Intestinal Disaccharidase Deficiency in Children with Coeliac Disease

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In a recent paper on their experiences with intestinal biopsy in children with coeliac disease, Sheldon and Tempny (1966) included evidence of a reduction in disaccharidase activity in many of the biopsies, thus confirming recently published observations (Plotkin and Isselbacher, 1964; Shmerling, Auricchio, Rubino, Hadorn, and Prader, 1964; Nordio, Lamedica, Vignolo, and Berio, 1965; Lifshitz, Klotz, and Holman, 1965).

In the present study certain aspects of the quantitative method of Plotkin and Isselbacher (1964) for estimation of disaccharidase activity have been investigated more fully. Disaccharidase activities were assayed, using a slight modification of their method, and histological examination of duodenal or jejunal mucosa was carried out in children who were suffering from coeliac disease (both treated and untreated) and they were compared with the findings in a group of children suffering from miscellaneous conditions resulting in malabsorption, and in a group with normal small intestinal mucosae.

Enzyme studies were also carried out in one case using necropsy material. The methods and results in this case are discussed separately.

Material and Methods

Coeliac disease: Studies were carried out on 12 children with coeliac disease aged 9 months to 10½ years. In each case the diagnosis of coeliac disease was confirmed by evidence of malabsorption, mucosal histology, and therapeutic response to a gluten-free dietary régime. Of these patients, 6 were new cases assessed before treatment, 3 had been on gluten-free diets for a variable period of time up to 2 years, 2 had relapsed following a return to normal diet, and 1 had been treated with a lactose-free diet for 8 months before the diagnosis of coeliac disease was made.

Miscellaneous malabsorption states: There were 4 patients with miscellaneous conditions: post-gastro-enteric (2), steatorrhoea from exocrine pancreatic insufficiency (1), and glucose-galactose intolerance (1).

Normal mucosae: 6 patients, who were found after investigation to have normal or virtually normal (grade I) histology, served as normal controls.

Biopsy of distal duodenal or proximal jejunal mucosa was carried out in all 3 groups using a Crosby per-oral biopsy capsule. The specimens were examined under a dissecting microscope, and a portion was reserved for histological examination.

The remaining tissue was immediately rinsed in 0.85% saline and blotted dry to remove excess mucus, frozen on solid CO₂, and stored at -20° C. until assayed. Assays were usually performed within 24-72 hours, though storage for at least two weeks was shown to have no deleterious effect on disaccharidase activities (see Table I).

TABLE I
*Effect on Disaccharidase Activities of Storage
at -20° C. (Necropsy Material)*

Period of Storage (days)	Disaccharidase Activity (units/g. mucosa, wet weight)			
	Lactase	Sucrase	Palatinase	Maltase
2-7	5.51	4.93	1.37	18.50
9-17	5.42	5.99	1.52	19.57
37-39	4.85	4.95	1.40	15.84

Figures represent means of triplicate assays from three sites, i.e. of 9 estimations.

Histology. Specimens were formalin-fixed, embedded in paraffin, sectioned, and stained with haematoxylin and eosin. Appearances under the dissecting microscope were correlated with the histological findings, all specimens being reviewed by Dr. C. L. Berry and graded according to the following criteria.

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TABLE II
Disaccharidase Activities and Mucosal Histology of Patients Studied

