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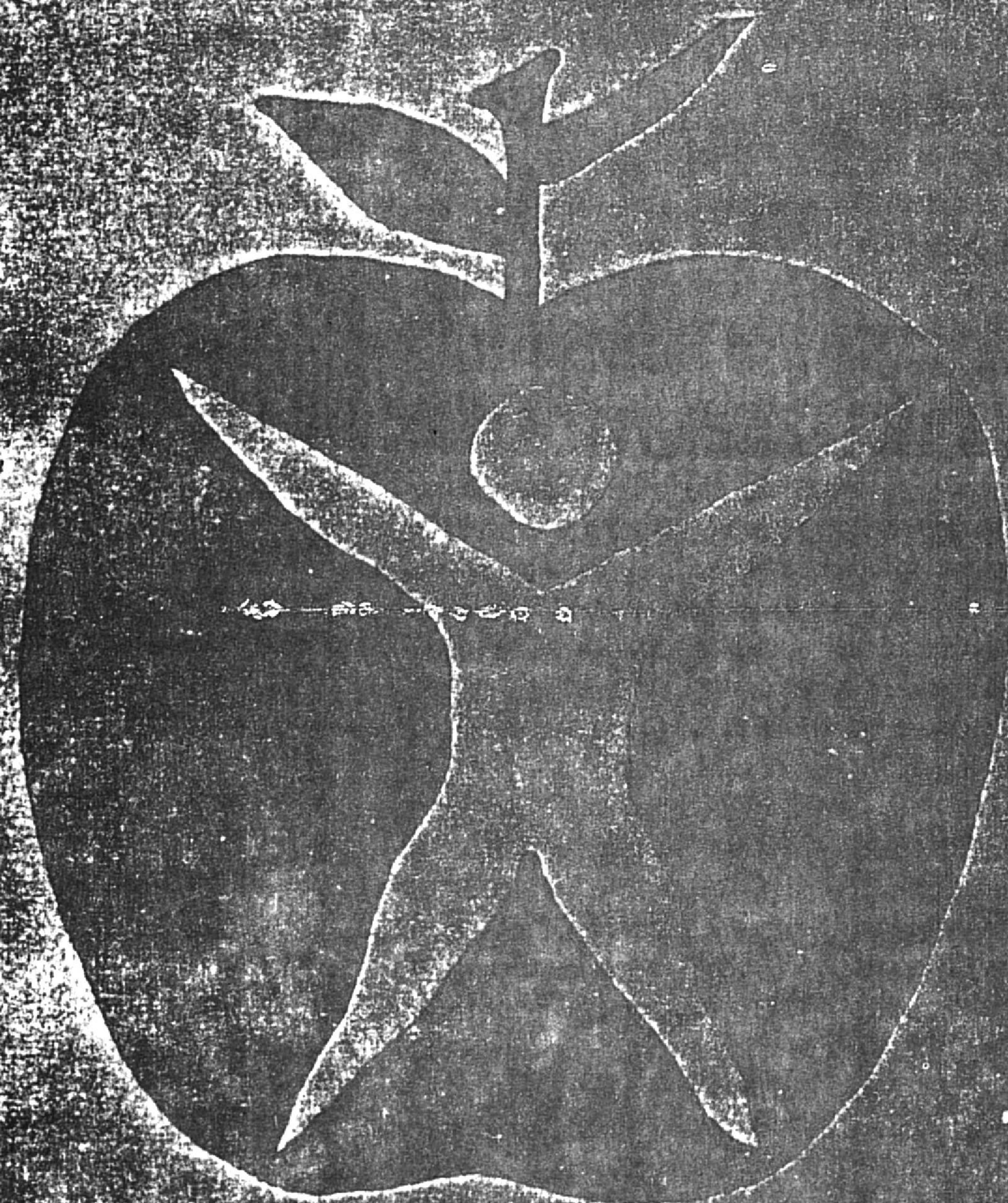

Clinical Consequences of Lactase Deficiency

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Evaluation of Carbohydrate Absorption: The Hydrogen Breath Test in Clinical Practice

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Evaluation of Carbohydrate Absorption: The Hydrogen Breath Test in Clinical Practice

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During the last decade, the breath-analysis test based on measurement of hydrogen (H_2) gas in expired air has moved to the forefront as a clinical and investigative tool. Its simplicity of concept and application, combined with noninvasive collection techniques, improved instrumentation, and versatility, have projected the H_2 breath test to a position of prominence in the field of gastrointestinal diagnosis. It has many applications that go beyond the determination of carbohydrate absorption. Either for application in clinical practice or for understanding the voluminous and expanding literature, the clinician should have a working knowledge of the H_2 breath test. In fact, even companion articles in this issue of *Clinical Nutrition* refer to studies employing the H_2 breath test. The present article endeavors to summarize the principles, pitfalls, and applications of the H_2 breath analysis methodology in gastroenterology and nutrition, with special emphasis on its use in quantifying carbohydrate absorption.

