Development of an Interval Sampling Hydrogen (H₂) Breath Test for Carbohydrate Malabsorption in Children: Evidence for a Circadian Pattern of Breath H₂ Concentration

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Summary

We have applied the gas chromatographic analysis of hydrogen (H₂) in expired air to the determination of carbohydrate malabsorption in children. A compact, inexpensive, and simple gas chromatograph was specifically adapted to the measurement of low concentration of H₂. A collection procedure using the sampling of expired air at evenly spaced intervals with a low resistance facemask was used to obviate the need for closed, continuous rebreathing systems of breath collection.

Under different experimental conditions, H₂ concentrations ranged from 11-166 ppm. During fasting, however, H₂ concentrations in preschool children were extraordinarily stable and uniform. The velocity of H₂ excretion from graded doses of 1.5, 3, and 6 g of the nonabsorbable disaccharide, lactulose, was linear with a response of 1.2 cc H₂/2 hr/g nonabsorbed carbohydrate. Formal clinical lactose tolerance tests were devised using the oral administration of 1.75 g lactose/kg body weight. The increase in H₂ concentration was compared with the rise in plasma glucose. Maximum increases in H₂ concentration of less than 15 ppm corresponded to a rise in plasma glucose greater than 20 mg/dl; increases in H₂ of more than 20 ppm were uniformly accompanied by flat glucose curves. Increases in H₂ concentration between 15 and 20 ppm comprised a borderline zone in which both flat and normal glucose curves were seen. The normal absorption of the monosaccharide constituents of lactose, glucose, and galactose, as demonstrated by increments of less than 15 ppm in H₂, indicates that the lactose intolerance was due to failure of digestion of the disaccharide rather than to decreased mucosal absorption of the monosaccharide products.

When H₂ concentration was measured at 120-min intervals over periods of 24 hr in children on a normal diet, a 2.5-fold or greater increment in breath H₂ concentration was observed at some time during nocturnal sleep. Moreover, the increase in H₂ concentration to a standard dose of 6 g lactulose was greater during induced sleep than in the awake subject.

Various pitfalls to the interpretation of clinical carbohydrate absorption tests using breath H₂ were identified. These included an occasionally elevated baseline concentration of H₂, delayed gastric emptying, and previous administration of broad spectrum oral antibiotics. The test is noninvasive, well tolerated, semiquantitative, and ideally suited for use in children.

Speculation

The noninvasive, interval sampling of H₂ breath test will provide a more versatile, widely applicable, and better tolerated index of

carbohydrate malabsorption in the pediatric population than conventional methods using blood glucose determinations, and will prove to be the preferred method for testing carbohydrate tolerance in children.

The methods for studying carbohydrate tolerance generally involve procedures which can be considered mildly to moderately invasive, e.g. sampling of capillary or venous blood for glucose or galactose determination (23), or intestinal intubation and perfusion (16, 29), or unsuitable for use in children, e.g., the use of ¹⁴C-labeled carbohydrates (2, 24). Moreover, in children the blood sampling technique can be associated with falsely abnormal tolerance tests due to unpredictable gastric emptying irregularities: in one study, many children who showed flat glucose curves when given oral lactose later demonstrated an adequate rise in glycemia with the intrainstestinal instillation of the carbohydrate (13). The determination of fecal reducing substances has been offered as an alternative, noninvasive method (4, 12), but intracolonic metabolism of nonabsorbed carbohydrates (3, 8) and variations in the water content of stools makes such methods inherently nonquantitative.

The exposure of carbohydrate to certain bacteria in the intestine results in their fermentation accompanied by the evolution of hydrogen (H₂) gas. Although a majority of this H₂ is passed as flatus, a certain percentage is absorbed and is excreted by the lungs (5, 14). Over the last decade, this phenomenon has been exploited to measure the malabsorption of carbohydrate in human subjects. Early clinical studies used closed, rebreathing systems for collection of expired air (1, 15), but a procedure of sampling breath at fixed intervals after an oral carbohydrate dose has been employed in adult patients to diagnose hypolactasia (6, 9, 21), hyposucrasia (20), and bacterial overgrowth (19). Such interval sampling of expired air is inherently noninvasive, and ideally suited for use in children, even infants (17). Moreover, the technique has proven to be versatile in that it allows for the study of a wide range of carbohydrate dosages and for observations over extended periods of time, neither of which is practical with the standard methods for determining carbohydrate malabsorption. Furthermore, H₂ breath excretion can provide semiquantitative information on intestinal fermentation of carbohydrates and has been suggested to be the most valid indirect index of carbohydrate malabsorption (2, 24). In the present study, we present the development and application of an interval sampling H₂ breath test for use in children. The test is compared with the standard lactose tolerance test of blood glucose determination, and observations on