Clinical Details	Patient	Sex	Age		Histology of Mucosa	Disaccharidase Activity (units*/g. mucosa wet weight)			
			Yr.	Mth.		Lactase	Sucrase	Palatinase	Maltase
Patients with Normal Mucosa									
Normal mucosa	L.T.	F	—	7	Normal	11.65	7.29	1.98	19.87
	A.L.	M	1	6	†Normal	9.43	8.77	3.03	19.89
	M.P.	F	2	—	Normal	8.82	9.21	2.87	22.05
	N.B.	M	2	6	Grade I	8.31	8.14	2.91	—
	A.G.	F	3	9	Normal	8.05	5.46	2.39	24.53
	L.J.	F	4	1	Normal	4.44	5.83	2.00	17.90
Patients with Coeliac Disease									
Untreated	P.P.	F	—	9	Grade II	< 0.5	< 0.5	< 0.5	3.27
	T.W.	M	—	10	Grade IV	< 0.5	1.11	< 0.5	7.86
	S.T.	M	1	—	Grade IV	< 0.5	0.59	< 0.5	3.29
	T.L.	M	1	1	Grade IV	< 0.5	0.60	< 0.5	2.30
	K.McD	M	1	2	Grade IV	< 0.5	0.68	< 0.5	6.14
	C.G.	F	4	—	Grade IV	0.74	1.76	0.60	8.25
Lactose-free diet 8 mth. (gluten not excluded)	A.W.	M	—	10	Grade IV	1.98	2.31	—	—
‡Normal diet 6 mth.	S.C.	F	3	—	Grade IV	< 0.5	< 0.5	< 0.5	2.70
‡Normal diet 12 mth.	A.K.	F	10	7	Grade IV	nil	0.89	< 0.5	4.22
Gluten-free diet { 6 mth.	K.B.	F	1	7	Grade IV	0.55	—	—	—
	L.R.	F	3	5	Grade II	2.66	2.30	1.02	—
	24 mth.	A.M.	F	7	Grade III	—	4.00	0.83	9.00
Patients with Miscellaneous Conditions									
Post-gastro-enteritis	S.V.	M	—	7	†'Leaf villi'	2.6	1.4	0.50	8.5
Post-gastro-enteritis	M.K.	M	1	5	†'Leaf villi'	—	1.5	—	—
Monosaccharide intolerance	P.B.	M	1	—	†'Leaf villi'	0.67	2.26	0.61	7.40
Pancreatic hypoplasia	M.B.	M	9	9	Grade I	3.70	8.70	4.20	—

* Units = μ moles substrate hydrolysed per minute. † Appearance under dissecting microscope only. ‡ Diet previously gluten-free. — Not estimated.

Normal: no abnormality detected by dissection microscopy or conventional histological methods.

Grade I: predominantly normal villous pattern on dissection microscopy; slight infiltration of the lamina propria with inflammatory cells seen histologically.

Grade II: thickened, leaf-like villi with some convoluted areas on dissection microscopy; heavy inflammatory cell infiltration of the lamina propria histologically, associated with shortened, oedematous villi.

Grade III: convoluted villous ridges on dissection microscopy; histological changes of partial villous atrophy.

Grade IV: 'flat' mucosa on dissection microscopy; histological changes of subtotal villous atrophy.

Disaccharidase activities. Lactase, sucrase (invertase), palatinase, and maltase were estimated after the method of Plotkin and Isselbacher (1964), with slight modification (as designed by Dr. A. D. Patrick). Sucrose, maltose (British Drug Houses Ltd., Poole), and palatinose (T. J. Sass and Sons Ltd., London), were prepared at concentrations of 0.056 M in 0.1 M maleate buffer pH 6.25, lactose (Hopkin and Williams Ltd., Chadwell Heath) being prepared in similar concentration in 0.1 M maleate buffer pH 5.8. Specimens of mucosa (usually 5–8 mg.) were homogenized in 49 volumes of ice-cold 0.15 M KCl. Mixtures containing 50 μ l. homogenate (12.5 μ l. + 37.5 μ l. water in the case of maltase) and 50 μ l. disaccharide were incubated for 60 minutes at 37° C., together with appropriate blanks, and after rapid dilution with 0.9 ml. water, the reaction was stopped by heating in a boiling-water bath for 4 minutes.

The liberated glucose was estimated with glucose oxidase ('Fermcozyme'—Hughes and Hughes Ltd. London), using TRIS-buffer (after Dahlqvist, 1961), in a total volume of 3 ml. After incubation for 30 minutes, 2 ml. concentrated HCl was added and extinctions were read at 530 m μ . on a 'Unicam SP 500' spectrophotometer. The HCl addition produces an optically stable red dye over a linear range of 5–60 μ g. glucose (A. E. Whitfield, 1965, unpublished observations).

Disaccharidase activity was expressed in units (μ -moles of disaccharide hydrolysed per minute) per g. wet weight of mucosa.

Results

Histology. The histological findings and the results of enzyme assays are shown in Table II.

