

# Intestinal permeability and screening tests for coeliac disease

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**SUMMARY** In disease states of the small intestine—for example, gluten-sensitive enteropathy—there is an increased permeability to large molecules. This increased permeability extends to polar molecules of intermediate size such as disaccharides, whereas small polar molecules are malabsorbed. A recently-developed oral test, based on the simultaneous administration of two test substances, cellobiose (a disaccharide) and mannitol (a small polar molecule) has been used to investigate permeability in a variety of gastrointestinal diseases, the result of the test being expressed as the ratio (cellobiose/mannitol) of the five hour urinary recoveries of the two probe molecules. Results for patients with pancreatic insufficiency, intestinal bacterial overgrowth, primary hypolactasia, ileocolic or colonic Crohn's disease, and ulcerative colitis were comparable with those in normal controls, whereas in 23 out of 24 untreated coeliacs, and five out of eight patients with Crohn's disease involving the more proximal small bowel, the cellobiose/mannitol ratio was clearly abnormal. A study of its application as a screening procedure for coeliac disease showed that the test was both sensitive and accurate, with fewer false-positive and false-negative results than other recognised screening tests—namely, the xylose test, reticulín antibodies, and blood folate estimations.

In coeliac disease, there is an increase in passive intestinal permeability to large polar molecules, ranging in size from proteins<sup>1</sup> down to oligosaccharides.<sup>2</sup> In contrast, small polar molecules are malabsorbed. We have described<sup>3,4</sup> our preliminary findings using a test of intestinal permeability (known as the cellobiose/mannitol test or 'sugar test' for short), based on the simultaneous oral administration and five-hour urinary recoveries of two probe molecules. The larger molecule used was cellobiose, a disaccharide (molecular radius 5 Å), and the smaller was mannitol, a polyhydric alcohol (molecular radius 4 Å). We showed that untreated coeliacs absorbed significantly more cellobiose, and less mannitol than control patients, and, when the result was expressed as a ratio (cellobiose recovery/mannitol recovery), the discrimination was greatly enhanced. We postulated that, by expressing the result in this way, the sensitivity of the test as a screening procedure would be increased, and that, providing the two molecules were not too dissimilar

in their behaviour, the influence of extraneous factors such as renal impairment would be eliminated or at least minimised. This would appear to be the case; certainly, patients with renal failure or liver disease have ratios within normal limits, and there is no significant correlation between values for the ratio and those for the serum creatinine, gastric emptying, or intestinal transit times<sup>5</sup>.

This paper describes the test results in a variety of gastrointestinal conditions, and compares the diagnostic accuracy and sensitivity of the test with other screening procedures for coeliac disease.

## Methods

### CELLOBIOSE/MANNITOL TEST

#### *Technique*

The test solution comprised 5 g cellobiose and 2 g mannitol dissolved in 100 ml water, to which were added 20 g lactose and 20 g sucrose to render the solution hypertonic (c. 1500 mOsmol). This has been shown to enhance the absorption of the larger molecule and increase the discrimination between controls and coeliacs,<sup>2,5</sup> the use of two sugars being dictated by considerations of solubility and palata-

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bility. The solution was drunk after fasting overnight and urine collected for five hours. Cellobiose and mannitol were assayed as previously described,<sup>3</sup> the results being expressed as the percentage recoveries of the two molecules in five hours. The ratio was derived from the absolute recoveries (in milligrams)—that is, cellobiose recovery (mg)/mannitol recovery (mg).

*Patients*

Tests were carried out by both hospital patients and outpatients at home. The following groups were studied.

*Controls*

Fifty-five control patients were identified who had performed the test, had had a normal jejunal biopsy, and in whom no significant gastrointestinal pathology was found on further investigation. Nineteen were referred with severe oral aphthae, four with unexplained anaemia, and two were relatives of known coeliacs. The remaining 30 presented with various combinations of diarrhoea, weight loss, and abdominal pain for which no cause was found. Many were diagnosed as suffering from the irritable bowel syndrome, on the basis of the negative tests and symptomatic response to high-fibre diet.

