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Intestinal permeability in coeliac disease: the response to gluten withdrawal and single-dose gluten challenge

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SUMMARY Intestinal permeability has been studied in 21 patients with coeliac disease in relapse and after gluten withdrawal using an oral test of intestinal permeability based on the simultaneous oral administration of two probe molecules. The increased absorption of the larger molecule (cellobiose) and the decreased absorption of the smaller (mannitol) found in untreated coeliac disease both returned to normal within five months of starting treatment, the abnormality in cellobiose absorption correcting more rapidly than that of mannitol. After exposure to a single oral dose of gluten, the intestinal permeability of six patients with treated coeliac disease became transiently abnormal with an increased absorption of cellobiose, returning to normal within one week. The possible structural and functional implications of these findings are discussed. The cellobiose/mannitol ratio appears to be of value in assessing the response to gluten withdrawal in coeliac disease, and also in monitoring patients who are already established on a gluten free diet by detecting dietary lapses and 'non-responding coeliac disease'. It may also offer an alternative to jejunal biopsy in patients subjected to gluten challenge.

The abnormal intestinal permeability of untreated coeliac disease is characterised by a reduced absorption of small hydrophilic molecules[1] with a paradoxical increase in absorption of larger molecules.[2 3] We have used the simultaneous oral administration of two water-soluble probe molecules, mannitol (molecular radius 0·4 nM) and cellobiose (molecular radius 0·5 nM) to demonstrate these changes, and have shown that patients with coeliac disease excrete more cellobiose and less mannitol in their urine than controls, after oral ingestion of these molecules in hypertonic solution.[4] Expression of the result as a ratio of cellobiose recovery to mannitol recovery allows clear separation of normal subjects from coeliacs, a finding confirmed by others using a similar test system.[5] The absorption of mannitol from the normal small bowel is 10–200 fold greater than that of cellobiose, suggesting, in the absence of active transport,[3 6] that, while mannitol may be absorbed through classical transcellular aqueous pores, cellobiose is excluded by its size. The effective pore radius must, therefore, lie between 0·4 and 0·5 nM, in agreement with earlier estimates,[7 8] and much smaller than the estimate of 0·8 nM by Fordtran *et al*,[9] whose calculations relied on the complete failure of mannitol absorption, while we can confirm the previously reported significant absorption of mannitol in man.[4 10] The paradoxical changes in absorption of the two probe molecules which occur in coeliac disease also suggest that they are absorbed through different routes, and the presence of more than one route of diffusion of hydrophilic molecules across cell membranes has been previously postulated.[11]

The alternative route of absorption for large molecules may be through the intercellular 'tight junction'[11] or through epithelial discontinuities such as the cell extrusion zones of the villous tips,[12] and the increased cellobiose absorption in disease may reflect non-specific epithelial injury, increased cell shedding, or changes in the tight junction. The reduced absorption of mannitol may result from a reduction in the number of aqueous pores available for diffusion secondary to a reduction in absorptive surface area. These permeability changes of coeliac disease are similar to those occurring in a rat nematode infestation, in which the histological lesion resembles that of coeliac disease.[13]

The simultaneous administration of two probe molecules, and expression of the urinary recoveries as a ratio, reduces the influence of factors other than intestinal absorption—for example, gastric emptying, intes-

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tinal transit, and renal function, on the result, and we have demonstrated the reliability of this test as a screening test for proximal small bowel disease.[14] The changes in intestinal permeability occurring during treatment of coeliac disease have not been described, and this paper demonstrates the use of the cellobiose/mannitol recovery ratio in monitoring the changes of intestinal permeability after treatment of coeliac disease with a gluten free diet, and after a single dose gluten challenge given to six coeliacs in remission.

Methods

PATIENTS

Response to gluten withdrawal

Eighty-nine individuals were studied, these comprised:

Untreated group Twenty-one patients, mean age 41 years (range 16–64), had partial (nine) or subtotal (12) villous atrophy on jejunal biopsy. Three had been diagnosed as having coeliac disease several years previously on the basis of an abnormal jejunal biopsy and had made a satisfactory clinical response to a gluten free diet, but were studied at a time of recurrent symptoms associated with dietary relaxation. The remaining 18 were newly diagnosed. All patients were treated by gluten withdrawal, and all those with symptoms responded clinically. Twelve have had a post-treatment biopsy which has confirmed histological response, and nine have refused a further biopsy. Intestinal permeability was measured in each of these patients before, and at a mean time of four months (range three to eight months) after the institution of a gluten free diet. Permeability changes in 10 of the patients were studied more closely by testing them initially weekly, and subsequently at increasing intervals.

