Breath hydrogen test for detecting lactose malabsorption in infants and children

Prevalence of lactose malabsorption in Japanese children and adults

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SUMMARY The breath hydrogen test (BHT) was adapted for use in young infants and children. The diagnostic criterion of sugar malabsorption in the BHT was determined by oral administration of 0.5 g/kg of unabsorbable sugar (lactulose) to 21 healthy infants and children. A maximum increase in breath hydrogen >0.05 ml/min per m^2 was observed in all subjects. A good correlation between results by the BHT and by the ordinary lactose tolerance test was obtained after oral administration of 2 g/kg lactose to 21 healthy infants and children, 2 congenital lactase-deficient infants, and 7 adults.

Using this test, 80 healthy Japanese infants and children (aged between one month and 15 years) and 18 adults were examined for lactose malabsorption after a dose of 1 g/kg lactose. All infants and children under 2-years old absorbed lactose completely. The incidence of lactose malabsorption was 30% in 3-year, 36% in 4-year, 58% in 5-year, and 86% in 6-year-old children, 85% in school-children, and 89% in adults. Thus the incidence of lactase deficiency gradually increases with age from 3 years, and about 90% of all normal Japanese adults are lactase-deficient.

At birth, humans have abundant lactase activity in the small intestine but, in most ethnic groups, there is a pronounced decrease in lactase activity during early childhood (Huang and Gayless, 1967; Johnson *et al.*, 1977). The definitive diagnosis of lactase deficiency requires the collection of biopsy specimens from the small intestine and demonstration of decreased lactase activity therein. Attempts have therefore been made to develop indirect methods for detecting lactase deficiency. Tolerance tests, although commonly used for this purpose, are influenced by many factors—such as, the rate of gastric emptying and intermediary glucose metabolism. In addition, these tests need a large amount of lactose and therefore give no information on the ability of the intestine to absorb

smaller, more physiological quantities. Moreover, the results are often unreliable (Krasilnikoff *et al.*, 1975).

Recently, many investigators have shown that the breath hydrogen test (BHT) (change in the concentration in the breath of hydrogen formed by bacterial metabolism of unabsorbed lactose in the colon) is a simple, accurate, and sensitive indirect test for lactase deficiency in adults (Levitt, 1969; Bond and Levitt, 1972; Newcomer *et al.*, 1975). There have been only a few reports of this test on children under 5 years (Maffei *et al.*, 1977).

We report the application of this test in children, of from one month old upwards, and in adults to determine the incidence and age of onset of lactase deficiency in Japanese children.

Subjects and methods

Subjects. Hydrogen excretion was studied in 80 healthy children (between one month and 15 years old), 2 infants with congenital lactase deficiency (diagnosed by per-oral intestinal biopsy), and 18 normal adults (between 20 and 50 years old). Of the

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80 children, 19 were infants (between one and 12 months old), 42 were preschool children (between 2 and 6 years old), and 19 were schoolchildren (between 7 and 15 years old). Informed consent was obtained from the adults and the children's parents.

Methods. The ability of the colonic flora of infants to produce H_2 during lactose fermentation was studied using faecal homogenates as described by Bond and Levitt (1972). Fresh faecal specimens (2 g) from 50 infants (15 newborn infants feeding with breast milk, 6 newborn babies with formula, 25 infants with formula, and 4 children) were homogenised in 4 ml of 0.10 mol/l phosphate-buffered saline (pH 7.0). All infants examined were healthy and not receiving treatment with antibiotics. The homogenates were placed in a series of 10 ml test-tubes and 1 ml 1.25% (mass/vol.) lactose solution was added to each. The tubes were mixed, sealed with rubber stoppers, and placed in a water bath at 37°C. A needle attached to a 50-ml syringe was inserted through the rubber stopper. After one hour, the tubes were vigorously stirred, and the gas in each tube was displaced into the syringe by injecting water into the test-tube through a second syringe. The concentration of H_2 was determined by gas chromatography.

Respiratory H_2 excretion

The subjects were starved overnight, and then the rate of respiratory H_2 excretion was determined at 0, 1, 2, 3, and 4 hours after carbohydrate loading by analysis of 3-min samples of expired air. As the H_2 concentration of expired air was often too low to measure accurately, a rebreathing technique, a modification of the method of Levitt (1969), was used to concentrate the breath H_2 . An infant circle, which is normally used for anaesthesia of infants, was used as the collecting system. The apparatus contains a flutter valve, CO_2 absorbent, 3-litre neoprene bag, and a 2nd flutter valve. Hydrogen was considered to be evenly distributed in a volume of gas equal to that present in the collecting system, plus the residual lung volume of the subject (estimated from a nomogram for normal Japanese children (Nakajima, 1967)). The volume of the infant circle (except for bag volume) was measured by the dilution method and the average value in 20 determinations was 980 ± 20 ml. After the collection period, the bag volume was measured. The total volume was taken as 980 ml, plus the bag volume, plus the residual lung volume. This total volume multiplied by the concentration of H_2 in the collecting system was defined as the volume of H_2 excreted during a 3-min period. The system was flushed with O_2 between collections. With infants

of <6 months old, expired air was collected by a modification of the to-and-fro rebreathing method of Krauss and Auld (1970). For this, a neoprene rubber bag of 1.5 litres was used, and the volume of the collecting system was nearly equal to the bag volume, because the dead space was very small (about 20 ml).