the changes in breath H₂ concentration over the course of 24 hr are recorded.

PATIENTS, PROCEDURES, AND METHODS

PATIENTS

The subjects were male preschool children, ranging in age from 18–44 months, hospitalized in the Clinical Research Center of the Division of Human Nutrition and Biology of INCAP. The criterion for admission had been protein-energy malnutrition of the edematous type (kwashiorkor). The dietary measures for stabilization and recovery of the subjects were those previously described for our unit (30), and subjects were studied on admission, during recuperation, and after complete nutritional recovery. In many cases, the same individual participated in various studies during several phases of the progress of his nutritional recovery. Informed consent was obtained from parents or guardians on admission of the children to the Clinical Research Center.

PROCEDURES

Studies were initiated after an overnight fast. After the consequences of the circadian pattern were appreciated, the subject was awakened an hour before receiving the test dose of carbohydrate. For the studies of baseline variability of breath H₂ concentration, the subjects were given 250 ml water by mouth on waking, vigorously exercised by playing games on the floor, and continued in the fasting state during the subsequent collection period. During periods, subjects were maintained awake and at minimum activity in their cribs; they were allowed to drink water *ad libitum*, but were otherwise fasted during the duration of the sampling. Study periods were 5 or 6 hr with breath samples collected at 30-min or 60-min intervals.

For the circadian studies, breath samples were collected at 2-hr intervals beginning at 7 AM, and continuing for 24 hr until 7 AM of the following day. During circadian studies, the usual daily routine of diet, activity, and sleep were maintained. Meals were served at 8 AM, 11 AM, 2 PM, 5 PM, and 8 PM, and periods of sleep generally lasted from 12 noon to 2 PM, and from 8:30 PM to 6 AM. The diets consisted of milk alone, milk and casein, or corn (tortillas) and black beans (frijoles).

In the studies designed to evaluate the effect of sleep, the child was kept awake with various games until 11 PM, then aroused again at 4 AM. At 5 AM, the basal breath sample was collected, and a dose of chloral hydrate, 40 mg/kg, was administered by mouth along with a test dose of nonabsorbable carbohydrate, lactulose (31). The child was then placed in a darkened, quiet room for half-hourly breath collection for 6 hr or until the subject awakened spontaneously. Lactulose was also used as the nonabsorbable test carbohydrate for the calibration of the linearity of the H₂ response. Doses of 6 g, 3 g, and 1.5 g lactulose were given on alternate days.

In clinical lactose tolerance tests, lactose (32) was administered on the first day, and its constituent monosaccharide components, galactose (Fisher Scientific Co., Fair Lawn, NJ) and glucose (J. T. Baker Chemical Co., Phillipsburg, NJ) (in equimolar proportions and equivalent in total weight to the lactose dose) were administered on the subsequent day to test monosaccharide absorption. All sugars were dissolved in 200 ml water, and administered after zero-time samples of expired air and plasma had been obtained. The subjects were encouraged to ingest the carbohydrate in the minimum time possible.

Expired air was collected with a pediatric anesthesia facemask attached to a 5-liter capacity gas bag via a low resistance, one-way Rudolph valve and transferred directly to a gas-tight container. The dead-space in the apparatus as measured by water displacement varied from 80–120 ml depending upon the size of the mask and valve used. Dead space error can be eliminated by flushing the system with a few breaths before clamping the outlet, or minimized by collecting volumes of expired air in excess of 3000

cc. It should be remembered that this multiple respiration, non-breathing, interval collection system samples *mixed air*, which, due to the factor of anatomic dead space, would theoretically have an H₂ concentration of approximately 70% of the corresponding *alveolar* air. The mechanics of our collection procedure and theoretical considerations have been discussed in detail elsewhere (28).