*Untreated coeliacs*

Twenty-four untreated adults with villous atrophy performed the test. Thirteen had subtotal, and 11 had partial villous atrophy of varying degrees but always

sufficient to suggest a diagnosis of coeliac disease. Thirteen have had a diagnosis of coeliac disease confirmed by histological response to gluten withdrawal (11) or challenge (two). Four have responded clinically to a gluten-free diet but have declined a follow-up biopsy, and two completely asymptomatic patients have not yet had a repeat biopsy. Two patients were lost to follow-up, and three were unresponsive to gluten-withdrawal: one has been shown to have a lymphoma, and this diagnosis is suspected in the other two.

*Pancreatic steatorrhoea*

Tests were performed on six patients with steatorrhoea in whom pancreatic exocrine insufficiency was the eventual diagnosis. Three had carcinoma and two had chronic pancreatitis, diagnosed by ERCP and Lundh meal. One patient had had a pancreatectomy for carcinoma. Two of the six patients had had a jejunal biopsy which was normal.

*Blind-loop syndrome*

Nine patients (two with surgical blind-loops, seven with intestinal scleroderma) had evidence of intestinal bacterial overgrowth with a high bacterial count ( $>10^6$ /ml) on jejunal culture. Five had a positive result with a  $^{14}\text{CO}_2$  or hydrogen breath-test for intestinal bacterial overgrowth.<sup>6</sup> Seven were known to have a normal jejunal biopsy.

*Gastric surgery*

Eight tests were carried out on seven patients with diarrhoea after gastric surgery. Five had had a

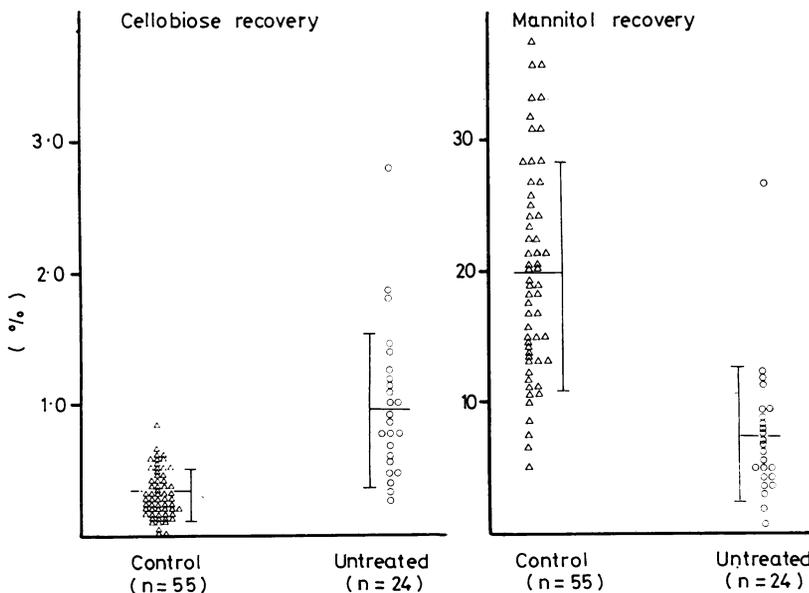


Fig. 1 Five-hour urine recoveries (mean  $\pm$  1 SD) of cellobiose (left) and mannitol (right) in controls and patients with villous atrophy.

partial gastrectomy and two had had a vagotomy and gastroenterostomy: patients with small bowel contamination were not included in this group, having already been counted in the previous group. Five patients had had a normal jejunal biopsy.

#### *Hypolactasia*

Two patients had primary hypolactasia, the diagnosis being based on lactase assay of a biopsy in one, and on a lactose/hydrogen breath test<sup>7</sup> in the other. Both had appropriate symptoms and a normal jejunal biopsy.

#### *Proctitis/colitis*

Ten patients with idiopathic proctitis or classical ulcerative colitis were studied. Three patients with a proctitis had had a normal jejunal biopsy at some stage in their investigations.