Previously treated group Ten patients were already established on a gluten free diet, a diagnosis of coeliac disease having previously been made on the basis of an abnormal jejunal biopsy and a satisfactory clinical response to treatment. Seven had shown a histological response on a post-treatment jejunal biopsy, and three had refused further biopsy. The mean age of these patients was 37 years (range 19–42 years) and the mean duration of treatment was 6·5 years (range three–14 years).

Non-responding group These three patients had been shown to have partial or sub-total villous atrophy on jejunal biopsy, and had not responded clinically or histologically to a gluten free diet. The diagnosis of lymphoma has been confirmed in one, and is suspected in the other two.

Control group Fifty-five patients were identified who had carried out the test and had subsequently been shown to have a normal jejunal biopsy, and no evidence of significant gastrointestinal pathology. These patients form the control population.

Gluten challenge

Six patients with coeliac disease who were well controlled on a gluten free diet agreed to take part. Their mean age was 50·6 years (range 40–64 years) and they had been treated for a mean of two years (range six months–six years). Each patient was tested one day before, and daily for five days after a single oral dose of 30 g gluten taken in milk at bedtime, otherwise continuing with their gluten free diet.

Three normal controls were tested in the same way, taking 30 g of gluten, while on a normal diet.

PROCEDURE

The test drink comprised 5 g cellobiose and 2 g mannitol dissolved in 100 ml water. Twenty grams of lactose and 20 g sucrose were added to increase the osmolality of the solution to approximately 1500 mOsmol, which enhances the cellobiose absorption and increases the discrimination between normal and abnormal mucosae.[12]

After an overnight fast, subjects emptied their bladder to provide a baseline urine sample, and drank 100 ml test solution undiluted. All urine passed over the next five hours was collected into 25 μM thiomersal.

Mannitol was assayed by a spectophometric method, with an accuracy of 94–106% and the coefficient of variation was ± 2·5%.[15] Cellobiose was assayed by quantitative paper chromatography.[16] The accuracy of the assay technique was 94–110% and coefficient of variation ± 2·0% for both samples and standard aqueous solutions.[15] The urinary recovery of each molecule was expressed as the percentage of the administered dose recovered, and the cellobiose/mannitol percentage recovery ratio was the ratio of the percentage quantities of each probe molecule recovered.

This expression of the cellobiose/mannitol ratio differs from that used in our previous papers,[4 13-15] which was the ratio of the absolute quantities of each probe molecule recovered, but has the advantage of automatically relating the recovery of each of the molecules to the administered dose.

Statistical comparisons were performed using the Mann-Whitney U test.

Results

RESULTS IN NORMAL CONTROLS (Table)

Mean cellobiose recovery was 0·32 ± 0·20%, mean mannitol recovery 19·6 ± 8·3%. The mean cellobiose/mannitol recovery ratio in normal subjects is 0·0172 ± 0·009, with an absolute range of 0·002–0·04. Distribution is skewed towards lower values, however, and the accepted upper limit of the normal range is 0·03.

Table *Urinary recoveries of cellobiose and mannitol, and cellobiose/mannitol recovery ratios, in controls, patients with newly diagnosed coeliac disease before and after treatment, patients with previously treated coeliac disease, and patients with non-responding coeliac disease*

	% Cellobiose recovery (mean ± SD)	*% Mannitol recovery (mean ± SD)*	*Cellobiose mannitol ratio (mean ± SD)*
Controls (n = 55)	0·32 ± 0·20	19·6 ± 8·30	0·0172 ± 0·009
Coeliac disease newly diagnosed (n = 21)			
Before treatment	0·96 ± 0·61	8·38 ± 6·32	0·23 ± 0·09
After treatment	0·29 ± 0·24	22·10 ± 14·00	0·019 ± 0·01
Previously treated (n = 10)	0·57 ± 0·50	14·63 ± 4·94	0·032 ± 0·02
Non-responsive (n = 3)	0·66 ± 0·22	10·50 ± 5·60	0·09 ± 0·056

