

## Hydrogen breath test in schoolchildren

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**SUMMARY** The frequency of negative hydrogen breath tests due to colonic bacterial flora which are unable to produce hydrogen was determined after oral lactulose challenge in 98 healthy Dutch schoolchildren. There was a negative result in 9.2%. The probability of a false normal lactose breath test (1:77) was calculated from these results together with those from a separate group of children with lactose malabsorption (also determined by hydrogen breath test). A study of siblings and mothers of subjects with a negative breath test did not show familial clustering of this condition.

Faecal incubation tests with various sugars showed an increase in breath hydrogen greater than 100 parts per million in those with a positive breath test while subjects with a negative breath test also had a negative faecal incubation test.

The frequency of a false negative hydrogen breath test was higher than previously reported, but this does not affect the superiority of this method of testing over the conventional blood glucose determination.

Measurement of hydrogen in expired air may be used to screen for carbohydrate malabsorption. Though indirect, it is highly specific in detecting this condition. Hydrogen is one of the products of bacterial fermentation of unabsorbed sugars in the lower gastrointestinal tract but is not found in protein or fat malabsorption. Furthermore, human cells do not produce hydrogen and atmospheric air contains only a trace. During the past decade the hydrogen breath test for determining carbohydrate absorption has gained popularity for a number of reasons: the procedure is non-invasive; it is more sensitive than measurement of the increase in the blood glucose concentration after an oral sugar load;<sup>1, 2</sup> and, recently, devices for rapid estimation of breath hydrogen, not requiring expert personnel, have been developed.<sup>3, 4</sup>

The reliability of the hydrogen breath test, however, may be biased by subjects with colonic bacterial flora that seem unable to produce hydrogen from unabsorbed carbohydrates. Based on one small series in adults, the frequency of this condition was estimated as 2 to 4%.<sup>5</sup> In a larger population of Swedish adults, however, hydrogen breath test after lactulose challenge gave a frequency of 8% for this condition.<sup>6</sup> There are few reports on the inability to produce hydrogen and on the reliability of the breath test in children.<sup>7</sup>

The present study establishes the frequency of

children unable to produce hydrogen, as well as the relation between the capacity to produce hydrogen during a breath test and the ability of the colonic bacterial flora to produce either hydrogen or methane, or both.

### **Patients and methods**

The study was carried out during 1983 and included 103 healthy schoolchildren of Caucasian origin, aged 6 to 12 years. Informed consent was obtained from the children as well as their parents. Five of the 103 children were excluded from the study because they had taken oral antibiotics within four weeks of the study. In the remaining 98 subjects the hydrogen breath test was performed after an overnight fast, with lactulose (1 g/kg bodyweight) given as a syrup (Duphalac, Duphar Nederland). The hydrogen concentration in expired air was estimated in duplicate samples as 0, 60 and 90 minutes by means of a recently described hydrogen analyser.<sup>4</sup> An increase in breath hydrogen of less than 20 parts per million (ppm) was considered a negative test result. Clinical signs of discomfort were noted during and for some time after the procedure.

To calculate the probability of a false negative test, the results of the hydrogen breath test in 747 children with either chronic diarrhoea or recurrent abdominal pain, or both, were included in this

study. The age of the children in this group ranged from 6 months to 16 years but most with lactose malabsorption were of preschool age. Postgastroenteritis enteropathy, lambliaisis, or cows' milk protein allergy were the prevailing causes of their condition; primary lactose malabsorption was found in only 13%.

To assess the possibility of familial clustering of this inability to produce hydrogen, a lactulose test was performed in 11 siblings and six mothers of the children with negative results as well as in a control group of 11 siblings and eight mothers of eight randomly assigned children with positive test results.

A second lactulose hydrogen breath test was carried out in the children with negative tests and in 11 hydrogen producing children six to eight months after the first to find out whether the capacity to produce hydrogen after lactulose challenge is a permanent condition. Since no correlation was found between the dosage of lactulose and the concentration of expired hydrogen in those with a positive result, the second test was performed with a standard dose of 19 g lactulose made up to 200 ml with water. Hydrogen was measured at intervals of 30 minutes for 150 minutes.