Blood samples were taken either as venous blood from an indwelling catheter or as capillary blood from a small finger incision at intervals of 0, 30, 60, 90, 120, 180, and 240 min after administration of the carbohydrate. The samples were transferred to heparinized tubes, centrifuged, and the plasma stored at –20° until analysis.

MATERIALS AND METHODS

Chromatographic analytic methods have previously been presented in detail (28). A thermal conductivity chromatograph, Quintron model S (QuinTron Instruments Co., Inc., Milwaukee, WI) was specifically adapted for H₂ analysis with an increased sample loop capacity of 16 cc, and a 13× molecular sieve column lengthened to 366 cm. Expired air was injected directly for analysis with a 60 cc syringe, or stored in gas-tight Mylar foil (Champion Paper Co., Des Plaines, IL (33))-coated gasbags. Argon, the carrier gas, was used at flow rates from 16–22 cc/min. Chromatograms were recorded with a model L 101 single pen recorder (QuinTron Instruments). A full scale deflection of 25.4 cm was equivalent to a 1-mV input signal.

Hydrogen concentration was determined by comparison of the height of the unknown H₂ peak from a breath sample with the peak height of a precalibrated reference gas containing 55 ppm H₂ in room air (Union Carbide Corp., Linde Division, Chicago, IL). For graphic display of the data, the change in breath H₂ concentration from the zero-time concentration has been termed the "excess H₂ concentration." A simple concentration criterion of the maximum increase seen during a 6-hr postdose observation period has been used for lactose absorption tests. During the circadian pattern studies, no reference hydrogen was available, and relative H₂ concentrations have been expressed as the absolute height of the H₂ peak on the chromatogram.

The area under the discontinuous curve of interval excess breath H₂ determinations can be integrated using an estimation for total volume of expired air to derive an approximation for total volume of H₂ in excess of the basal pulmonary excretion rate attributable to the fermentation of the nonabsorbable carbohydrate, lactulose. Tidal volumes have been determined from the Radford nomogram (26) and corrected for the altitude above sea level. Total excess pulmonary excretion of H₂ from a series of interval samples was estimated by the formula (28)

$$\begin{aligned} \text{Excess H}_2 &= \frac{(T_0 - T_0) + (T_1 - T_0)}{2} + \frac{(T_1 - T_0) + (T_{ii} - T_0)}{2} \\ &+ \frac{(T_{ii} - T_0) + (T_{iii} - T_0)}{2} + \dots + \frac{(T_{n-1} - T_0) + (T_n - T_0)}{2} \quad (1) \\ &\times \text{respirations/min} \times \text{min/interval} \\ &\times \text{tidal volume in cc (corrected)} \times 10^{-6} \end{aligned}$$

where T₀ = H₂ concentration in ppm at time zero (before dose); T₁ = H₂ concentration in ppm at the end of first interval; T_{ii} = H₂ concentration in ppm at the end of second interval; T_{iii} = H₂ concentration in ppm at the end of third interval; T_{n-1} = H₂ concentration in ppm at the end of the penultimate interval; T_n = H₂ concentration in ppm at the end of the final interval. By our conventions, the sampling interval was 30 min and an average respiratory rate of 35 respirations/min was used throughout. For comparison with the studies of Bond and Levitt (1), summation of excess hydrogen volumes during the 2 hr following the sharp increase in H₂ production has been considered the excess H₂ excretion in response to fermentation of the nonabsorbable carbohydrate in the colon.

Plasma glucose was determined by a glucose oxidase method (7) or by an automated AutoAnalyzer (Technicon Instruments Corp., Tarrytown, NY) technique (11).