INTRODUCTION

During the past 15 years, the capacity to measure carbohydrate malabsorption and other aspects of its intestinal metabolism for both clinical and investigative purposes has been given a strong impetus by the development and perfection of the hydrogen breath-analysis test. Early in the history of tests based on expired air, a bright future was predicted for the H_2 breath test.[1,2] Recent reviews of the subject attest to the validity of this early optimism[3-8]; in fact, the variety and versatility of application of H^2 breath-analysis have far outstripped the initial projections of the pioneers in this area. In the context of solving clinical problems related to carbohydrate absorption, the H_2 breath test may be our most useful resource. Its simplicity makes it ideal for administration in the office or clinic; the noninvasive nature of the collection procedures makes it ideal for application in pediatrics, even in the neonatology unit. Much of the information in the accompanying articles in this issue of *Clinical Nutrition* was derived from studies employing the H_2 breath test, and a truly comprehensive understanding of the topic cannot be developed without a thorough familiarity with the technique and its interpretation. In the present article, the fundamental principles, applications, and pitfalls of the modern H_2 breath test are discussed as a basis for further reading and for clinical use of the technology.

INTESTINAL HYDROGEN PRODUCTION

Under the appropriate conditions of pH and stasis, certain species of the normal anaerobic flora of the human intestinal tract, usually located in the proximal large bowel, can ferment carbohydrate with the evolution of hydrogen.[9] In the late 1960s, this principle was applied to the diagnosis of carbohydrate malabsorption in man.[10,11] Under ordinary circumstances, orally ingested carbohydrates are quantitatively removed from the intestinal lumen of the small intestine. The upper segments of the gut, moreover, have low concentrations of anaerobes under normal, healthy conditions. However, if there is impaired digestion of disaccharides or complex carbohyrates, or if the conditions of mucosal health or transit time do not permit efficient absorption of simple sugars, or if fecal flora are colonizing the superior portions of the gastrointestinal tract, carbohydrate will eventually come into contact with a fermenting bacterial flora, and H_2 will evolve. Ordinarily, the major part of this gas will be eliminated as flatus, but 14–21% will be absorbed into the bloodstream and eliminated by the lungs.[12] As little as 2 g of carbohydrate reaching the human colon will produce a detectable increment in pulmonary hydrogen excretion.[12] Most individuals harbor the appropriate species for H_2 production in their colons, and the mass of these bacteria determine the H_2 response to a given dose of nonabsorbed carbohydrate. Most sugars—monosaccharides and disaccharides—produce equivalent H_2 volumes both *in vitro* and *in vivo*.[13] Having established these principles, it was a direct extension to collect expired air and quantify the concentrations and/or excretion volumes of breath H_2 to monitor the completeness of absorption of oral loads of carbohydrate.[10,11]

BREATH COLLECTION PROCEDURES

The first objective in an H_2 breath test is to obtain material for analysis—specifically, expired air. Manolis[14] points out the necessity for standardization of breath samples:

> A standardized, reproducible breath sample is critically important for quantitative breath analysis. Without efforts to standardize the breathing and(or) collection technique, the proportion of alveolar air and dead-space air will vary from breath sample to breath sample, leading to highly variable quantitative data.

Early studies from Levitt's lab used a closed rebreathing system in which the rate of production of H_2 (ml/min) could be monitored continuously.[11] This required immobilization of the subject and sophisticated life-support systems for oxygen provision and carbon dioxide removal. It was an invasive system. An alternative strategy was to collect air at fixed serial intervals. Calloway's lab first used this approach, expressing the H_2 values as the absolute concentration (in parts per million).[10,15] This allowed the subject freedom and mobility between collections. To provide representative samples of alveolar air, several investigators introduced open rebreathing systems based on the principles of the Haldane-Priestley tube for use in adults.[16,17]

The open, interval-collection system now made the approach acceptably nonthreatening and noninvasive for application in children; several methods have been described for pediatric application.[18-25] A complex *rebreathing* system analagous to that of adults has been used in children,[18] but it requires consistent cooperation from patient and is unsuitable for infants and toddlers. Direct intubation of the oropharynx was described by Maffei et al.,[19] and validated against a rebreathing procedure, but this approach is somewhat threatening. More comfortable were collections of air made through a nasal catheter and synchronized to the end-expiratory phase of the respiratory cycle, as validated by Perman et al.[20] A modification of a party toy—the 'Wiggin's blow out'—can be used to encourage children to collect air.[23]