#### *Crohn's disease*

Fifteen patients had Crohn's disease. In seven the most proximal involvement radiologically was in the terminal ileum, while eight had evidence of disease in the jejunum or proximal ileum.

#### COMPARISON WITH OTHER SCREENING TESTS

Ninety-eight patients were identified who had had a jejunal biopsy and had carried out the test. This total comprised 24 patients with villous atrophy and 74 with normal histology (55 controls, plus 19 with other conditions) (see above). Of the patients with villous atrophy 15 had had a standard 5 g xylose tolerance test (12 assessed by five hour urine recovery, three by a one hour blood level) and 18 were investigated for the presence of reticulins antibodies in their serum. Serum folate was measured in 21 and red-cell folate in 15.

Of the patients with normal biopsies, 24 had carried out a xylose test (21 urine, three blood), reticulins antibodies had been sought in 45, serum folate measured in 47, and red-cell folate in 38.

A xylose recovery of less than 24% in five hours, or a blood level below 1.30 mmol/l were regarded as abnormal by the chemical pathology department. A serum folate of over 5.0  $\mu\text{g/l}$  was considered to be normal, and less than 4.0  $\mu\text{g/l}$  abnormal, by the Haematology Department. A borderline value (4.0–5.0  $\mu\text{g/l}$ ) was designated as a normal result for

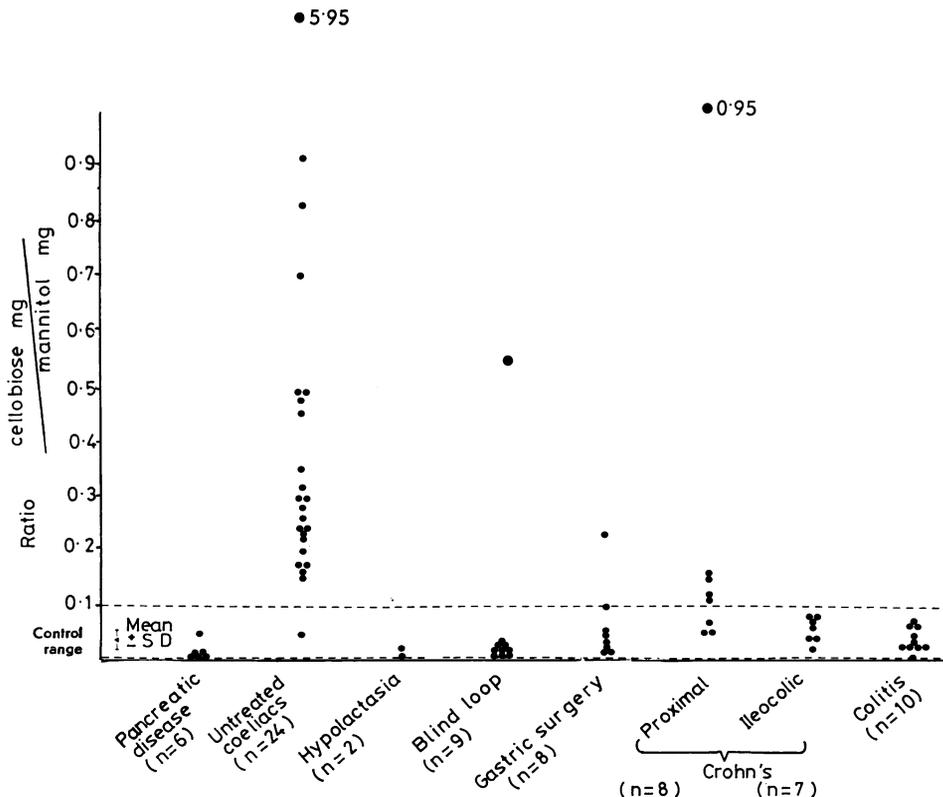


Fig. 2 Cellobiose/mannitol ratios in controls (between dashed lines) and patients with other gastrointestinal conditions.

the patients with normal histology, and abnormal for patients with villous atrophy. Red-cell folate levels of 160 µg/l or over were regarded as normal.