RESULTS IN UNTREATED COELIAC DISEASE AND EFFECT OF GLUTEN WITHDRAWAL

Before treatment, mean cellobiose recovery was 0·96 ± 0·61%, mannitol recovery 8·36 ± 6·32%, and the mean ratio 0·23 ± 0·09. Only one patient had a cellobiose/mannitol recovery ratio within the absolute range of normal and none was within the accepted normal range. All values differed significantly from controls (P = <0·05. Table).

After treatment mean cellobiose recovery in these patients fell to 0·29 ± 0·24 % (P = < 0·05), mannitol recovery rose to 22·1 ± 14% (P = < 0·05), and the mean ratio fell to 0·019 ± 0·01 (P = < 0·05). None of these mean post-treatment values differed significantly from controls (Table).

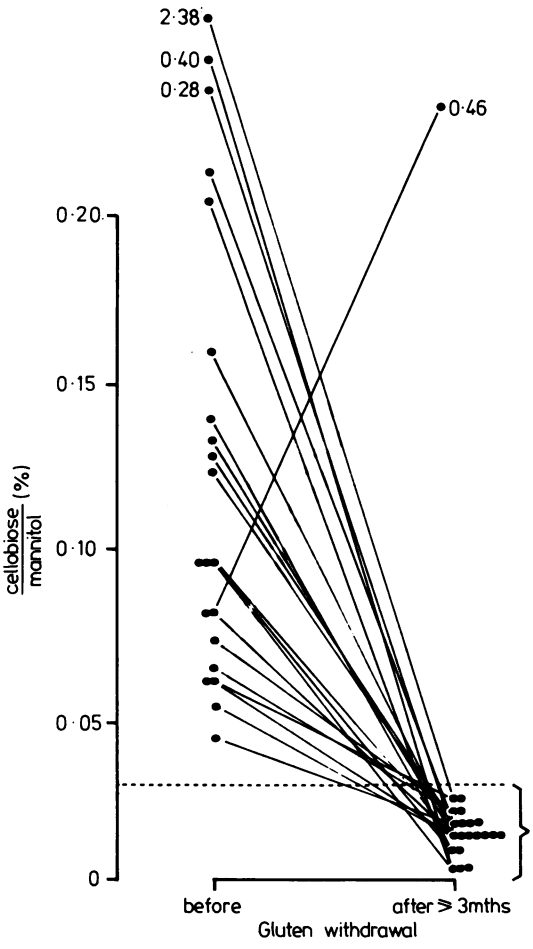

Fig. 1 *Cellobiose/mannitol percentage recovery ratio in 21 patients with coeliac disease before, and a minimum of three months after treatment with a gluten free diet. Bracket indicates normal range.*

The changes in cellobiose/mannitol recovery ratios of the individual patients are shown in Fig. 1. The ratio of 20 of the 21 patients fell from a clearly abnormal value to lie within the normal range after a mean period of four months' dietary treatment.

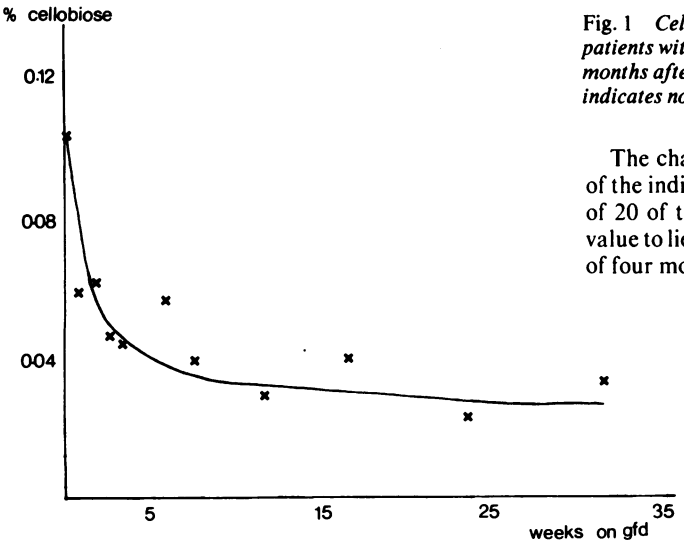

Fig. 2 *Mean cellobiose recovery in 10 patients with coeliac disease against duration of treatment with a gluten free diet.*

