Simultaneous assessment of intestinal permeability and lactose tolerance with orally administered raffinose, lactose and L-arabinose

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SUMMARY
1. In order to develop an improved differential sugar absorption test for simultaneously assessing intestinal permeability and lactose intolerance, methods were established for determining raffinose, lactose and L-arabinose in human urine. Using NAD(P)H-coupled enzymatic assays and fluorimetry, each sugar was measurable over a concentration range of approximately 3-300 μmol/l in diluted urine specimens.

2. After an overnight fast, 40 normal volunteers drank an iso-osmotic solution containing raffinose, lactose and L-arabinose. The median 5 h urinary sugar excretion was 0.26% of the ingested raffinose, 0.05% of lactose and 17.5% of L-arabinose.

3. In 143 patients with gastrointestinal disease, excretion of both ingested raffinose and lactose was significantly increased in coeliac disease in relapse or in partial remission and in Crohn’s disease, but not in the irritable bowel syndrome, coeliac disease in remission or ulcerative colitis. Excretion of lactose, but not raffinose, was increased in patients with mucosal lactase deficiency, whereas excretion of L-arabinose was reduced in all disease groups except ulcerative colitis.

4. Discrimination between diseases was poor when based on individual sugar recoveries, but improved dramatically when excretion was expressed relative to that of L-arabinose. The raffinose/L-arabinose excretion ratio, an index of intestinal permeability, was >0.08 in 15/15 untreated coeliac patients but <0.06 in all normal subjects and in 9/9 lactase-deficient patients, 15/16 recovered coeliac patients, 5/6 patients with ulcerative colitis, 13/16 patients with Crohn’s disease and 61/62 patients with irritable bowel syndrome.

5. The lactose/L-arabinose excretion ratio, an index of lactose maldigestion, was >0.01 in 9/9 lactose-deficient patients and 14/15 untreated coeliac patients, but <0.008 in all normal subjects, recovered coeliac patients and ulcerative colitis patients, and in 11/16 patients with Crohn’s disease and 58/62 patients with irritable bowel syndrome.

6. The test reliably detected the altered intestinal permeability of coeliac disease and small-bowel Crohn’s disease, and differentiated between lactosuria due to lactase deficiency and that secondary to villous atrophy. With automation of the analysis, it would be suitable for widespread use in screening for intestinal disease.

Key words: L-arabinose, coeliac disease, Crohn’s disease, intestinal permeability, lactase deficiency, lactose, raffinose.

Abbreviation: IBS, irritable bowel syndrome.

INTRODUCTION
During the past decade, the non-invasive assessment of intestinal permeability by the use of orally administered, passively absorbed probe substances has increasingly been applied to the detection and management of intestinal disease [1-9]. Current procedures include differential sugar absorption [1-5], absorption of labelled chelates such as 51Cr-ethylenediaminetetraacetate [6, 7] or absorption of polymers such as poly(ethylene glycol) [8, 9], and all depend on measuring the urinary recovery of the metabolically inert probe(s). However, most differential sugar absorption tests use hyperosmolar sugar loads which impose substantial osmotic stress on the intestine [10] and rely on technically demanding chromatographic [1-3, 5] or relatively non-specific chemical [4] analysis. Similarly, ethylene glycol polymers may suffer from unreliable quantification [11], and those of lower mol. wt. display anomalous permeation due to their lipid solubility [12-14]. Although easily measured, 51Cr-ethylenediaminetetraacetate requires a 24 h urine collection for adequate diagnostic accuracy [6, 15] and is absorbed to a
significant extent via the large intestine [13, 14]; more seriously, it appears to be excessively sensitive to small changes in permeability in normal intestine [15, 16] but less reliable in detecting coeliac disease than a sugar test [17].

The aim of this study was to develop an improved differential sugar absorption test based on the use of an isosmotic mixture of the monosaccharide L-arabinose, the disaccharide lactose and the trisaccharide raffinose, which could be measured fluorimetrically in urine by a specific enzymatic assay. As well as providing an index of intestinal permeability based on raffinose excretion, it was anticipated that the test could also be used to detect lactosuria due to lactose maltidigestion in patients with lactase deficiency. A preliminary account of part of this work has been communicated to the Medical Research Society of Great Britain [18].