RESULTS

SENSITIVITY AND STABILITY OF H_2 ANALYSES

The 55 ppm of H_2 in room air precalibrated reference gas was recorded on 47 occasions over a 2-month period while a constant carrier gas flow of 16 cc/min was maintained. The mean peak height was 5.6 ± 0.4 cm (mean ± 1 SD) with a coefficient of variation of 0.7%. Thus a change in H_2 concentration of 1 part/million was equivalent to approximately 0.1 cm on the chromatogram. In the present series of over 650 individual deter-

Fig. 1. The mean ± 1 SD of absolute breath hydrogen concentration for hourly samples taken over a 5-hr fasting period in five recovered children.

minations, the H_2 concentration in expired air ranged between 11 and 166 ppm. When children were awakened, given 250 ml water by mouth and exercised for 1 hr prior to 5 hr of subsequent observation during fasting, negligible variation from baseline H_2 concentration was observed (Fig. 1).

LINEARITY OF H_2 RESPONSE TO NONABSORBABLE CARBOHYDRATES

Previous workers (1) using a closed rebreathing system of H_2 collection and adult subjects demonstrated a linearity of the total excretion of H_2 over the first 2 hr of increased H_2 production with oral doses of 6.5, 13, and 26 g of the nonabsorbable carbohydrate, lactulose. Analogous experiments were conducted in preschool children using graded doses of 1.5, 3, and 6 g, using interval collection of breath samples. The total excess excretion of breath H_2 for the 2-hr period after the onset of the increase in H_2 concentration was calculated according to the formula outlined above. Figure 2a illustrates the H_2 concentration curves after the assigned doses of lactulose in one of the subjects. Figure 2b shows the mean 2-hr excess H_2 excretion for each dose in three recovered children studied at all three dosages. Dose-related linearity of the H_2 response to a nonabsorbable carbohydrate is thus demonstrated. The mean response of excess H_2 excretion was 1.2 ± 0.3 cc H_2 /2 hr/g lactulose ingested (mean $\pm$ SD).

LACTOSE TOLERANCE TESTS

A clinical lactose tolerance test based on H_2 excretion was developed and compared to a lactose tolerance test using the conventional measurement of blood glucose. Simultaneous analysis of the change in breath H_2 concentration and the change in plasma glucose was performed after an oral dose of 1.75 g lactose

Fig. 2. a: the changes in breath H_2 concentration above the zero-time level (excess breath H_2 concentration) at 0.5-hr intervals with doses of 6, 3, and 1.5 g lactulose in one recovered child are shown. The area under the curve for the 2-hr period following the increase in H_2 excretion rate has been integrated using formula 1. b: the mean $\pm$ SEM of excess breath H_2 volume during the first 2 hr of increased H_2 production after the three graded doses of lactulose for three recovered children is shown along with the linear regression line, $R = 0.95$; $m = 1.31$; $b = -0.23$.

or of its constituents, glucose and galactose. Figure 3 illustrates two representative studies. The graph in Figure 3a shows studies in a lactose-intolerant subject as defined by a maximum rise in plasma glucose concentration of less than 20 mg/dl after a lactose load. A maximum increase in breath H_2 concentration of 93 ppm was observed. The curve in Figure 3b shows typical results in a lactose-tolerant subject. Increases in plasma glucose of 64 mg/dl corresponded to net negative changes in breath H_2 concentration. In the accompanying glucose-galactose (monosaccharide) absorption tests a rise in plasma glucose of greater than 20 mg/dl was seen in all cases; neither showed an increase in H_2 concentration in excess of 15 ppm.

Simultaneous H_2 and glucose data are available for paired analysis in 15 studies (excluding those tests discussed under *Pitfalls*). In all four instances in which the maximum rise in H_2 concentration was less than 15 ppm, the corresponding increase in plasma glucose was greater than 20 mg/dl. In the eight subjects in which the increase in H_2 concentration was greater than 20 ppm, the increase in plasma glucose was less than 20 mg/dl. In three studies, the maximum increase in H_2 concentration was between 15 ppm and 20 ppm; of these, one had an increment greater and two had increments less than 20 mg/dl in plasma glucose (Fig. 4). Thus, an increase in H_2 concentration of more than 20 ppm can be considered indicative of lactose malabsorption following a 1.75-g/kg dose of carbohydrate in water.

On 14 monosaccharide absorption studies using equimolar glucose-galactose solutions, there was no rise in H_2 of greater than 15 ppm. In 12 of these studies, the increase in plasma glucose exceeded 20 mg/dl, whereas in 2 tests the plasma glucose increment was less than 20 mg/dl.