TIME COURSE OF RESPONSE TO GLUTEN WITHDRAWAL

The mean cellobiose recovery fell sharply from an initially high value, the fall occurring over the first eight–10 weeks of the diet (Fig. 2). The mean mannitol recovery rose more slowly, reaching a plateau over the first 20 weeks of treatment (Fig. 3). Mean recovery of each of the probe molecules remained substantially unchanged thereafter.

The cellobiose/mannitol recovery ratio (Fig. 4) of all 10 patients fell from an initially high value to lie within the normal range. In eight this fall took place rapidly, and normal values were reached within three months of starting treatment. The fall in the other two patients was more delayed, but the ratio became normal by the fifth month of treatment.

Once the normal range was achieved, the ratio tended to remain normal. The abnormal values could usually be related to an admitted dietary lapse.

PREVIOUSLY TREATED GROUP (Table)

Mean cellobiose recovery was 0·57 ± 0·5%, lower than in the untreated patients ($P = < 0·05$) but not significantly different from the controls or the post-treatment values in newly diagnosed coeliacs.

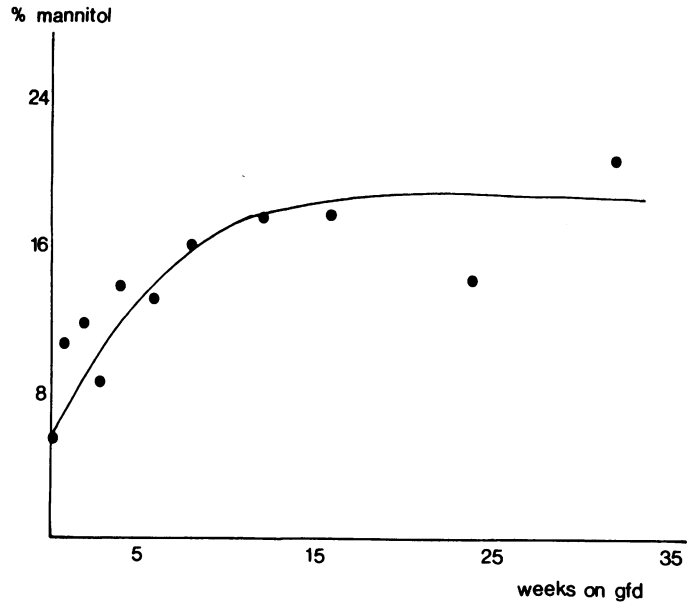

Fig. 3 *Mean mannitol recovery in 10 patients with coeliac disease against duration of treatment with a gluten free diet.*

