Combined assessment of intestinal disaccharidases in congenital asucrasia by differential urinary disaccharide excretion

D G Maxton, S D Catt, I S Menzies

Abstract
Investigation of intestinal disaccharide hydrolysis and permeability by means of a non-invasive differential sugar absorption test was performed in a family containing two siblings with primary sucrase-isomaltase deficiency. The procedure, which depends on measurement of urinary excretion ratios after the oral administration of lactose, sucrose, palatinose, lactulose and L-rhamnose, is capable of simultaneous determination of intestinal lactase, sucrase, and isomaltase activity and lactulose:rhamnose permeability. The results corresponded well with those of disaccharidase assay and histological findings in jejunal biopsy tissue obtained from the patients. Palatinose proved a satisfactory substrate for in vivo assessment of intestinal isomaltase activity.

The method described provides a reliable and comprehensive assessment of intestinal disaccharide hydrolysis, and simultaneous estimation of permeability assists discrimination of primary from secondary disaccharidase deficiency. The ability to assess three different disaccharidase activities in addition to intestinal permeability by means of a single test, and the simplicity of preservation and transport of urine samples for sugar analysis, makes this a convenient, definitive method for the investigation of defective sugar absorption in both patients and population groups.

Methods
A 10 year old boy presented with a five year history of intermittent diarrhoea and flatulence, passing up to four voluminous stools a day. His mother thought the symptoms were aggravated by sweet foods and possibly milk. He was well built and physical examination yielded normal results. A differential disaccharide absorption test gave abnormal urinary excretion ratios, suggesting intestinal asucrasia with normal lactase activity and permeability. As a result the entire family (mother, father, paternal uncle and two brothers) requested investigation. One brother, aged 13 years, had similar though milder symptoms.

Differential urinary excretion of ingested disaccharide (figure)

Principle
A small fraction of ingested disaccharide permeates across the intestinal mucosa by unmediated diffusion. The amount is determined by intestinal absorptive area, permeability, and transit rate, in addition to factors such as dilution and rate of hydrolysis which modify the intraluminal concentration. As most disaccharides are completely excreted by the kidney after reaching the blood stream the fraction of an ingested dose recovered as intact disaccharide in the urine is, assuming normal renal clearance, determined by these gastrointestinal factors. Oral administration of lactulose, which resists mucosal hydrolysis, together with "test disaccharides" such as lactose, sucrose, and palatinose, provide a correction for all the variables other than the rate of small intestinal hydrolytic degradation (figure). Urinary lactose:lactulose, sucrose:lactulose, and palatinose:lactulose excretion ratios can therefore be calculated as specific indices, respectively, of intestinal lactase, sucrase, and...
isomaltase activity. In the absence of intestinal hydrolytic activity the fraction of "test disaccharide" permeating the intestine becomes the same as that of lactulose, and the "test disaccharide":lactulose ratio of percentages excreted in the urine rises to a value of 1:0. Hydrolytic degradation of the "test disaccharide" reduces this ratio; values below 0.3 indicate efficient hydrolysis, while intermediate values suggest corresponding impairment of disaccharidase activity.3, 5

Procedure
All the subjects ingested a test solution containing lactose 20 g, sucrose 20 g, lactulose 6-7 g (as 10 ml "Duphalac") lactulose syrup, Duphar Laboratories Ltd, Southampton, England, and L-rhamnose 1-0 g, dissolved in 300 ml water (500 mmol/l) after an overnight fast during a 24 hour period of dietary lactose and sucrose exclusion. Baseline urine samples were collected immediately before starting each test to verify elimination of lactose and sucrose and absence of other interfering sugars. A complete urine collection was made for 10 hours after administration of the test solution, the volume recorded, and an aliquot preserved with thiomersal (10 mg/100 ml urine, minimum) for sugar analysis. On a second occasion palatinose (isomaltulose, α1-6 glucopyranosyl fructose) which, like isomaltose and α-dextrin, is a substrate for intestinal isomaltase, was substituted as the "test disaccharide" and administered to the two boys suspected of asucrasia. All the tests were managed on an outpatient basis.