However, since conscious, voluntary breathing can alter the pattern of respiration, and crying or sleep-hypoventilation may be a factor in neonates and infants, various pediatric interval-sampling approaches that normalize the H_2 concentration with reference to another respiratory gas have been reported. Niu et al.[24] and Flatz et al.[25] suggest the simultaneous measurement of carbon dioxide for normalization. Robb and Davidson[22] use oxygen and nitrogen for reference. Such procedures complicate the analyses, requiring splitting of the samples and a gas chromatograph capacity for other gas(es) besides H_2. Despite the admonition of Manolis[14] and the various proposed collection modifications, we[26] and others[27] have used the simplest of collections in children, namely, the collection of *mixed* air into a rubber anesthesia gas-bag via a low-resistance, Hans Rudolf, one-way valve fitted to a pediatric anesthesia mask. A variant is the collection of "*total expiratory* air" via a mask and valve into a curved plastic tube designed by Douwes et al.[21] This rigid tube-collector has been modified by Robb and Davidson.[22] Theoretically, in the mixed or total air systems, a contribution of nonexchanged air in the anatomical dead space of the mouth and tracheobronchial tree would dilute the H_2 concentration, but, in practice, the involuntary *hyper*ventilation attendant to conscious breathing washes out alveolar H_2, compensating for the dead-space dilution effect. Moreover, removing the mask and allowing the patient to fill the bag by breathing directly into the inlet of the valve serve as a simple method for collection in *adults*. The practitioner should select the most comfortable and acceptable collection approach consistent with the age of the patient and the diagnostic goals.

Table 1. Steps in the Chromatographic Analysis of Expired Air for Hydrogen

1. The breath sample is collected. End-expiratory samples and samples from a Haldane-Priestley tube or a closed, rebreathing system reflect alveolar air.
2. The sample is injected into a sample loop with a fixed, predetermined volume.
3. A carrier gas flowing at a fixed rate is passed through the sample loop, driving the sample of unknown gas into and through the chromatographic column.
4. By gas-solid chromatography, the gaseous components of the sample are distributed between the two phases and separated.
5. A detector system determines the quantity of hydrogen in the gas passing by the detector.
6. The signal is converted into an analog or digital signal and registered or recorded.
7. The signal produced by the unknown sample is compared to that for the gas in question (hydrogen) in a reference gas mixture with a precisely determined hydrogen concentration.

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STORAGE OF GAS SAMPLES

Hydrogen is the most diffusable of all gases. Storage of a breach sample from the time of collection to the time of analysis—which may range from minutes to weeks—must be accomplished in a vessel that is suitably hermetic, to prevent loss of H_2 concentration. Evacuated rubber-stoppered glass tubes have been used[16,24,28]; they preserve the H_2 concentration up to 3 weeks. Gas-bags of Mylar-impregnated foil have conserved a stable concentration of H_2 for 47 days.[26] In practical terms, however, it is most often only necessary to maintain a sample for the duration of a breath test, i.e., up to 6 hrs. Perman et al.[20] found plastic syringes to be adequate for 8–12 hr: the overall rate of loss of H_2 concentration from plastic syringes is about 5% per day.[29,30] Thus, they make practical and convenient vessels for short-term storage.

QUANTIFICATION OF BREATH HYDROGEN

The principles of gas chromatography relevant to the analysis of expired air have been reviewed.[31] Table 1 summarizes the stages of a typical quantitative determination of H_2 for a clinical breath test. There has been an evolution of the analytical instrumentation for H_2 breath tests in the past decade. In the past, hydrogen analysis based on helium ionization detectors was employed.[10,15,32] It was sensitive enough to detect and discriminate the trace quantities of H_2, but it was a complex, expensive, and difficult technology. Thermal conductivity detection was also available. It, too, was expensive, but it was less sensitive and required rebreathing systems to concentrate the H_2.[12] Major improvements in thermal conductivity gas chromatographs for breath tests were developed,[26,33,34] allowing the determination of H_2 concentrations in whole breath with simplified procedures and at reduced cost. More recently, electrochemical[35,36] and electronic[37,38] detectors have been developed, simplifying the process of breath H_2 analysis even further. Some apparati, once calibrated with a standard reference gas of known H_2 concentration, will provide a digital read-out of the unknown concentration of H_2 in a sample of expired air in a matter of seconds.[38]

It has been demonstrated that the rates of pulmonary H_2 excretion in the fasting state are low and relatively stable.[11,12,26,34,39]. When continuous, closed collection systems are used, the absolute rates of H_2 production and the total volume produced can be measured. With *interval* collection systems, the concentration of H_2 is the index. By simultaneous comparisons of the change in blood glucose[16,26,34,40] and other correlative observations,[41] it has been established that a rise of ≥20 ppm represents a biologically significant fermentation of carbohydrate. It has been argued that the stability of basal H_2 levels allows an increment criterion of ≥10 ppm,[42] but significant overlap with the intrinsic variability of breath H_2[39] would militate for a more ample criterion, such as the ≥20 ppm increment, for diagnosing incomplete carbohydrate absorption.