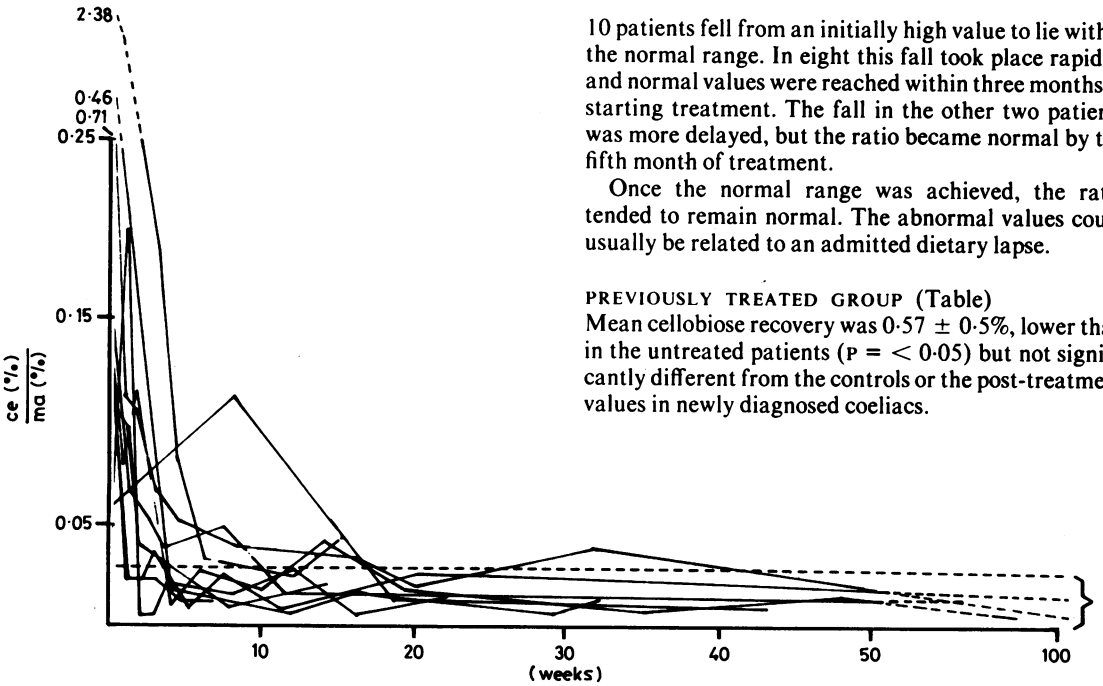

Fig. 4 *Cellobiose/mannitol percentage recovery ratios of 10 patients with coeliac disease, against duration of treatment with a gluten free diet. Bracket indicates normal range.*

Mean mannitol recovery was 14·63 ± 4·94%, higher than in untreated patients (P = < 0·002), and lower than controls (P = < 0·05) or newly diagnosed coeliacs after treatment (P = < 0·05).

Mean cellobiose/mannitol recovery ratio was 0·032 ± 0·02, lower than in untreated patients (P = < 0·05), but not significantly higher than in controls or newly diagnosed coeliacs after treatment.

NON-RESPONDING GROUP (Table)

Six tests were performed on three patients during treatment with a gluten free diet to which they did not respond clinically or histologically.

Mean cellobiose recovery was 0·66 ± 0·22%, which is higher than that found in controls or treated patients of either group (P = < 0·05) but not significantly different from that of untreated patients. Mannitol recovery was 10·5 ± 5·6% lower than in treated patients (P = <0·05) but not significantly different from untreated patients. The cellobiose/mannitol recovery ratio was 0·09 ± 0·056, and again was higher than in treated (P = < 0·05) but not untreated patients—that is, treated non-responders behaved like untreated coeliacs.

GLUTEN CHALLENGE

Before taking gluten, mean mannitol recovery in six patients was 19·8 ± 11·7%, mean cellobiose recovery

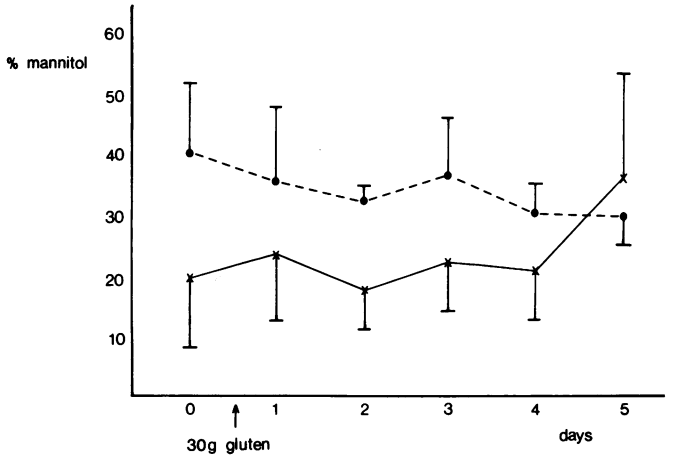

Fig. 5 *Mean (+ or − SD) urinary recovery of mannitol in six patients with coeliac disease (● - - - - ●) and three normal controls (X ——— X) before and after gluten challenge.*