CIRCADIAN PATTERN OF H_2 CONCENTRATION

Four children were studied on a total of six occasions over a period of 24 hr with bihourly breath samples. Taking the average

peak height of the H_2 peak in centimeters of the 7 AM samples as a baseline, a 2.5-fold or greater rise in the absolute peak height of H_2 was seen in each study during some hour during nocturnal sleep, usually around 1 AM or 3 AM (Fig. 5). In some cases, a suggestion of a discrete rise in the 1 PM sample can be discerned; this corresponds to the midpoint of the customary 2-hr midday nap period from 12 noon to 2 PM.

SLEEP STUDIES

Three fully recovered subjects were given a dose of 6 g lactulose in water while maintained awake and while induced to sleep for 240–360 min during daylight hours. The peak values for H_2

Fig. 4. A scattergram of the maximum increase in glucose concentration against the maximum increase in H_2 concentration following a dose of 1.75 g/kg lactose for 15 studies in severely malnourished, recuperating, and fully recovered children.

Fig. 3. Left: the upper graph shows the changes in breath H_2 concentration at 0.5-hr intervals over 6 hr after lactose administration, and glucose and galactose administration in a lactose-intolerant child. The lower graph shows the corresponding changes in plasma glucose over 4 hr. Right: the same changes in breath H_2 concentration and plasma glucose are shown for a lactose-tolerant child.

Fig. 5. The relative changes in the peak height of hydrogen curves on the chromatogram of breath samples taken at 2-hr intervals over the course of 24 hr in six children consuming various solid and liquid diets (indicated above each curve). A consistent circadian pattern of rise during the hours of nocturnal sleep is demonstrated.

concentration during the awake studies were 56, 79, and 79 ppm, respectively, whereas the corresponding maximum H_2 levels during sleep were 126, 133, and 166 ppm. Figure 6 shows the composite 4 hour H_2 concentration curves for the three children studied with lactulose under waking and sleeping conditions.

PITFALLS

HIGH INITIAL ZERO-TIME BREATH H_2 CONCENTRATION

Occasionally, as in the case of subject, N.L., illustrated in Figure 7, H_2 concentrations well above usual fasting levels are encountered in the basal breath sample. N.L. had breath H_2 concentrations of 59 ppm and 60 ppm, respectively, on 2 successive days. The dose of lactose, 1.75 g/kg, was withheld until the concentration had declined somewhat, but even after the administration of the substrate, there was a spontaneous tendency toward recovery of a normal basal level, superimposed upon a rise in H_2 due to lactose malabsorption. In this subject, the lowest concentration of H_2 observed during the first three hours was arbitrarily taken as the baseline. Using this approximation, peak increases in H_2 concentration of 19 ppm and 1 ppm, which corresponded to maximum increments in glucose of 20 mg/dl and 9 mg/dl, respectively, were attributed to carbohydrate fermentation. Thus, the interpretation of the H_2 concentration changes uniquely due to the fermentation of lactose was obscured by an elevated and declining initial H_2 concentration.

DELAYED GASTRIC EMPTYING

Just as with carbohydrate tolerance tests based on blood glucose determinations (13), prolonged gastric retention and delayed gastric emptying of an orally administered substrate can affect the validity of absorption tests based on breath H_2 . This phenomenon

Fig. 6. The changes in breath H_2 concentration (mean $\pm$ SEM) over 4 hr in three recovered children receiving 6 g lactulose in 100 ml water. During one occasion, the children were awake throughout the study. On the other occasion, they were asleep.