Even with interval-sampling procedures, additional quantification of the total production of H_2 following a dose of carbohydrate can be achieved by integrating the area under the discontinuous curve of changes in breath H_2, adding a factor for total ventilation.[26,34] This approach has been used extensively in studies employing interval breath H_2 collections.[43–45] An even simpler quantitation procedure involves triangulation of the graphic display of the H_2 concentration data, or the expression of the cumulative sum of interval changes in breath H_2 levels during the period of observation.[46]

The unsolved problem is relating total excess H_2 production to the *absolute amount* of carbohydrate that is not absorbed in the small bowel and hence is fermented in the colon. Each individual has a different mass of colonic bacteria, and consequently a different response of H_2 per gram of carbohydrate. Some have suggested that a reference dose of a non-absorbable sugar, such as lactulose, be given.[47] Indeed, stoichiometric relationships of graded levels of administered lactulose and total breath H_2 elimination have been demonstrated within individual persons.[13,34] However, when more complex carbohydrates are used, or when a food like milk is the test dose, the lactulose response fails to reflect the test carbohydrate in question. Thus, at the present time, the use of the ≥20 ppm criterion to decide whether significant malabsorption has occurred, and some form of integration of the interval values into an expression of net H_2 volume excreted (if serial, intrasubject comparisons are to be made), represent the most appropriate systems for quantifying the H_2 response to an oral dose of carbohydrate.

CLINICAL APPLICATION OF THE HYDROGEN BREATH TEST

Carbohydrate Absorption Tests

Various sugars have been used in conjunction with the H_2 breath test in a clinical context. These diagnostic uses are listed in table 2.

Lactose Digestion. By far the greatest single use of the H_2 breath test has been in evaluation of the completeness of lactose digestion, and the uptake of the hydrolysis products, glucose and galactose. Techniques for clinical application have been reported

Table 2. Clinical Applications of Hydrogen Breath Analysis In Assessing Carbohydrate Absorption

Lactose malabsorption
Sucrose malabsorption
Glucose malabsorption
D(-)xylose absorption
Inborn errors of monosaccharide metabolism
Investigation of "functional" gastrointestinal complaints
Evaluation of intestinal adaptation after gut resection

Studies failed to support any relationship between irritable bowel symptoms and impaired lactose digestion.

for both adults[11,13,15,32,39,40,47-50] and children.[19,27,24,51-56] The test was rated as a successful approach in all but one[56] study. In two independent reports, breath H_2 excretion was found to be the indirect measure of lactose absorption that most closely reflected direct mucosal lactase assay results[16] and intestinal perfusion data on lactose digestion and uptake.[47]

The conventional dosage of lactose for a "lactose tolerance test" based on the rise in blood glucose has been 2 g/kg for children up to 25 kg, and 50 g for all other children, adolescents, and adults. The dosage is given in a concentrated (generally 20%) aqueous solution.[4,57] This form of administration has been carried over, to some extent, into breath H_2 studies.[16,40] Fifty grams of lactose represent the sugar content of a liter of milk, and no food (except whey solids) has a lactose concentration of greater than 7%.* Given the sensitive detection threshold of the H_2 breath test *there is no longer any necessity to use unphysiological amounts or concentrations of lactose*. Milk itself can be used,[15,32,39,41,43,44,52,54] and the dosage of lactose can be reduced to the physiological (dietary) range, such as the 12 g in an 8-oz glass of cow's milk.[39,47] Admittedly, intestinal lactase levels have been correlated with the response to a 50-g dose of lactose with both blood glucose[57] and breath H_2,[16,40] but a lactose absorption test in clinical practice most often seeks information of *dietary relevance*. In this context, a physiological amount of whole milk might be the most commonly justified dosage form for a clinical lactose H_2 breath test. The lower dosage, however, obligates the collection of at least three postdose breath samples to obtain acceptable sensitivity.[58] As discussed below, lactose digestion/absorption has been evaluated by the H_2 breath test not only in the strict clinical context, but also with functional gastrointestinal complaints, disease states, and dietary issues.

Digestion/Absorption of Other Sugars. Sucrose maldigestion as a consequence of sucrase-isomaltase deficiency (hyposucrasia) has been evaluated using the H_2 breath analysis approach in adults[59] and children.[20,60,61] In one study, it failed to correlate with the biopsy-proven disaccharidase status.[61] As a cause of gastrointestinal complaints, hyposucrasia appears to be rare[60] the test's routine application, in the absence of specific clinical suspicion, will have a low positive yield among patients with gastrointestinal symptoms of unknown origin.