**Results**

**CELLOBIOSE/MANNITOL TEST ('sugar test')**

*Controls* (Figs. 1 and 2)

The mean cellobiose recovery was 0.32±0.20% and the mannitol recovery 19.6±8.3%. The mean cellobiose/mannitol ratio was 0.043±0.023 with an absolute range from 0.005 to 0.10.

*Coeliacs* (Figs. 1 and 2)

The mean cellobiose recovery was 0.97±0.57% for the patients with villous atrophy. This is significantly higher than for the controls (P<0.0001, Mann-Whitney U test). The mean mannitol recovery was 7.5±5.1%. This is significantly lower than for the controls (P<0.002). However, there is overlap between the groups for both substances. The ratios are significantly higher (P<0.0001), but of greater import is the fact that 23 of the 24 were clearly above the highest control value, ranging from 0.15 to 5.95.

*Other diseases (excluding Crohn's)*

Patients with pancreatic insufficiency, small bowel bacterial overgrowth, and colitis had normal ratios (Fig. 2). One patient who had had a gastroenterostomy had a ratio which was clearly abnormal (ratio 0.24), but repeat testing gave a normal result (0.07). All other post-surgery patients were normal. Cellobiose and mannitol values are not shown; most were clearly normal, although a few were over two standard deviations outside the control means.

*Crohn's disease* (see Fig. 2)

Five patients with Crohn's disease had ratios above the upper limit for the control group. All had evidence of proximal or mid-bowel disease, whereas those with disease apparently confined to the terminal ileum or beyond had normal ratios. The abnormality usually involved an increase in cellobiose recovery, but one had a very low mannitol recovery (2.7%).

**COMPARISON WITH OTHER SCREENING TESTS** (Fig. 3)

Taking the upper limit of normal for the cellobiose/mannitol ratio as 0.10, and excluding patients with Crohn's disease, the false-negative rate was 1/24. Although the repeat test on the gastroenterostomy patient was normal, for the purposes of comparison the first result must be taken, giving a false-positive rate of 1/74. Corresponding false-negative rates were 5/15 for the xylose test, 8/21 for serum folate, 8/15

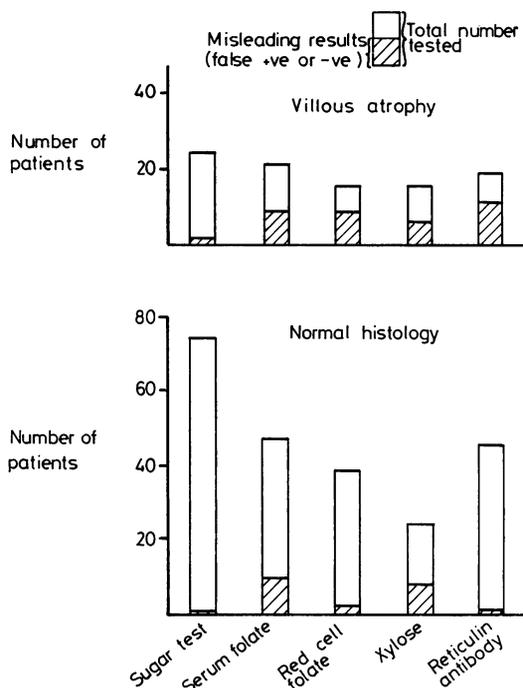


Fig. 3 Incidence of misleading results of screening tests in patients with normal (lower) and abnormal (upper) biopsies. (Sugar test=cellobiose/mannitol ratio).

for red-cell folate, and 10/18 for reticulin antibodies. The false-positive rates were 8/24 for the xylose test, 10/47 for serum folate, 2/38 for red-cell folate, and 1/45 for reticulin antibody.