**Faecal incubation studies.** Freshly passed stools were obtained from 17 children and one mother. Within one minute of defecation a suspension was prepared of 20 ml faeces in 60 ml, 0.15 M phosphate buffered saline (pH 7.0). After homogenising and sieving, 3 ml samples of the suspension were pipetted into a 23 ml vacuum container (Venoject, Terumo Europe, Belgium). Duplicate incubations were prepared by adding 1 ml of a 50 mg/ml solution of pure lactulose (Legendal, Inpharzam Nederland), lactose, sucrose, or glucose using a 1 ml glass pipette with a standard error of 0.015 ml.

After sealing the container, incubation was performed in upright position at 37°C in a temperature controlled stirred waterbath. After 60 minutes samples of the gas above the suspension were collected by needle aspiration through the stopper into a 20 ml plastic syringe and subsequently injected into 13 ml vacuum containers for transport and storage before gaschromatographical analysis. The remaining suspension was examined by Clinitest and Clinistix (Ames, Miles Laboratories, USA) in order to establish the presence of sugar residue, and by paperstrip (Panpeha, Schleicher and Schüll, Germany) to determine the pH. The result of the faecal incubation test was regarded as positive if the concentration in the test tube exceeded the concentration in control suspensions without sugar added by 100 ppm or more. This concentration was

chosen because of the finding that about 20% of the hydrogen present in the gut lumen is expired via the lungs, and a rise in breath hydrogen of 20 ppm or more is generally considered to indicate the presence of sugar malabsorption.

**Gas chromatographic analysis.** For estimation of hydrogen and methane, 4 ml gas samples derived from the stool incubations were injected into a Packard 428 Gas Chromatograph fitted with a thermal conductivity detector. The detector response was estimated by peak height measurement and was calibrated with standard mixtures of 29, 68, and 180 ppm hydrogen in air and 100 and 300 ppm methane in air (BOC Gases). The carrier gas was argon with a flow rate of 17 ml/minute. The stainless steel column (1.5 m × 3 mm diameter) was packed with 60 to 80 mesh pretested molecular sieve 5 A (Applied Science Laboratories, USA).

## Results

**Breath tests.** The dose of lactulose (1 g/kg) varied according to bodyweight between 20 and 50 g. Though the younger children tended to have a higher increase in breath hydrogen compared with older children, this difference did not reach statistical significance. Of the 98 children, 74% had abdominal discomfort, flatulence, or diarrhoea during or shortly after the test. No correlation was found between the occurrence of clinical symptoms and the magnitude of the hydrogen concentration in expired air or with the absence of hydrogen production. Six of nine children with negative tests experienced these symptoms.

In those subjects who produced hydrogen the test became positive by 60 minutes in 89%, and by 90 minutes in 11%. Breath hydrogen remained below 20 ppm in nine of the 98 children (9.2%, 95% confidence limits 4.3 to 16.7%).

Ninety five of the 747 (12.7%) children previously tested either because of chronic diarrhoea or recurrent abdominal pain, or both, were shown to have lactose malabsorption. Assuming that the frequency of children whose bacterial flora are unable to produce hydrogen in this population was also 9.2%, leaving 90.8% of correct negative test results, the frequency of missed lactose malabsorbers was calculated from the equation  $12.7 \times \frac{9.2}{90.8} = 1.29\%$ . The probability for a false negative test because of failure to produce hydrogen is therefore 1:77.

A second lactulose test was performed 6 to 8 months after the first one in the nine children with negative results, and in eight randomly selected subjects with positive tests, as well as in most of their siblings and mothers—comprising 53 subjects

in total. Breath hydrogen tests in the subjects who had had positive results became positive at 60 minutes in 59%, at 90 minutes in 30%, and after 90 minutes in 11%. Seven of nine of those who had previously had negative results now had a positive result at 90 minutes or before, except for one whose test became positive at 150 minutes. A previously positive test became negative in two of 11 children who had been included in the first part of the study. Hence, a change in outcome was found in nine of 20 children tested twice. Though three siblings of subjects whose bacterial flora were unable to produce hydrogen were also found to have this condition, significant clustering or a relation with the result of the test in the mothers could not be found when compared with families of children who had a positive result (Table 1).

**Faecal incubation studies.** Immediate incubation of freshly passed stools was performed in 18 subjects—seven of nine with negative results, 10 siblings, and one mother, three months before the second hydrogen breath test.

The mean coefficient of variation between the duplicate incubations from subjects with positive results ( $n=53$ ) was 13%, and 7% for the duplicate methane producing specimens ( $n=16$ ), indicating the reproducibility of the procedure.