Glucose malabsorption results from both acquired gastrointestinal defects and congenital conditions. The H_2 breath test has been used diagnostically in both contexts. The most common cause of incomplete glucose absorption in adults is gastric surgery. The H_2 breath test can be used to determine the magnitude of glucose malabsorption in gastrectomy patients.[12,62,63] The malabsorption of monosaccharides has also been studied in infants with primary glucose-galactose malabsorption.[36,61,64]

D(-)Xylose absorption is used as a marker of mucosal health and integrity even though D(-)xylose is a *pentose* sugar and does *not* share the same mechanism of intestinal uptake with the common dietary hexoses (glucose, galactose, or fructose). Its uptake is measured by the rise in blood levels or by 6-hr urinary excretion. A logical application of the H_2 breath test would be the quantification of malabsorbed D(-)xylose. This has been attempted[11,65] (BS Kirschner, C Lahr, D Lahr, unpublished), but because a large fraction of D(-)xylose is not absorbed even by the normal intestine, the discrimination between normals and defectives is difficult. Modification of the approach might permit a useful D(-) xylose breath test to be developed in the future.

Sugar Absorption and Gastrointestinal Function. The etiological role of sugar malabsorption in syndromes of gastrointestinal discomfort has been explored using the H_2 breath test as the arbiter of incomplete carbohydrate absorption. Recurrent abdominal pain of childhood is a common clinical problem in pediatrics. Studies at the Boston Children's Hospital suggested that impaired digestion of lactose was an etiological factor in this syndrome,[66-68] but studies from England[69] and France[70] failed to confirm this association. In adults, lactose maldigestion has been suspected of having an association with the "irritable bowel syndrome" (functional bowel disease). Careful studies at the Mayo Clinic, assisted by breath analysis, failed to support any relationship between symptoms and impaired digestion of lactose.[71]

The H_2 breath test has been instrumental in elucidating an obscure form of gastrointestinal pain, that due to malabsorption of sorbitol. Sorbitol is a sweetening agent used in "sugar-free" chewing gum, and, as a sugar-alcohol, is fermented. Children consuming large quantitites of gum experienced abdominal pain, which was traced to the colonic fermentation of non-absorbed sorbitol.[72,73]

The ability of the residual small intestine to absorb dietary carbohydrate after substantial resection is variable, but usually a significant compensatory adaptation occurs in patients with short-bowel syndrome. The evolution and magnitude of this adaptation has been measured longitudinally in infants who had intestinal ablation,[74] and this approach to monitoring the completeness of absorption of dietary carbohydrates may serve as a guide to dietary therapy.[74]

Other Diagnostic Uses of the Hydrogen Breath Test

In clinical gastroenterology, the H_2 breath test has a series of applications that deal with intraintestinal metabolism of carbohydrate and pathophysiology, but not specifically in relation to the absorption of dietary carbohydrates (table 3). The foremost use is in the detection of bacterial overgrowth of the normally sterile superior segments of the gastrointestinal tract. Metz et al.[75] used 50 g of oral glucose, whereas Rhodes et al.[76] used 10 g of oral lactulose. An *early* postdose peak in H_2 elimination must be sought. The H_2 breath test complements the cholylglycine (bile salt deconjugation) breath test, but is not as reliable as direct intubation and culture for diagnosis of upper intestinal bacterial colonization.

The transit time of a meal or a solution from mouth to colon can be determined by observing the interval between the ingestion of a nonabsorbable carbohydrate, such as lactulose (or lactose in a lactose-malabsorber), and a detectable rise in breath H_2 excretion, which signifies the initiation of colonic fermentation.[77-83] It has application in patients with reduced or accelerated transit of food.

Table 3. Clinical Applications of Hydrogen Breath Analysis in Gastrointestinal Diagnosis

Bacterial overgrowth of the small intestine
Gastrointestinal transit time (mouth-to-colon)
Monitoring neonatal flora
Surveillance for necrotizing enterocolitis
Diagnosis of pneumatosis cystoides intestinalis
Monitoring the safety of colonoscopic surgery

*Bovine milk and full-milk ice-cream have lactose contents of about 5%; human breast milk has a lactose concentration of 7%

The H_2 breath test can also be used to monitor the changes in bacterial colonization of the gastrointestinal tract at the level of the *large* intestine. The evolution of the mass of fermenting bacterial flora has been studied in premature infants.[84] It has been projected that a sudden increase in H_2 production by preterm infants is a harbinger of necrotizing enterocolitis,[85] but further confirmation of this observation is needed.

