Electrical assessment of functional lactase activity in conscious man

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SUMMARY Using an electrical technique for measuring transjejunal potential differences (PDs) in conscious man, we have estimated the electrogenic absorption of the hexoses liberated by hydrolysis of lactose which was infused into the jejunum of one normal control and 21 patients with diarrhoea. The results were compared with jejunal lactase levels determined from biopsy specimens taken from the recording site immediately after infusion. The PD evoked by 100 mM lactose was very significantly lower in patients with lactase levels below 4 units (lactase deficient) compared with subjects with normal lactase levels. There was also a significant correlation ($r = 0.87$, $P < 0.005$) between the magnitude of the lactose potential (expressed as the ratio of the maximum glucose transfer potential) and the mucosal lactase level in the hypolactasic subjects but not in patients with normal lactase levels. Thus, in the subjects with lactase deficiency, the electrogenic transfer of hexose is clearly limited by the rate of lactose hydrolysis. Unlike other assessments of functional lactase activity, the electrical test provides a specific index of jejunal function and, moreover, can be adapted to investigate the possible disorders of small intestinal motility and secretion associated with hypolactasia.

Studies in the rat (Kohn *et al.*, 1968) have shown that while maltose and sucrose increased the transjejunal potential difference (PD) *in vitro*, no change in PD occurred with lactose. Similar results have been obtained in the jejunum of the 14 week human foetus (Levin *et al.*, 1968). These data are best explained if the transfer potential evoked by disaccharides is caused by the electrogenic transport of the hexoses released by hydrolysis. The failure of lactose to produce a transfer PD in the jejunum of the adult rat and human foetus is then presumably due to the very low lactase levels in their jejunal mucosae (Doell and Kretchmer, 1962; Dahlqvist and Lindberg, 1966). It seems possible therefore that the magnitude of the lactose potential may reflect the functional lactase activity in the small intestine. We tested this hypothesis in man by comparing the transjejunal PD produced during infusion of lactose with the lactase activity estimated in a jejunal biopsy taken from the recording site immediately after infusion.

Methods

Studies were undertaken on one young, healthy volunteer and 21 patients (10 male and 11 female) who were referred for jejunal biopsy with symptoms which included diarrhoea. The clinical diagnosis of each patient is given in Table 1.

ELECTRICAL MEASUREMENTS

The method for intubating the proximal jejunum and recording the PDs in conscious human subjects has been described previously (Read *et al.*, 1974, 1976a, 1976b). The intestinal tube incorporated a biopsy capsule (Crosby and Kugler, 1957) as well as channels for the infusion and siphonage of solutions (Read *et al.*, 1976a, 1976b). The solutions shown in Table 2 were infused into the jejunal lumen at a constant rate of 5 ml per minute and allowed to siphon continuously from the infusion site into a receptacle at ground level. Infusion of the solution containing 100 mM mannitol provided a baseline PD against which the transfer PDs generated by the solutions containing lactose or glucose were compared. Because of possible variations in the absolute magnitudes of the transfer PDs between patients due to differences in both electrical conductivity and

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Table 1 Clinical diagnosis and jejunal lactase levels of 21 patients with diarrhoea and of one healthy volunteer

Patient	Sex	Age (yr)	Diagnosis	Lactase activity (Units)
Lactase deficient				
F.D.	M	30	Constitutional hypolactasia	0
A.S.	M	18	Constitutional hypolactasia	0
E.E.	F	56	Coeliac disease, lymphoma	0
R.K.	M	53	Tuberculosis, treated with isoniazid	0.6
J.E.	M	34	Total colitis, colectomy	0.4
G.M.	M	59	Post-gastrectomy diarrhoea	1.2
E.H.	F	70	Anaemia, diarrhoea	2.0
J.M.	F	31	Coeliac disease	2.4
E.B.	F	36	Total colitis, colectomy hypopituitarism	2.6
B.W.	F	33	Coeliac disease	3.7
Normal lactase activity				
T.B.	M	70	Distal colitis	6.7
J.S.	M	46	Irritable bowel syndrome	8.0
C.H.	F	69	Pernicious anaemia, diarrhoea	8.2
M.W.	F	23	Irritable bowel syndrome	8.4
D.B.	F	42	Anaemia, hyposplenism, visual failure	9.2
C.H.	F	21	Normal control	9.6
D.B.	F	76	Megaloblastic anaemia, osteomalacia	9.8
L.M.	M	70	Distal colitis, hypertension, carcinoma of bronchus	10.0
D.F.	F	14	Irritable bowel syndrome	10.4
G.D.	F	51	Purgative abuse	12.3
B.M.	M	75	Post-gastrectomy diarrhoea	13.0
A.M.	M	78	Tuberculosis, renal failure, weight loss, anaemia	14.5

The subjects, arranged in ascending order of their lactase activities, fall into two groups; those with lactase activities below 4 units (lactase deficient) and those with lactase activities above 6 units (normal). Although only two patients in the lactase deficient group have been diagnosed as constitutional hypolactasias, it is nevertheless possible that other patients in this group have constitutional hypolactasia in addition to the stated diagnosis.