Fig. 7. Changes in the absolute breath H_2 concentration on 2 consecutive days in subject, N.L., who had unusually elevated concentrations before his lactose absorption studies. The black arrow indicates the time at which the 1.75 g/kg lactose was ingested. The white arrow indicates the lowest concentration of breath H_2 observed during the first 3 hr, which was taken arbitrarily as the baseline. Maximum calculated increases above baseline were 19 and 11 ppm, respectively.

was suggested in our patient, O.R., who demonstrated clinical evidence of lactose intolerance including watery diarrhea and acid stools on a milk-based diet, while having both flat H₂ concentration curves and flat plasma glucose curves during his admission lactose and glucose:galactose tolerance tests. The possibilities considered to explain these findings were: 1) malabsorption of both lactose and monosaccharides in a subject lacking the flora necessary to produce H₂, 2) delayed gastric emptying of the carbohydrate doses. As clinical lactose intolerance persisted, studies were re-initiated later in the hospitalization. Multiple attempts at direct intrainestinal instillation of carbohydrates were thwarted by failure of the tube to pass the pylorus, and on one such attempt, a large gastric residual was encountered 2.5 hr postprandially. These observations further confirmed our clinical suspicion of functional gastric outlet problems. An oral dose of 6 g lactulose at this time, however, produced an expected H₂ response, assuring the presence of an adequate bacterial flora for fermentation. Therefore, repeat oral carbohydrate tolerance tests were attempted on the 92nd and 93rd hospital days. These showed a marked increase in H₂ after lactose with a flat glucose curve, while monosaccharide absorption was normal (see the first series in Fig. 3). The most consistent explanation of the negative lactose H₂ breath test on admission would seem to be gastric retention and delayed emptying of the test solutions, although the absence of the appropriate bacterial flora on admission has not been ruled out.

PRIOR ORAL ANTIBIOTIC ADMINISTRATION

The evolution of breath hydrogen is dependent upon bacterial fermentation. Antibiotics reduce the apparent deconjugation of bile salts as measured by the ¹⁴CO₂ breath test (27), and alter the H₂ response to carbohydrates in adults (22). Figure 8 illustrates two breath tests in subject E.G.d.L., using an oral dose of 6 g lactulose. The first study was conducted on the sixth day of treatment during a 10-day course of oral antibiotics (80 mg trimethoprim and 400 mg sulfamethoxazole daily) for a resistant otitis, and shows a minimal increment in H₂ concentration. Sixteen days after suspension of antibiotics, the same dose of lactulose elicited a maximum increase in breath H₂ concentration of 50 ppm. Thus, oral antibiotics dampened the expected H₂ response for a dose of nonabsorbable carbohydrate, presumably by reducing the number of intestinal bacteria available to ferment the substrate.

DISCUSSION

Interval collection and chromatographic analysis of H₂ concentration of samples of expired air has been applied to the evaluation

Fig. 8. The changes in breath H₂ concentration at 0.5-hr intervals over 6 hr observed after 6 g lactulose was administered to subject E.G.d.L. The first study was performed after 6 days of oral antibiotic therapy, the second study 16 days after suspension of the drugs.

of intrainestinal metabolism of carbohydrate in preschool children. This technique was designed to allow sensitive determination of carbohydrate malabsorption with little discomfort to or invasion of the pediatric subject. In practice, it has been found to be well tolerated by preschool children. Minor errors can be introduced into the method by dead space in the collection apparatus and by baseline corrections for peak height measurements on the chromatograms (28), but they are relatively small in view of the constancy of breath H₂ concentration in the basal state, and in relation to the magnitude of changes seen with significant malabsorption.

To estimate the total excess excretion of H₂ in expired air, we have integrated the area under the discontinuous concentration curve in a manner analogous to the calculations used in ¹⁴CO₂ breath tests (10, 25). In this manner, we have demonstrated the same finding of linearity of dose response of respiratory H₂ to nonabsorbable carbohydrates using interval sampling collection techniques and the estimation of excess H₂ excretion as was originally shown by Bond and Levitt (1) with total H₂ excretion from a closed, rebreathing collection system. Our calculated response of 1.2 cc H₂ (excess)/2 hr/g lactulose ingested differs from the volume determined by the previous workers of 1.9 cc H₂ (total)/2 hr/g lactulose (1). However, the difference can be partially explained by the factor of anatomical dead space, and the fact that we were analyzing mixed air while in the previous study alveolar H₂ concentrations were measured.