MATERIALS AND METHODS

Biochemicals

L-(+)-Arabinose, D-(+)-raffinose pentahydrate, β-galactosidase type X, β-fructosidase (‘Invertase’) type VII, hexokinase/glucose-6-phosphate dehydrogenase, phosphoglucone isomerase type III and glucose oxidase type V were obtained from Sigma Chemical Company Ltd, Poole, Dorset, U.K., β-galactose dehydrogenase S and catalase suspension were from Boehringer Corporation, Lewes, E. Sussex, U.K., and α-lactose monohydrate (Analar) was from BDH Chemicals, Poole, Dorset, U.K. The arabinose, raffinose and lactose used were all chromatographically homogeneous. All other chemicals were of analytical grade.

Subjects

A group of patients (56 male, 87 female; median age 42 years, range 16–81 years) with gastrointestinal symptoms and clearly defined diagnosis was selected for study. Sixty-two patients in whom no pathological process could be found were diagnosed as having irritable bowel syndrome (IBS). Patients with coeliac disease were classified as untreated (15 patients, either newly diagnosed or in relapse, not adhering to a gluten-free diet and with subtotal villous atrophy proven by intestinal biopsy), in partial remission (19 patients, maintaining a gluten-free diet and with histologically normal biopsy). Sixteen patients had Crohn’s disease, of whom ten were judged on radiological grounds to have upper-small-bowel disease, and five had only terminal ileal or colonic involvement. There were six patients with ulcerative colitis and nine cases of lactase deficiency (jejunal lactase activity <1 units/g of tissue). Healthy volunteers (hospital laboratory and medical staff; 27 male, 13 female; median age 28.5 years, range 20–50 years) were used as a reference group and were taking no medication at the time of the study. This study was approved by the Ethical Committee of the Central Manchester Health Authority.

Test protocol

After an overnight fast, subjects emptied their bladder and ingested a solution of 2.0 g of L-arabinose, 20 g of lactose and 8.0 g of raffinose in 250 ml of water. Urine was collected for 5 h thereafter. Subjects consumed no food during the test, but water was allowed ad libitum after 2 h had elapsed. After recording the urine volume, a 25 ml sample was mixed with thiomersalate (25 µmol/l final concentration) as a preservative and stored at −20°C for analysis. A sample of the pre-test urine was tested for glucose with a test strip (Diabur-2000; Boehringer).

Urine analysis

Before analysis, frozen urine specimens were thawed and clarified by centrifugation at 3000 rev./min for 10 min. Urine was then pretreated to remove endogenous fluorophores and other contaminants by passage through C18-silica solid-phase extraction columns (BondElut LRC; Jones Chromatography Ltd, Hengoed, Mid-Glamorgan, U.K.). Columns were prepared for use by activation with acetonitrile, followed by rinsing with twice-distilled water. A 10 ml aliquot of urine was passed through each column and the final 2–4 ml was collected for analysis. The pretreated urine was diluted 20- to 200-fold for L-arabinose determination, one- to eight-fold for lactose determination, and two- to ten-fold for raffinose determination, depending on the total urine volume. In preliminary experiments, urinary fluorophores were removed by treatment with AG501-X8 mixed ion-exchange resin (Bio-Rad Laboratories Ltd, Watford, Herts, U.K.) in the acetate form, essentially as described by Schersten & Tibbling [19].

If present, endogenous glucose was removed from the urine by treatment with glucose oxidase and catalase. Pretreated urine (0.2 ml) was mixed with 0.2 ml of 0.5 mol/l 4-morpholine-ethanesulphonic acid buffer, pH 7.0, containing glucose oxidase (500 units/ml) and catalase (2.0 mg/ml), and incubated at 37°C for 3 h under continuous gassing with 95% O2/5% CO2. After incubation, the mixture was boiled to inactivate the enzymes and the precipitated protein was removed by centrifugation at 11 000 g for 5 min. Water lost by evaporation was monitored by weighing the incubation tubes and was replaced after boiling.

