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Note

Value of breath hydrogen test in detection of hypolactasia in patients with chronic diarrhoea

NAJAT A. HASSAN

Chemistry and Biochemistry Department, Saddam College of Medicine, Baghdad (Iraq)

MAJEED R. AL-ANI*

Physiological Chemistry Department, College of Medicine, University of Baghdad, Baghdad (Iraq)

ARIF M. LAFTA

Oil Research Center, Scientific Research Council, Baghdad (Iraq)

and

ZUHAIR A. KASSIR

Department of Medicine, College of Medicine, University of Baghdad, Baghdad (Iraq)

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It has been known for over 100 years that hydrogen (H_2) may be present in high concentration in the flatus [1]. In 1920, Dodds [2] applied breath analysis for the diagnosis of gastrointestinal diseases. He reported a rise in breath CO_2 after a meal. Neilson [3] reported that H_2, a gas not normally present in high concentrations, was present in the breath of individuals following the consumption of bean meal. Calloway and co-workers [4,5] showed that H_2 was produced in the colon following degradation of malabsorbed carbohydrates by intestinal bacteria. In 1969, Levitt [6] introduced the breath hydrogen test (BHT) as a tool for the diagnosis of gastrointestinal disorders.

Chronic diarrhoea is a major cause of morbidity in infants and children, and secondary sugar malabsorption is a frequent perpetuating factor [7]. Yet most methods for diagnosing sugar malabsorption generally involve procedures that may be considered mildly to moderately invasive, or involve exposure to radiation [8–10].

Hydrogen gas in human breath derives exclusively from bacterial fermentation in the intestinal lumen. In the expired air the rise of H_2 concentration, monitored at intervals after administration of dietary carbohydrates, indicates malabsorption of sugar [11]. In the present study hydrogen gas was measured in the breath of patients with chronic diarrhoea by thermal conductivity gas chromatography (GC) of normalized to alveolar air concentrations. This method can detect hypo-

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lactasia in these patients and measure the time of onset of the mean maximum rise in breath H_2 concentration after an oral dose of lactose.

EXPERIMENTAL

Patients

The study comprised 52 patients (23 females and 29 males), aged 9 months to 86 years, with the primary complaint of chronic diarrhoea. Those suffering from diarrhoea gave case records of liquid stools for a period ranging from 1.5 months to 5 years. Each patient underwent an intraluminal jejunal biopsy and breath hydrogen test after an oral dose of lactose on the subsequent days, except two which could not be followed up. A further 11 patients aged 12–52 years (6 females and 5 males), none of them complaining of gastrointestinal diseases or having a history of lactose intolerance, were used as controls.

Breath hydrogen test

This test is based on the fact that when malabsorbed carbohydrates are fermented by colonic bacteria, a proportion of H_2 gas diffuses to the circulation, is transported to the lungs and is then exhaled. After an overnight fast for adults and of *ca.* 8 h for younger children and infants, expiratory alveolar air samples were collected for 1–3 min through a face-mask sampling device. Subjects were required not to smoke before or during sampling. The breath sampling was repeated after the oral administration of 2 g of lactose per kg body weight at 30-min intervals over the next 3 h. Samples were immediately injected into 10-ml vacutainers and then stored in the refrigerator to be analysed within 45 days.

Gas chromatography

Hydrogen, oxygen, and nitrogen in 5-ml samples of expired air were analysed by manual injection into a gas chromatograph (Pye Unicam, Series 204), equipped with a thermal conductivity detector and a 3 × 4 mm I.D. stainless-steel column packed with 60–80 molecular sieve 13X (BDH, Poole, U.K.). The column was conditioned before use at 250°C for 8 h with the carrier gas (argon 99.999%) at a flow-rate of 40.5 ml/min and pressure of 0.72 bar. The chromatograph was operated under the following temperature conditions: column and injector port, 70°C; detector, 125°C. The carrier gas flow-rate was 40.5 ml/min.

The concentration of H_2 was determined by comparison of the height of the unknown peak from breath samples with the peak heights from a standard gas mixtures containing 10–200 ppm H_2 in room air. Complete elution of a gas sample at this carrier gas flow-rate took 10 min. Carbon dioxide was absorbed on the 60–80 mesh molecular sieve 13X, and therefore could not be traced. A detection limit of 3 ppm H_2 was achieved at an attenuation of 1; for detection of O_2 and N_2 the attenuation was set at 256.