The rare and usually benign interstitial accumulation of gas in the bowel wall, *pneumatosis cystoides intestinalis*, produces a massive fermentation and evolution of breath H_2 when oral glucose is administered.[86] Accumulation of a potentially explosive mixture of hydrogen (or methane) in the colon is a less benign consequence if colonoscopic electrocauterization is contemplated. Fatal explosions during intracolonic endoscopic procedures have been reported. Mannitol, a fermentable sugar-alcohol, has been used as a purgative to cleanse the colon prior to colonoscopy. LaBroody et al.[87] attempted to use breath H_2 as an indirect index of intracolonic concentrations of that gas. Their results indicated that the breath H_2 test was poorly reflective of the intraluminal concentration, but further exploration of breath H_2 monitoring of colonoscopy patients is warranted. A practical guide to the administration of the more commonly used H_2 breath tests in clinical practice is presented in table 4.

INVESTIGATIVE APPLICATION OF THE HYDROGEN BREATH TEST IN GENETIC, GASTROINTESTINAL, AND NUTRITIONAL RESEARCH

In addition to the clinical applications discussed above, H_2 breath analysis is being applied widely in research. To better understand the scientific literature, an appreciation of the trends in investigative uses of the technology is useful. An outline of such applications is presented in table 5.

Surveys of Lactase Status of Various Regional and Ethnic Groups

Because of the nonthreatening, painless, and noninvasive nature of breath collections, the H_2 breath test has clear advantages over blood glucose tests or intestinal biopsy for investigation of lactase status in field studies. The H_2 breath-analysis procedure has been used to determine the prevalence of lactase deficiency in various regional and ethnic groups, including: North American Native Americans,[41,88-92] Bangladeshi villagers,[93] Japanese,[19] Greeks,[81] German,[25,94] Austrians,[95] Australian aborigines,[96] and a Sepik population of Papua New Guinea.[28] Several technical considerations, including breath collection techniques[97] and spacing of sampling intervals,[58] have been adapted for survey use.

Relationship of Carbohydrate Absorptive Capacity to Age

The relationship of carbohydrate absorptive capacity to chronological age has been investigated using the H_2 breath test. The ability of premature infants to absorb lactose has been examined.[43,44] The evolution of this capacity in the preschool years has also been investigated,[19,93] and it has been studied in the elderly.[98,99] An inability to completely absorb the carbohydrates of a mixed meal has also been identified as a consequence of intestinal senescence in geriatric subjects.[29]

Surveys of Carbohydrate Absorptive Capacity in Specific Disease Conditions

The H_2 breath test has been used to determine carbohydrate absorption with respect to specific disease states. An important application has been in assessing the prevalence of lactose digestion impairment in diarrheal diseases in children, both chronic diarrhea[52,56,100] and acute gastroenteritis.[101,102] Another application has been the relationship of lactose malabsorption and milk rejection to the dietary calcium deficit that may contribute to osteoporosis.[103-5] Recently, Cochet et al.[106] determined patients' lactose digestion capacity using the H_2 breath test, and they showed impaired calcium absorption in lactose-malabsorbers who took the isotope in the presence of dietary lactose.

The H_2 breath test has been used to clarify the relationship between intestinal parasitoses and lactase activity. In studies from Mexico[107] and Panama,[108] deworming of *Ascarsis*-infected children improved the digestion of an oral dose of lactose.

Finally, the effect of cancer chemotherapy on intestinal lactose digestion was investigated by Hyams et al.[109] Using serial H_2 breath tests, they observed a deterioration of lactose absorption capacity in children undergoing intensive antitumor regimens.

Evaluation of the Absorbability of Colonic Fermentation of Specific Carbohydrates in Foods and Diets

Since the early studies of Calloway et al.,[15] foods—specifically milk and cheese—have been used as the source of carbohydrates for H_2 breath tests. Milk, alone[15,32,39,41,43,44,52,54] or with other foods, such as wheat[110] or rice,[111] has been evaluated. It was demonstrated that dietary fiber could be fermented, giving rise to a detectable H_2 response,[112-114] as could beans[79,115,116] and certain fruits.[117] Even products from refined wheat flour, such as breads and pasta, produce a measurable in-

Table 4. Guide to the Clinical Application of the Hydrogen Breath Test in Certain Diagnostic Situations in Gastroenterology

Condition	Administer	Draw Breath Samples	Positive Reaction
Lactose malabsorption	50 g in 250 ml H_2O[a,b] 18 g in 100 ml H_2O 18 g as 360 ml milk	Every hr for 6 hr	Increase > 20 ppm at any sampling period
Sucrose malabsorption	50 g in 250 ml H_2O[c]	Every hr for 6 hr	Increase > 20 ppm at any sampling period
Bacterial overgrowth	50 g glucose in 250 ml H_2O or 10 g lactulose in 20 ml H_2O	Every 10–15 min over 4 hr (glucose) or until appearance of colonic peak (lactulose)	Increase>20 ppm with glucose or lactulose
Gastrointestinal transit time	10 g lactulose in 100 ml H_2O	Every 5–10 min until appearance of colonic peak	Mouth-to-colon transit time <50 min

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[a] The 50-g pharmacologic dose is only for genetic or stress test studies; the lower, "physiologic," dose is recommended in other cases.