All faecal suspensions showed a sugar residue after 60 minutes of incubation, showing that the production of gases had not been affected by depletion of substrate. Measurement of pH at 60 minutes showed a mean decrease in pH of 0.5, with no incubations below pH 6.0.

Concentrations of hydrogen in incubations from subjects with a positive result showed large individual differences at 60 minutes (range 126 to 17 503 ppm). Differences in hydrogen concentrations in samples from the same subject, incubated with the various sugars, were within the range shown in Table 2 and were found to be related to the sugars involved. With one exception, in which the lactose incubation exhibited the highest hydrogen concentration, sucrose or glucose, or both, always yielded

Table 1 Frequency of inability to produce hydrogen in families of subjects with this condition (group A) and those of subjects with a positive hydrogen breath test (group B)

|          | Group A families ( $n=8$ ) |               | Group B families ( $n=8$ ) |               |
|----------|----------------------------|---------------|----------------------------|---------------|
|          | Positive test              | Negative test | Positive test              | Negative test |
| Siblings | 8                          | 3             | 11                         | 0             |
| Mothers  | 6                          | 0             | 5                          | 3             |
| Total    | 14                         | 3             | 16                         | 3             |

Table 2 Results of faecal incubation test and its relation with the hydrogen breath test (HBT)

| Case no | Hydrogen | Methane | Outcome of HBT |
|---------|----------|---------|----------------|
| 1       | ****     | 0       | +              |
| 2       | ****     | 0       | +              |
| 3       | ***      | 0       | +              |
| 4       | ***      | 0       | +              |
| 5       | ***      | 0       | +              |
| 6       | ***      | 0       | +              |
| 7       | ***      | ***     | +              |
| 8       | **       | 0       | +              |
| 9       | **       | 0       | +              |
| 10      | **       | 0       | +              |
| 11      | **       | 0       | +              |
| Mother  | **       | ****    | +              |
| 12      | *        | 0       | -              |
| 13      | .        | **      | -              |
| 14      | 0        | 0       | -              |
| 15      | 0        | 0       | +              |
| 16      | 0        | ***     | +              |
| 17      | 0        | ***     | +              |

0=no increase in concentration.

\*=10 to 100 ppm.

\*\*=100 to 1000 ppm.

\*\*\*=1000 to 10 000 ppm.

\*\*\*\*=10 000 to 100 000 ppm.

the highest hydrogen concentrations compared with the lactose and lactulose incubated specimens. The same relation between concentration and kind of sugar used was found for methane.

The hydrogen concentrations in 12 of 18 incubations exceeded those of the control suspensions by 100 ppm; in 14 of 18 of the latter there were only trace amounts of hydrogen, and the former finding corresponded with a positive second hydrogen breath test in all cases. In six of 18 incubations the hydrogen concentration in the test tubes remained below 100 ppm (range 0 to 94) which corresponded with a negative breath test in three cases, the remaining three showing discordance with the outcome of the breath test. Two incubations showed complete failure to produce both hydrogen and methane. Methane was detected in three of six negative faecal incubations with concentrations ranging from 1.166 to 6.642 ppm. In two incubations methane was found together with a considerable concentration of hydrogen and a positive breath test (Table 2).

## Discussion

The validity of the hydrogen breath test as a sensitive method for the detection of carbohydrate malabsorption has been disputed.<sup>7 8</sup> Gardiner *et al* described six patients with primary sugar malabsorption who had no increase in breath hydrogen or in stool incubations after oral sucrose or glucose, suggesting that the breath test is not a reliable means

of diagnosing primary sugar malabsorption. They postulated that the failure of the hydrogen breath test in their subjects had been caused by altered bacterial metabolism.

The results of these authors, however, contrast with those of other investigators reporting on 18 patients with primary sucrase-isomaltase deficiency.<sup>1 9-11</sup> Therefore, altered bacterial metabolism does not seem to be a feature specifically correlated with primary sugar malabsorption. Inability of the colonic flora to generate hydrogen from carbohydrates is also found in the absence of sugar malabsorption syndromes (this study).