Since the concentration of nitrogen does not vary appreciably in the human

system, it can serve as an internal standard for GC analysis. Variation in the manual injection volume and leakage from the septum or syringe plunger could be corrected to constant atmospheric N_2 levels according to the formula:

$$\text{corrected result} = \frac{\text{atmospheric } N_2}{\text{observed } N_2} \times \text{observed result}$$

Normalization of H_2 values to a constant expired O_2 level was tested by using the relationship:

$$\text{normalized } H_2 = \text{observed } H_2 \frac{(\text{atmospheric } O_2 - \text{lowest measured } O_2)}{(\text{atmospheric } O_2 - \text{observed } O_2 \text{ level})}$$

Therefore, the variation in H_2 recordings due to the sampling technique was reduced, eliminating the need for rebreathing or end-expiratory collection procedure. A positive H_2 response was defined as a peak rise of at least 20 ppm (0.2 ml/min) above the fasting level, as suggested by Metz *et al.* [12]. A chromatogram showing serial rises in breath H_2 peaks over different time intervals in a lactose-

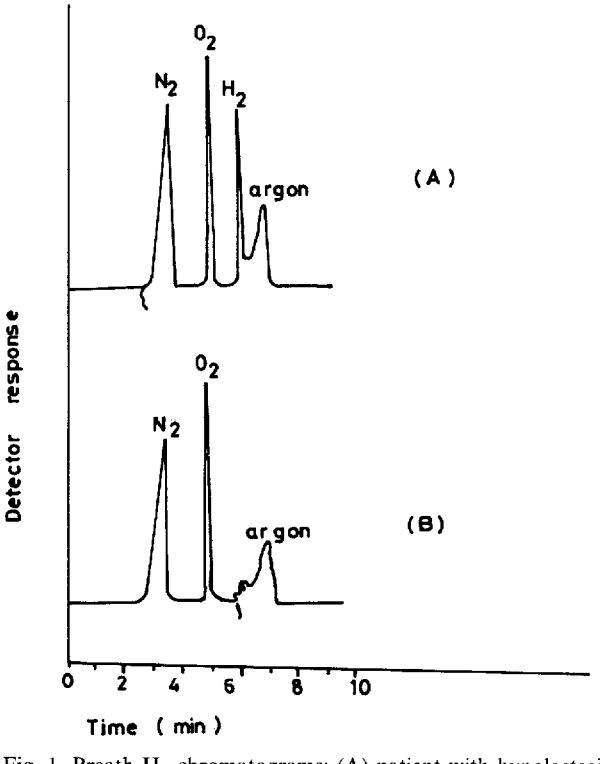

Fig. 1. Breath H_2 chromatograms: (A) patient with hypolactasia showing a large H_2 peak; (B) normal person.

intolerant patient and compared with that of a normal person is illustrated in Fig. 1.

Enzyme assay

Jejunal biopsies were obtained using the Watson modification of the Crosby capsule. Formalin-fixed specimens were processed and stained with hemotoxylin and eosin, and graded histologically under a light microscope into normal, partial, and total villous atrophy. The method of the assay of disaccharidase activity was adopted from that of Dahlqvist [13]. The enzyme activity was expressed as units of disaccharide hydrolysed per gram of protein [14].

RESULTS AND DISCUSSION

Fig. 2 depicts the relationship between lactase activity (U/g protein) and the maximum rise in breath H_2 levels in 61 patients. The 13 patients with normal lactase activity (> 5.9 U/g protein) had normal breath H_2 values, with the exception of 2 patients who exhibited a maximum increase in breath H_2 concentrations greater than 0.2 ml/min (20 ppm). The 48 patients with hypolactasia displayed

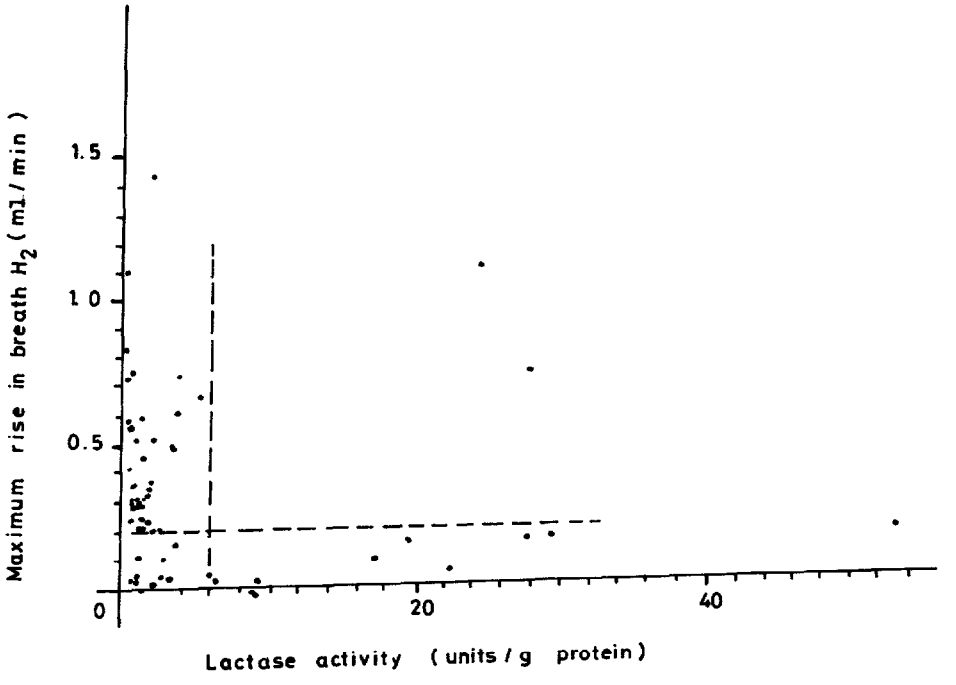

Fig. 2. Relationship between lactase activity (U/g protein) and the maximum rise in breath H_2 concentration (ml/min). The horizontal and vertical dashed lines represent upper and lower normal levels of breath H_2 and lactase activity.