[b] Pediatric procedure: 2 g/kg body weight up to 50 g as 20% solution or milk (12 g, first year; 15 g, second year; and 18 g, third year); sample every 30 min for 6 hr.

[c] Pediatric procedure: 2 g/kg body weight up to 50 g as 20% solution; sample every 30 min for 4 hr.

Table 5. Investigative Application of the Hydrogen Breath Test in Genetic, Gastroenterological, and Nutritional Research

Surveys of lactase status of various regional and ethnic groups
Surveys of carbohydrate absorption capacity in specific disease conditions or age groups.
Evaluation of the absorbability or fermentability of specific carbohydrates in foods and diets.
Study of dietary manipulations and interventions to improve carbohydrate tolerance.
Correlation with other diagnostic indices of carbohydrate absorption.

crement in pulmonary excretion of H_2.[118] Douwes et al.[119] described the evolution of carbohydrate absorption in neonates, comparing breast milk with a low-lactose and high-lactose infant formula. It has recently been shown by breath H_2 analysis that fructose—the major constituent of the sweetener for carbonated beverages—is not completely absorbed.[120]

Study of Dietary Manipulation and Interventions to Improve Carbohydrate Tolerance

After it was amply established that food carbohydrate metabolism could be evaluated using the H_2 breath test, the obvious departure was to determine to what extent manipulation of the diet could favor the utilization of carbohydrates. Investigations have been focused primarily on lactose digestion by exogenous lactose hydrolases and on dietary fiber sources. Beta-galactosidase enzymes are available from several microbial sources. A number of investigators have shown that *in vitro* incubation of milk with these microbial 'lactases' will convert a positive breath H_2 response into a negative (flat) one in lactase-deficient persons.[120–23] The use of *Acidophilus*-treated milk, however, had no effect on lactose maldigestion.[123] It has subsequently been demonstrated using the breath H_2 technology that *in vivo* administration of exogenous beta-galactosidases *at mealtime* can provide an effective "enzyme replacement therapy" in adult lactose-malabsorbers.[124–27] Even the intrinsic lactase produced by the microbes in a culture of yogurt will achieve substantial activity when raised to body temperature *in vivo* in the human intestinal tract.[128,129]

The potential of dietary fiber to alter the pattern of carbohydrate absorption has also been examined. Psyllium, the common bulking agent, has been shown to reduce the breath H_2 response to the oral administration of lactose in lactase-deficient subjects[130] and of glucose in postgastrectomy patients with dumping syndrome.[131] Breath H_2 analysis has also been used in concert with blood glucose and plasma insulin determinations to demonstrate that the reduced rate of glucose uptake in the presence of legumes was not associated with incomplete absorption of the sugar.[132,133]

LIMITATIONS AND PITFALLS OF BREATH HYDROGEN ANALYSIS AND INTERPRETATION OF BREATH TEST RESULTS

The application of breath H_2 analysis in clinical diagnosis and investigation is not without limitation. A number of potential pitfalls and caveats must be understood. These are listed in table 6 and discussed in the present section.

Table 6. Limitations and Caveats in Application and Interpretation of Hydrogen Breath-Analysis Tests

Idiopathic absence of appropriate bacterial flora
Prior use of oral antibiotics
Prior use of high-colonic enemas
Chronically acid colonic pH due to continuous fermentation
Active diarrheal disease
Elevated basal H_2 concentrations in the fasting state
Delayed gastric emptying
Cigarette smoking
Sleeping during the test
Administration of the test carbohydrate with a dietary fiber-containing meal
Administration of the test with a glycoprotein-rich meal
Storage of samples in contaminated evacuated glass tubes

The application of breath H_2 analysis in clinical diagnosis is not without limitation.