Gilat found that 11 of 55 (20%) of subjects had colonic flora unable to produce hydrogen but nine of the 11 were studied after antibiotic treatment, preparation for colonoscopy, or colonic surgery.<sup>4</sup> A recent study in 100 Swedish adults undergoing the lactulose hydrogen breath tests assesses the frequency of inability to produce hydrogen to be 8%.<sup>6</sup> The present study, which gives a frequency of 9.2%, shows that the occurrence of this condition in children is comparable with that in adults. This frequency suggests that the chance of a false negative hydrogen breath test in an already documented group with lactose malabsorption is 4 to 17%, assuming that sugar malabsorption in itself does not alter the hydrogen producing capacity of the colonic flora.

According to our study, the sensitivity of the hydrogen breath test compares favourably with the 22 to 53% false normal blood glucose determinations in lactose intolerant children.<sup>2</sup> When used as a screening procedure in children with chronic diarrhoea or recurrent abdominal pain, or both, the mean probability of a false normal result after lactose challenge is only 1:77. In case of doubt, a lactulose test will show if the negative result is caused by inability to produce hydrogen.

Most patients with lactose malabsorption have secondary malabsorption, however, and we cannot deny that blood glucose determination shows more concordant results in the group with primary lactase deficiency. The chance of a false positive hydrogen breath test is virtually zero, however, whereas the number of false flat glucose curves was found to be 13% in 109 children.<sup>2</sup>

One interesting finding is the change in the outcome of the hydrogen breath test after a six month interval in nine of 20 children tested twice. This phenomenon was also noticed in a recent study in four of eight subjects with negative results who were retested within a few weeks.<sup>6</sup>

Since previous use of antibiotics has been excluded in our study, we speculate that changes in the diet may influence the capacity of the colonic

bacterial flora to produce or to utilise hydrogen. Theoretically, this may be explained by two mechanisms—a change in the composition of the colonic microflora or in the metabolic activity of the resident flora, or both. The first possibility is supported by the finding that an increased consumption of crude fibre over three weeks caused a profound increase in the ratio of anaerobic: aerobic flora, from 2.9 to 5.14.<sup>12</sup>

Changes of short duration in feeding habits may affect the hydrogen producing capacity by altering the metabolism. Faecal incubations with sugars from one of the authors (ACD) invariably resulted in the generation of large amounts of hydrogen but this changed to exclusive production of methane after the consumption of 500 g french cheese during a 24 hour fast. It has also been shown that ammonia, produced by colonic microflora, may be utilised when a readily utilisable carbohydrate is added to the faecal incubation.<sup>13 14</sup> The production of bacterial metabolites such as hydrogen, methane, and ammonia may, therefore, depend on the combination of different substrates presented to the microflora, rather than on the log count of the various anaerobic strains. That the colonic flora are capable of a rapid substrate-induced shift into another metabolic pathway seems to us a reasonable explanation for this change in the outcome of the hydrogen breath test.

The faecal incubation system used in this study is a modification of the procedure described by Levitt.<sup>5</sup> This rapid incubation test is based on the following considerations: maximum hydrogen production in vivo as well as in vitro has been shown to occur after 60 minutes;<sup>15 16</sup> the presence of living bacteria does not seem to be essential, provided that bacterial enzymes are present,<sup>17</sup> thus obviating the need for strictly anaerobic incubation; and prolonged incubation procedures induce hydrogen catabolism. After seven hours of incubation the hydrogen concentration decreases to half of the concentration found in the test tube after 60 minutes and this decrease is accompanied by the appearance of methane (ACD, unpublished results).

Since the process of hydrogen production and diffusion from the colon into the circulation and the lungs is rapid, we believe that the results of hydrogen estimations in expired air should be correlated with the production of hydrogen in faecal incubations of short duration.

The question arises whether an anaerobic, aerobic, or a semi-aerobic incubation system should be preferred when one seeks to correlate breath test results with the outcome of faecal incubation studies. In contrast to the anaerobic system<sup>6</sup> which failed to generate methane in subjects producing methane,

the aerobic system used in the present study produced methane in five of 18 incubations. Though methane in expired air has not been estimated in this study, the results of the faecal incubation test show that its production does not exclude the generation of considerable amounts of hydrogen or a positive hydrogen breath test. The relation between hydrogen and methane production by the colonic microflora seems to be more complicated than has been suggested in previous studies.<sup>6 18 19</sup>

A major problem in the interpretation of the results of faecal incubation studies with regard to bacterial metabolites is our lack of knowledge of the micro-environmental circumstances prevailing in the human colon. Though methanogenic bacteria are considered to be strict anaerobes,<sup>20</sup> this study shows that methane production is possible under aerobic conditions.

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