Sugar analysis
Estimation of urinary sugars was by a method of thin layer chromatography adapted to measure urinary sugars with improved sensitivity and precision. It entails measurement of peak heights by scanning densitometry and incorporates raffinose and arabinose as internal standards to correct for application errors relating to the estimation of disaccharides and monosaccharides, respectively. Sugar separations are achieved by multiple development disaccharides on a three quarter (15 x 20 cm), and monosaccharides (L-rhamnose) on a half (10 x 20 cm), plate of F1500 plastic backed silica gel (Schleicher & Schull, Dassell, West

### Differential Absorption

After oral administration the behaviour of lactose, sucrose, and palatinose differs from that of lactulose only in respect of intestinal hydrolytic action: lactose:lactulose, sucrose:lactulose, and palatinose:lactulose urinary excretion ratios therefore relate specifically to intestinal lactase, sucrase, and isomaltase activity. The behaviour of lactulose and of L-rhamnose differ only in respect of mucosal permeation so that the lactulose:rhamnose excretion ratios relate specifically to intestinal permeability. Doses given are appropriate for the complete version of this test, the five sugars being dissolved in 300 ml water as a single test solution.
Germany). Three consecutive ascending runs with two different solvents—(A) butan-1-ol: ethanol: acetic acid: water, 60:30:10:10 and (B) ethyl acetate: butan-1-ol:pyridine: acetic acid: water, 70:5:15:10:10 by volume, in the sequence ABA (each rise 13.5 cm) are required for the disaccharides, while L-rhamnose separation is obtained using three ascending runs (each of 8.5 cm) with solvent B. The layers are dried for at least 30 minutes between each run and then for at least four hours (preferably overnight) to remove pyridine before performing a 4-aminobenzoic/phosphoric acid colour reaction at 120–130°C for 10 minutes. The chromatographic procedures require refinements of technique that improve precision and are described in detail elsewhere.39 They are accurate and sensitive, recovery being above 90%, with minimum level of detection, requiring multiple applications for low concentration samples, below 2 mg/ml for most sugars. The precision lies between 2 and 8% (coefficient of variation), without replication, over the test range of sugar concentration. Sugar excretion was calculated and expressed as ratios of the percentages of lactose:lactulose, sucrose: lactulose, palatinose:lactulose, and lactulose: rhamnose excreted in each 10 hour urine collection.

A peroral jejunal biopsy specimen was obtained from the two patients suspected of impaired disaccharidase activity for histological examination and in vitro disaccharidase assay.10

**Results**

The 10 hour urinary excretion ratios for lactose:lactulose, sucrose:lactulose, and palatinose:lactulose are given in table 1. The sucrose:lactulose ratios were raised to 1-08 and 0·66, in case 4 (index case) and one brother, case 6, respectively, suggesting intestinal sucrase deficiency. In both these subjects the sucrose test solution induced diarrhoea and flatulence characteristic of sugar malabsorption.

All other family members had normal intestinal sucrase activity as indicated by low sucrose:lactulose ratios (< 0·3), and were asymptomatic during and after the test. The entire family had low lactose:lactulose excretion ratios (< 0·3) consistent with efficient intestinal lactose hydrolysis.5 Inclusion of L-rhamnose allowed lactulose:rhamnose excretion ratios to be calculated: all family members, including the patients, had values within the normal range (< 0·05, mean lactulose:rhamnose ratio of percentages excreted + 2SD), suggesting that mucosal integrity was intact.36

The two subjects with raised sucrose:lactulose ratios also had high palatinose:lactulose excretion ratios (0·75 and 0·65), which indicated an associated deficiency of small intestinal isomaltase activity.

Results of in vitro jejunal disaccharidase assays are given in table 2. Sucrase activity was undetectable in the two subjects with raised urinary sucrose:lactulose excretion ratios, while lactase activity was within the normal range. Intestinal maltase activity was also reduced in both these subjects, as has been reported in other cases of asucrasia.11 Jejunal histology was normal in both subjects, confirming a diagnosis of primary sucrase-isomaltase deficiency.

**Discussion**

The principles involved for assessing intestinal disaccharidase activity by differential urinary excretion of intact disaccharide following ingestion have been described previously.35 In support of previous reports23 the present findings show good agreement between intestinal disaccharidase activity assessed in vivo by differential disaccharide excretion and by in vitro assay of jejunal biopsy tissue. Recent improvements in the sensitivity and resolution of the quantitative thin layer sugar chromatography method described now permit simultaneous evaluation of lactase, sucrase, and isomaltase activity: for this purpose lactose, sucrose, and palatinose (10 g each), with lactulose (6·7 g), are administered together in the same oral test solution. Response ranges are not significantly different from those obtained when 20 g amounts of test disaccharide are given, provided results are expressed as ratios of *percentages* of the oral sugar doses excreted in a 10 hour urine collection. It should be mentioned that this method cannot be extended further to assess intestinal maltase and trehalase because, unlike most other disaccharides, maltose4 and trehalose (Menzies 1S, unpublished observations) undergo metabolic degradation after intravenous administration in man and are therefore incompletely recoverable from the urine.