L-Arabinose was measured by reaction with galactose dehydrogenase. Pretreated urine (100 µl) was incubated at 37°C for 30 min with 1.0 ml of 0.1 mol/l potassium phosphate buffer, pH 7.7, containing 0.25 units of galactose dehydrogenase and 0.67 mmol/l NAD. Lactose was measured as glucose released by hydrolysis with β-galactosidase. Pretreated urine (50 µl) was mixed with 50 µl of 0.1 mol/l potassium phosphate buffer, pH 7.7, containing 12.5 units of β-galactosidase and incubated at 37°C for 30 min. After boiling for 2 min to inactivate the galactosidase, 1.0 ml of hexokinase reagent was added and incubation was continued at 37°C for a further 30 min. Hexokinase reagent contained 1 unit of hexokinase/ml, 0.5 unit of glucose-6-phosphate dehydrogenase/ml, 5
mmol/l adenosine 5'-triphosphate, 0.84 mmol/l NADP and 8.0 mmol/l MgSO\textsubscript{4} dissolved in 0.3 mol/l Tris-HCl buffer, pH 7.6. Precipitated protein was removed after incubation by centrifugation at 3000 rev./min for 10 min. Raffinose was measured as fructose released by hydrolysis with β-fructosidase. Pretreated urine (50 μl) was incubated at 37°C for 30 min with 50 μl of 0.4 mol/l sodium citrate buffer, pH 4.6, containing 12.5 units of β-fructosidase. After boiling to inactivate the fructosidase, 1.0 ml of hexokinase reagent containing phosphoglucose isomerase (10 units/ml) was added and incubation was continued at 37°C for 30 min.

For each sugar, fluorescence was measured at 25°C at an excitation wavelength of 345 nm and an emission wavelength of 475 nm, using a Perkin-Elmer LS-3B fluorescence spectrophotometer fitted with a 20 μl flow cell and coupled to a Gilson model 222 automatic sample changer. Assays were calibrated with sugar standards carried through the entire analysis.

**Calculation of results**

Samples of the sugar drink were analysed in parallel with urine samples and the urinary excretion of each sugar was expressed as a percentage of the oral dose. Excretion ratios for raffinose and lactose were calculated as the percentage urinary recovery of each relative to that of L-arabinose. The statistical significance of results was evaluated with a two-tailed Mann–Whitney U-test, since most of the data were not normally distributed.

**RESULTS**

**Removal of endogenous glucose**

Although glucosuria was detected in less than 1% of patients studied, the amount of urinary glucose present in such cases (up to 200 mmol/l) was sufficient to preclude the determination of both lactose and raffinose. A procedure for the removal of endogenous glucose from urine was therefore developed, based upon the coupled oxidation of glucose by glucose oxidase and catalase [21]. In order to reduce the amount of endogenous glucose by the requisite four orders of magnitude within a reasonable time period, it was essential to use relatively large amounts of the two enzymes, to continuously oxygenate the incubation mixture, and to include sufficient buffering capacity to prevent acidification due to gluconic acid formation during the procedure. Under these conditions, an initial concentration of 200 mmol/l of urinary glucose could be reduced to an acceptable 55 ± 23 μmol/l (mean ± SD, n = 5) within 3 h with good recovery of the probe sugars (L-arabinose 93.2 ± 1.5%, lactose 86.6 ± 1.7%, raffinose 88.7 ± 1.0%).

**Removal of endogenous glucose**

Validation of assays

Although the individual methods are all well established in other contexts, each enzymatic procedure was optimized for the microfluorimetric determination of probe sugars in urine. Incubation conditions were adjusted so that each reaction was essentially complete within 30 min and each sugar produced at least 90% of the stoichiometric equivalent of NAD(P)H. For each procedure there was a linear relationship between relative fluorescence and sugar concentration extending over two orders of magnitude (Table 2) and deviating from linearity at final NAD(P)H concentrations greater than approximately 30 μmol/l. Reproducibility of each method was better than 5% at half-maximum concentration (Table 2), falling to between 5% and 9% at concentrations below 5% of maximum.