As soon as possible after collection, samples were analysed for H_2 by gas chromatography using a thermal conductivity detector (Shimadzu Seisakusho, Ltd, Model GC-4B, Kyoto, Japan). A 2-ml sample of gas was introduced through a gas sample valve into a stainless steel column, 2 m $\times$ 0.4 cm (inside diameter), packed with 80-mesh molecular sieve 5A. Oven temperature was 75°C. N_2 (flow rate 20 ml/min) was used as carrier gas. The smallest detectable amount of H_2 was 5 ppm (0.05 ml in 10 litres), corresponding to the excretion of 0.0025 ml/min (as the minimum volume of the collecting system was about 1.5 litres). A single H_2 determination took only a few minutes.

Plasma glucose

Venous blood samples were obtained at 0, 30, 60, 90, and 120 min after glucose and lactose loading. Plasma glucose was determined by the glucose oxidase method.

Results

Out of faecal specimens from the 50 infants, 48 samples produced considerable hydrogen. The mean fasting breath H_2 output of 80 subjects was 0.027 ± 0.016 ml (1 SD)/min per m^2 (range 0.000 to 0.068). Usually the peak level for H_2 concentration occurred 2 or 3 hours after oral sugar administration. The fasting levels were subtracted from the peak levels, and the results were expressed as the increase above the fasting level. The reproducibility of the technique for collecting breath H_2 was studied in 4 infants and 4 children during a lactulose (unabsorbable sugar) loading test by comparing their rates of breath H_2 excretion during 2 consecutive 3-min periods ($n = 16$). The mean difference between the 2 determinations was 10.7% with a range of 1.3 to 15% (range of H_2 concentration was 27 to 167 ppm). The change in breath H_2 excretion in healthy subjects (7 infants, 7 preschool children, and 7 schoolchildren) after oral ingestion of lactulose or glucose (an absorbable sugar) is shown in Fig. 1. Breath H_2 excretion increased markedly (>0.05 ml/min per m^2) in each of the 21 subjects after ingestion of 0.5 g/kg lactulose (10% solution), but did not increase above the basal level after ingestion of 2 g/kg glucose. In fact, in the subjects given glucose, breath H_2 excretion decreased gradually.

In these 21 subjects, 2 congenital lactase-deficient

Fig. 1 Maximum increase of breath H_2 excretion after oral administration of glucose (2 g/kg) and lactulose (0.5 g/kg), in children of various ages. Increase in H_2 is expressed as ml/min per m^2 body surface area.

infants, and 7 normal adults, we compared the relationship between the maximum increase of breath hydrogen excretion and the maximum increase of the blood sugar level after oral ingestion of 2 g/kg (maximum 50 g) lactose (10% solution).

Fig. 2 shows the maximum increase in blood sugar in each subject plotted against the maximum increase in breath H_2 excretion. Subjects who had a normal tolerance test—that is, >20 mg/100 ml (1.1 mmol/l) increase in blood sugar—showed less

than 0.05 ml/min per m^2 increase in H_2 excretion, and no clinical response. On the other hand, those who had <20 mg/100 ml increase in blood sugar showed an increase in breath H_2 excretion of >0.05 ml/min per m^2 and some of these subjects developed clinical symptoms—such as diarrhoea, cramp, bloating, flatulence, or borborygmi—particularly the 6 adults and 2 congenital lactase-deficient infants.

To examine the effect of age on H_2 excretion, we carried out the breath hydrogen test after oral ingestion of 1 g/kg lactose (10% solution) in 80 healthy infants and children and 18 adults. The maximum increase in breath H_2 is plotted against age in Fig. 3. All the infants and children of under 2 years could absorb lactose completely, but 30% of the 3-year-old children showed an increase of breath H_2 >0.05 ml/min per m^2 , indicating lactose malabsorption. The incidence of lactose malabsorption was 36% in 4-year, 58% in 5-year, 86% in 6-year-old children, 85% in schoolchildren, and 89% in adults.

Discussion

A simple, reliable, and noninvasive test of sugar absorption is required for diagnosis and for studying malabsorption syndromes and adult-type lactose malabsorption in children. The technique of intermittent breath sampling for analysis of breath H_2 was developed by Levitt (1969) and is now established as the most accurate indirect test of disaccharide intolerance in adults. However, this test had not been applied to infants and children with a total collection method, so we adapted it for use on children.

We first tested whether the intestine of children contains the H_2 -producing bacteria that are essential

Fig. 2 Relationship of maximum increase in breath H_2 excretion with maximum increase in blood sugar level after ingestion of 2 g/kg lactose (▲ infants, ■ preschool children, ● schoolchildren, ○ congenital lactase-deficient infants, ○ adults).

Conversion: traditional units to SI—blood glucose 1 mg/100 ml $\approx$ 0.0555 mmol/l.