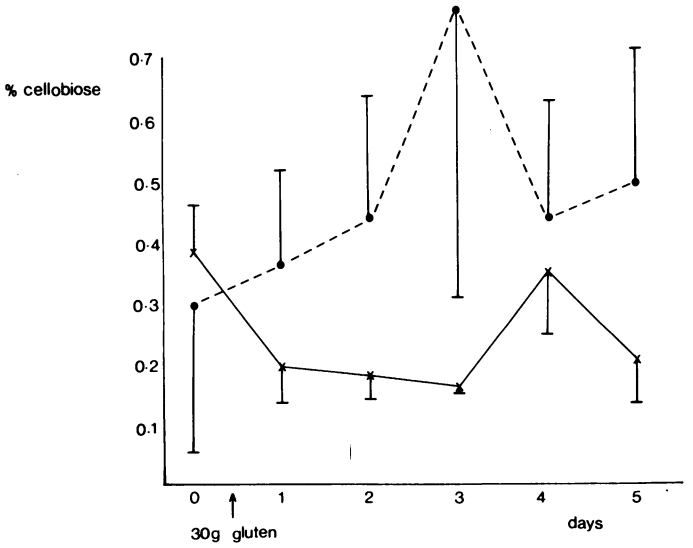

Fig. 6 *Mean (+ or − SD) urinary recovery of cellobiose in six patients with coeliac disease (● - - - - ●) and three normal controls (X ——— X) before and after gluten challenge.*

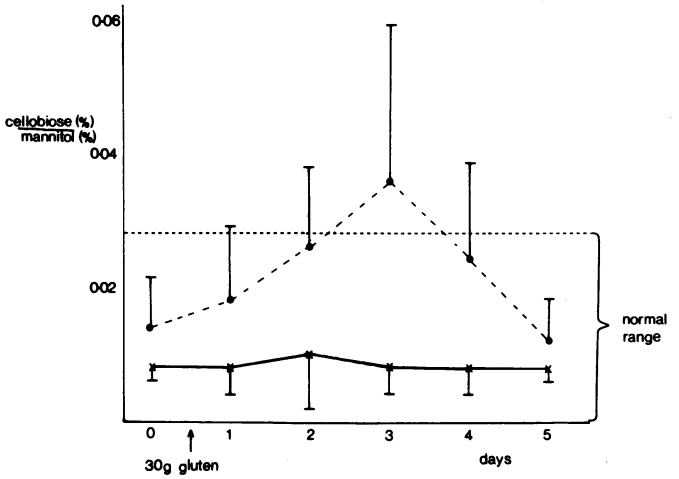

Fig. 7 *Mean (± SD) cellobiose/mannitol percentage recovery ratio in six patients with coeliac disease (●----●) and three normal controls (X————X) before and after gluten challenge.*

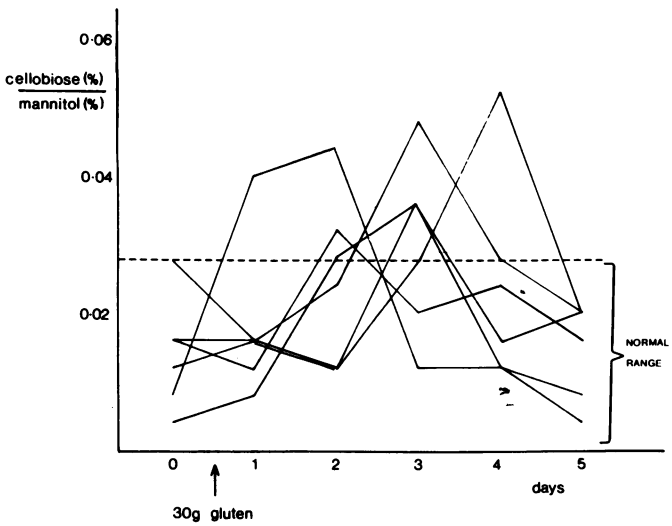

Fig. 8 *Cellobiose/mannitol percentage recovery ratios in six patients with coeliac disease before and after gluten challenge.*