The specificity of each procedure was investigated by measuring the detectability of, and interference from, each sugar in the assay for another using ‘worst-case’ combinations of sugars formulated from the data obtained in the patient study. At the concentrations tested (see Table 3), only L-arabinose was detectable in the assays for lactose and raffinose (at an apparent concentration of 1.5 and 1.8

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Reduction in fluorescence (%)</th>
<th>Sugar recovery (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>L-Arabinose</td>
</tr>
<tr>
<td>Ion-exchange resin (n = 5)</td>
<td>73.9 ± 1.15</td>
<td>77.9 ± 3.5</td>
</tr>
<tr>
<td>BondElut C18-silica (n = 6)</td>
<td>78.9 ± 1.39</td>
<td>89.9 ± 2.2</td>
</tr>
</tbody>
</table>

**Table 1. Comparison of ion-exchange and solid-phase extraction for the removal of endogenous urinary fluorescence**

The fluorescence of pooled urine containing known amounts of each sugar probe was measured before and after extraction with AG501-X8 mixed ion-exchange resin or with BondElut C18 columns as described in the text. Sugar recovery was determined after extraction. Results are means ± SD.
μmol/l, respectively) and no extraneous sugar was detected in the L-arabinose assay; the amounts of endogenous fructose and galactose in urine specimens were insufficient to interfere in the raffinose or L-arabinose determinations. As shown in Table 3, limited cross-interference between sugars was observed in these worst-case experiments, but such sugar combinations were rarely encountered in practice and in no case would allowing for their effect have altered the outcome of the test.

Metabolism of sugars

The metabolic stability only of L-arabinose was investigate, since published data concerning that of raffinose and lactose is already available [22]. After intravenous injection of a sterile, pyrogen-free solution containing 500 mg of L-arabinose in 5 ml of water in five normal volunteers, 63.3 ± 4.1% (mean ± std) was excreted in the urine within 5 h and 73.1 ± 4.5% within 12 h.

Excretion of ingested sugars in normal subjects

The median concentration of probe sugar excreted in the 5 h urine in the normal volunteers was 8.92 mmol/l (range 2.41–26.51 mmol/l) for L-arabinose, 110.9 μmol/l (32.1–703.4 μmol/l) for raffinose and 85.9 μmol/l (22.6–587.0 μmol/l) for lactose, all well within the linear range of the appropriate assay. The corresponding percentage recoveries (Table 4) indicate that absorption of L-arabinose was at least 68-fold greater than that of raffinose, which in turn was over five times that of intact lactose. However, there was considerable variation in both concentration and recovery between subjects, with an approximately tenfold range for each sugar. For raffinose, the spread of the data was not altered when excretion was expressed as a ratio relative to L-arabinose (Fig. 1), although there was some improvement for lactose (Fig. 1 and Table 4).

Sugar excretion in patients with gastrointestinal disease

Coeliac disease. In patients with untreated coeliac disease there was a highly significant reduction in L-arabinose recovery and an increase in both raffinose and lactose recoveries (Table 4). There was thus a highly significant increase in both the raffinose/L-arabinose and lactose/L-arabinose excretion ratios. However, whereas the recovery data for individual sugars, especially raffinose, overlapped with the normal results, there was a complete separation from the normal group for both ratios (Figs. 1 and 2). In patients in partial remission all parameters remained significantly different from normal, although much less so than in untreated patients and with considerable overlap with the corresponding normal data. In fully recovered patients, L-arabinose recovery remained significantly depressed, although raffinose and lactose recoveries had returned to normal (Table 4), and both excretion ratios were thus significantly greater than, but overlapping with, normal (Figs. 1 and 2).

Lactase deficiency. In patients with proven lactase deficiency, not only was urinary lactose recovery significantly elevated but L-arabinose recovery was significantly lower than, although overlapping with, normal (Table 4). Because of this both excretion ratios were significantly raised. However, the raffinose/L-arabinose excretion ratios all lay within the range of the normal group (Fig. 1), whereas the lactose/L-arabinose excretion ratios were completely separated from those of the normal group (Fig. 2).

Inflammatory bowel disease. There was a clear difference between patients with ulcerative colitis and those with Crohn's disease, the former having a decreased

Table 2. Performance characteristics for fluorimetric determination of probe sugars in urine

Sugars were dissolved in pooled urine to give a broad range of final concentration and each solution was analysed in triplicate. Sensitivity is expressed as a concentration equivalent to the blank fluorescence + 3 SDs (n = 5), linearity as the upper limit of the linear range, and precision as the coefficient of variation for a half-maximal sugar concentration (n = 10).