Preschool children were given oral doses of 1.75 g lactose/kg body wt in the lactose tolerance tests. Increments in breath H₂ concentration of greater than 20 ppm were found to correspond to flat glucose curves whereas increases of less than 15 ppm in H₂ corresponded to normally expected rises in plasma glucose. A borderline "gray area" between 15 and 20 ppm was observed in which the change in H₂ concentration cannot be interpreted in terms of the 20 mg/dl increment in plasma glucose. In no instance of lactose intolerance was there a rise in breath H₂ concentration with the monosaccharide constituents, galactose and glucose. This suggests that the lactose intolerance was due to failure to hydrolyze the disaccharide rather than inability to absorb the monosaccharide components. Throughout the whole series, a rise in H₂ of greater than 15 ppm was never observed with the monosaccharides although in two instances the expected rise in plasma glucose failed to occur. These latter two subjects probably had a delayed gastrointestinal transit of the substrate. The virtual absence of monosaccharide malabsorption in this group of subjects which included severely malnourished children has further clinical implications. Metz *et al.* (19) have shown in adults that an increase in breath H₂ after a load of 50 g glucose can be diagnostic of upper intestinal bacterial overgrowth. Severely malnourished Guatemalan children have previously been shown to have bacteria counts in the upper small intestine of 10⁷ or 10⁸ (18). Although none of the children in this series were directly cultured on admission, they were identical to the population previously reported. It is conceivable that the monosaccharides may have been removed rapidly from the lumen before they encountered significantly large concentrations of upper intestinal bacteria. However, the lack of an H₂ response to a monosaccharide load in recently admitted preschool children suggests to us that not only is the absorptive capacity for monosaccharides relatively unimpaired in severe protein-energy malnutrition, but that in the absence of stasis or anatomic abnormalities, upper intestinal bacteria may not metabolize carbohydrates in amounts sufficient to be detectable by an H₂ breath test.

Bi-hourly observations over a 24-hr period in recovering and fully recovered children consuming their assigned solid or liquid diets revealed a consistent rise in H₂ concentrations at some time during nocturnal sleep. It is tempting to ascribe this phenomenon to an increase in pulmonary excretion secondary to a net increase in intestinal production of H₂, due perhaps to a subclinical carbohydrate malabsorption combined with a corresponding circadian cycle in small intestinal emptying. These factors have not

been ruled out. However, subsequent experiments demonstrated that sleep, itself, has an independent effect on the H_2 concentration change with a given dose of nonabsorbable carbohydrate. The precise mechanism for this sleep effect has not been delineated. A reduced minute ventilation due to hypoventilation during sleep would increase the concentration of H_2 in the breath for a given production rate, but would not account for the magnitude of increases observed in our circadian pattern and induced sleep studies. As pulmonary excretion has been reported to be proportional to intraluminal concentration of H_2 (14), a decrease in colonic motility with a concomitant decrease in the passage of flatus could be postulated as a mechanism. An additional change in the absorption rate of intestinal H_2 secondary to changes in intestinal permeability occurring with sleep could also conceivably play a role. These observations impose a consideration on the performance of breath tests in young children. The subjects should not be allowed uninterrupted periods of sleep after ingestion of their carbohydrate test dose. It has been our experience, however, that brief periods of frequently interrupted sleep do not introduce the sleep artifact into routine tests in infants (unpublished data).

A number of pitfalls in the routine clinical application of the interval sampling H_2 breath tests have been encountered. Firstly, an elevated zero-time concentration of H_2 has been observed which most likely represents a persistence of the nocturnal circadian rise. The spontaneous tendency for this concentration to reequilibrate to usual fasting levels in the face of increasing intestinal hydrogen production due to carbohydrate malabsorption may obscure a diagnosis based on the magnitude of the increase in H_2 concentration. However, since only 5 min are required to determine the H_2 peak on the chromatogram, this artifact can be avoided by analyzing the baseline breath sample prior to administering the dose, and by withholding the carbohydrate until a reasonably normal baseline concentration has been registered. Secondly, extraordinarily delayed gastric emptying or retarded intestinal transit of the administered carbohydrate solution can obscure the breath test results. Variable gastric emptying has previously been found to influence the validity of conventional (blood glucose) lactose tolerance tests in children up to 14 years of age (13). In that report, the artifact was overcome by direct intestinal instillation of the carbohydrate, but such invasive measures defeat the noninvasive purpose of the breath test, itself. Alternatively, prolongation of the observation periods beyond the standard 6 hr may detect a delayed arrival of nonabsorbed carbohydrates to the lower intestine due to retarded transit.

