Problems in analysis of faecal sugar

Sugar malabsorption may be suspected on clinical grounds but the diagnosis must be founded on tolerance tests and chemical analyses. We describe here some studies on methods of examining faeces for evidence of sugar malabsorption.

**Methods**

All faecal samples were transferred to test tubes quickly to prevent the loss of the sugar-containing fluid phase of the faeces. Samples were then immediately frozen and kept at $-15$ to $-20$ °C. Before analysis one part of the thawed faeces was mixed with two parts water, homogenized, and centrifuged. The following analyses were made on the supernatant.

**pH.** Lyphan pH-indicator paper was used. The results were intermediately checked with the readings obtained using a glass electrode pH meter, and found to be accurate.

**Reducing sugars.** The Clinitest method (Ames & Co) was used (Kerry and Anderson, 1964).

**Paper chromatography.** This was performed essentially as described by Durand, Martino, and Lamedica (1961) and later by Soeaparto, Stobo, and Walker-Smith (1972), which was as follows. 10–30 μl of supernatant or of reference solutions were applied to a Whatman No. 1 paper, 2 cm from its lower edge. The chromatograms were developed by ethylacetate: pyridine : water, 13 : 5 : 4, over 16 hours. The papers were dipped in a mixture of 250 ml acetone and 3 ml saturated silver nitrate, air-dried, and dipped in ethanol with 5% of a 40% sodium hydroxide in water. After a second air drying, the spots were fixed by a solution of 25% sodium thiosulphate and 4% potassium metabisulphite. By this technique 0.25% sugar could easily be shown by the supernatant.

**Patients**

Faecal samples were obtained from 135 children admitted to our paediatric department. Most of them were under 2 years of age and none was less than one month old. All children had acute or chronic diarrhoea. A total of 180 samples was studied. Faecal samples were obtained for comparison from 10 subjects with no gastrointestinal disease. No sugar or pathogenic organisms could be detected in these samples.

**Results**

**Freezing.** First, the effect of freezing on sugar-containing faecal samples was studied. During storage at $-15$ to $-20$ °C no change was found in pH, Clinitest, or chromatography findings.

**Clinitest and pH.** All 180 samples were studied of which 39 were found to contain at least 0.5% reducing sugar. Only 20 of these showed pH 5.5 or below and four additional samples pH 6.0–5.6 (Table I).

**TABLE I**

Relation between pH and stool reducing substances
(Clinitest)

<table>
<thead>
<tr>
<th>Clinitest</th>
<th>pH</th>
</tr>
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<tbody>
<tr>
<td>$&lt;0.5%$</td>
<td>15</td>
</tr>
<tr>
<td>$&gt;0.5%$</td>
<td>8–6.1</td>
</tr>
<tr>
<td></td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>20</td>
</tr>
</tbody>
</table>

**Paper chromatography.** This was performed on 177 samples. The samples which were found to be free of reducing sugar by the Clinitest method (<0.25%) were also sugar-free at chromatography. In 12 samples the Clinitest showed 0.25–0.5 reducing sugar. At chromatography evidence of sugars could be seen but the spots were faint. In four samples the Clinitest showed more than 0.5% reducing sugar which could not, however, be shown by chromatography. Otherwise, sugars were always shown by chromatography if the Clinitest showed more than 0.25% reducing sugars. Accordingly, if the chromatography was positive, Clinitest was also positive.

**Sugars found by chromatography.** Various sugars were found in 31 children. Monosaccharides only were found in 29 of these, that is glucose only in 13 children, glucose and galactose in 7. Three children showed only galactose, 5 children showed glucose and fructose, and 1 fructose and galactose. Disaccharide was found only twice, namely sucrose, fructose, and glucose in 1 patient and lactose, glucose, and galactose in another. Thus a disaccharide was never found without its component monosaccharides. These findings prompted the following study.

**Disaccharidase effect of the faecal flora.** Fresh samples of sugar-free faeces were rapidly mixed with various disaccharides and dissolved in water to a final concentration of about 2%. Part of this mixture was applied on chromatographic paper as soon as possible and the paper dried. This procedure took about 2 minutes. At chromatography large amounts of the added disaccharide had already been split into its monosaccharide components (Table II). Other parts of the mixture were incubated at room temperature for various
TABLE II  
Sugar-free stools mixed with different disaccharides and incubated for various periods

<table>
<thead>
<tr>
<th>Disaccharide added</th>
<th>Saccharides found by chromatography</th>
<th>Incubation time (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>2</td>
</tr>
<tr>
<td>Lactose</td>
<td>Lactose</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Glucose</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Galactose</td>
<td>+</td>
</tr>
<tr>
<td>Maltose</td>
<td>Maltose</td>
<td>(+)</td>
</tr>
<tr>
<td></td>
<td>Glucose</td>
<td>+</td>
</tr>
<tr>
<td>Sucrose</td>
<td>Sucrose</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Glucose</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Fructose</td>
<td>+</td>
</tr>
</tbody>
</table>

periods of time and their sugar components studied by chromatography. After 30 minutes the disaccharide had been completely split (Table II). Incubation at 37°C did not seem to change the rate of the disaccharide-splitting. All samples of faeces tested had the same sugar-splitting capability. The concentration of monosaccharides seemed to decrease during prolonged incubation. After 180 and 240 minutes only small amounts were found.

**Monosaccharide fermentation.** For a further study of the disappearance of monosaccharides, larger amounts (10% final concentration) were incubated with faeces. After 24 hours both glucose and galactose had disappeared. At the same time pH fell to between 4.6 and 5.0. These findings were reproducible with several faecal samples. Fructose, however, was not affected in the same way and pH did not fall.