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Dilution factor</th>
<th>Sensitivity (μmol/l)</th>
<th>Linearity (μmol/l)</th>
<th>Precision (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>L-Arabinose</td>
<td>100</td>
<td>100</td>
<td>3.0 × 10^4</td>
<td>0.93</td>
</tr>
<tr>
<td>Lactose</td>
<td>2</td>
<td>4.0</td>
<td>600</td>
<td>4.57</td>
</tr>
<tr>
<td>Raffinose</td>
<td>3</td>
<td>7.5</td>
<td>900</td>
<td>1.75</td>
</tr>
</tbody>
</table>

Table 3. Interference between sugars in fluorimetric analysis

Each sugar was dissolved in pretreated urine at a standard concentration of 100 μmol/l and analysed without dilution in the presence of a high concentration of another added sugar. Results are means ± 30 (n = 3). Statistical significance (Mann–Whitney U-test): *P < 0.05 compared with additive-free control.

<table>
<thead>
<tr>
<th>Additive</th>
<th>Concentration added (μmol/l)</th>
<th>Concent. found (μmol/l)</th>
<th>L-Arabinose</th>
<th>Lactose</th>
<th>Raffinose</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>100.0 ± 0.6</td>
<td>100.0 ± 1.2</td>
<td>100.0 ± 3.1</td>
</tr>
<tr>
<td>L-Arabinose</td>
<td>2.5 × 10^3</td>
<td></td>
<td></td>
<td>100.9 ± 0.9</td>
<td>89.3 ± 2.5*</td>
</tr>
<tr>
<td>Lactose</td>
<td>600</td>
<td></td>
<td>99.5 ± 0.2</td>
<td></td>
<td>91.4 ± 0.8*</td>
</tr>
<tr>
<td>Raffinose</td>
<td>700</td>
<td></td>
<td>98.8 ± 0.5*</td>
<td>90.5 ± 0.6*</td>
<td></td>
</tr>
</tbody>
</table>
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Table 4. Urinary recovery of probe sugars in normal subjects and in patients with gastrointestinal disease

The Table shows the amount of each sugar excreted in a 5 h urine collection as a percentage of the oral dose (see the text). Results are median and range. Significance levels for patient groups compared with normal subjects were calculated using the Mann--Whitney U-test. Abbreviation: Ns, not significant.

<table>
<thead>
<tr>
<th>Group</th>
<th>Recovery (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>L-Arabinose</td>
</tr>
<tr>
<td>Normal (n = 40)</td>
<td>17.53 (4.08–35.45)</td>
</tr>
<tr>
<td>IBS (n = 62)</td>
<td>15.14 (6.35–31.91)</td>
</tr>
<tr>
<td>Coeliac</td>
<td></td>
</tr>
<tr>
<td>Untreated (n = 15)</td>
<td>5.18 (1.97–8.92)</td>
</tr>
<tr>
<td>Partial remission (n = 19)</td>
<td>14.78 (1.37–33.06)</td>
</tr>
<tr>
<td>Complete remission (n = 16)</td>
<td>14.01 (4.52–21.65)</td>
</tr>
<tr>
<td>Lactate deficiency (n = 9)</td>
<td>8.24 (5.91–19.30)</td>
</tr>
<tr>
<td>Ulcerative colitis (n = 6)</td>
<td>16.00 (9.97–23.66)</td>
</tr>
<tr>
<td>Crohn's disease (n = 16)</td>
<td>14.64 (6.03–24.55)</td>
</tr>
</tbody>
</table>

lactose recovery as the only significant difference from normal. In Crohn's patients as a whole, L-arabinose recovery was slightly but significantly less than normal and raffinose recovery was substantially increased, giving a significantly raised raffinose/L-arabinose excretion ratio. However, neither lactose recovery nor the lactose/L-arabinose excretion ratio was affected. When the results in Crohn's disease were analysed according to site, it was clear that only patients with extensive small-bowel involvement showed any change from normal, having increased raffinose and lactose recoveries and excretion ratios, whereas patients with disease confined to the terminal ileum and large intestine had entirely normal probe excretion (Figs. 1 and 2).

IBS. Patients with IBS showed minimal alteration in sugar probe excretion, with a small decrease in L-arabinose recovery (Table 4) and a slight increase in the lactose/L-arabinose excretion ratio (Fig. 2) as the only significant changes.