In some cases inadequate material was available for histological sections, and the superficial appearance under the dissecting microscope alone was included. The majority of the untreated cases showed severe mucosal damage, with the exception of patient P.P.—the latter perhaps surprisingly in view of the severity of the disaccharidase deficiency. Severe lesions were seen in patients S.C. and A.K. following relapse on return to normal diet. The cases treated with gluten-free diets for 19 and 24 months, respectively, showed persistent mucosal abnormality, in keeping with the findings of Madanagopalan, Shiner, and Rowe (1965). Enzyme

activity in the 2 patients with grade I mucosae appeared normal.

Disaccharidase activities. (1) *Patients with coeliac disease.* Results of assays in patients with coeliac disease are shown in Table II. Very low levels of disaccharidase activity (particularly affecting lactase) were seen in all untreated patients. In these, lactase activity ranged from < 0.5 to 0.74 units/g. (controls $4.44-11.65$), sucrase from < 0.5 to 1.76 units/g. (controls $5.46-9.21$), palatinase from < 0.5 to 0.60 units/g. (controls $1.98-3.03$), and maltase from 2.30 to 8.25 units/g. (controls $17.90-24.53$). The 3 treated cases showed increasing levels of activity, approximately in keeping with the duration of dietary exclusion of gluten.

The enzyme activities in the 2 patients (S.C. and A.K.), who, following clinical remission of symptoms on gluten-free diet, had reverted to a normal diet for periods of 6 and 12 months respectively, were at least as severely depressed as those in the untreated cases.

(2) *Patients with miscellaneous malabsorption states.* Two patients with recent histories of gastroenteritis showed decreased disaccharidase activities, with evidence of mucosal damage on dissection microscopy. An unusual feature of patient S.V., particularly as there had been some relief of symptoms with a low-lactose diet, was that the sucrase and palatinase activities were relatively more affected than that of lactase. In contrast with the report of Anderson, Kerry, and Townley (1965), the case of glucose-galactose intolerance (P.B.) showed decreased activities, especially of lactase, almost certainly explained by secondary mucosal damage during 10 months of severe intestinal symptoms before diagnosis. A further case (L.T.), of shorter duration, showed normal histology and is included among the controls. Case M.B., with steatorrhoea due to exocrine pancreatic insufficiency in association with neutropenia (Bodian, Sheldon,

and Lightwood, 1964), showed normal enzyme activities, as would be anticipated.

(3) *Children with normal mucosae.* The enzyme activities in these 6 children were comparable with those previously observed in normal children (Burgess, Levin, Mahalanabis, and Tonge, 1964), and in normal adults (Dunphy, Littman, Hammond, Forstner, Dahlqvist, and Crane, 1965; Cuatrecasas, Lockwood, and Caldwell, 1965), as shown in Table III.

Studies on Necropsy Material

Specimens of tissue were obtained within two hours after death from a case of Wolman's disease. Approximately 2 cm. segments of whole-thickness bowel wall were excised from the duodenum (3rd part), mid-jejunum, and mid-ileum, immediately frozen, and stored at -20°C . Mucosal scrapings were obtained as required, using a clean glass slide, and assayed as described above. Assays were performed in triplicate in an effort to ascertain the reproducibility of results, and were repeated at intervals up to 39 days after collection to determine the effect of prolonged storage on enzyme activity. A further aim of these studies was to compare the disaccharidase activities of the different sites along the small intestine. An analysis of variance was performed to investigate the differences between sites, and the effect of duration of storage (Appendix). In view of the time lapsing between death and the collection of specimens, and of the lethal nature of the disease, recorded activities have no absolute meaning but the comparative values are considered valid.

Results

Localization of enzyme activity. Each disaccharidase was shown to be widely distributed throughout the small intestine, the highest activities, except for maltase, being found in the mid-jejunum (Table IV). Lactase activity was significantly greater at mid-jejunum than at 3rd part of the duodenum ($p < 0.001$) or mid-ileum ($p < 0.001$), duodenal activity being significantly greater than ileal ($p < 0.001$). Sucrase activity was also