Idiopathic absence of sufficient numbers of bacterial flora to ferment carbohydrates and mount an H_2 response to malabsorbed sugars occurs. The frequency varies from region to region. Estimates range from 2% of the population in Minnesota[134] to 21% in Israel.[135] Oral antibiotics and rectal enemas can disrupt the fermenting flora. This occurs when patients are prepared for colonic surgery.[135] Broad-spectrum antibiotics will eliminate the H_2-producing species,[34,115] but Neomycin was shown to *favor* the fermenting flora and to *increase* the H_2 response.[115]

In addition to elimination of or changes in colonic flora, the intracolonic conditions can determine the efficiency of carbohydrate fermentation and determine H_2 production. Perman et al.[136] demonstrated that acidification of intestinal flora *in vivo* and *in vitro* reduced the rate of H_2 production. Thus, chronic malabsorption of carbohydrates might suppress colonic flora and produce false negative responses. We now allow at least 48 hr between successive absorption tests to allow the acidifying effect of the former procedure to abate.

Active diarrheal disease also reduces the magnitude of the breath H_2 response.[137–42] This might explain the poor yield of malabsorbers in one study,[56] although many investigators have evaluated carbohydrate absorption in patients during diarrheal episodes.[52,100–102] It has been suggested that the diagnostic criterion for a positive test might be altered (downward),[139] but we feel that diarrhea reduces the sensitivity of the diagnosis of malabsorption.[141] The most prudent course is to avoid using H_2 analysis until the patient has recovered from the diarrhea.[141,142]

In any of the aforementioned conditions, idiopathic absence of flora, antibiotic and enema treatments, chronic acidification of colonic contents, and gastroenteritides, the capacity of the individual to mount an appropriate H_2 response can be tested by administering an oral dose of lactulose. Only if the patient has a normal rise with the nonabsorbable sugar should the test carbohydrate be administered in a formal test.

When the *interval*-sampling approach is used, the diagnosis of malabsorption hinges on the *change* in H_2 concentration with respect to the fasting baseline level. This basal concentration of H_2 is often elevated,[34,143] with an inherent tendency to readjust (downward) during the ensuing hours. This can confound and obscure the increase in H_2 excretion attendant to carbohydrate malabsorption. Kolter et al.[143] avoid this pitfall by preparing the patient with a special carbohydrate-free diet on the day prior to a breath test.

Since sample collections proceed for a defined postdose interval (2–6 hr), delayed gastric emptying can interfere with the interpretation of absorption tests. When the assay is the rise in blood glucose, gastric retention produces false-positive (abnormal) results.[144] When the assay is breath H_2, failure to empty the stomach could lead to false-negative (normal) findings in a patient indeed destined to malabsorb and produce breath H_2, but *after* the collection period had elapsed. We have documented the confounding influence of prolonged gastric retention on the diagnosis of lactose malabsorption in a child recovering from severe protein-energy malnutrition.[145]

Cigarette smoke contains H_2 and other reducing gas(es) that register on gas chromatograph detectors.[146,147] An interval of 12–15 min between the cigarette and the collection of a breath sample is usually sufficient for re-equilibration to the breath H_2 concentration that reflects endogenous (intestinal) production, but the most prudent approach is to proscribe smoking during the entire duration of a breath test procedure.

Sleeping during an H_2 breath test causes a false increase in the *concentration* of breath H_2[148,149] as compared to the same test in the alert, mobile subject. This might be due, in part, to hypoventilation, but changes in the partition of gas elimination between blood and flatus or a circadian pattern of small bowel emptying cannot be ruled out. Patients should remain awake throughout the duration of the test, or at least be aroused periodically, as in the case of young infants, to eliminate this artifact.

If the test carbohydrate is given with a meal, or if food is allowed during the study, it is advisable to scrutinize the content of the meal. Fiber-containing foods[112] and foods rich in glycoproteins[150,151] are likely to provide substrate for bacterial fermentation and H_2 excretion independent of the fate of the test sugar. Two sources of potentially nonabsorbable carbohydrate and the superimposition of their respective contributions to breath H_2 confuse the interpretation of the absorption test.

Finally, the storage of H_2 in evacuated glass tubes is not without its own pitfall. Heat-sterilized tubes—both silicone-coated and uncoated—release a reducing gas that produces false elevations of the measured quantity of H_2.[152] Concentrations of H_2 up to 3000 ppm have been obtained from unused sterilized Vacutainer tubes.

CONCLUSION

The analysis of expired air for H_2 content as a reflection of intestinal carbohydrate fermentation is a powerful and versatile diagnostic tool, and is perhaps the most useful indirect approach to the evaluation of carbohydrate absorption. It can be applied to the assessment of glucose absorption and sucrose and lactose digestion. Dietary items (foods) themselves can be used instead of concentrated aqueous solutions. The results can give guidance to diet formulation or can clarify the origins of a syndrome of gastrointestinal discomfort. In clinical gastroenterology and in nutritional research, a host of other novel applications of breath H_2 have been developed. A thorough familiarity with the H_2 breath-analysis technology and the scientific literature that employs it will greatly aid the practitioner and investigator in understanding advances in carbohydrate metabolism.

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