DISCUSSION

Differential sugar absorption tests depend on the use of two or more sugars which differ only in respect of their intestinal absorption, so that a ratio of the amounts excreted in the urine provides an index of a particular intestinal function [22, 23]. In this way the influence of extraneous factors, such as gastric emptying, intestinal transit and renal function, can be largely eliminated. The probe sugars should be essentially exogenous, excreted in the urine quantitatively and unchanged after absorption and, in the context of permeability assessment, they should also be absorbed only by unmediated permeation and be resistant to intestinal metabolism [23, 24]. The trisaccharide raffinose (Galpa1-6Glcra1-2Fru) and the monosaccharide L-arabinose satisfy these essential requirements and are easily measured enzymatically. They are plant sugars and thus components of a normal diet [25, 26], but neither is present to a significant extent in fasting urine (R. W. Lobley et al., unpublished work) and both resist systemic metabolism and have adequate renal clearance, as shown by their near quantitative recovery after intravenous administration (this study and [22]). Raffinose is not hydrolysed by intestinal disaccharidases and is absorbed by unmediated diffusion [22, 27]; furthermore L-arabinose is transported with minimal affinity in hamster intestine in vitro [28] and has absorption characteristics in human jejunum in vitro indistinguishable from those of mannitol [29].

Intestinal permeation of hydrophilic molecules of radius 0.4 nm or less, e.g. L-arabinose (mol. wt. 150, radius approx. 0.3 nm), is believed to occur predominantly by free diffusion through numerous small aqueous pores, probably located in the enterocyte luminal surface membrane [23, 29–33]. Larger molecules, such as raffinose (mol. wt. 504, radius 0.59 nm), appear to be excluded from this transcellular pathway and penetrate the mucosa more slowly through larger, less numerous channels which are believed to be paracellular in location [23, 33–37]. Expressing the urinary recovery of the probes as a ratio relative to L-arabinose corrects not only for extraneous factors such as transit, but also for changes in intestinal surface area [23, 24, 27]. The raffinose/L-arabinose excretion ratio thus constitutes an index of paracellular permeability to raffinose in vivo, as demonstrated in vitro [27].
Fig. 1. Raffinose/L-arabinose excretion ratios in normal subjects and patients with gastrointestinal disease, expressed as a ratio of the percentage of the ingested dose of each sugar recovered in a 5 h urine collection. The median for each group is marked with an arrow, and the upper limit of normal is denoted by the broken horizontal line. Significance values (Mann–Whitney U-test) for each patient group compared with the normal group are shown in parentheses.

In practice, the two probes behaved as predicted, the recovery of L-arabinose being almost 70 times that of raffinose. Despite the different sugars and osmotic conditions, the median raffinose/L-arabinose excretion ratio of 0.015 in our healthy volunteers is almost identical to the cellobiose/mannitol excretion ratio of approximately 0.017 [2, 4, 17, 38, 39] and the lactulose/mannitol excretion ratios of 0.01–0.02 [3, 14, 39–42] found under hyperosmotic conditions. Reported disaccharide/rhamnose excretion ratios in control subjects [1, 5, 13, 43, 44] are higher (approximately 0.03), possibly because of the poorer urinary recovery of rhamnose [23], which may thus be inferior to mannitol or L-arabinose as a permeability marker.

As in other studies, individual sugar recoveries were of no diagnostic use, despite the often highly significant differences between subject groups. However, the raffinose/L-arabinose excretion ratio clearly discriminated between untreated coeliac patients and most of the remaining subjects, being below 0.06 in normal subjects and in 61/62 IBS patients, but greater than 0.08 in all untreated coeliac patients (Fig. 1). Taking these values respectively as the upper limit of normality and the lower limit of abnormality, the excretion ratio correctly identified 100% of untreated coeliac patients as having abnormal sugar permeability, while only two treated coeliac patients (both in partial remission) were frankly abnormal. Although within normal limits, the excretion ratio was significantly raised even in fully recovered coeliac patients (median 0.023, range 0.009–0.060 compared with 0.015, range 0.006–0.049, P < 0.01), consistent with the view that a persistent permeability defect is a feature of the disease [6, 27, 45]. The discrimination of our iso-osmotic procedure is unexpectedly good, as hyperosmotic sugar solutions are usually necessary for adequate separation of coeliac patients from control subjects [23]. This could be due to our choice of sugars, since raffinose is significantly larger than the conventional disaccharides and may be more selective for paracellular permeation, whereas L-arabinose is smaller than other monosaccharide probes and possibly a better transepithelial marker.