**Discussion**

Normally sugars are not found in the faeces of healthy persons past the neonatal stage. Reducing substances can be detected, however, in amounts corresponding to less than 0.2%. In acute diarrhoea and other states of disturbed intestinal function, sugars are often found (Anderson, 1971; Davidson and Mullinger, 1970; Ford and Haworth, 1963). The use of faecal pH as a screening procedure for sugar malabsorption is not suitable, as shown by our study as well as by others (Soeparto et al., 1972; Walker-Smith, 1973). The Clinitest method, however, seems to be a reliable one for the detection of unabsorbed sugars though a few Clinitest positive samples showed no sugar on chromatography, probably because other reducing substances were present.

The present finding of an extremely rapid splitting of disaccharides by the faecal flora does not seem to have been noted in earlier published reports. Probably the disaccharide splitting has already started during passage through the lower part of the gut and cannot be avoided however rapidly the faecal samples are frozen. This explains why disaccharide-deficient children usually excrete not only the disaccharide in the faeces but also its monosaccharide components. Occasionally the disaccharide disappears completely. Only Durand et al. (1961) have found pure lactose-excretion in lactase-deficient children.

The present study includes patients in whom a sucrase-defect was suspected on clinical grounds. The faeces of these children contained fructose and glucose and, in one sample, also sucrose. The diagnosis of sucrase deficiency has attracted special attention because it has been thought that the Clinitest method for reducing sugars would be negative. Thus, Soeparto et al. (1972) suggest acid hydrolysis of the faecal samples. We find this unnecessary, since in our experience sucrose is always split rapidly into fructose and glucose.

**Summary**

Significant amounts of sugar were found in 22% of 180 faecal samples from 135 children with acute or chronic diarrhoea. The methods used were the Clinitest method and paper chromatography. There was very good correlation between the results of these methods. Screening by pH was less reliable. Various di- and monosaccharides were found. However, a disaccharide was never found without the simultaneous finding of its component monosaccharides. In vitro studies showed that the faecal flora has the ability to split
disaccharides very rapidly. Within a few minutes much of the disaccharide had been split and no traces could be found after 30 minutes. Since the same process is assumed to take place in the lower gut, children with disaccharidase deficiency cannot be expected to excrete disaccharide alone in their faeces without the corresponding monosaccharides. The lack of a disaccharide in the faeces does not exclude the possibility of disaccharidase deficiency. Acid hydrolysis of faecal samples in cases of suspected sucrose deficiency seems not to be necessary.

REFERENCES

BO L. LINDQUIST* and L. WRANNE
Department of Paediatrics, Regional Hospital, S-701 85 Örebro 1, Sweden.

*Correspondence to Dr. Bo L. Lindquist.

Effect of gestational length on albumin content of meconium

A raised protein level in the meconium of an infant with cystic fibrosis (CF) was described by Buchanan and Rapoport (1952) in an infant with meconium ileus. This finding was confirmed by Green, Clarke, and Shwachman (1958) who showed that the protein was predominantly albumin. Wiser and Beier (1964) and Green and Shwachman (1968) subsequently showed that the meconium of infants with CF contained high levels of protein even in the absence of meconium ileus. Estimation of the protein content of meconium was suggested as a possible screening procedure for CF by Schutt and Isles (1968) and the results of such surveys have been reported (Cain, Deall, and Noble, 1972; Prosser et al., 1974).

In late 1971 we began to screen the newborn of North Monmouthshire and Breconshire for CF using an immunochemical method for the detection of albumin in meconium (Bull, Gladwin, and Griffiths, 1974). We found that false-positive results (i.e. detection of raised levels of meconium albumin in infants later shown to be clinically healthy and to have normal sweat electrolytes) did occur but the overall rate was low, being approximately 1%. It was our impression that such false-positive results were commoner in premature infants, and the present paper investigates this possibility.

Materials and methods

During the 3\frac{1}{2}-year period between January 1972 and June 1975 meconium samples from 6552 infants were tested for the presence of albumin using an immunochemical method previously reported (Bull et al., 1974). In this method a 1/5 dilution of meconium is prepared and a positive result recorded if the meconium albumin level reaches 20 mg/100 ml of this extract. In absolute terms this is equivalent to a level of 4 mg/g dried meconium, as the solid content of meconium averages 24·5% (average of 10 analyses).

During 1973 we began to test all meconium samples which gave positive results for albumin for the additional presence of occult blood using a modification of the method described by Kolmer, Spaulding, and Robinson (1952). In the modified method 2 g powdered guaiac are dissolved in 10 ml absolute alcohol and the solution filtered (this saturated solution is stable for one month). One drop of the solution is added to a smear of meconium on filter paper and followed by one drop of glacial acetic acid and one drop of hydrogen peroxide (20 vol). An intense green or blue colour developing within one minute indicates a positive result. This method will detect blood in a dilution of 1 : 10 000.

Of the 6552 infants screened, 212 were preterm, having gestational ages of <37 weeks.

Results

Meconium samples from 6552 infants were examined and 70 were found to contain raised albumin levels. 8 infants persistently defaulted for their follow-up appointment despite home visits by the health visitor, and though a further 4 infants have remained clinically well insufficient sweat (<100 mg) has been obtained for satisfactory analysis.

The remaining 58 infants have had negative sweat tests and have remained clinically healthy with no evidence of CF. In 17 of these infants gestational age was <37 weeks, in 35 it was ≥37 weeks, while in the remaining 6 it was in doubt and they were therefore excluded from further analysis.

The Table gives the distribution of raised meconium levels obtained from 52 healthy infants,
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B L Lindquist and L Wranne

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