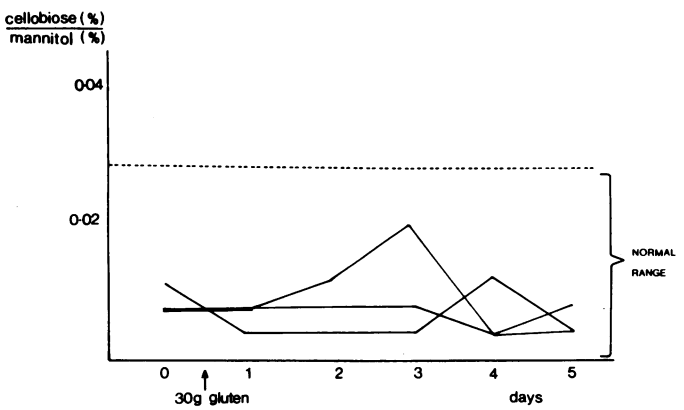

Fig. 9 *Cellobiose/mannitol percentage recovery ratios in three normal controls before and after gluten challenge.*

0·30 ± 0·28%, and the cellobiose/mannitol recovery ratio was 0·035 ± 0·02. All values were similar to the normal controls and the ratio was within the normal range.

After ingestion of a single dose of 30 g commercial gluten, there was little change in mannitol recovery in either patients or controls (Fig. 5), while cellobiose recovery showed a progressive rise, reaching a peak after three days and falling again by day 5 (Fig. 6), a change not seen in controls. Because of the wide variation between individuals and the small group of patients studied these results did not achieve statistical significance.

The mean cellobiose/mannitol recovery ratio in patients rose progressively over the three days after gluten ingestion to an abnormal value of 0·037 ± 0·023 on day 3, and fell to 0·014 ± 0·006 by day 5 (Fig. 7). The value on day 3 was significantly greater than that before gluten ($P = < 0·05$), and five days after gluten ($P = < 0·05$).

Of greater importance was the change in individual ratios (Fig. 8). Each patient had a normal ratio before gluten challenge, but in each case the ratio rose to reach a clearly abnormal value between the second and fourth day after taking gluten. The ratio for all patients had returned to normal by day 5.

None of the controls showed any significant change in cellobiose/mannitol recovery ratio after gluten, and the control value on day 3 differed significantly from that of patients ($P = < 0·05$) (Fig. 9). The mean ratio of controls was consistently within the range 0·008–0·01.

Discussion

This simple and non-invasive test of intestinal permeability has enabled us to demonstrate the improvement in one aspect of small bowel function which occurs during the first six months of treatment of coeliac disease with a gluten free diet, and also demonstrates the transient abnormality of permeability resulting from a single exposure to gluten in susceptible individuals.

The abnormal passive permeability of the small intestine in coeliac disease rapidly returns to normal after treatment with a gluten free diet, as is shown by the change in the cellobiose/mannitol percentage recovery ratio (Figs. 1 and 4). The only patient in whom the ratio did not return to normal was asymptomatic at the time of diagnosis, and has subsequently admitted that she did not adhere to a strict gluten free diet. Patients already established on a gluten free diet had results which were intermediate between normals and untreated coeliacs, possibly indicating a less rigid dietary adherence, since they had been on treatment for considerably longer, and did not have the incentive of being closely followed up for research purposes. The improvement in permeability after treatment occurs during the period when the earliest morphological indices of response, the surface cell height and mitotic index, are also improving,[17] but before complete morphological response can be expected,[18] in agreement with earlier reports that functional improvement precedes histological recovery.[18] Although ultrastructural changes in the basement membrane and sub-epithelial deposition of immune complexes have been observed within 48 hours of gluten challenge,[19 20] the maximal change in intestinal permeability occurs two to four days after a single dose of gluten. This discrepancy may reflect the time taken for damaged cells to mature to a point on the mucosa where they have a greater influence on passive permeability, or it may be that permeability changes are the result of a separate mechanism, more delayed than immune complex deposition. Light microscopic evidence of a reduction in surface cell height and an increased mitotic index may be seen within seven days of gluten challenge,[17] coinciding with the increased permeability to cellobiose we have demonstrated, and the fall in cellobiose recovery after treatment of coeliac disease and rise after gluten challenge may be due to changes in epithelial integrity or cell turnover, affecting the 'leakiness' of the mucosa. The slow change in mannitol recovery after treatment may reflect the time taken for villous architecture and absorptive surface area to return to normal.