TABLE III
Disaccharidase Activities in Normal Patients

Series	Disaccharidase Activity (*units/g. mucosa, wet weight)			
	Lactase	Sucrase	Palatinase	Maltase
Burgess <i>et al.</i> (1964)	8.7	17.4	6.7	62.6
Children—5 cases	(6.6-12.4)	(12.4-20.2)	(6.5-6.9) (2 cases)	(43.2-88.9)
Dunphy <i>et al.</i> (1965)	4.73	9.77	—	29.68
Adults—22 cases	(1.0-7.8)	(2.94-16.4)	—	(11.2-50.5)
Cuatrecasas <i>et al.</i> (1965)†	2.65	9.29	—	28.08
Adults—7 cases	(1.67-4.20)	(5.65-13.58)	—	(15.74-45.6)
Present series	8.45	7.45	2.53	20.85
Children—6 cases	(4.44-11.65)	(5.46-9.21)	(1.98-3.03)	(17.90-24.53)

* Units = μ moles of substrate hydrolysed per minute. † Converted from dry weight (= 17.2% of wet weight—author's figures).

maximal at mid-jejunum, being significantly greater than duodenal activity ($p < 0.001$), the difference between duodenum and ileum not being significant. Maltase activity was maximal in mid-ileal samples, being significantly greater than mid-jejunum ($p < 0.001$), which in turn was significantly greater ($p < 0.001$) than 3rd part of duodenum. Differences in distribution of palatinase activity were not significant. These studies suggest that the exact site of a mucosal biopsy is not of great practical significance in regard to disaccharidase estimations, particularly as there was virtually no overlap between normal and abnormal values in the clinical studies. It is emphasized, however, that these findings concern material from one case only.

TABLE IV
*Localization of Disaccharidase Activities
(Necropsy Material)*

Site of Specimen	Disaccharidase Activity (units/g. mucosa, wet weight)			
	Lactase	Sucrase	Palatinase	Maltase
Duodenum (3rd part) ..	5.52	5.02	1.44	15.85
Mid-jejunum ..	5.85	5.56	1.52	18.65
Mid-ileum ..	4.48	5.36	1.34	19.43

Figures represent means of triplicate assays carried out on 3 occasions, i.e. of 9 estimations.

Effect of storage. There was some decrease in activity by the sixth week of storage at -20°C . (Table IV), probably accentuated by thawing and refreezing to obtain successive samples. Loss of activity appeared considerable for lactase and maltase and negligible for sucrase and palatinase: variable durations of storage before assay made the results unsuitable for formal statistical analysis. Activities after storage for two weeks were unimpaired.

Reproducibility of results. Analysis of the results of assays performed in triplicate on the same homogenate showed the coefficient of variation (standard error expressed as a percentage of the mean) to be $\pm 2.64\%$ for lactase, $\pm 3.48\%$ for sucrase, $\pm 5.47\%$ for palatinase, and $\pm 1.36\%$ for maltase, i.e. reproducibility of results was good. Greater variation was found between assays of adjacent samples of tissue, partly explained by inevitable variation in the depth of the mucosal scrapings, further sources of possible error being in the weighing and dilution of samples before homogenization.

Discussion

Coeliac disease was described by Gee in 1888, yet over 60 years elapsed before the vital role of the gluten fraction of wheat flour in the aetiology of a hitherto puzzling disease was recognized (Dicke, 1950). Despite this major advance, difficulty was experienced in many cases in successfully establishing severely affected children on a gluten-free diet, the initial clinical response to exclusion of gluten often being disappointing (Sheldon, 1959). A possible explanation, i.e. associated intolerance to disaccharides, was suggested by the demonstration that diarrhoea could be caused by deficiency of sugar-splitting enzymes (Weijers, Van de Kamer, Dicke, and Ijsseling, 1961), and by the demonstration in coeliac patients of intolerance to milk (Cooke, Fone, Cox, Maynell, and Gaddie, 1963), and to sucrose (Sheehy and Flock, 1964).

Histological studies have shown that in coeliac disease the characteristic pathological feature is mucosal flattening with villous atrophy, principally affecting the proximal small intestine (Shiner and Doniach, 1960). Demonstrated initially in adults (Paulley, 1954), this was first confirmed in children by Sakula and Shiner (1957). Once thought to be a finding specific to coeliac disease, it is now shown to be found in a variety of disorders (Hindle and Creamer, 1965).