Fig. 3 Maximum increase in breath H_2 excretion after ingestion of 1 g/kg lactose (maximum 50 g) at various ages.

for application of this test. *In vitro* tests on the faeces of 50 infants of various ages showed that almost all (96%) infants have colonic flora that produce H_2 . Thus the breath H_2 test may be applied to infants. The breath H_2 collecting technique used by Newcomer *et al.* (1977) is an accurate method for older children, but the apparatus is too big and complex for children under 5 years and the rebreathing time too long, so that accuracy may be poor. The methods of end-expiratory breath H_2 sampling reported by Maffei *et al.* (1977) and Caskey *et al.* (1977) can give good information, but it is less precise than the total collection method (Metz *et al.*, 1976). However, as the total volume of collecting system is small in our method, and a detectable increase in concentration of H_2 gas in the system can be obtained in 3 minutes, we achieved satisfactorily accurate quantitative sampling of breath H_2 . To reduce the total volume of the collecting system for infants under 6 months, the expiratory air was sampled using a to-and-fro rebreathing system. Our technique for collecting breath H_2 gave reproducible results, even with children under 5 years.

According to Newcomer and McGill (1977), 25 g (0.3–0.5 g/kg) of unabsorbed sugar may be enough to provide a distinct increase in breath H_2 in adults. On the other hand, we found that some patients began to develop clinical symptoms—such as diarrhoea, borborygmi, and abdominal distention—after administration of >0.5 g/kg lactulose, suggesting that the presence of more than 0.5 g/kg of unabsorbed sugar in the large intestine may cause clinical symptoms. So we tentatively made the presence of 0.5 g/kg unabsorbed sugar in the large intestine a diagnostic criterion of sugar mal-

absorption. For example, if an infant of 10 kg was given 10 g lactose, half of which was not absorbed (absorption rate 50%), a certain amount of H_2 would be produced. On the other hand, if a man of 50 kg was given 50 g lactose, and 5 g lactose was not absorbed (absorption rate 90%), H_2 excretion derived from unabsorbed sugar may be the same, since the amount of breath H_2 may reflect the absolute amount of unabsorbed sugar (Bond and Levitt, 1976). We thus thought it reasonable to establish a criterion of sugar malabsorption in BHT, by dividing H_2 excretion/min by body surface area. We first attempted to demonstrate an appreciable maximum increase in breath H_2 after oral administration of 0.5 g/kg lactulose to healthy infants and children of various ages. As shown in Fig. 1, even in infants, a definite increase of breath H_2 excretion (>0.05 mL/min per m²) was detected after administration of 0.5 g/kg of unabsorbable sugar (lactulose). On the other hand, when an absorbable sugar (glucose) was administered (2 g/kg) to the same subjects, no increase of breath H_2 was observed in any age group. These findings show that our method is reliable and accurate for evaluating sugar absorption. Moreover, as Fig. 2 shows, there was good correlation between the results of the ordinary lactose loading test and the results of the breath H_2 test.

We then carried out the breath H_2 test after oral ingestion of 1 g/kg lactose to determine the incidence of lactose malabsorption. We chose this dose of lactose for the following reasons: (1) it is more physiological than that ordinarily used (2 g/kg), because uptake of lactose, at one time, from the diet does not usually exceed 1 g/kg (Hegsted, 1976; Newcomer *et al.*, 1978); (2) the absorption of less

than half the administered lactose may be reasonably defined as lactose malabsorption. Thus, judging from the fermentation of various sugars (Bond and Levitt, 1972) and our results with lactulose loading, if half the lactose is not absorbed after a load of 1 g/kg, there should be an increase of breath hydrogen >0.05 ml/min per m^2 .

Our results showed that all infants and children under 2 years could absorb lactose completely. Lactase deficiency seems to appear at 3 years and it gradually increases with age; 85% of the children of over 6 years, and about the same percentage of adults, showed lactose malabsorption.

There have only been two previous reports on the age of onset and prevalence of lactose intolerance in Japanese people. Shibuya *et al.* (1970) reported a somewhat earlier age of onset of lactose intolerance and 100% incidence of lactose intolerance after 8 years by using the ordinary lactose tolerance test. As the ordinary lactose tolerance test gives about 20% false-positive results (Krasilnikoff *et al.*, 1975), their results are not essentially different from ours. Naito *et al.* (1973) investigated the effect of age on lactase activity, and found an adult-type pattern of lactase activity in children of over 6 years.

There are two reports of studies on American Indians using breath H_2 analysis. Newcomer *et al.* (1977) observed lactase deficiency by age 5, but they did not obtain satisfactory results on younger children; Caskey *et al.* (1977) made similar studies but their results are not comparable with ours because of the difference in method.

The breath H_2 test has many advantages (Metz *et al.*, 1976; Fernandes *et al.*, 1978) and should have wide application for elucidating problems of sugar absorption in children.

We thank Mrs Ichihara and Mrs Nose for their help in preparing the manuscript. This investigation was supported in part by grant from the Ministry of Education in Japan.

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Received 10 October 1978