Another way of looking at these results is to consider the frequency with which a test result correctly predicted the biopsy findings (Table). For the cellobiose/mannitol ratio, nearly 99% of normal results were associated with a normal biopsy, the other tests being only about 80% accurate. Similarly, an abnormal test result was indicative of villous atrophy in nearly 95% of cases for the cellobiose/mannitol result, the figures for other tests being markedly inferior, with the possible exception of the reticulin antibody.

Table Correlation between screening test results and biopsy findings

Test result	Predictive value of test results—uncorrected				
	Sugar test*	Serum folate	Red cell folate	Xylose	Reticulin antibody
Normal % accurate	98.6	82.2	81.8	76.2	81.5
Abnormal % accurate	95.8	56.5	77.8	55.5	88.7

\*Sugar test=cellobiose/mannitol ratio.

## Discussion

The cellobiose/mannitol test (sugar test) fulfils many of the requirements for a screening test. It is non-invasive, simple to perform, and can be carried out easily by both inpatients and outpatients. The technique is very similar to that of the five hour urine xylose test. However, unlike the latter, the test result is normal in the presence of renal failure, liver disease, or intestinal bacterial overgrowth, and is not affected by changes in gastrointestinal transit.<sup>5</sup> It is abnormal in untreated coeliac disease and tends to return to normal with treatment, although abnormal ratios have been found in some treated patients who were assumed to be on a strict gluten-free diet, and who were apparently well at the time of the test.<sup>4</sup>

The possible explanations for the paradoxical behaviour of the two probe molecules in coeliac disease have been discussed in previous publications.<sup>3</sup> Similar mechanisms probably apply in Crohn's disease, which is known to be associated with both malabsorption and an increased trans-epithelial leakage of protein.<sup>8</sup> The results of the test in Crohn's disease demonstrate this, but also indirectly confirm that the test mainly reflects changes in proximal intestinal function. It has been used to follow the response to a gluten-free diet,<sup>4</sup> and it is interesting to consider the results in one patient with jejunal Crohn's disease and an abnormal ratio, who was studied serially before and after a small bowel resection: his initial postoperative improvement and later recrudescence of disease were reflected by corresponding changes in the test ratio.<sup>5</sup>

As a screening test for coeliac disease, it would appear to be very sensitive and specific. The only false-negative result was in a man who had dermatitis herpetiformis and who was otherwise totally asymptomatic: no other screening test was abnormal. The one false-positive result was not confirmed on repeat testing, and the reason for it is unknown. We have generally found the ratio to be reproducible in the same subject (Fig. 4). It could have been a labelling or dilutional error in one of the assays: we do not consider it as evidence that the test result is altered after gastric surgery, but we have counted it as a false-positive result for the purposes of comparison.

When considering screening tests for a disease, what matters to a clinician is how much reliance he can place on the result, and it is possible to be misled as to the value of a test if the study does not take into account the actual incidence of the disease in the population which it is wished to screen. For example, to compare results in equal numbers of control and diseased patients would be wrong, if in practice the diagnosis was confirmed in only a small

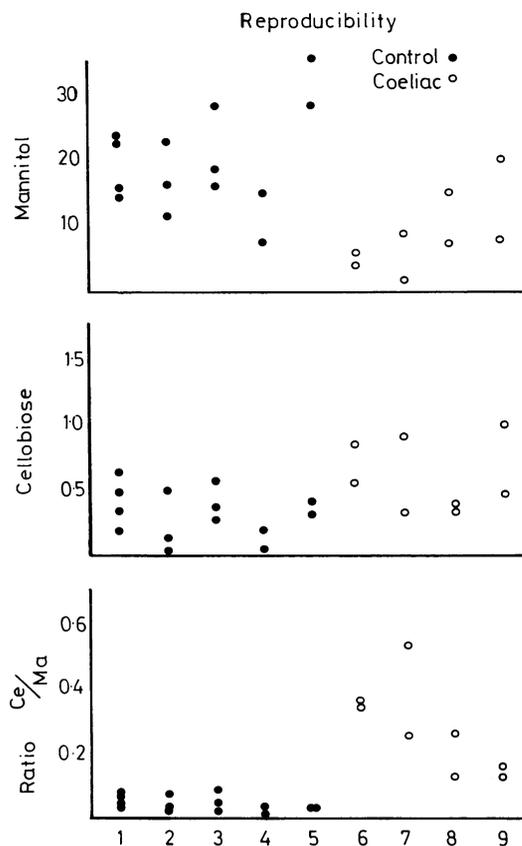


Fig. 4 Reproducibility of 'sugar test'. Five control subjects and four untreated coeliacs repeated the test as shown. Although there was variation between individual cellobiose and mannitol recoveries in the same subject, the ratio was much more reproducible.