Inclusion of L-rhamnose permits intestinal

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**Table 1**  Urinary disaccharidase and permeability excretion ratios

<table>
<thead>
<tr>
<th>Relationship to index case</th>
<th>Sucrose/lactulose (normal &lt; 0·3)</th>
<th>Lactose/lactulose</th>
<th>Palatinose/lactulose (normal &lt; 0·05)</th>
<th>Symptoms</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 (paternal uncle)</td>
<td>0·11</td>
<td>0·20</td>
<td>0·04</td>
<td>nil</td>
</tr>
<tr>
<td>2 (Father)</td>
<td>0·08</td>
<td>0·15</td>
<td>0·03</td>
<td>nil</td>
</tr>
<tr>
<td>3 (Mother)</td>
<td>0·09</td>
<td>0·13</td>
<td>0·03</td>
<td>nil</td>
</tr>
<tr>
<td>4 (index case)</td>
<td>1·08</td>
<td>0·16</td>
<td>0·75</td>
<td>+ +</td>
</tr>
<tr>
<td>5 (Brother)</td>
<td>0·11</td>
<td>0·13</td>
<td>0·04</td>
<td>nil</td>
</tr>
<tr>
<td>6</td>
<td>0·66</td>
<td>0·10</td>
<td>0·05</td>
<td>+</td>
</tr>
</tbody>
</table>

**Table 2**  In vitro intestinal disaccharidase activity (μmol glucose min"1 g")

<table>
<thead>
<tr>
<th>Reference ranges</th>
<th>Maltase</th>
<th>Lactase</th>
<th>Sucrase</th>
</tr>
</thead>
<tbody>
<tr>
<td>Case 4 (Index case)</td>
<td>2·1</td>
<td>3·1</td>
<td>0</td>
</tr>
<tr>
<td>Case 6 (Brother)</td>
<td>1·8</td>
<td>5·1</td>
<td>0</td>
</tr>
</tbody>
</table>

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lactulose:rhamnose permeability, a useful index of mucosal integrity to be estimated. Primary disaccharidase deficiency not only presents a characteristic profile (affecting lactase, or sucrase-isomaltase activities alone) but is also usually associated with normal intestinal permeability. It can therefore be distinguished from disaccharidase deficiency secondary to small intestinal disease, in which lactulose: rhamnose permeability is increased, by means of this single non-invasive test.

The validity of any method for investigating disaccharidase hydrolysis in patients with gastrointestinal disease should be considered in relation to any impairment of monosaccharide transport that may also be present. Tests that depend on a rise in blood concentration of the monosaccharide products generated by hydrolysis of ingested disaccharide become unreliable when the absorption of such products is defective. Therefore, it is a particular advantage of this method that ratios of intestinal permeation of intact disaccharide are not affected by changes in the mediated transport of monosaccharides. Methods such as the lactose-hydrogen breath test may also become unreliable when malabsorption causes retention of monosaccharide in the gut; furthermore, failure of colonic bacteria to generate hydrogen from unabsorbed sugar, another possible source of unreliability in the breath test, does not affect the disaccharide permeation ratio.

The x1–6 links of isomaltose, palatinose, and a-/d-xetraos are broken by a brush-border isomaltase that is associated with sucrase activity on the same molecular complex. Consequently, in congenital ascarasias defects of both sucrase and isomaltase activity are usually combined. Sucrase-isomaltase deficiency is inherited as an autosomal recessive condition, and normal intestinal disaccharidase activity would therefore be expected in both parents and the paternal uncle, as was shown.

Although hydrolysis of palatinose by normal human jejunal mucosa is reported to be considerably less efficient than that of isomaltose or sucrose (1.4–40 v 4.0–14 and 6.0–17 μmol/min/g wet weight, respectively) the excretion ratios obtained from our normal subjects suggest that palatinose is a very satisfactory substrate for in vivo assessment of human intestinal isomaltase activity, being hydrolysed with similar efficiency to sucrose. Palatinose shows pronounced "substrate inhibition"—that is, inhibition produced by the presence of palatinose itself—in certain in vitro assay systems, which may offer some explanation for the observed discrepancy. In conclusion, both our present and previously published experiences with the differential urinary disaccharide excretion test indicate that it is a clinically convenient method for assessing intestinal disaccharide hydrolysis. It is non-invasive and can provide a reliable estimate of lactase and, as shown in this study, of sucrase and isomaltase activity, together with mucosal integrity indicated by a lactulose:rhamnose permeability ratio, from a single test procedure. As a measure of small intestinal hydrolytic performance it complements, and could replace, in vitro disaccharidase assay which requires tube biopsy. It is an effective method for investigating individual patients with symptoms suggestive of primary or secondary disaccharidase intolerance, but is also particularly suitable for studying profiles of intestinal disaccharidase activity in population groups.

We are grateful to Tate & Lyle Industries Ltd, Reading, Berkshire, for the gift of palatinose. We also thank Dr GS Clayden for permission to report one of his patients.

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