The transient absence of an H_2 producing flora must also be considered in explaining an unexpected flat breath H_2 curve. Bond and Levitt (1) described a subject with the idiosyncratic absence of a flora which would generate H_2 either *in vivo* or in *in vitro* fecal homogenates. We have not identified any such patients in our series. Another factor to be considered in practice, however, is the previous use of broad spectrum oral antibiotics which was observed to obliterate the normal H_2 rise for a nonabsorbable carbohydrate although a baseline production of H_2 was preserved. The expected response to the same dose of lactulose was seen on the 16th day after suspension of the antibiotics. Alterations in H_2 production with oral antibiotics have been reported by Murphy and Calloway (22). In routine practice, therefore, a history of recent oral antibiotic usage should be obtained prior to scheduling of H_2 breath tests, and the test can be postponed until antibiotics have been discontinued. Alternately, the ability to generate an H_2 response could be assessed with a nonabsorbable carbohydrate such as lactulose.

The application of breath hydrogen determinations to the measurement of carbohydrate malabsorption using noninvasive, discontinuous sampling of expired air at intervals has been shown to be well tolerated by preschool children. It has an advantage of reflecting the amount of carbohydrate which is *not absorbed*, which is not the case with conventional tests based on blood glucose. Therefore, any carbohydrate, poorly absorbable or nonabsorbable, as well as readily absorbable, can be used as a

substrate. Moreover, the observations can be extended over longer periods of time, up to 24 hr. A technician can learn the chromatographic techniques in a matter of hours, and the test could be administered by parents in the comfortable setting of home if desired. The precise nutritional consequences to the host of the fermentation of carbohydrates not absorbed in the small intestine is still in question. Bond and Levitt (3) have suggested recently that carbohydrates which reach colonic bacteria are not only fermented, with the evolution of H_2 , but also converted into short chain fatty acids which are absorbed by colonic mucosa. Thus, many of the carbons originating from malabsorbed carbohydrates may eventually participate in useful energy metabolism. The production of H_2 from carbohydrate substrates, however, can be safely interpreted as a failure of small bowel absorption, with or without the participation of abnormally located bacteria, *i.e.*, bacterial overgrowth, and as such the test reflects physiologic abnormalities of clinical significance.

CONCLUSION

A noninvasive H_2 breath test for carbohydrate malabsorption in children using the interval collection and gas chromatographic analysis of expired air following an oral load of carbohydrate has been developed. It is based on the increased pulmonary excretion of intraluminally produced H_2 when nonabsorbed carbohydrates are fermented by certain bacteria of the intestinal flora. Although total pulmonary excretion of H_2 is not determined with interval sampling, both the maximum rise in H_2 concentration and the area under the discontinuous H_2 concentration curve, expressed as *excess* excretion, can be used to quantify the increase in breath H_2 . Lactose intolerance as measured by the conventional technique of blood glucose determination was compared with that determined by increase in breath H_2 excretion. As a clinical test of lactose malabsorption, the breath test is more versatile and less invasive. The validity of routine clinical breath tests can be affected by elevated zero-time concentrations of H_2 , extraordinarily delayed gastric emptying, and by the prior administration of antibiotics which destroy the necessary flora.

The inherently noninvasive and open-ended nature of interval sampling of expired air allows patients to be studied for extended periods of time. Analysis of breath hydrogen at 2-hr intervals over 24-hr periods revealed a circadian pattern with a consistent 2-fold or greater rise in H_2 concentration during the hours of nocturnal sleep. Induced sleep during daylight hours substantially increased the H_2 concentration response to a given dose of nonabsorbable carbohydrate. The present data do not fully explain the mechanism of the sleep-related increase in breath H_2 concentration.

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31. D-Lactulose syrup, Calbiochem, La Jolla, California, USA.
32. Locally purchased commercial lactose in bulk from the same lot was used.
33. Designed for H₂ collection and storage in the Department of Nutrition, University of California at Berkeley.
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