percentage of those suspected of having the disease. Excluding two coeliacs from other hospitals whom we were kindly allowed to study, this means that 96 patients presented to us in whom we felt that a jejunal biopsy was indicated, and that 22 of these (23%) had villous atrophy. A review of our previous biopsy figures showed that approximately 18% of biopsies were found to be abnormal: this suggests that the 96 patients represent a genuine population for screening, and that our indications for performing a biopsy were not greatly influenced by the study. The proportions of normal to coeliac patients studied are not the same for the other tests.

It might therefore be thought fairer to compare results only for those 30 patients (10 coeliac, 20 normal) who have carried out all of the tests. The figures are little changed, however, except that now

an abnormal xylose or serum folate was associated with villous atrophy less than half the time.

The poor showing of the urinary xylose test is in keeping with the findings of others.<sup>9,10</sup> One hour blood xylose tests have been claimed to be much more accurate,<sup>11</sup> although this has been denied by others.<sup>12</sup> Recently, the correlation of one hour blood xylose with body surface area has produced very promising results,<sup>13</sup> and it may be that the blood xylose would have proved to be as discriminating as the cellobiose/mannitol ratio. It is unlikely to be much better, particularly as, of the six one-hour blood xylose results incorporated into the figures, five correctly predicted the biopsy findings, but one was a false-positive. The serum folate fared little better than the urine xylose test and would probably have made a much worse showing if borderline results had not been given the benefit of the doubt. Red-cell folate was better, but still significantly worse than the cellobiose/mannitol ratio. Reticulin antibodies proved to be highly specific, with only one false-positive, but were absent in a number of patients with villous atrophy. Of the 13 patients in whom gluten sensitivity has been confirmed histologically, at least six had no detectable reticulin antibodies. This is a rather lower incidence than was found by Mallas and his colleagues<sup>14</sup> (about 80%); their results in control patients (not biopsied) were similar to ours—1/56 false-positive, but they did detect antibodies in over 25% of normal relatives of coeliacs, producing a much higher false-positive rate.

The cellobiose/mannitol ratio is abnormal in some cases of proximal Crohn's disease, and, clearly, if these results are taken as false-positives, the specificity of the test for coeliac disease is reduced. On the other hand, it might be considered an advantage, in that it would draw the attention of the clinician to the small bowel as the source of a patient's symptoms. The correct diagnosis would then be fairly simply made on radiological or histological grounds. Reticulin antibodies may be more specific, and would be less likely to be abnormal in these patients, but would almost certainly be less accurate in screening asymptomatic coeliac relatives, for example.

It is probably more important for a screening test to be sensitive rather than specific, as the consequences of failing to pick up a case are usually more serious than a misdiagnosis, which will be corrected by the appropriate confirmatory test. We would naturally anticipate that, as the numbers studied increase, the boundary between the cellobiose/mannitol ratios for controls and coeliacs will in time become more blurred. However, it should be noted that 51 of our 55 controls had ratios of 0.07 or

less, and only four were between 0.08 and 0.10. In the future, therefore, it might prove advantageous to lower the upper limit of normal in order to pick up more coeliacs. This should not greatly increase the number of false-positive results, although even a corrected normal range—for example, 0.0–0.08—would still have misdiagnosed our one coeliac patient with a low ratio (0.05).

In conclusion, the cellobiose/mannitol test assesses two aspects of proximal intestinal permeability. We have found it to be more sensitive, and at least as specific as other screening tests for coeliac disease.

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