Table 2 Solutions infused into jejunal lumen

Solution 1	104 mM NaCl + 100 mM mannitol
Solution 2	104 mM NaCl + 100 mM glucose
Solution 3	104 mM NaCl + 100 mM mannitol
Solution 4	104 mM NaCl + 100 mM lactose

the current induced by ion-linked hexose transfer, the potentials evoked by 100 mM lactose were normalised by being expressed as a ratio of the transfer potential generated by 100 mM glucose. This concentration was employed because it saturated the electrogenic glucose transport mechanism, thus avoiding problems in interpretation caused by possible differences in 'apparent K_m ' between patients.

ESTIMATIONS OF LACTASE ACTIVITY

Before the intestinal tube was finally withdrawn a specimen of jejunal mucosa was obtained by means of the biopsy capsule. The biopsy was detached from the capsule, spread mucosa upwards on a clean piece of blotting paper, and divided into two portions with a sharp scalpel. One portion was immersed in formalin and sent for routine histology, while the other was assayed for lactase activity by a modification of the method described by Burgess *et al.* (1964). Briefly, this consisted of incubating a homogenate

of the biopsy with 40 mM lactose for 20 minutes and then estimating the glucose liberated, using the glucose oxidase method (Boehringer Test Kit; catalogue number 15756). The results were expressed as lactase units where 1 unit = $1 \mu\text{mol}$ lactase split/g of tissue wet weight⁻¹ minute⁻¹.

Results

The lactase activities for the subjects included in this study cluster into two groups (Table 1); those below 4 units (range 0.3-3.7 units) and those above 6 units (range 6.6-14.5 units). The normal range for lactase activity reported by different authors varies widely, but our upper group appears to correspond approximately with the normal ranges of McMichael *et al.* (1966) (3.4-18.6 units) and Preston (1970) (4.0-12.5 units), who have both used the same method for lactase assay described in this present paper. We have thus classified all of our patients with lactase activity below 4 units as being lactase deficient. It can be seen from Table 1 that these are a heterogeneous group, containing patients in whom hypolactasia may be secondary to another condition such as coeliac disease as well as two subjects with alactasia for which there was no obvious cause. It was interesting that these two patients, who have been credited with the diagnosis of constitutional

Fig. 1 The PD evoked by 100 mM lactose (dotted bars) and the PD generated by 100 mM glucose (open bars) in subjects with normal lactase levels and patients with lactase deficiency (see Table 1). The results are expressed as the mean $\pm$ SEM.

Fig. 2 A graph of the lactose potential expressed as a ratio of the glucose transfer potential (see methods) plotted against the lactase activity in the biopsy specimens. The regression lines have been inserted for the groups with lactase deficiency and normal lactase activity. There is a significant correlation ($r = 0.87$, $P < 0.005$) between the ratio of the potentials and the mucosal lactase levels in the patients with lactase deficiency but not in those with normal lactase levels.

hypolactasia, were the only subjects to have no demonstrable lactose potential.

It can be seen from Fig. 1 that the lactose potentials from the lactase deficient group are significantly lower than the corresponding values from the group with normal lactase activities, while the glucose potentials are not significantly different. If the lactose transfer potential, expressed as a ratio of the glucose PD is plotted against the lactase activity (Fig. 2) then there is a highly significant correlation ($r = 0.87$, $P < 0.005$) between the two sets of data in the subjects with lactase deficiency. However, in

those patients with normal lactase activity the correlation is not significant ($r = 0.06$), the slope of the regression line being very close to zero.

Discussion

The results of this study clearly indicate that, in patients with lactase deficiency, including three with coeliac disease, the electrogenic uptake of hexose from lactose is limited by the rate of lactose hydrolysis. As such, they confirm and extend the findings of McMichael *et al.* (1967) who used perfusion studies in which the uptake of hexose from lactose infused into the human jejunum was estimated chemically. Measurement of absorption by these perfusion techniques is, however, subject to considerable errors when absorption is very depressed (Holdsworth and Dawson, 1965), which presumably accounts for the absence of published data on the variations in hexose absorption from lactose within the hypolactasic group. With the electrical technique it is possible to obtain a definitive index of active hexose absorption even when this is low and thus we have been able to demonstrate a significant correlation between the electrogenic transfer of hexoses and the mucosal lactase levels in patients with lactase deficiency (Fig. 2).

Although most of the tests available for the clinical determination of hypolactasia are non-invasive, they provide only indirect estimates of the functional lactase activity of the intestinal mucosa. The lactose tolerance test depends on the functional integrity of the liver, pancreas and kidneys, while the more recent breath tests (Sasaki *et al.*, 1970; Bond and Levitt, 1972) require relatively normal intestinal motility and respiratory function and appropriate colonic bacteria. Even the estimation of lactase levels in biopsy specimens may not provide a true index of functional activity because of possible differences between the lactase activity in homogenates and intact brush border (Zoppi *et al.*, 1966). The measurement of the transfer PDs from the hydrolysis products of lactose, while providing an index of the functional lactase activity of the jejunal mucosa *in situ*, is, at the present, a research technique rather than a routine clinical test. Although an intrusive method, it can be easily undertaken during the procedure for jejunal biopsy. Moreover, the recording of the PD can also be adapted for the electrical investigation of the putative secretory activity of the small intestine associated with motility (Read *et al.*, 1976c). This could provide a new research approach to investigate the pathophysiology of diarrhoea and abdominal discomfort associated with hypolactasia.

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