Of the remaining patients, only one case of ulcerative colitis and two of Crohn's disease with small intestinal involvement gave an abnormal excretion ratio. Our results
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Fig. 2. Lactose/l-arabinose excretion ratios in normal subjects and patients with gastrointestinal disease, expressed as a ratio of the percentage of the ingested dose of each sugar recovered in a 5 h urine collection. The median for each group is marked with an arrow, and the upper limit of normal is denoted by the broken horizontal line. Significance values (Mann-Whitney U-test) for each patient group compared with the normal group are shown in parentheses.

in Crohn's disease patients are in broad agreement with some studies, where an abnormal sugar excretion ratio [2-5] or \(^{51}\text{Cr}-\text{ethylenediaminetetraacetate}\) permeation [7] was associated with small-bowel involvement, but not others, where abnormal permeability to sugars [40] or poly(ethylene glycol) [9] was found in patients with Crohn's disease apparently confined to the colon. This discrepancy may be partly attributed to differing test protocols and diagnostic criteria, but much may be due to inter-patient variation and the small size of the groups studied. In their more extensive study, Andre et al. [42] concluded that the prevalence of abnormal sugar permeability in Crohn's disease was too low to be clinically useful except in patients in relapse.

It has long been known that lactosuria is associated with impaired lactose digestion [46, 47]. Menzies and co-workers [22, 43] convincingly demonstrated that, when expressed as a ratio relative to a non-hydrolysable disaccharide such as lactulose, the urinary excretion of ingested lactose provided a good index of lactose intolerance. We found that, despite the excellent theoretical arguments [22, 43] in favour of a lactose/oligosaccharide excretion ratio, diagnostic sensitivity is much improved by making use of the lactose/monosaccharide excretion ratio. Thus lactose/raffinose excretion ratios in patients with proven lactase deficiency (median 0.73, range 0.37-1.07) were substantially greater than normal (0.2, 0.07-0.75, \(P<0.0001\)), but with appreciable overlap between the groups. Furthermore, the lactose/raffinose excretion ratio in untreated coeliac disease was essentially normal (0.26, 0.08-0.59, \(P=0.058\)). The lactose/l-arabinose excretion ratio, however, not only detected lactose maldigestion in all the patients with lactase deficiency, but also clearly indicated its presence in 93% of the untreated coeliac group. It can be shown from the data of Noone et al. [43] that the expression of lactosuria as a lactose/rhamnose excretion ratio would have improved the discrimination of their combined procedure in patients with rotaviral gastroenteritis (range 0.33-1.00 in infection versus 0.13-0.41 in control subjects for the lactose/lactulose excretion ratio, compared with 0.033-0.59 versus 0.0015-0.011 for the lactose/rhamnose excretion ratio). The reason for this improvement is unclear, but lactose hydrolysis is a mucosal surface phenomenon and expressing lactose recovery relative to monosaccharide provides a correction for mucosal surface area [27], so that the
excretion ratio may reflect inversely a 'specific activity' for lactose hydrolysis relative to mucosal surface.

Whatever the reason, the improvement in diagnostic sensitivity is considerable and enables the lactose intolerance of untreated coeliac disease [47, 48] to be reliably detected. The lactose/l-arabinose excretion ratio also detected lactose malabsorption in 3/32 cases of IBS, a condition with which lactose intolerance may be associated [49, 50], and in 3/16 Crohn's disease patients, where reduced lactase activity has also been demonstrated [51, 52].

The use of ingested raffinose, lactose and l-arabinose in combination thus provides a simple and reliable means for the non-invasive assessment of intestinal permeability and lactose tolerance. It can distinguish between lactose malabsorption due to lactase deficiency, where only the lactose/l-arabinose excretion ratio is raised, and that secondary to villous atrophy where both excretion ratios are abnormal. The procedure is well tolerated and inherently safe, and has the advantage over other sugar tests of analytical simplicity, complex pretreatment of urine specimens being rarely necessary. The use of lactose in this way (or as an osmotic filler) is not compatible with other enzymatic procedures [4, 53], where it would interfere in the disaccharide determination after hydrolysis with β-galactosidase. Although we have used fluorimetry, conversion to spectrophotometry [4, 53] and thence to automated analysis [14] should not be difficult, enabling the procedure to be used routinely in any district general hospital.

ACKNOWLEDGMENT

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REFERENCES