shedding of 'mature' enterocytes into the lumen.

The results in our centrifugation study showed that almost all of the maltase activity was present in free solution and not associated with sedimentable

cell structural material. The mechanism responsible for the release of brush border enzyme activity into the intestinal fluid is not known. It may be caused by desquamation of enterocytes from the villous tips followed by autolysis but intraluminal factors may also be responsible. The detergent function of bile acids, pancreatic proteases, the gut microflora, and gastrointestinal hormone release¹¹⁻¹⁸ may all be contributory. Gotze *et al.*¹¹ studying the release of enterokinase, alkaline phosphatase, and sucrase in the rat found that the absence of bile from the intestinal perfusate increased the amount of sucrase and alkaline phosphatase sedimented by centrifugation. Both enzymes were 85% sedimented whereas in the presence of bile only 25% of sucrase and 5% of alkaline phosphatase sedimented. Boedeker¹⁹ showed that solubility was greater with the more lipophilic secondary bile acid, deoxycholic acid.

Nordstrom¹⁶ also investigated the release of enzyme in the rat duodenum. In addition to bile, pancreatic juice, and crystalline pancreatic proteases he showed that intravenous cholecystokinin also increased soluble enzyme concentrations in the lumen. He concluded, however, that this was not a direct effect of the hormone but secondary to the provoked secretion of bile acid and pancreatic proteases.

The appreciably higher fluid:tissue ratio for maltase in the children with normal histology and the higher lactase fluid:tissue ratios than sucrase fluid:tissue ratios may have been the result of differential susceptibility to proteolysis. In *in vitro* experiments Seetheran *et al.*²⁰ showed that incubation with trypsin had no effect on brush border maltase activity but reduced lactase and sucrase activities by 26% and 40% respectively. This observation agrees with our findings and suggests that changes in the relative concentration of the enzymes in the fluid compared with tissue were a result of proteolytic degradation. Other factors, however, may influence the relative enzyme concentrations in the fluid including the maturity of the enterocytes from which they are derived. In the children with STVA fluid sucrase:lactase ratios were higher and this would be consistent with transfer of enzyme from the less mature enterocytes that are found after mucosal damage.

The fluid samples may have contained micro-organisms that may in theory have contributed to the hydrolysis of substrate in the enzyme assay. We think this is unlikely because fluid from the upper small intestine contains only a sparse microflora that is reduced to almost nil viable organisms by a brief period of storage at -20°C .²¹ The removal of only a very small part of total fluid maltase

activity by high speed centrifugation also suggests that micro-organisms did not contribute appreciably to the hydrolysis of substrate. The significant positive correlation between fluid and tissue enzyme shown by our data is in itself further evidence that any non-mucosal disaccharidase activity in the fluid was too small for the fluid tissue relation to be obscured.

The tissue disaccharidase method of Dahlqvist¹ was used without modification in this study for measuring activities in intestinal fluid. Our comparison of fluid and tissue enzyme kinetics confirmed that the conditions described by Dahlqvist¹ for disaccharide hydrolysis by the tissue enzymes were also appropriate for enzyme measurements in fluid. Dahlqvist,²² in an earlier study using pig small intestine also found the same K_m value for the sucrase activity of homogenised mucosa and the non-sedimentable sucrase prepared by treating the mucosa with deoxycholic acid. The value he reported (25 mM) was very similar to our mean tissue value and almost identical to that of our fluid sucrase after isoelectric focusing. The lower K_m values for fluid lactase that were reversed by purification by isoelectric focusing suggest the presence of factors enhancing lactase activity in fluid—a feature that may also lead to the lower sucrase:lactase ratios characteristic of fluid.

We attribute the minor loss of linearity shown by our investigations to the proteolytic activity of the fluid. Earlier methodological investigations showed that pre-incubation of fluid at 37°C for 1 hour resulted in the loss of 5–10% of activity when compared with an aliquot of the same fluid that had not been pre-incubated, and that the loss could be largely (but not completely) prevented by trypsin inhibitors.

Messer and Dahlqvist⁴ reported a lag phase in tissue disaccharidase activity when using their single stage procedure that was not seen when using the Dahlqvist¹ 2 stage method. Our experience with the fluid enzymes was identical providing further evidence of the close tissue fluid enzyme relation.

Although not conclusive, these results suggest that neither the change from membrane bound to free solution nor the luminal factors discussed above modify the enzyme molecules sufficiently to invalidate their measurement by Dahlqvist's¹ tissue enzyme procedure. The close relation that we have shown between values of disaccharidase activities in intestinal fluid and the adjacent mucosa may have applications in paediatric research. In circumstances in which studies of disaccharidase enzymes would be important but where jejunal biopsy is not feasible, the assay of intestinal fluid enzyme may give the information required.

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