Disaccharide hydrolysis is a specific enzymatic function, the disaccharidases being located in the vicinity of the brush-border of the intestinal mucosal epithelium (Miller and Crane, 1961). Some contention exists regarding the exact distribution of the different disaccharidases along the intestinal tract, but both disaccharidase activity (Auricchio, Rubino, Tosi, Semenza, Landolt, Kistler, and Prader, 1963) and disaccharide absorption (Dahlqvist and Borgström, 1961; Gray and Ingelfinger, 1965) appear to be maximal in the jejunum, i.e. in the region of maximal mucosal damage in coeliac disease.

Recent work (Plotkin and Isselbacher, 1964; Shmerling *et al.*, 1964; Nordio *et al.*, 1965; Sheldon and Tempny, 1966) has clearly demonstrated decreased activities of mucosal disaccharidases in untreated patients with coeliac disease, and the return towards more normal values associated with mucosal regeneration in response to dietary treatment, thus providing a convincing explanation for the disaccharide intolerance often accompanying acute coeliac disease. The present investigation, on a further 12 patients, confirms these findings. Lactase activity was most affected, and was slowest to return toward normal values in response to treatment with a strict gluten-free diet. Shmerling *et al.*

(1964), reporting on biopsies from 13 children, showed that the activity of sucrase, isomaltase, and maltase increased in parallel with the clinical improvement, but that lactase activity remained selectively depressed in about half the cases subjected to serial biopsy. Lifshitz *et al.* (1965) have recently shown that lactose intolerance, as manifested by a poor glycaemic response to oral lactose loading and production of symptoms such as diarrhoea and abdominal cramps, was present in 4 out of 6 patients with coeliac disease, and that lactase activity in mucosal biopsy specimens was absent (as estimated chromatographically) in all patients before treatment. Rapid clinical improvement followed exclusion of lactose from the diet of those previously showing poor response to a gluten-free régime, though lactase activity returned to normal in only one patient after the start of dietary treatment.

The studies on necropsy material showed that the method used for estimation of disaccharidase activity gave consistent results within reasonable limits of accuracy, the coefficient of variation for triplicate assays performed on the same homogenate ranging from $\pm 1.36\%$ for maltase to $\pm 5.47\%$ for palatinase.

Complete assays were readily determined on 5-8 mg. wet weight of mucosa, and satisfactory use of smaller samples could also be made by minimizing the number of blank estimations.

Studies on the distribution of activity within the small intestine were in agreement with those of Auricchio *et al.* (1963), in that maximal activity was demonstrated in the jejunum, but indicated that enzyme activity was widely distributed. Determination of the precise site of a per-oral biopsy is difficult, and these studies suggest that the exact localization of a biopsy specimen does not have great practical importance in regard to disaccharidase estimation.

Summary

The disaccharidase activities of the mucosa of the small intestine of 12 patients with coeliac disease are reported, comparison being made with 6 normal subjects.

Disaccharidase activity was greatly diminished in untreated patients, lactase being the most severely affected; a slow return of activity, accompanying mucosal regeneration, was seen following treatment with a gluten-free diet. These findings are in agreement with the recently published reports of other workers.

Biopsy specimens could be stored at -20°C . for at least two weeks without loss of activity, and the quantitative method employed gave reproducible results on 5-6 mg. samples of material.

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was a nested one, and a parametric model for the analysis of variance was assumed.

An analysis of variance (see Table below) was performed to investigate differences between sites, and differences due to time of storage. Since pairwise comparisons of sites were also of interest, the three comparisons of each site with every other site were made, in such a way as to keep the significance level of the over-all test of difference among the three sites at not more than 5% (see, for example, Gabriel, 1964).

Appendix

Statistical Analysis. The design of the experiment

Analysis of Variance Table

	Source	Sum of Squares	Degrees of Freedom	Mean Squares
Lactase	Times	8·9797	6	1·4966
	Sites	11·0527	2	5·5264
	Residual	0·9871	17	0·0581
	Total	21·0195		
Sucrase	Times	10·7437	6	1·7906
	Sites	1·2713	2	0·6357
	Residual	1·7350	17	0·1021
	Total	13·7500		
Palatinase	Times	0·2222	6	0·0370
	Sites	0·1336	2	0·0668
	Residual	0·3153	17	0·0185
	Total	0·6711		
Maltase	Times	148·4796	6	24·7466
	Sites	63·7230	2	31·8615
	Residual	3·1328	17	0·1843
	Total	215·3354		