The variation between subjects in the speed with which they respond to a gluten challenge has been related to the quality of dietary control,[19] the dose and form of gluten given,[21] age[22] and the duration of treatment before challenge, those patients treated most recently responding more rapidly.[17] Speed of response was not related to age or duration of treatment in our patients, and they were all given the same dose of commercial gluten, but the possibility cannot be excluded that those patients showing the most rapid changes in permeability were habitually consuming small quantities of gluten, although all denied knowingly doing so.

Low molecular weight polyethylene glycol (PEG 400) has been advocated as a probe molecule to demonstrate changes in intestinal permeability, the various subfractions showing a decreased absorption as the molecular weight increases.[23] In coeliac disease there appears to be a general reduction in absorption with no increased permeability to the high molecular weight fractions[24] in contrast with our findings. All patients studied with PEG, however, had been treated with a gluten free diet, so the failure to demonstrate increased permeability to large molecules in these patients may have been the effect of treatment.

Measurement of mannitol and cellobiose recoveries by the use of separate assays may be considered inconvenient, and quantitative paper chromatography has been criticised for its inaccuracy.[25] In our hands, how-

ever, the technique is both accurate and reliable, and the precision is similar to that found by Menzies when using the technique to measure similar disaccharides.[16]

The use of cellobiose as a probe molecule is open to criticism, as it is partially hydrolysed by intestinal disaccharidases; this effect, however, is small and unlikely to affect these findings, as we have been unable to demonstrate any difference between recoveries of cellobiose and lactulose in normal or coeliac subjects,[26] and patients with intestinal hypolactasia have normal test results.[14] Hence the increased cellobiose absorption in untreated coeliac disease is not due to associated hypolactasia. Furthermore, the change in cellobiose recovery after treatment is unlikely to be due to recovery of intestinal disaccharidase, as enzyme levels revert to normal only after a prolonged period of gluten exclusion.[22]

The advantage of simultaneous administration of two probe molecules of different sizes is clearly demonstrated by the response to gluten challenge in which the cellobiose/mannitol recovery ratio of all patients became abnormal, whereas the effect on the recovery of either probe molecule alone failed to demonstrate such changes. Even the considerable increase in cellobiose recovery does not achieve statistical significance in this small group of patients, because of the wide variation between individuals, and it is unlikely that any other probe molecule used alone—as, for example, the xylose absorption test—would be sufficiently sensitive to demonstrate such unequivocal changes in permeability. The reproducibility of this test system is emphasised by the constant ratio of the controls, suggesting that changes in ratio reflect true changes in permeability.

Reliable oral tests of intestinal permeability may have important applications in coeliac disease. As well as being useful in reaching a diagnosis,[14] such a test is of value in monitoring the effect of treatment, either confirming an early response or recognising 'non-responding coeliac disease'. In the long-term it will detect those patients who relapse.

The test described here may be a major advance in the context of a gluten challenge; conventional gluten challenge is made difficult by several factors. Many patients are unwilling to abandon their diet, or to undergo further jejunal biopsy, and the optimum dose and nature of gluten to be given, and the duration of challenge, are unknown. The belief that failure to demonstrate relapse after three months' gluten challenge excludes the diagnosis of coeliac disease[27] is no longer tenable, as relapse may occur after much longer periods.[28 29] The timing of a post-challenge jejunal biopsy is therefore difficult; symptomatic relapse is of little value in predicting histological relapse, which may be symptomatic,[21 27 29] and previous screening tests—for example, one hour blood xylose[21]—have not proved universally reliable.[30] The cellobiose/mannitol ratio may be a sufficiently sensitive screening test to aid in the timing of a post-challenge biopsy.

The changes in intestinal permeability after a single dose gluten challenge, which we have demonstrated in each of a small group of patients with coeliac disease, are likely to be a direct result of gluten ingestion, although a double-blind trial comparing the effects of gluten on intestinal permeability with those of placebo may be necessary to confirm this. The use of this test to demonstrate such changes after a brief period of exposure to gluten may, if confirmed, offer a convenient and non-invasive alternative to jejunal biopsy in diagnosing gluten sensitivity.

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