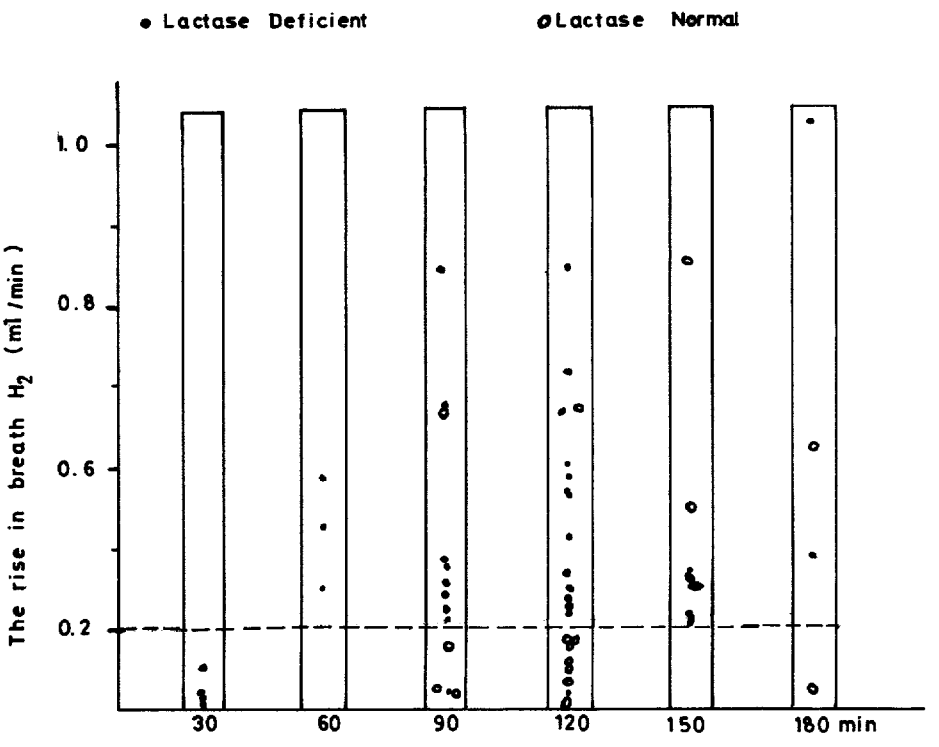

Fig. 3. Time of peak exhalation breath H_2 after an oral dose of 50 g of lactose. The horizontal dashed line represents the upper limit of normal.

abnormal breath H_2 values, except 10 patients who had an increment of less than 0.2 ml/min. Thus, the BHT gave two (3.5%) false-positive and ten (17.5%) false-negative values, with an 80% sensitivity. Similar results with 100% specificity had been achieved by Rosado and Solomons [15]. Yet the efficiency of the BHT may be negatively affected by sources of false-positive results, *e.g.* inadequate fasting, sleep, smoking, and bacterial overgrowth of the small intestine [16–21], or false-negative results, *e.g.* absence of H_2-producing bacteria, prior antibiotic use, hyperventilation, delayed gastric emptying, or acute diarrhoea [18,21].

The mean maximum rises of breath H_2 level (ml/min) were compared with the mean lactase activities (U/g protein) in all patients. There was a positive correlation between the BHT and lactase activity ($r = +0.43$), with a confidence limit in the range from $+0.52$ to $+0.23$.

A clear discrimination between lactose absorber and lactose malabsorber was noted by breath H_2 analysis at 90–120 min (Fig. 3). A late increase in hydrogen response (after 120 min) was found in three lactase normal subjects, and seven patients with lactase deficiency. These findings may represent incomplete lactose

absorption in lactase-sufficient subjects, or malabsorption of small amounts of a large lactose load in patients with partial lactase deficiency secondary to the mucosal injury [11].

It can be concluded, therefore, that the use of GC for the breath H_2 analysis to detect intestinal sugar malabsorption holds great promise for wide-spread clinical applications, since it is simple, rapid, and relatively inexpensive. Patient acceptance and safety of this non-invasive interval collection method is better than for those procedures that require the use of radioisotopes. It is suitable for use in infants and younger children. Moreover, the elimination of the extraintestinal influences would further improve the reliability of the test as a sensitive indirect alternative test for the disaccharidase assay in the jejunal biopsy.

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